Research Article

First Generation Amperometric Glucose Biosensor For Determination Of Glucose At Room Temperature Using Glucose Oxidase

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Accepted on: 15-04-2016; **Finalized on:** 31-05-2016.

ABSTRACT

Glucose biosensors are used to detect the glucose concentration in a sample. In the first generation amperometric biosensor, an enzyme glucose oxidase is used which is reduced on reacting with glucose yielding Hydrogen Peroxide as a product which then breaks down to Water and Oxygen. The latter reaction is studied by electrolysis. Two electrons are released in the process which produces current. The amount of current produced by the system and due to the release of the electrons is detected by an ammeter. This is directly proportional to the concentration of glucose present. Glucose measurements are commonly based on interactions with one of three enzymes: hexokinase, glucose oxidase (GOx) or glucose-1-dehydrogenase (GDH). A linear graph plots the current and voltage. All the values were plotted in the form of a tabular column and these values were obtained by using Faraday's law of electrolysis and efficiency equations. The efficiency was shown in the tabular column with the first trial (V=1) showing maximum efficiency.

Keywords: Diabetes, Glucose oxidase, electrolysis, signal, biosensor.

INTRODUCTION

Diabetes or diabetes mellitus (DM) is a metabolism disorder in which the quantity of glucose in the blood is highly elevated due to the inability of the body to produce enough insulin[1]. Type 2 diabetes, characterized by hyperglycemia, has become a significant concern and 8.4% of the total deaths are caused by this form of disease[2]. This excess blood glucose eventually passes out of the body in urine. As of 2013, an estimated 382 million people have diagnosed with diabetes worldwide, with type 2 diabetes making up about 90% of the cases[3]. It has been studied that such glycemic concentration is also associated with early menarche and cardiovascular risk factors[4]. Thus, it is essential to understand the causes of this disease and to devise more effective strategies for its treatment[5]. There are many conventional ways of diagnosing a patient for diabetes[6] but these are very slow and cumbersome and clinically accurate glycemic monitoring still poses a major challenge[7]. A fuzzy hierarchical model has been developed to detect early signs of positive DM[8]. The advent of biosensors has greatly increased the scope of detection of blood glucose level with the help of glucose biosensors. There are three generations of glucose biosensors. In this paper we discuss the process and design of a first generation glucose biosensor.

Biosensors

Biosensor is a compact analytical device that uses specific biochemical reactions to detect and measure target compounds in biological samples[9]. There are three main parts of a biosensor (i) the sensitive biological recognition elements that recognizes the target molecules in the presence of various chemicals such as receptors, enzymes, antibodies, nucleic acids, microorganisms and lectins (ii) a bio transducer converts a biochemical signal to an electronic or optical signal that can be more easily measured and quantified and (iii) a signal processing system that converts the signal into a readable form.

Biosensors are mainly used for screening of diseases. It monitors the blood glucose level in diabetic patients and detects the hCG protein in urine for pregnancy tests. The selection of a particular food by a consumer is largely based on sensory perceptions with taste, which depend on factors like saltiness, sweetness, bitterness and acidity as the most important factors. Some biosensors are used for food analysis (to measure carbohydrates, alcohols and acids), study of bio molecules and their interaction with gold nanosensors[10], crime detection and other medical diagnosis. Biosensors help in controlling environmental pollution by measuring the toxicity of water bodies and microbial contamination of natural resources[11]. The devices may also be used to check fermentation during the production of beer, yoghurt and soft drinks.

Due to their numerous attributes, biosensors offer an accurate, fast, relatively cheap, stable, portable, easy to calibrate and user-friendly device for in situ monitoring of product maturity and quality along with robustness and ruggedness[12].

Glucose and Glucose oxidase

In a solution of neutral pH, glucose exists in a cyclic hemiacetal form (63.6% β-D-glucopyranose and 36.4% α-D-glucopyranose). The GOx binds specifically only to β-D-glucopyranose form. It is able to oxidise all of the glucose in solution because the equilibrium between the α and β

anomers is driven towards the β side which is consumed in the reaction.

For all practical purposes, glucose measurements are detected based on interactions with one of the three enzymes: hexokinase[13], glucose oxidase (GOx) or glucose-1-dehydrogenase (GDH). GOx is the most common enzyme for biosensors as it has a relatively higher selectivity for glucose in comparison to the others. It is inexpensive, easy to obtain and can withstand extremes of pH and temperature. Certain amperometric biosensors use combination of these enzymes to determine glucose concentration[14].

