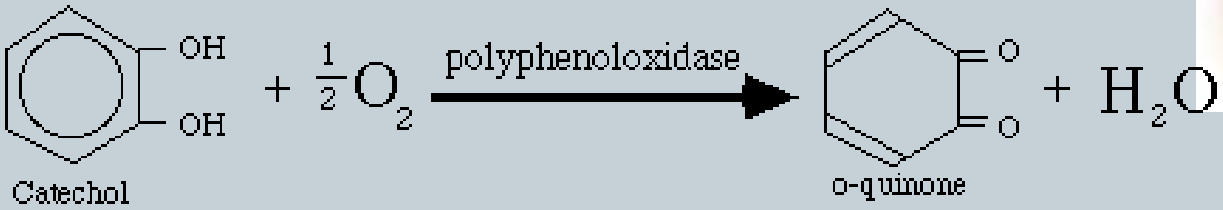
Some factors affecting polyphenol oxidase activity

Poly phenol Oxidase Enzyme

-It is a **copper**-containing enzyme with an optimum pH of **6.7**. It catalyses the **oxidation** of **di- and tri-hydroxyl phenol** to the corresponding **quinine**.

-This oxidation-reduction reaction is accompanied by a color change (quinines absorb light in the visible region of the spectrum).



This reaction commonly occurs in nature and accounts for the "browning" of peeled potatoes and bruised fruits.

You will familiarize yourself with the reaction catalyzed by the enzyme polyphenol oxidase, as it occurs removed from the intact living cell, i.e. in a test tube.

Objectives :

1- To demonstrate ***activity of the enzyme*** polyphenol oxidase in crude extract prepared from potato.

2- To demonstrate the ***chemical nature*** of the enzyme.

3-To investigate the ***substrate specificity*** of the enzyme.

4- To investigate the ***effects of various temperatures*** on the activity of the enzyme.

This experiment is in four parts:

A) Enzyme Activit

B) Chemical Nature of polyphenol oxidase

C) Substrate Specificity

D) Temprature and Enzymetic Activity.

A) Enzyme Activity :

Occurrence of an enzymatic reaction which is the conversion of one molecule into another; a chemical reaction catalyzed at the active sites on the enzyme

Method:

a) Label three clean test tubes A, B and C.

A1	B1	C1
Enzyme(15 drops)	Enzyme(15 drops)	Catechol(15 drops)
Catechol (15 drops)	distilled water (15 drops)	distilled water(15 drops)
Place the tube in a water bath at 37 ^{o}C.		
Shake the tube every 5 min to aerate, thereby adding oxygen to the solution.		
Every 5 minutes, after shaking, hold the tubes up to the light and examine. **Record the color** in each tube. Continue for 25 minutes.		

B) Chemical Nature of poly phenol oxidase

All known enzymes are **proteins**. They are high molecular weight compounds made up principally of chains of **amino acids** linked together by **peptide bonds.**

Therefore it expected that every factor effect on protein will effect the enzyme relatively.

These factors could be :

Temperature, PH value, conc. of certain substances e.g. [S] ,[E] or [I].

Method:

a) Label four clean test tubes A, B, C and D.

A2	B2	C2	D2
15 drops of enzyme	10 drops of enzyme	10 drops of enzyme	15 drops of enzyme
15 drops of **catechol.**	**2ml of trypsin**	10 drops of **trichloroacetic acid**	few crystals of **phenylthiourea**
Shake tube → place in water bath at 37 °C for ⑩ min	Shake tube thoroughly→ Place tube in a **water bath at 37 °C for ⑩ min**	Shake tube thoroughly and wait ⑤ min	Shake tube thoroughly and continue shaking it frequently during a period of ⑤ min.
	in the same water bath add 10 drops of catechol	10 drops catechol	15 drops of catechol
Set tube aside as control with which to compare results of tubes B, C and D	Re place in the same water bath for ⑩ min. compare with tube A.	Place tube in water bath at 37 °C for ⑩ min. compare with tube A.	Place tube in water bath at 37 °C for ⑩ min. compare with tube A

Discussion

What is trypsin?

It is a protease E found in the digestive system , where it hydrolyses proteins .It is produced in the pancreas as inactive E proE (trypsinogen).

Trypsin **cleaves peptide** and used for many biotechnological processes.

The process is referred to as trypsin **proteolysis** or trypsinization, and proteins that have been **digested/treated** with trypsin are said to have been trypsinized.

How do you think it will effect the ppo activity?

Discussion

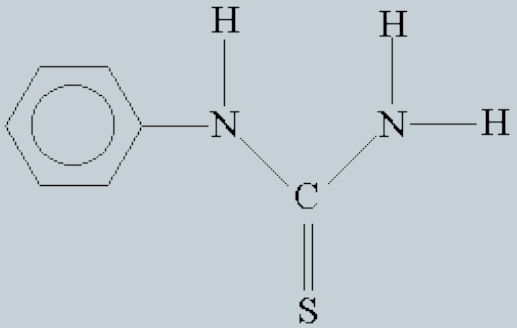
what is trichloroacetic acid?

