

**Solutions to Exercises – chapter 5**

**5.1) Which electrons within molecules are normally involved in absorption of UV and visible radiation?**

It is the valence electrons that are normally involved in absorption of UV and visible light.

**5.2) A student performs a set of UV visible determinations on a set of unknown samples for an analyte,  $a$ , with a known  $\lambda_{\max}$ . All of the student's results give absorbances of between 2 and 3. Why may these results not be used for the quantitative determination of  $a$ , and what must the student do next?**

From the Beer-Lambert law an absorption of 2 represents an absorption of 99% of the incident light; and absorption of 3 corresponds to an absorption of 99.9%. The instrumentation will struggle to follow or record the concentration of  $a$  when absorption at these levels are occurring.

In this case the student should dilute the samples by a known amount(s) until the absorption is below a value of 1. An absorption of 1 corresponds to an absorption of 10% of the incident light which the instrumentation should be able to determine far more easily. In this way the concentration of  $a$  may be determined.



**5.3) Explain why absorbance is a unitless quantity.**

Absorbance is a unitless quantity since the term :  $A = \log_{10} \left( \frac{I_0}{I} \right)$

involves a ratio of radiation intensities for the numerator and denominator.

These cancel each other out and so absorbance is left as a unitless quantity.

**5.4) Why will many UV-visible spectrophotometer possess more than one lamp?**

UV-visible spectrophotometers are known as Double Beam Spectrophotometers, and employ two light beams of equal intensity together with two photomultipliers to record and subtract the baselines from spectra. One light beam irradiates the cell containing the analyte. The other irradiates a cuvette containing the appropriate solvent only. The two spectra are recorded simultaneously as the wavelength of light is scanned through the desired range. The baseline spectrum is then subtracted from the spectrum corresponding to the analyte sample and a normalised UV-visible spectrum is obtained.

**5.5) Sodium chloride and potassium chloride both act as quenching agents for a fluorometric determination of quinine. Explain (a) why this is the case and (b) which salt will act as the most effective quenching agent.**

The fluorometric emission of light involves the relaxation of excited electrons together with the dissipation of heat as a result of molecular collisions. The more molecular collisions that occur in the fluorescent mixture, the less fluorescence will occur. Any process that increases the frequency at which molecular collisions occur will therefore quench the fluorescence.

The majority of fluorescent reactions occur in solution, in which case molecular collisions between (a) the fluorescent molecules, (b) the solvent molecules and / or (c) any other solutes, (including other fluorescent molecules), will all serve to quench the fluorescence to some extent.

Ions and / or other solutes may be added to the mixture to act as quenching agents; larger molecules will be involved with a greater number of molecular collisions than smaller solutes. In this case  $K^+$  will act as a more effective quenching agent than  $Na^+$  and so it follows that potassium chloride will act as a more effective quenching agent than sodium chloride.

- 5.6) A cuvette with a path length of 1cm and solution containing 8.96g ppm of a dye gives an absorbance of 0.8. Calculate the molar absorptivity of the dye.

It should first be noted that in this example that concentration is quoted in terms of ppm and so must the molar absorptivity,  $\epsilon$ .

8.96ppm of dye gives rise to an absorbance of 0.8

It follows that  $A = \epsilon c_n l$

So  $l = 1\text{cm}$

$$\therefore 0.8 = \epsilon \times c_n$$

$$\therefore \epsilon = \frac{0.8}{c}$$

$$\therefore \epsilon = \frac{0.8}{8.96} = 0.02 \text{ ppm dm}^{-3}\text{cm}^{-1}$$

- 5.7) A compound of molecular weight 245 is found to have an absorptivity of  $298 \text{ g dm}^{-3} \text{ cm}^{-1}$ . Calculate its molar absorptivity,  $\epsilon$ .

