spectrophotometry

Explain Beer-Lambert's law Derivation and deviations from Beer-Lambert's law.

Beer-Lambert's law - statement :

Beer-Lambert's law (also called Beer's law) relates the absorbance (A) of light to the concentration (c) of the absorbing substance and the path length (l) of the sample through which the light passes.

$$A = \varepsilon c l$$

where :

- A = Absorbance (no unit)
- ε $(L \cdot mol^{-1} cm^{-1})$ = Molar absorp lar extinction coefficient.
- c = concentration of the solution $(mol \cdot L^{-1})$
- l = path length of the sample (cm).

Derivation of Beer-Lambert's law :-

step-1 :- Lambert's law (effect of path length)

Lambert stated that:

The decrease in intensity of light (dI) as it passes through an absorbing medium is Proportional to the intensity of incident light (I) and the thickness (dx) of the medium.

$$-\frac{dI}{dx} \propto I$$

`-\ frac{dI}{dx} = kI □`

Integration from $x = 0$ to $x = l$ and $I = I_0$ to $I = I$

$$\int_{I_0}^{I} \frac{dI}{I} = -k \int_{0}^{l} dx$$

$$In \left(\frac{I}{I_0}\right) = -l$$

If the absorbing species concentration is c then:

$$k \propto c \Rightarrow k = \alpha c$$

So,

$$I = I_0 e^{-\alpha c l}$$

Taking logarithm base 10:

$$\log_{10} \left(\frac{I_0}{I} = \frac{\alpha c l}{2.303}\right.$$

Define absorbance

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

Hence,

$$\boxed{A = \varepsilon c l}$$

Deviations from Beer - Lamberts law :-

Although theoretically linear, the relationship b/w A & C sometimes deviates from linearity. These deviations are classified into three types:

1. Real (Fundamental) Deviations :-

- Occur due to the chemical or physical nature of the absorbing species.

- Example :-
  - Association or dissociation of solute at higher concentrations.
  - Change in refractive index with concentration

2. Instrumental Deviations :-

- Caused by limitations or imperfections in the instrument.

- Example :-
  - Use of polychromatic (non-monochromatic) light instead of monochromatic light.
  - stray light entering the detector.
  - Non-linear detector response or incorrect calibration of the instrument.

3. Chemical Deviations :-

- Arise due to interactions b/w solute molecules or changes in equilibrium at different concentrations.

* Examples :-
  - Change in oxidation state of an absorbing species.
  - Solvent effects altering absorption characteristics.

DOUBLE BEAM SPECTROMETER

A Double beam Spectrometer is an instrument used to measure the absorbance or transmittance of a sample by comparing the light intensity passing through the sample with that passing through a reference.

Working principle :-

- * Light from a source is split into two beams using a beam splitter or rotating mirror.
- * One beam passes through the sample.
- * The other passes through a reference (blank).
- * Both beams then go to a detector, which compares their intensities.
- * The instrument continuously measures the ratio of sample to reference intensities, giving accurate absorbance values.

Components :-

1. Light source - Emits radiation (UV, visible or IR).
2. Monochromator - selects a specific wavelength of light
3. Beam splitter - divides the light into two beams.
4. Sample and Reference cells - holds the sample and blank.
5. Detectors - Measure the intensity of both beams.
6. Read out system - Display absorbance or transmittance.

Advantages :-

* compensates for fluctuations in light source intensity.
* provides greater accuracy and stability than single-beam instruments.
* Allows simultaneous measurement of sample and reference.

Applications :-

* used in UV-visible spectroscopy, molecular absorption studies, and quantitative analysis of solutions.

In short :-

A double beam spectrometer compares light intensities of a sample and a reference simultaneously, ensuring more precise and stable measurements.

Determination of Mn^{+2} by Periodate Method

The periodate Method is used to determine manganese in the +2 oxidation state (Mn^{+2}). It is based on the oxidation of Mn^{+2} to Permanganate ion (MnO_4^-) by periodate (IO_4^-) under specific conditions. The resulting Permanganate ion is purple and can be Quantitatively measured by using a spectrophotometer.

Principle:-

Manganese (II) is oxidized by periodate (IO_4^-) in an acidic medium to form permanganate ion (MnO_4^-) which is intensely purple and absorbs strongly at 525 - 545 nm. The intensity at the purple colour is proportional to the concentration of Mn^{+2} in the sample.

The reaction can be simplified as

$$2Mn^{2+} + 5IO_4^- + 3H_2O \longrightarrow 2MnO_4^- + 5IO_3^-$$

1. Sample Solution containing Mn^{+2}
2. Potassium Periodate (KIO_4) Sol^n - oxidizing agent.
3. Sulfuric acid (H_2SO_4) or Nitric acid (HNO_3) for acidic Medium.
4. Distilled water.

Procedure :-

* Take an aliquot of the Mn^{+2} containing Sol^n Add acid Carefully dilute Sulfuric acid to make the Sol^n acidic (pH - 1-2)

* Add a known volume of freshly prepared Potassium Periodate Solution.

* Heat gently (around 60 to 70°C) for a few minutes to ensure complete oxidation.

* Cool the Sol^n to room temperature.

* Measure the absorbance of the resulting Purple Sol^n at 525 - 545. using a UV - vis Spectro-Photometer against a reagent blank.

Calibration :-

Prepare a Series of Standard Mn^{+2} Solution.

* Treat each with Periodate under identical condition.

