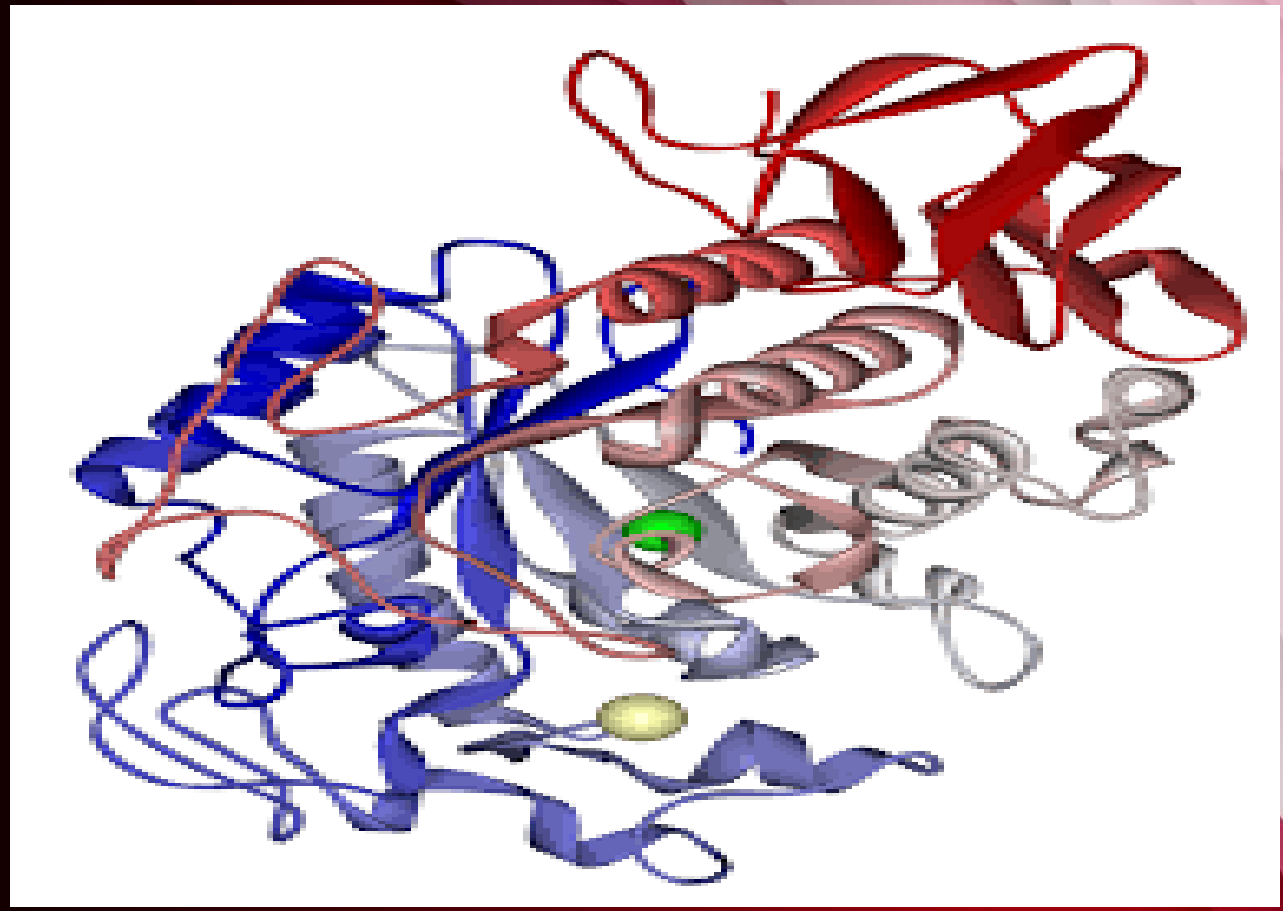
1. Isolation of beta amylase from sweet potato
2. Determination of alpha amylase activity in saliva.