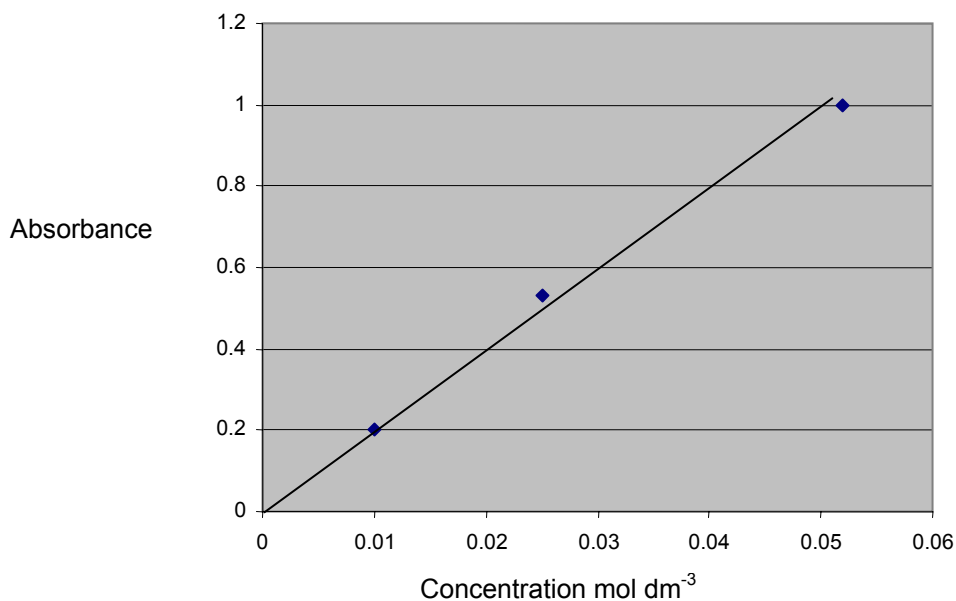
$$\text{Molar absorptivity} = \frac{298}{245} = 1.216 \text{ mol dm}^{-3} \text{ cm}^{-1}$$

- 5.8) Three standard  $\text{Fe}^{2+}_{(\text{aq})}$  solutions are found to have absorbance values as shown below:

Conc $\text{Fe}^{2+}$ ( $\text{mol dm}^{-3}$ )	Absorbance
0.010	0.21
0.025	0.53
0.052	1.00

- (a) Show whether or not the absorbances recorded for these solutions obey the Beer-Lambert law.

If the Beer-Lambert law is obeyed, then a plot of absorbance vs concentration should follow a straight line. The plot below shows a good correlation with a straight line and so it can be concluded this system does obey the Beer Lambert law.



- (b) An iron salt containing solution is found to exhibit an absorption of 0.2 at 510nm following the addition of 1,10 phenanthroline (together with excess hydroxylamine) to form a coloured complex. The molar absorptivity,  $\epsilon$ , for the iron-phenanthroline complex is  $1.08 \times 10^4 \text{ mol dm}^{-3} \text{ cm}^{-1}$ . Determine the concentration of iron within this solution.

An absorbance of 0.2 will correspond to:

$$0.2 \times \frac{1}{1.08 \times 10^4} \text{ mol dm}^{-3} \text{ Fe}$$

**5.9) A solution of the drug Tolbutamine is found to exhibit an absorbance of 0.85 in a 1cm path length cuvette. The molecular weight of tolbutamine is 270 and the molar absorptivity at 262nm is  $703 \text{ g}^{-1} \text{ dm}^{-3} \text{ cm}^{-1}$ . What is the molar concentration of tolbutamine?**

$$\text{Molar absorptivity} = \frac{703}{270} \text{ mol dm}^{-3} \text{ cm}^{-1}$$

$$= 2.603 \text{ mol dm}^{-3} \text{ cm}^{-1}$$

If there is an absorbance of 0.85 then the molar concentration of Tolbutamine

$$= \frac{0.85}{2.603} \text{ mol dm}^{-3} \text{ cm}^{-1}$$

**5.10) A 0.15M solution within a 1cm pathlength placed within a UV visible spectrophotometer shows an absorbance of 0.62. Calculate the molar absorptivity for this compound.**

$$A = \epsilon c l$$

$$\therefore 0.62 = \epsilon \times 0.15 \times 1$$

$$\therefore \epsilon = \frac{0.62}{0.15} = 4.13 \text{ mol dm}^{-3} \text{ cm}^{-1}$$

**5.11) A compound with a molar absorptivity of  $32667 \text{ mol}^{-1} \text{ dm}^{-3} \text{ cm}^{-1}$  (at 740nm) exhibits an absorbance of 0.81 when placed within a 1cm pathlength cuvette in a UV visible spectrophotometer. Calculate the concentration of the compound.**

$$\epsilon = 32667 \text{ mol}^{-1} \text{ dm}^{-3} \text{ cm}^{-1}$$

$$A = 0.81$$

$$A = \epsilon cl$$

$$\therefore c = \frac{A}{\epsilon l}$$

$$\therefore c = \frac{0.81}{32667}$$

$$\therefore c = 2.48 \times 10^{-5} \text{ M}$$

**5.12) You have a compound which you know has  $\lambda_{\max}$  at 215 and 244 nm and you wish to determine its  $\epsilon$  value in a range of solvents. It is soluble in acetone, acetonitrile, benzene, carbon tetrachloride, dioxan, methanol, toluene and water. Which solvents can you use for this experiment?**

The  $\epsilon$  value for both peaks can be determined in acetonitrile, methanol and water and for the higher (244 nm) peak only in dioxin. The other solvents absorb too strongly above 250 nm to be suitable.

