Problem 4-06 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 $5.52 \cdot 10^{-4}$ mol dm^{-3}, at pH = 7.6. Michaelis constant is $K_M = 7 \cdot 10^{-4}$ 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? [$(c_{E0})_2 = 1.4\ (c_{E0})_1$]

Solution:

$c_S = 0.84\ c_{S0}$ at $(c_{E0})_1$ .... $\tau_1 = 28$ min

at $(c_{E0})_2$ .... $\tau_2 = 20$ min

$c_{S0} = 5.52 \cdot 10^{-4}$ mol dm^{-3}

$K_M = 7 \cdot 10^{-4}$ mol dm^{-3} ..... c_{S0} comparable in magnitude with K_M :

$$\upsilon_0 = -\frac{\mathrm{d}c_S}{\mathrm{d}\tau} = \frac{k_2 \cdot c_{E0} \cdot c_S}{K_M + c_S}$$

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

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

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

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

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

$$\frac{k_2 \cdot (c_{E0})_2 \cdot \tau_2}{k_2 \cdot (c_{E0})_1 \cdot \tau_1} = \frac{-K_M \cdot \ln(1-\alpha) + c_{S0} \cdot \alpha}{-K_M \cdot \ln(1-\alpha) + c_{S0} \cdot \alpha} \quad \Rightarrow \quad (c_{E0})_2 = \frac{\tau_1}{\tau_2} \cdot (c_{E0})_1 = \frac{28}{20} \cdot (c_{E0})_1$$

$(c_{E0})_2 = 1.4\ (c_{E0})_1$