

## BIO 201 Lab

### Experiment 2

#### Estimation of Glucose in Grapes by enzymatic method using a standard curve

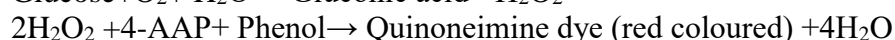
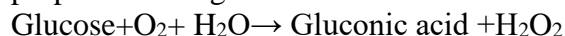
##### Background:

Glucose is the single most important energy source of the brain and the primary energy supplier of the body.

Numerous foods function as sources of glucose. Most dietary carbohydrates contain glucose, either as their only building blocks, as in starch and glycogen or together with other monosaccharide. Since ancient times, fruits have been an important part of human diet, supplying us with sugars, fibres, vitamins, minerals, water and even fat. Despite fructose being the principal sugar in fruits, they also contain significant amount of glucose, which is important for people suffering from fructose malabsorption, diabetes etc. Some fruits containing more fructose than glucose, e.g. apples, pears, watermelon may be more of a problem than ones with less fructose than glucose like berries, bananas and oranges.

##### Principle:

This procedure is based on Trinder method in which aldehyde group of glucose is oxidized by glucose oxidase (GOD) to gluconic acid. In the presence of peroxidase (POD), the formed hydrogen peroxide reacts with chromogenic oxygen acceptor, phenol-aminophenazone (4-AAP) and resultant is the red coloured quinone. The intensity of the colour produced is directly proportional to glucose concentration.



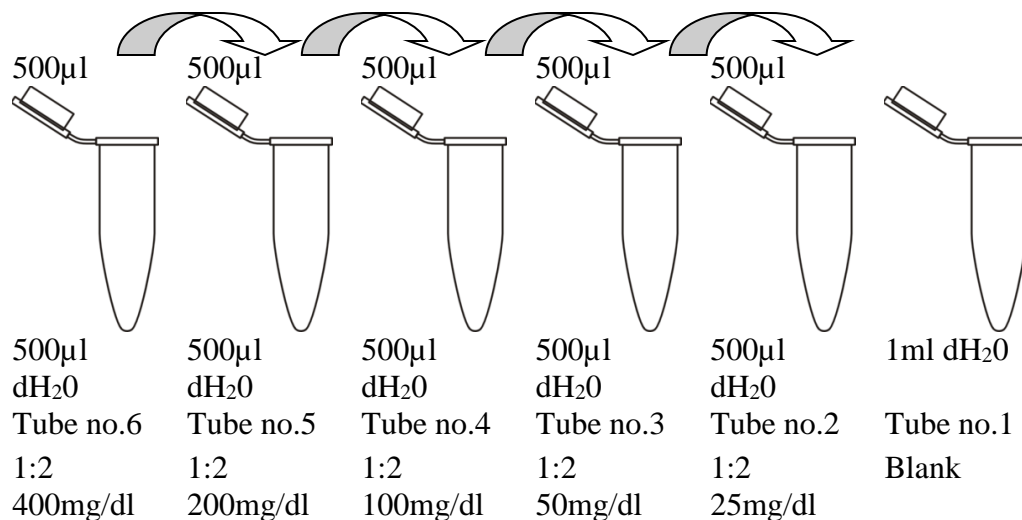
##### Reagents:

Buffer R	Tris pH 7.4	92 mmol/l
	Phenol	0.3 mmol/l
	Glucose oxidase (GOD)	15000 U/l (unit/liter)
	Peroxidase (POD)	1000 U/l
	4-aminophenazone (4-AP)	2.6 mmol/l

##### Procedure:

**Specimen:** grape juice

**Standard preparation:** Using the given glucose standard, prepare 5 standards of different concentrations (25, 50, 100, 200 and 400 mg/dl) using serial dilution from a stock solution of 800 mg/dl.



	Desired Concentration (mg/dl)	Dilution	Volume of D-Glucose solution (µl)	dH <sub>2</sub> O (µl)	Total volume (ml)
5.	400	1:2	500	500	1.0
4.	200	1:2	500	500	1.0
3.	100	1:2	500	500	1.0
2.	50	1:2	500	500	1.0
1.	25	1:2	500	500	1.0
Blank	0	1	0	1000	1.0

### Sample preparation:

For Sample A prepare a .....X dilution and Sample B with a .....X dilution of the fruit juice by diluting the juice in distilled H<sub>2</sub>O.

### Assay preparation:

Take 8 test tubes and level them as B (Blank), Std1, Std2, Std3, Std4, Std5, Smpl A and Smpl B.

Prepare the test tubes as shown in the table below:

	Blank (B)	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Sample A	Sample B
RT	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml
Specimen	10µl of distilled water	10µl of 25 mg/dl solution	10µl of 50 mg/dl solution	10µl of 100 mg/dl solution	10µl of 200 mg/dl solution	10µl of 400 mg/dl solution	10µl of ..... X diluted fruit juice	10µl of .....X diluted fruit juice
Absorbance								

1. Mix the contents well and incubate for 10 minutes at 37°C or 20 minutes at room temperature.
2. Measure the absorbance of the specimen and standard against the blank at 505nm using Biochemistry analyzer.