Deacon's Challenge No. 16 Answer

A buffer is required for an enzymatic assay which has a pH of 7.4 and total phosphate concentration of 100 mmol/L. Calculate the amounts of anhydrous sodium dihydrogen phosphate and disodium hydrogen phosphate which need to be weighed in to make 1 L of buffer. The pK of the dissociation is 6.82 (Atomic weights: Na = 23, P = 31).

MRCPath November 2001

The relationship between the concentrations of an acid, its conjugate base and the pH of the solution is described by the Henderson Hasselbalch equation:

$$pH = pK_a + \log_{10} \frac{[salt]}{[acid]}$$

The required pH of the phosphate buffer is close to the second pK_a so that the dissociation to be considered is:

$$H_2PO_4^- \longleftrightarrow HPO_4^{2-} + H^+$$

Substitute [salt] = $[HPO_4^{2-}]$, [acid] = $[H_2PO_4^-]$, pH = 7.40 and pK_a = 6.82 into the Henderson Hasselbalch equation:

$$7.40 = 6.82 + \log_{10} \frac{[HPO_4^{2-}]}{[H_2PO_4^-]}$$

Rearranging: $\log_{10} \frac{[HPO_4^{2-}]}{[H_2PO_4^-]} = 7.40 - 6.82 = 0.58$

Taking antilogs: $\frac{[HPO_4^{2-}]}{[H_2PO_4^-]} = \text{antilog } 0.58 = 3.80$ ……..…..(i)

Since the required total phosphate concentration is 100 mmol/L (i.e. 0.1 mol/L)

$$0.1 = [H_2PO_4^-] + [HPO_4^{2-}]$$

Rearranging: $[HPO_4^{2-}] = 0.1 - [H_2PO_4^-]$ ……...……(ii)

Substitute for $[HPO_4^{2-}]$ in equation (i) and solve for $[H_2PO_4^-]$:

$$0.1 - \frac{[H_2PO_4^-]}{[H_2PO_4^-]} = 3.80$$

$$0.1 - [H_2PO_4^-] = 3.80\,[H_2PO_4^-]$$

$$3.80\,[H_2PO_4^-] + [H_2PO_4^-] = 0.1$$

$$4.80\,[H_2PO_4^-] = 0.1$$

$$[H_2PO_4^-] = \frac{0.1}{4.80} = 0.0208 \text{ mol/L}$$

Substitute $[H_2PO_4^-]$ = 0.0208 in equation (ii) and solve for $[HPO_4^{2-}]$:

$$[HPO_4^{2-}] = 0.1 - 0.0208 = 0.0792 \text{ mol/L}$$

Now calculate the weights required for each phosphate salt:

$$\text{Conc (g/L)} = \text{Conc (mol/L)} \times \text{MW}$$

For anhydrous sodium dihydrogen phosphate, NaH_2PO_4:

MW = 23 + (2 x 1) + 31 + (4 x 16) = 120

Weight required per litre = 0.0208 x 120 = 2.50g

For anhydrous disodium hydrogen phosphate, Na2HPO4:

MW = (2 x 23) + 1 + 31 + (4 x 16) = 142

Weight required per litre = 0.0792 x 142 = 11.2g ■

Question No. 17

25 mg of bilirubin ($C_{33}H_{36}O_6N_4$) were dissolved in 4 mL of dimethyl sulphoxide; 200mL of this solution was diluted to 250mL with chloroform. This solution gave an absorbance of 0.502 when measured in a 1 cm cell against a chloroform blank.

Given that the molar absorbtivity of bilirubin under these conditions is 6.07×10^4, calculate the percentage purity of the bilirubin.

MRCPath May 1995