**5.13) You have three dilute solutions of equal molarity of pentane, 1,3-pentadiene and 1,4-pentadiene. How would you be able to tell them apart using UV spectrometry?**

The spectra of these would differ greatly. The pentane would display negligible absorbance at 200 nm. Both the dienes would show significant absorption, however the conjugated 1,3-system would have the higher  $\lambda_{\max}$  and  $\epsilon$  value.

**5.14) Define what is meant by a hypsochromic shift and a bathochromic shift.**

A shift in the  $\lambda_{\max}$  from a shorter to a longer wavelength is known as a bathochromic shift.

A shift in the  $\lambda_{\max}$  from a longer to a shorter wavelength is known as a hypsochromic shift.

**5.15) A solution that absorbs radiation with a  $\lambda_{\max}$  at 475nm is placed within a UV visible spectrophotometer. An absorbance of 0.82 is recorded. Calculate the percentage of the incident light that is being absorbed.**

$$A = \log_{10} \frac{I_0}{I}$$

$$A = 0.82 = \log_{10} \frac{I_0}{I}$$

$$6.606 = \frac{I_0}{I} = \frac{1}{I}$$

$$I = \frac{1}{6.606} = 0.1514$$

0.1514 of the incident radiation is transmitted.

$\therefore 1 - 0.1514 = 0.8486$  of the incident radiation is absorbed.

It follows that 0.8486 of the incident radiation is absorbed. This corresponds to 84.86% of the incident radiation being absorbed.

**5.16) A solution within a UV visible spectrophotometer shows an absorbance of 0.72 at a wavelength of 489nm. Calculate the percentage of radiation that is transmitted.**

$$A = 0.72$$

$$A = \log_{10} \frac{I_0}{I}$$

$$5.258 = \frac{I_0}{I}$$

$$\therefore I = \frac{1}{5.248} = 0.1905$$

$\therefore$  0.1905 of the incident radiation is transmitted. This corresponds to 19.05% of the incident radiation being transmitted.

**5.17) The co-enzyme nicotinamide adenosine dinucleotide (NADH) fluoresces when irradiated with radiation of wavelength 340nm. A fluorescence emission maximum is observed at 465nm. An analyst measured the relative intensities of fluorescent radiation at 465 nm for varying concentrations of NADH as shown below:**

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Conc. NADH ( $\mu\text{mol dm}^{-3}$ )	Relative intensity
0.2	8.92
0.4	18.00
0.6	27.43
0.8	35.85

**Plot a best-fit calibration curve using the least-squares approach.**

**Calculate the concentration for an unknown NADH sample exhibiting a fluorescence with a relative intensity of 20.**

Plot a best fit calibration using a least squares approach:

$y = mx + c$ . It follows that  $\bar{y} = m\bar{x} + c$

First calculate  $m$

$$m = S_{xy} / S_{xx}$$

$x$  values correspond to concentration of NADH.  $y$  values correspond to relative intensity.

$$S_{xy}: \sum x_i y_i - \frac{\sum x_i \sum y_i}{N} \text{ and } S_{xx} = \sum x_i^2 - \frac{(\sum x_i)^2}{N}$$

