Protocol 8: Effect of starch concentration on alpha amylase activity (*K*max & *V*max) determination

AIM: To determine the K_m & V_{max} of salivary alpha amylase for starch.

Principle:

In 1913 Michaelis and Menten showed that enzyme forms complex with substrate forming an enzyme-substrate complex, later the complex breaks down to liberate the enzyme and release the product. This can represented as

$$E+S \underset{k-1}{\overset{k1}{\rightleftharpoons}} ES \xrightarrow{k2} E+P$$

Where *k1*, *k2* & *k-1* are the rates of respective reaction. Based on this they derived an equation,

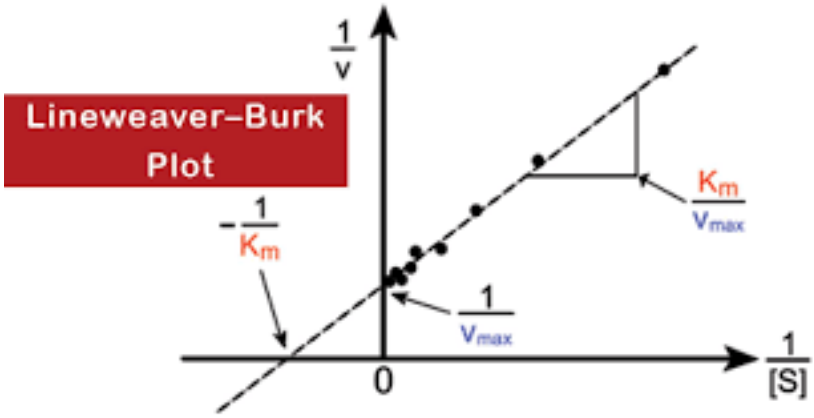
$$v = \frac{v_{max}[S]}{[S] + K_m}$$

Where *vo*=Initial rate of reaction, Vmax = max velocity of the reaction, Km= Michaelis Menten constant, [S]= concentration of the substrate.

Line weaver Burk in 1934 modified the above equation as

$$\frac{1}{V_O} = \frac{K_m}{V_{max}(S)} + \frac{1}{V_{max}}$$

By plot tin 1/v vs. 1/[S] a straight line is obtained from which Km & Vmax value is calculated. Km & Vmax values are essential for selecting a suitable substrate for a given enzyme and vice versa.



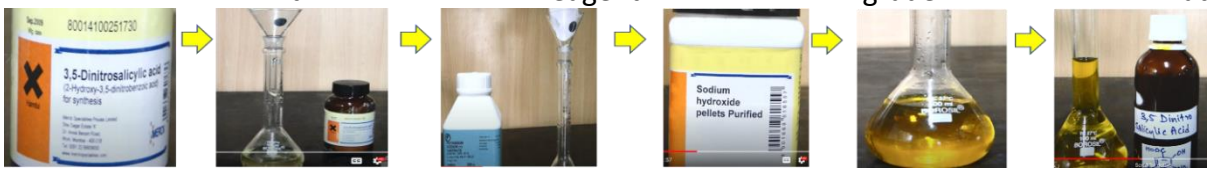
REAGENTS REQUIRED:

1.**Buffer:** 20 mM Sodium Phosphate Buffer with 6.7 mM Sodium Chloride, pH 6.9 at 20°C. Prepare 100 mL in purified water using Sodium Phosphate, Monobasic, Anhydrous, and Sodium Chloride. Adjust to pH 6.9 at 20°C with 1 M NaOH.

2. **1.0 % (w/v) Soluble Starch Solution** (Starch): Prepare 25 mL in Reagent using Starch Potato Soluble, Facilitate solubilization by heating the starch solution in a glass beaker directly on a heating/stir plate using constant stirring. Bring to boil and maintain the solution at this temperature for 15 minutes. Allow the starch solution to cool to room temperature with stirring. Return the starch solution to its original volume (25 mL) by the addition of purified water and dispense aliquots for assay with stirring.

