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Reference for this materials :

1. https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Bulletin/gago20bul.pdf

2. https://www.nzytech.com/files/brochures/AK0016_D-Glucose%20GOD-POD,%20UV%20method.pdf

3. Internet sources and wikepedia

Estimation of glucose by glucose oxidase (GOD) and peroxidase (POD) method.

Introduction

Glucose oxidase enzymatic method is a highly specific method for measuring glucose in starch, serum or plasma by reacting the test fluid with glucose oxidase in which gluconic acid and hydrogen peroxide are formed. This enzymatic method yields maximum specificity for glucose estimation. **The glucose oxidase binds specifically to β-D-glucopyranose**. In solution, the glucose is having cyclic form (at pH7, 63.6% of β-D-glucose and 36.4% of α-D-glucose) and the oxidation displaces the equilibrium to β-D-glucose. GOx catalyzes the oxidation of β-D-glucose into D-glucono-1, 5-lactone, which then hydrolyzes to gluconic acid. Glucose can be measured by its reaction with glucose oxidase, in which gluconic acid and hydrogen peroxide are formed. Hydrogen peroxide than reacts with an oxygen acceptor, such as ortho-dianisidine, phenylamine-phenazone or 4-aminoantipyrine, in a reaction catalysed by peroxidase to form a colour.

This highly specific method is widely used in the field of clinical chemistry and for food analysis. Glucose is the major carbohydrate present in the peripheral blood. Oxidation of glucose is the major source of cellular energy in the body. Glucose derived from dietary sources is converted to glycogen for storage in the liver or to fatty acids for storage in adipose tissue. The concentration of glucose in blood is controlled within narrow limits by many hormones, the most important of which are produced by the pancreas. The most frequent cause of hyperglycemia is diabetes mellitus resulting from a deficiency in insulin secretion or action. A number of secondary factors also contribute to elevated blood glucose levels. These include pancreatitis, thyroid dysfunction, renal failure, and liver disease. Hypoglycemia is

less frequently observed. A variety of conditions may cause low blood glucose levels such as insulinoma, hypopituitarism, or insulin induced hypoglycemia. Glucose measurement in urine is used as a diabetes screening procedure and to aid in the evaluation of glucosuria, to detect renal tubular defects, and in the management of diabetes mellitus. Glucose measurement in cerebrospinal fluid is used for evaluation of meningitis, neoplastic involvement of meninges, and other neurological disorders. In clinical chemistry, the enzymatic analysis of glucose in blood and urine has been modified as dipstick test. Glucose oxidase is widely used, coupled to peroxidase reaction that visualizes colorimetrically the formed H_2O_2, for the determination of free glucose in sera or blood plasma for diagnostics, using spectrometric assays manually or with automated procedures, and monitoring glucose levels in fermentation, bioreactors, and to control glucose in vegetal raw material and food products. Enzyme electrode biosensors detect levels of glucose by keeping track of the number of electrons passed through the enzyme by connecting it to an electrode and measuring the resulting charge. This has a possible application in the world of nanotechnology when used in conjunction with tiny electrodes as glucose sensors for diabetics.

Principle:

Glucose is oxidized to gluconic acid and molecular oxygen gets reduced to hydrogen peroxide by glucose oxidase. Hydrogen peroxide reacts with o-dianisidine in the presence of peroxidase enzyme to form oxidized o-dianisidine, which further reacts with sulfuric acid to form a more stable 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.

In cells, it aids in breaking the sugar down into its metabolites.

Peroxidases are a large family of enzymes that typically catalyze a reaction of the form:

$$ROOR' + \text{electron donor } (2\ e^-) + 2H^+ \rightarrow ROH + R'OH$$

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

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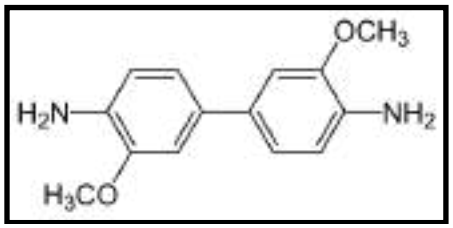
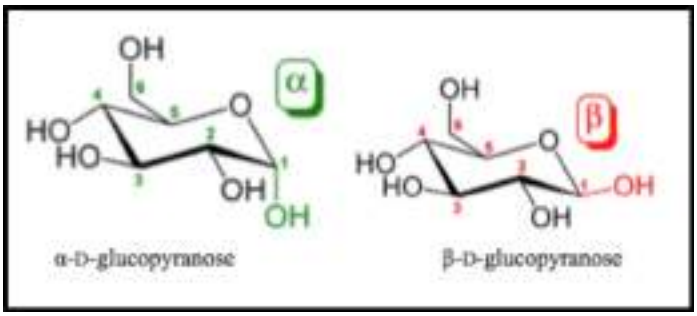
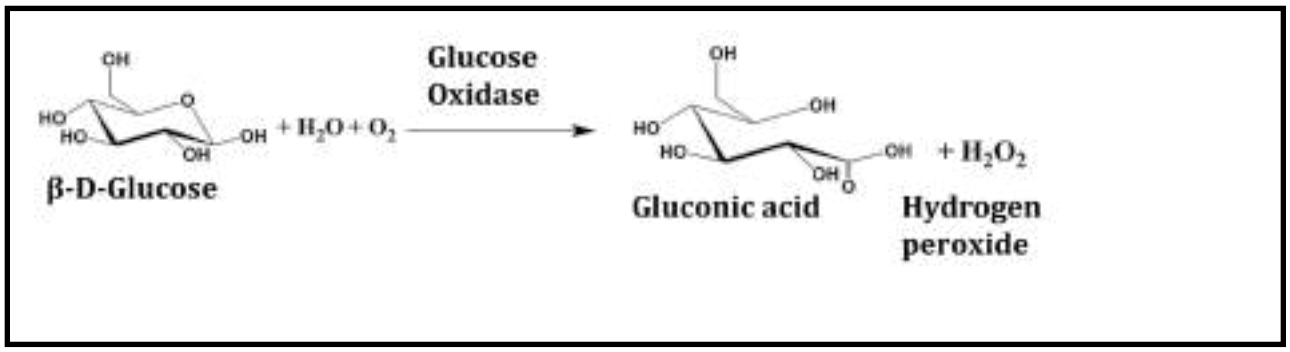


o-Dianisindine





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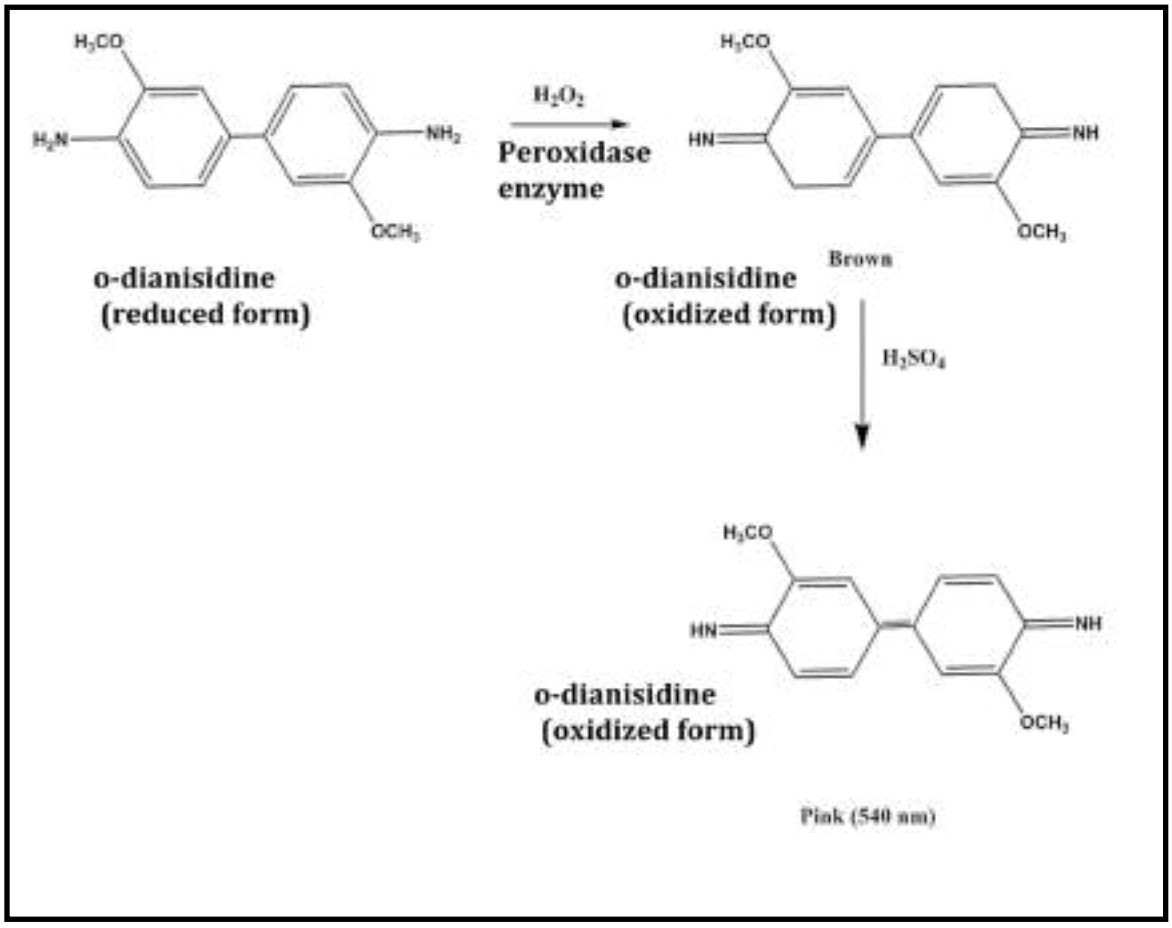