Electrolysis

A biocatalyst generates or consumes an electro active species (ex. O_2 or H_2O_2) during electrolysis, whose amount can be calculated by stoichiometric means[15]. Thus, the measured current intensity, generated by the electrochemical reaction is proportional to the analyte concentration. Amperometric biosensors are based on redox reactions of such electro active species generated in an enzymatic reaction[16]. During an amperometric measurement, the electrode or sensor is held at a constant potential and the current is monitored. The current is then used to calculate the concentration of the analyte present.

The amperometric glucose sensors have advanced over three generations:

The first generation: This method uses the amount of H_2O_2 produced in the reaction to determine the concentration of glucose presen[3]. This reaction takes place in the presence of glucose oxidase (GOx)[17] which contains the group FAD (flavine adenine dinucleotide). FAD is reduced to $FADH_2$ and oxygen is released in the process. However, the main drawback of such biosensors is that the amperometric measurement of hydrogen peroxide required a high operation potential for high selectivity. Another problem faced is the limited solubility of oxygen in biological fluids, which produce fluctuations in the oxygen tension, also known as the "oxygen deficit".

Biocatalyst

$$FADH_2\text{-oxidase} + O_2 \longrightarrow FAD\text{-oxidase} + H_2O_2$$

Electrode

$$H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^-$$

Hydrogen peroxide is oxidized at a catalytic, platinum (Pt) anode[18]. The electrode recognizes the number of electron transfers with great efficacy and this electron flow determines the number of glucose molecules present in blood.

The second generation: This system replaced the oxygen with a synthetic electron acceptor called a mediator. These are small redox active molecules (e.g., ferrocene derivatives, ferrocyanide and quinones). These mediators react with the active site of the enzyme and with the electrode surface, shuttling the electrons. However, the redox mediators are not that selective causing major hindrance to the process. Glucose detection can be carried out at concentrations from 0mM to 20mM[19].

The third generation: They are reagent less and require no mediator[20]. There is a direct electron transfer between proteins[21] and this gives them high selectivity. If incorporated in certain membranes of proteins, the electron transfer can be greatly enhanced, offering greater selectivity[22].

MATERIALS AND METHODS

Glucose powder was purchased and 0.09 g, 0.018 g and 0.009 g of glucose were weighed using electronic weighing machine. The above mentioned quantities were dissolved in 100 ml of distilled water giving 0.05 M, 0.001 M, 0.005 M glucose solutions respectively. 3 ml of glucose isomerase enzyme (figure 1) was added to each beaker.

Figure 1: Glucose isomerase enzyme

The glucose concentration was determined through electrolysis as shown in figure 2. A platinum electrode (figure 3), coupled with a standard electrode were used and potentials of 1 V, 5 V, 0.5 V respectively were supplied to the circuit. The glucose oxidase added to the solution started the flow of electrons and the current produced was measured through a multimeter. The time taken for the reaction to occur at a constant current level was measured using a stopwatch for each glucose solution.

Figure 2: Experimental Setup

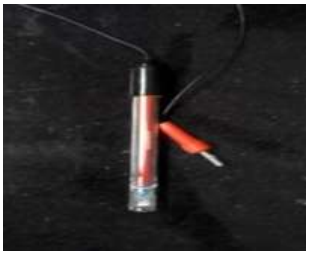

Figure 3: Platinum electrode

Charge, $Q = I * t$ and mass, $m = \frac{(Q \times M)}{(F \times z)}$, (Faraday's law of electrolysis)

Where,

Q = Charge

I = Current produced in the circuit

M = Molar mass of Glucose

F = Faradays constant

z = Number of electrons transferred in a single reaction of glucose

Efficiency $\eta = \frac{Output\ level\ of\ Glucose}{Input\ level\ of\ Glucose} \times 100$

Observation

On application of various voltages, the current produced for the conversion of H_2O_2 to H_2O and O_2 was recorded with the help of a multimeter. The exact time that it takes for the reaction to complete under the given voltage was observed. The net charge transfer is given by the formula Q=It where both I and t are measured values. Using Faraday's second law the mass of a material that has been consumed in a reaction at an electrode is directly proportional to its equivalent mass. Since we know Q, F (faraday's constant), M and z for a particular reaction and Q can be measured using the aforementioned formula, the mass of substance that has been consumed in this electro-chemical reaction can be calculated. This mass of glucose calculated experimentally can then be verified with the mass of glucose that was initially supplied into the system, giving us the efficiency.

