DIRECTORATE OF DISTANCE & CONTINUING EDUCATIONS

MANONMANIAM SUNDARANAR UNIVERSITY

TIRUNELVELI – 627012

OPEN AND DISTANCE LEARING(ODL) PROGRAMMES

(FOR THOSE WHO JOINED THE PROGRMMES FROM THE ACADEMIC YEAR 2023 – 2024)

M.Sc. CHEMSITY
COURSE MATERIALS
ANALYTICAL CHEMISTRY PRACTICAL
SCHP32

By

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ANALYTICAL CHEMISTRY PRACTICAL

Unit I

1. Potentiometric titration of HCl Vs NaOH
2. Determination of pKa of weak acid by EMF method.
3. Potentiometric titration of FAS Vs $K_2Cr_2O_7$
4. Potentiometric titration of KI Vs $KMnO_4$.
5. Potentiometric titration of a mixture of Chloride and Iodide Vs $AgNO_3$.
6. Determination of the pH of buffer solution by EMF method using
1. Quinhydrone and Calomel electrode.
7. Study of the inversion of cane sugar in the presence of acid by Polarimetric method.

Unit II

1. Estimation of Fe and Ni by colorimetric method.
2. Determination of spectrophotometrically the mole ratio of the ferrithiocyanate complex and
   equilibrium constant for the complex formation.
3. Determination of the amount (mol/L) of ferricyanide present in the given solution using
   cyclic voltammetry.
4. Determination of the standard redox potential of ferri-ferrocyanide redox couple using cyclic
   voltammetry.
5. Estimation of the amount of nitrate present in the given solution using spectrophotometric
   method.
6. Analysis of water quality through COD, DO, BOD measurements.
7. Assay of Riboflavin and Iron in tablet formulations by spectrophotometry
8. Estimation of chromium in steel sample by spectrophotometry

9. Separation of (a) mixture of Azo dyes by TLC (b) mixture of metal ions by Paper chromatography.
10. Estimation of chlorophyll in leaves and phosphate in waste water by colorimetry.

Unit III

Interpretation and identification of the given spectra of various organic compounds arrived at

from the following spectral techniques.

1. UV-Visible
2. IR
3. NMR
4. ESR

ANALYTICAL CHEMISTRY PRACTICAL

Unit I

1. Potentiometric titration of HCl Vs NaOH

Aim:

To determine the strength of hydrochloric acid potentiometrically by titration with sodium hydroxide.

Principle:

The end point of the neutralization titration may be found by direct measurement of the emf of the solution after each addition of alkali to acid. The change of emf for the addition of a given amount of titrant is maximum at the equivalence point and so the later can be identified.

The potential of any hydrogen electrode is given by

$E = E° - 0.059 \log a_{H^+}$. The change in electrode potential or the emf of a cell made up of the hydrogen and a standard electrode is thus proportional to the change in pH during titration. At the end point $\frac{\Delta E}{\Delta V}$ is maximum, where ΔE is the change in potential (or) emf resulting from the addition of a definite volume (eg. 0.1ml) of titrant. The accuracy with which this point can be deducted depends on the magnitude of the inflexion in the pH neutralization curve.

Procedure:

An approximately 0.05N HCl is prepared, 10ml of which is pipetted out in to a beaker. A pinch of quinhydrone is added. A platinum electrode is dipped into the

solution and it is connected to reference electrode (Saturated calomel electrode). A standard solution of (0.05N) sodium hydroxide is taken in aburette and added in 1 cc portions. Emf is measured and the range of equivalence point is noted. The exact end point is found out by noting the emf value for each addition of 1 cc portion of the titrant and nearing the equivalence point 0.5cc, 0.2cc and 0.1cc portions. The following graphs are drawn.

1. The value of emt Vs Volume of NaOH
2. The value of $\frac{\Delta E}{\Delta v}$ Vs mean volume of NaOH

Result:

The strength of hydrochloric acid is _____N

2. Determination of pKa of weak acid by EMF method.

Aim

To determine the pKa of a weak acid using the Electro Motive Force (EMF) method.

Principle

The EMF method is based on the measurement of the electrochemical potential difference (EMF) in a weak acid solution when titrated with a strong base. The relationship between the pH of the solution and the EMF can be used to determine the pKa of the weak acid. The EMF at different points of titration is plotted against the pH to determine the pKa.

For a weak acid, HA, dissociation occurs as:

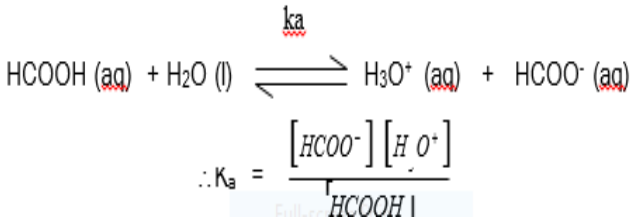
$$HA \rightleftharpoons H^+ + A^-$$

At the equivalence point, the concentrations of H^+ and A^- are equal. The pKa of the weak acid is determined from the point where the buffer capacity is at its maximum.

THEORY:

The strength of an acid is experimentally measured by determining its equilibrium constant or disassociation constant (Ka). Strong acids are completely ionized in aqueous solution and hence it is impossible to determine the disassociation constant of strong el ectrolytes. But in case of weak electrolytes are partially ionized in aqueous solution and hence the equilibrium constant of weak electrolytes can be experimentally determined.

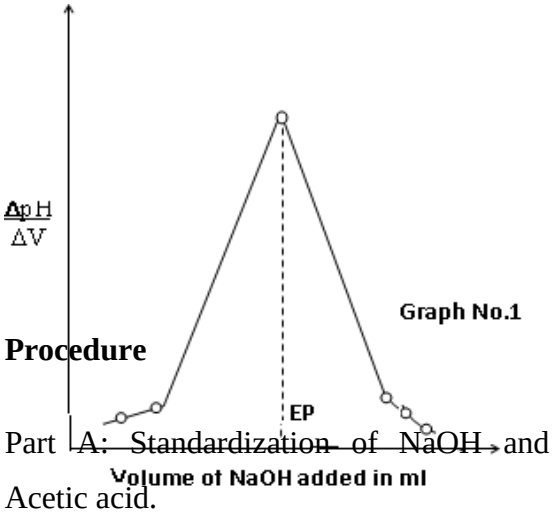
Let us consider weak acid like formic acid, which ionizes, freely in aqueous solution as,



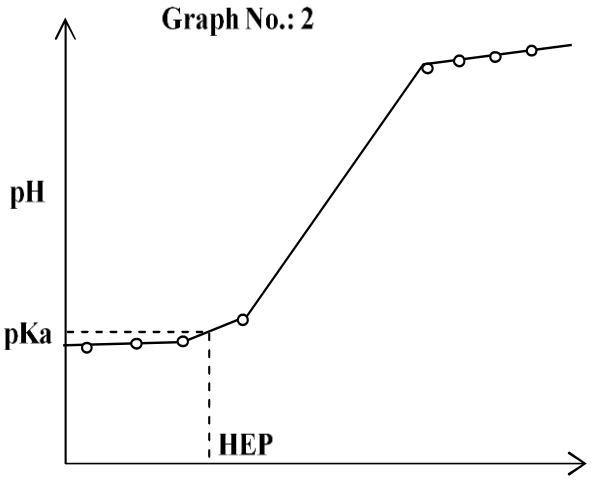
pKa is a modern method of expressing the strength of weak acid , ie., pKa = $-\log_{10}$ Ka

pKa value is determined by measuring the changes in pH of acid solutions at different amount of the base added. In the titration of an acid with a base, the pH of the solution rises gradually at first and then more rapidly. At the equivalence point there is a very sharp increase in pH for a very small quantity of base added. Beyond the equivalence point, the pH increases slightly on addition of base. The titration curve is obtained by plotting change in pH against different volume of the base added and from the graph, determined the equivalence point of an acid. The pKa value of an acid can be calculated using Henderson equation ,

$$pH = pKa + \log_{10} \frac{[Salt]}{[Acid]}$$



Graph No.1



Procedure

Part A: Standardization of NaOH and Acetic acid.

1. Prepare oxalic acid solution (0.1 M) by dissolving appropriate amount of oxalic acid to 100 cm^3 of distilled water in a volumetric flask of 100 cm^3 . Using this solution as primary standard, standardize NaOH solution (0.1N) using phenolphthalein indicator. Report your observations in the observation Table 1. 2.
2. Similarly, using above NaOH solution as secondary standard, standardize given acetic acid solution. Report your observations in the observation Table 2.

Part B: Determination of the Ka of Acetic Acid.

1. Perform a titration by taking 10 cm^3 acetic acid against NaOH using phenolphthalein indicator to know the position of end point.
2. Again take 25 cm^3 acetic acid in a 100 cm^3 beaker add 20-25 cm^3 of distilled water and dip the pH electrode, find the initial pH using pH meter (you pH meter should be pre-calibrated with standard buffers). Add standardized NaOH from the burette in small instalments of 1-2 cm^3 and stir well. Record the pH after each addition. Near

the end point, NaOH should be added in very small instalments of 0.1-0.5 cm^3 . At the end point, you will observe sudden change in the pH and continue the titration until pH readings remain relatively constant at a pH of 10- 12. Record your observation in the observation Table 3. Your pH reading should provide a smooth titration curve. If not, try again.

3. Plot a graph of pH (y-axis) vs. volume NaOH added (x-axis). Determine the equivalence point from the graph. Find out the NaOH volume used in complete neutralization of acetic acid. From plotted curve find out the cm3 of NaOH and pH at ½ at the neutralization of acetic acid. Calculate the value of Ka using Eq. pKa = Ph.

Observations

Observation Table 1: Standardization of NaOH and Acetic Acid.

S.No.	Volume of oxalic acid (cm^3)	Volume of NaOH(cm^3)
1	10	
2	10	
3	10	

Volume of NaOH added at end point……

Molarity of NaOH ……

Observation Table 2: Standardization of Acetic acid

S.No.	Volume of Acetic acid (cm^3)	Volume of NaOH(cm^3)
1	10	
2	10	
3	10	

Volume of NaOH added at the end point……..

Molarity of Standardized NaOH…….

Calculated the molarity of Acetic acid ……

Observation Table 3: Determination of the Ka of CH_3COOH

S.No.	Volume of NaOH (cm^3)	pH

Result

The pKa of the weak acid = _____

3. Potentiometric estimation of FAS using standard $K_2Cr_2O_7$ solution.

Aim:

Estimation of potentiometric titration-estimation of FAS using standard $K_2Cr_2O_7$ solution

Principle:

The procedure of using a measurement of emf to determine the concentration of ionic species in solution is called as potentiometry. The principle involved in potentiometric titration is the measurement of emf between two electrodes, an indicator electrode, (the potential of which is function of the concentration of the ion to be determined) and a reference electrode of constant potential. In this titration, the measurement of emf is made while the titration is in progress. The equivalence point of the reaction is revealed by a sudden change in potential in the plot of emf readings against the volume of titrant.

The determining factor in oxidation and reduction reactions is the ratio of the concentration of oxidized and reduced forms of certain species

Ecell = Eo + 0.0591/n log $[Fe^{3+}]$ / $[Fe^{2+}]$

Where Eo = standard potential of the system.

The potential of the immersed electrode is controlled by the ratio of these concentrations. During the oxidation of a reducing agent or the reduction of an oxidizing agent, the ratio changes, hence the emf changes more rapidly in the vicinity of the end point.

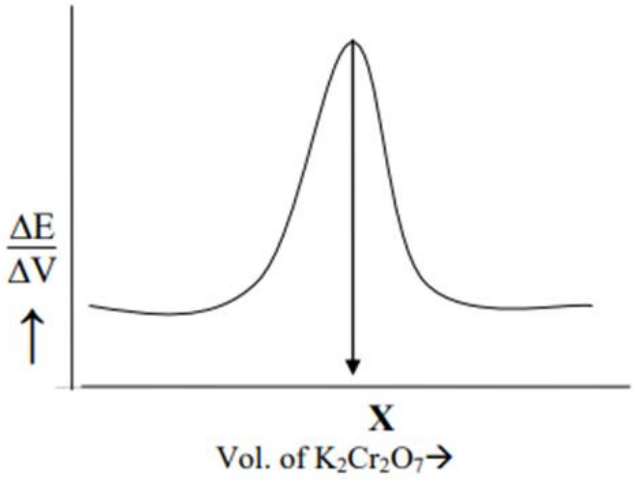
Procedure:

Pipette out 25ml of the given FAS solution into a beaker. Add one test tube of dilute sulphuric acid. Immerse the platinum and calomel electrode assembly into it. Connect the electrodes to potentiometer and measure the potential. Add $K_2Cr_2O_7$ from the burette in increments of 0.5ml and measure the potential after each addition. Plot a graph of $\Delta E / \Delta V$ against volume of $K_2Cr_2O_7$ and determine the equivalence point. From the normality of $K_2Cr_2O_7$, calculate the normality and weight of FAS present in the given solution.

Observation and Calculation:

Volume of $K_2Cr_2O_7$ (V)	**Emf (E) mv**	**ΔV**	**ΔE**	**$\Delta E / \Delta V$**
0.0			-	-
0.5		0.5		
1.0		0.5		
1.5		0.5		
2.0		0.5		
2.5		0.5		
3.0		0.5		
3.5		0.5		
4.0		0.5		

4.5		0.5		
5.0		0.5		
5.5		0.5		
6.0		0.5		
6.5		0.5		
7.0		0.5		
7.5		0.5		
8.0		0.5		
8.5		0.5		
9.0		0.5		
9.5		0.5		
10.0		0.5		



Where, X = Equivalence point

$$N_{FAS} = \frac{N_{K_2Cr_2O_7} \times V_{K_2Cr_2O_7}}{\text{Volume of FAS}}$$

$$= \frac{0.5 \times}{25}$$

$$= \text{----------}$$

Weight of FAS present in a dm^3 of the given solution = N_{FAS} x Eq.weight of FAS (392.14)

= --------- X 392.14 g

= ---------- g

Weight of FAS present in a 25 Cm^3 of the given solution = $\frac{\text{----------} 25}{1000}$

= ---------- g

Result:

Weight of FAS present in 25 cm^3 of the given solution = g

4. Potentiometric estimation of FAS using standard $K_2Cr_2O_7$ solution.

Aim:

Estimation of potentiometric titration-estimation of FAS using standard $K_2Cr_2O_7$ solution

Principle:

The procedure of using a measurement of emf to determine the concentration of ionic species in solution is called as potentiometry. The principle involved in potentiometric

titration is the measurement of emf between two electrodes, an indicator electrode, (the potential of which is function of the concentration of the ion to be determined) and a reference electrode of constant potential. In this titration, the measurement of emf is made while the titration is in progress. The equivalence point of the reaction is revealed by a sudden change in potential in the plot of emf readings against the volume of titrant.

The determining factor in oxidation and reduction reactions is the ratio of the concentration of oxidized and reduced forms of certain species

$E_{cell} = E_o + 0.0591/n \log [Fe^{3+}] / [Fe^{2+}]$

Where Eo = standard potential of the system.

The potential of the immersed electrode is controlled by the ratio of these concentrations. During the oxidation of a reducing agent or the reduction of an oxidizing agent, the ratio changes, hence the emf changes more rapidly in the vicinity of the end point.

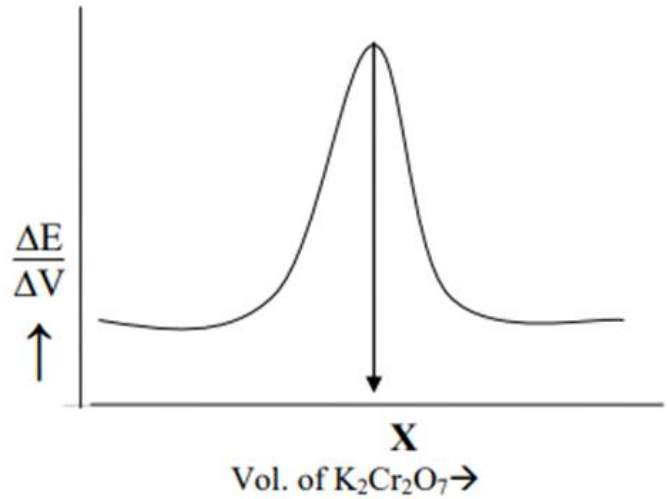
Procedure:

Pipette out 25ml of the given FAS solution into a beaker. Add one test tube of dilute sulphuric acid. Immerse the platinum and calomel electrode assembly into it. Connect the electrodes to potentiometer and measure the potential. Add $K_2Cr_2O_7$ from the burette in increments of 0.5ml and measure the potential after each addition. Plot a graph of $\Delta E / \Delta V$ against volume of $K_2Cr_2O_7$ and determine the equivalence point. From the normality of $K_2Cr_2O_7$, calculate the normality and weight of FAS present in the given solution.

Observation and Calculation:

Volume of $K_2Cr_2O_7$ (V)	**Emf (E) mv**	**ΔV**	**ΔE**	**$\Delta E / \Delta V$**

0.0			-	-
0.5		0.5		
1.0		0.5		
1.5		0.5		
2.0		0.5		
2.5		0.5		
3.0		0.5		
3.5		0.5		
4.0		0.5		
4.5		0.5		
5.0		0.5		
5.5		0.5		
6.0		0.5		
6.5		0.5		
7.0		0.5		
7.5		0.5		
8.0		0.5		
8.5		0.5		
9.0		0.5		
9.5		0.5		
10.0		0.5		



Where, X = Equivalence point

$$N_{FAS} = \frac{N_{K_2Cr_2O_7} \times V_{K_2Cr_2O_7}}{\text{Volume of FAS}}$$

$$= \frac{0.5 \times}{25}$$

$$= \text{----------}$$

Weight of FAS present in a dm^3 of the given solution = N_{FAS} x Eq.weight of FAS (392.14)

= --------- X 392.14 g

= ---------- g

Weight of FAS present in a 25 Cm^3 of the given solution = ---------- $\frac{25}{1000}$

= ---------- g

Result:

Weight of FAS present in 25 cm^3 of the given solution = g

5. Potentiometric titration of a mixture of Chloride and Iodide Vs $AgNO_3$.

Aim:

To determine the strength of the halides in a mixture using standard silver nitrate solution.

Apparatus required:

Silver electrode, Standard calomel electrode, Potentiometer etc.

