

Model answer for T.D. no. 4

(Enzyme kinetics with one substrate)

Exercise n° 1 :

We have : $K_m = 1 \cdot 10^{-3} \text{ M} = 1 \text{ mM}$

- **Calculation of V_m :**

According to the Michaelis-Menten equation : $V_i = V_m \cdot [S] / K_m + [S]$

So : $V_m = V_i (K_m + [S]) / [S]$

numerical calculation: $V_m = 0,15 \text{ mM/min}$

Substrate (mM)	Initial speed ($\mu\text{M/min}$)
1	75
2	100
3	112,5
10	136,36

Exercise n° 2 :

We have :

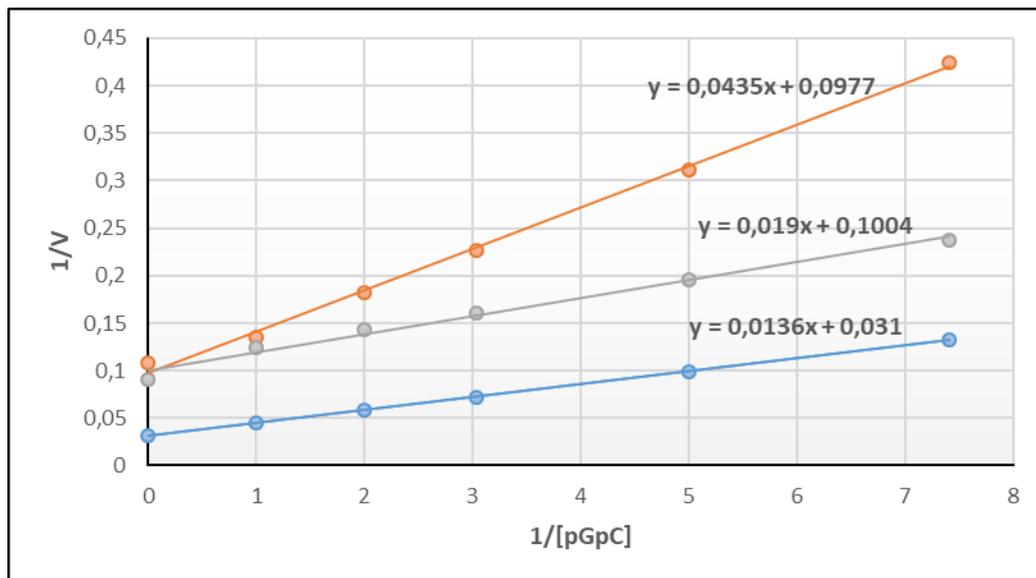
The enzyme : ribonuclease T₁

The substrate : le pGpC.

- ✓ To determine the values of maximum rate (V_m) and Michaelis constant (K_m), we plot a graph of **Lineweaver-Burk** : $1/V_i = f(1/[S])$.
- ✓ To determine the values of the catalytic rate constant (k_{cat}), the following formula is used :
 $V_m = k_{cat} \times [E_t]$ so : $k_{cat} = V_m/[E_t]$

	1/[pGpC] (mM^{-1})				
	7,41	5,00	3,03	2,00	1,00
Asp-58	0,132	0,099	0,072	0,058	0,045
Gln-58	0,425	0,312	0,227	0,182	0,135
Ala-58	0,238	0,196	0,161	0,143	0,125

$1/V_m$ is expressed in $\mu\text{M}^{-1} \cdot \text{min}$



For ribonuclease Asp-58 :

Graphically :

- $-1/K_m = -2,3 \text{ mM}^{-1}$ so : **$K_m = 0,43 \text{ mM}$**
- $1/V_m = 0,031 \text{ } \mu\text{M}^{-1} \cdot \text{min}$ so : **$V_m = 32,26 \text{ } \mu\text{M} \cdot \text{min}^{-1}$**
- $k_{\text{cat}} = 32,26/5,4 \times 10^{-3}$ so : **$k_{\text{cat}} = 5974 \text{ min}^{-1}$**

For ribonuclease Gln-58 :

Graphically :

- $-1/K_m = -2 \text{ mM}^{-1}$ so : **$K_m = 0,5 \text{ mM}$**
- $1/V_m = 0,089 \text{ } \mu\text{M}^{-1} \cdot \text{min}$ so : **$V_m = 11,23 \text{ } \mu\text{M} \cdot \text{min}^{-1}$**
- $k_{\text{cat}} = 11,23/9 \times 10^{-3}$ so : **$k_{\text{cat}} = 1248 \text{ min}^{-1}$**

For ribonuclease Ala-58 :

Graphically :

