Enzyme Based biosensor

for blood glucose detection

Progress Report

In fulfillment of the requirements for the

NU 302 R&D Project

~~**At NIIT University**~~

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CERTIFICATE

This is to certify that the present research work entitled "Enzyme Based Biosensors" being submitted to NIIT University, Neemrana, Rajasthan, in the fulfillment of the requirements for the course at NIIT University, Neemrana, embodies authentic and faithful record of original research carried out by Varuni Agarwal, Gourav Thapa, Hradyansh Prashar, students of B Tech at NIIT University, Neemrana. She/He has worked under our supervision and that the matter embodied in this project work has not been submitted, in part or full, as a project report for any course of NIIT University, Neemrana or any other university.

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TABLE 3: EXPERIMENT 3:

PHASE I:

CONC OF SOLUTION (IN MOLAR)	PRISM(FIRST TIME) REFRACTED LIGHT(IN MV)	PRISM(SECOND TIME) REFRACTED LIGHT (IN MV)
0.1	299	360
0.2	301	362
0.3	314	363
0.4	316	365

PHASE II:

CONC OF SOLUTION (IN MOLAR)	PRISM(FIRST TIME) REFRACTED LIGHT(IN MV)	PRISM(SECOND TIME) REFRACTED LIGHT (IN MV)
0.12	315	316
0.14	350	318
0.16	362	329
0.18	345	333

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RATIONALE OF PROJECT:

Blood glucose monitoring has been established as a valuable tool in the management of diabetes. Since maintaining normal blood glucose levels is recommended, a series of suitable glucose *biosensors have been developed. During the last 50 years, glucose biosensor technology including point-of-care devices, continuous glucose monitoring systems and noninvasive glucose monitoring systems has been significantly improved. However, there continues to be several challenges related to the achievement of accurate and reliable glucose monitoring. Further technical improvements in glucose biosensors, standardization of the analytical goals for their performance, and continuously assessing and training lay users are required. This project analyze one of the component found from glucose and make some relationship between them which can be used in future approach of biosensors.

Note: * A biosensor is an analytical device, used for the detection of a chemical substance, that combines a biological component with a physicochemical detector.

LITERATURE REVIEW:

Non-Invasive Methods-

1. Glucometer[1]-
    - A glucose meter is a medical device for determining the approximate concentration of glucose in the blood.
    - It is a key element of home blood glucose monitoring (HBGM) by people with diabetes mellitus or hypoglycemia.
    - A small drop of blood, obtained by pricking the skin with a lancet, is placed on a disposable test strip that the meter reads and uses to calculate the blood glucose level.
    - The meter then displays the level in units of mg/dl or mmol/l.
    - It is invasive and not continuous.
    - And also gives reading with 20% error w.r.t. gold standard

2. Mid Infrared Spectroscopy[2]-
    - Location: Oral Mucosa
    - Mid-infrared (Mid-IR) spectroscopy is based on light in the 2500–10,000 nm spectrum. The physical principle is similar to that of NIR.
    - However, due to the higher wavelengths, Mid-IR exhibits increased absorption thus the tissue penetration of light can reach a few micrometers.
    - Compared to NIR is that the Mid-IR bands produced by glucose, as well as other compounds, are sharper than those of NIR, which are often broad and weak.

3. Optical Coherence Tomography[3]:
    - Location: Forearm
    - Tissue scattering properties are highly dependent on the ratio of the refractive index of scattering centers to the refractive index of the interstitial fluid.
    - According to Beer-Lambert law, light attenuation inside tissues is exponential
    - The slope of this exponential is directly proportional to attenuation coefficient of ballistic photons
    - Because only ballistic photons are backscattered, one can estimate tissue properties by coefficient profile
    - Limitations: OCT technique can be sensitive to motion artefacts.

4. Near Infrared Spectroscopy[4]-
    - Location: Forearm
    - Near infrared (NIR) spectroscopy is based on focusing on the body a beam of light in the 750– 2500 nm spectrum.
    - NIR spectroscopy allows glucose measurement in tissues in the range of 1–100 mm of depths, with a decrease in penetration depth for increasing wavelength values.
    - Transmission or reflectance (localized or diffuse) of the light can be measured by proper detectors.
    - In NIR, the weak glucose spectral bands not only overlap with the stronger bands of water, but also of hemoglobin, proteins and fats.
    - Which is not a good predictor of glucose concentration.