* Plot a calibration curve of absorbance vs Mn^{+2} concentration.

use the calibration curve to determine Mn^{+2} concentration in unknown sample.

This method is highly sensitive and suitable for the analysis.

Interface from other oxidizable ions (like Fe^{+2}, Cr^{+3}) should be Minimized.

Applications

Determin , soil,

duct &

biological

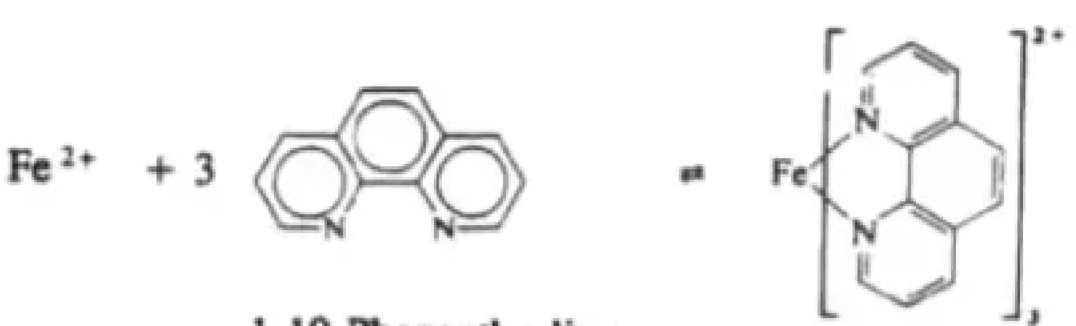
pollution.

Environme

DETERMINATION OF IRON WITH 1,10 -PHENANTHROLINE: A SPECTROPHOTOMETRIC ANALYSIS

BACKGROUND

The reaction between Fe(II) and 1,10-phenanthroline to form a red complex serves as a sensitive method for the determination of iron. The reaction is:



1,10-Phenanthroline

The molar absorptivity of the complex is 11,100 $M^{-1}cm^{-1}$ at a wavelength of 508 nm. The intensity of the color is independent of pH between 2 to 9. The colored complex is very stable and intensity of the color does not change over a long period of time. Beer's law is obeyed.

The iron must be in the +2 oxidation state, and hence a reducing agent is added before the color is developed. Hydroxylamine, as its hydrochloride, can be used for the purpose. The reaction is:

$$2Fe^{3+} + 2NH_2OH + 2OH^- \rightarrow 2Fe^{2+} + N_2 + 4H_2O$$

The pH is adjusted to a value between 6 and 9 by addition of sodium acetate.

PROCEDURE

PART I. Prepare the following solutions separately

1. Dissolve 0.1 g of 1,10-phenanthroline monohydrate in 100 mL of distilled water, warm if necessary.

2. Dissolve 10 g of hydroxylamine hydrochloride in 100 mL distilled water.

3. Dissolve 10 g of sodium acetate in 100 mL distilled water.

4. Weigh precisely about 0.07 g of pure iron(II) ammonium sulfate (ferrous ammonium sulfate), dissolve in water, and transfer the solution to a 1 L volumetric flask. Carefully add

2.5 mL of concentrated sulfuric acid and dilute the solution to the mark. Calculate the concentration of the solution in mg of iron per mL of solution. Into five 100 mL volumetric flasks, precisely pipet 1, 5, 10, 25, and 50 mL portions of the standard iron solution. Put 50 mL of distilled water in another flask to serve as the blank. To each flask add 1 mL of the hydroxylamine solution, 10 mL of 1,10-phenanthroline solution, and 8 mL of sodium acetate solution. Then dilute all the solutions to the 100 mL mark and allow them to stand for 10 minutes.

Measure the absorbance of one of the middle iron solution at wavelengths between 400 to 600 nm vs. the blank as the reference. Take readings about 20 nm apart except in the region of maximum absorbance where intervals of 5 nm should be used. Plot the absorbance vs. wavelength and connect the points to a smooth curve (use spreadsheet graphics). Select the proper wavelength to use for the determination of iron with 1,10-phenanthroline.

Measure the absorbance of each of the iron solution prepared above using the selected wavelength. Plot the absorbance vs. the iron concentration (mg/mL) of the standards. Note whether Beer's law is obeyed.

PART II. Preparation of iron-tablet solution

Dissolve an iron tablet in 50 mL water and add 2.5 mL of concentrated sulfuric acid. Filter the solution into a 100.00 mL volumetric flask and rinse the filter paper with water. Dilute the solution to the mark. Dilute 10 mL of this solution in a 1.00 L volumetric flask; add 2 mL of concentrated sulfuric acid and dilute to the mark. Take 10 mL of this solution, add 1 mL of hydroxylamine solution, 10 mL of 1,10-phenanthroline solution, and 8 mL of sodium acetate solution in a 100 mL volumetric flask and dilute to the mark. Allow the solution to stand for 10 minutes and measure its absorbance at the proper wavelength. Calculate the mg of iron per tablet.

TREATMENT OF DATA AND RESULTS

Your report should include the following in the data and results section:

1. A plot of absorbance vs. wavelength (absorption spectrum). Identify the wavelength of maximum absorbance

2. A plot of absorbance vs. mg of iron in the standards (calibration graph). Show the linear least squares fitting line, the line equation, and the error of slope and intercept.

3. Calculation of mg iron per tablet. Use the above data to calculate the measurement error.

QUESTIONS

1. A plot of absorbance vs. concentration at constant cell-length gives a straight line if Beer's law is obeyed. What property of the line is represented by the absorptivity?