MARRI LAXMAN REDDY INSTITUTE OF PHARMACY

(Approved by AICTE & PCI, New Delhi and Affiliated to JNTUH)

Dundigal - Gandimaisamma (V) &(M), Medchal (Dt), Hyderabad, Telangana - 500 043.

INSTRUMENTAL METHODS OF ANALYSIS

LAB MANUAL

B. PHARMACY IV-I

About MLRIP

VISION To be an educational Institute of par excellence and produce competent pharmacy professionals to serve the community through research and the ever-increasing needs of Industry.

MISSION
1. Imparting quality education and innovative research for various career opportunities.
2. Creating conducive academic environment to produce competent pharmacy professionals.
3. Indoctrination of students adorned with high human values and make them aware of their responsibility as health care professionals.

Program Educational Objectives
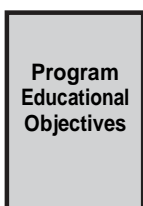
PEO 1: To produce graduates with sound theoretical knowledge and technical skills required for their career opportunities in various domains.

PEO 2: To incite the students towards research and to address the challenges with their innovative contributions for the benefit of the mankind.

PEO 3: To instill the essence of professionalism, ethical commitment to become a health care professional with sound integrity and adherence to the core human values in the service of the society.

PROGRAM OUTCOMES

1. **Pharmacy Knowledge:** Possess knowledge and comprehension of the core and basic knowledge associated with the profession of pharmacy, including biomedical sciences; pharmaceutical sciences; behavioral, social, and administrative pharmacy sciences; and manufacturing practices.

2. **Planning Abilities:** Demonstrate effective planning abilities including time management, resource management, delegation skills and organizational skills. Develop and implement plans and organize work to meet deadlines.

3. **Problem analysis:** Utilize the principles of scientific enquiry, thinking analytically, clearly and critically, while solving problems and making decisions during daily practice. Find, analyze, evaluate and apply information systematically and shall make defensible decisions.

4. **Modern tool usage:** Learn, select, and apply appropriate methods and procedures, resources, and modern pharmacy-related computing tools with an understanding of the limitations.

5. **Leadership skills:** Understand and consider the human reaction to change, motivation issues, leadership and team-building when planning changes required for fulfillment of practice, professional and societal responsibilities. Assume participatory roles as responsible citizens or leadership roles when appropriate to facilitate improvement in health and well-being.

6. **Professional Identity:** Understand, analyze and communicate the value of their professional roles in society (e.g. health care professionals, promoters of health, educators, managers, employers, employees).

7. **Pharmaceutical Ethics:** Honour personal values and apply ethical principles in professional and social contexts. Demonstrate behavior that recognizes cultural and personal variability in values, communication and lifestyles. Use ethical frameworks; apply ethical principles while making decisions and take responsibility for the outcomes associated with the decisions.

8. **Communication:** Communicate effectively with the pharmacy community and with society at large, such as, being able to comprehend and write effective reports, make effective presentations and documentation, and give and receive clear instructions.

9. **The Pharmacist and society:** Apply reasoning informed by the contextual knowledge to assess societal, health, safety and legal issues and the consequent responsibilities relevant to the professional pharmacy practice.

10. **Environment and sustainability:** Understand the impact of the professional pharmacy solutions in societal and environmental contexts, and demonstrate the knowledge of, and need for sustainable development.

11. **Life-long learning:** Recognize the need for and have the preparation and ability to engage in independent and life-long learning in the broadest context of technological change. Self-assess and use feedback effectively from others to identify learning needs and to satisfy these needs on an ongoing basis.

R17 B. PHARMACY IV YEAR

PS709: INSTRUMENTAL METHODS OF ANALYSIS LAB

B.Pharm. IV Year I Sem.   L/T/P/C
                                                                                                              0/0/4/ 2

List of Experiments:
1. Determination of absorption maxima and effect of solvents on absorption maxima of organic compounds
2. Estimation of dextrose by colorimetry
3. Estimation of sulfanilamide by colorimetry
4. Simultaneous estimation of ibuprofen and paracetamol by UV spectroscopy
5. Assay of paracetamol by UV- Spectrophotometry
6. Estimation of quinine sulfate by fluorimetry
7. Study of quenching of fluorescence
8. Determination of sodium by flame photometry
9. Determination of potassium by flame photometry
10. Determination of chlorides and sulphates by nephelo turbidometry
11. Separation of amino acids by paper chromatography
12. Separation of sugars by thin layer chromatography
13. Separation of plant pigments by column chromatography
14. Demonstration experiment on HPLC
15. Demonstration experiment on Gas Chromatography

Recommended Books (Latest Editions):
1. Instrumental Methods of Chemical Analysis by B.K Sharma
2. Organic spectroscopy by Y.R Sharma
3. Text book of Pharmaceutical Analysis by Kenneth A. Connors
4. Vogel's Text book of Quantitative Chemical Analysis by A.I. Vogel
5. Practical Pharmaceutical Chemistry by A.H. Beckett and J.B. Stenlake
6. Organic Chemistry by I. L. Finar
7. Organic spectroscopy by William Kemp
8. Quantitative Analysis of Drugs by D. C. Garrett
9. Quantitative Analysis of Drugs in Pharmaceutical Formulations by P. D. Sethi
10. Spectrophotometric identification of Organic Compounds by Silverstein

1. SEPARATION AND IDENTIFICATION OF AMINO ACIDS BYASCENDING PAPER CHROMATOGRAPHY

Aim: To separate and identify the given amino acids by Ascending Paper Chromatography.

Apparatus and glass ware: Chromatographic chamber, spraying gun, capillary tubes and whatman grade filter paper

Chemicals:

    Solvent System: N-butanol, Acetic acid and Water

    Visualizing Agent: Ninhydrin solution

    Standard references: Aminoacids

Principle:

The principle involved is **partition**, where the substances are distributed or partitioned between to liquid phases. One phase is the water which is held in pores of filter paper used and other phase is that of mobile phase which moves over the paper. The compounds in the mixture get separated due to differences in their affinity towards water (in stationary phase) and mobile phase during the movement of mobile phase under the capillary action of pores in the paper

The principle can also be adsorption chromatography between solid and liquid phases, where in the stationary phase is the solid surface of paper and the liquid phase is of mobile phase. But most of the applications of paper chromatography work on the principle of partition chromatography i.e. partitioned between to liquid phases. Identification of amino acids in the given mixture is determined by R_f value

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

    R_f value is less than one for all compounds.

Preparation of solutions:

    Solution A: 0.1 gm of Methionine in required quantity of suitable solvent

Solution B: 0.1 gm of Valine in required quantity of suitable solvent

Solution C: 0.1 gm of in required quantity of suitable solvent

Solution D: unknown mixture

Mobile phase: N-butanol : Acetic acid : Water (4 : 1 : 5)

Ninhydrin Solution: 0.2% (200mg in 100ml of N-butanol)

Procedure:

a. **Preparation of mobile phase**: 40 ml of N-butanol, 10 ml of acetic acid and 50 ml of water were taken in a beaker and mixed well.

b. **Preparation of sample**: 0.1 gm of ariginine, 0.1 gm of valine and 0.1 gm of methionine were taken separately and dissolved in required quantity of ethanol .

c. **Application of sample**: One drop of individual sample solutions were applied to chromatographic paper with capillary tube.

Procedure of development of chromatogram

- Take a whatman filter paper and draw a thin straight line of about 2cm from the bottom of the paper.
- Mark four points with equidistance on the straight line and number them.
- Prepare sample solutions and place a drop of these solutions on the straight line by using capillary tube.
- Hang the chromatographic paper in chamber containing mobile phase, Run the chromatogram till the mobile phase travels on ¾th of the chromatographic paper.
- Remove the paper from the chamber and mark the solvent distance with a pencil and dry in air for 15 minutes
- Now spray ninhydrin solution to the chromatogram and dry it in oven for 10-15 minutes
- Measure the distance of purple colour spots from the baseline and also the distance of travelled by mobile phase.
- Calculate the R_f values for the identification of amino acids in the given mixture.

R_f = Distance travelled by solute/ Distance travelled by solvent

Observation:

S.No	Aminoacid	Distance travelled By amino acid	Distance travelled By solvent front	R_f value

Report:

The R_f values of **standard or reference samples** are:

Arginine :

Valine:

Methionine:

R_f Vlues of amino acids in the mixture are: 2.

Based on the R_f Values , the mixture was found

Questions

1. Give the name and composition of mobile phase?

2. What is Retention factor and write the formula for Retention factor?

3. What are the units of R_f value ?

4. How do you detect amino acids in chromatography?

5. What is the principle involved in paper chromatography?

2. SEPARATION AND IDENTIFICATION OF AMINO ACIDS BY

RADIAL PAPER CHROMATOGRAPHY

Aim: To separate and identify the given amino acids by Radial Paper Chromatography.

Apparatus and glass ware: Chemicals: : Chromatographic chamber, spraying gun, capillary tubes and whatman grade filter paper

Chemicals:

Solvent System: N-butanol, Acetic acid and Water (**BAW System)**

Visualizing Agent: Ninhydrin solution

Standard references: Aminoacids

Principle:

The principle involved is partition .where in the substances are distributed or partitioned between to liquid phases. One phase is the water which is held in pores of filter paper used and other phase is that of mobile phase which moves over the paper. The compounds in the mixture get separated due to differences in their affinity towards water (in stationary phase) and mobile phase during the movement of mobile phase under the capillary action of pores in the paper

The principle can also be adsorption chromatography between solid and liquid phases, where in the stationary phase is the solid surface of paper and the liquid phase is of mobile phase. But most of the applications of paper chromatography work on the principle of partition chromatography

i.e. partitioned between to liquid phases. Identification of amino acids in the given mixture is determined by R_f value.

$$Rf = \frac{Distance\ travelled\ by\ solute}{Distance\ travelled\ by\ solvent}$$

R_f value is less than one for all compounds.

Preparation of solutions:

Solution A: 0.1 gm of Methionine in required quantity of suitable solvent

Solution B: 0.1 gm of Valine in required quantity of suitable solvent

Solution C: 0.1 gm of Methionine in required quantity of suitable solvent

Solution D: unknown mixture

Mobile phase: N-butanol : Acetic acid : Water (4 : 1 : 5)

Ninhydrin Solution: 0.2% (200mg in 100ml of N-butanol)

Procedure:

    d. **Preparation of mobile phase**: 40 ml of N-butanol, 10 ml of acetic acid and 50 ml of water were taken in a beaker and mixed well.

    e. **Preparation of sample**:0.1 gm of ariginine,0.1 gm of valine and 0.1 gm of methionine were taken separately and dissolved in required quantity of ethanol .

    f. **Application of sample**: One drop of individual sample solutions were applied to chromatographic paper with capillary tube.