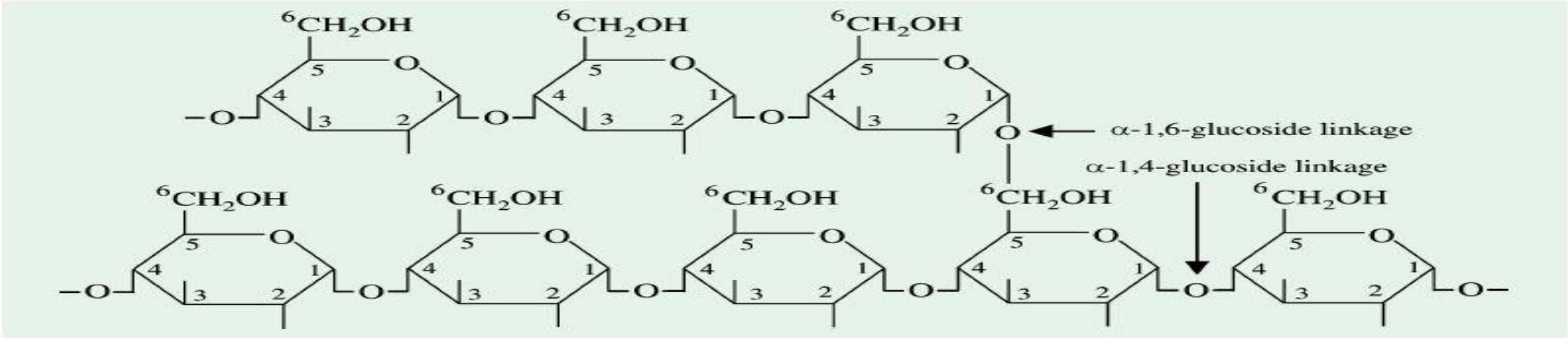
18BTL57

BIOKINETICS AND ENZYME TECHNOLOGY LABORATORY

Experiment 1: Isolation of beta amylase from sweet potato

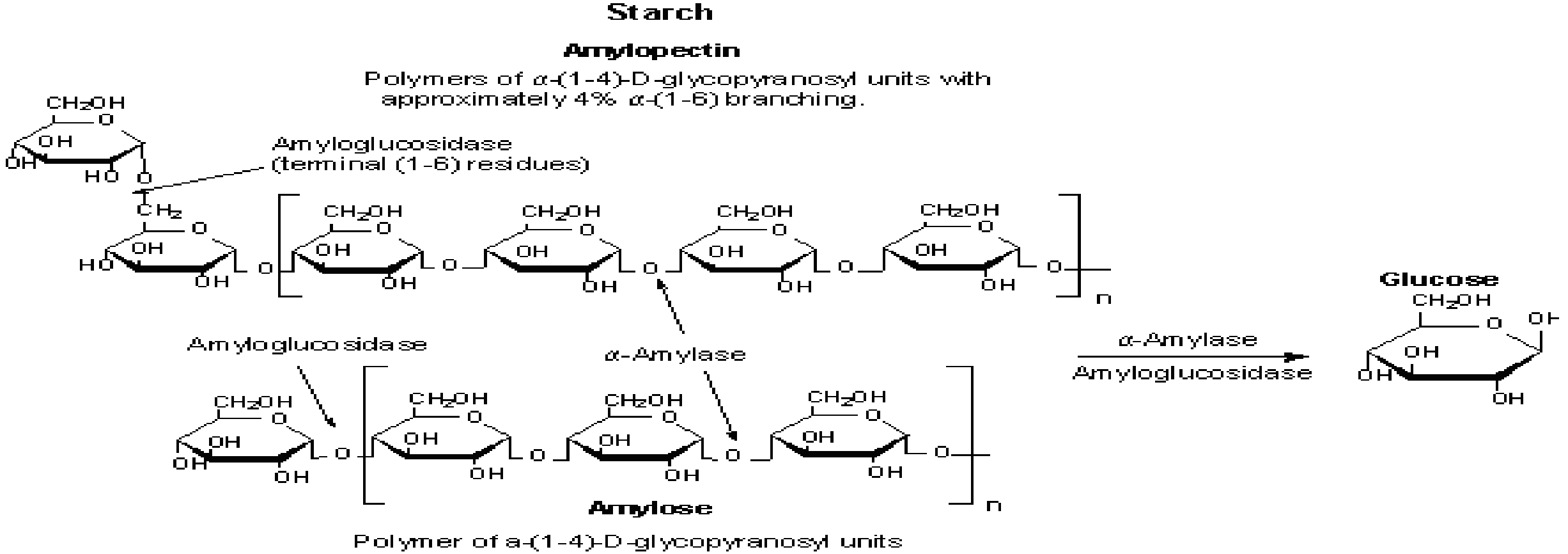
Objective: To isolate the β -amylase using sweet potato as a source.

- Carbohydrates is the major storage form of energy in plants and in animals.
- Starch is a homopolysaccharide which is the most important storage polysaccharides in plant cells.
- It is composed of two types of glucose polymer, amylose and amylopectin.
- The **amylose** consists of **long, unbranched chains of D-glucose residues joined by (α-1,4) linkages**.
- The successive glucose residues in **amylopectin** are joined by **α-1,4 glycosidic linkages and α-1,6 linkages for branch points**.



Structure of amylopectin (= B-fraction)

- Amylases are enzymes that hydrolyze starch.
- The enzyme β-amylase catalyses the hydrolysis of α-1,4 glycosidic linkages from the non-reducing end of the polysaccharides (starch –amylose, amylopectin), to yield maltose units.
- The glucose residues at the nonreducing ends of the outer branches are removed enzymatically to facilitate the mobilization of starch for energy production. Thus it is also known as 1-4-α-D-Glucan maltohydrolase.
- β-Amylase is specific for amylose chains of six glucose units.



Materials Required:

1. Sweet potato.
2. Knife/peeler.
3. Mortar and Pestle.
4. A Blender.
5. Blue capped tubes.
6. 20mM sodium Phosphate buffer at pH 7.
7. Vortexer.

Procedure:

1.Take a clean sweet potato and peel the skin off.

Procedure:

2. Weigh the peeled sweet potato and note the weight.

Procedure:

3. The sweet potato is cut into small pieces and transferred into a mortar and pestle.

Procedure:

4. The pieces are crushed and then transferred into a blender.

Procedure:

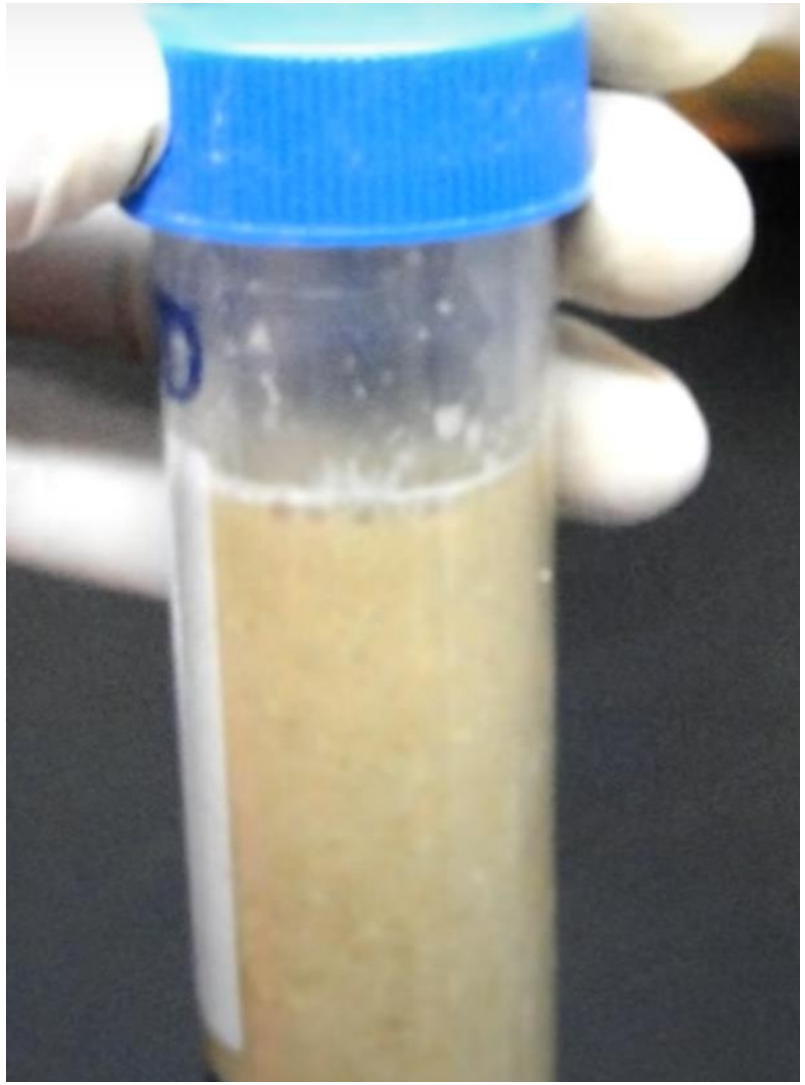
The pieces are crushed and then transferred into a blender.

Add 40 ml of cold **20mM sodium phosphate buffer saline**. Blend it until it forms a paste.

Procedure:

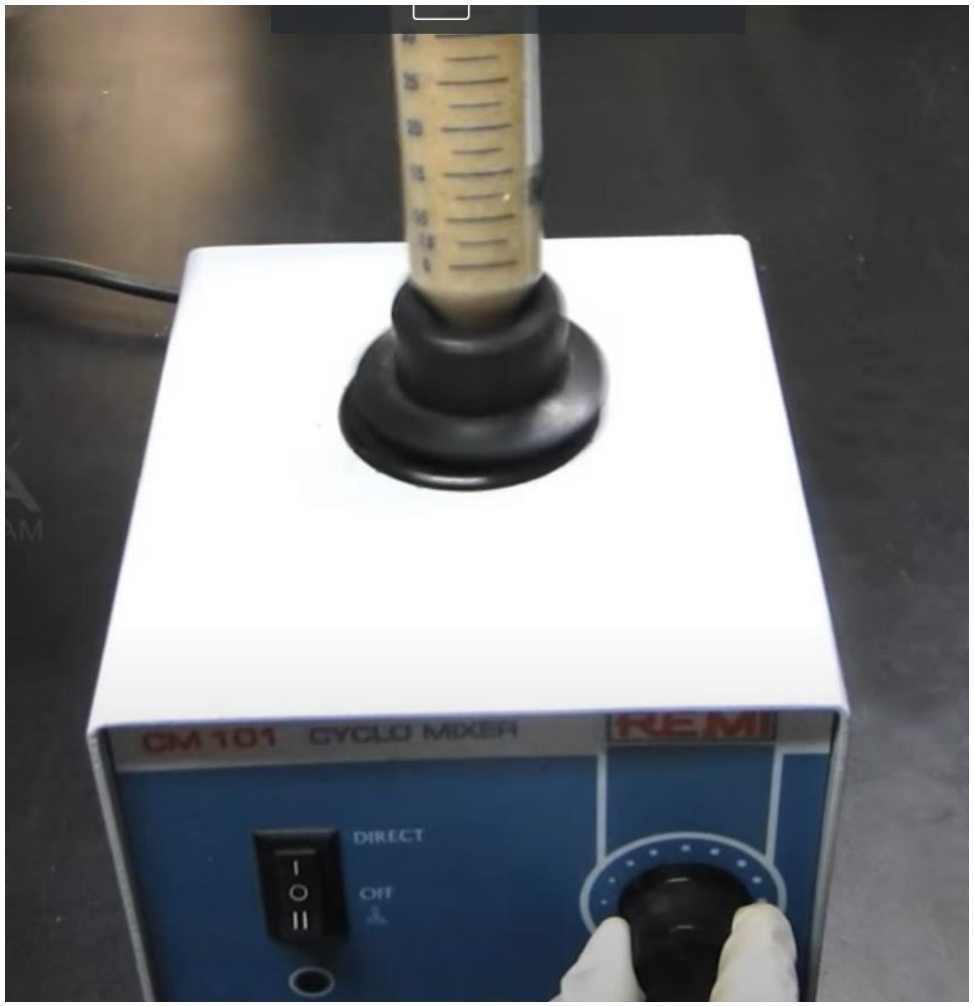
Gently transfer the potato slurry into a blue capped tube.

Procedure:



Allow the enzyme to extract over a 1 hour period at room temperature, with frequent vigorous stirring on a vortex mixer.

