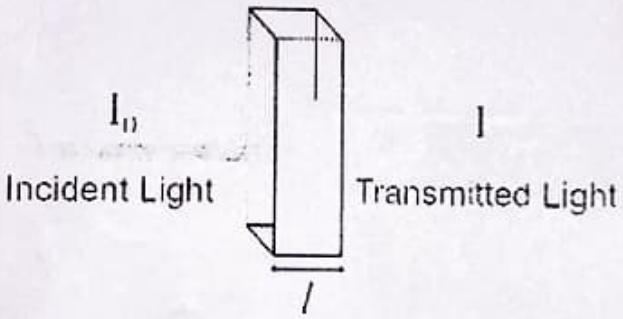
Himanshu Raj
AIDS-B2
076

LAB MANUAL: EXPERIMENT 1

Aim: To verify Lambert-Beer's law using a given solution of potassium permanganate at the wavelength of its maximum absorption (λ_{max}) and consequent determination of the unknown concentration of a solution of potassium permanganate.

Theory:

The Beer-Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length (cuvette length), UV/Vis spectroscopy can be used to determine the concentration of the absorber in a solution. The absorbance changes with concentration. Thus, a higher concentration of the colored solution absorbs more light (and transmits less) than a solution of lower concentration.



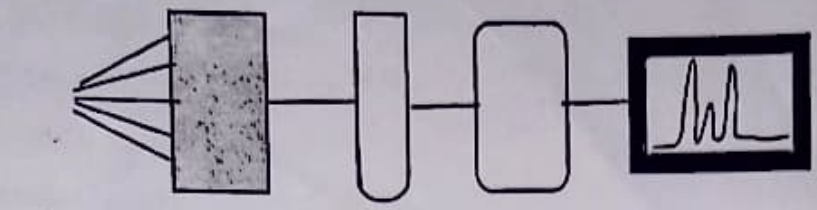
$$\log(I_0 / I_t) = A = \varepsilon c l$$

According to Beer–Lambert law,

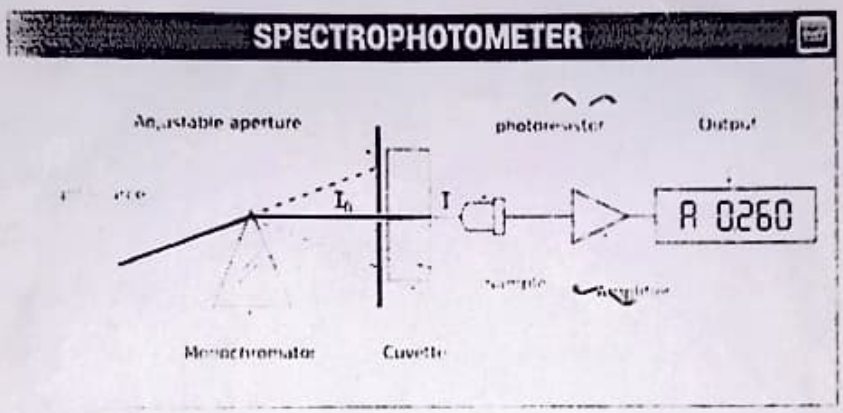
$\log(I_0/I_t)=A=\varepsilon cl$ where I_0 and I_t are the incident and transmitted intensities,

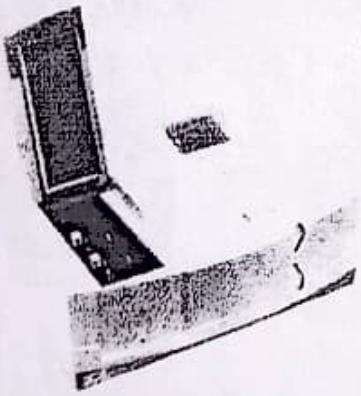
A = absorbance and ε is a constant i.e. absorptivity (also called the extinction coefficient).

If the concentration is measured in $molL^{-1}$, the absorptivity is called molar absorptivity. $A= \varepsilon cl$. At constant length $A \propto c$