Procedure of development of chromatogram

- Take a Whatman filter paper and draw a round circle in the middle of paper and make a wick exactly in the centre of the circle.
- Mark four points with equidistance on the circle line and number them.
- Prepare sample solutions and place a drop of these solutions on the points using capillary tube.
- Dip the wick of chromatographic paper in chamber containing mobile phase.
- Run the chromatogram till the mobile phase travels ¾th of the chromatographic paper.
- Remove the paper from the chamber and mark the distance travelled by solvent with a pencil and dry in air for 15 minutes
- Now spray ninhydrin solution to the chromatogram and dry it in oven for 10-15 minutes
- Measure the distance of purple colour spots, from the baseline and also the distance of travelling of mobile phase.
- Calculate the R_f values for the identification of amino acids in the given mixture.

    R_f = Distance travelled by solute/ Distance travelled by solvent

Observation:

S.No	Aminoacid	Distance travelled By amino acid	Distance travelled By solvent front	R_f value

Report:

The R_f values of **standard or reference samples** are:

Arginine :

Valine:

Methionine:

R_f Vlues of amino acids in the mixture are

Based on the R_f Values , the mixture was found

Questions

1) What is the difference between partition and adsorption chromatogarphy?

2) Give the name and composition of mobile phase?

3) What are the different development technique in paper chromatogarphy?

4) Discuss applications of paper chromatography techniques ?

3. SEPARATION AND IDENTIFICATION OF ALKALOIDS BY

THIN LAYER CHROMATOGRAPHY

Aim: To separate and identify alkaloids by thin layer chromatography

Apparatus and chemicals: Chromatographic chamber, TLC plates, capillaries, dryer, spray gun

Adsorbent: Silica gel G

Mobile phase: Methanol, Ammonia

Visualizing agent: Dragendroff's reagent

Standard references: Ephedrine, Atropine and Quinine

Principle:

TLC is based on the principle of adsorption. The stationary phase used in TLC is adsorbents like silica gel coated onto a inert solid support such as glass plate, mobile phase is either single solvent or mixture of solvents based on the chemical nature of sample i.e. polarity . Sample should be dissolve in mobile phase. Silicagel contains some free Si-OH groups these groups form hydrogen bonds or other vanderwaal interactions with the analyte components, thus adsorption takes place. Identification of amino acids in the given mixture is determined by R_f value.

$$Rf = \frac{Distance\ travelled\ by\ solute}{Distance\ travelled\ by\ solvent}$$

Perpration of solutions:

Solution A: 0.1 g of Atropine in required quantity of ethanol

Solution B: 0.1 g of Quinine in required quantity of ethanol

Solution C: 0.1 g of A & B

Mobile phase: Methanol: Ammonia(200: 3)

Visualizing Agent: Dragendroff's reagent

Procedure:

    a. **Preparation of mobile phase:** Take 200 ml of methanol and 3 ml of ammonia in a beaker and mix well, keep a side for 20-30 min to get saturation of mobile phase

    b. Preparation of sample: Take 0.1 g of ephedrine, 0.1 g of atropine and 0.1 g of quinine in individual separate volumetric flasks and dissolved in required quantity of methanol.

    c. Application of sample: Make a straight horizontal line 1cm above from the bottom of TLC plate. Apply one drop of individual sample solutions to respective spots on TLC plate with capillary tube.

Procedure for development of TLC plate

- Sample solutions are spotted on the plate by using capillary tubes. After drying of spots TLC plate has to dip in the development chambers which contain mobile phase.

Note: Dip the plate in such a way that the straight line on the TLC plate should not be immersed.

- Remove the plate from the chamber and mark the distance travelled by solvent with a pencil and dry in air for 5 minutes

- The sample s were visualized using Dragendroff's reagent after getting the solvent front.
- TLC plates were dried in an oven to visualize the samples
- The R_f values were measured and reported.

Report: The R_f values of **standard reference samples** are:

    Ephedrine

    Atropine:

    Quinine:

    R_f values of alkaloids in the mixture are: 1.   2.

    Based on the R_f Values , the mixture was found to contain   and

Questions

1. Write the principle for Thin layer Chromatography.
2. Writ the chemicals and reagents required for Thin layer chromatography.
3. Write the adsorbents required for preparation of TLC plates.
4. Write the visualizing agent & mobile phase required for TLC
5. Discuss the applications of TLC.

6. What are the other methods to detect the spots.

4. **HIGH PERFORMANCE LIQUID CHROMATOGRAPHY**

It is a technique in analytical chemistry used to separate the components in a mixture, to identify each component, and to quantify each component. The placement(injection) of a small volume of a liquid sample into a tube packed with porous particles(stationary phase).Where individual components of a sample are transported along the column by a liquid moved by pressure. This is called High Performance Liquid Chromatography as this technique is simple and able to detect components at nanogram level.

It is also called High Pressure Liquid Chromatography as mobile phase is pumped with high pressures

Principle involved in separation by HPLC

> ➢ The separation of compounds is due to their relative differences in travel through the column on application of pressure exerted through mobile phase or carrying liquid

> ➢ The compounds of the mixture travel with different rates due to their relative affinities with the solvent and stationary phase.

> ➢ Compound with higher affinity towards stationary phase of the column travels slowly and vice-versa.

> ➢ The above principle is similar to that of column chromatography but in HPLC, the separation is more effective due to greater surface area achieved due to very small particle size of stationary phase in comparison to that used in column chromatography.

Types of HPLC techniques

Based on modes of chromatography

1. Normal phase : Stationary phase is polar, mobile phase is non polar
2. Reverse phase: Stationary phase is non polar, mobile phase is polar

Different columns used include ODS,C_{18},C_8

Based on elution technique

1. Isocratic separation: Polarity of mobile phase is same in entire procedure
2. Gradient separation: Polarity of mobile phase is gradually increasing, to get better separation.

Components of HPLC system

- ✓ Mobile phase reservoirs

- ✓ Degassing units

- ✓ HPLC Pumps

- ✓ Mixing valves

- ✓ Sample injector (manual or auto)

- ✓ Guard columns

- ✓ Column

- ✓ Column ovens

- ✓ Detector

- ✓ Recorder and integrator

- ✓ Mobile phase waste container
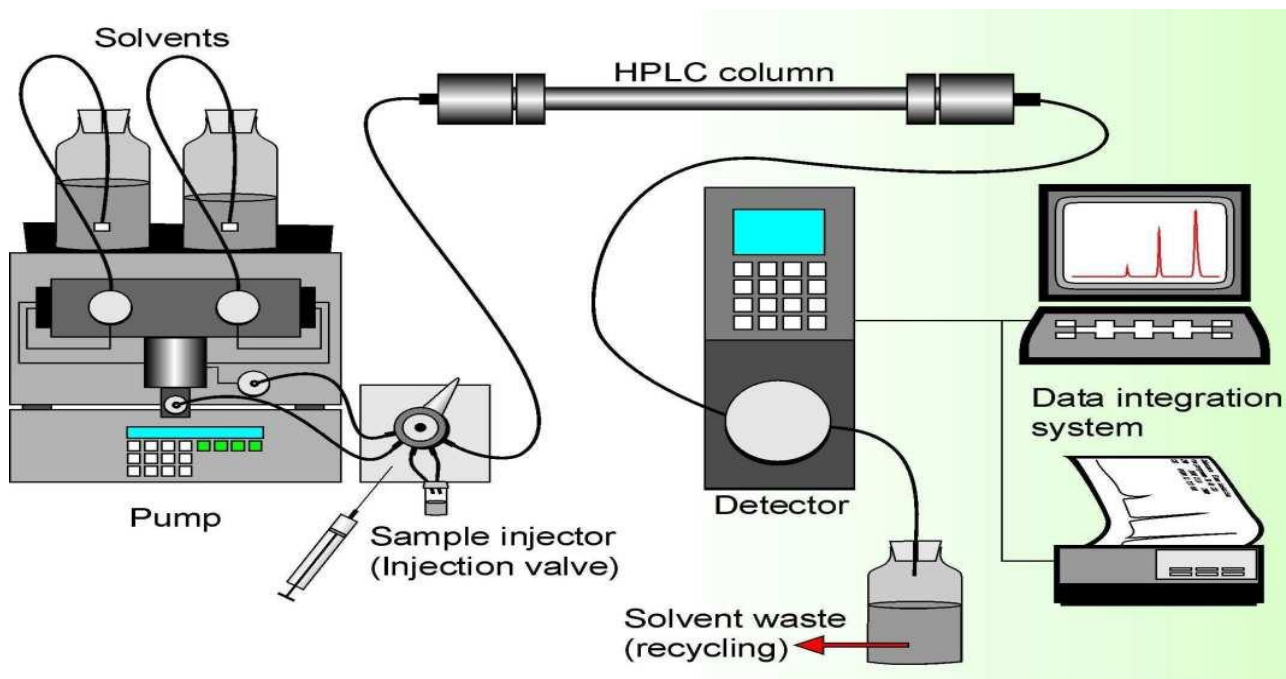
BLOCK DIAGRAM OF HPLC
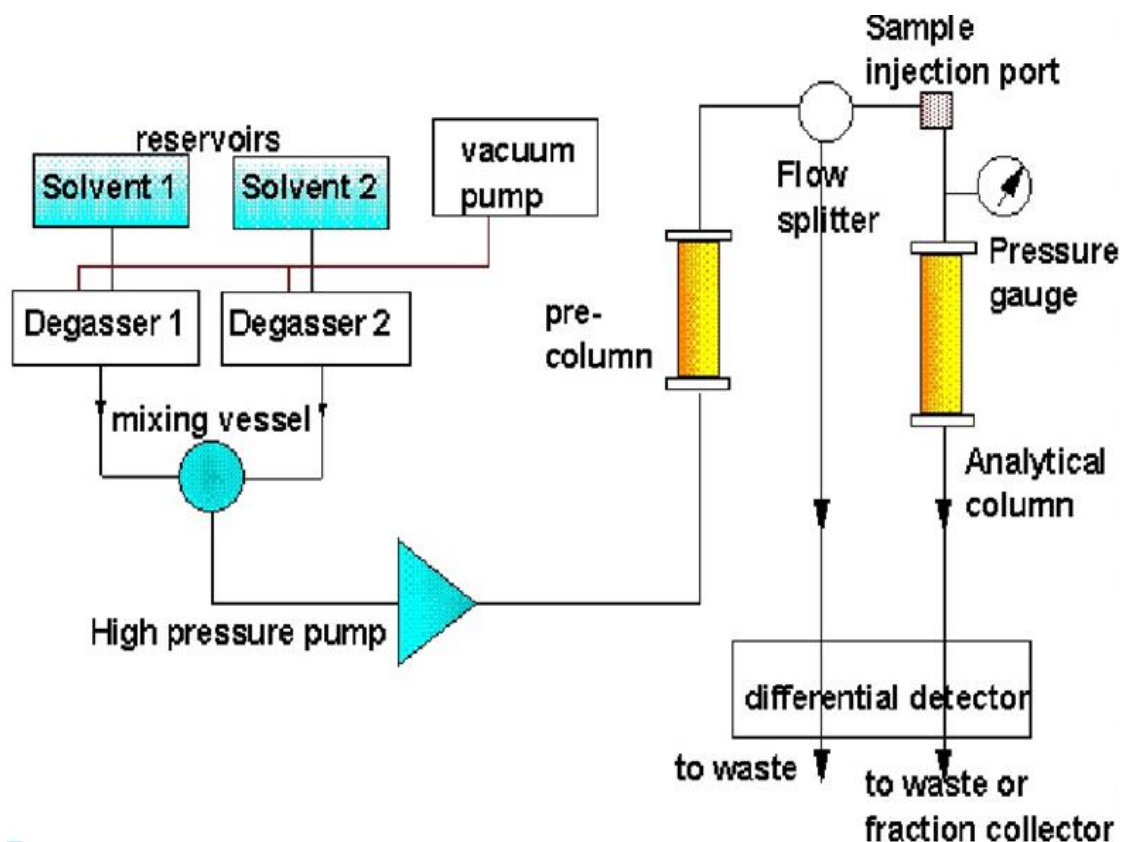
Degassing units

