Name:

Roll no.:

Glucose Sample no.:

Estimation of Glucose concentration by GOD-POD Assay

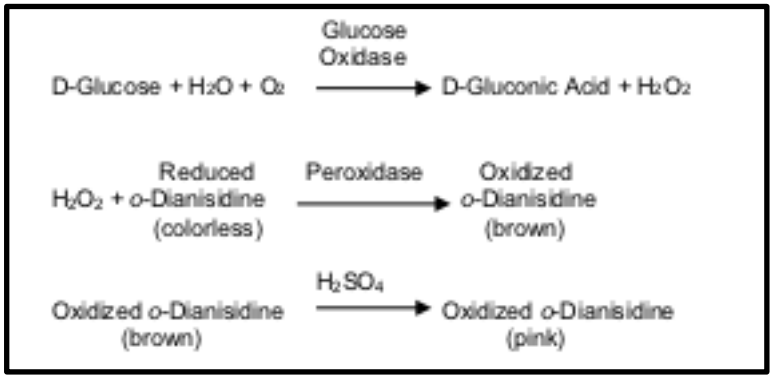
Principle:

Glucose gets oxidized to gluconic acid and molecular oxygen gets reduced to hydrogen peroxide by glucose oxidase enzyme. Next, hydrogen peroxide reacts with o- dianisidine (reduced form) in the presence of peroxidase enzyme to form brown coloured oxidized o-dianisidine, which further reacts with concentrated sulfuric acid to form a more stable pink colored product. The intensity of the pink color measured at 540 nm is proportional to the original glucose concentration.

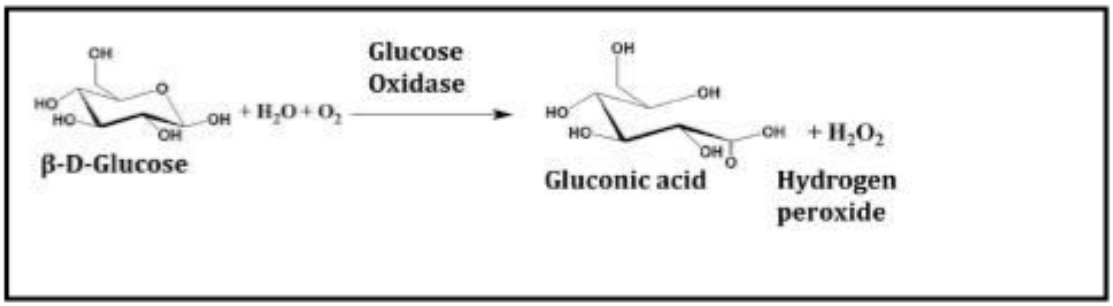
The glucose oxidase enzyme is an oxido-reductase that catalyses the oxidation of glucose to D-glucono-δ-lactone and molecular oxygen gets reduced to hydrogen peroxide. Peroxidases are a large family of enzymes that typically catalyze a reaction of the form:

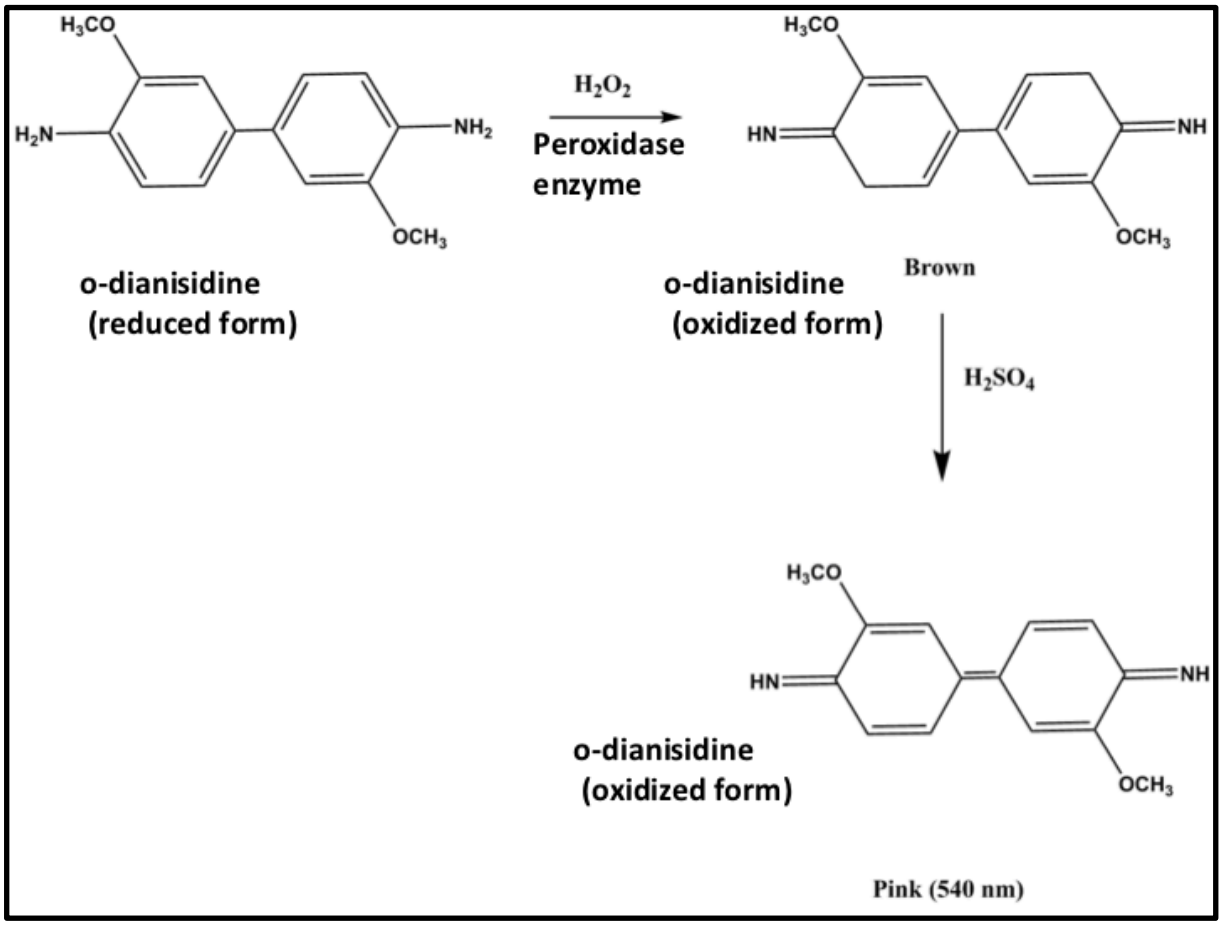
ROOR' + electron donor (2 e^-) + 2H^+ → ROH + R'OH

The series of reactions involved in the assay system is as follows:



Scheme 1. Schematic representation of the chemical reactions of glucose estimation by GOD-POD method.


Glucose
Oxidase
β-D-Glucose
+ H2O + O2
Gluconic acid
+ H2O2
Hydrogen
peroxide


H2O2
Peroxidase
enzyme
o-dianisidine
(reduced form)
o-dianisidine
(oxidized form)
Brown
H2SO4
o-dianisidine
(oxidized form)
Pink (540 nm)

Concentration of Glucose solution (given) = _____ µg/mL to prepare the standards.

Data Table 1: Preparation of standards

Sample No.	Required conc. for preparation of stanadards (µg/mL)	Required volume of glucose soln (µL)	Buffer volume (µL)
1A 1B	5	12.5	487.5
2A 2B	10	25	475
3A 3B	15	37.5	462.5
4A 4B	20	50	450
5A 5B	25	62.5	437.5

Data Table 2: Recorded absorbance data

Sample No.	Absorbance
1A	0.087
1B	0.090
2A	0.235
2B	0.235
3A	0.320
3B	0.307
4A	0.343
4B	0.336
5A	0.434
5B	0.471
Unknown A	0.368
Unknown B	0.359