4. **3,5-Dinitrosalicylic Acid Solution:**

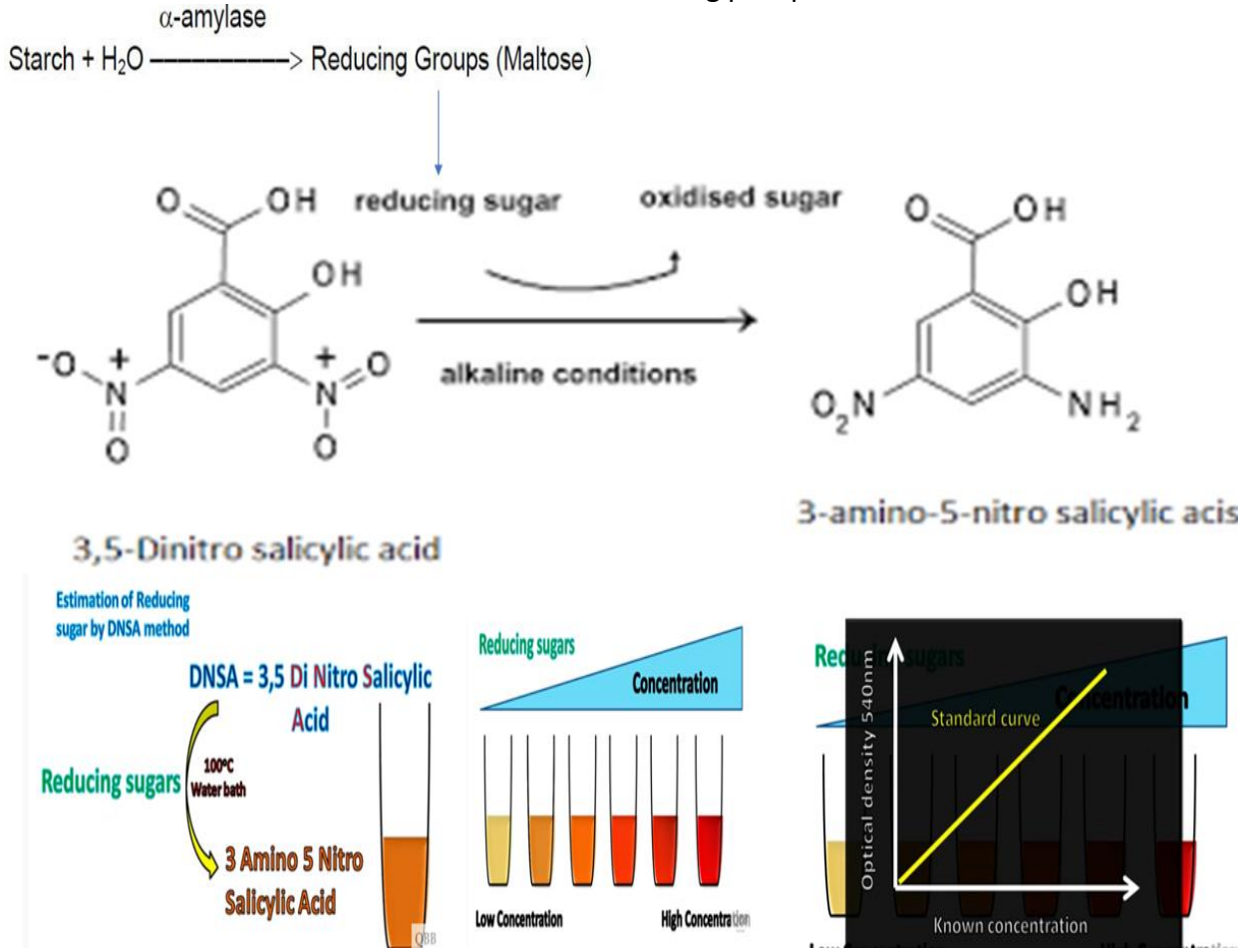
Dinitrosalicylic acid color reagent. Prepare by dissolving 1.0 g of **3,5-dinitrosalicylic acid** in 50 ml of reagent grade **water**. Add slowly 30.0 g **sodium potassium tartrate tetrahydrate which** turns milky yellow colour (Which gives more stability and sensitivity). Add 20 ml of 2 N NaOH which turns the solution to transparent orange yellow colour. Dilute to a final volume of 100 ml with reagent grade wat



er and mix. Complete process of dssolving might take 1 hour. Protect from carbon dioxide and store no longer than 2 weeks.

