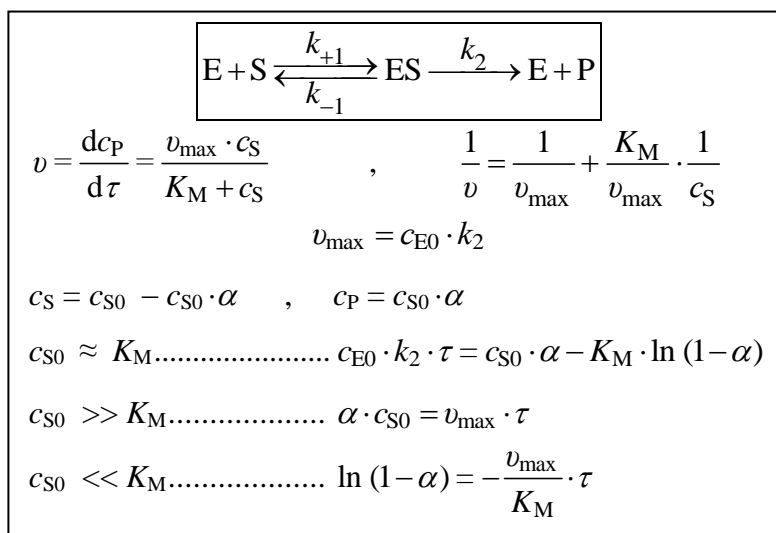


4. ENZYME KINETICS



Problem 4-01 Constants of Michaelis-Menten equation from differential data; integrated equation ($c_{S0} \ll K_M$)

The values of initial reaction rates of enzyme reaction according to the above mentioned scheme for two different substrate concentrations are given in the following table:

$c_S / (\text{mmol dm}^{-3})$	0.25	76.923
$10^8 v_0 / (\text{mol dm}^{-3} \text{ s}^{-1})$	1.852	27.78

- (a) Determine the constants of Michaelis-Menten equation K_M and v_{\max} .
 (b) How long will it take to react 25 % of substrate, if its initial concentration was $c_{S0} = 2 \cdot 10^{-7} \text{ mol dm}^{-3}$?

[(a) $v_{\max} = 2.9106 \cdot 10^{-7} \text{ mol dm}^{-3} \text{ s}^{-1}$, $K_M = 3.679 \cdot 10^{-3} \text{ mol dm}^{-3}$, (b) $\tau = 1.01 \text{ h}$]

Problem 4-02 Constants of Michaelis-Menten equation from differential data; integrated equation ($c_{S0} \gg K_M$)

Polarimetric study of enzymatic hydrolysis yielded the dependence of the initial reaction rate on the substrate concentration. Some of the gained values are given in the following table:

$c_S / (\text{mol dm}^{-3})$	0.062	1.82
$10^6 v_0 / (\text{mol dm}^{-3} \text{ s}^{-1})$	4.96	5.48

- (a) Calculate the constants of Michaelis-Menten equation K_M and v_{\max} .
 (b) What will be the conversion of optically active substrate after 6 hours from the instant when the enzyme was added to the substrate solution, the concentration of which was 2.2 mol dm^{-3} ?

[(a) $K_M = 6.754 \cdot 10^{-3} \text{ mol dm}^{-3}$, $v_{\max} = 5.5 \cdot 10^{-6} \text{ mol dm}^{-3} \text{ s}^{-1}$; (b) $\alpha = 0.054$]

Problem 4-03 Constants of Michaelis-Menten equation from integral data

Determine the kinetic parameters K_M and v_{\max} describing the action of chymotrypsin from bovine pancreas on N-acetyl-L-tyrosine ethyl ester (substrate S) using these data measured at 25°C and $\text{pH} = 7.8$:

τ / min	0	17.2	32.5
$c_S / \text{mol dm}^{-3}$	$2.0 \cdot 10^{-4}$	$1.4 \cdot 10^{-4}$	$1.0 \cdot 10^{-4}$

[$K_M = 6.966 \cdot 10^{-4} \text{ mol dm}^{-3}$, $v_{\max} = 1.7933 \cdot 10^{-5} \text{ mol dm}^{-3} \text{ s}^{-1}$]

Problem 4-04 Integrated Michaelis-Menten equation; $c_{S0} \approx K_M$; the amount of enzyme