Invasive Methods-

1. GOD POD Method[5]:
    - Principle:

      Glucose + $H_2O + O_2$ → Gluconic acid + H_2O_2 (hydrogen peroxide)

      4-Amino-Phenazone + Phenol + H_2O_2 → Quinoneimine(pink)
    - Intensity is determined at on 505 nm filter.
    - Sample: Blood Serum.
    - The device used for intensity filter is spectrophotometer.
    - Even though this method is precise but requires lot of equipment

2. HexoKinase Method[6]:
    - Principle:

      Glucose + ATP + Hexokinase → Glucose-6-Phosphate + ADP

      Glucose-6-Phosphate + NAD + GLUC-6-PD → 6-Phophogluconate +NADH
    - NADH production measured photometrically at 340 nm.
    - Conversion of NADH from NAD at 340 nm, increase in O.D. is measured at fix interval
    - Increase O.D. /min is directly proportional to the conc. of glucose in the specimen
    - Plasma glucose =[Delta O.D./min(test)/Delta O.D./min(Std.)] × 100

OBJECTIVE:

Estimation of glucose, by measuring the light absorbed by Hydrogen Peroxide produced, when Glucose-Pyranose is reacts with glucose.

This was done by passing the light through different concentrations of Hydrogen peroxide solution, and measure the intensity of refracted light coming from the sample. To get a linear relation between refracted light, concentration of hydrogen peroxide and concentration of glucose.

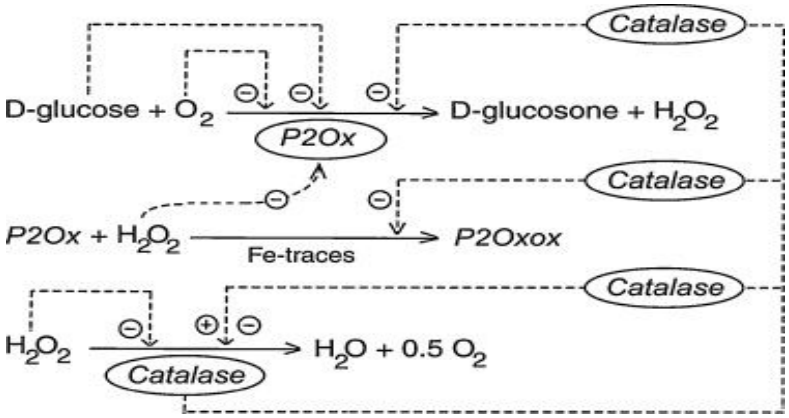
NOTE:

Assumption (confirmed by mentor):

Concentration of glucose is linearly prop. Concentration of Hydrogen Peroxide

METHODOLOGY:

❖ Producing Hydrogen Peroxide solution: (done by Ismile of biotechnology)



❖ Experiment 1:

1. Apparatus:
    - Light source: Green coloured laser light (~ 560–520 nm)
    - Sample Container: Appendroff
    - Receiver: A photodetector from physics lab. Output range (100mV - 2V)
    - And adjustable stand
    - Power supply for the light source(for continuous volts).

2. Steps:
    - Make the solution of 0.1 M, 0.2 M, 0.3 M, 0.4 M of hydrogen peroxide.
    - Take 1 ml of each solution.
    - Then keep laser light (vertically), photo detector and eppendorf parallel and pass the light through it.
    - Observe the intensity of refracted light(in mV) for each sample.

❖ Experiment 2:

1. Apparatus:
    - Light source: Green coloured laser light (~ 560–520 nm)
    - Sample Container: Appendroff
    - Receiver: A photodetector from physics lab. Output range (100mV - 2V)
    - Adjustable stand
    - Power supply for the light source(for continuous volts).

2. Steps:
    - Make the solution of 0.1 M, 0.2 M, 0.3 M, 0.4 M of hydrogen peroxide.
    - take 2 ml of each solution.
    - Then keep laser light (horizontally), photo detector and eppendorf parallel and pass the light(diagonally) through it.
    - Observe the intensity of refracted light(in mV) for each sample.

❖ Experiment 3:

Apparatus:

- A prism and its holder (made in Workshop Lab).
- Laser light along with its power supply and Stand.
- A photo Detector (provided by physics lab).
- Hydrogen Peroxide Sample (of different concentration).