Principle:

A mixture of iodide and chloride may be titrated with $AgNO_3$ solution potentiometrically using a silver electrode. The first point of the inflection is the equivalence point of iodide ions and the second is that for chlorine ion reaction. The indicator electrode must be reversible to the halides and silver electrode serves this purpose. The following cell is constructed,

$$\underset{(E_1)}{Pt, Hg/Hg_2Cl_{2(S)}, KCl} // \underset{(E)}{Ag^+/Ag}$$

$$E_{obs} = E - E_1$$

$$= E^0 + \frac{RT}{F}\log[Ag^+] - E_1$$

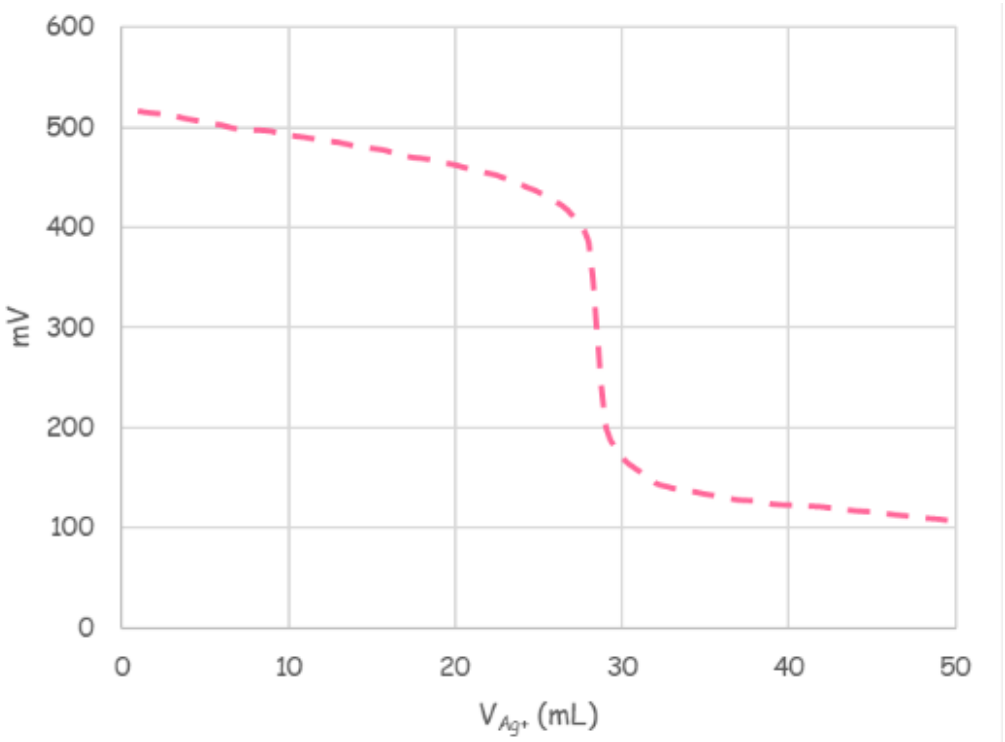
Therefore when $AgNO_3$ is added to the system, e.g. KCl, KI etc., AgCl and AgI is formed which in turn dissociates to produce Ag^+ ions and this becomes reversible to the silver electrode.

Procedure:

The cell is set up and connections are made carefully, calomel electrode is used as the other half cell along with ammonium nitrate salt bridge. At the beginning of the titration

calomel electrode is positive, while the silver electrode dipping in the mixture of halides is negative. When all the iodide ions have been precipitated as AgI, the first equivalent point is noted and the electrode terminals are reversed indicated by change in sign of electrode potential such that the silver electrode becomes positive when the titration is being continued. 20 ml of the mixture of the halides is taken in a beaker and the clean silver electrode is immersed in the solution. The e.m.f reading are taken as the volume of silver nitrate added. The addition causes the e.m.f to change and consequently the e.m.f changes are measured. The addition of silver nitrate is carefully done such that the equivalence points are clearly found out.

The volume of silver nitrate is plotted in a graph against the e.m.f and the first derivative graph of E_{obs} vs volume of silver nitrate is made. Two maxima are observed, one for the iodide and the other for the chloride ion. Knowing the strength of silver nitrate, the strength of halides can be calculated.



Results:

Strength of iodide ion in the mixture =_____N

Strength of chloride ion in the mixture = _____N

7. Determination of the pH of buffer solution by EMF method using Quinhydrone and Calomel electrode.

Aim:

To determine the pH of a buffer solution using the EMF method with a quinhydrone electrode and a calomel electrode.

Theory:

The quinhydrone electrode is a type of redox electrode which can be used to measure the hydro-gen ion concentration (pH) of a solution in a chemical experiment. It provides an alternative to the commonly used glass electrode in a pH-meter.

Procedure:

1. Take off electrodes from the storage solution. Rinse them with distilled water, don´t dry.

2. Fill the beaker with measured solution. Approximate values pH of solutions are written on the flask. Measure temperature of solution (t) and write it down to the Table.

3. Take a little bit quinhydrone (black powder) using a wood spatula, and add to the measured solution. Mix the solution carefully using a glass stick to obtain solution saturated with quinhydrone.

4. Put the electrodes to the solution in the beaker.

5. The quinhydrone electrode is the positive terminal, and the calomel electrode is negative terminal.

6. Read the electromotoric force (E in mV) from the display of potentiometer when its value is stable.

7. Write the E value down to the Table.

8. Calculate the standard potential of quinhydrone electrode at laboratory temperature.

9. Calculate pH of the solution.

10. Repeat the procedure for each solution where the use of the quinhydrone electrode is possible.

11. Clean booth electrodes with distilled water.

Measured and calculated results for system quinhydrone-calomel electrodes.

Measured solution	***E* [V]**	***pH***

Results:

The calculated pH of the buffer solution is

UNIT-II

1. Estimation of Fe and Ni by colorimetric method.

Aim:

To estimate the amount of fe in a given solution using the colorimetric method

Principle:

When a monochromatic light of intensity I_o is incident on a transparent medium, a part I_a of it is absorbed, a part I_r is reflected & the remaining part I_t is transmitted.

$I_o = I_a + I_r + I_t$

For a glass-air interface I_r is negligible.

Therefore $I_o = I_a + I_t$

$I_t / I_o = T$ called the transmittance $\log 1/T = \log I_o / I_t$ is called the absorbance or optical density. The relation between absorbance A, concentration C & path length I is given by Beer-Lambert's law.

$A = \log I_o / I_t = €Ct$

Where '€' is the molar extinction coefficient, 't' is the path length. 'C' is a constant for a given substance at a given wave length. If 't' is the path length is kept constant, then A α C. Hence a plot a graph of absorbance against concentration gives a straight line.

The colorimetric method is an analytical technique based on the principle that the intensity of color in a solution is proportional to the concentration of the substance causing the color. In this method, iron ions react with a suitable reagent, such as potassium thiocyanate (KSCN), to form a colored complex. The intensity of the color, measured using a spectrophotometer, is directly proportional to the concentration of iron in the solution, following Beer's Law.

Procedure:

- Prepare a stock solution of ferric ammonium sulfate by dissolving an appropriate amount in distilled water.
- Prepare standard solutions of known iron concentrations (e.g., 1 ppm, 2 ppm, 3 ppm) from the stock solution.

- Pipette a known volume (e.g., 10 mL) of each standard solution into separate test tubes.
- Add a fixed volume of potassium thiocyanate solution to each test tube.
- Allow the solutions to stand for 5-10 minutes to ensure complete color development. A reddish-colored ferric thiocyanate complex is formed.
- Take a sample solution (e.g., from a water source or an unknown sample) and treat it similarly by adding potassium thiocyanate to develop the color.
- Calibrate the spectrophotometer at 490 nm (the wavelength of maximum absorbance for ferric thiocyanate).
- Measure the absorbance of the blank solution and standard solutions.
- Record the absorbance values for each sample solution.
- Plot the absorbance of the standard solutions against their concentrations to construct the calibration curve.
- Measure the absorbance of the sample solution.
- Use the calibration curve to determine the concentration of iron in the sample solution.

Volume of ferric ammonium sulfate (ml)	Concentration of ferric	Absorbance (Optical density)
Blank Solution		
2.0		
4.0		
6.0		
8.0		

10.0		
Test Solution		

From graph, volume of the given test solution, a = _____ml

Ferric present in the given test solution =

= ----------mg

Result: The given test solution contains ----------mg of Fe

Aim:

To estimate the amount of Ni in a given solution using the colorimetric method

Principle:

When a monochromatic light of intensity I_o is incident on a transparent medium, a part I_a of it is absorbed, a part I_r is reflected & the remaining part I_t is transmitted.

$I_o = I_a + I_r + I_t$

For a glass-air interface I_r is negligible.

Therefore $I_o = I_a + I_t$

$I_t / I_o = T$ called the transmittance $\log 1/T = \log I_o / I_t$ is called the absorbance or optical density. The relation between absorbance A, concentration C & path length I is given by Beer-Lambert's law.

$A = \log I_o / I_t = €Ct$

Where '€' is the molar extinction coefficient,'t' is the path length. 'C' is a constant for a given substance at a given wave length. If 't' is the path length is kept constant, then A α C. Hence a plot a graph of absorbance against concentration gives a straight line.

The colorimetric method is based on the principle that the intensity of the color produced in a chemical reaction is directly proportional to the concentration of the analyte, in this case, nickel. Nickel reacts with dimethylglyoxime (DMG) under alkaline conditions to form a pink-red complex. The absorbance of this colored complex is measured using a spectrophotometer at a specific wavelength, and the concentration of nickel is determined using a calibration curve constructed from standard solutions of nickel.

Procedure:

- Prepare a stock solution of nickel sulfate by dissolving a known amount in distilled water.

- From the stock solution, prepare standard nickel solutions of known concentrations (e.g., 1 ppm, 2 ppm, 3 ppm, etc.).
- Take 10 mL of each standard nickel solution into separate test tubes.
- Add a few drops of ammonium hydroxide (NH_4OH) to each test tube to create an alkaline medium.
- Add 1 mL of dimethylglyoxime (DMG) reagent to each test tube. A pink-red complex forms.
- Mix well and allow the solution to stand for 5-10 minutes for complete color development.
- Treat the unknown nickel solution similarly by adding NH_4OH and DMG to develop the color.
- Calibrate the spectrophotometer at 450 nm (the wavelength of maximum absorbance for the nickel-DMG complex).
- Measure the absorbance of the blank solution, standard solutions, and the sample solution.
- Record the absorbance values.
- Plot a graph of absorbance versus concentration using the standard nickel solutions.
- Use the calibration curve to determine the concentration of nickel in the sample solution by comparing its absorbance value.

Volume of nickel sulfate (ml)	Concentration of nickel	Absorbance (Optical density)
Blank Solution		
2.0		

4.0		
6.0		
8.0		
10.0		
Test Solution		

From graph, volume of the given test solution, a = _____ml

Nickel present in the given test solution =

= ----------mg

Result: The given test solution contains ----------mg of nickel.

2. Determination of spectrophotometrically the mole ratio of the ferrithiocyanate complex and equilibrium constant for the complex formation.

Aim:

To determine the mole ratio of the ferrithiocyanate $[Fe(SCN)]^{2+}$complex and calculate the equilibrium constant for the complex formation using spectrophotometry.

Principle:

Ferric ions (Fe^{3+}) react with thiocyanate ions (SCN^-) in aqueous solution to form a red-colored ferrithiocyanate complex $[Fe(SCN)]^{2+}$. The intensity of the color is proportional to the concentration of the complex and can be measured using a spectrophotometer. By analyzing the absorbance of solutions containing varying molar ratios of Fe^{3+} and SCN^- the stoichiometry of the complex can be determined. Additionally, the equilibrium constant for the complex formation can be calculated.

Procedure:

- Prepare a stock solution of ferric nitrate (Fe^{3+}) in 1 M nitric acid.
- Prepare a stock solution of potassium thiocyanate (SCN^-) in distilled water.
- Prepare a series of reaction mixtures with varying molar ratios of (Fe^{3+}) and (SCN^-), keeping one of the reactants in excess. For example:
  - Constant (Fe^{3+}) with varying (SCN^-): Mix 10 mL of ferric nitrate solution with increasing volumes of KSCN (e.g., 0.5 mL, 1 mL, 1.5 mL, etc.).
  - Constant (SCN^-) with varying (Fe^{3+}): Mix 10 mL of potassium thiocyanate solution with increasing volumes of (Fe^{3+})
- Maintain the total volume of each solution by adding 1 M HNO_3 as the diluent.
- Measure the absorbance of each reaction mixture at 490 nm (wavelength of maximum absorbance for the $[Fe(SCN)]^{2+}$complex) using a spectrophotometer.
- Use a blank solution (1 M HNO_3) to calibrate the spectrophotometer.
- Plot the absorbance values against the mole ratio of (Fe^{3+}) to (SCN^-).
- The mole ratio corresponding to the maximum absorbance indicates the stoichiometry of the complex.

- Using the stoichiometric ratio, prepare a solution with equimolar concentrations of (Fe^{3+}) and (SCN^{-}).
- Measure the equilibrium concentration of the complex from the absorbance using Beer's Law: $A= \epsilon \cdot c \cdot l$ where A is absorbance, ϵ is the molar extinction coefficient, c is concentration, and l is the path length (usually 1 cm).
- Calculate the equilibrium concentrations of (Fe^{3+}), (SCN^{-}), and $[Fe(SCN)]^{2+}$.
- Use the equilibrium concentrations to calculate the equilibrium constant for the reaction: $Keq= [Fe(SCN)]^{2+} / [(Fe^{3+}) +][(SCN^{-})]$.

Mole Ratio	**Absorbance**

Result:

• The mole ratio of Fe^{3+} to SCN^{-} in the ferrithiocyanate complex is determined to be _____.

• The equilibrium constant for the formation of the ferrithiocyanate complex is calculated to be _____.

3. Determination of the Amount (mol/L) of Ferricyanide in a Given Solution Using Cyclic Voltammetry

Aim

To determine the concentration (mol/L) of ferricyanide ($[Fe(CN)_6]^{3-}$) in a given solution using cyclic voltammetry (CV).

Principle:

Cyclic voltammetry is an electrochemical technique that involves sweeping the potential of a working electrode linearly with time while measuring the resulting current. Ferricyanide undergoes a reversible redox reaction:

$$Fe(CN)_6]^{3-}+e^{-}\leftrightarrow[Fe(CN)_6]^{4}$$

By analyzing the peak current (I_p) from the voltammogram, the concentration of ferricyanide can be determined using the Randles–Ševčík equation:

$$I_p=(2.69\times10^5)n^{3/2}\,AD^{½}\,C\nu^{1/2}$$

Where,

- ✓ I_p = Peak current (A)
- ✓ n = Number of electrons transferred (1 for ferricyanide)
- ✓ A = Electrode area (cm^2)
- ✓ D = Diffusion coefficient of ferricyanide (cm^2/s)
- ✓ C = Concentration of ferricyanide (mol/L)
- ✓ ν = Scan rate (V/s)

Procedure:

Prepare a 0.1 M potassium chloride (KCl) solution as the supporting electrolyte. Prepare standard solutions of ferricyanide ($[Fe(CN)_6]^{3-}$) with known concentrations (e.g., 1 mM,

2 mM, 3 mM, etc.) in the supporting electrolyte. Use the given ferricyanide solution for the analysis without dilution (or dilute if necessary).

Clean the glassy carbon working electrode by polishing it with alumina slurry on a polishing cloth. Rinse the electrode thoroughly with distilled water and sonicate it in distilled water to remove residual polishing particles.

Assemble the three-electrode system in the electrochemical cell: place the glassy carbon electrode as the working electrode, immerse the platinum wire as the counter electrode, and place the Ag/AgCl electrode as the reference electrode. Fill the electrochemical cell with the test solution.

Set up the electrochemical workstation and input the following parameters: potential range from 0.4 V to -0.4 V (or adjust based on the redox behavior of ferricyanide), a scan rate of 50 mV/s, and 2-3 cycles to ensure stable voltammograms. Record the voltammograms for the standard solutions and the test solution.

Identify the anodic peak current (I_{pa}) from the voltammogram for each standard solution. Plot a calibration curve of I_pa vs. concentration (C) of the standard ferricyanide solutions. Use the calibration curve to determine the concentration of ferricyanide in the given solution based on its anodic peak current.

Ferricyanide Concentration (mM)	**Anodic Peak Current (I_{pa} (µA))**

Result:

The concentration of ferricyanide in the given solution is determined to be _____ **mol/L**.

4. Determination of the standard redox potential of ferriferrocyanide redox couple using cyclic voltammetry.

Aim

To determine the standard redox potential (E^0) of the ferri/ferrocyanide ($[Fe(CN)_6]^{3-}$) / $[Fe(CN)_6]^{4-}$) redox couple using cyclic voltammetry.

Principle:

Cyclic voltammetry (CV) is a widely used electrochemical method for studying redox reactions. In this experiment, the ferri/ferrocyanide redox couple undergoes a reversible redox reaction:

$$([Fe(CN)_6]^{3-}) + e^- \leftrightharpoons [Fe(CN)_6]^{4-}$$

The peak anodic potential (E_{pa}) and cathodic potential (E_{pc}) from the cyclic voltammogram are used to calculate the formal redox potential (E^0) using the equation:

$$E^0 = E_{pa} + E_{pc} / 2$$

The E^0 value represents the standard redox potential of the ferri/ferrocyanide redox couple.

Procedure:

- Prepare a mixture of 0.001 M ferricyanide $K_3[Fe(CN)_6]$ and 0.001 M ferrocyanide ($K_4[Fe(CN)_6]$ in a 0.1 M potassium chloride (KCl) supporting electrolyte.
- Polish the glassy carbon electrode (GCE) with alumina slurry on a polishing cloth to achieve a mirror-like surface.
- Rinse the electrode with distilled water and sonicate to remove polishing residues.
- Assemble the three-electrode system in an electrochemical cell:

        - Use the glassy carbon electrode as the working electrode.
        - Use a platinum wire as the counter electrode.
        - Place an Ag/AgCl electrode as the reference electrode.
    - Fill the cell with the prepared ferri/ferrocyanide solution.
    - Set the initial parameters on the electrochemical workstation: potential range (-0.2 V to +0.6 V), scan rate (50 mV/s), and step potential (10 mV).
    - Record the cyclic voltammogram by sweeping the potential in both forward and reverse directions.
    - Repeat the experiment to ensure consistent and reproducible data.
    - Identify the anodic peak potential (E_{pa}) and cathodic peak potential (E_{pc}) from the voltammogram.

Result:

The standard redox potential (E^0) of the ferri/ferrocyanide redox couple is determined to be **_____ V**.

5. Estimation of the amount of nitrate present in the given solution using spectrophotometric method.

Aim

To determine the concentration of nitrate (NO_3^-) ions in the given solution by the spectrophotometric method.

Principle

Nitrate ions react with specific reagents to form a colored complex. In acidic conditions, nitrate reacts with sulfanilic acid and N-(1-naphthyl) ethylenediamine dihydrochloride (NED reagent) to form a pink azo dye. The intensity of the color produced is proportional to the concentration of nitrate in the solution.

Using a UV-visible spectrophotometer, the absorbance of the solution is measured at 540 nm (wavelength of maximum absorption for the azo dye). A calibration curve is prepared using standard nitrate solutions, and the concentration of nitrate in the unknown solution is determined by comparing its absorbance with the curve.

Procedure

- Sulfanilic Acid Reagent: Dissolve 5 g of sulfanilic acid in 1 L of 1% hydrochloric acid.
- NED Reagent: Dissolve 0.5 g of N-(1-naphthyl) ethylenediamine dihydrochloride in 500 mL of distilled water.
- Prepare a series of standard nitrate solutions with known concentrations (e.g., 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm) using potassium nitrate (KNO_3) dissolved in distilled water.
- Take an aliquot of the given nitrate solution and dilute it to an appropriate concentration for analysis.
- Pipette 10 mL of each standard nitrate solution and the unknown solution into separate beakers.
- Add 2 mL of sulfanilic acid reagent and 2 mL of NED reagent to each solution.

- Mix thoroughly and allow the solutions to stand for 15 minutes to develop the pink azo dye.
- Calibrate the spectrophotometer using a blank solution (distilled water with the reagents but no nitrate).
- Measure the absorbance of each standard solution and the unknown solution at 540 nm.
- Record the absorbance values.
- Plot a graph of absorbance (y-axis) versus nitrate concentration (x-axis) using the standard solutions.
- Determine the nitrate concentration in the unknown solution by comparing its absorbance with the calibration curve.

Concentration of Standard Nitrate Solution (ppm)	**Absorbance**

Result

The concentration of nitrate in the given solution is determined to be **_____ ppm**.