It was observed that for high and low voltage values the efficiency of the glucose sensor reduced while for a median voltage value there was a maximum efficiency of 98.8%.

A graph was then plotted using the current and voltage values with current in milli amperes on the y-axis and voltage in volts on the x-axis.

The readings taken were calculated and the results obtained are shown below in the table and the graph was plotted taking the x-axis as voltage and y-axis as current (figure 4).

Table 1: Determination of glucose concentration by Faraday's Law

Conc. of glucose	Voltage	Current	Time	Charge	Mass of Glucose	Efficiency
(M)	(V)	(mA)	(sec)	(Coulomb)	(g)	(%)
0.05	1	9	10700	99	0.089	98.8
0.001	5	30	630	18.9	0.017	94.4
0.005	0.5	6	1500	9	0.0083	92.2

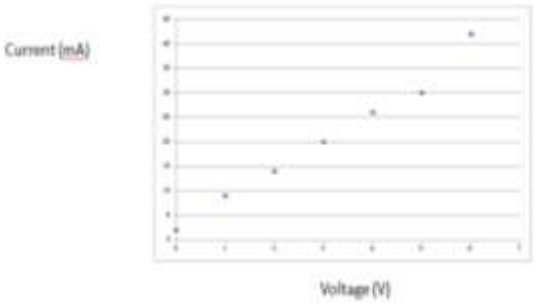

Figure 4: Graph depicting the relationship between voltage applied and current generated.

RESULTS AND DISCUSSION

The significance of the project is the prevention and control of diabetes as it is a disease causing millions of lives every year. DC Voltage source provided potential for the dissociation of hydrogen peroxide to form water and oxygen. The rate of conversion of hydrogen peroxide was directly linked to the potential applied. Platinum electrode coupled with a standard electrode was used and the reaction rates determined by the current flow, and detected by a multimeter. The current flow is in direct proportion with the glucose concentration[15]. It is due to the fact that the oxygen consumption linearly increases with the increase in glucose concentration[13]. On increasing the concentration of glucose, the rate of reaction slowed while an increase in potential increased the rate of reaction. Thus the factors affecting the rate of reaction were concentration of glucose, concentration of glucose oxidase and the voltage applied. The trials were conducted in a cell free environment. Concentration of glucose in the blood is much lesser in comparison to the concentrations used in the experiment. The accurate measurement of glucose in minor concentration is still under study. For a cell free system an average efficiency of 95.13% was achieved.

CONCLUSION

The primary objective of the experiment was to demonstrate the concept and working of bio-sensors in the detection of glucose concentration and its application in the detection and monitoring of diseases such as diabetes. The lab scale model that we worked on showed an encouraging result in accordance to our primary aim. The detection of glucose levels in our samples was obtained with a high degree of accuracy. This experiment hence proves that the idea of usage of bio-sensors for

detection of various diseases is possible and also very viable. Our secondary objective was to popularize the concept of bio-sensors which is still at a very nascent stage. With new diseases propping up every day it is essential that we have a cost effective, efficient and accurate solution for a monitoring and detection system, we believe that bio-sensors would be the ideal choice for playing such a role in the medical industry. Glucose bio-sensors as mentioned in the paper has come a long way from the first generation that we have tested and is now commercially available, but this is not the case for other bio-sensor devices. Thus we would like to conclude hoping that our paper inspires researchers from the fields of medicine, electronics, computer science and biotechnology to come together so as to shine greater detail and find innovative solutions to our problems by developing various applications in the field of bio-sensors which is still nascent and shows plenty of potential.

Acknowledgement

The authors thank the management of Vellore Institute of Technology, for the constant encouragement, help and support to carry out this work.