Procedure:

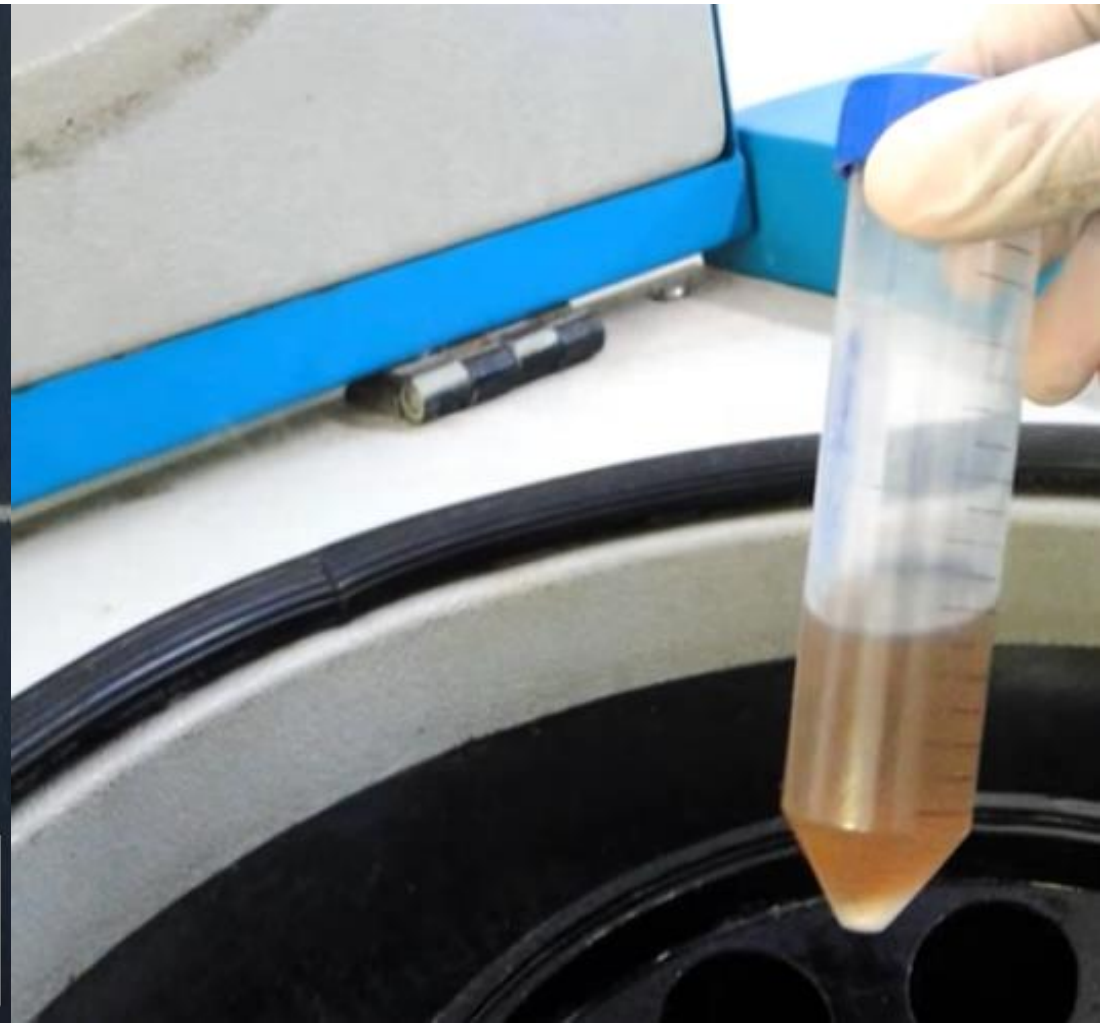


Allow the enzyme to extract over a 1 hour period at room temperature, with frequent vigorous stirring on a vortex mixer.

Procedure:

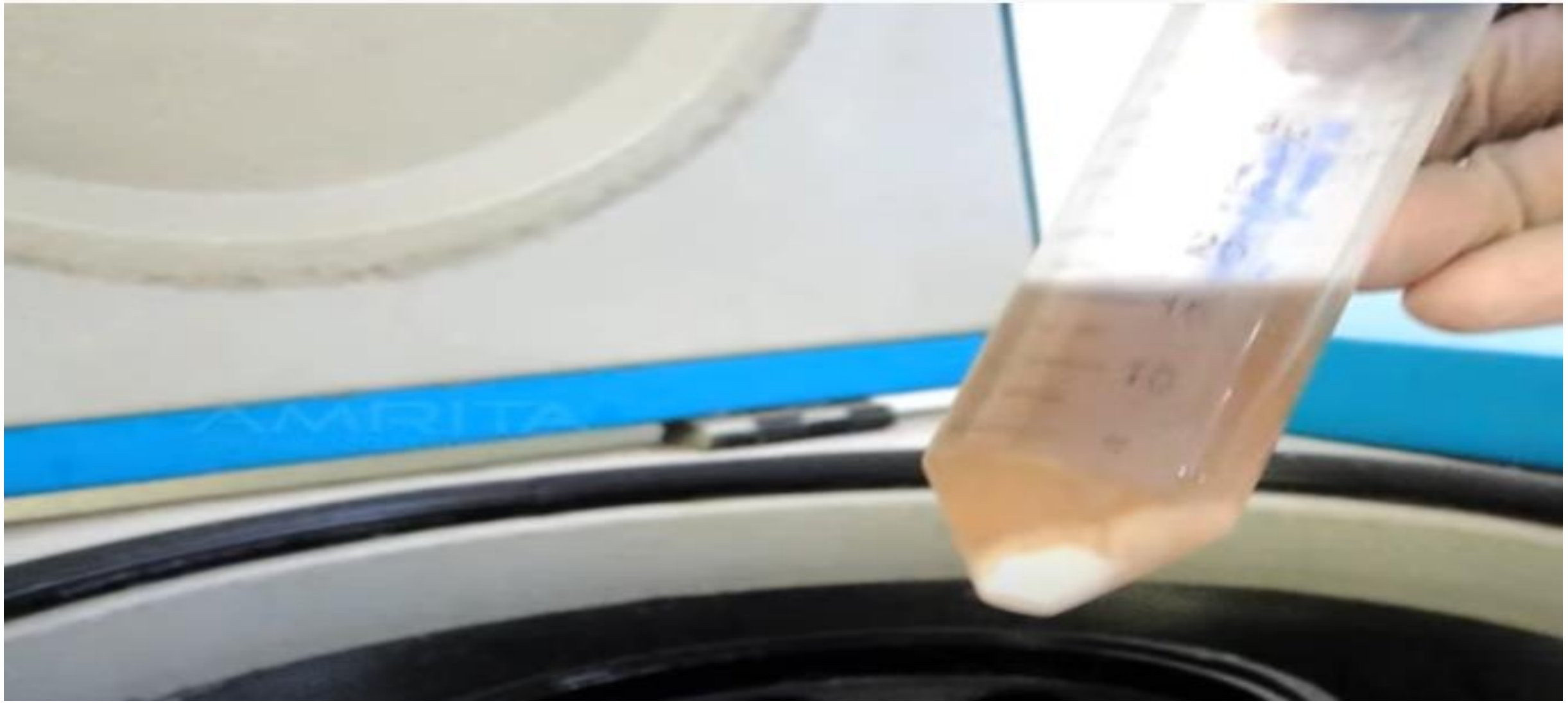
Then the extract is filtered using a filter and the filtrate is collected in a new blue capped tube.