Calculate  $S_{xy}$

$$\sum x_i y_i = (0.2 \times 8.92) + (0.4 \times 18.00) + (0.6 \times 27.43) + (0.8 \times 35.85)$$

$$= 1.784 + 7.2 + 16.458 + 28.68$$

$$\therefore \underline{\sum x_i y_i = 54.122}$$

$$\sum x_i \sum y_i = (0.2 + 0.4 + 0.6 + 0.8) \times (8.92 + 18.00 + 27.43 + 35.85)$$

$$\sum x_i \sum y_i = 2 \times 90.2$$

$$\underline{\sum x_i \sum y_i = 180.4}$$

$$\text{It follows } S_{xy} = 54.122 - \frac{180.4}{4}$$

$$S_{xy} = 54.122 - 45.1$$

$$\underline{S_{xy} = 9.022}$$

$$\text{So } \sum x_i^2 - \frac{(\sum x_i)^2}{N}$$

$$\sum x_i^2 = (0.0)^2 + (0.4)^2 + (0.6)^2 + (0.8)^2$$

$$= 0.04 + 0.16 + 0.36 + 0.64$$

$$\underline{\sum x_i^2 = 1.2}$$

$$(\sum x_i)^2 = (0.2 + 0.4 + 0.6 + 0.8)^2$$

$$= (2)^2$$

$$\therefore (\sum x_i)^2 = 4$$

It follows  $S_{xx} = 1.2 - 1$

$$\underline{\therefore S_{xx} = 0.2}$$

$$m = S_{xy} / S_{xx}$$

$$\text{so } m = \frac{9.022}{0.2}$$

so  $m = 45.11$  relative intensity units  $\mu \text{ mol dm}^{-3}$

Step 2: Calculate best fit line:

$$\bar{y} = m\bar{x} + c$$

$$\bar{y} = (8.92 + 18.00 + 27.43 + 35.85) / 4$$

$$\therefore \bar{y} = 90.2 / 4$$

$$\therefore \bar{y} = 22.55$$

$$\bar{x} = (0.2 + 0.4 + 0.6 + 0.8) / 4 = 2 / 4$$

$$\therefore \bar{x} = 0.5$$

By substituting:  $22.55 = 0.5m + c$

So:  $22.55 = 45.11 \times 0.5 + c$

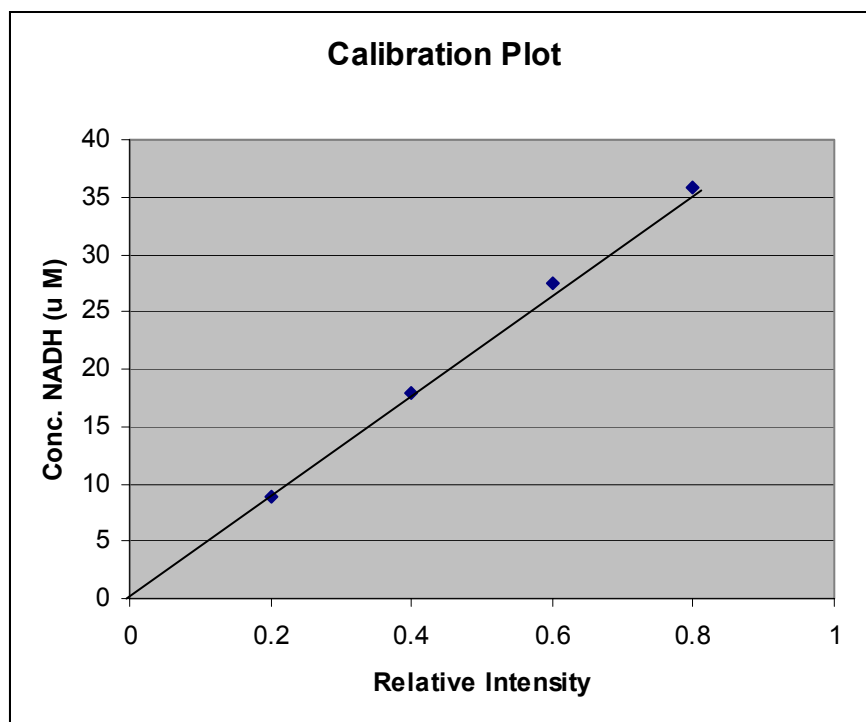
$$22.55 = 22.555 + c$$

$$\therefore c = 22.55 - 3.759$$

$$\therefore c = -0.005 \text{ relative intensity}$$

It follows that the equation for the best fit line =

$$y = 45.11x - 0.005$$



Step 3: Substitute in value for unknown

The unknown sample gives a fluorescence with a relative intensity of 20

It follows  $20 = 45.11x - 0.005$

$$\therefore 45.11x = 19.995$$

$$\therefore x = \frac{19.995}{45.11}$$

$\therefore$  unknown sample is equivalent to 0.4432

$$\therefore [\text{NADH}] = 0.44 \mu \text{ mol dm}^{-3}$$

**5.18) Quinine fluoresces with an emission maximum of 450nm. A series of quinine samples prepared to known concentrations and the fluorescence recorded as in the table below:**