- $-1/K_m = -6 \text{ mM}^{-1}$ so : **$K_m = 0,167 \text{ mM}$**
- $1/V_m = 0,107 \text{ } \mu\text{M}^{-1} \cdot \text{min}$ so : **$V_m = 9,34 \text{ } \mu\text{M} \cdot \text{min}^{-1}$**
- $k_{\text{cat}} = 9,34/9 \times 10^{-3}$ so : **$k_{\text{cat}} = 1038 \text{ min}^{-1}$**

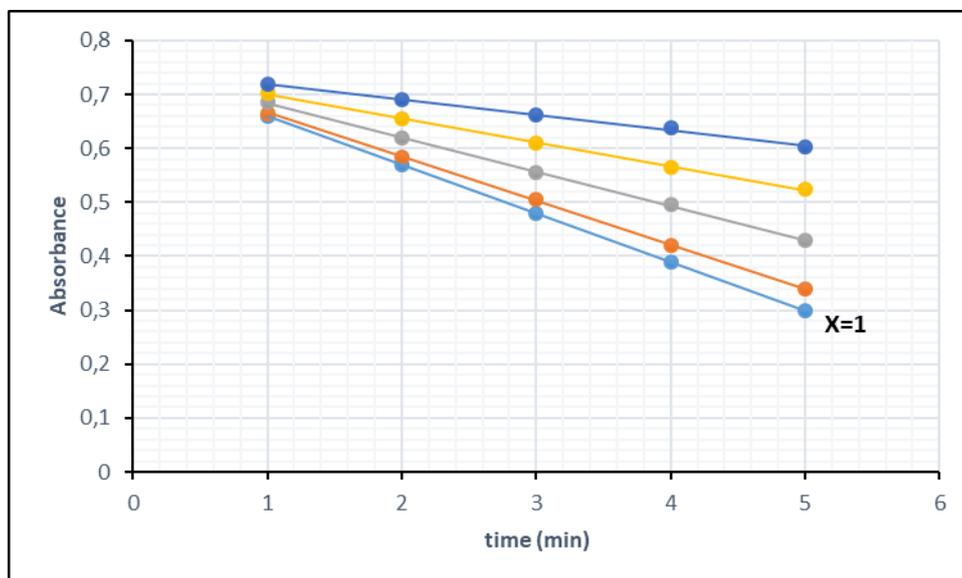
Exercise n° 3 :

we have :

**Calculation of V_i for each $[X]$:**

- ✓ To determine the initial rate, plot the curve of absorbance measured as a function of time : $A = f(t)$ for each concentration of X and calculate the slope. V_i values are expressed in $(\Delta A \cdot \text{min}^{-1})$.
- ✓ Using the law of **Beer-Lambert ($A = \epsilon \cdot l \cdot C$)** so : $C = A/\epsilon \cdot l$; we obtain V_i in $(\mu\text{M} \cdot \text{min}^{-1})$ knowing that : $l = 1 \text{ cm}$ and $\epsilon = 6220 \text{ M}^{-1} \cdot \text{cm}^{-1}$:

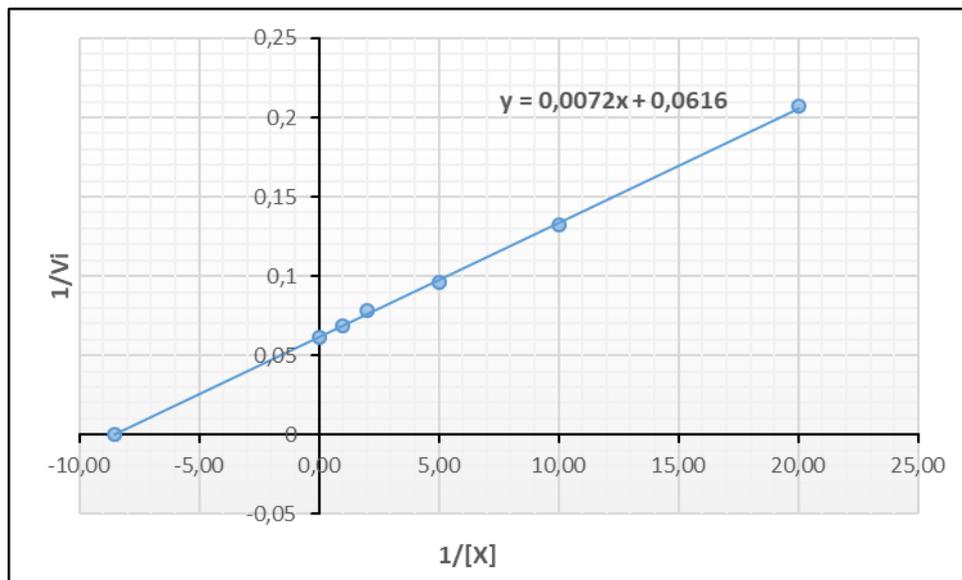
$$V_i (\mu\text{M} \cdot \text{min}^{-1}) = V_i (\Delta A \cdot \text{min}^{-1}) / \epsilon \cdot l$$