Calculate what amount of enzyme (μg) should be added to 15 cm^3 of the solution, containing 0.168 mmol of substrate S, in order that the conversion of 35 % of initially present substrate was reached in 40 seconds from the beginning of the reaction. The molar mass of the enzyme is 38 kg mol^{-1} and its catalytic activity is $k_2 = 8 \cdot 10^5 \mu\text{mol}$ of substrate the enzyme converts per $1 \mu\text{mol}$ of the enzyme per one second. Michaelis constant for this system has the value $K_M = 8.13 \text{ mmol dm}^{-3}$.

$$[m_{E0} = 1.322 \cdot 10^{-10} \text{ kg} = 0.132 \mu\text{g}]$$

Problem 4-05 Integrated Michaelis-Menten equation; $c_{S0} \approx K_M$; the amount of enzyme

The digestion of casein by the action of trypsin was studied at the temperature of 0°C using casein solutions of concentration $5.52 \cdot 10^{-4} \text{ mol dm}^{-3}$, at $\text{pH} = 7.6$. Michaelis constant is $K_M = 7 \cdot 10^{-4} \text{ mol dm}^{-3}$. It was found that after 28 minutes from the beginning of the experiment the concentration of casein (i.e. substrate) dropped to 84 % of the initial value. At these conditions the deactivation of trypsin is negligible. How much greater concentration of enzyme was used in next experiment with the solution of the same concentration if the same casein conversion was reached already in 20 minutes?

$$[(C_{E0})_2 = 1.4 (C_{E0})_1]$$

Problem 4-06 Catalytic activity of an enzyme

For the enzymatic action of ribonuclease isolated from bovine pancreas on a substrate solution of initial concentration 0.18 mol dm^{-3} at the temperature of 28°C the value of Michaelis constant was found to be $0.0087 \text{ mol dm}^{-3}$. The following table presents the time dependence of the degree of substrate conversion obtained at initial enzyme concentration $c_{E0} = 3.6 \cdot 10^{-7} \text{ mol dm}^{-3}$:

τ / h	1.2	2.35	4.5	6.2
α	0.147	0.287	0.549	0.758

Calculate the molar activity of ribonuclease.

$$[k_2 = 16.97 \text{ mol}_{\text{substrate}} (\text{mol}_{\text{ribonuclease}})^{-1} \text{ s}^{-1}]$$

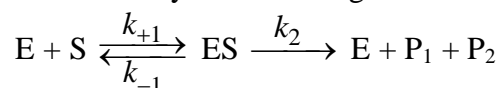
Problem 4-07 Integrated Michaelis-Menten equation; $c_{S0} \approx K_M$; molar enzyme activity

The study of pepsin enzymatic action on 1-carboxy-1-glutamyl tyrosin led to the value of Michaelis constant $K_M = 1.73 \cdot 10^{-3} \text{ mol dm}^{-3}$. In one experiment, in which $8.8 \cdot 10^{-8} \text{ mol}$ of pepsin was added to 20 cm^3 of solution containing $1.24 \cdot 10^{-4} \text{ mol}$ of substrate, was found that a substrate conversion of 50 % was reached after 1.2 min. Calculate the molar activity of pepsin.

$$[k_2 = 13.57 \text{ mol}_{\text{substrate}} (\text{mol}_{\text{pepsin}})^{-1} \text{ s}^{-1}]$$

Problem 4-08 Integrated Michaelis-Menten equation; $c_{S0} \ll K_M$; molar enzyme activity

Michaelis constant $K_M = 226 \text{ mmol dm}^{-3}$ was evaluated from the measurements of initial reaction rates for the enzymatic reaction described by the following scheme:



At enzyme concentration $2.17 \cdot 10^{-6} \text{ mol dm}^{-3}$ the concentration of substrate ($M_S = 150 \text{ g mol}^{-1}$) decreased to 40 % of the initial value $3 \mu\text{g cm}^{-3}$ in 1.2 hour. Calculate the molar activity of the enzyme catalyzing the above mentioned reaction.

$$[k_2 = 22.09 \text{ s}^{-1}]$$

Problem 4-09 Integrated Michaelis-Menten equation; $c_{S0} \gg K_M$; substrate conversion

The action of pepsin on 1-carboxy-1-glutamyl tyrosin (substrate S) at the temperature of 38°C and $\text{pH} = 4$ is characterized by the kinetic parameters

$$K_M = 1.73 \cdot 10^{-4} \text{ mol dm}^{-3} \quad \text{a} \quad v_{\text{max}} = 9.2 \cdot 10^{-7} \text{ mol dm}^{-3} \text{ s}^{-1}$$

Calculate the percentage of the substrate converted after 10 hours from the beginning of the reaction in case that the initial concentration of the substrate was 0.8 mol dm^{-3} .

$$[4.14 \text{ \%}]$$