Procedure:



Centrifuge the filtrate at 12000rpm for 20 minutes at 4 degree Celsius.

Procedure:



After the centrifugation, take out the blue capped tube.

Procedure:

Carefully transfer the supernatant into a new blue capped tube and is stored at 4 degree Celsius in refrigerator and discard the pellet.

Experiment 2: Determination of alpha amylase activity in saliva

AIM: To standardize a procedure for determining the enzymatic activity of α-Amylase

Principle:

- **Alpha Amylase** is the **hydrolytic enzyme** that breaks down many polysaccharides such as starch which is the polymer of glucose units linked by **α-1, 4 bond to yield the disaccharide**, **maltose** as the end product.
- **The units of the amylase activity can be expressed as the quantity of the maltose formed in unit time** and the **specific activity of the amylase is expressed as the units of enzyme activity in relation to the concentration of the total protein present in the sample under standard conditions**.
- Here, the substrate starch and the product maltose are colorless and cannot be estimated directly, and hence converted to colored product.
- Quantitative estimation of α –amylase is done based on the product formed after the enzyme activity.

Starch + H2O > α-Amylase > Reducing Groups (maltose**)**

Experiment 2: Determination of alpha amylase activity in saliva

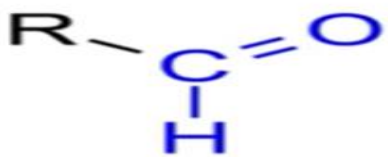
AIM: To standardize a procedure for determining the enzymatic activity of α-Amylase

Principle:

- **Alpha Amylase** is the **hydrolytic enzyme** that breaks down many polysaccharides such as starch which is the polymer of glucose units linked by **α-1, 4 bond to yield the disaccharide**, **maltose** as the end product.
- **The units of the amylase activity can be expressed as the quantity of the maltose formed in unit time** and the **specific activity of the amylase is expressed as the units of enzyme activity in relation to the concentration of the total protein present in the sample under standard conditions**.
- Here, the substrate starch and the product maltose are colorless and cannot be estimated directly, and hence converted to colored product.
- Quantitative estimation of α –amylase is done based on the product formed after the enzyme activity.

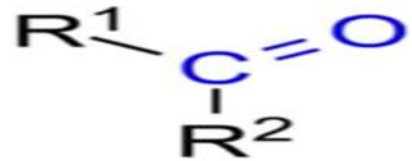
Starch + H2O > α-Amylase > Reducing Groups (maltose**)**

$$\text{Starch} + H_2O \xrightarrow{\alpha\text{-amylase}} \text{Reducing Groups (Maltose)}$$