> ➢ Degassing of the mobile phase can be done with reservoir of inert gases He or N_2
> ➢ By applying vacuum
> ➢ Ultrasonication (converts ultra high frequency to mechanical vibrations)

SOLVENT DELIVERY PUMPS

To produce an appropriate pressure to push solvent into the column.

A pump capable of pumping solvent up to a pressure of 4000 psi and at flows of up to 10 ml/min

Types of pumps:

➢   Direct Gas-Pressure pumps

➢   Syringe type pumps
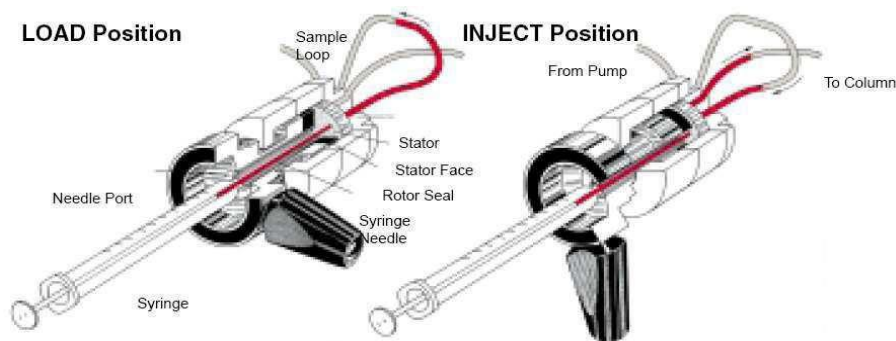
➢   Pneumatic pumps

➢   Reciprocating pumps

Reciprocating pumps are widely used as they maintain accurate flow rate

Solvent delivery systems

➢   Isocratic systems

➢   Low pressure gradient systems

➢   High pressure gradient systems

Injection systems

➢   Rheodyne injectors

➢   Syringe injectors

Rheodyne injector

Columns

The heart of the system is the column. Many different reverse phase columns will provide excellent specificity for any particular separation. It is therefore best to routinely attempt separations with a standard C8 or C18 column (e.g. Zorbax RX C8) and determine if it provides good separation. Types of columns include:

Guard column: Protect the analytical column from impurities and foreign substances, no

separation will be occurred in guard column

Analytical column: Separation of compounds will take place in analytical column.

- ➢ Length (5-15 cm) much shorter than GC column

- ➢ Diameter (4 mm to 50mm)

- ➢ Particle size (3- 10 mm)

Based up on the type of column material columns are classified into\

- ➢ Normal phase columns

- ➢ Reverse phase columns

- ➢ Size exclusion columns

- ➢ Ion exchange columns

- ➢ Chiral columns

HPLC Detectors

The **HPLC detector**, located at the end of the column, must register the presence of various components of the sample, but must not detect the solvent. For that reason there is no universal detector that works for all separations. A common HPLC detector is a **UV absorption detector**, as most medium to large molecules absorb UV radiation. Detectors that measure fluorescence and refractive index are also used for special applications. A relatively new development is the combination of an HPLC separation with an **NMR detector**. This allows the pure components of the sample to be identified and quantified by nuclear magnetic resonance after having been separated by HPLC, in one integrated process.

List of most common HPLC detectors

- • UV-Visible

- • Refractive Index

- • Fluorescence

- • Conductivity (for ion chromatography)
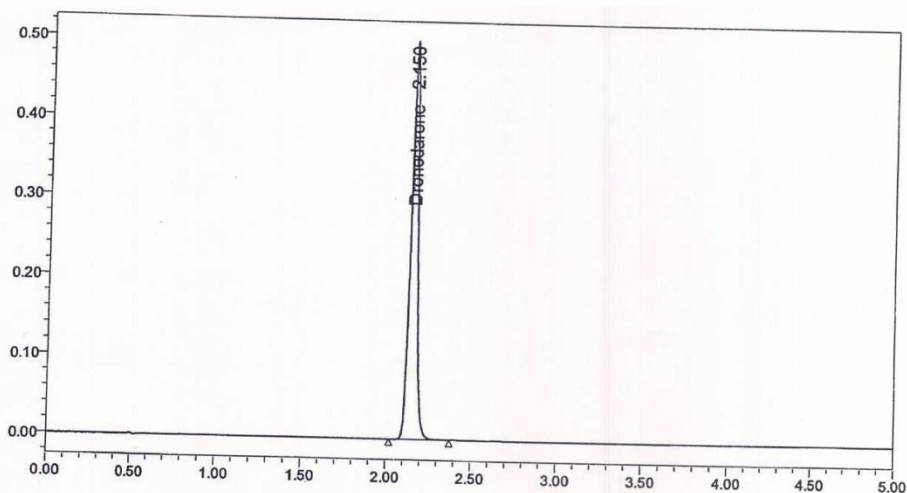
- • Photodiode array detector

- Amperometric detector

- Mass spectroscopy detectors

- NMR detectors

RECORDERS&INTEGRATORS

Recorders : Used to record the responses

Integrators: Used to data processing, record individual peaks with R_t, height, width of peaks, peak area, % of area

Typical HPLC chromatogram

APPICATIONS OF HPLC

➢ Pharmaceutical field

➢   Chemical and Petrochemical industry

➢   Forensic studies

➢   Biochemical separations

➢   Food analysis

- ➢ Qualitative analysis : Checking the purity of a compound

- ➢ Quantitative Analysis

  - ✓ Direct Comparison method

  - ✓ Calibration curve method

  - ✓ Internal standard method

- ➢ HPLC is used to analyze raw materials and finished products to assure that pre-established quality levels are being met.

- ➢ Multi component analysis

- ➢ Isolation and identification of drugs

- ➢ Stability studie

5. DETERMINATION OF λ MAX OF KMNO4 BY COLORIMETRY

Aim: To determine λ max of KMnO4 solution.

Instrument: colorimeter

Apparatus: 100ml volumetric flask, test tubes, beakers

Chemicals: KMnO4 solution, distilled water

Principle:

Spectroscopy is the tool for study of atomic and molecular structure. It deals with interaction of electronic radiation with matter involving the measurement and interpretation of the extension of absorption or emission of electromagnetic radiation by molecule.

λ_{max} is defined as wavelength at which maximum absorption of radiation takes place. The extent to which a sample absorbs light depends strongly upon the wavelength of light and type chromophore present in the analyte. Chromophore is a functional group or a part of the molecule responsible for the absorption of electromagnetic radiation at a specific frequency. λ_{max} is characteristic of a compound and provides information of the electronic transitions occurs in the analyte. In order to obtain the highest sensitivity and to minimize deviations from Beer's Law, analytical measurements are made using light with a wavelength of λ_{max}.

NOTE: λ_{max} *is* independent from the concentration of the analyte.

Procedure:

➢ Prepare a solution of 100 µg/ml of $KMnO_4$
➢ Switch on the instrument and warm up for 15 minutes.
➢ Using distilled water as blank adjust to 100% transmittance or zero absorbance.
➢ Take the solution of $KMnO_4$ in cuvette and measure the absorbance from 400-800 nm with interval of 40nm.
➢ Plot the graph between wavelength v_s absorbance and note the wavelength at which maximum absorbance is observed.

Observation:

S.No	Wavelength (nm)	Absorbance
1	400	
2	440	
3	480	
4	520	
5	560	
6	600	
7	640	
8	680	
9	720	
10	760	
11	800	

Report:

The λ $_{max}$ of potassium permanganate ($KMnO_4$) was found to be …… nm.

Reference:

A Practical Approach to Pharmaceutical Analysis, Nema et al, CBS Publications, P5-6.

Viva questions:

1. Define λ $_{max}$.
2. What is mean by wave length and write the units.
3. Write the wave length range of UV and Visible light.
4. Define chromophore.
5. Write the wavelength ranges in VIBGOYR

6. DETERMINATION OF λ $_{MAX}$ OF PARACETAMOL SOLUTION BY UV-VISIBLE SPECTROPHOTOMETER

Aim: To determine λ $_{max}$ of paracetamol solution

Instrument: UV-Visible Spectrophotometer

Apparatus: 100ml volumetric flask, test tubes, beakers

Chemicals:, paracetamol, distilled water

Principle:

Spectroscopy is the tool for study of atomic and molecular structure. It deals with interaction of electronic radiation with matter involving the measurement and interpretation of the extension of absorption or emission of electromagnetic radiation by molecule.

