Model answer for T.D. no. 5

(Enzyme inhibitors)

Exercise n° 1 :

Knowing that a competitive inhibitor increases the Michaelis constant by a factor of (1+[I]/Ki), the expression for rate becomes :

$$\mathbf{v = v_m [S] / [K_M (1 + [I] /K_I) + [S]]}$$

This implies :

$K_M (1 + [I] /K_I) + [S] = v_m [S]/v \Longrightarrow K_M + K_M[I] /K_I + [S] = v_m [S]/v$

$\Longrightarrow (K_M + [S]) + K_M[I] /K_I = v_m [S]/v$

$\Longrightarrow K_M[I] /K_I = v_m [S]/v - (K_M + [S])$

$\Longrightarrow K_M[I] /K_I = (v_m [S]-v(K_M + [S])/v$

$\Longrightarrow K_I/ K_M[I] = v/(v_m [S]-v(K_M + [S])$

So : $\mathbf{K_I = K_M[I] v/(v_m [S]-v(K_M + [S])}$

C.N. : $\mathbf{K_I = 1,8 . 10^{-6} M = 1,8 \mu M}$

Exercise n° 2 :

Having 75% inhibition means that the initial speed decreases by 75%, so :

Vi' = (100% – 75%) Vi = 25% Vi = 0,25 Vi so : Vi'/Vi = 0,25

By replacing each speed with its mathematical expression, we obtain :

$Vi'/Vi = 0,25 = (v_m [S] / [K_M (1 + [I] /K_I) + [S]])/ (v_m [S] / [K_M + [S]])$

$0,25 = (K_M + [S])/ (K_M (1 + [I] /K_I) + [S])$

$(K_M (1 + [I] /K_I) + [S]) = (K_M + [S])/0,25$

$K_M + K_M[I] /K_I + [S] = (K_M + [S])/0,25$

$K_M[I] /K_I = (K_M + [S])/0,25 - (K_M + [S])$

$K_M[I] /K_I = ((K_M + [S]) - 0,25(K_M + [S]))/0,25$

$K_M[I] /K_I = 0,75(K_M + [S])/0,25$

So : $\mathbf{[I] = 0,75(K_M + [S]).K_I /0,25.K_M}$

C.N. : $\mathbf{[I] = 3,7.10^{-4} M}$

Exercise n° 3 :

We have :

Enzyme : S-adenosyl-homocycteinase

Substrate : S-adenosyl-homocycteine (SAH)