REFERENCES

1. Ngugi MP, Njagi JM, Kibiti CM, Ngeranwa JJN, Njagi ENM. Diagnosis of Diabetes Mellitus. International Journal of Diabetes Research. 1(2), 2012, 24-27.
2. Idf Diabetes Atlas Group. Update of mortality attributable to diabetes for the IDF Diabetes Atlas: Estimates for the year 2013. Diabetes Res Clin Pract. 109(3), 2015, 461-465.
3. Shi Y, Hu FB. The global implications of diabetes and cancer. Lancet (London, England). 383(9933), 2014, 1947-1948.
4. Gomes MB, Negrato CA, Calliari LEP. Early age at menarche: A risk factor for overweight or obesity in patients with type 1 diabetes living in urban areas? Diabetes Res Clin Pract. 107(1), 2015, 23-30.
5. Yoo E-H, Lee S-Y. Correction: Yoo E.H., Glucose Biosensors: An Overview of Use in Clinical Practice. Sensors. 10, 2010, 4558-4576.
6. Krzyczmonik P, Socha E, Skrzypek S. Immobilization of glucose oxidase on modified electrodes with composite layers based on poly(3,4-ethylenedioxythiophene). Bioelectrochemistry. 101, 2015, 8-13.
7. Wang J. Glucose biosensors: 40 Years of advances and challenges. Electroanalysis. 13(12), 2001, 983-988.
8. Budi R, Irwansyah E. The Early Detection of Diabetes Mellitus (DM) Using Fuzzy Hierarchical Model. Procedia Comput Sci. 59, 2015, 312-319.
9. Rana JS, Jindal J, Beniwal V, Chhokar V. Utility Biosensors for applications in Agriculture – A Review. J. Am. Sci. 6(9), 2010, 353-375.
10. Xu J, Zhao G. A Third-Generation Biosensor Based on the Enzyme-Like Activity of Cytochrome c on a Room Temperature Ionic Liquid and Gold Nanoparticles Composite Film. International Journal of Electrochemical Science. 3, 2008, 519-527.
11. Park S, Boo H, Chung TD. Electrochemical non-enzymatic glucose sensors. Anal Chim Acta. 556(1), 2006, 46-57.
12. Shamsipur M, Amouzadeh Tabrizi M. Achieving direct electrochemistry of glucose oxidase by one step electrochemical reduction of graphene oxide and its use in glucose sensing. Material Science and Engineering C. 45, 2014, 103-108.
13. Ureta T, Fernández WY, Centelles JJ, Cascante M. *In vivo* measurements of control coefficients for hexokinase and glucose-6-phosphate dehydrogenase in Xenopus laevis oocytes. FEBS Lett. 475(2), 2000, 145-149.
14. Kucherenko IS, Soldatkin OO, Lagarde F, Jaffrezic-Renault N, Dzyadevych SV, Soldatkin a. P. Determination of total creatine kinase activity in blood serum using an amperometric biosensor based on glucose oxidase and hexokinase. Talanta. 144, 2015, 604-611.
15. Turner A. Diabetes mellitus: biosensors for research and management. Biosensors and Bioelectronics. 1(1), 1985, 85-115.
16. Pisoschi AM. Analytical Biochemistry Glucose Determination by Biosensors. Biochemistry and Analytical Biochemistry. 1(6), 2012, 1-2.
17. Koopal CGJ, Nolte RJM. Highly stable first-generation biosensor for glucose utilizing latex particles as the enzyme-immobilizing matrix. Enzyme Microb Technol. 16(5), 1994, 402-408.
18. Wang J. Electrochemical glucose biosensors. Electrochem Sensors, Biosens their Biomed Appl. 2008, 57-69.
19. Mayorga Martinez CC, Treo EF, Madrid RE, Felice CC. Real-time measurement of glucose using chrono-impedance technique on a second generation biosensor. Biosens Bioelectron. 29(1), 2011, 200-203.
20. Chu Z, Liu Y, Xu Y, Shi L, Peng J, Jin W. In-situ fabrication of well-distributed gold nanocubes on thiol graphene as a third-generation biosensor for ultrasensitive glucose detection. Electrochim Acta. 176, 2015, 162-171.
21. Jindal K, Tomar M, Gupta V. Inducing electrocatalytic functionality in ZnO thin film by N doping to realize a third generation uric acid biosensor. Biosens Bioelectron. 55, 2014, 57-65.
22. Freire RS, Pessoa CA, Mello LD, Kubota LT. Review Direct Electron Transfer : An Approach for Electrochemical Biosensors with Higher Selectivity and Sensitivity. Journal name. 14(2), 2003, 230-243.

Source of Support: Nil, **Conflict of Interest:** None.