λ$_{max}$ is defined as wavelength at which maximum absorption of radiation takes place. The extent to which a sample absorbs light depends strongly upon the wavelength of light and type chromophore present in the analyte. Chromophore is a functional group or a part of the molecule responsible for the absorption of electromagnetic radiation at a specific frequency. $λ_{max}$ is characteristic of a compound and provides information of the electronic transitions occurs in the analyte. In order to obtain the highest sensitivity and to minimize deviations from Beer's Law, analytical measurements are made using light with a wavelength of $λ_{max}$.

NOTE: $λ_{max}$ *is* independent from the concentration of the analyte.\

Procedure:
- ➢ Prepare a solution of 100 µg/ml of paracetamol.
- ➢ Switch on the instrument and warm up for 15 minutes.
- ➢ Using distilled water as blank adjust to 100% transmittance or zero absorbance.
- ➢ Take the solution of paracetamol in cuvette and scan from 200-400 nm.
- ➢ Repeat its for 3-6 times and consider the wavelength at which maximum absorption of radiation takes place. It is known as $λ_{max.}$

Report:

The λ $_{max}$ of potassium paracetamol was found to be …… nm.

7. EFFECT OF SOLVENTS ON ABSORPTION MAXIMA OF DELTIAZEM HCl

Aim: To determine the effect of solvents on absorption maxima of deltiazem HCl

Apparatus: Volumetric flask, pipette, beaker, glass rod

Chemicals: Deltiazem HCl, distilled water, methanol, NaoH

Principle:
λ_{max} is a constant value in a particular solvent as the solvent system varies, the λ_{max} also varies, The shift in absorption maxima depends on the polarity of solvent and the pH of the solvent.

Molecule which undergo π-π^* transition, the π^* state is more polar and stabilized more in polar solvent relative to nonpolar, thus in going from nonpolar to polar solvent there is a **red shift or bathochromic shift** (increase in λ_{max}).

For n-π^* transition, the n state is much more easily stabilized by polar solvent (H-bonds and association), so in going from nonpolar to polar solvent there is a **blue shift or hypsochromic shift** (decrease in λ_{max}).
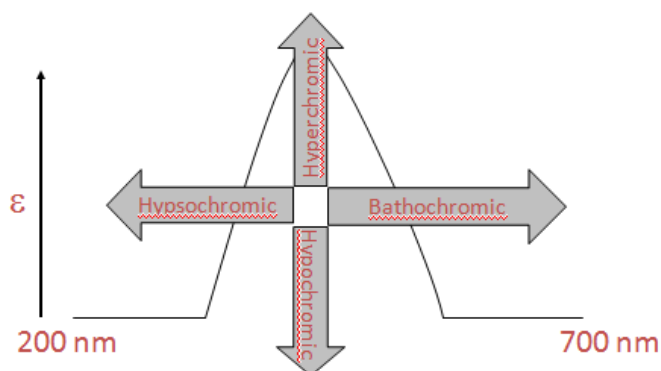
Procedure:

Weigh accurately 100mg of deltiazem HCl powder and add distilled water to make it 100ml in a volumetric flask, shake well and take 10 ml of above solution and dilute to 100ml with distilled water. Scan the above solution to get spectrum against the distilled water as blank. Similarly make the deltiazem HCl solution by using methanol and NaOH as solvents. Scan the above solution to get spectrum against the methanol and NaOH as blanks . Finally overlap all the spectras and observe the shift in λ_{max}

Report:

The absorption maxima using water was found at   nm

The absorption maxima using methanol was found at   nm

The absorption maxima using NaOH was found at   nm

Questions:

1.Discuss the effect of solvent on absorption maxima.

2. Define Hypsochromic shift with examples.

3. Define Bathochromic shift with examples.

4.Write the principle of Beer-Lamberts law.

5.Explain about different types of electronic transitions involved in Absorption spectroscopy.

8. ESTIMATION OF SALICYLIC ACID BY COLORIMETRY

AIM: To estimate the amount salicylic acid in given sample by colorimetric method.

CHEMICALS: Salicylic acid, Hydrochloric acid (1%v/v), Ferric chloride, Distilled water.

PRINCIPLE:

The increasing concentrations of salicylic acid is treated with 1% ferric chloride reagent (1g FeCl3 in 100 mL of 1% Hydrochloric acid). The free phenolic hydroxyl group present in salicylic acid reacts with the reagent and forms a violet colored complex i.e., ferric salicylate which is proportional to the concentration of salicylic acid.

PROCEDURE: Ferric chloride reagent is prepared by adding 1 gm of FeCl3 to 100 ml of 1% HCl (1mL concentrated hydrochloric acid added to 100mL of distilled water). Stock solution of salicylic acid (1mg/ml) is prepared by dissolving 100 mg of salicylic acid in few ml of methanol and made up to 100 ml with distilled water in a volumetric flask. 10 ml of this stock solution is diluted with 100 ml distilled water to get 100 μg/ml salicylic acid solution. Take the respective samples in each test tube, add the reagent and distilled water to make total volume of 10 ml (as per mentioned in table) and measure the absorbance of the violet colored complex usingUV-Visible spectrophotometer or colorimetry at wavelength of 525 nm against blank sample (without salicylic acid).

Volume of stock solution (mL)	Volume of reagent (mL)	Distilled water to make 10 mL	Concentration of Salicylic acid (μg/mL)	Absorbance
0	1	9	0	0
1	1	8	10	
2	1	7	20	
3	1	6	30	
4	1	5	40	

5	1	4	50	
6	1	3	60	

Plot a graph taking concentration on X-axis and observed absorbance values on Y-axis, draw a best fit line and record r2 value (regression coefficient) and equation of straight line.

REPORT:

9.ESTIMATION OF PARACETAMOL IN TABLETS BY USING

STANDRAD ABSORPTIVITY VALUE

Aim: To estimate the percentage purity of Paracetemol percent in the given sample

Apparatus: Mortar and pestle, beaker, standard flasks, funnel

Chemicals: Paracetemol tablets, water, NaOH

Principle:

Estimation of paracetamol by UV- Visible spectrophotometer depends on the Beer's -Lambert's law

Beer's law :States that "the intensity of a beam of monochromatic light decreases exponentially with increase in the concentration of absorbing species arithmetically"

- $dI / dc \, \alpha \, I$

Lambert's law : states that the rate of decrease of intensity (monochromatic light) with the thickness of the medium is directly proportional to the intensity of incident light
$-dI / dt \, \alpha \, I$

Beer – lamberts law

$I = I_o \, e^{- \, kct}$

$I = I_o \, 10^{- \, kct}$ (converting natural algorithm to base 10)

$I / I_o = 10^{-kct}$ (rearranging terms)

$I_o / I = 10^{kct}$ (inverse on both side)

$Log \, I_o / I = kct$ (taking log on both sides) --- Equation 4

It can be learnt that transmittance $(T) = I / I_o$ and Absorbance $(A) = \log 1 / T$

Hence $A = \log 1 / I/ I_o$

$A = \log I_o /I$ --- Equation 5,   by Using Equation 4 & 5 , Since $A = \log I_o /I$ and $\log I_o /I = Kct$ we can infer that $A = Kct$ (instead of K, we can use ε)

A= εct or A= act or A= $A^{1\%}_{1cm}$ ct

Where:

A = Absorbance or optical density

ε = Molecular extinction coefficient

c = Concentration of the drug (mol/lit)

t = Path length (normally 10mm or 1cm)

Paracetemol is chemically known as Para-acetyl-aminophenol. Estimation of Paracetemol concentration in tablets can be alone done by U.V. spectrophotometry by using standard Absorptivity value ($A^{1\%}_{1cm}$). In this the absorbance of the diluted sample solution is observed at

257nm. From this calculate the concentration of Paracetemol taking 715 on A $^{1\%}_{1cm}$ value at maximum of about 257nm

Procedure:

- Weigh and powder 20 tablets
- Take the powder equivalent to 0.15gm
- Add 50ml of 0.1 M NaOH and dilute to 100ml with water
- Shake the mixture for 15 minutes and add sufficient water to produce 200ml
- The resulting solution is mixed and filtered
- Take 10ml of above solution and dilute to 100ml with water
- To 10ml of resulting solution add 10ml of 0.1 N NaOH
- Make up the volume to 100ml with water and mix it
- Measure the absorbance of resulting solution at 257nm by using 715 as A $^{1\%}_{1cm}$ value

Report:

The purity of Paracetemol in the given sample of tablet powder was found to be

Questions:

1. Write the principle for standard absorptivity value of Paracetamol in tablets.
2. Write the absorptivity value of Paracetamol
3. Write the λ max of Paracetamol
4. Write the dilution factor in the above given procedure.
5. Explain application of spectrophotometery.

10.ESTIMATION OF PARACETAMOL BY USING CALIBRATION CURVE METHOD

AIM: To estimate the percentage purity of Paracetamol present in the given sample

APPRATUS: Beaker, glass rod, volumetric flask, measuring cylinder, pipette

CHEMICALS: Paracetamol powder, Paracetamol tablets, NaOH, water

PRINCIPLE:

The assay of light absorbing substance may be quickly carried out by using Beer – Lamberts Law.

Beer's law :States that "the intensity of a beam of monochromatic light decreases exponentially with increase in the concentration of absorbing species arithmetically"

$- dI / dc \, \alpha \, I$

Lambert's law : states that the rate of decrease of intensity (monochromatic light) with the thickness of the medium is directly proportional to the intensity of incident light
$-dI / dt \, \alpha \, I$

Beer – lamberts law
$I = I_o \, e^{-kct}$
$I = I_o \, 10^{-kct}$ (converting natural algorithm to base 10)
$I / I_o = 10^{-kct}$ (rearranging terms)
$I_o / I = 10^{kct}$ (inverse on both side)
$\text{Log } I_o / I = kct$ (taking log on both sides) --- Equation 4
It can be learnt that transmittance $(T) = I / I_o$ and Absorbance $(A) = \log 1 / T$
Hence $A = \log 1 / I / I_o$
$A = \log I_o / I$ --- Equation 5,   by Using Equation 4 & 5 , Since $A = \log I_o / I$ and $\log I_o / I = Kct$
we can infer that $A = Kct$ (instead of K, we can use ε)

$$A = \varepsilon ct \text{ or } A = act \text{ or } A = A^{1\%}_{1cm} \, ct$$

Where:
A = Absorbance or optical density
ε = Molecular extinction coefficient
c = Concentration of the drug (mol/lit)
t = Path length (normally 10mm or 1cm)

Paracetamol is chemically known as *N*-(4-hydroxyphenyl)acetamide. Estimation of paracetamol concentration in tablet can be done by UV- spectrophotometry using calibration curve method. In

this the absorbance of the diluted sample solution is observed at 257nm. From this concentration is calculated.

