Problem 4-07 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 mol dm^{-3}. The following table presents the time dependence of the degree of substrate conversion obtained at initial enzyme concentration c_{E0} = 3.6·10^{-7} 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 mol$_{substrate}$ (mol$_{ribonuclease}$)$^{-1}$ s^{-1}]

Solution:

Substrate concentration (0,18 mol dm^{-3}) is much higher than the value of Michelis constant (0,0087 mol dm^{-3}), $c_{S0} >> K_M$, $\Rightarrow$ zero order kinetics:

$$v_0 = v_{max} \quad , \quad -\frac{dc_S}{d\tau} = k_2 \cdot c_{E0}$$

Balance:

$$c_S = c_{S0} - c_{S0} \cdot \alpha \quad , \quad dc_S = -c_{S0} \cdot d\alpha$$

$$c_P = c_{S0} \cdot \alpha \quad , \quad dc_P = c_{S0} \cdot d\alpha$$

$$\int_0^{\alpha} c_{S0}\, d\alpha = k_2 \cdot c_{E0} \int_0^{\tau} d\tau$$

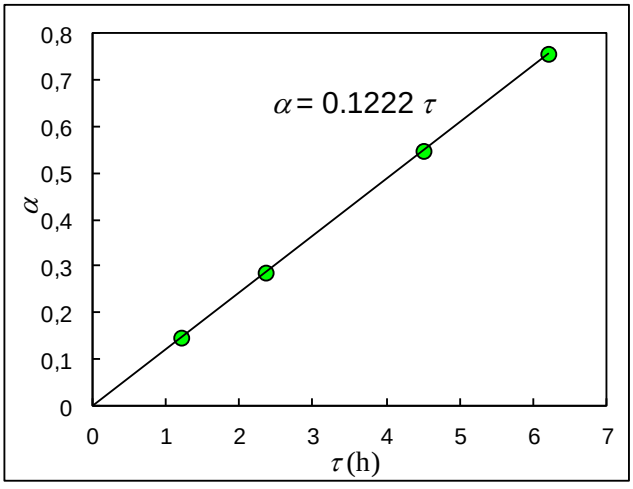
$$c_{S0} \cdot \alpha = k_2 \cdot c_{E0} \cdot \tau \quad \Rightarrow \quad k_2 = \frac{c_{S0} \cdot \alpha}{c_{E0} \cdot \tau}$$

$$k_2 = \frac{0.18 \cdot \alpha}{3.6 \cdot 10^{-7} \cdot \tau} = 5 \cdot 10^5 \cdot \frac{\alpha}{\tau} \quad \left[\frac{\text{mol}_{substrate}\ \text{dm}^{-3}}{\text{mol}_{enzyme}\ \text{dm}^{-3}} \cdot \frac{1}{\text{h}} = \frac{1}{3600} \text{mol}_{substrate}\ (\text{mol}_{enzyme})^{-1}\ \text{s}^{-1} \right]$$

Computational solution:

τ / h	1.2	2.35	4.5	6.2
α	0.147	0.287	0.549	0.758
k_2 /(mol$_{substrate}$ (mol$_{enzym}$)$^{-1}$ h^{-1})	61250	61063.8	61000	61290
k_2 /(mol$_{substrate}$ (mol$_{enzym}$)$^{-1}$ s^{-1})	17	16.962	16.944	16.98

Average: k_2 = 16.97 mol$_{substrate}$ (mol$_{enzyme}$)$^{-1}$ s^{-1}



Graphic solution:

$$\alpha = \frac{k_2 \cdot c_{E0}}{c_{S0}} \cdot \tau$$

$$\frac{k_2 \cdot c_{E0}}{c_{S0}} = 0.1222$$

c_{S0} = 0.18 mol dm^{-3}

c_{E0} = 3.6·10^{-7} mol dm^{-3}

$$k_2 = \frac{c_{S0}}{c_{E0}} \cdot 0.1222 = \frac{0.18}{3.6 \cdot 10^{-7}} \cdot 0.1222$$

k_2 = 61100 mol$_{substrate}$ (mol$_{enzyme}$)$^{-1}$ h^{-1}

= 16.972 mol$_{substrate}$ (mol$_{enzyme}$)$^{-1}$ s^{-1}