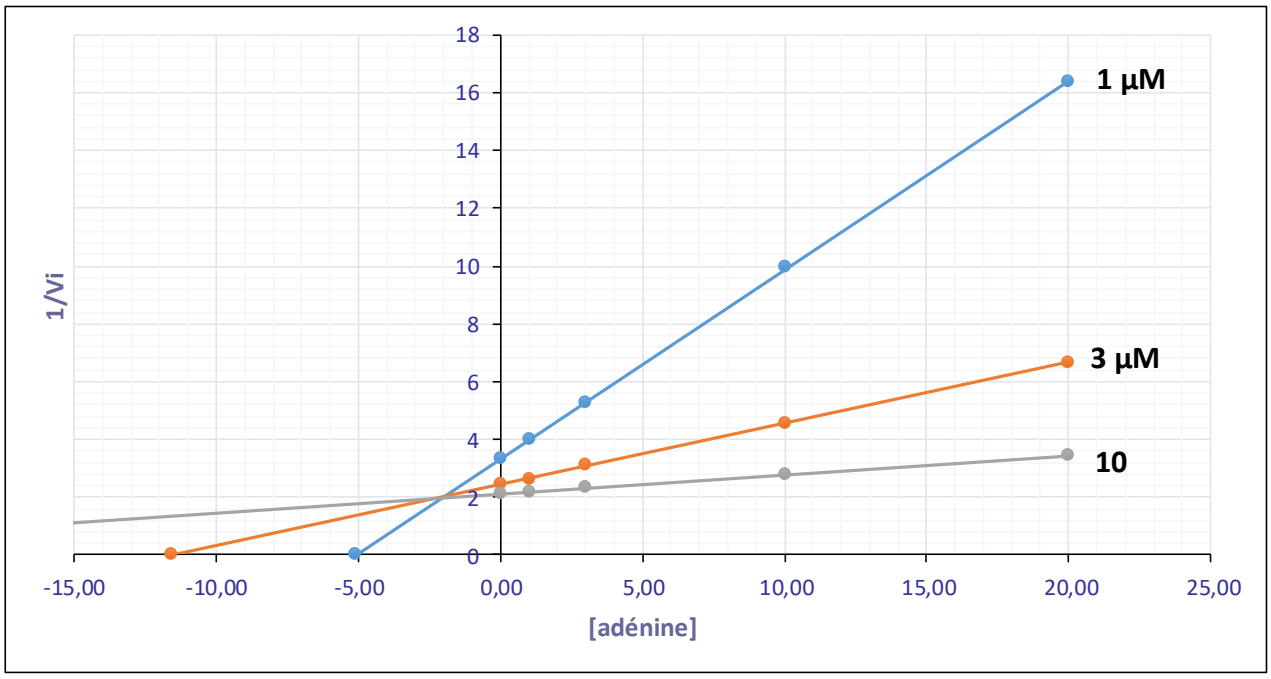
Inhibitor : adenine

Dixon's graphical representation consists of plotting 1/v as a function of [I], thus :

1/v = f([adenine])

[SAH] (µM)	[adenine] (µM)				
	0	1	3	10	20
1	3,33	4,00	5,26	10,00	16,39
3	2,44	2,63	3,13	4,54	6,67
10	2,13	2,17	2,33	2,78	3,45

Knowing that : v(µM/min) so 1/v (μM^{-1}.min)



Graphically :

We can see that the lines intersect above the x-axis, so inhibition is **competitive**.

From which : -Ki = -2 µM so : **Ki = 2 µM**

Exercise n° 4 :

We have :

Enzyme : glucose-oxydase

Substrates : D-glucose and O_2

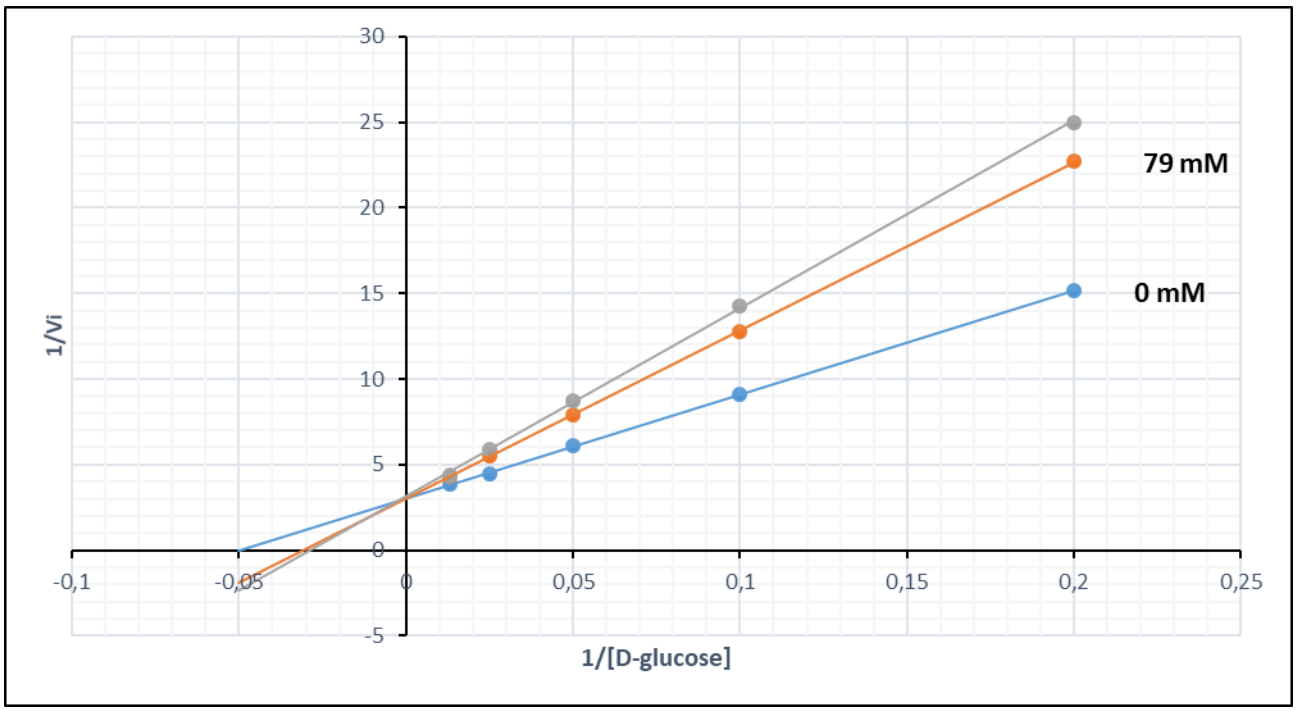
Products : D-glucono-δ-lactone and H_2O_2