CALIBRATION CURVE: It is a standard graph or linear plot of serial standard dilutions.Graph plotted between concentration(x-axis) Vs response(Y-axis). The curve should be passed through the origin and r^2 value should be greater than 0.99 and less than 1.

PROCEDURE:

Preparation of stock solution: 100mg of paracetamol powder is dissolved in 100ml of 0.1N NaOH (1mg/ml)

Working standard: From the stock solution, 10 ml is taken and diluted to 100 ml with 0.1N NaOH (100µg/ml)

Dilutions of Linearity Range: From the working standard 1ml,2ml.3ml, 4ml,5ml is taken into individual volumetric flasks (10ml) and make up the volume by usimg 0.1N NaOH to get the concentrations about 10,20.30,40,50 µg/ml respectively.

Estimation of amount of Pracetamol in tablets:

- Weigh and powder 20 tablets
- Take the powder equivalent to 0.15gm
- Add 50ml of 0.1 M NaOH and dilute to 100ml with water
- Shake the mixture for 15 minutes and add sufficient water to produce 200ml
- The resulting solution is mixed and filtered
- Take 10ml of above solution and dilute to 100ml with water
- To 10ml of resulting solution add 10ml of 0.1 N NaOH
- Make up the volume to 100ml with water and mix it
- Measure the absorbance of resulting solution at 257nm

REPORT:

The purity of Paracetemol in the given sample of tablet powder was found to be

QUESTIONS:

1. Write the chemical name of Paracetamol
2. Calculate the dilution factor of given Paracetamol sample
3. What is mean by r^2 value and what is the limit for r^2
4. Write the relationship between absorbance and transmittance

11. ASSAY OF CHLORAMPHENICOL CAPSULES BY USING UV-VISIBLE SPECTROPHOTOMETRIC METHOD

Aim: To estimate the percentage purity of chloramphenicol percent in the given capsules

Appratus: Mortar and pestle, beaker, standard flasks, funnel
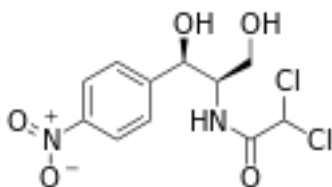
Chemicals: Chloramphenicol capsules, water

Principle:

The assay of an absorbing substance may be quickly carried out by using Beer – Lamberts Law.

$I = I_o\, e^{-kct}$

A= ε ct

Where: A = Absorbance or optical density. ε = Molecular extinction coefficient ,c = Concentration of the drug (mol/lit) ,t = Path length (normally 10mm or 1cm)

Chloramphenicol is chemically known as 2,2-dichloro-N-[(1R,2R)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]acetamide. Estimation of chloramphenicol concentration in capsule can be alone done by U.V. spectrophotometry by using standard Absorptivity value (A $^{1\%}_{1cm}$). In this the absorbance of the diluted sample solution is observed at 278nm. From this calculate the concentration of chloramphenicol by taking 298 as A $^{1\%}_{1cm}$ value.

Procedure:

- Weigh 20 capsules and mix the contents.
- Take the powder equivalent to 0.200gm of chloramphenicol
- Add 500ml of distilled water, warm gently to get a clear solution and dilute to 1000ml with distilled water
- Take 10ml of above solution and dilute to 100ml with distilled water
- To 10ml of resulting solution add 10ml of 0.1 N NaOH
- Measure the absorbance of resulting solution at 278 nm by using 298 as A $^{1\%}_{1cm}$ value

Report:

The purity of chloramphenicol in the given sample was found to be

Questions:

1. Write the limitations of Beer – Lambert's law.
2. Write the uses of chloramphenicol.
3. What is mean by A $^{1\%}_{1cm}$ value.
4. Define chromophore and find out the chromophore and auxochrome present in chloramphenicol .
5. Explain application of spectrophotometery.

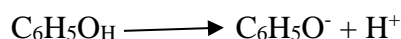## 12. EFFECT OF P^H ON ABSORPTION MAXIMA OF PARACETAMOL

AIM: To determine the effect of P^H on λ_{max} of paracetamol

APPRATUS: Volumetric flask, pipette, glass rod, beaker

CHEMICALS: Buffer – 4, 7, 9 capsules, Paracetamol, distilled water

PRINCIPLE:

High pH solutions absorb at higher wavelengths. Whereas low pH solutions absorb at lower wavelengths.

$$C_6H_5O_H \longrightarrow C_6H_5O^- + H^+$$

In the given equation when the equilibrium of the reaction lies to the right, then the wavelength of the absorbance would decrease, because the concentration of H^+ has increased (ie lower pH). The increased H^+ concentration stabilize the ground state as opposed to the excited electronic state leads to decrease in wavelength (hypsochromic (blue) shift), which in turn is an increase in energy. In other words, more energy is required for an electronic excitiation to occur (stabilised ground state by the H^+).

PROCEDURE:

- Weigh accurately 100mg of Paracetamol powder and add it to P^H-4 buffer in 100ml volumetric flask
- Shake well and mask it as solution A
- Similarly add 100mg of Paracetamol powder to P^H – 9 and P^H-7 buffer in 100ml volumetric flask
- Shake well and mark it as solution B and C respectively
- Prepare blank solutions of P^H 4, 7, 9
- Solution A is taken and spectrum is plotted by U.V. Visible spectrophotometer and λ_{max} is obtained against the blank P^H – 4
- Similarly plot for the solution B and C against P^H – 9 and P^H-7 as blank
- Overlap spectra and observe the shift in λ_{max} and report it

REPORT:

In neutral P^H – λ_{max} – ….. nm
In acidic P^H – λ_{max} – ….. nm
In alkaline P^H – λ_{max} – …. nm

13. EFFECT OF SOLVENT ON ABSORPTION MAXIMA OF PARACETEMOL

AIM: To determine the effect of solvent on absorption maxima

APPRATUS: Volumetric flask, pipette, beaker, glass rod

CHEMICALS: Paracetemol, distilled water, methanol

PRINCIPLE:

λ_{max} is a constant value in a particular solvent as the solvent system varies, the λ_{max} also varies,The shift in absorption band depends on the polarity of solvent and the solute. For $\pi \to \pi^*$ transition, the π^* state is more polar and stabilized more in polar solvent relative to nonpolar, thus in going from nonpolar to polar solvent there is a red shift or bathochromic shift (increase in λ_{max}). For $n \to \pi^*$ transition, the n state is much more easily stabilized by polar solvent (H-bonds and association), so in going from nonpolar to polar solvent there is a blue shift or hypsochromic shift (decrease in λ_{max}).

PROCEDURE:

Weigh accurately 100mg of Paracetemol powder and add distilled water to make it 100ml in a volumetric flask, shake well and scan the above solution to get spectrum against the distilled water as blank. Similarly make the paracetamol solution by using methanol as solvent. Scan the above solution to get spectrum against the distilled methanol as blank. Finally overlap both the spectra and observe the shift in λ_{max}

REPORT:

The absorption maxima using methanol was found at higher wavelength.

Questions:

1.Discuss the effect of solvent on absorption maxima.

2.Explain about Hypsochromic shift and Bathochromic shift.

3.Write the chemicals required for effect of solvent on Absorption maxima.

4.Write the principle for Beer-Lamberts law.

5.Explain about different electronic transitions involved in Absorption spectroscopy.

14. IR INTERPRETATION

Infrared spectroscopy (IR) measures the bond vibration frequencies in a molecule and is used to determine the functional groups and bonds present in chemical substance. The infrared region of the spectrum encompasses radiation with wave numbers ranging from about 12,500 to 50cm^{-1} (or) wave lengths from 0.8 to 200μ.

The infrared region constitutes 3 parts

- ✓ The near IR (0.8 -2.5 µm) (12,500-4000 cm^{-1})

- ✓ The middle IR (2.5 -15 µm) (4000-667 cm-^{1})

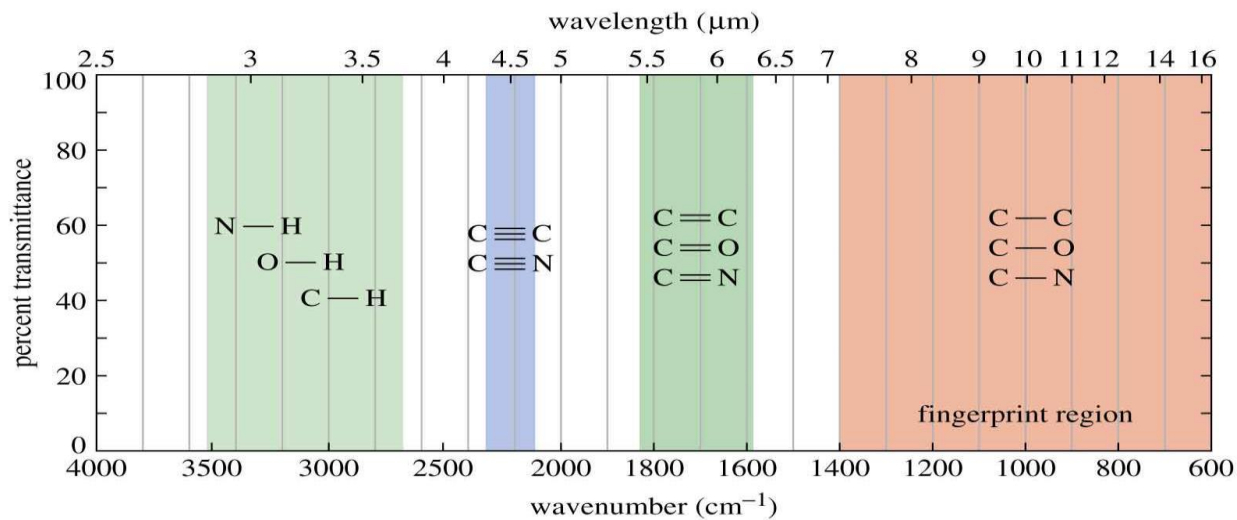- ✓ The far IR (15-200 µm) (667-50 cm-^{1})

PRINCIPLE

The absorption of infra red radiation leads to excitation of molecule and change in shape of the molecule because of stretching of bonds, bending of bonds, or internal rotation around single bonds. Vibrational transitions which are accompanied by a change in dipole moment of the molecule are called infrared active transitions and one which are not accompanied by change in dipole moment are not IR active transitions.