It is an analogue of acetic acid widely used in biochemistry **for precipitation of macromolecules, such as proteins, DNA, and RNA** .

How do you think it will effect the ppo activity?

Discussion

phenylthiourea (PTU), has a very strong chemical affinity for the element copper. It is able to bind with copper, even when the copper is attached to other chemical substances, as in the active site of polyphenol oxidase.



Phenylthiourea (PTU)

How do you think it will effect the ppo activity?

⚠ CAUTION

PTU and catechol are poisons

Avoid contact with solutions. Do not pipette any solutions by mouth.

Method:

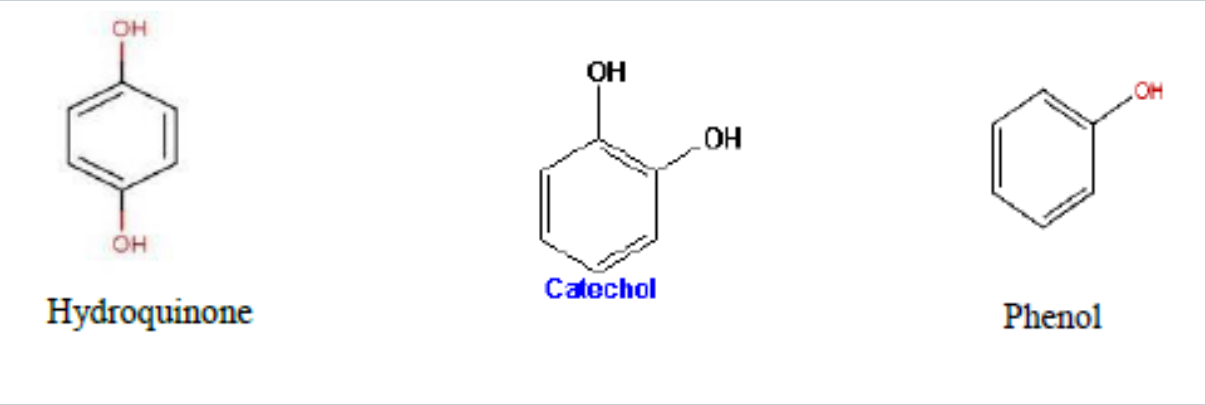
a) Label three clean test tubes A, B and C.

A3	B3	C3
15 drops of enzyme extract	15 drops of enzyme extract	15 drops of enzyme extract
15 drops of catechol solution	**15 drops of phenol solution**	**15 drops of hydroquinone**
Shake the tubes gently and place them in a water bath at 37 ^{o}C.		
Examine the tubes after 5 min and 10 min. Record the color in each tube,		

Discussion

The three compounds used as substrates in this part of the experiment are structurally related, as shown in the figure below. Each is capable of reacting with oxygen to form various colored products

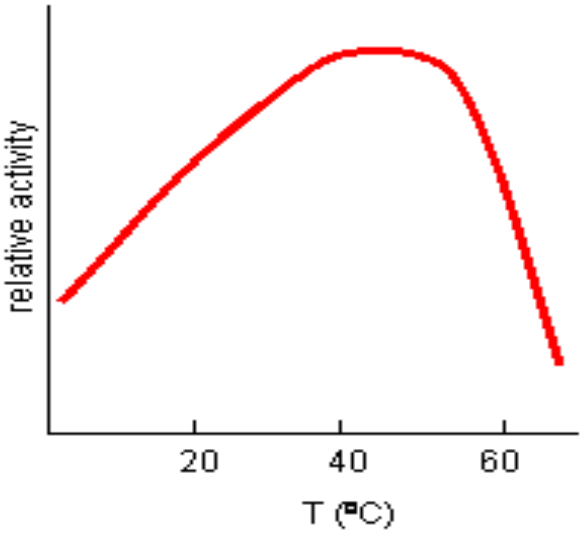
Which tube do you think will show best result ? And why?



D)Temprature and Enzymetic Activity.

The reaction **rate increases** with temperature to a maximum level, then suddenly declines with further increase of temperature. Because most animal enzymes rapidly become denatured at temperatures above 40°C.

Temperature and enzyme activity



Method

a) Label three clean test tubes A, B and C.

A	B	C
15 drops of enzyme extract	15 drops of enzyme extract	15 drops of enzyme extract
Place the tube in a water: 0 ºC for 10 min.	**Place the tube in a water:37 ºC for 10 min.**	**Place the tube in a water:70 ºC for 10 min.**
In the same water bath add to each tube 15 drops of catechol		
Shake the tube gently and quickly return it to its proper temperature condition.		
Wait for 15 min. →examine the tube, without removing it from its temperature condition, and record the color in each tube		

Discussion

Which tube do you think will show best result ? And why?

Thank You