

COMSATS Institute of Information Technology, Abbottabad

Course title and code

Analytical Techniques

Assignment number

01

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10 Numericals of Beer–Lambert law

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1. A solution of Tryptophan has an absorbance at 280 nm of 0.54 in a 0.5 cm length cuvette. Given the absorbance coefficient of trp is $6.4 \times 10^3 \text{ LMol}^{-1}\text{cm}^{-1}$. What is the concentration of solution?

Solution:

$$\text{As } \epsilon = A / l c$$

$$l = 0.5 \text{ cm}$$

$$A = 0.54$$

$$\epsilon = 6.4 \times 10^3 \text{ LMol}^{-1}\text{cm}^{-1}$$

$$C = ?$$

$$\text{So } c = A / \epsilon l$$

$$= 0.54 / 6.4 \times 10^3 \times 0.5$$

$$\text{Answer} = 0.000168 \text{ M}$$

2. A solution of thickness 2 cm transmits 40% incident light. Calculate the concentration of the solution, given that $\epsilon = 6000 \text{ dm}^3/\text{mol}/\text{cm}$.

Solution:

$$A = 2 - \log_{10} \%T = 2 - \log_{10} 40 = 2 - 1.6020 = 0.398$$

$$A = \epsilon l c$$

$$l = 2 \text{ cm}$$

$$\epsilon = 6000 \text{ dm}^3/\text{mol}/\text{cm}$$

$$A = 0.398$$

$$c = ?$$

$$\text{So } c = A / \epsilon l = 0.398 / 6000 \times 2$$

$$\text{Answer} = 3.316 \times 10^{-5} \text{ mol} / \text{dm}^3$$

3. A solution shows a transmittance of 20%, when taken in a cell of 2.5 cm thickness. Calculate its concentration, if the molar absorption coefficient is 12000 dm³/mol/cm.

Solution:

$$A = 2 - \log_{10} \%T = 2 - \log_{10} 20 = 2 - 1.301 = 0.698$$

$$A = \epsilon l c$$

$$l = 2.5 \text{ cm}$$

$$\epsilon = 12000 \text{ dm}^3/\text{mol}/\text{cm}$$

$$A = 0.698$$

$$c = ?$$

$$\begin{aligned} \text{So } c &= A / \epsilon l \\ &= 0.698 / 12000 \times 2.5 \end{aligned}$$

$$\text{Answer} = 2.33 \times 10^{-5} \text{ mol} / \text{dm}^3$$

4. Calculate the molar absorptivity of a 1×10^{-4} M solution, which has an absorbance of 0.20, when the path length is 2.5 cm.

Solution:

$$A = \epsilon l c$$

$$l = 2.5 \text{ cm}$$

$$A = 0.20$$

$$C = 1 \times 10^{-4} \text{ M}$$

$$\epsilon = ?$$

$$\text{So } \epsilon = A / l c$$

$$= 0.20 / 2.5 \times 1 \times 10^{-4}$$

Answer = 800 dm³/mol/cm.

5. The concentration of yeast t-RNA in an aqueous solution is 10 M. The absorbance is found to be 0.209 when this Solution is placed in a 1.00 cm cuvette and 258 nm radiations are passed through it.

- Calculate the specific absorptivity, including units, of yeast t-RNA.**
- What will be the absorbance if the solution is 5 M?**
- What will be the absorbance if the path length of the original solution is increased to 5.00 cm?**

Solution

5a

$$l = 1.00 \text{ cm}$$

$$c = 10.00 \text{ M}$$

$$A = 0.209$$

$$\begin{aligned} \text{So } \epsilon &= A / l c \\ &= 0.209 / 1.00 \text{ cm} \times 10 \text{ M} \end{aligned}$$

Answer = 0.0209 dm³/mol/cm.

5b

$$\epsilon = 0.0209 \text{ dm}^3/\text{mol}/\text{cm}.$$

$$l = 1.00 \text{ cm}$$

$$c = 5.00 \text{ M}$$

$$A = ?$$

$$\text{So } A = \epsilon l c$$

$$A = 0.0209 \text{ dm}^3/\text{mol}/\text{cm} \times 1.00 \text{ cm} \times 5\text{M}$$

$$\text{Answer} = 0.1045$$

5c

$$\epsilon = 0.0209 \text{ dm}^3/\text{mol}/\text{cm}.$$

$$l = 5.00 \text{ cm}$$

$$c = 10.00 \text{ M}$$

$$A = ?$$

$$\text{So } A = \epsilon l c$$

$$A = 0.0209 \text{ dm}^3/\text{mol}/\text{cm} \times 5.00 \text{ cm} \times 10.00 \text{ M}$$

$$\text{Answer} = 1.045$$

6. Calculate the molar absorptivity of a $0.5 \times 10^{-3} \text{ M}$ solution, which has an absorbance of 0.17, when the path length is 1.3 cm.