6. Analysis of water quality through COD, DO, BOD measurements.

Aim

To analyze the quality of water by determining its Chemical Oxygen Demand (COD), Dissolved Oxygen (DO), and Biochemical Oxygen Demand (BOD) levels.

Principle

The quality of water is assessed based on its oxygen demand, which reflects the presence of organic and inorganic pollutants. The parameters COD, DO, and BOD are widely used in water quality testing:

1. **Chemical Oxygen Demand (COD):** COD measures the total oxygen required to oxidize both organic and inorganic matter in water chemically using a strong oxidizing agent. It is expressed in mg/L.
2. **Dissolved Oxygen (DO):** DO measures the amount of oxygen dissolved in water, which is critical for aquatic life. The Winkler method is often used for DO determination, where oxygen reacts with manganese sulfate in an alkaline medium, forming a precipitate that is titrated.
3. **Biochemical Oxygen Demand (BOD):** BOD measures the amount of oxygen consumed by microorganisms during the degradation of organic matter in water over a specific time period, typically 5 days at 20°C.

Procedure

1. Determination of COD

1. Take 10 mL of the water sample in a reflux flask.
2. Add 5 mL of potassium dichromate solution ($K_2Cr_2O_7$) and 15 mL of concentrated sulfuric acid (H_2SO_4).
3. Reflux the mixture in a COD digestion unit for 2 hours.
4. Cool the mixture and titrate it with ferrous ammonium sulfate (FAS) using ferroin indicator.
5. Perform a blank experiment with distilled water instead of the sample.

6. Calculate COD using the formula:
   COD (mg/L)=$(V_b - V_s) \times N \times 8000$/Volume of sample (mL) where V_b = Volume of FAS used for blank, V_s = Volume of FAS used for the sample, and N = Normality of FAS.

2. Determination of DO

1. Collect a water sample in a BOD bottle, ensuring no air bubbles are present.
2. Add 2 mL of manganese sulfate ($MnSO_4$) solution and 2 mL of alkaline potassium iodide solution.
3. Stopper the bottle, mix well, and allow the precipitate to settle.
4. Add 2 mL of concentrated sulfuric acid, mix well, and titrate the liberated iodine with sodium thiosulfate solution using starch as an indicator.
5. Calculate DO using the formula: DO (mg/L)=$Vt \times N \times 8$/Volume of sample (mL), where V_t = Volume of sodium thiosulfate used, N = Normality of sodium thiosulfate.

3. Determination of BOD

1. Measure the initial DO (DO_i) of the water sample using the DO determination procedure.
2. Incubate the water sample in a BOD bottle at 20°C for 5 days.
3. After 5 days, determine the final DO (DO_f) using the DO procedure.
4. Calculate BOD using the formula: BOD_5 (mg/L)=$DO_i - DO_f$

Parameter	**Sample Reading (mL)**	**Blank Reading (mL)**	**Concentration (mg/L)**
COD			
DO (Initial)			
DO (Final)			
BOD			

Result

- COD of the given water sample: **_____ mg/L**
- DO of the given water sample: **_____ mg/L**
- BOD of the given water sample: **_____ mg/L**

7. Assay of Riboflavin and Iron in tablet formulations by Spectrophotometry

Aim

To determine the amount of riboflavin and iron present in tablet formulations using spectrophotometric analysis.

Principle

Riboflavin (vitamin B_2) absorbs light in the UV-visible region due to its conjugated aromatic system, and its concentration can be determined using spectrophotometry. For iron, the assay involves forming a colored complex, typically with orthophenanthroline or thiocyanate, which absorbs light at a specific wavelength, allowing its concentration to be measured spectrophotometrically. The absorbance of the solutions is directly proportional to the concentration of riboflavin or iron according to Beer-Lambert's law:

$$A=\varepsilon \cdot c \cdot l$$

where A = Absorbance, ε = Molar absorptivity, c = Concentration, and l = Path length of the cuvette.

Procedure

1. Assay of Riboflavin

- Weigh an accurately known amount of pure riboflavin and prepare a standard solution in distilled water.
- Crush a tablet formulation containing riboflavin and weigh an accurately known amount.
- Dissolve the powdered tablet in distilled water, filter, and dilute to a known volume.
- Measure the absorbance of the standard and sample solutions at the maximum absorbance wavelength (λmax) of riboflavin (approximately 445 nm).
- Record the absorbance values.

- Using the calibration curve obtained from the standard solution, determine the concentration of riboflavin in the sample solution.

2. Assay of Iron

- Prepare a standard solution of ferric iron (Fe^{3+}) in distilled water.
- To 10 mL of the standard and sample solutions, add orthophenanthroline reagent and a buffer solution (sodium acetate).
- Allow the complex to form by incubating the solution for 10 minutes.
- Measure the absorbance of the iron-orthophenanthroline complex at its maximum absorbance wavelength λmax of approximately 510 nm.
- Record the absorbance values.
- Add ammonium thiocyanate to the standard and sample solutions to form a ferric thiocyanate complex.
- Measure the absorbance at λmax of approximately 480 nm.
- Use the calibration curve obtained from the standard solution to determine the concentration of iron in the sample solution.

Component	**Sample Absorbance**	**Standard Absorbance**	**Concentration (mg/L)**
Riboflavin			
Iron			

Result

- The concentration of riboflavin in the tablet formulation: **_____ mg/tablet**
- The concentration of iron in the tablet formulation: **_____ mg/tablet**

8. Estimation of chromium in steel sample by spectrophotometry

Aim

To determine the amount of chromium present in a steel sample using spectrophotometric analysis.

Principle

Chromium in steel is typically present in the form of trivalent chromium (Cr^{3+}) or hexavalent chromium (Cr^{6+}). In this experiment, chromium is oxidized to the hexavalent state (Cr^{6+}) by using an oxidizing agent, typically ammonium persulfate or potassium permanganate. The hexavalent chromium forms a yellow-orange chromate ion (CrO_4^{2-}) in alkaline medium or dichromate ion ($Cr_2O_7^{2-}$) in acidic medium, which absorbs light in the visible region.

The absorbance of the solution is measured at its maximum wavelength (λmax), typically around 375 nm, using a UV-visible spectrophotometer. The concentration of chromium in the steel sample is determined by comparing the absorbance to that of a standard chromium solution, based on Beer-Lambert's law.

Procedure

1. Weigh an accurately known amount of the steel sample (approximately 0.5 g) and transfer it into a clean conical flask.
2. Add 20 mL of concentrated sulfuric acid and a few drops of phosphoric acid to the flask to dissolve the steel sample completely. Heat gently if required.
3. Cool the solution and filter it to remove any undissolved impurities. Transfer the filtrate into a 100 mL volumetric flask and dilute to the mark with distilled water.
4. Take a known aliquot (e.g., 10 mL) of the prepared sample solution into a conical flask.
5. Add ammonium persulfate or potassium permanganate as the oxidizing agent to oxidize Cr^{3+} to Cr^{6+}.
6. Ensure that the reaction is complete by heating the solution gently until no further color change is observed.

7. Prepare standard potassium dichromate ($K_2Cr_2O_7$) solutions of known concentrations to create a calibration curve.
8. Measure the absorbance of each standard solution and the prepared steel sample solution at the maximum wavelength (λmax) of approximately 375 nm using a UV-visible spectrophotometer.
9. Plot a calibration curve of absorbance vs. concentration for the standard solutions.

Calculation

Using the calibration curve, determine the concentration of chromium in the steel sample solution. The amount of chromium in the original steel sample is calculated using the formula:

Concentration of Chromium (mg/g)=Concentration from Calibration Curve (mg/L)×Di lution Factor/Weight of Steel Sample.

Component	**Sample Absorbance**	**Concentration (mg/L)**

Result

The concentration of chromium in the given steel sample is: **_____ mg/g**

9. Separation of (a) mixture of Azo dyes by TLC (b) mixture of metal ions by Paper chromatography

Aim

To separate a mixture of Azo dyes using Thin Layer Chromatography (TLC) and identify the individual components based on their Rf values.

Principle

Thin Layer Chromatography (TLC) is a chromatographic technique used for the separation of components in a mixture. It involves the use of a stationary phase (typically silica gel or alumina coated on a glass plate) and a mobile phase (solvent or solvent mixture). Azo dyes are a class of dyes containing the azo group (-N=N-) and are known for their bright colors. In TLC, the mixture of dyes is applied as a spot at the bottom of the plate, and the mobile phase moves up the plate by capillary action, separating the components based on their affinities for the stationary phase.

Procedure

- Cut the TLC plate to an appropriate size (usually 5 cm × 10 cm).
- Draw a pencil line 1 cm from the bottom of the plate to mark the origin.
- Using a capillary tube, spot a small amount of the Azo dye mixture onto the TLC plate at the marked origin. Allow it to dry and repeat the process if necessary to concentrate the sample.
- Prepare the mobile phase (solvent or solvent mixture) in a developing chamber (beaker or jar). Ensure the solvent is below the pencil line to prevent the sample from dissolving into the mobile phase.
- Place the TLC plate in the developing chamber and cover it. Allow the solvent to rise by capillary action until it reaches a distance of 2-3 cm from the top of the plate.
- Remove the plate from the chamber and allow it to dry.

- Visualize the separated components under UV light or by using an appropriate staining method if the dyes are colorless.
- Measure the distance traveled by the solvent front (Rf).
- Measure the distance traveled by each individual dye spot.
- Calculate the Rf value for each component using the formula: Rf=Distance traveled by dye/ Distance traveled by solvent

Results

The Rf values for each component (Azo dye) can be determined by measuring the distance traveled by each spot and the solvent front. Comparing these Rf values with those of known standards allows identification of the Azo dyes in the mixture.

(b) mixture of metal ions by Paper chromatography

Aim

To separate a mixture of metal ions using Paper Chromatography

Principle

Paper Chromatography is a method for separating dissolved substances based on their relative affinities for the stationary phase (the paper) and the mobile phase (a solvent or solvent mixture). When a sample of metal ions is applied to a strip of filter paper, the solvent moves up the paper by capillary action, and the metal ions separate based on their different affinities for the paper and solvent.

Procedure

- Cut the filter paper to a suitable size (approximately 10 cm long).
- Draw a pencil line 1 cm from the bottom of the paper as the origin.
- Using a capillary tube, apply a small drop of the metal ion mixture on the pencil line. Allow it to dry and repeat the process if necessary.
- Prepare the mobile phase (solvent or solvent mixture) in the developing chamber. The solvent should be below the pencil line to avoid dissolving the sample.
- Place the filter paper in the chamber, ensuring that the pencil line does not touch the solvent. Close the chamber and allow the solvent to rise by capillary action, carrying the metal ions along the paper.
- Once the solvent has moved sufficiently up the paper (about 3/4 of the paper), remove it and allow it to dry.
- Depending on the metal ions, the paper may be visualized using UV light or by spraying with specific reagents that color the metal ions. Common reagents include dimethylglyoxime for nickel and potassium ferrocyanide for iron.
- Compare the distance traveled by each metal ion to the distance traveled by the solvent front. The separation will depend on the metal ion's affinity for the solvent.

Results

The metal ions in the mixture will separate into distinct spots on the paper, each corresponding to a different metal ion based on its Rf value. The position of the spots can be compared with standards for metal ion identification.

10. Estimation of chlorophyll in leaves and phosphate in waste water by colorimetry.

Aim

To estimate the amount of chlorophyll in plant leaves using colorimetric method.

Principle

Chlorophyll is a green pigment found in plant cells, crucial for photosynthesis. The estimation of chlorophyll content is done by extracting the pigment from leaves using an organic solvent (usually acetone or ethanol). The concentration of chlorophyll is determined by measuring the absorbance of the solution at specific wavelengths, using a colorimeter or spectrophotometer. The amount of chlorophyll is calculated using the absorbance and the known extinction coefficient of chlorophyll.

Procedure

- Take 2-3 grams of fresh leaves, and weigh them accurately.
- Grind the leaves using a mortar and pestle with small amounts of acetone or ethanol to extract the chlorophyll.
- Filter the extract using filter paper into a clean test tube to remove solid plant material.
- Take a clean test tube and fill it with the chlorophyll extract.
- Using a colorimeter or spectrophotometer, measure the absorbance of the solution at two wavelengths: 645 nm (for chlorophyll a) and 663 nm (for chlorophyll b).
- Using the following formulae, calculate the concentrations of chlorophyll a and b: Chlorophyll a (mg/L)$=12.7\times A_{663}-2.69\times A_{645}$ Chlorophyll b (mg/L)$=22.9\times A_{645}-4.68\times A_{663}$
- Sum the values of chlorophyll a and b to get the total chlorophyll content.

Results

By calculating the chlorophyll content using the above formulas, the amount of chlorophyll in the leaf sample can be determined. The results are usually expressed in milligrams of chlorophyll per gram of fresh leaf material.

Aim

To estimate the concentration of phosphate ions in a wastewater sample using the colorimetric method.

Principle

Phosphates in wastewater can be determined colorimetrically by reacting with a reagent (such as ammonium molybdate) in an acidic medium to form a colored complex. The intensity of the color formed is proportional to the concentration of phosphate in the sample. The absorbance of the solution is measured at a specific wavelength, and the phosphate concentration is calculated by comparing the absorbance with that of a standard phosphate solution.

Procedure

- Prepare a series of standard phosphate solutions of known concentrations (e.g., 0, 1, 2, 5, and 10 ppm of PO_4^{3-}) using the standard phosphate solution.
- Prepare the colorimetric reagent solution by mixing ammonium molybdate solution and ascorbic acid in a suitable ratio to form a molybdenum blue complex.
- Pipette a known volume (e.g., 10 mL) of the wastewater sample into a clean test tube.
- Add a fixed volume of the colorimetric reagent solution to the test tube.
- Mix the contents thoroughly and allow the reaction to proceed for about 10 minutes, until the color develops.
- Set the colorimeter or spectrophotometer to the appropriate wavelength (usually around 880 nm).
- Measure the absorbance of the developed color for both the sample and the standard solutions.
- Prepare a calibration curve by plotting the absorbance values of the standard solutions against their known phosphate concentrations.

- Use the calibration curve to determine the phosphate concentration in the wastewater sample by comparing its absorbance with the curve.

Results

By comparing the absorbance of the sample with the standard curve, the concentration of phosphate in the wastewater can be calculated in parts per million (ppm).

Unit -3

UV-Visible spectroscopy

Spectroscopy and **spectrography** are the techniques based on the measurement of radiation intensity (absorbed or emitted or scattered by the sample or analyte) as a function of wavelength. Spectral measurement instruments are more commonly referred as spectrometers, spectrophotometers, spectrographs or spectral analyzers. These instruments measure the light intensity or resultant light from sample, (emitted or transmitted or scattered). Absorption is the measure of difference between incident and transmitted light (Figure 1.1).

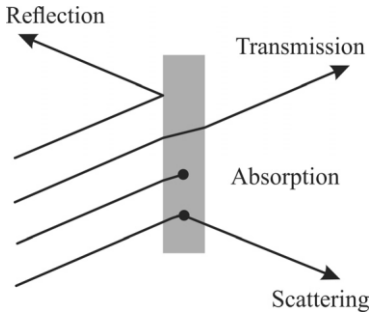


Figure 1.1 Various interaction of effect of light with matter (Analyte).

The output of scan measurement of sample from a range of wavelength is represented as 2D or 3D spectrum. In general X axis (Input) will be wavelength, Y axis (output) will be light absorbed (UV- Visible, IR, NMR or any absorption spectroscopy, Light emitted (fluorescence or any emission spectroscopy) or scattered (intensity of scatted light Raman spectroscopy). There are three types of Spectrum produced by spectroscopy

1. Continuous spectrum
2. Emission lines
3. Absorption lines

Selection Region based on Sample Nature

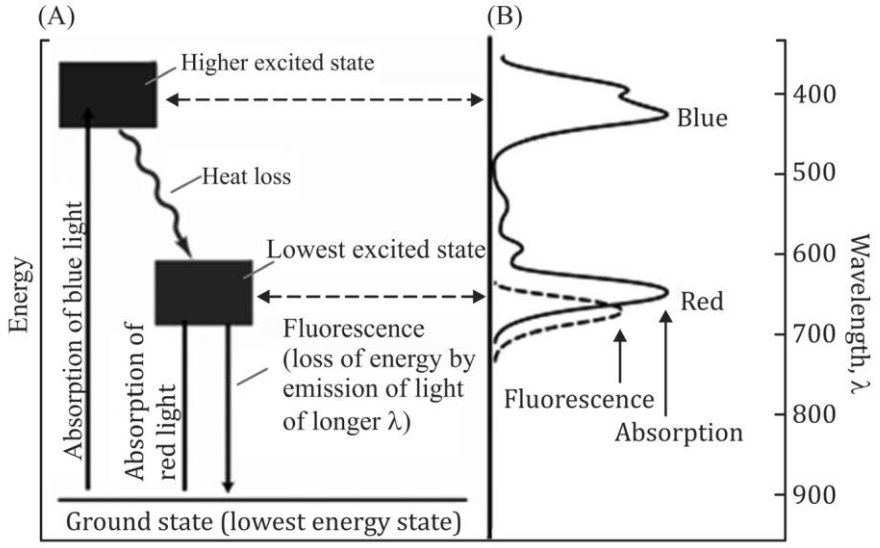
The difference between UV and Visible spectroscopy is the wavelength region used in measurement. UV region (190-380 nm) for colorless sample and visible region 380-790

nm for coloured sample. As result of scanning of sample using the light, the spectrum obtained is characteristic of sample / molecule nature, its highly depends on double bonds (pi electrons) configuration of the structure.

Absorption/Emission of Light by a Chemical Molecule

The mechanism behind the absorption of light by a molecule (analyte) or / and emission of light from the molecule (Analyte) is depends on the phenomena of electronic excitation / transition (Figure 1.2). The amount (probability) or type of the electron (sigma, pi, n) involved in the excitation determine the characteristic photon energy absorbed or emitted, will determine the shape and pattern of UV-Visible Spectrum.

As no two chemical molecules will have same number of electrons or configuration, the UV spectrum will be different for dif **Figure 1.2** Absorption and emission process of matter (Analyte).



• The above figure shows the electronic excitation process and relaxation process of electron of molecule when UV-Visible light is irradiated on sample.

ferent compounds.

- Blue light and red light is indicated to understand the wavelength comparison.
- *NOTE*: sample should be low concentration, pure and highly transparent for any qualitative and quantitative analysis

Types of Electronic Transitions

- There are different types of electronic transition take place in the molecule, and each molecule undergoes more than one electronic transition, and requires different energy (Figure 1.3).
- Sigma transitions are higher takes in very low wavelength of UV region or in Vacuum UV region (< 190 nm).
- The below diagram indicate the comparison of different electronic transition and energy in which n' electron transition require less energy (absorbs higher wavelength)

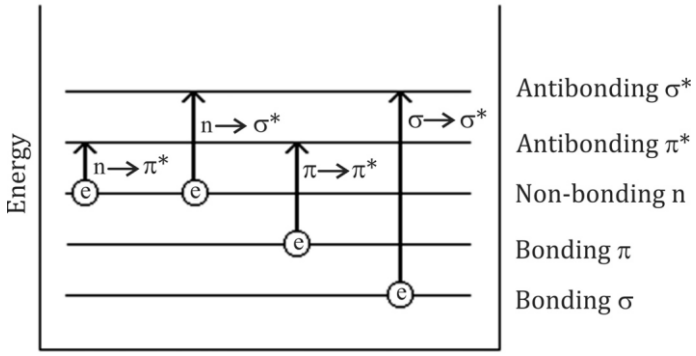


Figure 1.3 Types of electronic transition in a organic molecules upon UV- Visible light exposure.