Inhibitor : D-glucal

1. **In the presence of oxygen (0.27 mM) :**

The type of inhibition and Ki are determined by plotting the Lineweaver-Burk graph : **1/V = f(1/[D-glucose])**

[D-glucal] (mM)	1/[D-glucose] (mM^{-1})				
	0,20	0,10	0,05	0,025	0,013
0	15,2	9,1	6,1	4,5	3,9
79	22,7	12,8	7,9	5,5	4,3
113	25,0	14,3	8,7	5,9	4,4



Graphically :

We observe that Vm does not change but Km increases, so inhibition is **competitive** with respect to D-glucose.

so : $Km' = K_M (1 + [I] / K_I) \Longrightarrow Km'/Km = 1 + [I] / K_I$

$$Km'/Km - 1 = [I] / K_I$$

$$(Km' - Km)/Km = [I] / K_I$$

Thus : **$K_I = [I].Km / (Km' - Km)$**

$-1/Km = -0{,}05\ mM^{-1}$ so : **Km = 20 mM**

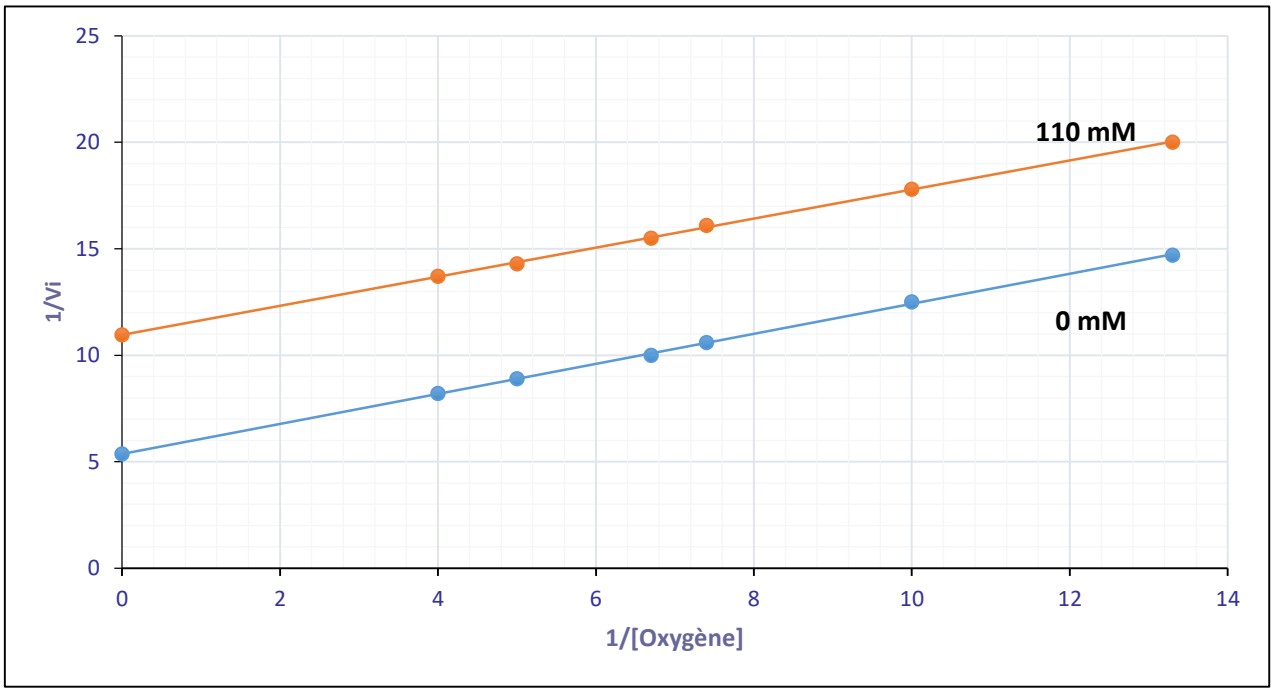
$-1/Km'(79) = -0{,}03\ mM^{-1}$ so : **Km' = 33,3 mM** from which : **K_I = 119 mM**