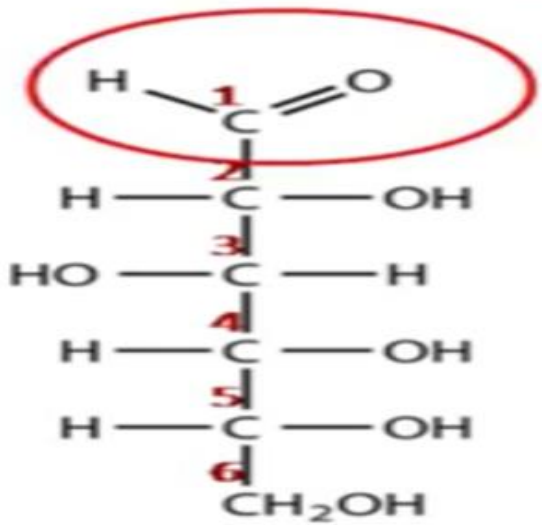
Aldehyde

Reducing sugars

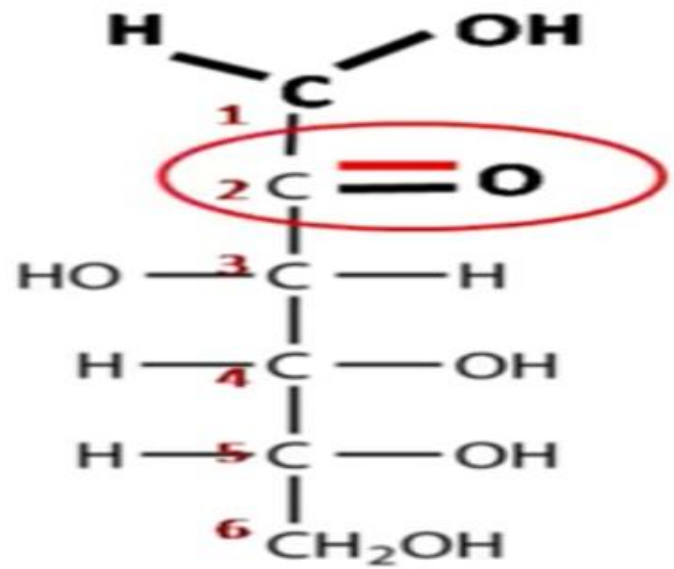


Ketone

Reducing sugars

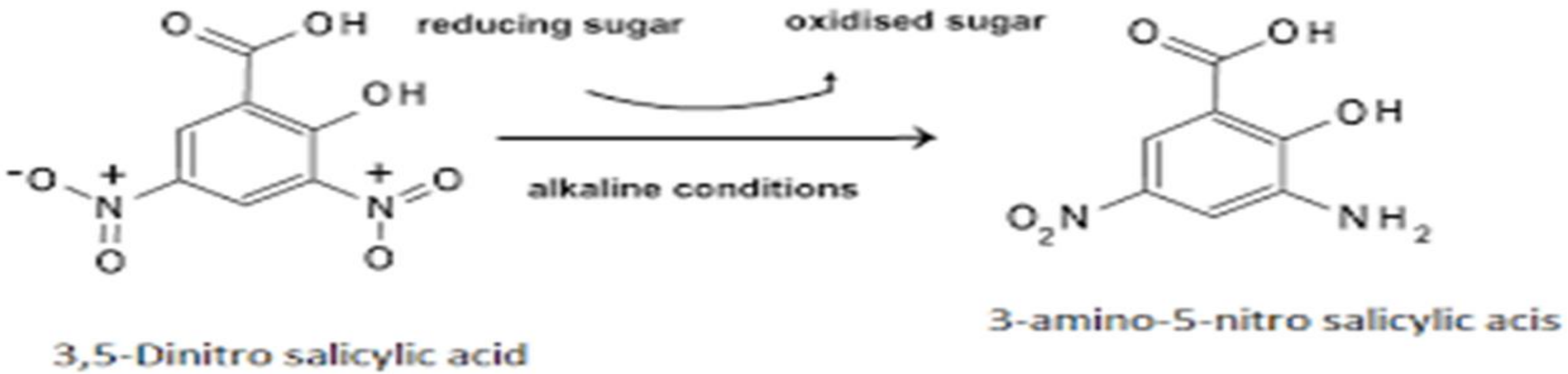


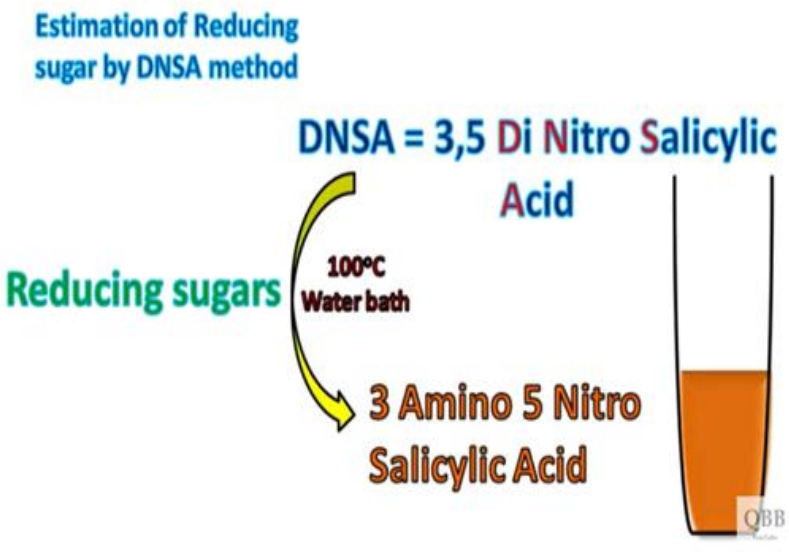
Glucose

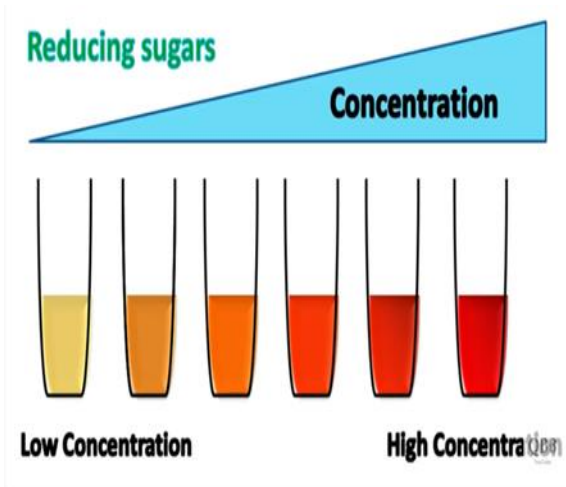


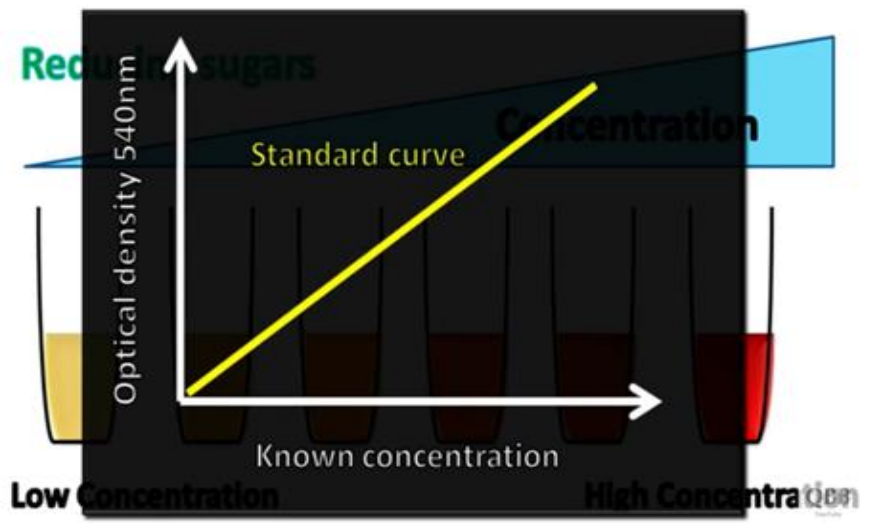
Fructose

$$\text{Starch} + H_2O \xrightarrow{\alpha\text{-amylase}} \text{Reducing Groups (Maltose)}$$