Solution:

$$A = \epsilon l c$$

$$l = 1.3 \text{ cm}$$

$$A = 0.17$$

$$C = 0.5 \times 10^{-3} \text{ M}$$

$$\epsilon = ?$$

$$\text{So } \epsilon = A / l c$$

$$= 0.17 / 1.3 \times 0.5 \times 10^{-3}$$

$$\text{Answer} = 261.53 \text{ dm}^3/\text{mol}/\text{cm}.$$

7. A CaCO_3 solution shows a transmittance of 90%, when taken in a cell of 1.9 cm thickness. Calculate its concentration, if the molar absorption coefficient is $9000 \text{ dm}^3/\text{mol}/\text{cm}$.

Solution:

$$A = 2 - \log_{10} \%T = 2 - \log_{10} 90 = 2 - 1.954 = 0.045$$

$$A = \epsilon l c$$

$$l = 1.9 \text{ cm}$$

$$\epsilon = 9000 \text{ dm}^3/\text{mol}/\text{cm}$$

$$A = 0.045$$

$$c = ?$$

$$\text{So } c = A / \epsilon l$$

$$= 0.045 / 9000 \times 1.9$$

$$\text{Answer} = 2.631 \times 10^{-6} \text{ mol} / \text{dm}^3$$

8. Extinction coefficient of NADH at 340 nm is $6440 \text{ L}/\text{mol}/\text{cm}$. whereas NAD does not absorb at 340nm. What absorbance will be observed when light at 340nm passes through a 1cm cuvette containing 10uM NADH and 10 uM NAD.

Solution:

$$\epsilon = 6440 \text{ L}/\text{mol}/\text{cm}.$$

$$l = 1.00 \text{ cm}$$

$$c = 10.0 \text{ uM} = 10 \times 10^{-6} \text{ M}$$

$$A = ?$$

$$\text{So } A = \epsilon l c$$

$$A = 6440 \text{ L}/\text{mol}/\text{cm} \times 1.00 \text{ cm} \times 10 \times 10^{-6} \text{ M}$$

Answer = 0.0644

Note: this absorbance is only for NADH because NAD do not absorb at 340nm.

9. A 1.00×10^{-4} M solution of an analyte is placed in a sample cell with a path length of 1.00 cm. When measured at a wavelength of 350 nm, the solution's absorbance is 0.139. What is the analyte's molar absorptivity at this wavelength?

$$l = 1.00 \text{ cm}$$

$$c = 1.00 \times 10^{-4} \text{ M}$$

$$A = 0.139$$

$$\epsilon = ?$$

$$\text{So } \epsilon = A / l c$$

$$= 0.139 / 1.0 \times 1.00 \times 10^{-4}$$

$$\text{Answer} = 1390 \text{ cm}^{-1} \text{ M}^{-1}$$

10. The absorbance of a Cu sulphate solution containing 0.500 mg Cu/mL was reported as 0.3500 at 440 nm.

- Calculate the specific absorptivity, including units, of Cu sulphate on the assumption that a 1.00 cm cuvette was used.**
- What will be the absorbance if the solution is diluted to twice its original volume**

Solution

$$\text{a) } l = 1.00 \text{ cm}$$

$$c = 0.500 \text{ mg/ml}$$

$$A = 0.3500$$

$$\epsilon = ?$$

$$\begin{aligned}\text{So } \epsilon &= A / l c \\ &= 0.3500 / 1.0 \times 0.500\end{aligned}$$

$$\text{Answer} = 0.7 \text{ cm}^{-1}(\text{mg/mL})^{-1}$$

b) $c = 0.250 \text{ mg/ml}$

$$\epsilon = 0.7 \text{ cm}^{-1}(\text{mg/mL})^{-1}$$

$$l = 1.00 \text{ cm}$$

$$A = ?$$

$$\text{So } A = \epsilon l c$$

$$A = 0.7 \text{ cm}^{-1}(\text{mg/mL})^{-1} \times 1.00 \text{ cm} \times 0.250 \text{ mg/ml}$$

$$\text{Answer} = 0.175$$