5. **0.2% (w/v) Maltose Standard (**STD**):** Prepare 10 mL in purified water using Maltose, Monohydrate .

6. **α-Amylase Solution (**Enzyme**):** Saliva is the best and easily available source for amylase. Collect some saliva in beaker and dilute with 1:20 using phosphate buffer.

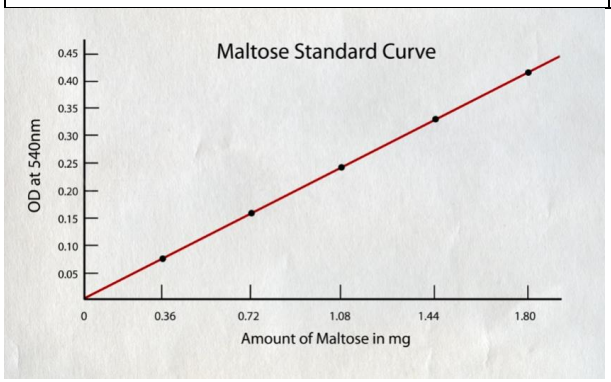


a. PLOTTING STANDARD GRAPH FOR MALTOSE:

Pipette out aliquots of 0, 0.2, 0.4, 0.6, 0.8, and 1.0 of standard maltose solution (1mg/mL). Make up the volume to 2 mL using distilled water. Add 2 mL of DNS reagents. Heat all the test tubes in boiling water for 10 minutes. Read the OD at 520 nm. Drown the standard graph for maltose by plotting concentration of maltose along X-axis and OD along Y-axis.

(Note: Write on observation page-side of the record book)

Test tubes	1(Blank)	2	3	4	5	6
Pipette out aliquots of maltose solution	0	0.2	0.4	0.6	0.8	1.0
Make up to 2 mL	2	1.8	1.6	1.4	1.2	1.0
DNS reagent mL	2	2	2	2	2	2
Heat for 10 min in boiling water bath						
Cool and take OD at 520						



b. ESTIMATION OF K_m AND V_{max} VALUE

1. Transfer aliquots of 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, and 2.8 mL of 1% starch solution ether with 5 mL phosphate buffer as blank.
2. Make up the volume to 5 mL using phosphate buffer and add 1mL of 1% NaCl solution to all the test tubes.
3. Pre incubate the tubes for the 10 min at 37oC.
4. Add 0.5 mL of distilled water to all the test tubes.
5. Add 0.5 mL of enzyme solutions to each test tube.
6. Mix well and incubate for 15 min at 37oC.
7. Arrest reaction by adding 0.5 mL of 2N NaOH solution.
8. Add DNS reagent to all the test tubes, boil in water bath for about 10 min, and cool.
9. Read the OD at 520 nm.
10. Plot the graph of 1/[V] versus 1/[S] and estimate *Km* and *Vmax* value.

(Note: Write on observation page-side of the record book)

Test Tubes	B	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Add 1 % of Starch	0	0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4	2.6
Make up the volume to 5 mL with H_2O	5	4.2	4.0	3.8	3.6	3.4	3.2	3.0	2.8	2.6	2.4
Add 1mL 1% NaCl solution	1	1	1	1	1	1	1	1	1	1	1
Con. of starch **[S] (mg/mL)**											
Pre incubate at 37°C for 10 min											
Distilled water (mL)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dilute saliva as Enzyme source (mL)	2	2	2	2	2	2	2	2	2	2	2
Mix well and incubate at 37°C for 15 min.											
2N NaOH mL	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
DNS reagent	2	2	2	2	2	2	2	2	2	2	2
Mix well and incubate at 37°C for 15 min.											
OD at 520nm											
Activity of the enzyme V mg/min											
1/[S] mL/mg											
1/V min/mg											

RESULT:

Km & Vmax for the enzyme

Km = mg/mL

Vmax= mg/mL/min