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Chapter 30

Experimental validation of Lambert-Beer's law: Principles, spectrophotometric analysis, and applications

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1. Introduction:

Beer-Lambert law states that, the absorbance or transmittance value of any solution is directly correlated (proportional) with both the concentration of the absorbing substance within the solution and the distance light travels through it (Kellner et al., 2004; Srivastava et al., 2022). This relationship is fundamental in UV/Vis spectroscopy, where a fixed path length (typically the length of a cuvette) allows for precise determination of absorber concentration (Beer,1852). The concentration directly impacts absorbance: as concentration increases, so does light absorption, resulting in decreased transmission compared to solutions with lower concentrations (Swinehart, 1962; Lambert, 1970).

Expressed through the Beer-Lambert law formula, where I_o represents incident intensity, I_t denotes transmitted intensity, A stands for absorbance, and ε represents a constant known as absorptivity (formerly referred to as the extinction coefficient). When concentration is expressed in units of mol/L, the term used for absorptivity is molar absorptivity (Jenkins and White, 2001).

Absorbance (A) =Molar extinction coefficient (ε) x Concentration of solution(C) x Path length (l)

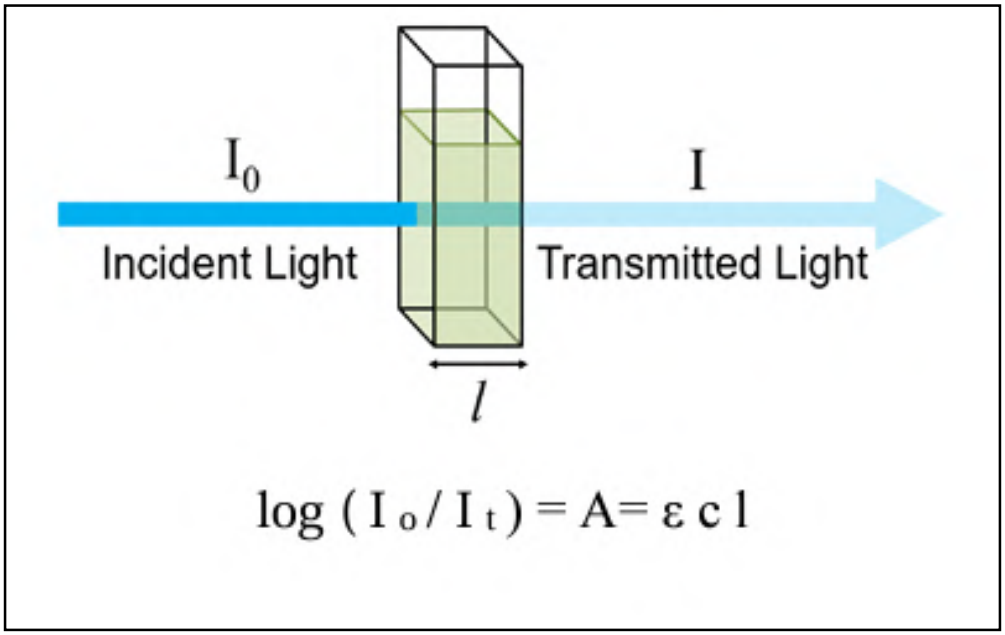

Figure 1: Transmission of light through the solution in a cuvette

Under the constant path length, absorbance of a solution is directly proportional to concentration of the solution:

$$A \propto c$$

2. Materials Required:

1. Colorimeter
2. Cuvettes
3. Test Tubes
4. Burettes or Graduated Cylinders
5. 100 Ml Beakers
6. 0.01m $KMnO_4$ Solution
7. Distilled Water
8. Test Tube Rack
9. Stirring Rod.

3. Procedure:

(a) Determination of λ_{max}

To identify the maximum absorbance wavelength (λ_{max}), one can either refer to established references like tables of molar extinction coefficients or, for greater accuracy, derive it from a calibration curve**.**

(b) Absorbance of different concentration solutions at λ_{max}

To determine the absorbance of solutions at their maximum absorption wavelength (λ_{max}), adhere to these guidelines:

1. Create a 100 ml solution of $KMnO_4$ at a concentration of 0.01M, serving as the stock solution, and transfer it into a burette.
2. Switch on the instrument and attached computer, allowing a 30-minute warm-up period.
3. Customize display settings on the instrument, such as selecting between % transmittance or absorbance, and specify the desired wavelength range.
4. Utilize a clean and dry cuvette (quartz or glass) with a defined path length (e.g., 1 cm) for visible range scanning.
5. Fill the cuvette approximately 3/4 full with distilled water to establish a blank. Clean the surface of the cuvette by a tissue paper.
6. Eliminate any air bubbles from the cuvette by gently tapping it on a solid surface, ensuring optimal light transmission through its transparent sides.
7. Perform colorimeter calibration using the blank cuvette filled with distilled water to set absorbance to 0 and transmittance to 100%.
8. After calibration, remove the blank cuvette.
9. Fill a second cuvette with stock solution and note the absorbance value in a table.
10. Repeat steps 5 and 6 for each filter within the designated wavelength range.
11. Analyze the recorded data to determine λmax, which indicates the wavelength at which the solution displays the highest absorbance or optical density (O.D.).

