

# Deacon's Challenge

## No 185 - Answer

A new drug (A) is inactivated by a deaminase in plasma to form metabolite (B). A rare deficiency in the enzyme can easily result in toxic plasma concentrations of the drug. To assay the enzyme 10  $\mu\text{L}$  of plasma was added to 2 mL of buffer then the reaction initiated by adding 100  $\mu\text{L}$  of substrate (A). The reaction was monitored by following the change in absorbance in a cell with a 1 cm path-length. Unfortunately both the product and substrate absorb strongly at the wavelength used with the following molar absorptivities:

$$\begin{aligned}\text{Drug (A)} &= 3.0 \times 10^5 \text{ L.mol}^{-1}.\text{cm}^{-1} \\ \text{Metabolite (B)} &= 1.2 \times 10^5 \text{ L.mol}^{-1}.\text{cm}^{-1}\end{aligned}$$

Calculate the enzyme activity (in  $\mu\text{mol/Lmin}$  in plasma) for an initial rate of change in absorbance of  $-0.021/\text{min}$ .

For each species (A or B) their absorbance (A) is given by:

$$A = a \times b \times c$$

where  $a$  = molar absorptivity in  $\text{L.mol}^{-1}.\text{cm}^{-1}$ ,  $c$  = concentration in  $\text{mol/L}$  and  $b$  is the path-length in cm

The change in absorbance ( $\Delta A$ ) is similarly related to the change in concentration ( $\Delta c$ ):

$$\Delta A = a \times b \times \Delta c$$

Since the molar absorptivity of A is greater than B the absorbance will decrease as the reaction proceeds.

Therefore the net change in absorbance will be due to the absorbance increase due to the formation of B minus the loss in absorbance as an *equivalent* amount of A is consumed. Since the rate of absorbance change due to each species is given by  $a \times \Delta c$  (ignoring  $b$  since it is unity) the following expression can be written:

$$\text{net } \Delta A = (a_B \times \Delta c_B) + (a_A \times \Delta c_A)$$

Since each mole of A is converted to B it follows that  $\Delta c_A = -\Delta c_B$  therefore:

$$\text{net } \Delta A = (a_B \times \Delta c_B) + (a_A \times (-\Delta c_B)) = \Delta c_B (a_B - a_A)$$

which can be rearranged to:  $\Delta c_B = \frac{\text{net } \Delta A}{a_B - a_A}$

Substituting for net  $\Delta A$ ,  $a_A$  and  $a_B$  and solving for  $\Delta c_B$ :

$$\Delta c_B = \frac{-0.021}{(1.2 \times 10^5) - (3.0 \times 10^5)} = \frac{-0.021}{10^5(1.2 - 3.0)} = \frac{-0.021}{-1.8 \times 10^5} = 1.17 \times 10^{-7} \text{ mol/L/min}$$

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This is the rate of formation of B in 1 L of reaction mixture not plasma. Therefore allow for dilution of plasma in the assay mixture by multiplying by the total reaction volume (0.01 mL + 2.0 mL + 0.1 mL = 2.11 mL) and dividing by the plasma volume (0.010 mL). Finally, multiply by 1,000,000 to convert the rate from  $\text{mol/L/min}$  to  $\mu\text{mol/L/min}$ :

$$\text{Enzyme activity} = \frac{1.17 \times 10^{-7} \times 2.11 \times 10^6}{0.01} = 25 \mu\text{mol/L/min} \quad (\text{to 2 sig figs})$$

## Question 186

Two solutions (A and B) have the following compositions:

- A: 6g anhydrous sodium dihydrogen orthophosphate in 500 mL water
- B: 7.1g anhydrous disodium hydrogen orthophosphate in 500 mL water
- a) 50 mL of A is mixed with 50 mL B and the measured pH is 6.82. Calculate the  $\text{pK}_2$ .
- b) 60 mL of A is mixed with 40 mL B. Calculate the expected pH.

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