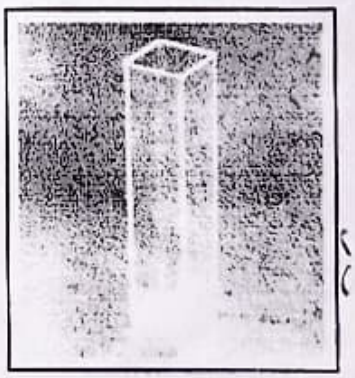


Working principle of spectrophotometer





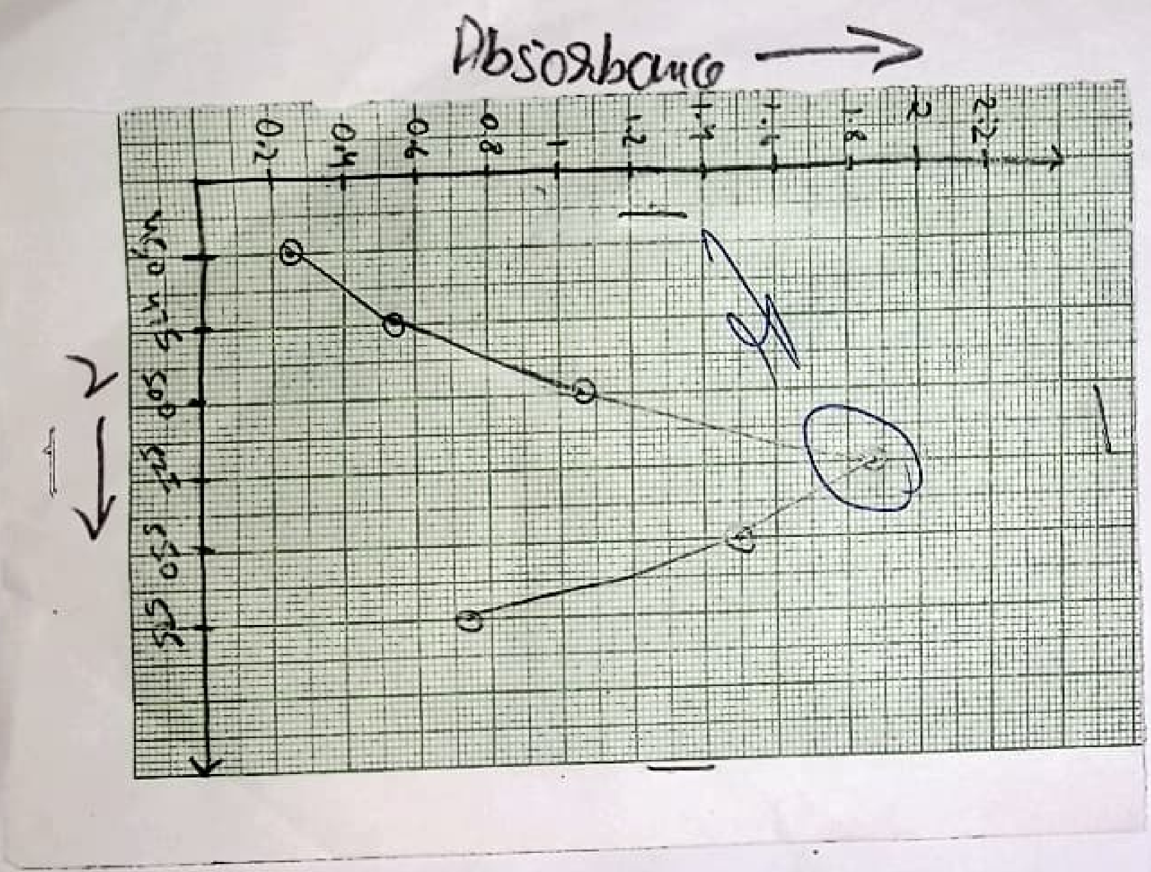
Spectrophotometer

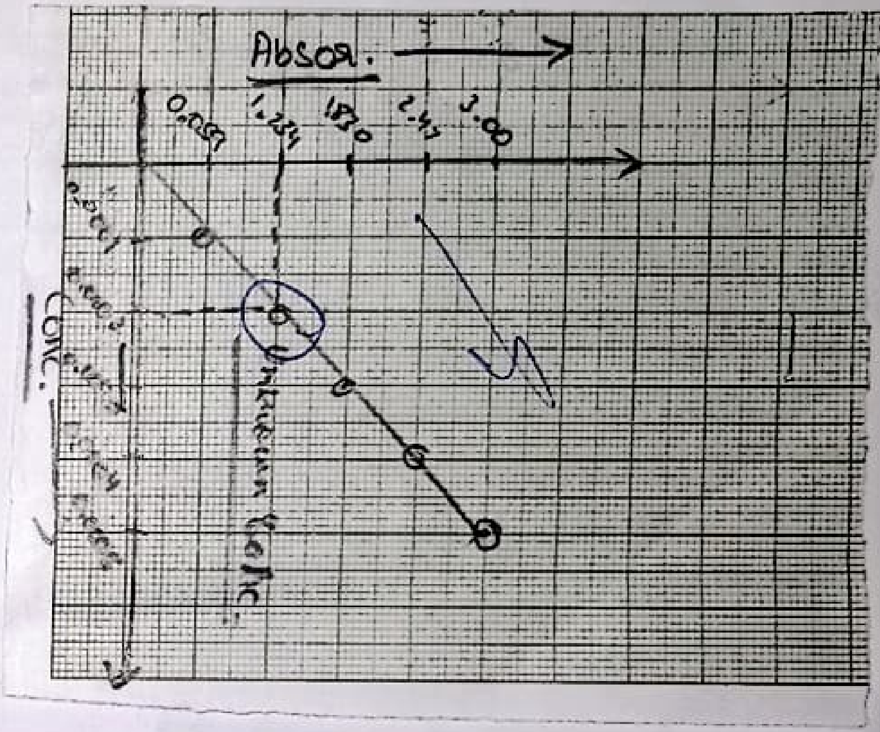


Cuvette

Requirements:

spectrophotometer, cuvette, six test tubes, Measuring cylinder, 10 mL pipette, 0.001M $KMnO_4$ solution, distilled water, test tube rack, and tissues (preferably lint-free).


Absorbance
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
2.2
450
475
500
525
550
575
λ


Absor.
0.059
1.234
1.830
2.47
3.00
Unknown Conc.
Conc.

Procedure & observation table:

Step 1: To record the absorbance of $KMnO_4$ solution at different wavelengths to determine the light wavelength for its maximum absorption (λ_{max}):

a) Prepare 100 mL of 0.001M $KMnO_4$ (**Molecular weight 158.03 gm/mol**) solution in distilled water.

b) Label five clean, dry, test tubes 1–5.

c) Use a 10 mL pipette to prepare five standard solutions according to Table 1.

d) Thoroughly mix each solution.

e) Calibrate the spectrophotometer with respect to the blank solution i.e. distilled water.

f) Fill any one of the prepared solutions (1-5) up to a certain level in the cuvette of the spectrophotometer.

g) Record the absorbance of the respective solution at different wavelengths as mentioned in Table 2.

h) Plot the absorbance data in the graph paper with respect to the wavelength and calculate the light wavelength for its maximum absorption (λ_{max}) in $KMnO_4$.

[Mn = 158]

Table 1:

Test-tube	0.001M $KMnO_4$ (mL)	Distilled water (mL)	Concentration (M)	
1	1	9	[redacted]	→ 0.0001
2	2	8	[redacted]	→ 0.0002
3	3	7	[redacted]	→ 0.0003
4	4	6	[redacted]	→ 0.0004
5	5	5	[redacted]	→ 0.0005

Table 2: The solution of the ...3..... **No.** test tube was chosen for the determination of the light wavelength for its maximum absorption (λ_{max}).

Entry	Wavelength (λ in nm)	Absorbance
1	450	0.224
2	475	0.526
3	500	1.156