Another regions in infrared spectrum are

Group frequency region (2.5 – 8.0 µm) (4000 - 1300 cm-^{1})

Finger print region (8.0 - 25 µm) (1300 – 400 cm-^{1})

- ✓ 4000 – 2500 cm^{-1}: Absorbance of single bonds formed by hydrogen and other elements e.g. O–H, N–H,C–H
- ✓ 2500 – 2000 cm^{-1}: Absorbance of triple bonds e.g. C≡C, C≡N
- ✓ 2000 – 1500 cm^{-1}: Absorbance of double bonds e.g. C=C, C=O
- ✓ 1500 – 400 cm^{-1}: This region often consists of many different, complicated bands. This part of the spectrum is unique to each compound and is often called the *fingerprint* region. It is rarely used for identification of particular functional groups

wavelength (μm)

| 2.5 | 3 | 3.5 | 4 | 4.5 | 5 | 5.5 | 6 | 6.5 | 7 | 8 | 9 | 10 11 12 | 14 16 |

N — H
O — H
C — H

C ≡ C
C ≡ N

C = C
C = O
C = N

C — C
C — O
C — N

fingerprint region

wavenumber (cm⁻¹)

BASE VALUES
(+/- 10 cm⁻¹)

These are the minimum number of values to memorize.

O–H	3600
N–H	3400
C–H	3000
C≡N	2250
C≡C	2150
C=O	1715
C=C	1650
C–O	~1100

large range

O-H STRETCH

The O-H stretching region
- O-H   3600 cm^{-1} (alcohol, free)
- O-H   3300 cm^{-1} (alcohols & acids, H-bonding)

broadens

shifts

FREE

H-BONDED

3600   3300

"Neat" solution.

Many kinds of OH bonds of different lengths and strengths This leads to a broad absorption.

Longer bonds are weaker and lead to lower frequency.

Hydrogen bonding occurs in concentrated solutions (for instance, undiluted alcohol).

C=O IS SENSITIVE TO ITS ENVIRONMENT
EACH DIFFERENT KIND OF C=O COMES AT A DIFFERENT FREQUENCY

acid chloride	ester	aldehyde	ketone	carboxylic acid	amide

R—C—Cl   R—C—OR'   R—C—H   R—C—R   R—C—OH   R—C—NH₂

1800   1735   1725   1715   1710   1690

BASE VALUE

anhydride

R—C—O—C—R

1810 and 1760
(two peaks)

THESE VALUES ARE WORTH LEARNING all are +/- 10 cm^{-1}

Bond	Type of Compound	Frequency Range, cm^{-1}	Intensity
C—H	Alkanes	2850–2970	Strong
		1340–1470	Strong
C—H	Alkenes ($\,>C=C<^H$)	3010–3095	Medium
		675–995	Strong
C—H	Alkynes (—C≡C—H)	3300	Strong
C—H	Aromatic rings	3010–3100	Medium
		690–900	Strong
O—H	Monomeric alcohols, phenols	3590–3650	Variable
	Hydrogen-bonded alcohols, phenols	3200–3600	Variable, sometimes broad
	Monomeric carboxylic acids	3500–3650	Medium
	Hydrogen-bonded carboxylic acids	2500–2700	Broad
N—H	Amines, amides	3300–3500	Medium
C=C	Alkenes	1610–1680	Variable
C=C	Aromatic rings	1500–1600	Variable

Bond	Type of Compound	Frequency Range, cm^{-1}	Intensity
C≡C	Alkynes	2100–2260	Variable
C—N	Amines, amides	1180–1360	Strong
C≡N	Nitriles	2210–2280	Strong
C—O	Alcohols, ethers, carboxylic acids, esters	1050–1300	Strong
C=O	Aldehydes, ketones, carboxylic acids, esters	1690–1760	Strong
NO$_2$	Nitro compounds	1500–1570	Strong
		1300–1370	Strong
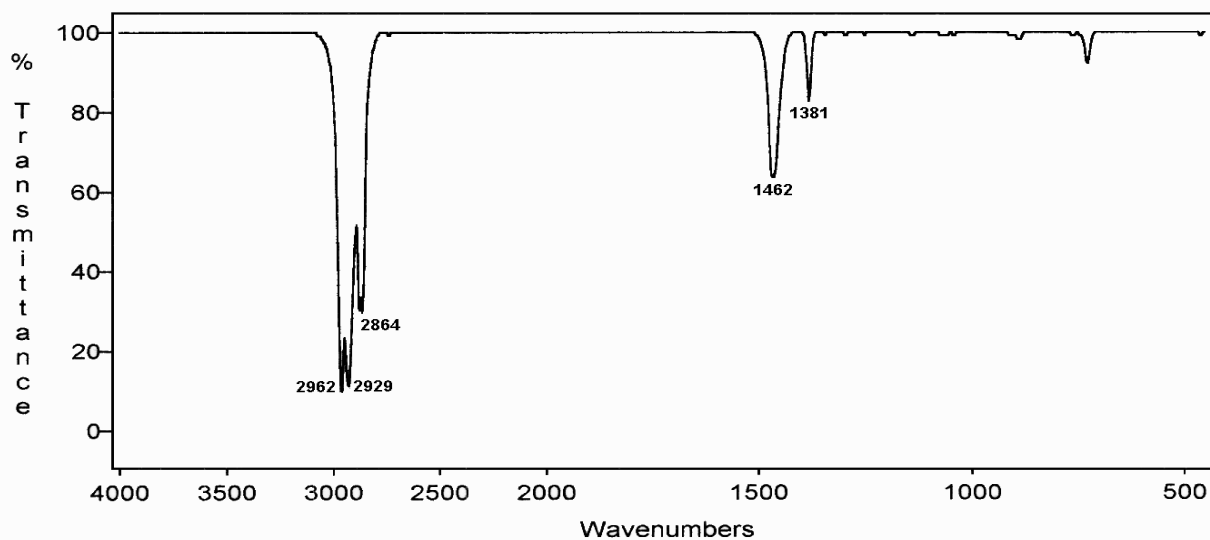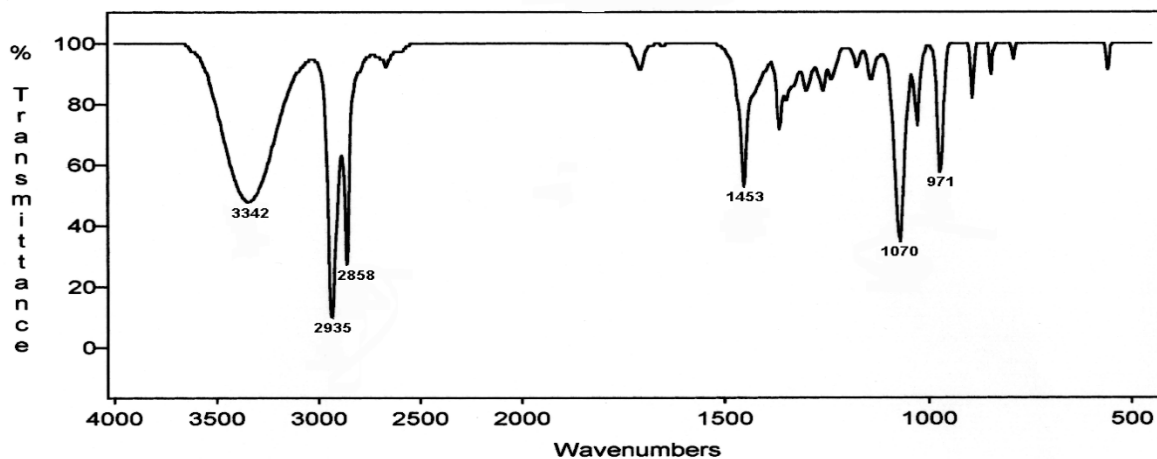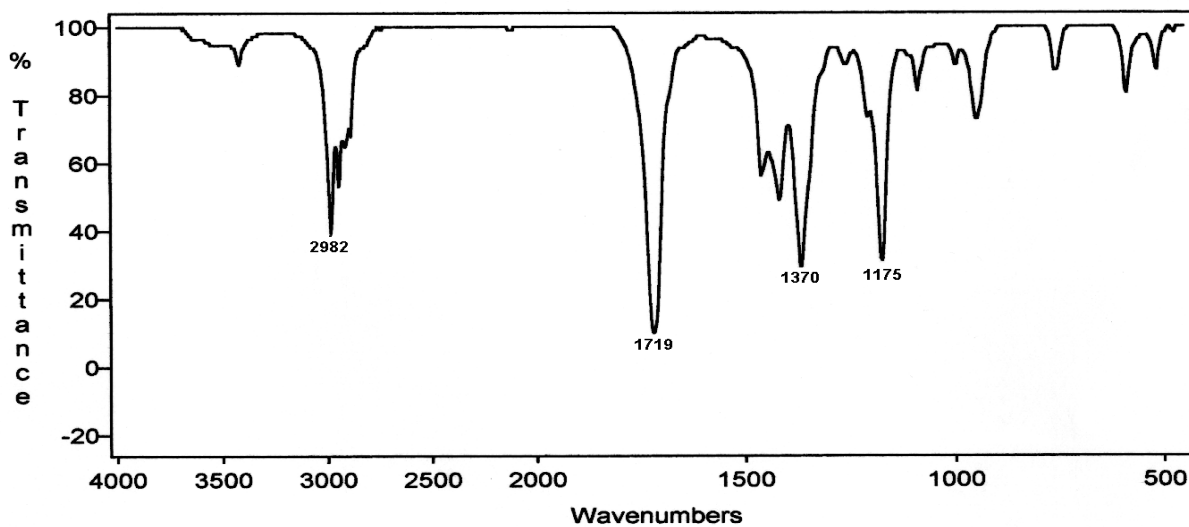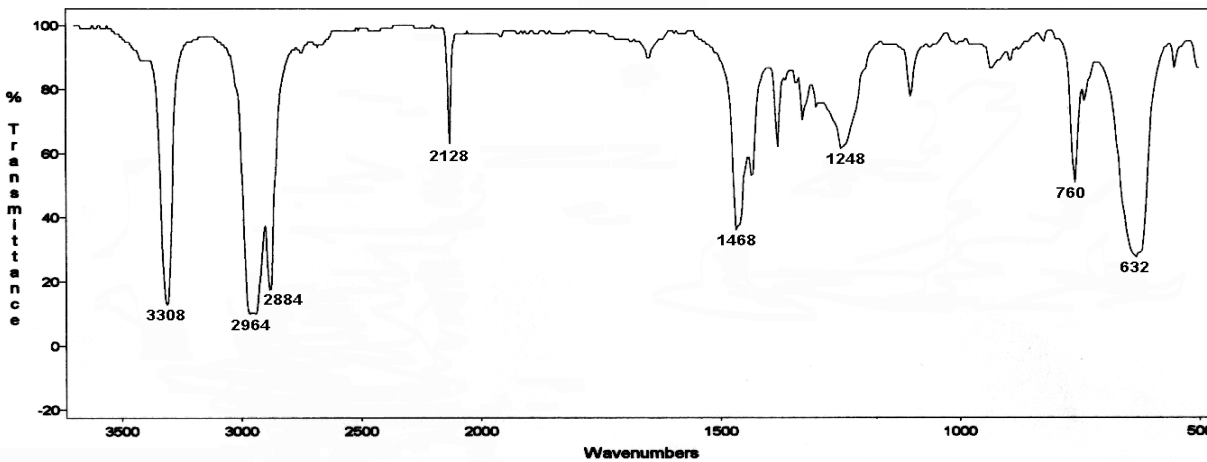
## 15.Estimation of Quinine sulphate by Fluorimetry

Aim : To estimate the amount of Quinine sulphate present in given sample by fluorimetry.

