Deacon's Challenge

No 170 - Answer

An assay mixture for the measurement of a dehydrogenase in plasma contains the following:

Reagent A (buffer + NADH)	*2.7 mL*
Reagent B (substrate)	*0.2 mL*
Plasma	*0.1 mL*

The course of the reaction is followed by measuring the decrease in absorption at 340 nm due to the oxidation of NADH in a cell with a 0.5 cm light path. The NADH concentration must be selected to achieve measurable absorbance values even if this means using a sub-optimal concentration. Calculate the weight of NADH disodium salt (formula $C_{21}H_{27}N_7O_{14}P_2Na_2$) required to prepare 500 mL of reagent A to give an initial absorbance in the assay mixture of 0.5.

The molar absorptivity of NADH at 340 nm is 6.3 x 10^3 L.mol^{-1}cm^{-1}.

Atomic weights: C = 12, N = 14, O = 16, P = 31, Na = 23.

Use the Beer-Lambert equation to calculate the required NADH concentration in mol/L:

$$A = \varepsilon \times L \times c$$

where A = absorbance = 0.5
ε = molar absorptivity = 6.3 x 10^3 L.mol^{-1}cm^{-1}
L = light path = 0.5 cm
c = concentration = ?

therefore: $0.5 = 6.3 \times 10^3 \times 0.5 \times c$

rearrange and solve: $c = \dfrac{0.5}{6.3 \times 10^3 \times 0.5} = 0.0001587$ mol/L

Calculate MW of NADH:

C_{21}	=	12 x 21	= 252
H_{27}	=	1 x 27	= 27
N_7	=	14 x 7	= 98
O_{14}	=	16 x 14	= 224
P_2	=	31 x 2	= 62
Na_2	=	23 x 2	= 46
Total	=		709

Therefore concentration in g/L = Concentration (mol/L) x MW
= 0.0001587 x 709
= 0.1125 g/L

This is the NADH concentration in the reaction mixture. Since Reagent A has been diluted by mixing with Reagent B and plasma the NADH concentration of Reagent A will be:

$$\frac{\text{Concentration in mixture} \times \text{Final reaction volume (2.7 mL + 0.2 mL + 0.1 mL)}}{\text{Volume of Reagent A (2.7 mL)}}$$

$$= \frac{0.1125 \times 3.0}{2.7} = 0.125 \text{ g/L}$$

Therefore to prepare 500 mL of reagent A weigh out $\frac{0.125}{2}$ = **0.0625 g** NADH (to 3 sig figs)

Question 171

The following data were obtained for a 26 year-old Caucasian male of average build at his routine six-monthly psychiatric clinic appointment:

Serum lithium	= 4.65 mmol/L
Serum creatinine	= 275 µmol/L

He was clinically well and told to stop taking his lithium. His psychiatrist has asked you to use the above information to estimate the time it will take for his serum lithium to return to the relatively safe value of 1.5 mmol/L by endogenous clearance alone.