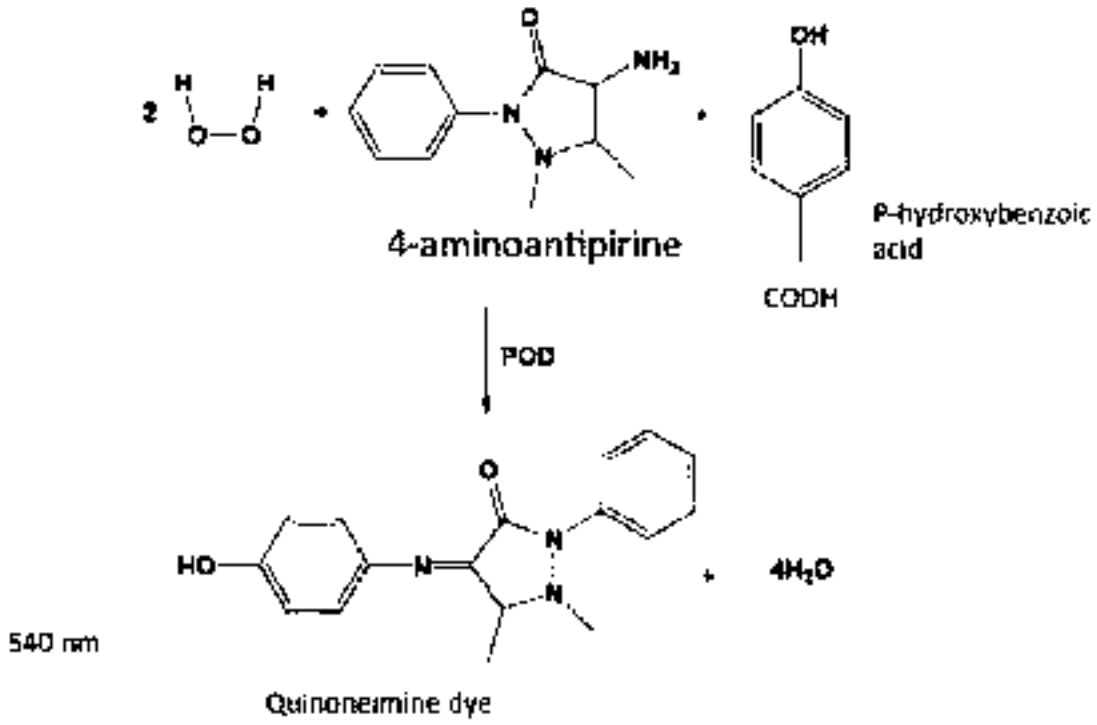
$$\text{D-Glucose} + H_2O + O_2 \xrightarrow[\text{Oxidase}]{\text{Glucose}} \text{D-Gluconic Acid} + H_2O_2$$

$$H_2O_2 + \text{Reduced } o\text{-Dianisidine (Colourless)} \xrightarrow{\text{Peroxidase}} \text{Oxidized } o\text{-Dianisidine (Brown)}$$

$$\text{Oxidized } o\text{-Dianisidine (Brown)} \xrightarrow{H_2SO_4} \text{Oxidized o-Dianisidine (Pink)}$$

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

When 4-aminoantipyrine and phenol are used instead of o-Dianisidine, then the following reaction occurs in presence of peroxide enzyme.