From above figure, we can observe that the energy required for sigma transition (alkane) is the highest which is not available in UV region, so sigma transition may not be a practically observed. sigma transition may be possible at vacuum UV region (<190 nm).

How to predict the possible electronic transition for the given structure?

To predict the possible transition, we need to know the chemical structure and type of electrons available.

Table 1.1 Transition and Chemical structures

Chemical class	Types electron	Possible transitions (theoretical)
Alkane	Sigma electron only	Sigma to sigma*
Alkene (C=C), Benzene, Alkynes	Sigma and Pi electrons	Sigma to sigma* Pi to Pi*
Aldehyde, ketones, heterocyclic, acids, esters, (all compounds containing C=O, C=S	Sigma, pi electrons n' electrons	All type of transitions
Complexes	n' electrons	Charge transfer mechanism

Schematic Procedure in Measuring Absorbance

- In the instrumentation, always a monochromatic light will be used to measure an absorbance (in quantitative analysis).
- The monochromatic light to be used has to be selected from the UV spectrum (spectrum as a result of scan for entire UV-Visible range).
- Sample cell need to be quartz in UV light measurement, because glass can absorb UV light. But for Visible light measurement (colorimetric) glass cuvettes can be used.
- The sample concentration should be low, and transparent to avoid scattering and beers law deviations.

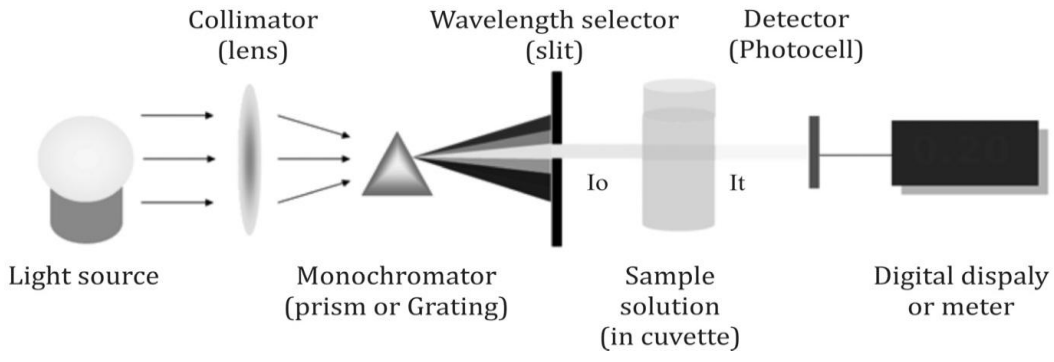


Figure 1.4 Basic Instrumentation of UV-Visible Spectrophotometer.

- Io is the intensity of incident light (monochromatic), $I_t$is the intensity of transmitted light.
- If Io = I_t sample inactive and indicate no absorption, and molecule structure may not have n and pi electrons.

- If Io is greater than I_t – indicate the reduction of intensity of transmitted light due to absorption of photon energy by the molecular electrons, by the process of electronic excitation.
- Hence Absorbance A = log (Io/I_t) or A = –log T; (where T = It/Io).

The UV Spectrum

For example (2D- UV spectrum) the following UV spectra shows absorption in UV region (190 – 380 nm) indicate the sample is colorless (Figure 1.5).

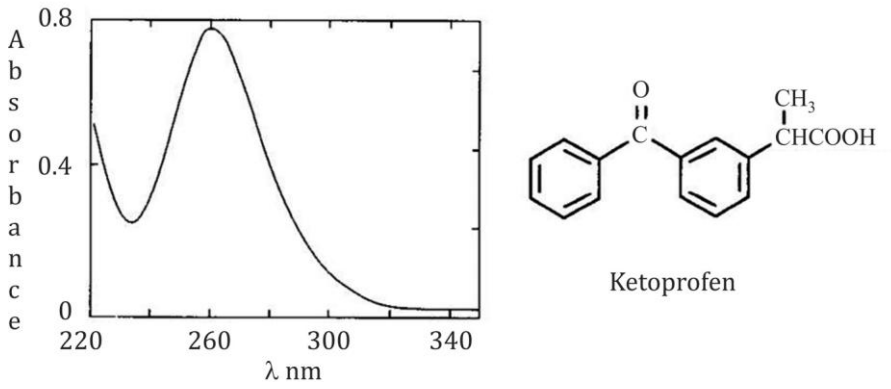


Figure 1.5 UV – Spectrum and UVmax (Lambda max) : Colourless Compound.

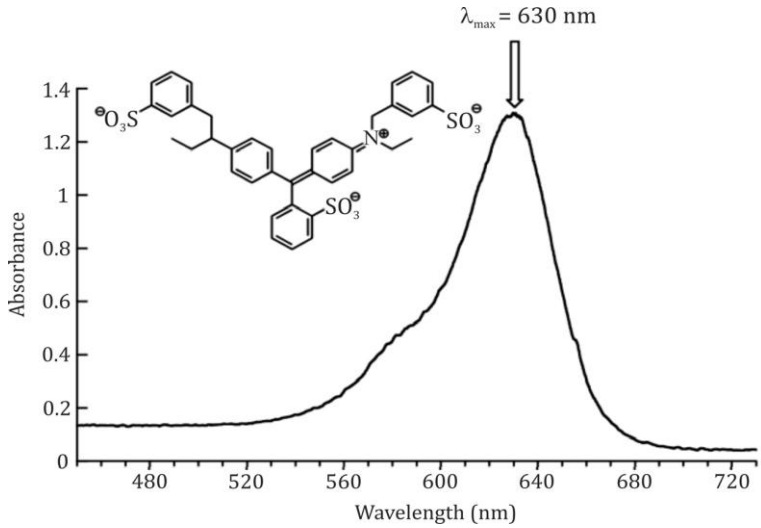


Figure 1.6 UV – Spectrum and absorption maximum: Coloured Compound.

The above UV spectrum is for Colour less sample shows a cut-off wavelength around 340 nm and lambda max of 260 nm. Thus the spectrum shows no absorbance in visible region

- In the same way coloured compound showed absorption maximum at 630 nm, but showed no absorbance in UV region (Figure 1.6).
- Visible spectrum of Coloured sample. Indicate the absorption characteristics of sample in the visible region (400-780 nm).
- The spectrum (Figure 1.7) shows that a spectrum characteristic does not affected by Concentration.

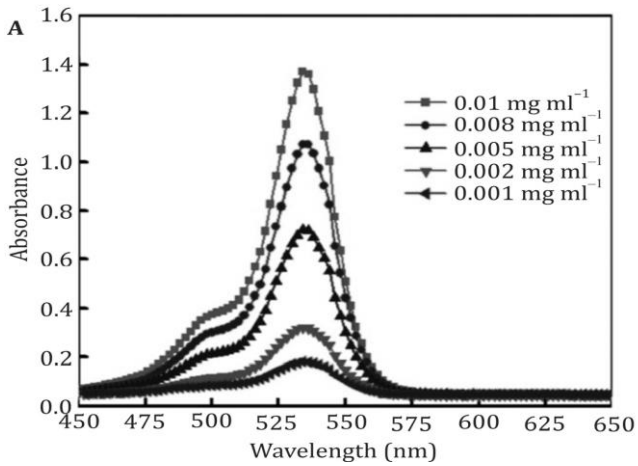
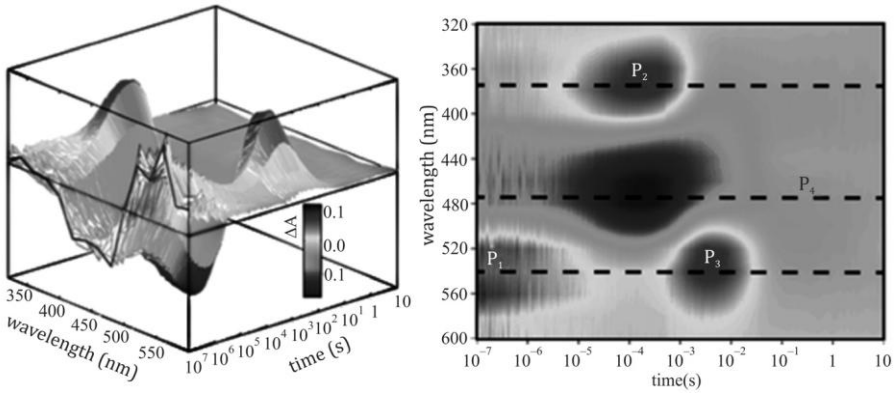


Figure 1.7 UV – Spectrum Vs Concentration (Shows that there is no change in spcetrum **chracteristics).**

3D UV Spectrum

It is more commonly employed in Chromatography as a function of detection using Photo-diode array detection (PDA). X-axis (wavelength), Y-axis (absorbance), Z axis (time), which can be more conveniently used for kinetic studies. PDA Detection is more commonly employed in stability and degradation of analyte. This detection can give peak purity when coupled with chromatography. This can also predict the response of



analyte at different wavelength.

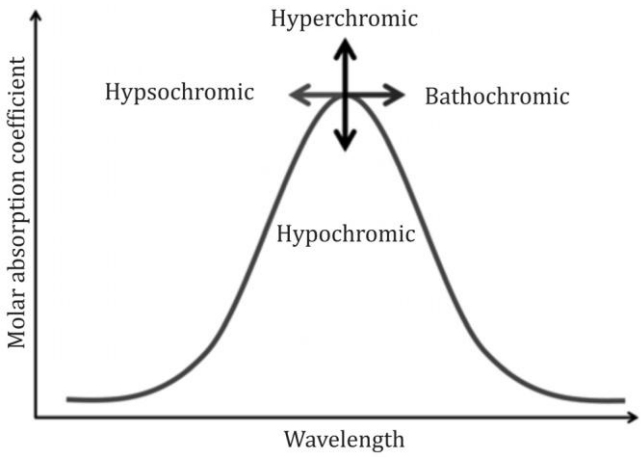


Figure 1.9 3D – UV Visible Spectrum (Example – PDA).

The comparison of absorption spectrum and emission spectrum

- The molecule which possesses more conjugation and exhibit tendency to emit light are called fluorescence. This molecule will have both absorption characteristic spectrum (excitation) and emission characteristic spectrum (emission).

- But emission spectrum will be bathochromic (red shift), it means the spectrum will shift to higher wavelength. The spectrum (Figure 1.9) shows that fluorescence wavelength shifted to higher wavelength than absorption wavelength.
- Maxima of absorption spectrum is excitation wavelength and maxima of emission spectrum is emission wavelength (In fluorimetry)
- If the wavelength shifts to lower wavelength, it's called hypsochromic shift. The hyper and hypo chromic shift are used to indicate the absorption to higher and lower value respectively (Figure 1.10). These effects are called auxochrome effect (UV Spectrum nomenclature).

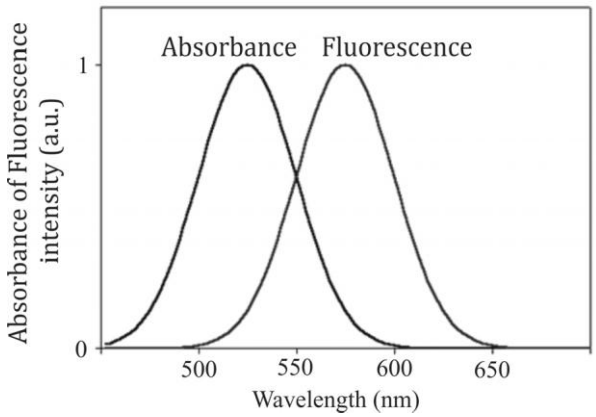


Figure 1.10 Absorption and Emission (Fluorescence) spectrum for an analyte (Stokes shift).

All these four effect is seen (Figure 1.10), when there is any change in the following,

- pH,
- solvent,
- scan speed,
- chemical decomposition,
- change in structure,
- isomerism,
- tautomer's

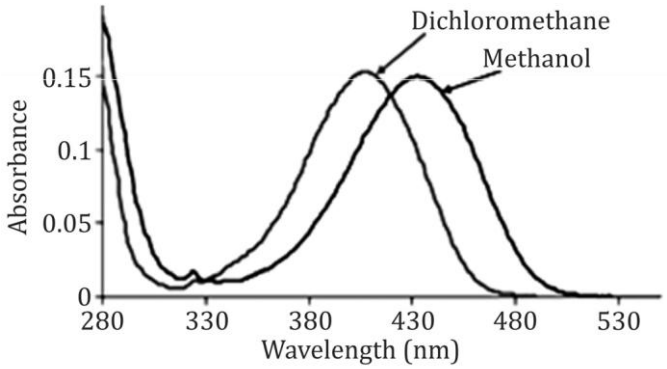


Figure 1.12 Effect of solvent on light absorption of an analyte.

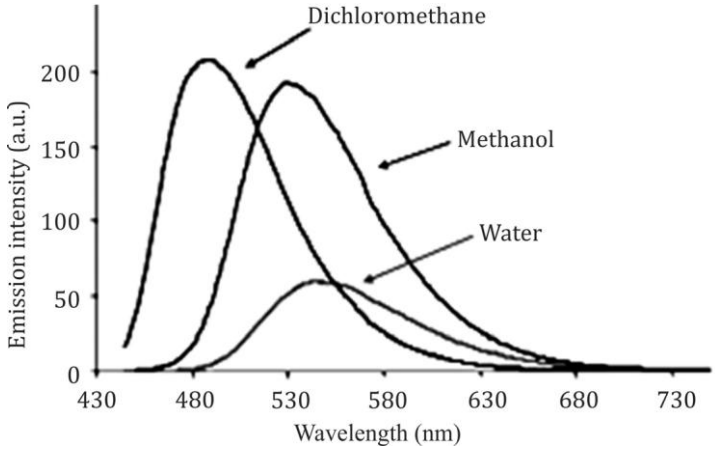


Figure 1.13 Effect of solvent on emission intensity of analyte (Fluorescence).

- The above spectrum (Figure 1.11) shows that there is a bathochromic shift for a molecule when polarity of solvent is increased. (Methanol is polar and dichloromethane is non-polar).

- The relation between polarity of solvent and shift of wavelength depends on the type electronic excitation (nothing but depends on structure and electron involved).
- Usually n- electrons excitation more affected by polar solvent (Hypochromic effect with increase in polarity) and vice versa for pi electrons (Hence, both "positive and negative solvatochromism" is possible)
- In the same way, solvent also affect the emission intensity of an analyte (Figure 1.12).

Solvent Effect and Absorption

Solvato chromism is the ability of a chemical substance to change color due to a change in solvent polarity. Negative solvate chromism corresponds to hypsochromic shift (or blue shift) with increasing solvent polarity. The corresponding bathochromic shift (or red) is termed positive solvatochromism.

In the UV spectrum the absorption from 200 nm to 220 nm,(sliding slope) is due to solvent or , due to both solvent and solute, hence, the wavelength should not be chosen in quantitative analysis. Furthermore, the absorption characteristics also changes in between 200 – 220 nm up on change in solvents. The various solvents and cut-off wavelength are listed in Table 1.2.

So it is always advisable to choose characteristic wavelength beyond the solvent absorption (I.e. above 230 nm). The following overlay spectrum shows the significant difference in the absorbance as well in wavelength for a same compound but in different solvents. It's all due various interactions exist between solute and solvent that ultimately affect the electron transition energies and transition probability. In the below UV-Visible spectrum dotted line (……) is due to solvent absorption. Solid lines are spectrum of sample with solvent / without solvent effect.

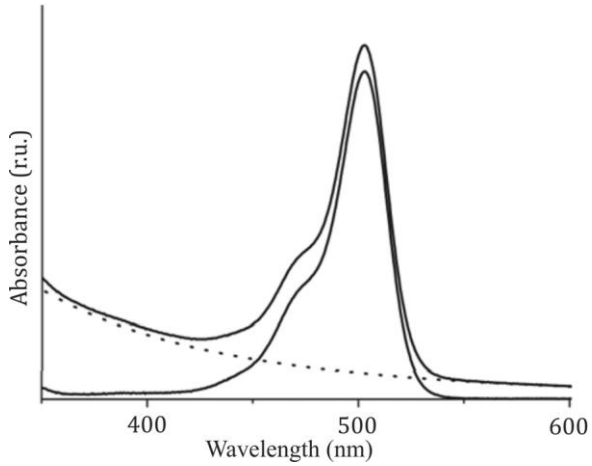


Figure 1.14 Solvent effect on UV-Visible light absorption.

The transparency limit of various UV solvent is given in the below Table 1.2.

Table 1.2 Transparency limit of commonly used Solvent in UV-Visible spectroscopy

Solvent	Cutoff Point (nm)	Solvent	Cutoff Point (nm)[a]
Water	200	Dichloromethane	233
Ethanol (95%)	205	Butyl ether	235
Acetonitrile	210	Chloroform	245
Cyclohexane	210	Ethyl proprionate	255
Cyclopentane	210	Methyl formate	260
Heptane	210	Carbon tetrachloride	265
Hexane	210	*N*,*N*-Dimethylformamide	270
Methanol	210	Benzene	280
Pentane	210	Toluene	285
Isopropyl alcohol	210	*m*-Xylene	290
Isooctane	215	Pyridine	305
Dioxane	220	Acetone	330
Diethyl ether	220	Bromoform	360
Glycerol	220	Carbon disulfide	380
1,2-Dichloroethane	230	Nitromethane	380

[a] Wavelength at which the absorbance is unity for a 1-cm cell, with water as the reference.

Structure and Spectrum Characteristics

- The absorption spectrum shape and pattern is depends on the structure, so that UV spectrum is considered as one of the identification tools in modern analytical chemistry.

• The electrons and bonds and their position (electronic configuration) determine the spectrum characteristics.

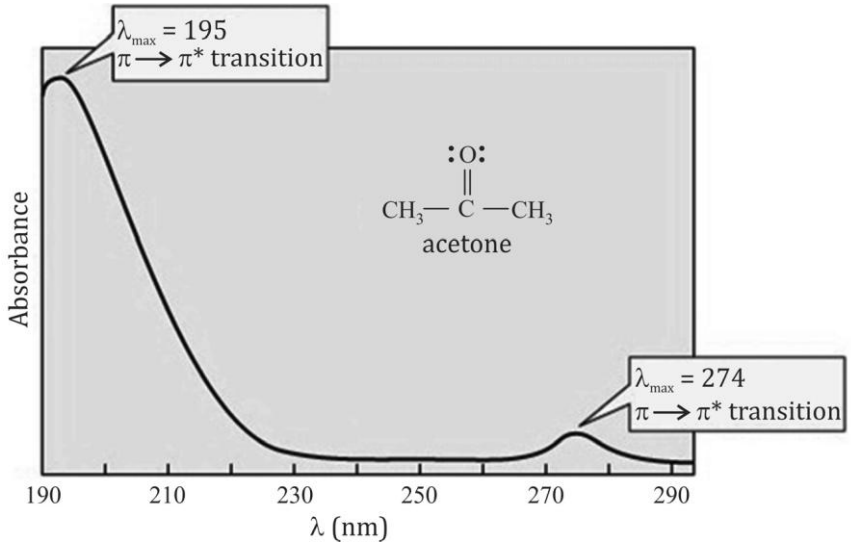


Figure 1.15 UV-Spectrum of Acetone.