Table1: Calculating the λmax

S.No.	Wavelength/Range	OD/Absorbance
1	**420**	
2	**440**	
3	**490**	
4	**520**	
5	**540**	
6	**570**	
7	**600**	
8	**650**	
9	**700**	

(c) Absorbance of different concentration solutions:

1. Evaluate the absorbance of solutions at different concentrations.
2. Utilize burettes to prepare five standard solutions, each labeled from 1 to 5 as per the provided chart.
3. Using a stirring rod, thoroughly combine each solution, being sure to clean the rod after each use.

(d) Concentration can be calculated by $M_1V_1 = M_2V_2$

S. No	Test Tube	0.01$KMnO_4$ (ml)	Distilled H_2O (ml)	Total Volume (ml)	Concentration (M)
1.	1	2	8	10	0.002
2.	2	4	6	10	0.004
3.	3	6	4	10	0.006
4.	4	8	2	10	0.008
5.	5	10	0	10	0.100

1. Start by powering on the computer and/or instrument, allowing them to warm up for 30 minutes.
2. Make use of the many capabilities of the instrument, which include changeable slit width, scan speed, and the choice to show % transmittance or absorbance, as well as several light sources including UV and visible. Make the wavelength range further customized to meet your needs.
3. Choose two spotless, ideally 1 cm-long glass or quartz cuvettes that must be totally dry.
4. Fill a cuvette with pure water primarily and a solution of KMnO4 at the lowest concentration to create a blank solution.
5. The first absorbance value shown on the meter should be noted.
6. Enter the absorbance's value into your data table as soon as it stabilizes.
7. Proceed as before with Test Tubes 2 through 5.
8. Measure the remaining samples, stepping up from the KMnO4 concentration that is lowest first. Remind yourself to wash the cuvette with solution that will be examined.
9. To see the connection between the two variables, create a concentration-absorbance curve.
10. Assess whether the plot exhibits a linear trend or not.

Test Tube	0.01M $KMnO_4$ (mL)	Distilled H_2O	Concentration (M)	Absorbance
	0	10	0.00	
1	2	8	0.002	
2	4	6	0.004	
3	6	4	0.006	
4	8	2	0.008	
5	10	0	0.0100	

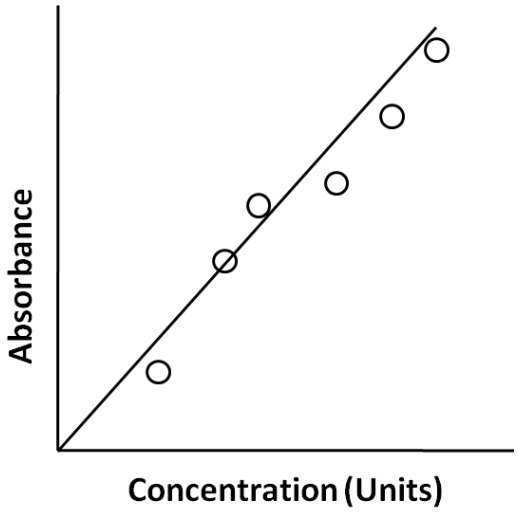

Figure: 22 Relation between absorbance and concentration.

4. Limitations of Beer-Lambert Law:

1. **Assumptions of linearity:** The law assumes a direct proportionality between absorbance and concentration, which may not hold true at high concentrations.
2. **Applicability limited to dilute solutions**: Accuracy diminishes in concentrated solutions or when solute-solute interactions occur.
3. **Dependency on monochromatic light:** Assumes incident light consists of a single wavelength, potentially leading to inaccuracies with polychromatic sources.
4. **Sensitivity to path length and wavelength:** Variations can cause deviations from expected absorbance values.
5. **Requirement for homogeneous samples:** Variations within the sample can lead to inaccuracies.
6. **Temperature sensitivity:** Changes affect the molar absorptivity coefficient and optical properties.
7. **Solvent absorption effects:** Interference from solvent absorption requires correction.
8. **Non-linear concentration effects:** Deviations occur at very low or high concentrations.
9. **Chromophore complexity:** Presence of multiple absorbing species or structural changes can affect accuracy.
10. **Instrumental errors:** Stray light, detector limitations, and wavelength accuracy impact measurements.

5. Precautions:

1. Ensure all spectrophotometric equipment is calibrated and maintained according to manufacturer specifications.
2. Use high-purity chemicals and reagents to prepare standard solutions and samples.
3. Handle solutions and samples carefully to avoid contamination or introduction of air bubbles.
4. Use spectrophotometer cuvettes that are clean and free of scratches or defects.
5. Ensure all solutions are mixed thoroughly and equilibrated to room temperature before measurement.
6. Perform blank measurements with the solvent used for dilution to account for any background absorbance.
7. Measure absorbance at the appropriate wavelength specific to the compound being analyzed.
8. Use standard solutions of known concentrations to generate a calibration curve.
9. Validate linearity by ensuring the absorbance values of standard solutions follow Lambert-Beer's law.
10. Perform measurements in triplicate or more to ensure reproducibility and reliability of results.
11. Calculate the molar absorptivity (ε) or concentration of unknown samples using the calibration curve.
12. Dispose of used reagents and contaminated materials according to laboratory waste disposal guidelines.

6. References:

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