Scheme 2. Reaction scheme for glucose estimation using 4-aminoantipirine.

Enzymatic methods for glucose determination were first described in 1948 by Keilin and Hartee using glucose oxidase in a manometric technique. Keston modified this method in the early 1950's using glucose oxidase/peroxidase enzyme system and o-dianisidine chromogen systems. The Trinder method replaces carcinogenic o-dianisidine with phenol plus 4-aminoantipirine.

Reagents required:

1. **Glucose Stock:** 200 ug/ml solution in 20 mM potassium phosphate (pH 7.0) buffer. It is stable at 2–8°C for at least six months. Discard if turbidity develops.

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2. **GOD- POD Reagent:** 500 units of glucose oxidase, 100 units of peroxidase were dissolved in 39.2 ml of 20 mM potassium phosphate (pH 7.0) buffer. Avoid exposing the reagent to light. The solution is stable up to one month at 2–8°C and for at least 6 months frozen at – 20°C. Discard if turbidity develops.

3. **o-Dianisidine Reagent:** 5mg of o –dianisidine was dissolved in 1.0 ml of 50% ethanol. Avoid exposing the reagent to light. Solution is stable for 3 months at 2–8°C.

4. **GOD- POD Assay Reagent:** Add 0.8 ml of the o -Dianisidine reagent to the bottle containing the 39.2 ml of GOD- POD Reagent. Invert bottle several times to mix. Minimize exposure to light. Solution is stable up to 1 month at 2 –8°C . Discard if turbidity develops or color forms.

Procedure:

1. Make standard glucose solution for five concentrations (5-25 ug/ml) using 20 mM potassium phosphate (pH 7.0) buffer.
2. Make each standard glucose solution in triplicate. The volume of solution in each test tube should be 500 ul.
3. For blank, take 500 ul of 20 mM potassium phosphate pH 7.0 solutions (here duplicates is good enough) .
4. Take 500 ul unknown solution in triplicate.
5. Add 500 ul of **GOD-POD assay reagent** in each test tube including the two for blank.
6. Mix well and incubate for 15 min at 37^0 C using hot air oven.
7. Add 500 ul of 12N H_2SO_4 in each test tube and mix well.
8. Take absorbance of the **pink colour** at 540 nm by using plastic cuvette.
9. Draw standard curve and calculate the amount of glucose present in the unknown solution.

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Estimation of Glucose by Glucose oxidase (GOD) and Peroxidase (POD) method

Reagents required:

1. **Glucose Stock:** 200 µg/ml solution in 20 mM potassium phosphate (pH 7.0) buffer. It is stable at 2–8°C for at least six months. Discard if turbidity develops.

2. **GOD- POD Reagent:** 500 units of glucose oxidase, 100 units of peroxidase were dissolved in 39.2 ml of 20 mM potassium phosphate (pH 7.0) buffer. Avoid exposing the reagent to light. The solution is stable up to one month at 2–8°C and for at least 6 months frozen at – 20°C. Discard if turbidity develops.

3. **O-Dianisidine Reagent:** 5mg of o –dianisidine was dissolved in 1.0 ml of 50% ethanol. Avoid exposing the reagent to light. Solution is stable for 3 months at 2–8°C.

4. **GOD- POD Assay Reagent:** Add 0.8 ml of the o -Dianisidine reagent to the bottle containing the 39.2 ml of GOD- POD Reagent. Invert bottle several times to mix. Minimize exposure to light. Solution is stable up to 1 month at 2 –8°C . Discard if turbidity develops or color forms.

Procedure:

1. Make standard glucose solution for five concentrations (5-25 µg/ml) using 20 mM potassium phosphate (pH 7.0) buffer.
2. Make each standard glucose solution in triplicate. The volume of solution in each test tube should be 500 µl.
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8. Take absorbance of the **pink colour** at 540 nm by using plastic cuvette.
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