V_i	$[X]$ (mM)				
	1	0,5	0,2	0,1	0,05
The slope	- 0,09	- 0,08	- 0,065	- 0,047	- 0,03
En $\Delta A \cdot \text{min}^{-1}$	0,09	0,08	0,065	0,047	0,03
En $\mu\text{M} \cdot \text{min}^{-1}$	14,52	12,86	10,45	7,56	4,82

Example :

When $[X] = 1 \text{ mM}$ we have : $V_i = 0,09/1 \times 6220 = 14,5 \times 10^{-6} \text{ M} \cdot \text{min}^{-1}$ from which $V_i = 14,5 \mu\text{M} \cdot \text{min}^{-1}$

**Km et Vm :**

In order to determine the kinetic parameters of the enzyme (K_m and V_m), a Lineweaver-Burk plot is constructed : $1/V_i = f(1/[S])$.

$1/V_i$ ($\mu\text{M}^{-1} \cdot \text{min}$)	0,069	0,078	0,096	0,132	0,207
$1/[X]$ (mM^{-1})	1	2	5	10	20

Graphically :

- $1/V_m = 0,062 \mu\text{M}^{-1} \cdot \text{min}$ so : **$V_m = 16,13 \mu\text{M} \cdot \text{min}^{-1}$**
- $-1/K_m = -9,09 \text{ mM}$ so : **$K_m = 0,11 \text{ mM}$**

Exercise n° 4 :

We have :

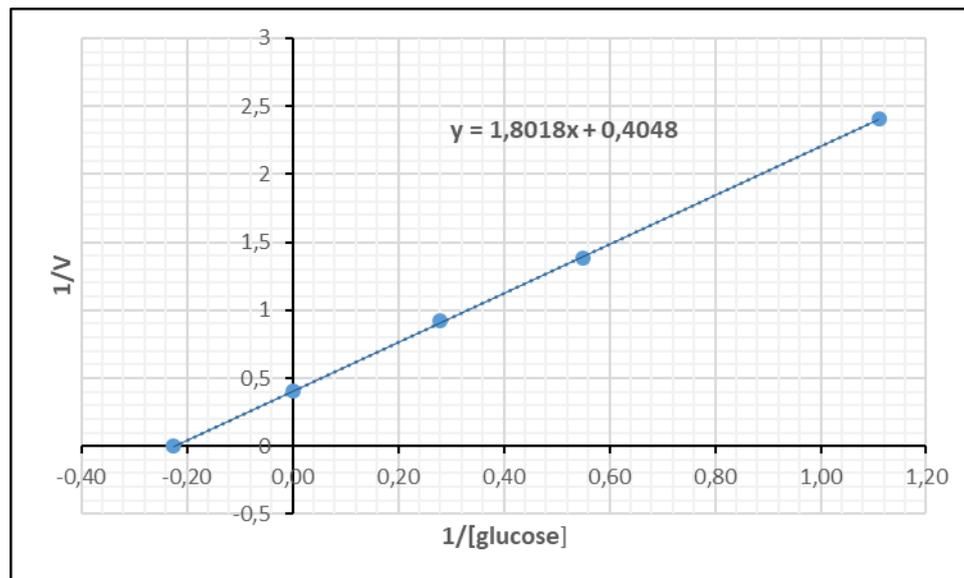
The enzyme : notatin

Substrates : glucose and oxygen gas

1. Km and Vm :

To determine K_m and V_m , we plot the Lineweaver-Burk graph :
 $1/V_i = f(1/[S])$ so : $1/V_i = f(1/[\text{glucose}])$

$1/[\text{glucose}]$ ($\text{mg}^{-1} \cdot \text{mL}$)	1,11	0,55	0,28
$1/V_i$ ($\text{mL}^{-1} \cdot \text{min}$)	2,41	1,38	0,92



- Graphically :

$$1/V_m = 0,4 \text{ mL}^{-1} \cdot \text{min} \text{ so : } V_m = 2,5 \text{ mL} \cdot \text{min}^{-1}$$

We have : molar volume of $O_2 = 24,5 \text{ L/mol}$ so the amount in moles contained in 2,5 mL is equal to 10^{-4} mol

Knowing that the volume of the reaction medium is 1mL, we obtain :

$$V_m = 10^{-4} \text{ mol/mL/min from which : } V_m = 0,1 \text{ mol} \cdot \text{L}^{-1} \cdot \text{min}^{-1}$$

