Problem 4-08 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 $K_M = 1.73 \cdot 10^{-3}$ mol dm^{-3}. In one experiment, in which $8.8 \cdot 10^{-8}$ mol of pepsin was added to 20 cm^3 of solution containing $1.24 \cdot 10^{-4}$ mol of substrate, was found that a substrate conversion of 50 % was reached after 1.2 min. Calculate the molar activity of pepsin.

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

Solution:

$$c_{E0} = \frac{8.8 \cdot 10^{-8}}{20 \cdot 10^{-3}} = 4.4 \cdot 10^{-6} \text{ mol dm}^{-3}$$

$$\left.\begin{array}{l} c_{S0} = \dfrac{1.24 \cdot 10^{-4}}{20 \cdot 10^{-3}} = 0.0062 \text{ mol dm}^{-3} \\ K_M = 1.73 \cdot 10^{-3} \text{ mol dm}^{-3} \end{array}\right\} \quad \boxed{\text{the same order of magnitude}}$$

$$v_0 = \frac{k_2 \cdot c_{E0} \cdot c_S}{K_M + c_S}$$

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

$$c_{S0} \cdot \frac{\mathrm{d}\alpha}{\mathrm{d}\tau} = k_2 \cdot c_{E0} \frac{c_{S0}(1-\alpha)}{K_M + c_{S0}(1-\alpha)}$$

$$k_2 \cdot c_{E0} \cdot \mathrm{d}\tau = \frac{K_M + c_{S0} \cdot (1-\alpha)}{(1-\alpha)} \mathrm{d}\alpha = \left(\frac{K_M}{(1-\alpha)} + c_{S0} \right) \mathrm{d}\alpha$$

$$k_2 \cdot c_{E0} \cdot \tau = \int_0^x \left(\frac{K_M}{(1-\alpha)} + c_{S0} \right) \mathrm{d}\alpha = -K_M \cdot \ln(1-\alpha) + c_{S0} \cdot \alpha$$

data: $\alpha = 0.5$, $\tau = 1.2$ min = 72 s

$$k_2 = \frac{-K_M \cdot \ln(1-\alpha) + c_{S0} \cdot \alpha}{c_{E0} \cdot \tau} = \frac{-1.73 \cdot 10^{-3} \cdot \ln(1-0.5) + 6.2 \cdot 10^{-3} \cdot 0.5}{4.4 \cdot 10^{-6} \cdot 72}$$

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