REAGENTS REQUIRED:

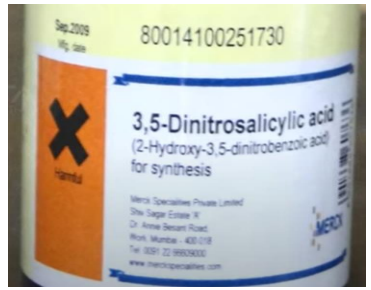
1. **Buffer: 20 mM Sodium Phosphate Buffer with 6.7 mM Sodium Chloride**, pH 6.9 at 20°C.Prepare 100 mL in purified water using Sodium Phosphate, Monobasic, Anhydrous, and Sodium Chloride. Adjust to pH 6.9 at 20°C with 1 M NaOH.

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.

3. **0.2% (w/v) Maltose Standard (**STD**):** Prepare 10 mL in purified water using Maltose, Monohydrate .

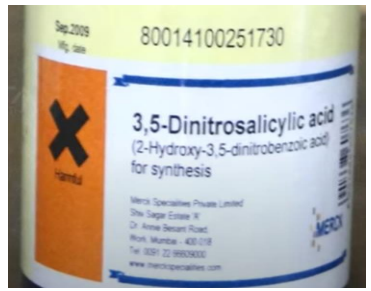
4. **α-Amylase Solution (**Enzyme**):** Saliva is the best and easily available source for amylase. Collect some saliva in beaker and dilute with 1:20 distilled water.

5. 3,5-Dinitrosalicylic Acid Solution: Prepare 20 mL in purified water, 50°C - 70°C, using 3,5-Dinitrosalicylic Acid,. Heat directly on a heating/stir plate with constant stirring to dissolve. **DO NOT BOIL**.



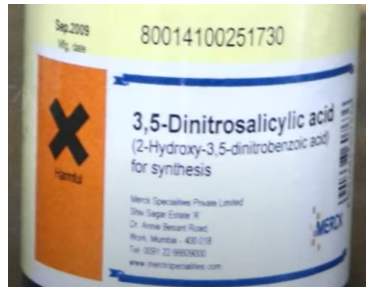
REAGENTS REQUIRED:

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



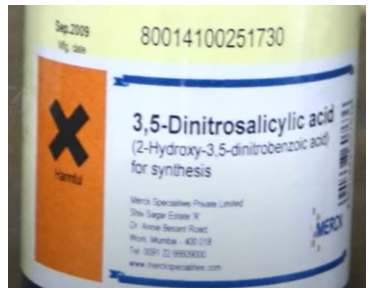
REAGENTS REQUIRED:

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.



REAGENTS REQUIRED:

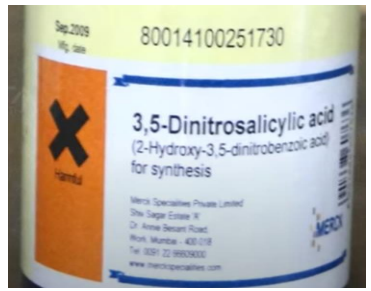
3. **0.2% (w/v) Maltose Standard (**STD**):** Prepare 10 mL in purified water using Maltose, Monohydrate .



REAGENTS REQUIRED:

.

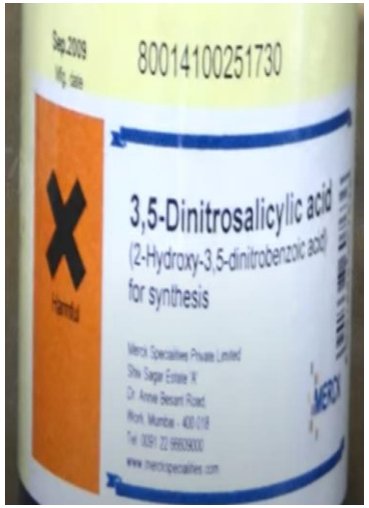
4. **α-Amylase Solution (**Enzyme**):** Saliva is the best and easily available source for amylase. Collect some saliva in beaker and dilute with 1:20 distilled water.



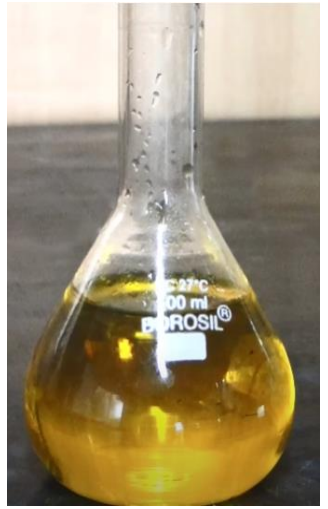
REAGENTS REQUIRED:

5. 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 water and mix. Protect from carbon dioxide and store no longer than 2 weeks.








