T.P. n°2 : Realization of a calibration curve for the DNS method

Calibration curve :

A calibration curve in spectrophotometry is a (linear) graph that relates the absorbance of a solution to its concentration. It is constructed by measuring the absorbance of several solutions of known concentrations, then used to determine the concentration of an unknown solution by comparing its absorbance to the curve.

The curve follows Beer-Lambert's law ($A = \varepsilon.l.C$) within a certain concentration range (linearity zone), where (A) is the absorbance and (C) is the concentration.

DNS reagent :

In an alkaline and warm environment, yellow 3,5-dinitrosalicylic acid (3,5-DNS) is reduced by reducing sugars to orange-red 3-amino 5-nitrosalicylic acid. It should be noted that the calibration curves do not always pass through the origin.

Thus, the enzymatic activity of invertase will be studied by measuring the reducing sugars released after hydrolysis using the DNS method.

Equipment :

- ✓ Test tubes ;
- ✓ Rack ;
- ✓ Pipettes and micropipettes ;
- ✓ Pipettors ;
- ✓ Beakers ;
- ✓ Balance ;
- ✓ Watch glass ;
- ✓ Spatula ;
- ✓ Water bath ;
- ✓ Agitator and magnetic stir bar ;
- ✓ Vortex agitator ;
- ✓ Spectrophotometers and cuvettes.

Reagents :

- ✓ Sodium acetate buffer pH 4.7;
- ✓ DNA reagent ;
- ✓ 0.1M sucrose solution (table sugar) ;
- ✓ 0.005 M glucose and fructose solution.

Preparations :

- ✓ Sucrose at 0.1 mol/L : 34.2 g of table sugar in one liter of distilled water.
- ✓ Glucose and fructose solution at 0.005 M : 1 g glucose + 1 g fructose in one liter of distilled water.
- ✓ Sodium acetate buffer at 0.05 M pH 4.7 : 4.1 g sodium acetate in 1 liter of distilled water + diluted acetic acid (5%).

Preparation of the calibration range :

Produce a calibration range according to the table below :

Tube number	**0**	**1**	**2**	**3**	**4**	**5**
0.005M glucose + fructose solution (ml)	0	0,2	0,4	0,6	0,8	1
Distilled water (ml)	1	0,8	0,6	0,4	0,2	0
Acetate buffer pH 4.7 (ml)	1					
0.1 M sucrose solution (ml)	1					
DNS reagent (ml)	2					
Incubation	✓ Homogenize, seal tubes with aluminum foil and heat in a boiling water bath for 5 min. ✓ Allow to cool, then add :					
Distilled water (ml)	6					

- ✓ Homogenize and leave to stand for 10 min at laboratory temperature.
- ✓ Read absorbances (OD) at 540 nm against blank (tube 0).

Work to do :

Trace the calibration curve : D0 = f([invert sugar] in µM).