Requirements : Quinine sulphate , Fluorimeter, Sulphuric acid.

Apparatus: Pipette, volumetric flask, measuring cylinder.

Principle :

Fluorescence is the emission of visible light by a substance that has absorbed light of a different wavelength. The emitted photon has a longer wavelength and lower energy. As the excitation of the molecule is due to the absorption of a photon (light), these types of luminescence are called photoluminescence. Fluorescence is also defined as the radiation emitted in the transition of a molecule from a singlet excited state to a singlet ground state.
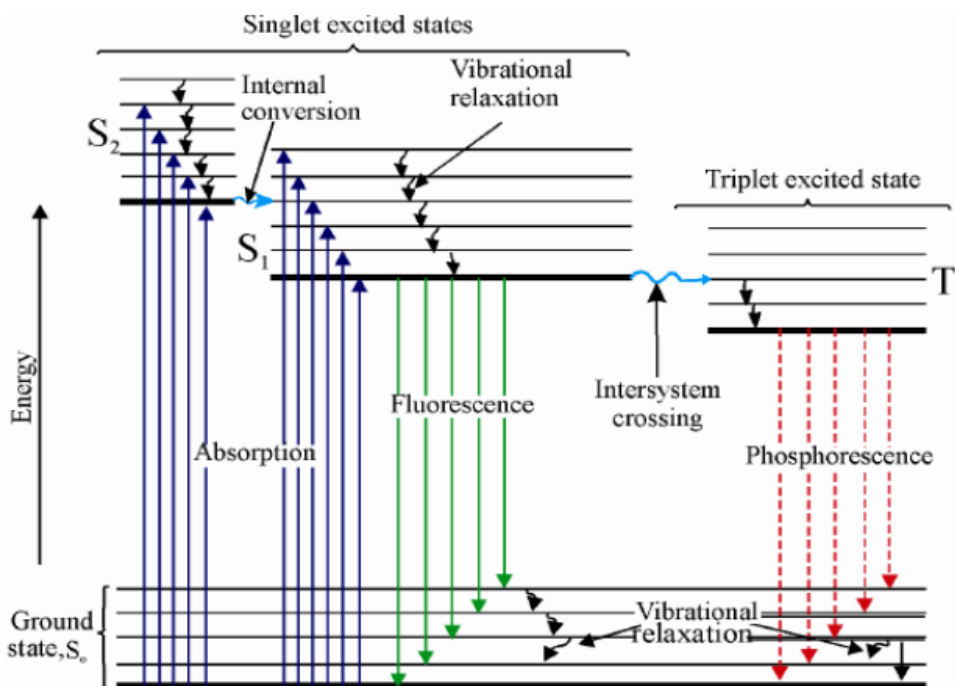
Fluorescence is also defined as the radiation emitted in the transition of a molecule from a singlet excited state to a singlet ground state. The intensity of the emitted radiation proportional to concentration of sample. such measurement form the basis of a sensitive method of analysis called the fluorometry. Fluorometric methods of analysis have found application in many situations of pharmaceutical interest such as in analysis of riboflavin, thiamine,reserpine, quinine sulphate in drug dosage forms.

Procedure;

Preparation of 0.1 N H$_2$SO$_4$:-
   Pipette out 2.7 ml of conc H$_2$SO$_4$ to 100 ml of water and then make it up to 1000 ml with water.

Preparation of standard Quinine sulphate solution

- Weigh accurately 100mg of Quinine sulphate powdered drug
- Dissolve in 100 ml of 0.1 N conc H$_2$SO$_4$ to get 1mg/ml
- Take 10 ml of above solution and dilute to100 ml with 0.1N H$_2$SO$_4$(**100µg/ml**)
- Again 10ml of above solution dilute to 100ml with 0.1N H$_2$SO$_4$(**10µg/ml**)
- Pipette out 0.5,1, 1.5, 2,2.5,3 ml of above diluted standard quinine sulphate solution in to a set of 10 ml volumetric flask and make up to 10 ml with 0.1N H$_2$SO$_4$ to get concentration of 0.5,1,1.5,2,2.5,3 µg/ml respectively.
- Switch on the instrument and stabilize for 10-15min.
- Set excitation and emissiom filters at the wavelengths 365 and 459nm respectively.
- Set the fluorescence intensity to 0% by using 0.1N H$_2$SO$_4$ as blank and 100% by using highest concentration of the standard solution.Measure the fluorescence of serial dilutions and and plot the calibration curve (fluorescence intensity Vs concentration).

Observation:

S.No	Concentration (µg/ml)	% Fluoroscence intensity
1	0.5	
2	1	
3	1.5	
4	2	
5	2.5	
6	3	
7	Unknown	

Report : The amount of Quinine sulphate present in the given sample was found to be ……..

QUESTIONS

1. Write the principle of Fluorimetry.

2.What is Fluorescence.Write the examples a for Fluoresent substance.

3.Write difference between UV-Visible spectroscopy and Fluorimetry.

4. What is the difference between Fluorescence and Phosphorescence.

5. Write the applications of Fluorimeter.

16. EFFECT OF QUENCHING ON THE FLUORESCENCE OF QUININE SULPHATE

Aim: To estimate the effect quenchingon the fluorescence of Quinine sulphate by Fluorimetry.

Requirements: Quinine sulphate , Fluorimeter, Sulphuric acid, KI.

Apparatus: Pipette, volumetric flask, measuring cylinder.

Principle: The reduction in fluorescence intensity due to certain ions is known as quenching. These processes can occur during the excited state or they may occur due to formation of complexes in the ground state. Quenching is due to various factors like pH, concentration, temperature, viscosity, and presence of certain ions like halides.

Quenching mainly is of four types:
1. Self quenching
2. Chemical quenching
3. Collisional quenching
4. Static quenching

Presence of halides leads to quenching due to Collision.

Procedure;

Preparation of 0.1 N H_2SO_4:-
  Pipette out 2.7 ml of conc H_2SO_4 to 100 ml of water and then make it up to 1000 ml with water.

Preparation of standard Quinine sulphate solution

- Weigh accurately 100mg of Quinine sulphate powdered drug
- Dissolve in 100 ml of 0.1 N H_2SO_4 to get **1mg/ml**
- Take 10 ml of above solution and dilute to100 ml with 0.1N H_2SO_4(**100μg/ml**)
- Again 10ml of above solution dilute to 100ml with 0.1N H_2SO_4(**10μg/ml**) from this finally prepare **1μg/ml** solution.

Preparation of KI solution

- Weigh accurately 100mg of KI, dissolve in 100 ml of 0.1 N conc H_2SO_4 to get 1mg/ml
- Take 10 ml of above solution and dilute to100 ml with 0.1N H_2SO_4(**100μg/ml**)
- ➢ In six 10 ml volumetric flasks, take 1ml of **1μg/ml** solution in each.
- ➢ Add 1,2,3,4 and 5ml of KI solution in each flask. Make up the volume with 0.1N H_2SO_4
- ➢ Switch on the instrument and stabilize for 10-15min.

➢ Set excitation and emissiom filters at the wavelengths about 365 and 459nm respectively.

➢ Set the fluorescence intensity to 0% by using 0.1N H_2SO_4 as blank and 100% by using highest concentration of the standard solution (not containing KI). Measure the fluorescence of serial dilutions and plot a graph between (volume of KI Vs fluorescence intensity).

Observation:

S.No	Vol. of KI added	% Fluoroscence intensity
1	0	
2	1	
3	2	
4	3	
5	4	
6	5	

Report :

Questions

1. Define quenching.

2. How temperature and viscosity effect the quenching.

3.Write difference between self quenching and chemical quenching.

4.What is the difference between Fluorescence and Phosphorescence.

5.Write the factors influence the fluorescence.

17. Estimation Of Sodium Concentration By Flame Photometry

Aim : To estimate the concentration of sodium present in given sample by flame photometry.
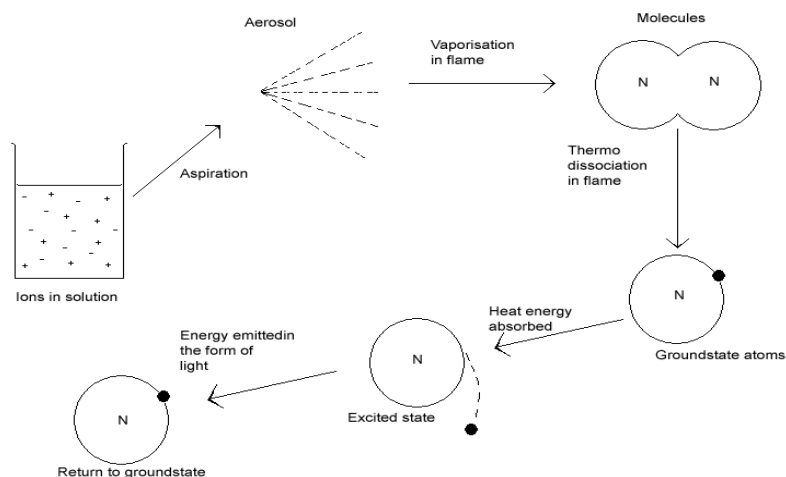
Requirements : Sodium chloride, Distlled water, Flame Photometer.