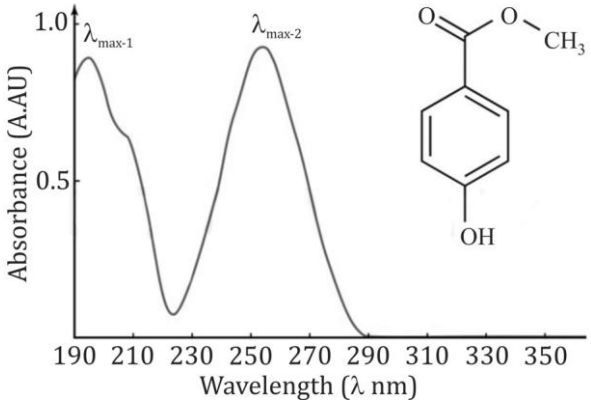


Figure 1.16 UV-Spectrum of aromatic ester.

1. For example above two spectrum are not similar indicate the compounds are different
2. Acetone UV spectrum (Figure 1.12) two maxima, one at 195 nm (for pi electron transition) and one at 274 nm (n' electron transitions), but the intensity for pi' (195 nm) is more than n' electron (274 nm). It indicate that the transition probability for pi is more in the structure when compare to n' electron. As a

3. result, the molar absorptivity (Epsilon value) will be more for Pi' and less for n' electron).
4. When look into the second spectrum (Figure 1.13), the intensity of peak absorption is increased due to conjugation. Furthermore, lambda max increases due to conjugation.
5. Hence the conjugation in structure will have greater effect on transition energy as well in wavelength absorbed.
6. The following UV spectrum is one more example for different transition in a molecule and its effect on UV spectrum. Where Pi to Pi is stronger and n' to pi is weaker. So always pi to pi transition yield greater absorbance than n' to pi transitions.
7. Pi to pi transitions always occur in low wavelength when compare to n' electrons due to the fact the energy of electron is in the order of Sigma > pi > n' electrons.

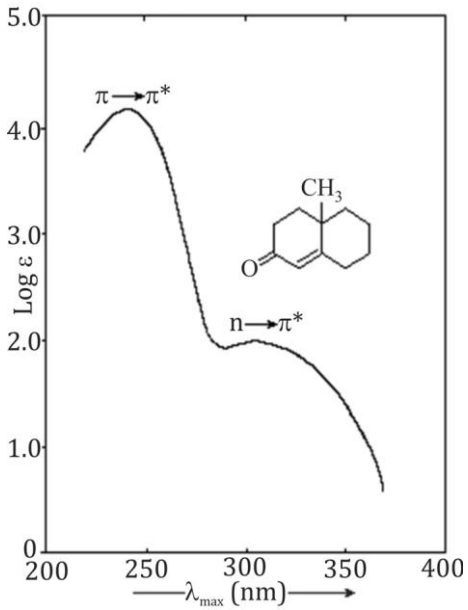


Figure 1.17 UV-Spectrum of Naphthalenone hexahydro methyl.

[1] The following overlay UV- Visible spectrum shows the effect of conjugation on UV-spectrum and number maxima in spectrum characteristics. As the conjugation increase in the structure the spectrum moves towards higher wavelength (bathochromic effect).

[2] Number of maxima is also increases as number conjugation increases.

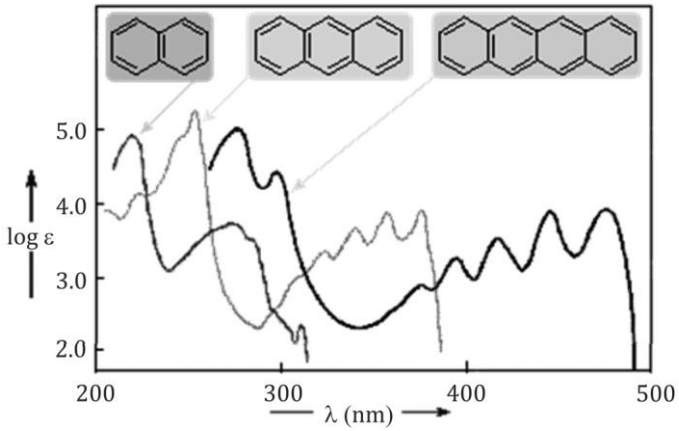
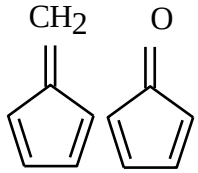


Figure 1.18 Effect of Conjugation on UV-Visible spectrum Characteristics.

1. **Cross Conjugation:** The below two structures are similar but differ in the electronic transition pattern as well in UV spectrum due to the n' electron contribute addition transition. Below structure also an example for cross conjugation.



Auxochrome effect: The below spectrum of quinones, shows the comparison of similar structures with same chromophoric system (conjugated system responsible for light absorption), but differ in substitution at NH2 group. Thus the substitution on chromophoric system has effect on both lambda max and absorbance (Figure 1.14 and Figure 1.15)

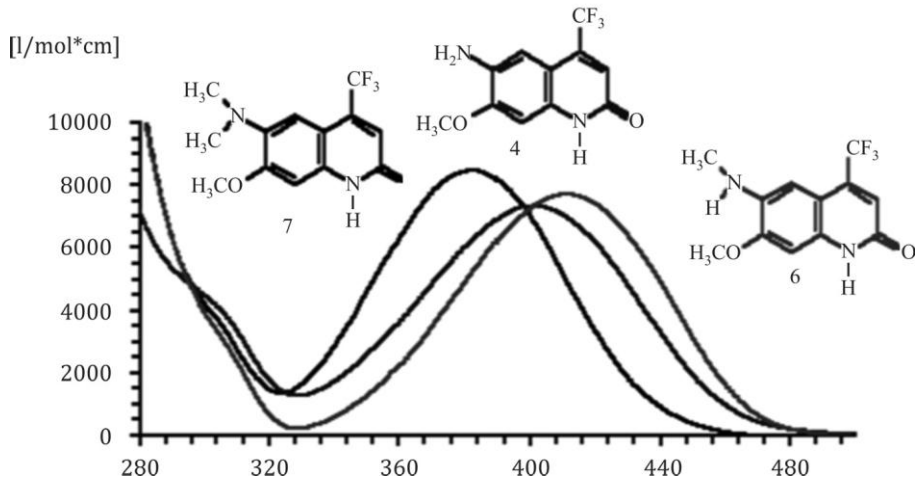


Figure 1.19 Auxochrome effect of UV-Visible spectrum Characteristics (Solvent: DMSO).

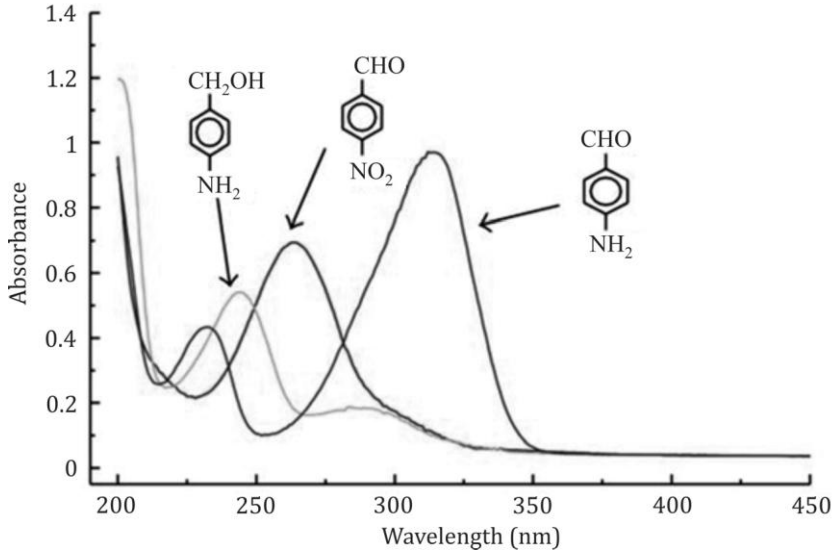


Figure 1.20 Auxochrome effect of UV-Visible spectrum Characteristics.

Order of Conjugation: The imidazole carboxylic acid (Figure 1.16) represents the order of conjugation in UV spectrum characteristics. The UV spectrum move higher wavelength when there is more conjugation. You can observe the change in the double bond position in imidazole nucleus of the structure.

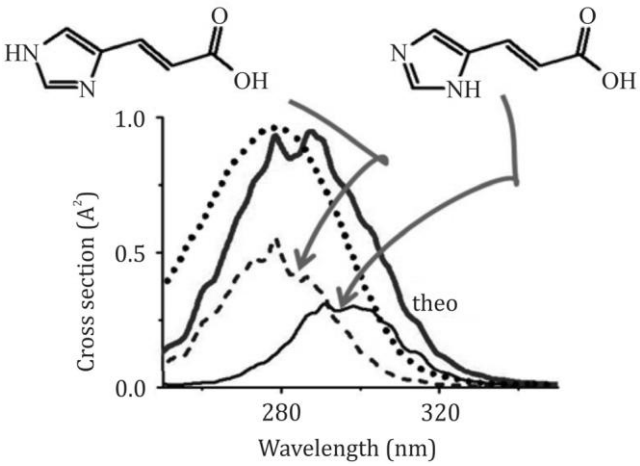


Figure 1.21 Effect of Order of Conjugation in UV-Visible spectrum Characteristics.

Position of Auxochrome in Chromophore: The below structure represent the importance of Auxochrome in benzene and their UV light absorption pattern. The presence of amine group may have significant effect on Lambda max and light absorption intensity. The ortho position in the below structure may contribute intra molecular hydrogen bonding and produce a addition cyclic residue to the structure as well as it also produce, inductive effect.

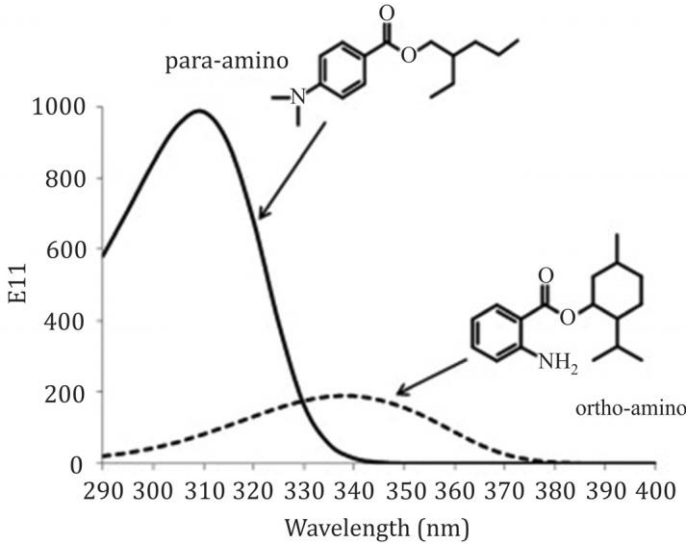
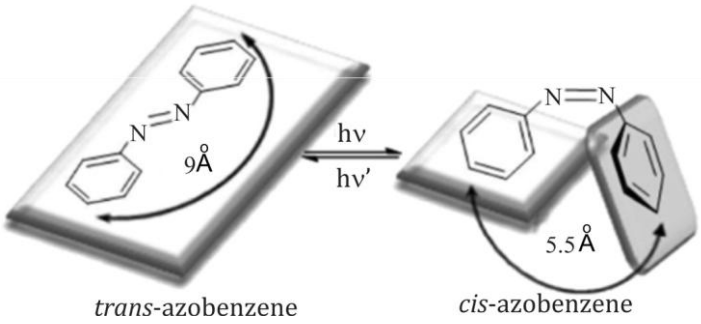


Figure 1.22 Effect of Auxochrome position on UV-Visible spectrum Characteristics.

Cis - Trans Isomerism and UV spectrum: The azobenzene is the best example of conformation transformation due to UV light and both are inter-convertible.



Like a C=C double bond, the azobenzenes have two geometric isomers (*Z*/*E*) around the N=N double bond, the *trans* isomer (*E*) is

~12 kcal·mol^{-1} more stable than the *cis* isomer (*Z*). The energy barrier of the photoexcited state is ~23 kcal·mol^{-1}, such that the *trans* isomer is predominant in the dark at room temperature. This energy barrier make different UV spectrum for trans (high intense) and Cis isomer.

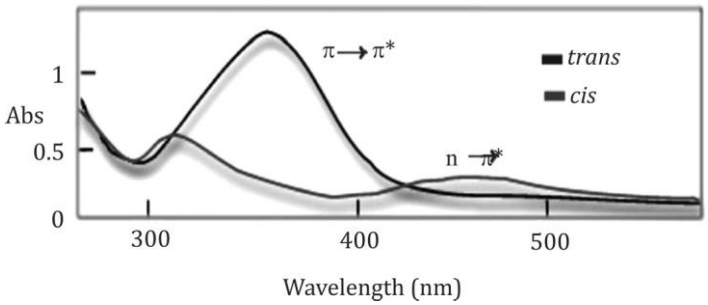


Figure 1.23 Effect of *Cis* /*Trans* isomer on UV-Visible spectrum Characteristics.

Keto-enol tautomer's in UV spectrum: The following figure shows the UV spectrum for same structure but exist in different tautomerism. B is Keto' form. A and C are enol form. For enol' structure spectrum is moved to higher wavelength due to induction of conjugation and extension of conjugation. Where as in structure B' both benzene is isolated not in conjugation.

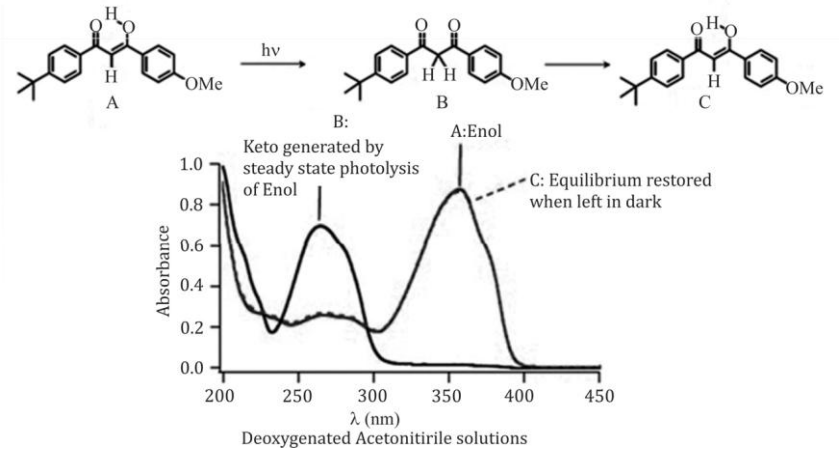


Figure 1.24 Effect of tautomerism on UV-Visible spectrum Characteristics.

Identification of Compound by UV Spectrum

For example: Ibuprofen, the following figure 1.19, shows the three UV spectra overlapped indicated that three (a, b, c) are same, as they have same UV spectrum and fingerprint matching of absorption characteristics.

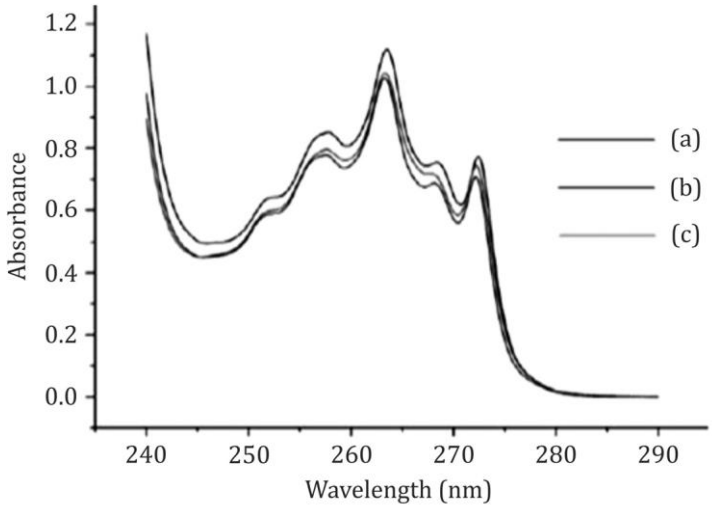


Figure 1.25 Overlay UV- Spectra of three different batch Ibuprofen samples.

The following UV spectrum (Figure 1.20) of four chrysene derivatives indicates that substitution (Auxochrome) will have impact on UV spectrum fingerprint (pattern of absorption), so no two derivatives of same chemical class would have same spectrum

Identification of degradation by UV spectrum

The degradation by UV spectrum can be identified by three ways

1. Change in absorption pattern and fingerprint (shift of maxima, appearance and disappearance of maxima, change in cut off wavelength): The following UV spectrum (Figure 1.21) shows the change in absorption pattern of compound after 90 minutes.

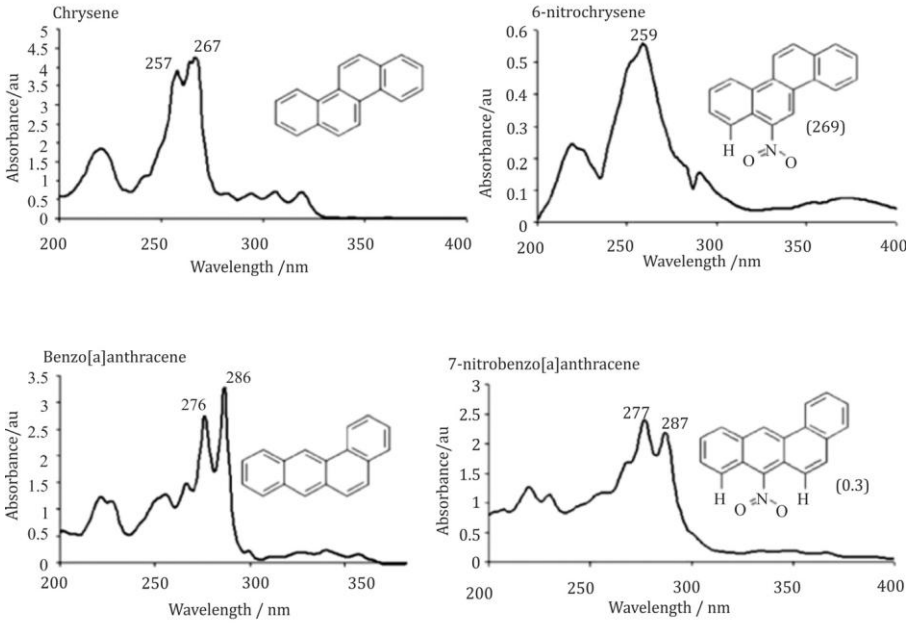


Figure 1.26 Overlay UV- Spectra of four derivatives of chrysene.

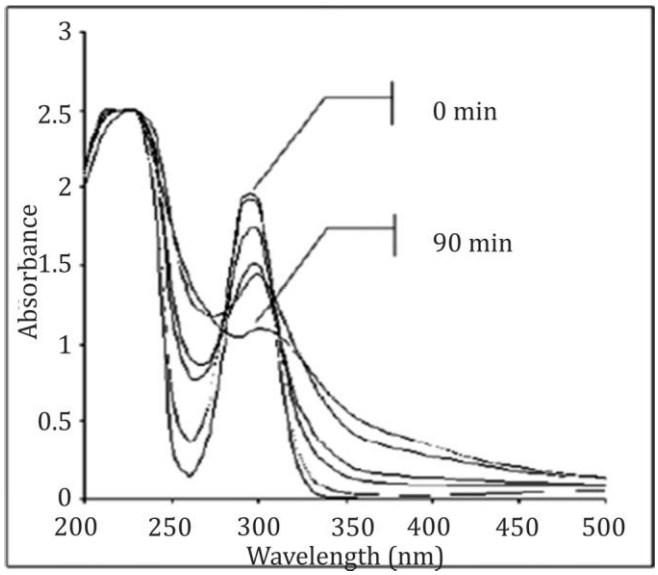


Figure 1.27 Overlay UV- Spectra of decomposed product at different time intervals.