ISOLATION OF ALPHA AMYLASE FROM SALIVA USING AMMONIUM SULFATE

PRECIPITATION METHOD

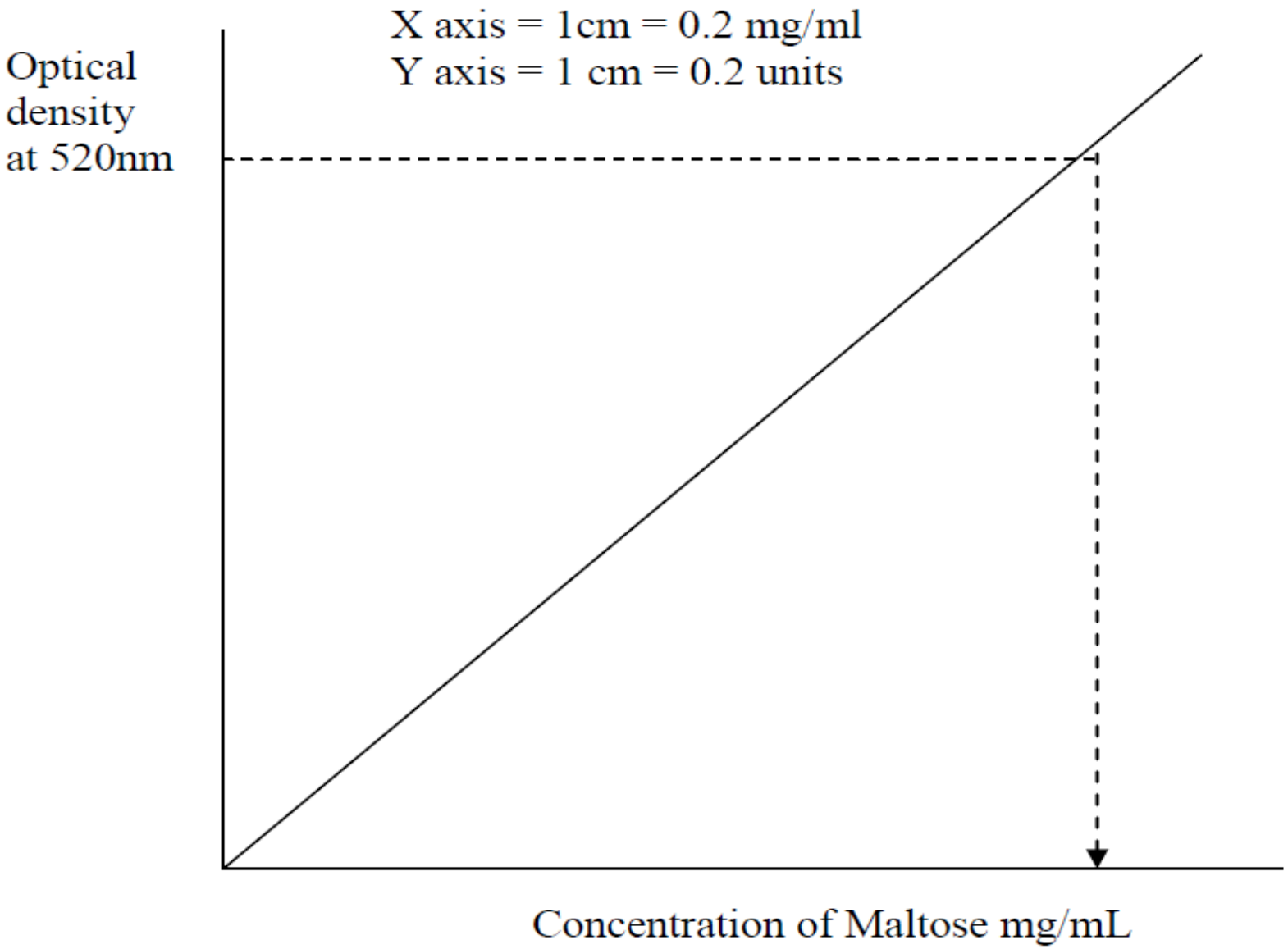
- Collect the fresh saliva by spitting in to a fresh beaker before meal. Into 100 mL of 1:20 diluted saliva 6 g finely ground pure ammonium sulfate is added slowly byconstant stirring to get 80% saturation, and allowed to stand for 30 min.
- Precipitate formed is pelleted by centrifugation at 5000 rpm for 10 min.
- Pellets formed is reconstituted using 2 mL of 0.001 M Phosphate Buffer.
- Enzyme activity was determined and compared with the crude.

b. PLOTTING STANDARD GRAPH FOR MALTOSE:

- Pipette out aliquots of 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 mL 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.
- Draw the standard graph

Test tubes	1	2	3	4	5	6	7	8
Pipette out aliquots of maltose solution	0	0.2	0.4	0.6	0.8	1.0	1.2	1.4
Make up to 2 mL	2	1.8	1.6	1.4	1.2	1.0	0.8	0.6
DNS reagent mL	2	2	2	2	2	2	2	2
Heat for 10 min in boiling water bath								
OD at 520								

Model graph


X axis = 1cm = 0.2 mg/ml
Y axis = 1 cm = 0.2 units
Optical density at 520nm
Concentration of Maltose mg/mL

c. ENZYME ASSAY:

- Isolated enzyme is incubated with phosphate buffer, 1% NaCl and 1% starch for 15 minutes.
- The amount of maltose formed after the enzyme activity is estimated by DNS method.
- The assay is carried out along with the enzyme blank and the substrate blank.
- The amount of maltose formed in 15 minutes by the amount of enzyme added is (OD of tube 4-OD of tube 2) - (OD of tube 5-OD of tube 3).
- From the standard graph of maltose, the corresponding amounts of maltose formed/ mL of saliva would be calculated.

ENZYME ASSAY:

Test tube	Blank	Test sample 1
Phosphate buffer mL	1	1
Starch solution [S]	2.5	2.5
1% NaCl	1	1

Mix well and incubate 10 min at 37°C

Distilled water	1	1
Dilute saliva [E]	-	0.5

Rest of tube incubated at 37°c for 15 mints

2N NaOH	0.5	0.5
DNS reagent	0.5	0.5

Boil in water bath 10 min, then Cool to room temperature

OD at 520 nm		
Enzyme activity (mg/mL/ 15 min)		
Unit (mg/mL/min)		

Result:

Enzyme activity in the test sample: mg of maltose formed/mL of sample/ 15 min

Enzyme unit: mg of maltose formed/mL of sample/min