Deacon's Challenge

No 196 - Answer

You need to prepare an isotonic (osmolality = 290 mmol/L) phosphate buffer with a pH of 7.4. Calculate the amounts of anhydrous sodium dihydrogen phosphate and anhydrous disodium hydrogen phosphate that need to be weighed into 1 litre of water. The pKs of phosphoric acid are pKa_1 *= 1.96, p*Ka_2 *= 6.82 and p*Ka_3 *= 12.32. Atomic weights: Na 23, P 31.*

Phosphoric acid is a tribasic acid but at pH 7.4 the first and third ionisations can be ignored. The reaction to be considered is:

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

The corresponding Henderson-Hasselbalch equation is:

$$\mathrm{pH} = \mathrm{p}Ka_2 + \log_{10}\frac{[HPO_4^{2-}]}{[H_2PO_4^-]}$$

Substitute pH = 7.40 and pKa_2 = 6.82 to obtain the ratio of the concentrations of both phosphate species:

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

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

$$\frac{[HPO_4^{2-}]}{[H_2PO_4^-]} = \mathrm{antilog}_{10}\,0.58 = 3.80$$

$[HPO_4^{2-}]$ has 2 cations associated with it making 3 osmotically active species altogether.

$[H_2PO_4^-]$ has 1 cation associated with it making 2 osmotically active species altogether.

These must add up to 390 mmol/L. Therefore:

$$3[HPO_4^{2-}] + 2[H_2PO_4^-] = 390$$

Since the ratio of both species is known, substitute $[HPO_4^{2-}] = 3.80[H_2PO_4^-]$ and solve for $[H_2PO_4^-]$:

$$3 \times 3.80[H_2PO_4^-] + 2[H_2PO_4^-] = 390$$

$$13.4[H_2PO_4^-] = 390$$

$$[H_2PO_4^-] = \frac{390}{13.4} = 29.1 \text{ mmol/L} \quad \text{(3 sig figs)}$$

Substitute this value into the ratio to obtain $[HPO_4^{2-}]$:

$$[HPO_4^{2-}] = 3.80 \times 29.1 = 110.6 \text{ mmol/L}$$

For each salt:

$$\text{Concn (g/L)} = \text{Concn (mol/L)} \times \text{MW} = \frac{\text{Concn (mmol/L)}}{1{,}000} \times \text{MW}$$

MW NaH_2PO_4 = 23 + (2 x 1) + 31 + (4 x 16) = 120

MW Na_2HPO_4 = (2 x 23) + 1 + 31 + (4 x 16) = 142

Wt NaH_2PO_4 to make 1L = $\frac{29.1}{1{,}000} \times 120$ = **3.49 g** (to 3 sig figs)

Wt Na_2HPO_4 to make 1L = $\frac{110.6}{1{,}000} \times 142$ = **15.7 g** (to 3 sig figs)

Question 197

Your laboratory provides a service for the assay of several red cell enzymes, most of which involve absorbance measurements at 340 nm. Recently results for your quality control material have been consistently lower than the target mean. As part of your investigation you carefully prepare a standard (100 mg/L) solution of potassium dichromate ($K_2Cr_2O_7$) in 0.001 M perchloric and measure its absorbance using a cell with a path-length of 5 mm with 0.001 M perchloric acid solution as the reference. Potassium dichromate has peaks at 257 nm and 350 nm and troughs at 235 nm and 313 nm. Your absorbance readings and the literature molar aborptivities at each wavelength are as follows:

Wavelength (nm)	235	257	313	350
Molar absorbtivity ($L.mol^{-1}.cm^{-1}$)	1841	2144	715	1585
Absorbance reading	0.335	0.329	0.134	0.257

Calculate the expected absorbance reading at each wavelength. What is the explanation for the low QC results? (Atomic weights: K = 39; Cr = 52; O = 16).