Quinine concentration (ppm)	Fluorescence (arbitrary units)
0.0	0.123
0.2	0.258
0.4	0.394
0.6	0.492
0.8	0.627
1.0	0.763

**If an unknown sample exhibits a fluorescence of 0.63 estimate the concentration of quinine within the sample.**

Plot a best fit calibration using a least squares approach:

$$y = mx + c. \text{ It follows that } \bar{y} = m\bar{x} + c$$

First calculate  $m$

$$m = S_{xy} / S_{xx}$$

$x$  values correspond to Quinine concentration.  $y$  values correspond to fluorescence (arbitrary units).

$$S_{xy}: \sum x_i y_i - \frac{\sum x_i \sum y_i}{N} \text{ and } S_{xx} = \sum x_i^2 - \frac{(\sum x_i)^2}{N}$$

Calculate  $S_{xy}$

$$\sum x_i y_i = (0.0 \times 0.123) + (0.2 \times 0.258) + (0.4 \times 0.394) + (0.6 \times 0.492) + (0.8 \times 0.627) + (1.0 \times 0.763)$$

$$= 0 + 0.0516 + 0.1576 + 0.2952 + 0.5016 + 0.763$$

$$\therefore \underline{\sum x_i y_i = 1.769}$$

$$\sum x_i \sum y_i = (0.0 + 0.2 + 0.4 + 0.6 + 0.8 + 1.0) \times (0.123 + 0.258 + 0.394 + 0.492 + 0.627 + 0.763)$$

$$\sum x_i \sum y_i = 3 \times 1.657$$

$$\underline{\sum x_i \sum y_i = 7.971}$$

$$\text{It follows } S_{xy} = 1.769 - \frac{7.971}{6}$$

$$S_{xy} = 1.796 - 1.3285$$

$$\underline{S_{xy} = 0.4405}$$

$$\text{So } \sum x_i^2 - \frac{(\sum x_i)^2}{N}$$

$$\sum x_i^2 = (0.0)^2 + (0.2)^2 + (0.4)^2 + (0.6)^2 + (0.8)^2 + (1.0)^2$$

$$= 0 + 0.04 + 0.16 + 0.36 + 0.64 + 1.0$$

$$\underline{\sum x_i^2 = 2.2}$$

$$(\sum x_i)^2 = (0.0 + 0.2 + 0.4 + 0.6 + 0.8 + 1.0)^2$$

$$= (3)^2$$

$$\therefore (\sum x_i)^2 = 9$$

$$\text{It follows } S_{xx} = 2.2 - \frac{9}{6}$$

$$\therefore S_{xx} = 2.2 - 1.5$$

$$\underline{\therefore S_{xx} = 0.7}$$

$$m = S_{xy} / S_{xx}$$

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$$\text{so } m = \frac{0.4405}{0.7}$$

$$\text{so } m = 0.629 \text{ arbitrary units ppm}^{-1}$$

Step 2: Calculate best fit line:

$$\bar{y} = m\bar{x} + c$$

$$\bar{y} = (0.123 + 0.258 + 0.394 + 0.492 + 0.627 + 0.763) / 6$$

$$\therefore \bar{y} = 2.657 / 6$$

$$\therefore \bar{y} = 0.4428$$

$$\bar{x} = (0.0 + 0.2 + 0.4 + 0.6 + 0.8 + 1.0) / 6 = 2 / 6$$

$$\therefore \bar{x} = 0.5$$

By substituting:  $0.4428 = 0.629 \times 0.5 + c$

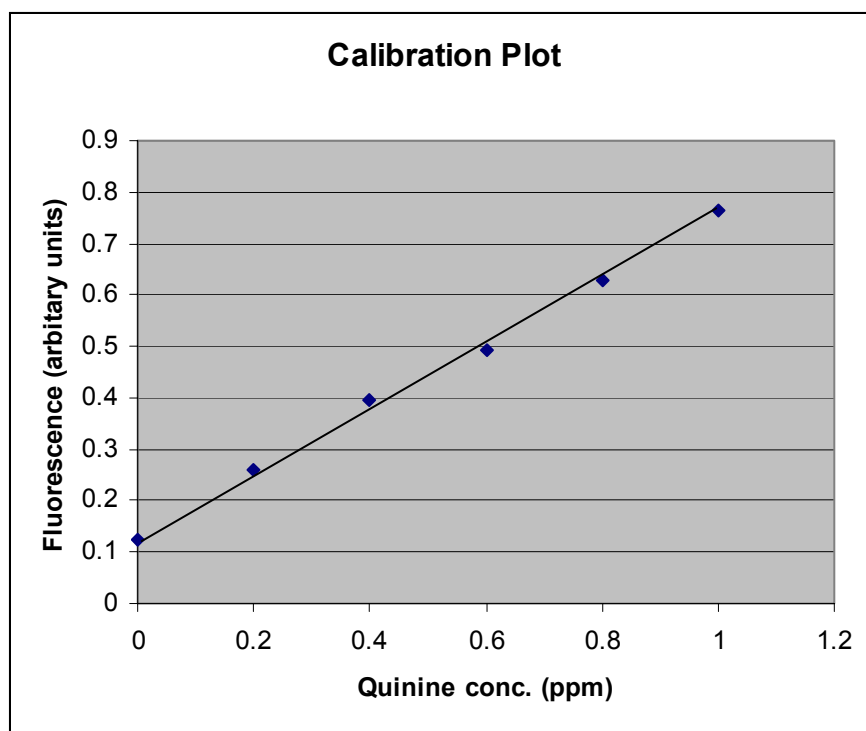
$$\text{So: } 0.4428 = 0.3145 + c$$

$$\therefore c = 0.4428 - 0.3145$$

$$\therefore c = 0.1283 \text{ arbitrary units}$$

It follows that the equation for the best fit line =

$$y = 0.629x + 0.1283$$



Step 3: Substitute in value for unknown

The unknown sample gives a fluorescence with a relative intensity of 0.63

$$\text{It follows } 0.63 = 0.629x + 0.1283$$

$$\therefore 0.629x = 0.63 - 0.1283$$

$$\therefore 0.629x = 0.5017$$

$$\therefore x = \frac{0.5017}{0.629}$$

$$\therefore x = 0.798 \text{ ppm Quinine}$$

**5.19) A solution of sucrose causes a  $42.07^\circ$  dextro rotation of plane polarised light within a polarimeter. Assume  $\alpha^t$  ( $\text{mol}^{-1} \text{dm}^{-3} \text{dm}^{-1}$ ) at  $20^\circ\text{C} = 66.5^\circ$ .**

Calculate the concentration of sucrose within the solution.

$$[\alpha]^t = \frac{\alpha}{lc}$$

$$\therefore [\alpha]^t = 66.5 = \frac{\alpha}{lc}$$

By re-arranging and substituting in values  $c = \frac{\alpha}{66.5} = \frac{42.07}{66.5}$

Therefore the concentration of sucrose =  $0.633 \text{ g cm}^{-3}$

Since the RMM of sucrose = 342, the concentration is equivalent to:

$$\frac{0.633}{342} \times 1000 \text{ mol dm}^{-3}$$

$$\therefore [\text{sucrose}] = 1.85 \text{ M}$$

**5.20) A fructose solution of unknown concentration causes a laevo-rotation of plane polarised light of  $62.76^\circ$ . What is the concentration of fructose? Assume  $\alpha^t$  ( $\text{mol}^{-1} \text{ dm}^{-3} \text{ dm}^{-1}$ ) at  $20^\circ\text{C}$  =  $-92.4^\circ$ .**

$$[\alpha]^t = \frac{\alpha}{lc}$$

$$\therefore [\alpha]^t = 62.76 = \frac{\alpha}{1xc}$$

$$\text{By re-arranging and substituting in values } c = \frac{\alpha}{62.76} = \frac{92.4}{62.76}$$

Therefore the concentration of fructose =  $1.269 \text{ g cm}^{-3}$

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Since the RMM of fructose = 180.15, the concentration is equivalent to:

$$\frac{1.269}{180.15} \times 1000 \text{ mol dm}^{-3}$$

$$\therefore [\text{Fructose}] = 7.044 \text{ M}$$

**5.21) A 0.24M glucose solution is analysed within a polarimeter. If  $\alpha^t$  for glucose at 20°C is 66.5°, calculate the expected angle of rotation for this sample.**

$$[\alpha]^t = \frac{\alpha}{lc}$$

$$\alpha = [\alpha]^t \times lc$$

By substituting in values,  $\alpha = 66.5 \times 1 \times 0.24$

$\therefore$  Expected angle of rotation  $\alpha = 15.95^\circ$