

T.P. n°3 : Study of the enzymatic activity of a medicine

Principle :

Enzymes are used for a variety of therapeutic purposes, including the treatment of genetic disorders and metabolic disorders, the elimination of toxic substances, acting as anti-inflammatory agents, aiding digestion, and, more recently, in cancer treatment and the fight against infectious diseases.

This study involves isolating α -amylase from a medicine and then studying the kinetics of starch hydrolysis by this enzyme. The kinetic study as a function of substrate concentration will allow us to estimate the kinetic parameters (K_m and V_m) of α -amylase. It should be noted that this enzyme follows the Michaelis-Menten kinetic law.

In this experiment, we will assume that the absorbance at 580 nm is due to both amylose and amylopectin. The hydrolysis of starch by amylases produces glucose, maltose, and limited dextrans (oligosaccharide residues with $\alpha(1-6)$ branches). Consequently, the reaction under study causes the reaction medium to decolorize, resulting in a decrease in absorbance at 580 nm. By monitoring the change in absorbance at 580 nm, we can determine the overall apparent kinetic constants of amylase.

Equipment :

- ✓ Mortar ;
- ✓ Test tubes ;
- ✓ Erlenmeyer flasks ;
- ✓ Beakers ;
- ✓ Funnel ;
- ✓ Graduated tubes ;
- ✓ Pipettes and micropipettes ;
- ✓ Hot plates ;
- ✓ Agitator ;
- ✓ Vortex agitator ;
- ✓ Balance, watch glass, and spatula ;
- ✓ UV-Vis spectrophotometer + cuvettes ;
- ✓ Filter paper.

Reagents :

- ✓ I_2 ;
- ✓ KI ;
- ✓ Starch ;
- ✓ Mega-mylase[®] tablet.

Procedure :

1. Preparation of the iodine reagent :

- ✓ Dissolve 2 g of potassium iodide (KI) and then 1 g of iodine (I_2) in 20 mL of distilled water; Complete with distilled water to a total volume of 100 mL.

2. Preparation of the starch solution :

- Dissolve 1 g of starch in 20 mL of distilled water ;
- Add 80 mL of boiling distilled water ;
- Gently agitate the mixture and continue boiling for 5 minutes on a hot plate (to obtain a clear solution) ;
- Cool the mixture and complete to a volume of 100 mL with distilled water.

3. Preparation of the enzymatic solution :

- Dissolve the coating of 3 Mega-mylase[®] tablets under a slow stream of water ;
- Once only the tablet cores remain, crush them in 50 mL of distilled water to obtain the enzymatic solution ;
- Filter the solution before use.

4. Study of the variation in initial speed as a function of substrate concentration :

	Tube 0 (blank)	Tube 1	Tube 2	Tube 3	Tube 4
Starch solution (mL)	0	1	3	6	10
Distilled water (mL)	10	9	7	4	0
Iodine reagent (mL)	0,02	0,02	0,02	0,02	0,02
Enzymatic solution (mL)	1	1	1	1	1
OD at 580 nm (every 30 seconds, then every minute)		t = 0 S t = 30 S t = 60 S t = 2min t = 3min t = 4min t = 5min t = 6min	"	"	"

Note : Add the enzymatic solution immediately before reading the DO.

Use the same blank for all measurements.

Work to do :

1. Plot the curve $DO = f(t)$ for each starch concentration and determine the initial rates for each concentration ;
2. Plot the curve $v_i = f([starch])$ and determine whether this kinetics follows the Michaelis-Menten equation or not ;
3. Plot the curve $1/v_i = f(1/[S])$ and calculate the kinetic parameters K_m and V_m .