

T. D. n° 4 (enzyme kinetics with a substrate)

Exercise n° 1 :

Using the Michaelis-Menten equation, complete the kinetic data table, knowing that K_m is equal to $1 \cdot 10^{-3}$ M :

Substrate (mM)	Initial speed ($\mu\text{M}/\text{min}$)
0,5	50
1	
2	
3	
10	

Exercise n° 2 :

The kinetic parameters of ribonuclease **T1** and three enzymes obtained by site-directed mutagenesis were studied using the dinucleotide pGpC as a substrate. The native enzyme is characterized by a catalytic rate constant of 75700 min^{-1} and a Michaelis constant of 0.54 mM. Directed mutagenesis was used to change the glutamic acid at position 58 to aspartate (Asp-58), glutamine (Gln-58), or alanine (Ala-58).

The table shows the substrate hydrolysis rates, expressed in $\mu\text{M}/\text{min}$, for different substrate concentrations and at the indicated modified enzyme concentrations :

	[pGpC] (mM)				
	0.135	0.2	0.33	0.5	1
5.4 nM de Asp-58	7.6	10.1	13.8	17.1	22.3
9 nM de Gln-58	2.35	3.2	4.4	5.5	7.4
9 nM de Ala-58	4.2	5.1	6.2	7	8

For the three enzymes, determine the values of :

1. Michaelis constants.
2. Maximum rates.
3. Catalytic rate constants.

Exercise n° 3 :

The action of a dehydrogenase that has NAD^+ as a coenzyme can be summarized as follows :



The kinetics of **X** reduction in the presence of NADH, H^+ at 0.12 mM (a highly saturating concentration for the enzyme) were studied at different concentrations of **X** and in the presence of enzyme at 0.1 μM . The decrease in absorbance at 340 nm due to the oxidation of NADH, H^+ was monitored (given that the molar extinction coefficient of NADH, H^+ is $\epsilon = 6220 \text{ M}^{-1} \cdot \text{cm}^{-1}$; NAD^+ is transparent at this wavelength) :

[X] (mM)	Absorbance measured at time				
	1 min	2 min	3 min	4 min	5 min
1	0,66	0,57	0,48	0,39	0,3
0,5	0,665	0,585	0,505	0,42	0,34
0,2	0,685	0,62	0,555	0,495	0,43
0,1	0,7025	0,655	0,61	0,565	0,525
0,05	0,72	0,69	0,6625	0,6375	0,6025

Knowing that the law linking absorbance to concentration is Beer-Lambert's law : $\mathbf{A = \epsilon \cdot l \cdot C}$ ($l = 1\text{cm}$), determine :

1. The initial reaction rate (in $\mu\text{M} \cdot \text{min}^{-1}$) for each substrate concentration **X**.
2. The Michaelis constant of **X** and the maximum reaction rate.

Exercise n° 4 :

The dehydrogenation of glucose by notatin (glucose oxidase : E.1.1.3.4) (an enzyme found in *Penicillium notatum*) corresponds to the following reaction :



The reaction rates are determined by measuring the volumes of O_2 gas consumed by a series of solutions containing 1 mg of pure enzyme per mL and different concentrations of glucose. The results obtained are recorded in the following table :

Glucose concentration in different reaction mixtures (mg/mL)	0,9	1,8	3,6
Volume of O_2 consumed per mL of reaction mixture per minute (mL)	0,415	0,725	1,090

1. Determine graphically K_m (in $\text{mol} \cdot \text{L}^{-1}$) and V_m (in $\text{mol} \cdot \text{L}^{-1} \cdot \text{min}^{-1}$).
2. Determine the specific molar activity of notatine.

Data :

- ✓ Molar volume of O₂ = 24.5 L/mol
- ✓ Molecular weight (notatin) = 152,000 Da
- ✓ Molar mass (glucose) = 180 g/mol

Exercise n° 5 :

Lactase (β -galactosidase) (EC.3.2.1.23) hydrolyzes lactose into glucose and galactose. The rate of lactose hydrolysis by lactase under initial conditions is determined. 0.672×10^{-2} moles of glucose appear in 10 minutes. 1 mL of the enzyme solution was added to the medium.

The protein content of this solution is 2.85 g/L.

1. Calculate the catalytic activity concentration of the enzyme preparation in (kat/mL) and in (U/mL).
2. Calculate the specific activity of the enzyme in (kat/mg) and in (U/mg).
3. Calculate the molar specific activity of the enzyme.

Data : $M_{lactase} = 135000 \text{ Da}$