2. Change in absorption (at maxima and valley): The UV spectrum (Figure 1.21) shows that there is an absolute shift of UVmax and valley point with respect to time.

3. Change in shape of the spectrum and AUC of the UV spectrum: The UV spectrum (Figure 1.22) shows the change in AUC and shape of UV spectrum up on degradation.

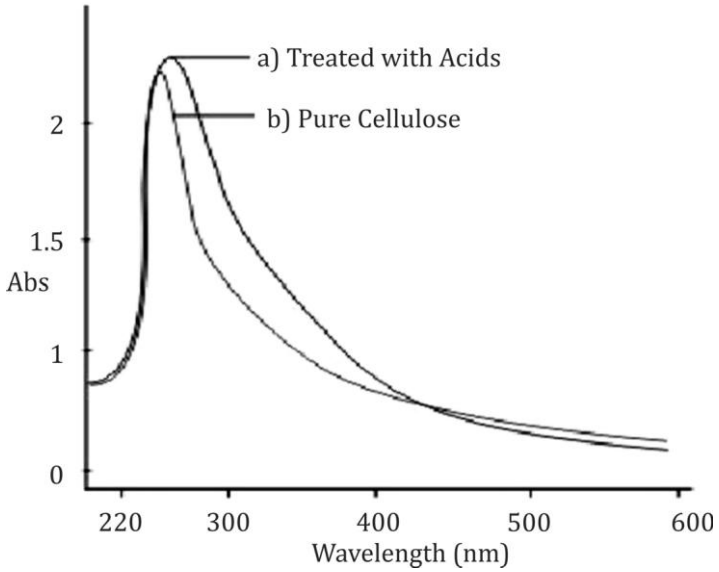


Figure 1.28 Overlay UV- Spectra of decomposed cellulose (Acid treated) and Pure Cellulose.

Differentiation of benzenoid and quinonoid by UV spectrum: It is more common with indication, at different pH the indicator colour will be different so the UV-visible spectrum also different for same indicator at different pH. The following is the example

(Figure 1.22) of Phenol red indicator; we can observe the shift of spectrum between acid form and base form.

Isosbestic point: However, there is a different absorption pattern for acidic /basic or ionized or unionized form of a chemical species, there a wavelength in which two compounds or different forms will have same absorbance. This particular wavelength is called as isosbestic point (Figure 1.22). The isosbestic point and its relevant absorbance are very import in simultaneous or multicomponent analysis (Q-analysis or absorbance ratio method).

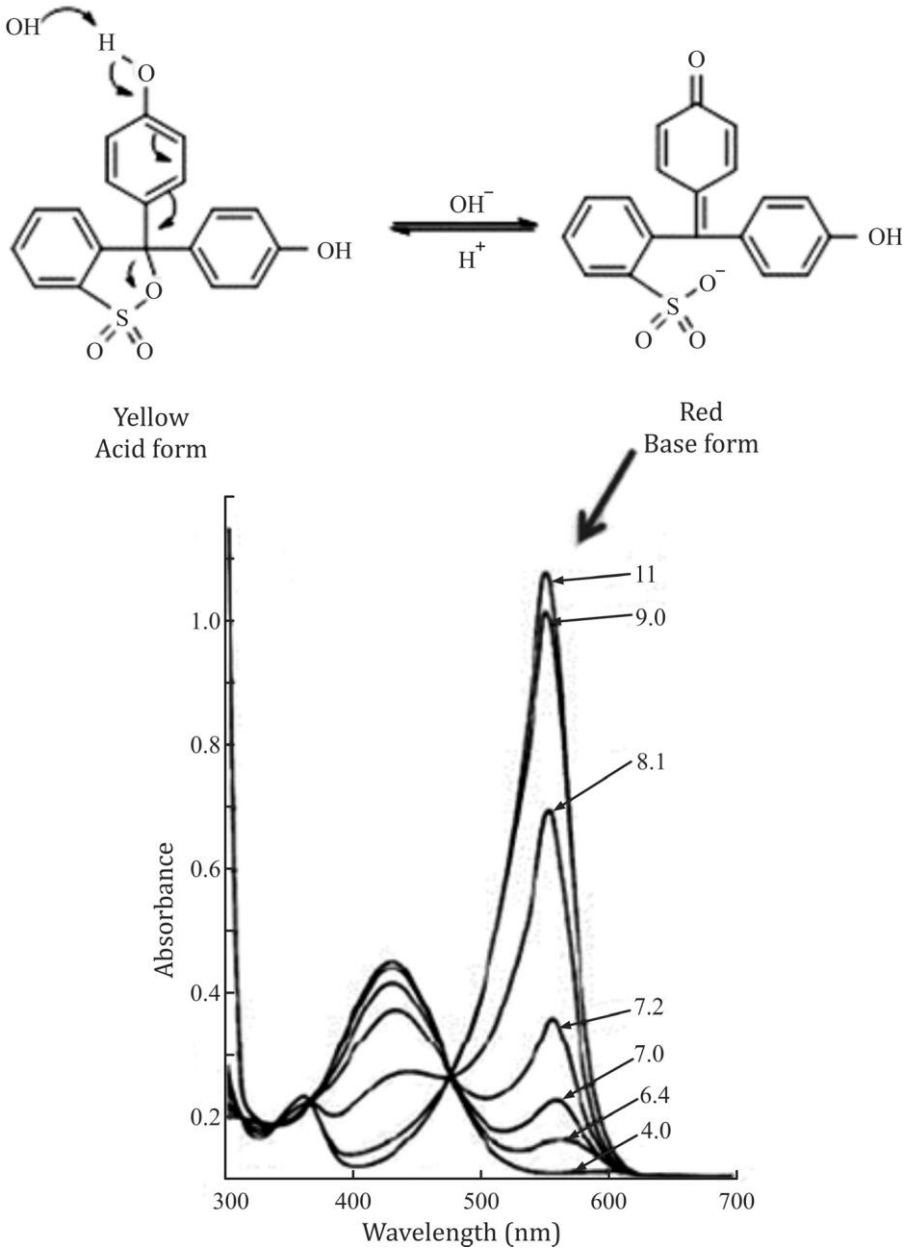


Figure 1.29 Overlay UV - Spectra of Phenol red indicators (at different pH).

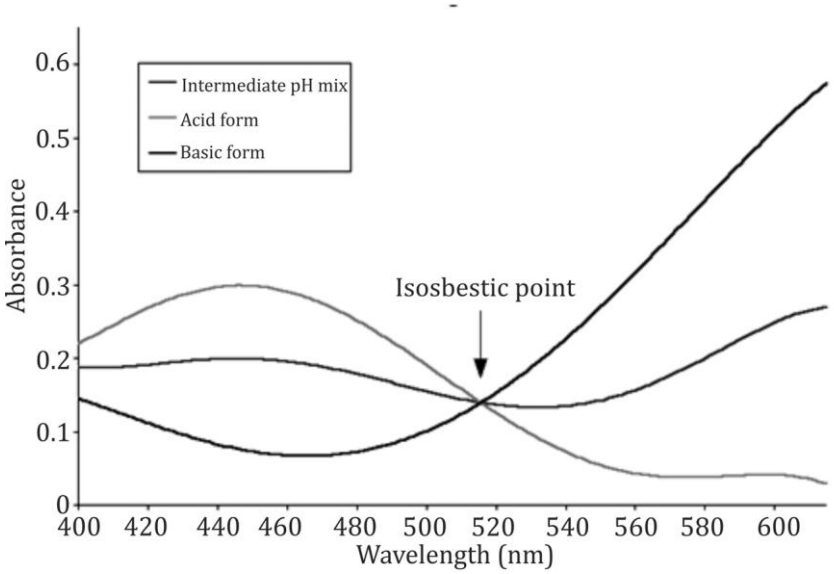
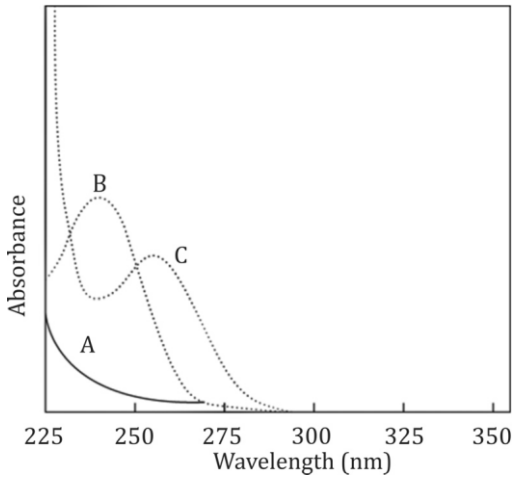


Figure 1.30 Overlay UV - Spectra of a compound at different pH (Isosbestic point).

Differentiation of ionized and unionized form of compounds (ex. Barbiturates): The following UV spectrum (Figure 1.23) represents the example of ionized and unionized spectrum of Barbiturate. It can be noticed that maxima was absent unionized form of barbiturate (A), Where Spectrum B' and C' are other ionized form of barbiturate. So, pH of solvent in quantitative analysis is very important factor for certain drugs.

Figure 1.31 Overlay UV- Spectra of Barbiturate (A: unionized; B &C are ionized at different proportions).



Determination of pKa by UV-spectrum

When there is a pH dependent absorbance changes takes place for a molecule, it is possible to determine the pKa by UV spectroscopy. The following figure 1.32 shows, the change absorbance with respect to change in pH values for isolapachol. The pKa was found to be 5.75 for isopachol

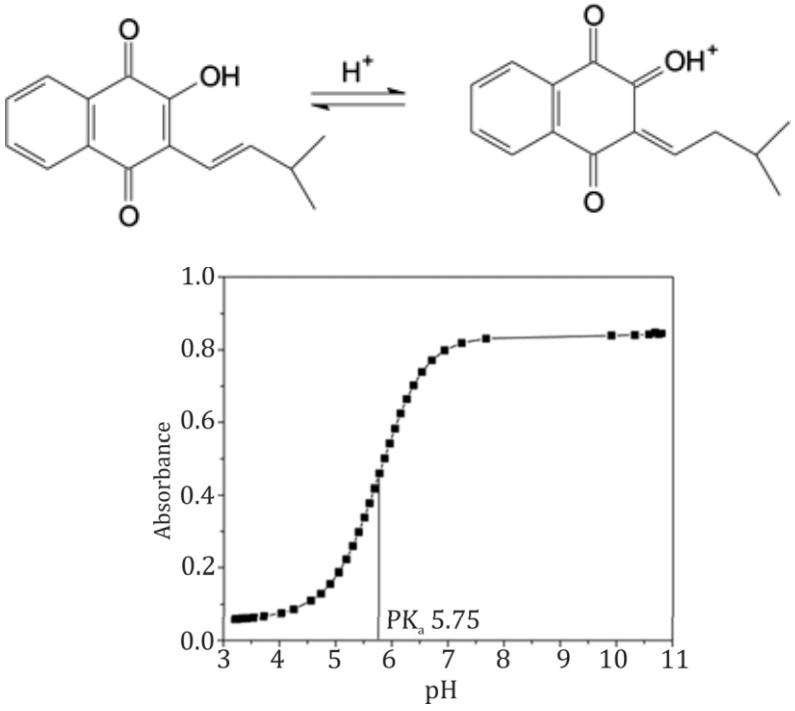


Figure 1.32 Effect of pH on UV- Visible light absorbance.

pKa is the pH in which both ionized and unionized forms are equal.
That exhibit direct relation in absorbance value.

Use of UV spectrum in Immune mediated complex: The following spectrum (Figure 1.33) shows the assay relies upon gold nanoparticles (AuNPs) based complex in the immunoreactions of antigen and antibody that can induce the aggregation of antibody-functionalized AuNPs. The UV spectrum shows significant shift in absorbance as well in wavelength to indicate the presence or absence of complex.

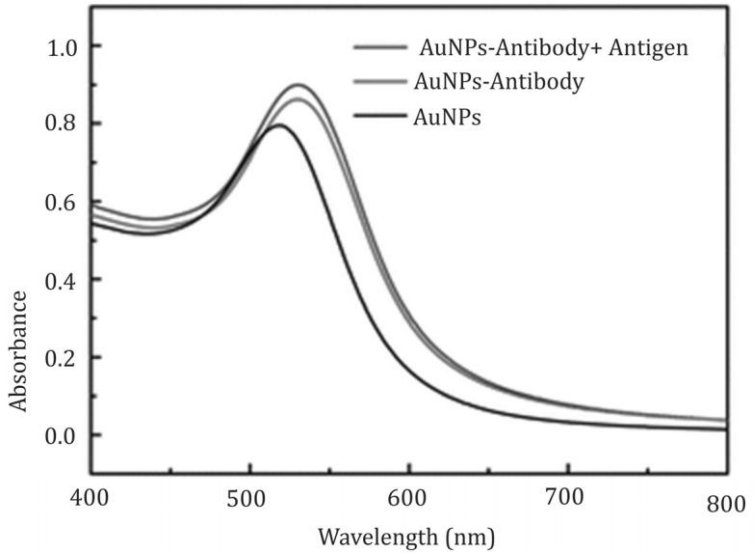


Figure 1.33 Overlay UV spectra in Immune mediated recation and its complex.

Identification of natural pigments: The UV spectrum is the good indicator to identify the natural pigments. The following figure 1.34 shows the UV- visible spectrum of several natural compounds which are distinct in their absorption characteristics.

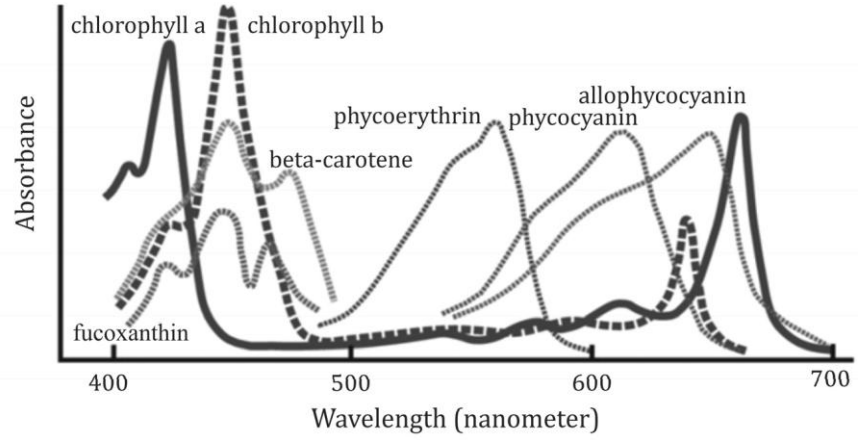


Figure 1.34 Overly UV spectra of natural pigments.

Detection of impurity by UV spectrum: There is a chance for predicting the presence of impurity (preferably at moderate level of concentration). The following UV spectrum (Figure 1.35) shows considerable shift of 2 nm for both maxima (275 nm) and Valley (248 nm) along with the maxima effect below 246 nm. This may be because of the presence of potential dissolved impurities.

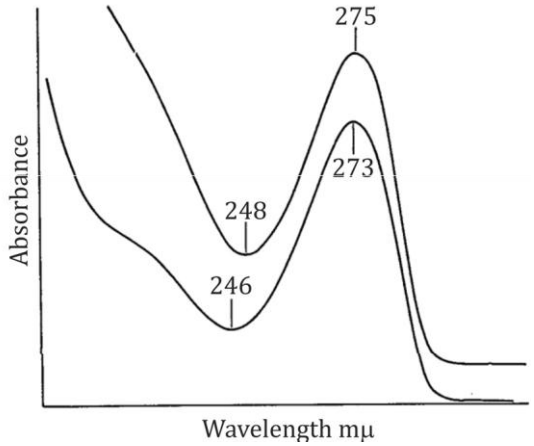


Figure 1.35 Detection of impurity by UV spectra.

Identification of Active pharmaceutical ingredients in Dosage form: It is done by comparison of Sample UV spectrum with reference UV spectrum (finger print matching; Figure 1.36). The excipient effects in UV absorption below 250 nm have to be considered or placebo correction may be required. The spectra A and C are sample, B is standard.

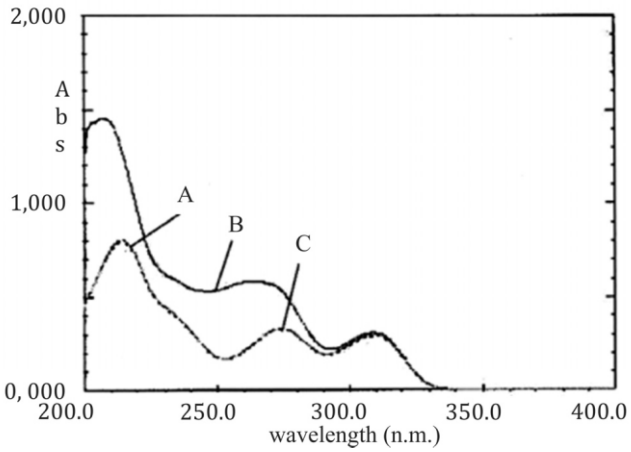


Figure 1.36 Identification of API in dosage form by UV spectrum.

Identification mixture using standards: If a UV spectrum is considered as mixture of two drugs (A and B), then the overlay spectra of pure drugs A and B and the mixture C can give us the information regarding authentication of drugs in the mixture. In the below figure 1.37, A and B are UV spectrum of pure drugs, where C (dotted line) is the mixture. The spectrum of C can be matched with spectrum of both A and B.

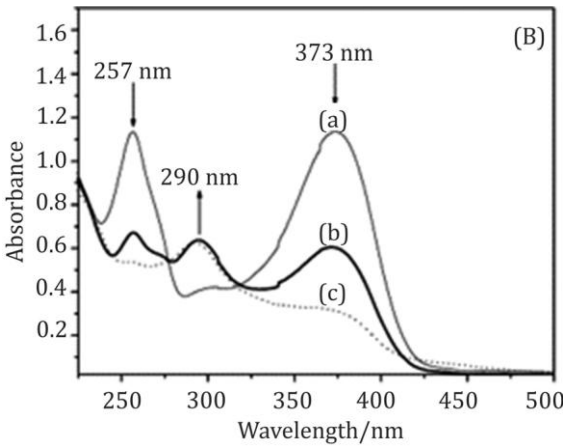


Figure 1.37 Identification of mixture using standard by UV spectra overlay.

WOODWARD RULES (Rules in calculation of UV max (lampta max) for chemical compounds)

"As lambda **(λ)** max increases that indicate the compound structure has more conjugation, with increase in pi-electron density. This lambda **(λ)** max vary from structure to structure (Figure 1.38 and 1.39) even with same number of double bond and conjugation, Lambda max **(λ)** is depends on position of the double bond rather than degree and number of double bond"

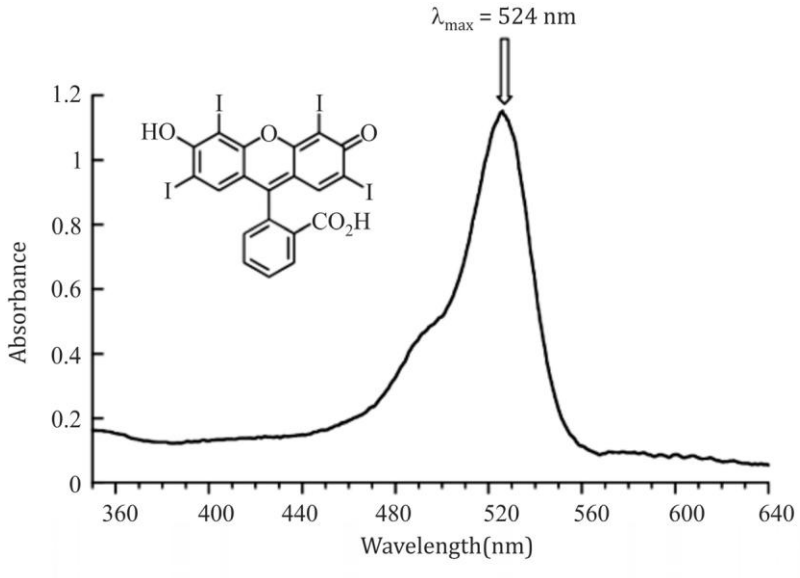


Figure 1.38 Determination of lambda **(λ)** max in UV Spectrum.