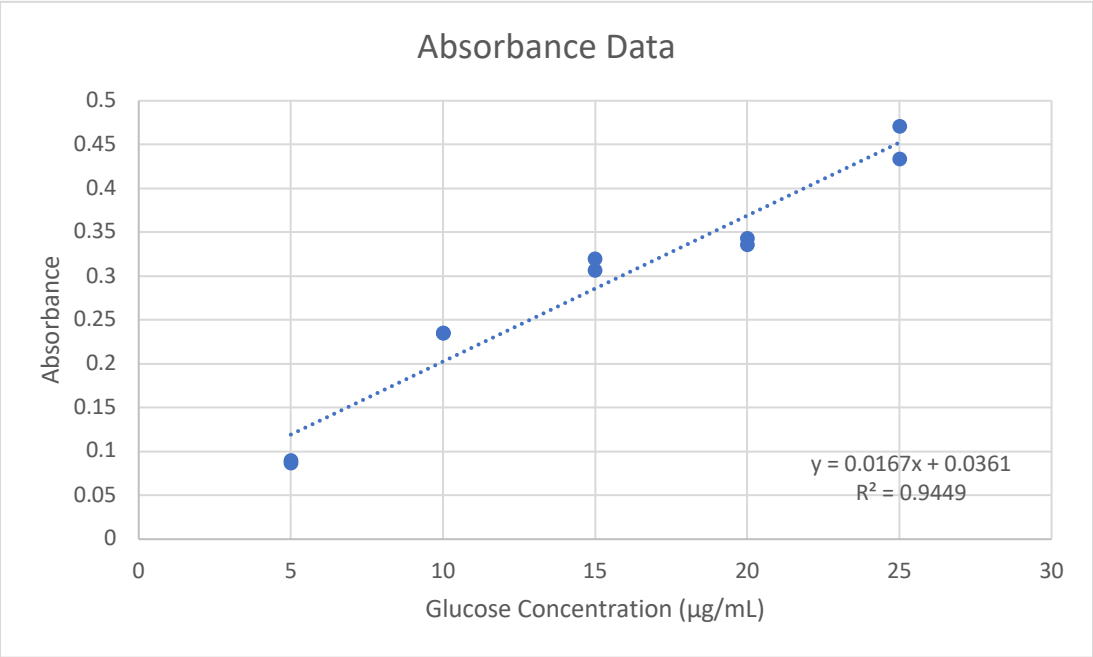
In the graph paper, please write

Name:

Roll No.:

Unknown Glucose solution No. :

Results



By plotting the graph and fitting the data, we get,

y = 0.0167x + 0.0361

for y = (0.368+0.359)/2

= 0.3635

Put all the absorbance values and then draw the line.

0.3635 = 0.0167x + 0.0361

0.3635 - 0.0361 = 0.0167x

0.3274 = 0.0167x

x = (0.3274/0.0167)

= 19.60 µg/mL

The concentration of unknown glucose solution is 19.60 µg/mL

You donot need to write these points during exam

Few important points:

1. Please take **THREE blank solutions** during GOD-POD experiment.

2. Donot forget to write your **name, roll no, sample no. in the graph**. Please staple the graph along with the answersheet.

3. In one kind of spectrophotometer, you need first measure the absorption of buffer and tare it to zero. In another kind of spectrophotometer, you can keep simultaneously both buffer and your sample. Please be careful about the use of spectrophotometer.

4. Hold the test tube racks carefully. Be careful while keeping the test tube racks at 37 degree and whie taking that out. If you break any test tube, your whole experiment will be spoiled. Please check that you have not taken any broken test tube.

5. After adding 500 µL of 12N H_2SO_4 in each test tube and mix well. Since, H_2SO_4 has higher density, if you forget to mix well with 1 mL pipette / glass dropper (not plastic dropper), you will get erroneous result.

6. We have pipettes from Fisher (black colour), Gilson (blue colour) and Eppendorf (white colour). Please learn their usage appropriately. They are different from each other.

7. Pipetting appropriately will be important for all qualitative tests, like GOD-POD and Enzyme Kinetics.

8. Absorbance value should be 0.1 – 0.9 . If you get absorbance value above 1, you need to dilute the sample. Please remember that Lambert-Beer's Law $A = \varepsilon.c.L$ is valid for 0.1-0.9 absorbance range. **Absorbance within range of 0.2 to 0.5 is ideal to maintain linearity in the Beer–Lambert law.**