<u>PHASE I:</u>

Steps:

- Make the solution of 0.1 M, 0.2 M, 0.3 M, 0.4 M of hydrogen peroxide.
- Take a drop of the solution on the prism.
- Keep the photo detector and laser light in the direction of refracted light and incident light respectively.
- Pass the light through the drop of sample.
- Observe the intensity of refracted light (in mV) for different samples

PHASE II:

Steps:

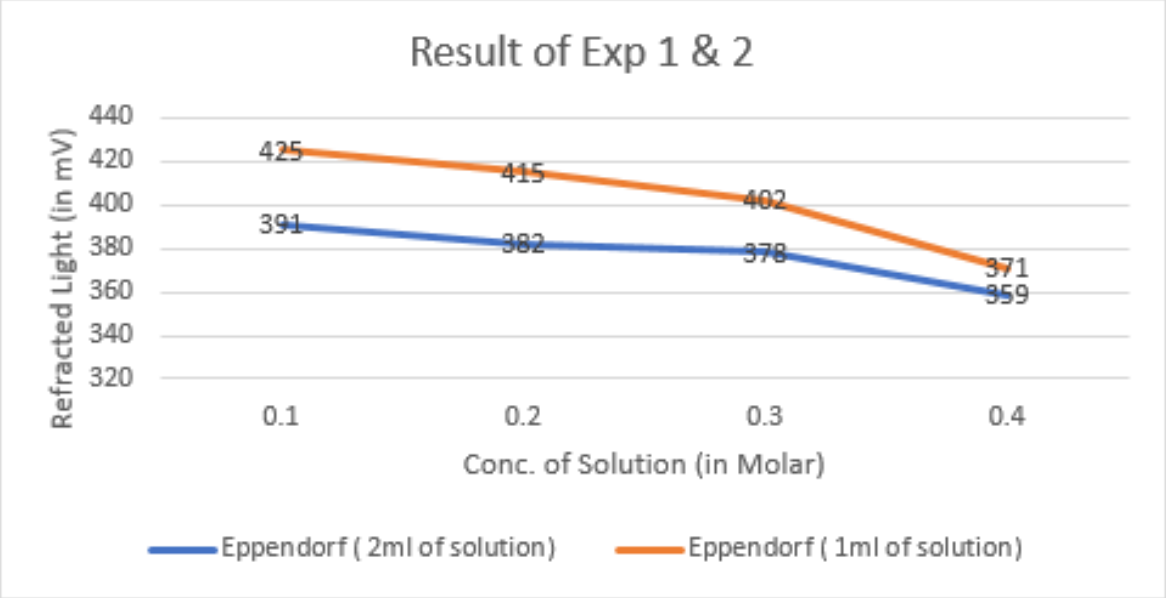
- Make the solution of 0.12 M, 0.14 M, 0.16 M, 0.18 M (i.e different concentration within 0.1 M) of hydrogen peroxide.
- Take a drop of the solution on the prism.
- Keep the photo detector and laser light in the direction of refracted light and incident light respectively.
- Pass the light through the drop of sample.
- Observe the intensity of refracted light (in mV) for different samples

❖ After all the readings has been taken compare them and using linear regression find a relationship between hydrogen peroxide and refracted light.

❖ Then the previous relation is used to find concentration of glucose from the relation stated before.

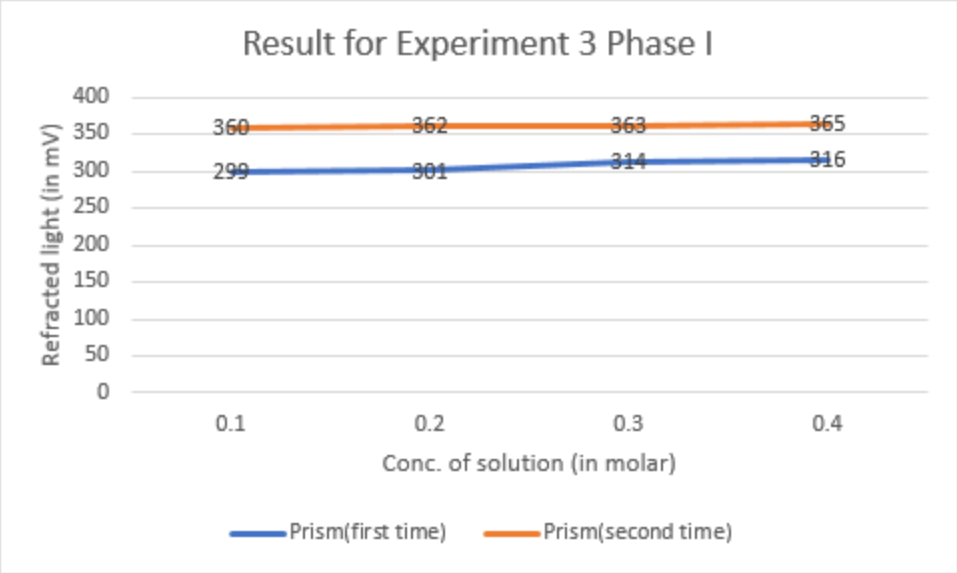
RESULTS:

Graph for Experiment 1 & 2:



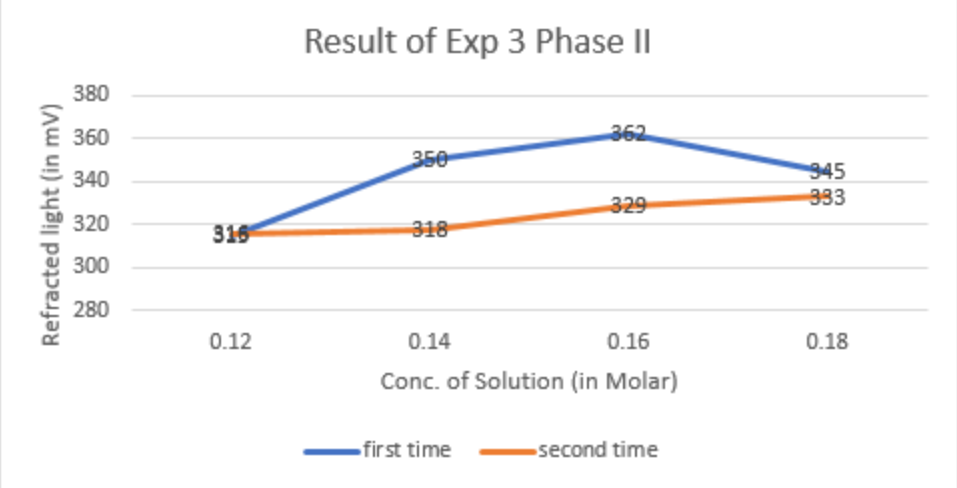
This graph is first observation which allow us to work further as it is giving us correlation between refracted light and concentration of solution. As we can see there is slight slope hence can be used for later analysis and formation of linear equation.

Graph for Experiment 3 Phase 1:



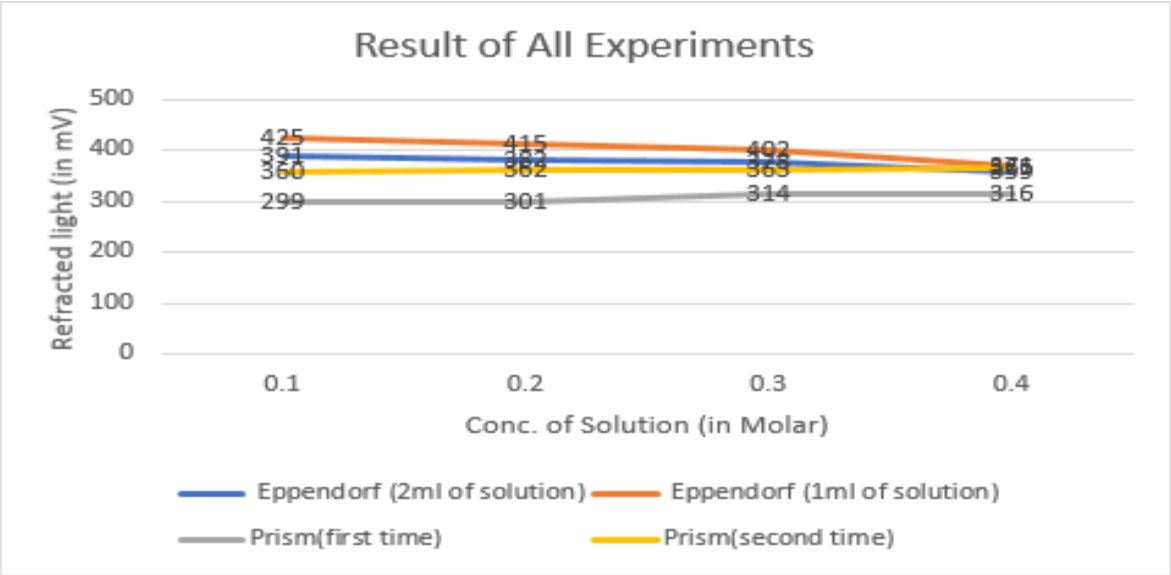
This graph confirms that small amount of solution also gives correlation between refracted light and concentration of solution, thus letting us experiment on further samples.