- Graphically :

$$-1/K_m = -0,22 \text{ mg}^{-1} \cdot \text{mL} \text{ so : } K_m = 4,54 \text{ mg} \cdot \text{mL}^{-1}$$

We have : the molar mass of glucose = 180 g/mol so the amount in moles contained in 4,54 mg is equal to $2,5 \cdot 10^{-5} \text{ mol}$

Knowing that the volume of the reaction medium is 1mL, we obtain :

$$K_m = 2,5 \cdot 10^{-5} \text{ mol/mL from which : } K_m = 0,025 \text{ mol} \cdot \text{L}^{-1}$$

- The specific molar activity of notatin (Z_m) :**

We know that : $Z_m = Z/[E]$ with $[E]$ is the molar concentration of the enzyme

We have : molecular weight of notatin = 152000 Da = 152000 g/mol

Knowing that the mass of the enzyme used is 1mg, the amount in moles contained in this mass is equal to $6,58 \cdot 10^{-9} \text{ mol}$

Where from : $[E] = 6,58 \cdot 10^{-9} \text{ mol/mL} = 6,58 \cdot 10^{-6} \text{ mol/L}$

Calculation :

$$Z_m = 0,1 \text{ mol} \cdot \text{L}^{-1} \cdot \text{min}^{-1} / 6,58 \cdot 10^{-6} \text{ mol} \cdot \text{L}^{-1}$$

$$\text{So : } Z_m = 15197 \text{ min}^{-1} = 253 \text{ S}^{-1}$$

Exercise n° 5 :

We have :

The enzyme : lactase (β -galactosidase)

The substrate : lactose

The products : glucose and galactose.

1. Calculation of catalytic activity (Z) in (kat/mL) and in (U/mL) :

We know that :

$$1 \text{ UI} = 1 \mu\text{mol}/\text{min}$$

$$1 \text{ kat} = 1 \text{ mol}/\text{S}$$

Knowing that catalytic activity is a rate and we have $0,672 \times 10^{-2}$ moles of glucose which appears in 10 minutes in 1 mL, so : $V = \text{concentration}/t$

$$\checkmark Z = 0,672 \times 10^{-2} \text{ mol}/1 \text{ mL}/10 \text{ min} = 672 \mu\text{mol}/\text{min}/\text{mL} \text{ d'où : } Z = 672 \text{ UI}/\text{mL}$$

$$\checkmark Z = 0,672 \times 10^{-2} \text{ mol}/1 \text{ mL}/10 \text{ min} \times 60 = 1,12 \times 10^{-5} \text{ mol}/\text{S}/\text{mL} \text{ d'où : } Z = 1,12 \times 10^{-5} \text{ kat}/\text{mL}$$

2. Calculation of specific activity (Zsp) in (kat/mg) and in (U/mg) :

We have : $Z_{sp} = Z / \text{protein mass}$

Knowing that $[Et] = 2,85 \text{ g}/\text{l}$ so the amount of enzyme contained in 1 ml is : $\text{mass} = 2,85 \text{ mg}$

So :

$$\checkmark Z_{sp} = 672 \text{ (UI/mL)}/2,85 \text{ mg} \text{ from which : } Z_{sp} = 235,79 \text{ UI}/\text{mg}$$

$$\checkmark Z_{sp} = 1,12 \times 10^{-5} \text{ (kat/mL)}/2,85 \text{ mg} \text{ from which : } Z_{sp} = 3,93 \times 10^{-6} \text{ kat}/\text{mg}$$

3. Calculation of specific molar activity (Zm) :

We have : $Z_m = Z / [\text{enzyme}]$ ($[\text{enzyme}]$ is a molar concentration).

- Calculation of $[\text{enzyme}]$:

We have : $M_{\text{lactase}} = 135000 \text{ Dalton}$

Knowing that $1 \text{ Dalton} = 1 \text{ g}/\text{mol}$ so the amount in moles contained in 2.85 g is $2,11 \times 10^{-5} \text{ mol}$

So : $[\text{enzyme}] = 2,11 \times 10^{-5} \text{ mol}/\text{L}$ from which : $[\text{enzyme}] = 2,11 \times 10^{-8} \text{ mol}/\text{mL}$

- Calculation of Z_m :

$$\checkmark Z_m = 672 \text{ (UI/mL)}/2,11 \times 10^{-8} \text{ mol}/\text{mL} \text{ from which : } Z_m = 318,48 \times 10^2 \text{ min}^{-1}$$

$$\checkmark Z_m = 1,12 \times 10^{-5} \text{ (kat/mL)}/2,11 \times 10^{-8} \text{ mol}/\text{mL} \text{ from which : } Z_m = 530 \text{ S}^{-1}$$