$-1/Km'\ (113) = 0{,}028\ mM^{-1}$ so : **Km' = 35,7 mM** from which : **K_I = 144 mM**

2. In the presence of D-glucose (20 mM) :

To determine the type of oxygen inhibition, a Lineweaver-Burk plot is performed : **$1/Vi = f(1/[O_2])$**

[D-glucal] (mM)	1/[oxygen] (μM^{-1}) x 10^{-3}					
	13,3	10,0	7,4	6,7	5,0	4,0
0	14,7	12,5	10,6	10,0	8,9	8,2
110	20,0	17,8	16,1	15,5	14,3	13,7



Graphically :

We can see that the slopes are parallel (Km and Vm decrease), so the inhibition is **non-competitive** with respect to oxygen.

From which :

$Km' = Km/1 + [I]/K_I$ and $Vm' = Vm/1 + [I]/K_I$

We have :

$Vm' = Vm/1 + [I]/K_I \Longrightarrow Vm/Vm' = 1 + [I]/K_I$

$\Longrightarrow (Vm - Vm')/Vm' = [I]/K_I$

$\Longrightarrow$ **$K_I = [I].Vm'/(Vm - Vm')$**

$1/Vm = 5{,}4.10^3$ S so : **$Vm = 0{,}18.10^{-3}$ S^{-1}**

$1/Vm' = 10{,}9.10^3$ S so : **$Vm' = 0{,}09.10^{-3}$ S^{-1}**

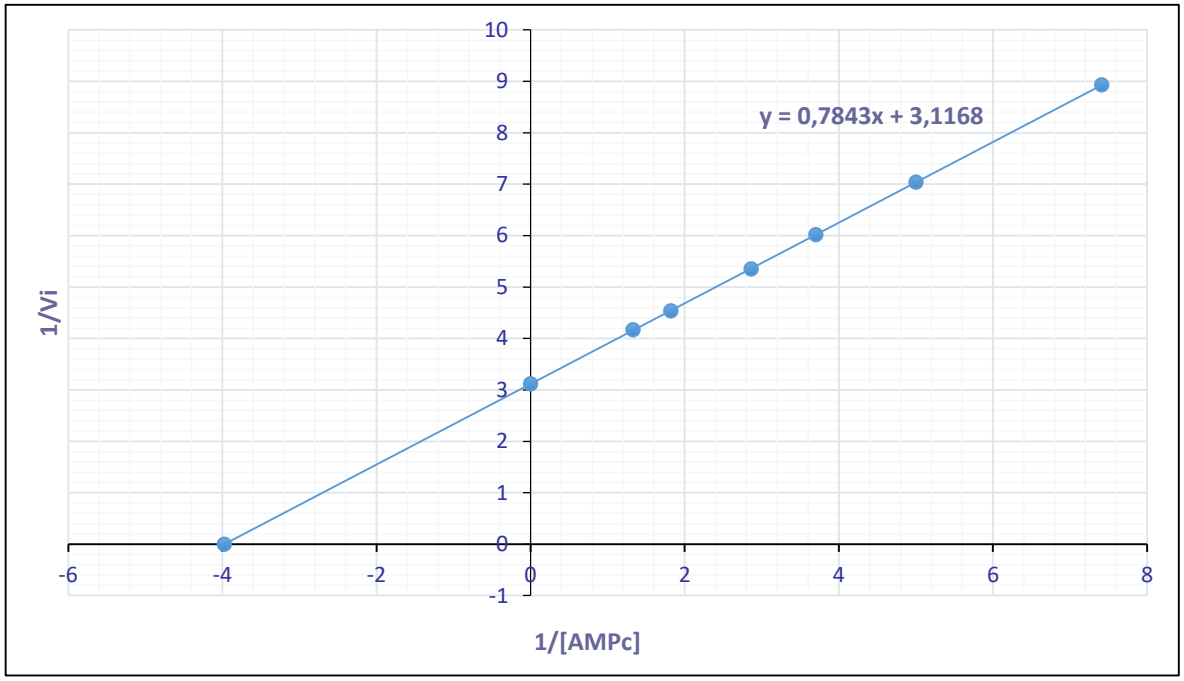
From which : **K_I = 110 Mm**

Exercise n° 5 :

1. *The Michaelis constant (Km) :*

To determine this constant, a Lineweaver-Burk plot is performed : **1/Vi=f(1/[AMPc])**