Graph for Experiment 3 Phase 2:



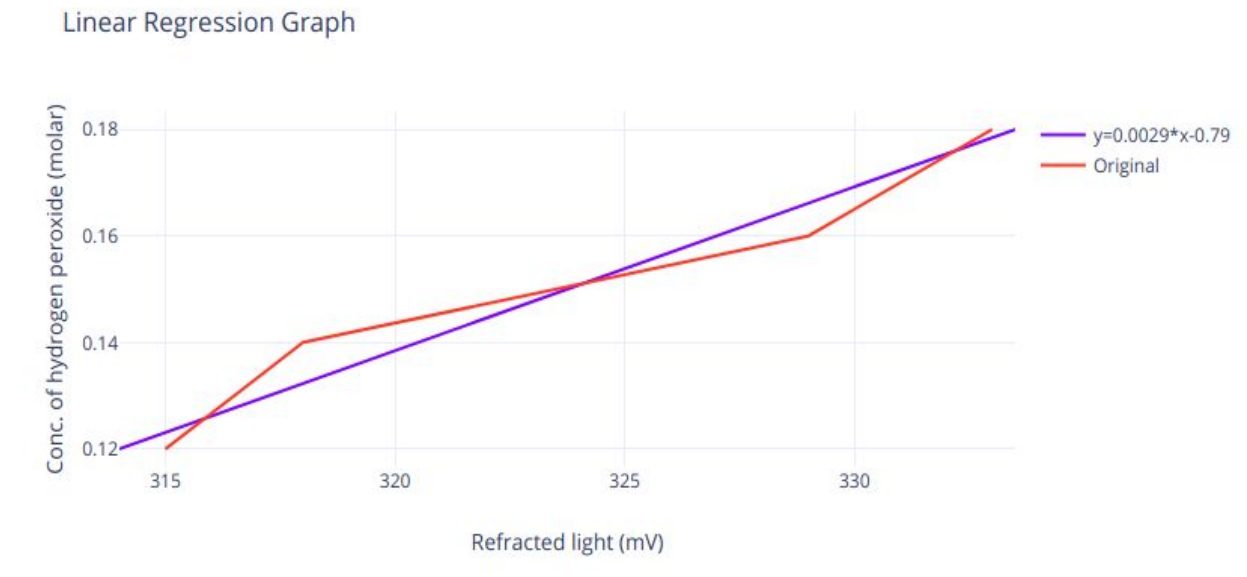
This result shows that when small amount of solution is taken we obtain a slope that give us co-relation between light and concentration.

Comparing Graphs of All Experiments:



As we can see there is a slight slope in graph confirming that there is a relation between the axis (refracted light and concentration of hydrogen peroxide), which will be further used in analysis.

Final Result:



Using above model the equation formed is:

Y = 0.002918*X - 0.7947

Where,
X = light refracted
Y = conc. Of Hydrogen Peroxide
slope= 0.002918

thus,

Glucose = k * Hydrogen Peroxide + c

where k is constant found from calibration method.

Conclusion:

This equation obtained can be used for finding value of concentration of Hydrogen peroxide thus we can find concentration of glucose with the help of assumption taken.

SUMMARY

We studied various methods for blood glucose estimation. There are lots of other methods available to do that but almost all of them rely on chemical properties of glucose like measuring the intensity of colour change in indicator or measuring the amount of current passed through sample(glucometer). But all those methods suffer from weakness that they need to be calibrated very often and their accuracy is questionable.

We wanted a model which will address these issues and give us high accuracy results, a model which is reusable unlike glucometer strips which wear off after certain number of use. So, after thorough research and experimentation, we arrived at a method where we take Glucose Pyranose enzyme and drop it in blood which creates fumes of Hydrogen peroxide, this Hydrogen peroxide was then allowed to absorb the light reflected from prism and residue light was measured by photodetector. The slope was satisfactory, and could be made more accurate by taking readings from couple more samples and then calibrated.

FUTURE WORK

This project can be further experimented on variety of blood samples thus finding relation for large volume of data (blood serum) confirming its reliability on large scale thus can we can apply this equation and implement a device with minimal resources available and saver to use and easy to handle.

We can apply this procedure on non invasive technique. Here we will be using a finger tip instead of the solution. First with the help of magnetic field, we will bring all the molecules near to the skin and pass the light through it in same way as done in this experiment. And observe the respected values for different type of fingers. And to find a linear relation.

REFERENCES:

[1] Source: https://en.wikipedia.org/wiki/Glucose_meter

[2],[3], [4] SOURCE: https://www.ncbi.nlm.nih.gov/pubmed/10471679

[5] Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5531325

[6] Source: https://acutecaretesting.org.

Some other sources to understand the topic:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5905919

https://onlinelibrary.wiley.com/doi/full/10.1002/adhm.201701150

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3292132