# 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 µL of plasma was added to 2 mL of buffer then the reaction initiated by adding 100 µL of substrate (A). The reaction was monitored by following the change in absorbance in a cell with a 1cm path-length. Unfortunately both the product and substrate absorb strongly at the wavelength used with the following molar absorptivities:*

*Drug (A) = $3.0 \times 10^5$ $L.mol^{-1}.cm^{-1}$*
*Metabolite (B) = $1.2 \times 10^5$ $L.mol^{-1}.cm^{-1}$*

*Calculate the enzyme activity (in µmol /min/L plasma) for an initial rate of change in absorbance of -0.021/min.*

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

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

where $a$ = molar absorptivity in $L.mol^{-1}.cm^{-1}$, $c$ = concentration in 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 $\Delta c_B$:

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





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 mol/L/min to µmol/L/min):

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

# 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 $pKa_2$.
b) 60 mL of A is mixed with 40 mL B. Calculate the expected pH.