Deacon's Challenge

No 197 - Answer

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 acid 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).

MW potassium dichromate ($K_2Cr_2O_7$) = (2 x 39) + (2 x 52) + (7 x 16) = 294

At each wavelength: $A = a \times b \times c$

where: a = molar absorptivity ($L.mol^{-1}.cm^{-1}$)

b = cell path length = 0.5 cm

c = molar concentration = $\frac{\text{Concn (mg/L)}}{\text{MW x 1,000}} = \frac{100}{294 \times 1{,}000}$ mol/L

Therefore $A = \frac{a \times 0.5 \times 100}{294 \times 1{,}000}$

Inserting the appropriate value for *a* at each wavelength gives the following expected absorbances which can be compared with the observed values:

Wavelength	235	257	313	350
Expected absorbance	0.313	0.365	0.122	0.270
Observed absorbance	0.335	0.329	0.134	0.257

The observed absorbance at 350 nm is lower than expected which suggests that a fault with the spectrophotometer is the source of the low QC results. It is unlikely that there was an error in preparing the potassium dichromate solution because both peak values are lower but the trough values higher than expected. These findings could be explained by calibration error of the instrument causing a shift of all wavelengths.

Question 198

A 25 year old anorexic female was admitted via A&E with marked emaciation and the following plasma results:

Urea	= 1.5 mmol/L	Sodium	= 120 mmol/L
Creatinine	= 30 µmol/L	Potassium	= 2.5 mmol/L

On the ward a repeat blood revealed her plasma osmolality to be 248 mmol/L. A 6 h urine collection yielded the following results:

Volume	= 0.185 L
Osmolality	= 55 mmol/L

Calculate the free water clearance (in mL/min) and comment on your result.