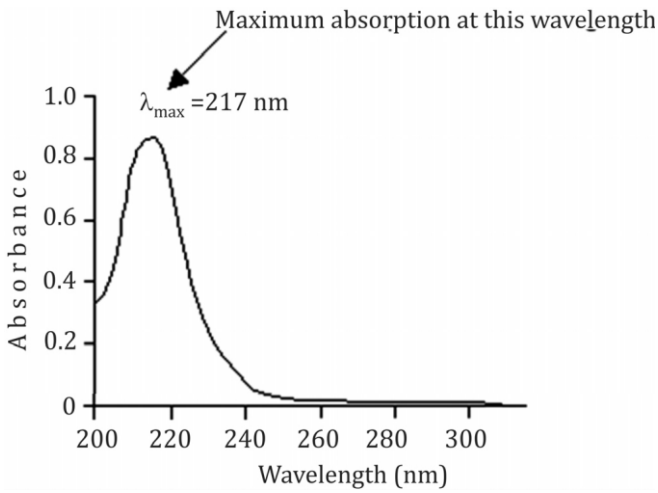


Figure 1.39 Maximum Absorption (lampta max) in UV absorption of diene compounds.

Infrared Spectroscopy

Infrared (IR) spectroscopy is a powerful analytical technique used to identify and characterize molecular structures by examining their vibrational transitions. This document provides a comprehensive overview of interpreting IR spectra and identifying functional groups.

1. Basic Principles of IR Spectroscopy

Infrared spectroscopy involves the interaction of infrared radiation with a molecule. The absorption of specific IR frequencies corresponds to the vibrational modes of molecular bonds. These vibrations fall within the mid-infrared region (4000–400 cm^{-1}) of the electromagnetic spectrum.

Types of Vibrations:

- Stretching vibrations: Changes in the bond length between two atoms (e.g., symmetric and asymmetric stretching).
- Bending vibrations: Changes in bond angles (e.g., scissoring, rocking, wagging, and twisting).
- The position and intensity of IR peaks provide critical information about the molecular structure and functional groups present.

2. Important Regions of the IR Spectrum

The IR spectrum is divided into three regions:

1. Functional Group Region (4000–1250 cm^{-1}):

- ✓ Contains characteristic absorption bands for different functional groups.
- ✓ Example: O–H stretching around 3200–3600 cm^{-1}, C≠O stretching around 1650–1750 cm^{-1}.
- ✓ Fingerprint Region (1500–400 cm^{-1}):
- ✓ Unique to each compound due to complex bending and stretching vibrations.

- ✓ Used for the identification of specific molecules by comparison with reference spectra.
- ✓ Overtone Region (>4000 cm^{-1}):
- ✓ Rarely used in interpretation, containing weak bands from overtones or combination bands.
- ✓ Interpretation of Key Functional Groups
    - o Hydroxyl (O–H) Groups:
- ✓ Alcohols and phenols: Broad absorption at 3200–3600 cm^{-1}.
- ✓ Carboxylic acids: Very broad band centered around 2500–3000 cm^{-1} due to hydrogen bonding.
    - o Carbonyl (C≠O) Groups:
- ✓ Sharp, intense peak in the range of 1650–1800 cm^{-1}.
- ✓ Subtypes:
- ✓ Ketones: 1705–1720 cm^{-1}.
- ✓ Aldehydes: 1720–1740 cm^{-1}.
- ✓ Carboxylic acids: 1700–1720 cm^{-1}.
- ✓ Esters: 1735–1750 cm^{-1}.
    - o C–H Stretching:
- ✓ Alkanes: 2850–3000 cm^{-1}.
- ✓ Alkenes: 3000–3100 cm^{-1}.
- ✓ Aromatics: 3050–3100 cm^{-1}.
    - o Nitrogen-Containing Groups:
- ✓ Amine (N–H): Two weak bands for primary amines at 3300 and 3400 cm^{-1}.
- ✓ Amides (C≠O and N–H): Strong C≠O band at 1650–1700 cm^{-1} and weak N–H bands at 3300–3500 cm^{-1}.
    - o Triple Bonds (C≡C, C≡N):
- ✓ C≡C: Weak peak around 2100–2250 cm^{-1}.
- ✓ C≡N: Strong peak around 2200–2250 cm^{-1}.
    - o Aromatic Compounds:
- ✓ Multiple weak to moderate absorption bands between 1450 and 1600 cm^{-1} (C≡C stretching).
- ✓ C–H bending bands in the range of 700–900 cm^{-1}.
- ✓ Steps to Interpret an IR Spectrum

- ✓ Step 1: Identify the Functional Group Region
- ✓ Look for broad or sharp peaks corresponding to O–H, N–H, or C≠O groups.
- ✓ Check the wavenumber range to determine the functional groups present.
- ✓ Step 2: Examine the Fingerprint Region
- ✓ Compare the pattern of peaks to reference spectra to confirm the compound.
- ✓ Note the unique combination of bending vibrations.
- ✓ Step 3: Correlate Peaks with Bond Types
- ✓ Assign specific peaks to corresponding bond vibrations.
- ✓ Consider peak intensity, width, and position.
- ✓ Step 4: Analyze Structural Information
- ✓ Confirm the presence of multiple functional groups.
- ✓ Use complementary data from other techniques (e.g., NMR, MS) for structural elucidation.

- ✓ Factors Affecting IR Absorption
- ✓ Several factors can influence the position and intensity of IR absorption bands:
- ✓ Bond Strength:
- ✓ Stronger bonds (e.g., triple bonds) absorb at higher frequencies compared to weaker bonds (e.g., single bonds).
- ✓ Mass of Atoms:
- ✓ Heavier atoms result in lower-frequency vibrations.
- ✓ Hydrogen Bonding:
- ✓ Broadens and shifts the absorption bands (e.g., O–H stretching).
- ✓ Conjugation:
- ✓ Lowers the absorption frequency of carbonyl groups due to electron delocalization.
- ✓ Substitution Effects:
- ✓ Alters the position and intensity of aromatic and alkene absorptions.

- ✓ Applications of IR Spectroscopy
- ✓ Functional Group Identification:
- ✓ Rapid identification of specific groups in organic and inorganic compounds.
- ✓ Quantitative Analysis:
- ✓ Determination of concentrations based on peak intensities.
- ✓ Polymer Analysis:
- ✓ Monitoring curing processes and identifying polymer functional groups.
- ✓ Quality Control:
- ✓ Ensures consistency in pharmaceuticals and chemical manufacturing.
- ✓ Environmental Analysis:
- ✓ Detects contaminants and pollutants (e.g., CO_2, SO_2).
- ✓ Limitations of IR Spectroscopy
- ✓ Complex Spectra: Overlapping peaks in the fingerprint region can complicate interpretation.
- ✓ Weak Absorptions: Some functional groups produce weak signals, making detection difficult.
- ✓ Sample Preparation: Impurities or improper sample handling can lead to artifacts.
- ✓ Complementary Techniques: IR spectroscopy often needs to be combined with NMR or MS for complete structural elucidation.

Raman spectroscopy

Principles and Applications

Raman spectroscopy is a sophisticated and widely used analytical technique that provides insights into the molecular vibrations and structural characteristics of materials. It relies on the phenomenon of inelastic scattering of monochromatic light, known as Raman scattering, which occurs when light interacts with the vibrational modes of molecules. This interaction results in energy shifts that are unique to the molecular structure, allowing precise identification and characterization of chemical bonds, molecular interactions, and crystallinity within a sample.

Key Parameters in Raman Spectroscopy

The position of Raman peaks, expressed as Raman shifts in units of cm^{-1}, is a critical parameter for interpreting the spectra. These shifts represent specific vibrational modes of the sample and are categorized into different regions based on the molecular or structural feature they represent. Low wavenumber peaks, typically below 500 cm^{-1}, are associated with lattice vibrations or metal-ligand bonds. These are particularly significant in materials such as metal oxides or composites where the vibrations of metal centers dominate. Mid-wavenumber peaks, spanning 500 to 1500 cm^{-1}, often correspond to bending or stretching vibrations of functional groups like C-C, C-N, or C-O bonds. High wavenumber peaks, above 1500 cm^{-1}, are characteristic of functional group vibrations, including C=C, C≡C, or C-H stretching modes. The precise location of these peaks provides direct information about the bonding environment within the sample.

Intensity and Width of Raman Peaks

In addition to peak positions, the intensity and width of Raman peaks offer valuable information about the material's structure and properties. The intensity of a Raman peak is proportional to the change in polarizability during the vibration and can be used to compare the relative concentration or bond strength of different components in the sample. For example, a higher intensity peak for a specific bond implies a greater contribution to the overall molecular structure or a stronger interaction. Peak width, on the other hand, can indicate the degree of order or crystallinity within a material. Narrow peaks are typical of highly ordered crystalline materials, whereas broader peaks suggest the presence of amorphous or disordered structures.

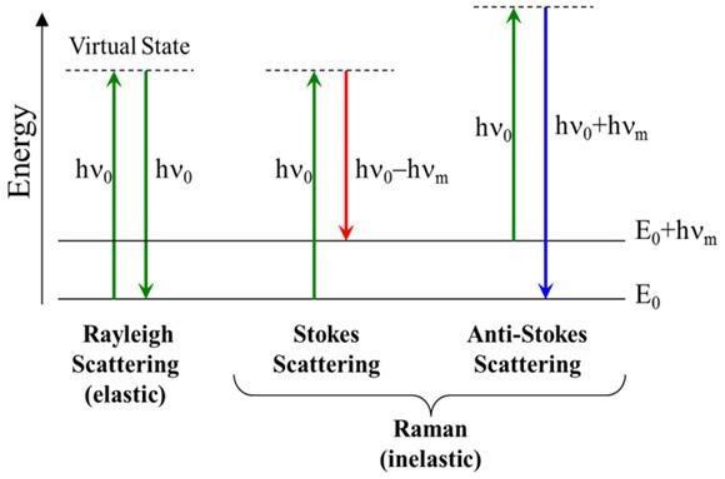
Material Identification Using Raman Spectroscopy

The identification of specific materials using Raman spectroscopy often involves the analysis of characteristic peaks. For instance, in carbon-based materials like g-C_3N_4 or graphene, the D band, located around 1350 cm^{-1}, is indicative of structural defects or disorder, while the G band at approximately

1580 cm^{-1} corresponds to sp^2-hybridized carbon vibrations. The 2D band, typically observed around 2700 cm^{-1}, provides information about the stacking order or number of graphene layers. Similarly, metal oxides such as ZrO_2 exhibit peaks in the range of 150 to 700 cm^{-1} due to Zr-O vibrations, while TiO_2 (anatase phase) shows strong peaks at 144, 197, 399, 519, and 639 cm^{-1} corresponding to various Ti-O-Ti stretching and bending modes. For hydroxyapatite (HAP), characteristic peaks include symmetric phosphate stretches around 960 cm^{-1} and asymmetric stretches between 1000 and 1100 cm^{-1}, along with broad OH^- vibrations near 3570 cm^{-1}.

Analysis of Organic Molecules

Raman spectroscopy is also extensively used to study organic molecules, where specific functional groups produce distinctive vibrational signatures. For example, carbonyl (C=O) groups typically exhibit strong peaks between 1650 and 1750 cm^{-1}, while C-H stretching vibrations are observed in the range of 2800 to 3100 cm^{-1}. N-H bending modes appear between 1550 and 1650 cm^{-1}. These characteristic vibrations help in identifying and confirming the presence of specific organic moieties within a complex matrix.



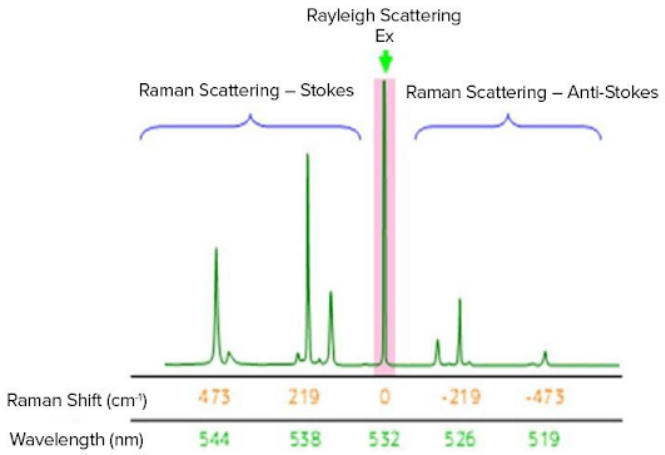
Advanced Analysis Techniques in Raman Spectroscopy

A key aspect of Raman spectral interpretation involves advanced analysis techniques such as peak shifts, mapping, and defect identification. Peak shifts often indicate changes in the chemical bonding environment, strain, or doping

effects. For instance, in ZrCN coatings, shifts in C≡N peaks may signify interactions between carbon nitride and zirconium or alterations in lattice structure. Raman mapping provides spatially resolved information, enabling the visualization of phase distribution or the identification of compositional gradients across a sample. This is particularly useful in heterogeneous systems such as composites or coatings. Furthermore, the analysis of defects is critical in materials like graphitic carbons, where an increase in the intensity of the D band relative to the G band suggests higher defect density or disorder.

Quantitative analysis

Quantitative analysis using Raman spectroscopy involves the comparison of relative peak intensities or areas to estimate compositional ratios or structural features. This is especially important in materials research, where the synthesis process often affects the stoichiometry or phase purity of the product. Additionally, baseline correction and noise reduction are essential preprocessing steps to ensure accurate peak identification and intensity measurements. By comparing experimental spectra with reference databases or computational models, researchers can assign peaks to specific vibrational modes and derive detailed insights into the material's structure and properties.



Applications and Complementary Techniques

Overall, Raman spectroscopy is a versatile tool for materials characterization, offering detailed information on chemical bonding, molecular interactions, and structural order. Its ability to provide non-destructive, high-resolution insights makes it indispensable in fields ranging from nanotechnology and catalysis to

biomedical research and advanced coatings. By combining Raman data with complementary techniques such as X-ray diffraction (XRD) or atomic force microscopy (AFM), a comprehensive understanding of material properties can be achieved, enabling the design of innovative solutions for various scientific and industrial challenges.

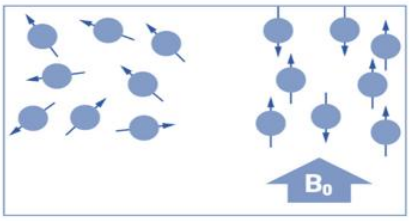
Nuclear Magnetic Resonance (NMR) Spectroscopy

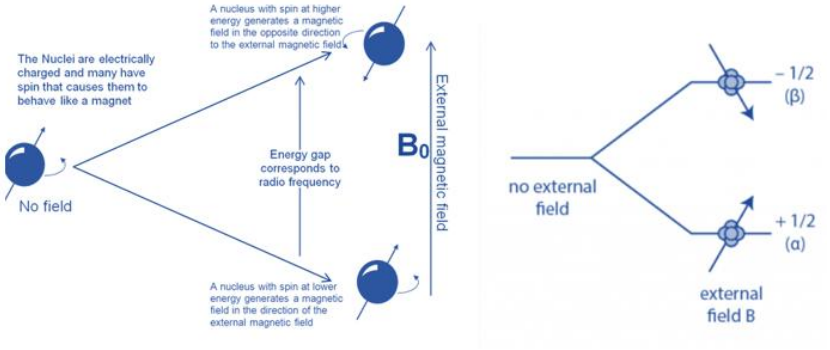
Principles and Applications

Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful and non-destructive analytical technique used to determine the molecular structure, dynamics, and interactions of compounds. This method is based on the magnetic properties of certain atomic nuclei, such as hydrogen (1H), carbon (13C), and phosphorus (31P). When placed in a strong external magnetic field and exposed to radiofrequency (RF) radiation, these nuclei absorb energy and resonate at characteristic frequencies, providing detailed information about their chemical environment and connectivity.

Fundamental Principles of NMR Spectroscopy

NMR spectroscopy exploits the magnetic properties of nuclei with non-zero spin, which behave like tiny magnets when subjected to an external magnetic field (B_0). The interaction between nuclear spins and the magnetic field creates discrete energy levels, with transitions between these levels occurring upon absorption of RF radiation. The resonance frequency is determined by the Larmor equation ($\nu = \gamma B_0/2\pi$), where γ is the gyromagnetic ratio of the nucleus. Each nucleus experiences a unique local magnetic environment influenced by electron density and neighboring atoms, resulting in chemical shifts, expressed in parts per million (ppm).





Chemical Shifts and Their Interpretation

Chemical shifts are a primary feature of NMR spectra, revealing the electronic environment around nuclei. They are influenced by factors such as electronegativity, hybridization, and molecular geometry. For instance:

Hydrogen (1H) NMR: Chemical shifts for aliphatic protons typically appear between 0 and 4 ppm, while aromatic protons resonate between 6 and 8 ppm. Deshielding effects from electronegative atoms or functional groups shift peaks downfield (higher ppm), whereas shielding effects shift peaks upfield (lower ppm).

Carbon (13C) NMR: Carbon atoms in sp3 hybridized environments resonate between 0 and 90 ppm, while sp2 carbons (e.g., aromatic or carbonyl groups) appear between 100 and 220 ppm. The position of these peaks provides insights into the bonding and hybridization of carbon atoms in the molecule.

Peak Splitting and Spin-Spin Coupling

Spin-spin coupling occurs when the magnetic fields of neighboring nuclei interact, causing splitting of NMR signals into multiplets. The number and pattern of peaks within a multiplet are determined by the number of equivalent neighboring nuclei (n), following the (n+1) rule. Coupling constants (J), measured

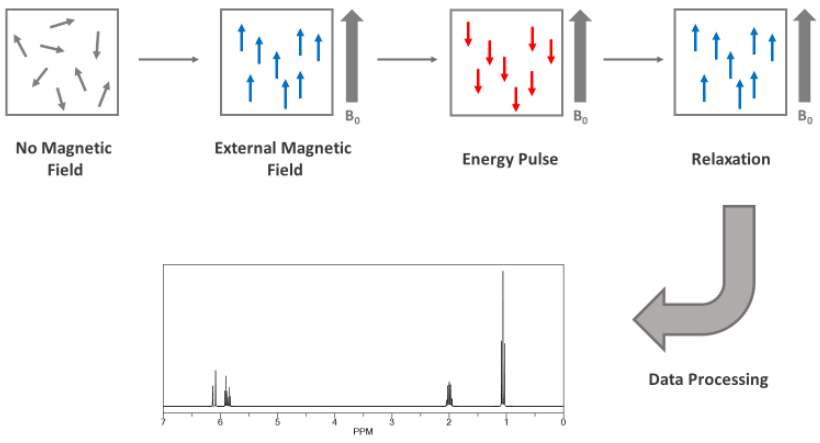
in Hz, quantify the strength of these interactions and provide information about the spatial arrangement of nuclei. For example:

In a simple CH_3-CH_2 fragment, the CH_3 protons split into a quartet due to coupling with two equivalent CH_2 protons, while the CH_2 protons split into a triplet.

