

Verification of Lambert-Beer's Law and Determination of Concentration using Spectrophotometer

Object

To test the validity of Lambert-Beer's law and determine the concentration of unknown solution spectrophotometrically.

Principle

When monochromatic light passes through a solution containing an absorbing substance, a part of the incident radiation is absorbed by the solution. According to Lambert-Beer's law, the absorbance of a solution is directly proportional to its concentration when the path length, wavelength, temperature and other experimental conditions are kept constant.

$$A = \epsilon l c$$

where A is the absorbance, ϵ is the molar absorptivity, l is the path length of the cuvette and C is the concentration of the solution.

The absorbance is related to the intensity of incident and transmitted radiation by:

$$A = \log_{10} \left(\frac{I_0}{I} \right)$$

where I_0 is the intensity of incident radiation and I is the intensity of transmitted radiation.

For a fixed path length and wavelength, Lambert-Beer's law can therefore be written as:

$$A \propto c$$

Thus, a plot of absorbance against concentration gives a straight line when Lambert-Beer's law is valid over the concentration range studied. The concentration of the unknown solution can then be determined directly from the calibration graph using its measured absorbance.

Chemicals Required

- Potassium permanganate ($KMnO_4$)
- Distilled water
- Unknown potassium permanganate solution

Apparatus Required

- UV-Visible spectrophotometer
- Suitable cuvettes
- Volumetric flasks
- Pipettes
- Beakers
- Measuring cylinder
- Wash bottle

Preparation of Standard Solutions

A 0.001 M stock solution of potassium permanganate was used to prepare standard solutions having concentrations from 0.0001 M to 0.0006 M by suitable dilution with distilled water. The final volume of each standard solution was kept at 10 mL.

S. No.	Required Concentration of $KMnO_4$ (M)	Volume of 0.001 M Stock Solution (mL)	Final Volume (mL)	Distilled Water (mL)
1	0.0001	1	10	9
2	0.0002	2	10	8
3	0.0003	3	10	7
4	0.0004	4	10	6

S. No.	Required Concentration of KMnO_4 (M)	Volume of 0.001 M Stock Solution (mL)	Final Volume (mL)	Distilled Water (mL)
5	0.0005	5	10	5
6	0.0006	6	10	4

Procedure

The spectrophotometer was switched on and allowed to warm up properly. The wavelength of maximum absorption (λ_{max}) for the potassium permanganate solution was selected.

Distilled water was used as the blank. A clean cuvette was rinsed with distilled water and then with a small quantity of the solution to be measured.

The cuvette containing distilled water was placed in the sample holder and the instrument was adjusted to zero absorbance or 100% transmittance according to the operating procedure of the instrument. The standard potassium permanganate solutions were taken one by one. The cuvette was rinsed with the respective standard solution and then filled with it. The outside surface of the cuvette was wiped carefully and the cuvette was placed in the spectrophotometer in the same orientation each time.

The absorbance of each standard solution was measured at the selected wavelength and recorded in the observation table. The absorbance values were plotted against their corresponding concentrations to obtain a calibration graph.

The unknown potassium permanganate solution was then taken in the same manner. The cuvette was rinsed with a small quantity of the unknown solution, filled with the solution and placed in the spectrophotometer. Its absorbance was measured at the same wavelength and under the same experimental conditions and recorded in the same observation table.

The absorbance of the unknown solution was then used to determine its concentration directly from the calibration graph.

Observations

A. Instrumental Conditions

Parameter	Observation
Analyte	KMnO_4
Blank	Distilled water

Parameter	Observation
Wavelength of maximum absorption, λ_{max}	_____ nm
Path length of cuvette	_____ cm
Temperature	_____ °C

B. Absorbance of Standard and Unknown Solutions

S. No.	Concentration of KMnO ₄ (M)	Absorbance
1	0.0001	
2	0.0002	
3	0.0003	
4	0.0004	
5	0.0005	
6	0.0006	
7	Unknown	

Calculations

A. Calculation for Preparation of Standard Solutions

The standard solutions were prepared using the dilution equation:

$$C_1V_1 = C_2V_2$$

where C_1 is the concentration of the stock solution, V_1 is the volume of stock solution taken, C_2 is the required concentration and V_2 is the final volume of the solution.

For example, for the preparation of 0.0001 M KMnO_4 solution from 0.001 M stock solution:

$$V_1 = \frac{C_2 V_2}{C_1}$$

$$V_1 = \frac{0.0001 \times 10}{0.001} = 1.0\text{ mL}$$

Therefore, 1 mL of 0.001 M stock solution was diluted to 10 mL with distilled water.

B. Verification of Lambert-Beer's Law

The absorbance values obtained for the standard potassium permanganate solutions were plotted against their corresponding concentrations. According to Lambert-Beer's law:

$$A = \epsilon l c$$

Since the wavelength and path length were kept constant:

$$A \propto c$$

Therefore, a straight-line relationship between absorbance and concentration confirmed the validity of Lambert-Beer's law over the investigated concentration range.

C. Determination of Concentration of Unknown Solution

The absorbance of the unknown solution was measured at the same wavelength used for the standard solutions. The measured absorbance of the unknown solution was marked on the Y-axis of the

calibration graph. A horizontal line was drawn from this point to intersect the calibration curve. From the point of intersection, a vertical line was drawn towards the X-axis. The concentration corresponding to this point was directly obtained from the X-axis.

Thus, the concentration of the unknown solution was determined directly from the calibration graph.

Graph

A graph was plotted between the concentration of potassium permanganate solution on the X-axis and absorbance on the Y-axis. A straight-line relationship was obtained for the standard solutions. The absorbance of the unknown solution was marked on the Y-axis, and its corresponding concentration was obtained directly from the X-axis of the calibration graph.

Result

Lambert-Beer's law was found to be valid for the investigated concentration range of potassium permanganate solution. The concentration of the unknown solution was determined spectrophotometrically from the calibration graph and was found to be _____ M.

Precautions

1. The spectrophotometer was allowed to warm up properly before taking measurements.
2. Distilled water was used as the blank.
3. The cuvette was cleaned and rinsed properly before use.
4. The outside surface of the cuvette was wiped clean before placing it in the instrument.
5. Air bubbles were avoided inside the cuvette.
6. The cuvette was placed in the same orientation for all measurements.
7. The same wavelength and path length were used for all solutions.
8. The standard solutions were prepared accurately by suitable dilution of the stock solution.
9. All absorbance measurements were taken under identical experimental conditions.
10. The solutions were kept within the suitable linear working range of the spectrophotometer.