4	525	1.830 → λmax
5	550	1.584
6	575	0.780

Step 2: To record the absorbance of different concentrations of solutions at the specified λ_{max}:

a) Set the operating wavelength of the spectrophotometer in the range of absorption maxima of aqueous KMnO4 solution (λ_{max}).

b) Calibrate the spectrophotometer with respect to the blank solution i.e. water.

c) Fill each of the solutions up to a certain level in the cuvette of the spectrophotometer.

d) Record the absorbance of the respective solutions as stated in Table 3.

e) Plot the absorbance data in the graph paper with respect to the concentration which should be a straight line passing through the origin.

Table 3:

Entry	Test-tube	Absorbance
1	1	0.654
2	2	0.234
3	3	1.830
4	4	2.427
5	5	3.000
Unknown	6th	1.235

Step 3: Determination of the unknown concentration of a given potassium permanganate solution:

- Fill the solutions up to a certain level in the cuvette of the spectrophotometer.
- Record the absorbance of the given solution of unknown concentration.
- Plot the absorbance data in the same graph obtained above (ideally it should be on the same straight line obtained from the plot of step 1)
- Draw a perpendicular line from the absorbance point to the concentration axis.
- Note down the corresponding unknown concentration.

Conclusion:

1) The light wavelength for its maximum absorption (λ_{max}) is found to be 525 nm.

2) The concentration of the unknown solution was found to be 0.0002M

Precautions:

- Always mix the standard solutions properly.
- Wipe the outside of the cuvette every time with a lint-free tissue.
- Handle cuvettes only by the top edge of the ribbed sides.
- Dislodge any bubbles by gently tapping the cuvette on a hard surface.
- Always position the cuvette so the light passes through the clear sides.
- Always set the light source of the instrument in the absorption maxima range of the given solution.

12/04/23