Large J values (e.g., >10 Hz) indicate vicinal coupling in rigid systems, whereas small J values (<5 Hz) are characteristic of long-range or geminal couplings.

Integration and Quantitative Analysis

The area under each NMR signal is proportional to the number of nuclei contributing to that signal. Integration of peaks in 1H NMR allows for the determination of relative proton counts, aiding in the elucidation of molecular formulas and structural connectivity. For example, a molecule with an aromatic ring may display a signal with an integration corresponding to five protons, indicative of a monosubstituted benzene derivative.



Advanced NMR Techniques

Advanced NMR methods extend the capabilities of basic 1D spectra to provide more detailed structural and dynamic information:

2D NMR Spectroscopy: Techniques such as COSY (Correlation Spectroscopy) and HSQC (Heteronuclear Single Quantum Coherence) establish correlations between nuclei through scalar or dipolar couplings, revealing connectivity and spatial relationships.

NOESY (Nuclear Overhauser Effect Spectroscopy): Provides insights into spatial proximity between nuclei, useful for determining three-dimensional molecular structures.

Solid-State NMR: Used for studying materials, polymers, and biological samples, offering insights into crystalline and amorphous regions.

Applications of NMR Spectroscopy

- NMR spectroscopy is indispensable across a wide range of scientific disciplines, including:
- Structural Elucidation: Identification of organic compounds, natural products, and biomolecules through comprehensive analysis of chemical shifts, coupling patterns, and integration.
- Dynamic Studies: Investigation of molecular dynamics, including conformational changes, reaction mechanisms, and diffusion properties.
- Material Science: Characterization of polymers, nanomaterials, and porous solids.
- Biomedical Applications: Metabolomics and drug discovery by analyzing complex mixtures of biomolecules.

Limitations and Complementary Techniques

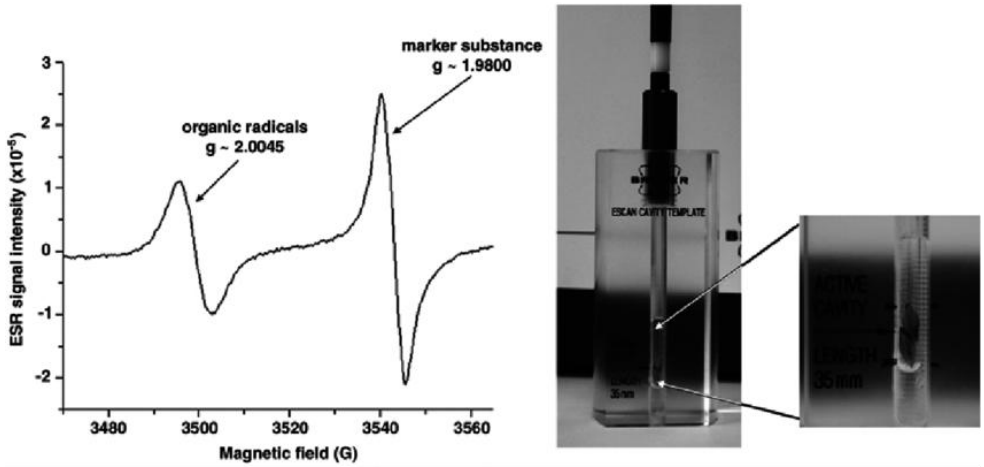
While NMR spectroscopy provides unmatched resolution for structural studies, it has limitations such as sensitivity to low-concentration samples and overlapping peaks in complex mixtures. These challenges can often be addressed by combining NMR with complementary techniques like mass spectrometry (MS) or X-ray crystallography, offering a holistic approach to molecular characterization.

Electron Spin Resonance (ESR) Spectroscopy

Principles and Applications

Electron Spin Resonance (ESR) spectroscopy, also known as Electron Paramagnetic Resonance (EPR), is a powerful analytical technique used to study systems with unpaired electrons. This includes free radicals, transition metal ions, and defects in materials. ESR provides insights into the electronic environment,

magnetic interactions, and dynamics of paramagnetic species, making it an essential tool in fields like chemistry, physics, and biology.

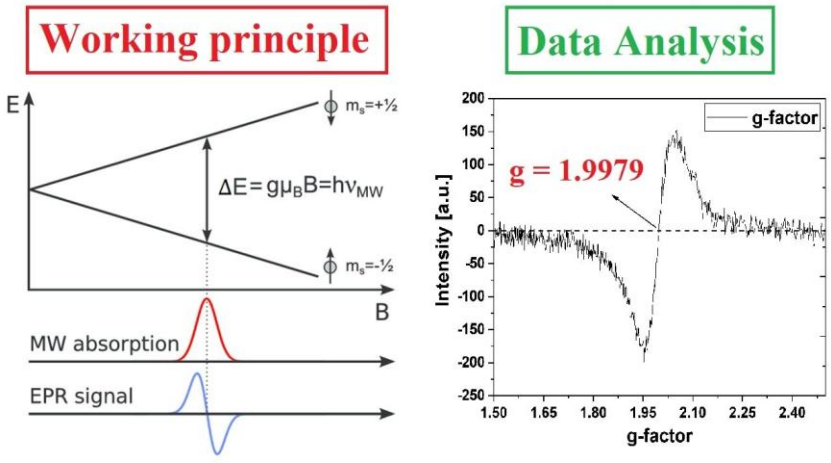


Fundamental Principles of ESR Spectroscopy

ESR is based on the magnetic properties of unpaired electrons, which possess intrinsic spin and magnetic moments. When placed in an external magnetic field (B_0), these electrons exhibit Zeeman splitting, where their energy levels split into two states: α (lower energy, spin-up) and β (higher energy, spin-down). By applying microwave radiation at a frequency (ν) that matches the energy difference between these states, transitions are induced, resulting in absorption signals. The resonance condition is given by the equation:

$$\Delta E = h\nu = g\beta B_0$$

where *g* is the g-factor, β is the Bohr magneton, and *h* is Planck's constant. The g-factor provides information about the electronic environment and is sensitive to the nature of the unpaired electron and its surroundings.



Hyperfine Interactions

A distinctive feature of ESR spectra is hyperfine splitting, which arises from interactions between unpaired electrons and nearby magnetic nuclei (e.g., 1H, 14N). This splitting provides detailed information about the local environment and the identity of interacting nuclei. The number of hyperfine lines follows the (2I+1) rule, where *I* is the nuclear spin quantum number. For example:

- A free radical with one proton (I = ½) shows a doublet.
- A nitroxide radical interacting with 14N (I = 1) displays a triplet.

The hyperfine coupling constant (A), measured in gauss or MHz, quantifies the interaction strength and provides structural and electronic insights.

ESR Spectral Parameters

Key parameters in ESR spectra include:

- ✓ g-Factor: The g-value, typically close to 2.0023 for free electrons, shifts depending on the electronic environment. Deviations from this value indicate interactions with ligands, spin-orbit coupling, or anisotropy in the magnetic field.
- ✓ Linewidth: The width of ESR signals reflects the relaxation processes and dynamics of the paramagnetic species. Broad lines suggest strong spin-spin or spin-lattice interactions, while narrow lines indicate isolated, fast-relaxing systems.
- ✓ Intensity: The signal intensity is proportional to the number of unpaired electrons, allowing for quantitative analysis of radical concentrations.

Advanced ESR Techniques

- ✓ Modern ESR employs advanced techniques to enhance spectral resolution and extract detailed information:
- ✓ Continuous Wave (CW) ESR: The most common method, providing steady-state spectra of paramagnetic species.
- ✓ Pulsed ESR: Time-domain techniques such as Electron Spin Echo (ESE) and DEER (Double Electron-Electron Resonance) reveal relaxation times and distances between spin centers, useful in structural biology.

- ✓ ENDOR (Electron-Nuclear Double Resonance): Combines ESR with nuclear magnetic resonance to resolve overlapping hyperfine interactions and identify specific nuclei.
- ✓ High-Field ESR: Utilizes high magnetic fields and frequencies to improve resolution and analyze complex systems.

Applications of ESR Spectroscopy

- ✓ Free Radical Chemistry: ESR is extensively used to detect and quantify free radicals in chemical reactions, combustion processes, and environmental samples.
- ✓ Biological Systems: Identifying radicals in biomolecules, studying oxidative stress, and characterizing metalloenzymes.
- ✓ Material Science: Investigating defects in semiconductors, conducting polymers, and paramagnetic centers in catalysts.
- ✓ Environmental Science: Monitoring pollutants and assessing radiation-induced radicals in materials.
- ✓ Spin Labeling: Using spin probes or spin labels to study molecular dynamics, conformational changes, and interactions in biological macromolecules.

Limitations and Complementary Techniques

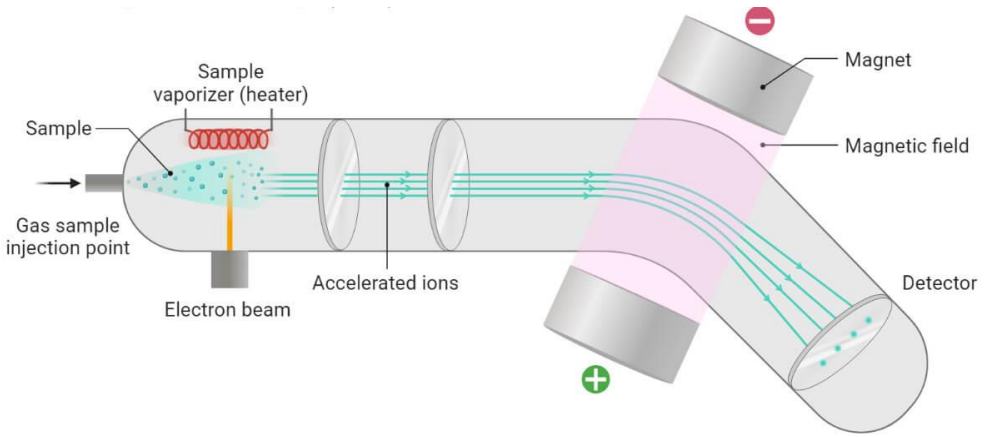
While ESR is highly sensitive to unpaired electrons, it cannot detect diamagnetic species. This limitation can be addressed by coupling ESR with complementary techniques such as UV-Vis spectroscopy for electronic transitions, or NMR spectroscopy for nuclear interactions. Additionally, low sensitivity to dilute systems and overlapping spectra in complex mixtures can be mitigated using advanced ESR methods or isotopic labeling.

Mass Spectrometry

Principles and Applications

Mass spectrometry (MS) is a powerful analytical technique used to measure the mass-to-charge ratio (m/z) of ions. It provides detailed information on the

molecular weight, structural composition, and chemical properties of analytes, making it indispensable in diverse fields such as chemistry, biology, and material science. The basic principle involves ionizing chemical compounds to generate charged species and separating them based on their mass-to-charge ratio using electromagnetic fields.



Ionization Techniques

The ionization process is critical in mass spectrometry, and various techniques are employed depending on the sample type and analytical requirement. Common ionization methods include:

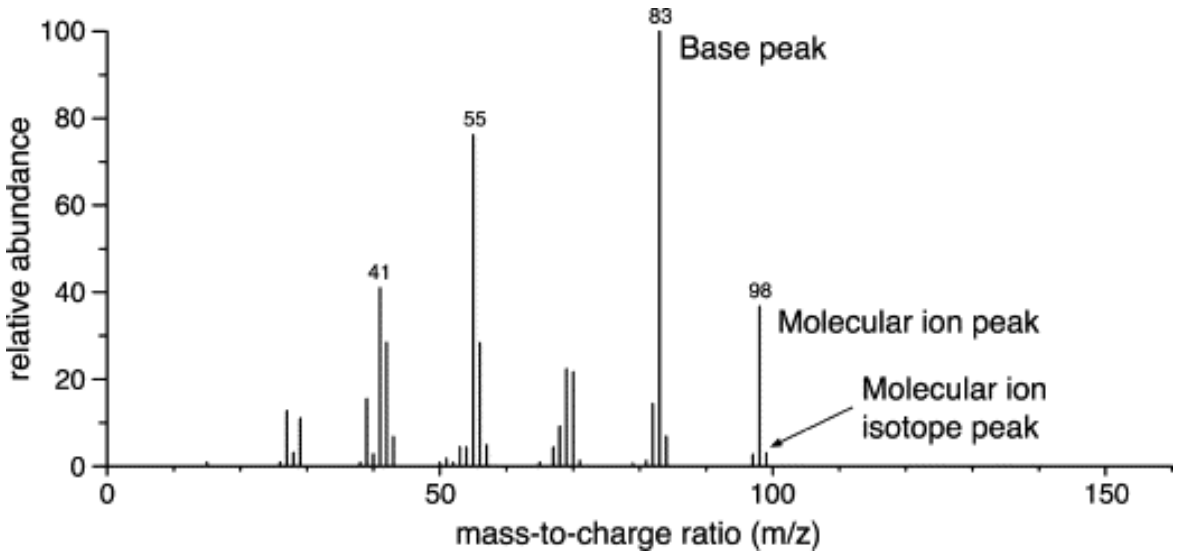
1. Electron Impact (EI): High-energy electrons collide with molecules, resulting in fragmentation. This technique provides detailed structural information.
2. Chemical Ionization (CI): A softer ionization method that produces fewer fragments, suitable for determining molecular weights.
3. Matrix-Assisted Laser Desorption/Ionization (MALDI): Used for large biomolecules like proteins and polymers, where a matrix absorbs laser energy to assist ionization.
4. Electrospray Ionization (ESI): Generates ions from liquid samples and is widely used for biomolecules and non-volatile compounds.
5. Field Ionization (FI): Involves the ionization of gaseous samples through high electric fields.

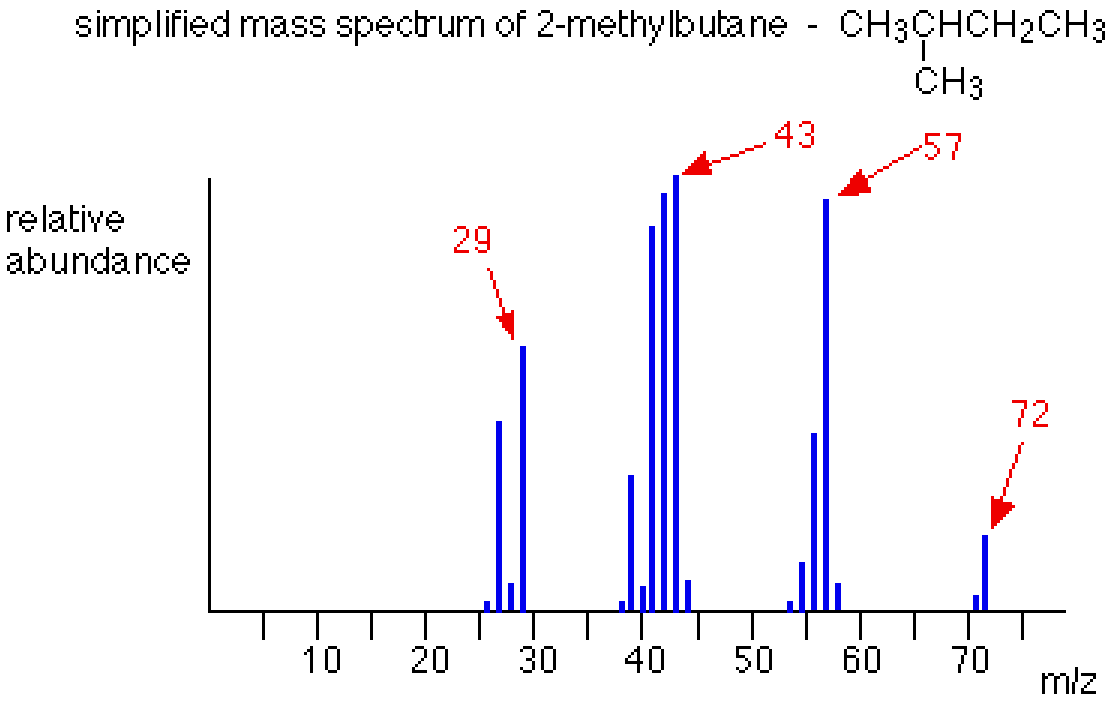
Mass Analyzers

Mass analyzers are the core components of a mass spectrometer, responsible for separating ions based on their m/z values. Different types of analyzers include:

1. Quadrupole: Employs oscillating electric fields to filter ions. It is compact and suitable for routine analysis.
2. Time-of-Flight (TOF): Measures the time it takes for ions to travel a fixed distance, providing high resolution and mass accuracy.
3. Ion Trap: Traps ions using electric or magnetic fields and releases them sequentially for analysis.
4. Fourier Transform Ion Cyclotron Resonance (FT-ICR): Offers ultra-high resolution by detecting ion cyclotron frequencies.
5. Orbitrap: Combines high resolution with accurate mass measurements, suitable for complex mixtures.

Data Interpretation in Mass Spectrometry





The interpretation of mass spectra involves analyzing the m/z values and relative intensities of peaks. Key features include:

1. Molecular Ion Peak (M^+): Represents the intact ionized molecule and provides the molecular weight.
2. Fragment Ions: Arise from the breakdown of the molecular ion, offering insights into the molecule's structure.
3. Isotopic Peaks: Reflect the natural abundance of isotopes, aiding in the identification of elements like Cl or Br.
4. Adduct Peaks: Formed by the association of analytes with ions like H^+, Na^+, or K^+, commonly observed in ESI and MALDI.

i. Quantitative and Qualitative Analysis

ii. Mass spectrometry is employed for both quantitative and qualitative purposes. Quantitative analysis involves measuring the concentration of analytes using calibration curves or internal standards. Qualitative analysis focuses on identifying unknown compounds, elucidating molecular structures, and studying reaction mechanisms. Tandem mass spectrometry (MS/MS) enhances the capability for structural elucidation by fragmenting specific ions and analyzing the resulting products.

Applications of Mass Spectrometry

1. Proteomics and Genomics: Identifying and characterizing proteins, peptides, and nucleotides.
2. Environmental Analysis: Detecting pollutants and analyzing complex environmental samples.
3. Pharmaceutical Industry: Drug discovery, pharmacokinetics, and quality control.
4. Material Science: Characterizing polymers, nanoparticles, and surface coatings.
5. Clinical Diagnostics: Biomarker discovery and metabolomics.

Advanced Techniques in Mass Spectrometry

1. High-Resolution Mass Spectrometry (HRMS): Provides precise mass measurements for exact mass determination and formula identification.
2. Tandem Mass Spectrometry (MS/MS): Enables the study of specific ions and their fragmentation patterns.
3. Imaging Mass Spectrometry (IMS): Maps the spatial distribution of compounds on surfaces, useful in biology and materials research.
4. Ambient Ionization Techniques: Allow real-time analysis of samples in their natural state without extensive preparation.

Limitations and Complementary Techniques

Despite its versatility, mass spectrometry has limitations, such as difficulties in analyzing non-ionizable or highly volatile compounds and the need for extensive sample preparation in certain cases. These challenges can be mitigated by combining MS with complementary techniques such as chromatography (GC-MS, LC-MS) for separation and Nuclear Magnetic Resonance (NMR) spectroscopy for structural confirmation. Additionally, advancements in software and databases enhance the interpretation and automation of mass spectrometric data, making it a cornerstone of modern analytical science.