

Determination of glucose concentration using trinder method



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The Trinder method is used to determine glucose concentration only, (Lott et al, 1975). This method was first described by Trinder in 1969 thus named after him, (Lott et al, 1975). It uses an enzyme glucose oxidase for the first reaction and peroxidase for the second reaction thus the name of the enzyme Glucose oxidase/peroxidase (GODPOD), (Meiattin, 1973).

Enzymes are biological or any chemical catalysts that speed up a reaction without it being used up, (Jan, 2010). It functions to catalyse a reaction by lowering the activation energy of the reaction. Activation energy is the energy needed to initiate the reaction. It is a point of high energy and requires more energy than the substrates. An enzyme also contains an active site for the substrate to catalyse the reaction. Its efficiency depends on the concentration of the substrate and conditions like temperature or pH, (Hames et al, 2005).

The Trinder method, ' is based on two sequential enzymatic reactions, the first one involves the oxidation of glucose to gluconic acid and H₂O₂, ' (Casabnon et al, 2005). This reaction is catalysed by the enzyme Glucose oxidase. ' Then, the H₂O₂ produced is quantified by a chromogenic reaction with peroxidase (POD), ' as the enzyme that catalyses the reaction with the reduced dye, (Casabnon et al, 2005). The oxidised dye changes colour to pink or red according to the glucose concentration. ' The colour formed is stable at room temperature for at least two hours after development, ' (Anon, 2010).

The main advantage of this method is that it is very specific. It doesn't target other sugars except glucose. It is also simple straight forward and easy to

manipulate. Its results are very reliable and specific, (Bauninger, 1974). Its final products are stable as they are not reactive at room temperature.

Other colorimetric methods to identify glucose include, 'oxidation of glucose in the presence of Cu^{+2} to give Cu_2O ,' and different types of Chromatography, (Casabnon et al, 2005). Glucose can be detected with o-toluidine or other amines, (Casabnon et al, 2005).

The experiment had to check for the specificity of the assay thus other carbohydrates were assayed. These were galactose, fructose, maltose and ribose. Maltose is a disaccharide which is made up of two glucose molecules joined together by a glycosidic bond.

Galactose is a major sugar found in milk, (Hames et al, 2005). Its structure consists of six carbons with a glycosidic bond to join the next glucose molecule, (Berg et al, 2007). Fructose is abundant in fruits. It is a monosaccharide with six carbons as its structure. Ribose is a pentose sugar molecule with 5 carbons. It is mostly abundant in the nucleotides.

The aim of the experiment was to determine glucose concentration in different concentrated solutions and unknown solutions. The specificity of the assay was to be determined by application of the assay on different sugar molecules. A standard curve was to be drawn from the absorbencies acquired from the spectrophotometer at 515nm

Materials

12 - Test tubes
2 - Long pipette tips

1ml of 0.5mM Fructose 1ml of 0.5mM Maltose

1ml of 0.5mM Galactose 1ml of Unknown Glucose

1ml of 0.5mM Ribose

5ml of 0.5mM Glucose 5ml of Distilled water

7ml of 0.1% Phenol 20ml of GODPOD Reagent

9 – pipette tips 8 – Disposable Cuvettes

Black Marker Stop watch

P100 Pipette Automatic pipette

Recording paper and pen

Spectrophotometer at 515nm Water bath at 37°C

Test tubes rack Blotting paper

Method

The test tubes were marked T1 to T6, for those that had to be inoculated with glucose and S1 to S6, for those that had to be inoculated with different types of sugars. They were arranged in order of concentration on the rack. One row was left out for agitating the test tubes.

Inoculation commenced by transferring glucose into different test tubes T1 being the least concentrated. 0.5mM of Glucose was transferred using a

P100 pipette and not changing the tip. 0.2 cm³ was inoculated into T2, 0.4 cm³ into T3, 0.6 cm³ into T4, 0.8 cm³ into T5 and 1.0 cm³ into T6.

Distilled water was then inoculated into the test tubes using a different tip to avoid cross contamination. One centimetre cubed was inoculated into T1, 0.8 cm³ into T2, 0.6 cm³ into T3, 0.4 cm³ into T4 and 0.2 cm³ into T5. There was no water inoculated into the last tube T6.

Phenol was then inoculated into all the twelve test tubes. It was transferred using a different tip to avoid cross contamination.

One centimetre cubed of different sugars were inoculated in specific S tubes. 0.5 mM of Galactose was inoculated into S1, 0.5 mM of Glucose was inoculated into S2, Glucose unknown was inoculated into S3, 0.5 mM of Fructose was inoculated into S4, 0.5 mM of Maltose was inoculated into S5 and then 0.5 mM of Ribose was inoculated into S6. These transfers were done with different tips for different sugars.

One and half millilitres of GODPOD reagent was then inoculated into all the test tubes using an automatic pipette and a long pipette tip. The test tubes were then agitated on the rack and incubated in the water bath for forty minutes. The temperature was constantly checked during incubation.

After forty minutes, the solutions changed colour from colourless to light pink according to the concentration. These different solutions were then read on a spectrophotometer in a cuvette. The spectrophotometer was zeroed at first then absorbencies of Glucose and other sugars were read and recorded.

A cuvette was wiped on the soft side to minimize absorbencies caused by contamination. These different absorbencies were recorded on a table.

Discussion

The reactions of glucose with the GODPOD were slow due to the fact that the enzyme was stored in ice thus it took long for the reaction to take place. The enzyme's structure was disrupted because it was kept in cold thus it took time to equilibrate with the conditions.

The reason why GODPOD was slow to react was because it is sensitive to its environment, (Teal et al, 1985). Enzymes are sensitive to pressure, temperature and pH. This added to the fact that the colour produced was not very dark because the enzyme was adjusting to the conditions. The enzyme also didn't denature because it was kept in ice at 4oC not in the heat above 40oC.

On the graph, the points that are not on the line of best fit might have appeared because there might have been a competitive inhibitor thus the reaction didn't go on well owing to reduced absorbance. The inhibitor might have been so because of cross contamination. The same pipette might have been used to transfer the solutions thus cross contamination.

The specificity of the enzymes might have caused the other solutions not to produce reliable results. Enzymes are sensitive to pH, (Jan, 2010). The pH of the test tube might have been so low or higher than the required thus some of the points are not in the line of best fit. This might have been avoided by carrying out the experiment repeatedly and then getting average values.

When the enzyme was applied to the other sugars, there was no absorbance at all because the enzyme is specific to one substrate thus it didn't catalyse the reaction of other sugars and GODPOD. There might have no absorbance because these sugars might have their own wavelengths they absorb the light. This might have been avoided by scanning the various wavelengths and determine the exact wavelength.

The unknown glucoses were determined by the use of the graph. The line of best fit was used to determine the glucose concentration. An equation was used also to determine the concentrations.

The other points not on the line of the best fit might be there because the transitional state might have been great, thus when thirty minutes had passed, the enzyme had not gone past the transitional stage. This might have been characteristic of the colours produced according to the concentration of the different solutions.

The unknown glucose solutions showed to have the same concentration as the stock solution. It might have the same compounds and properties as the stock solution.

The results of the experiment were according to the literature values. This was highlighted by the absorbencies of stock solutions and different sugars. The specificity of the reaction was achieved.

In conclusion, the aims of the experiment were achieved by obtain reliable data and results. The standard curve showed the absorbencies of the different solutions and unknowns.