Deacon's Challenge

No 169 - Answer

A direct-reading ion-selective electrode is calibrated at sodium values of 120 mmol/L and 165 mmol/L. Both calibrants contain 70 g/L of protein and also give sodium readings of 120 mmol/L and 165 mmol/L when analysed by flame photometry. Stating any assumptions you make, what sodium concentration would you expect to obtain by flame photometry for a plasma sample from a myeloma patient (containing 120 g/L of protein) which gives a sodium concentration of 120 mmol/L using the ion-selective electrode?

Direct-reading ion-selective electrodes (ISEs) measure sodium concentration (or rather activity) in plasma water whereas flame photometers (which involve a sample dilution step) measure the sodium concentration in plasma. Due to the displacement of water by plasma proteins the concentration in plasma water is higher than in whole plasma. Manufacturers calibrate direct-reading ISEs so as to give identical readings to flame photometry when the plasma protein concentration is "normal" – usually 70 g/L.

Assuming that 1 g of protein displaces 1 mL of water the direct-reading ISE calibrants containing 70 g/L of protein will contain only 1000 - 70 = 930 g water/L.

The concentration of sodium in the water of the 120 mmol/L calibrant will be:

$$\frac{120 \times 1000}{930} = 129.0 \text{ mmol/L}$$

The plasma from the myeloma patient gives an identical reading using the direct-reading ISE so its sodium concentration in plasma water must also be 129.0 mmol/L.

However, the flame photometer measures the sodium concentration in total plasma. Assuming that each g of paraprotein also displaces 1 mL of water, then the water content of the plasma will be 1000 - 120 = 880 mL/L.

Therefore the sodium concentration in plasma, which will be obtained by flame photometry, is:

$$\frac{129 \times 880}{1000} = \textbf{114 mmol/L}$$

Question 170

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 6.3×10^3 $L.mol^{-1}cm^{-1}$.

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