1/[AMPc] (μM^{-1}).10^{-2}	7,41	5,00	3,70	2,86	1,82	1,33
1/V ($pmol^{-1}$.min).10^{-3}	8,93	7,04	6,02	5,35	4,54	4,17



Graphically :

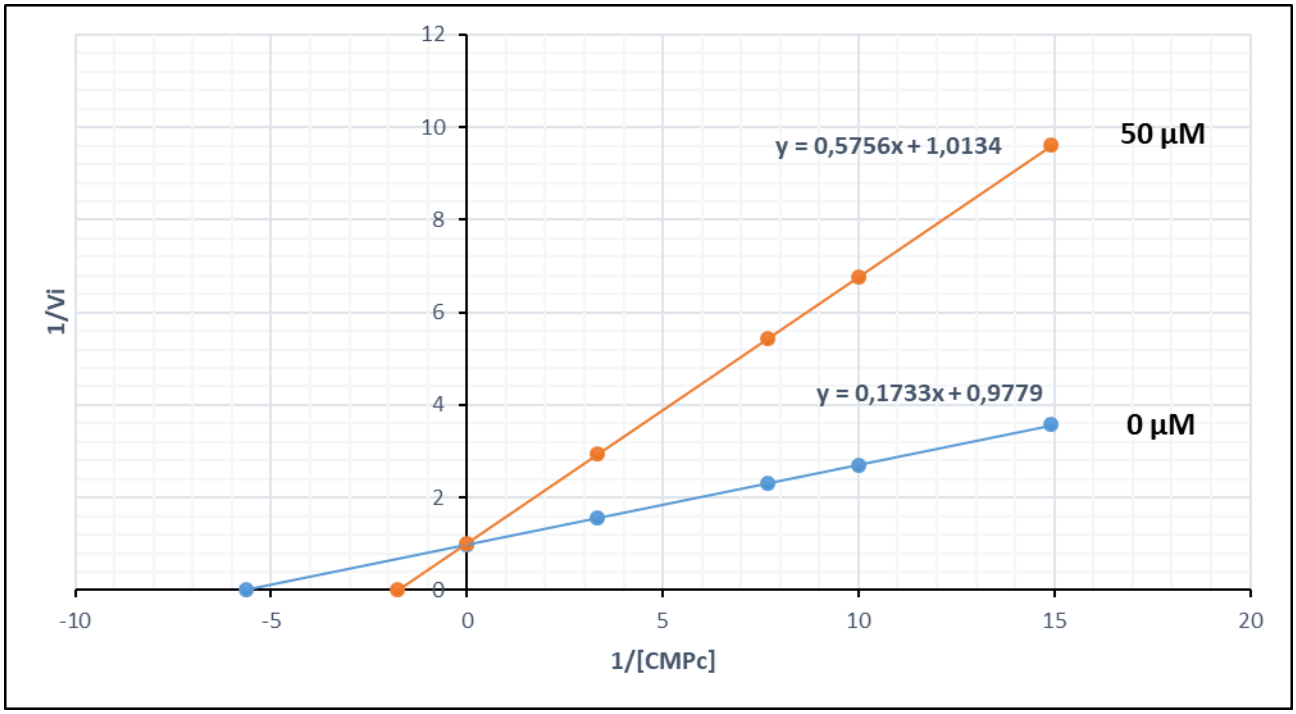
$-1/Km = -4.10^{-2}$ μM^{-1} so : **Km = 25 µM**

2. The inhibition constant (K_I) :

To determine this constant, a Lineweaver-Burk plot is performed in the absence and presence of inhibitor : **1/Vi = f (1/[CMPc])**

[AMPc] (µM)	1/[CMPc] (μM^{-1}).10^{-3}			
	14,92	10,00	7,69	3,33
0	3,57	2,70	2,31	1,56
50	9,61	6,76	5,43	2,94

1/Vi is expressed in ($pmol^{-1}$.min). 10^{-3}



Graphically :

We can see that the lines intersect on the y-axis, so AMPc is a **competitive inhibitor**.

-1/Km = -5.10^{-3} μM^{-1} so : **Km = 200 µM**

-1/Km' = $-1{,}6.10^{-3}$ μM^{-1} so : Km' = **625 µM**

In this type of inhibition : $Km' = Km (1 + [I]/K_I)$

So : **K_I = [I].Km / (Km' – Km)**

From this : **K_I = 23,53 µM**

Conclusion :

The Km and K_I values of cAMP are very close, suggesting that the same site binds cAMP in both cases.