Apparatus: Pipette, volumetric flask, measuring cylinder.

Principle : Flame photometry is also known as atomic emission spectrometry. It works on the basis of heating the metal, in this case sodium, such that the atoms of the metal travel from ground state to their excited state. The atoms then return to their ground state and release their energy as photons of ultraviolet radiation. The wavelength of the UV radiation is then measured. When a liquid sample consisting a metallic salt solution is introduced into a flame, the following steps takes place

 ➢ The solvent gets evaporated
 ➢ The solid salt gets converted to its gaseous state
 ➢ The dissociation of either portion or total gaseous molecules give rise to neutral atoms (or) free radicals
 ➢ The neutral atoms are excited by the thermal energy of the flame which are unstable
 ➢ They instantly emit photons and return to it's ground state

The measurement of photons (emitted radiations forms the fundamental basis of flame photometry

The intensity of radiation emitted by depends upon proportion of thermally exited atoms

Procedure;

- ➢ Weigh accurately 100mg of sodium chloride powdered drug
- ➢ Dissolve in 100 ml of distilled water to get 1mg/ml
- ➢ Take 10 ml of above solution and dilute to100 ml with distilled water (**100µg/ml**)
- ➢ Pipette out 5,10, 20,30,40,50 ml of above diluted standard solution in to a set of 100 ml volumetric flask and make up to 100 ml with to get concentration of 5,10, 20, 30,40, 50 µg/ml respectively.
- ➢ Switch on the instrument, select sodium filter and stabilize for 10-15min.
- ➢ Set the gas in flame in order to get the non-luminous flame and air pressure at 0.4 to 0.5Kg/cm^2
- ➢ Set the flame intensity to 0% by using distilled water as blank and 100% by using highest concentration of the standard solution. Measure the % flame intesity of serial dilutions and plot the calibration curve (% intensity Vs concentration).
- ➢ Find out the concentration of unknown sample from calibration curve.

Observation:

S.No	Concentration (µg/ml)	% Flame intensity
1	5	
2	10	
3	20	
4	30	
5	40	
6	50	
7	Unknown	

Report : The concentration of sodium present in the given sample was found to be ……..

QUESTIONS

1. Write the principle of flame photometry.

2. Write the examples for atomic emission spectrometry.

3.Write difference between UV-Visible spectroscopy and Flame photometry..

4. Write the applications of Flame photometry .

18. Estimation Of Potassium Concentration By Flame Photometry

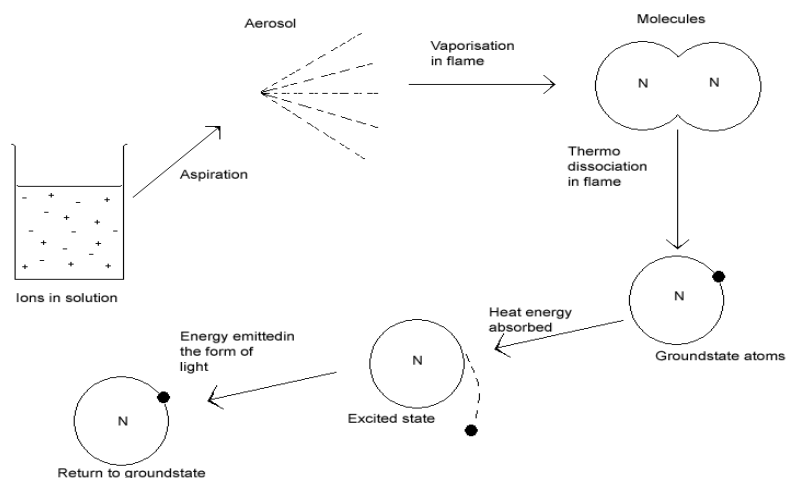**Aim** : To estimate the concentration of potassium present in given sample by flame photometry.

Requirements : Potassium chloride, Distlled water, Flame Photometer.

Apparatus: Pipette, volumetric flask, measuring cylinder.

Principle : Flame photometry is also known as atomic emission spectrometry. It works on the basis of heating the metal, in this case sodium, such that the atoms of the metal travel from ground state to their excited state. The atoms then return to their ground state and release their energy as photons of ultraviolet radiation. The wavelength of the UV radiation is then measured. When a liquid sample consisting a metallic salt solution is introduced into a flame, the following steps takes place

  ➢ The solvent gets evaporated
  ➢ The solid salt gets converted to its gaseous state
  ➢ The dissociation of either portion or total gaseous molecules give rise to neutral atoms (or) free radicals
  ➢ The neutral atoms are excited by the thermal energy of the flame which are unstable
  ➢ They instantly emit photons and return to it's ground state

 The measurement of photons (emitted radiations forms the fundamental basis of flame photometry

The intensity of radiation emitted by depends upon proportion of thermally exited atoms

Procedure;

> ➢ Weigh accurately 100mg of potassium chloride powdered drug
> ➢ Dissolve in 100 ml of distilled water to get 1mg/ml
> ➢ Take 10 ml of above solution and dilute to100 ml with distilled water (**100µg/ml**)
> ➢ Pipette out 5,10, 20,30,40,50 ml of above diluted standard solution in to a set of 100 ml volumetric flask and make up to 100 ml with to get concentration of 5,10, 20, 30,40, 50 µg/ml respectively.
> ➢ Switch on the instrument, select sodium filter and stabilize for 10-15min.
> ➢ Set the gas in flame in order to get the non-luminous flame and air pressure at 0.4 to 0.5Kg/cm^2
> ➢ Set the flame intensity to 0% by using distilled water as blank and 100% by using highest concentration of the standard solution. Measure the % flame intesity of serial dilutions and plot the calibration curve (% intensity Vs concentration).
> ➢ Find out the concentration of unknown sample from calibration curve.

Observation:

S.No	Concentration (µg/ml)	% Flame intensity
1	5	
2	10	
3	20	
4	30	
5	40	
6	50	
7	Unknown	

Report : The concentration of sodium present in the given sample was found to be ……..

QUESTIONS

1. Write the principle of flame photometry.

2. Write the examples for atomic emission spectrometry.

3.Write difference between UV-Visible spectroscopy and Flame photometry..

4. Write the applications of Flame photometry .

19. POTENTIOMETRIC TITRATION OF STRONG ACID Vs STRONG BASE

AIM: To determine the exact equivalent point and normality of 0.1 N HCl by titrating it with 0.1N NaOH solution potentiometrically.

CHEMICALS: NaOH, distilled water, HCl.

APPARATUS: pH meter, potentiometer, Burette, Beaker, Electrodes, Measuring cylinder.

PRINCIPLE: Potentiometric determination of end point depends upon the potential across to suitable reference and indicator electrodes immersed in solution changes sharply at the end point. This change is similar to colour change by ion by ion indicator by visual method. Potentiometric endpoint determination is more accurate and this titrations are useful when there is no suitable indictor available. End point will be accurately noted after plotting a graph between volume of titrant vs potential developed.

PROCEDURE:

Preparation of 0.1 N NaOH: 0.4 g of NaOH dissolved in 100 ml of H_2O.

Preparation of 0.1 N Oxalic acid: 0.63 g of oxalic acid dissolved in 100 ml of water.

Preparation of 0.1 N HCl: 0.84 ml of conc. HCl is dissolved in 100 ml water.

Standardization of 0.1 N NaOH:

 ➢ Pipette out 20 ml of 0.1 N oxalic acid solution into beaker
 ➢ Dip the electrodes (saturated calomel electrode as reference electrode and glass electrode as indicator electrode) into the solution which is connected to a potentiometer.
 ➢ Take the prepared 0.1 N NaOH solution into a burette and add 0.5 ml of this to the beaker until the equivalent point of the titration is obtained.

- ➤ Pipette out 20 ml of standardised NaOH solution into a beaker and take the prepared HCl solution into a burette.
- ➤ Proceed the titration in the same way like oxalic acid.
- ➤ A graph is plotted between the volume of titrant add on X-axis and the potential difference on Y-axis in mV.

S.No.	Vol of titrant	pHof solution	e.m.f
1	0 ml		
2	1 ml		
3	2 ml		
4	3 ml		
5	4 ml		
6	5 ml		
7	6 ml		
8	7 ml		
9	8 ml		
10	9 ml		
11	10ml		

REPORT: The endpoint in potentiometric titration of strong acid v_s strong base was found to be -------

With pH---- and e.m.f was found to be-------

VIVA QUESTIONS

1. What is a potentiometric titration?
2. What are the electrodes used in the experiment?
3. What is the indicator electrode?
4. What is the Reference electrode?

5. What is neutralization point?

6. How can you determine the strength of acid from neutralization point?

7. What is the principle involved in potentiometric titration.

8. What is the single electrode potential?

9. What is meant by e.m.f?

10. What are the electrodes used in potentiometric titration

11. How do you confirm the end point in the potentiometric titration?

12. What are the advantages of potentiometric titrations?

20. CONDUCTOMETRIC DETERMINATION EQUIVALENCE POINT OF TITRATION OF A SOLUTION OF HYDROCHLORIC ACID BY A STANDARD SOLUTION OF SODIUM HYDROXIDE.

AIM: To determination equivalence point of titration of a solution of hydrochloric acid by a standard solution of sodium hydroxide by Conductometry.

CHEMICALS: NaOH, oxalic acid, distilled water, HCl.

APPRATUS: Conductometer, Burette, Beaker, Electrodes, Measuring cylinder.

PRINCIPLE: Conductometric titrations works on the principle of Ohm's law. As current (i) is inversely proportional to Resistance (R) and the reciprocal of resistance is termed as Conductance, and its unit is Siemen (mho) cm $^{-1}$. The conductivity of a solution depends on the number of ions and their mobility towards their respective electrodes.

$$R= \frac{V}{I}$$

V= Potential difference

R=Resistance

I= Current flow

$$Conductance = \frac{1}{Resistance}$$

 The main principle involved in this experiment is when HCl is taken in beaker, initially the conductivity is high because of the complete dissociate of HCl in to H^+ and Cl^- and as NaOH is added, the OH^- of NaOH reacts with H^+ to produce water. As a result conductivity of solution is gradually decreases after every addition of NaOH, until all H^+ ions are reacted with OH^- to produce water. At the end point there is on H^+ ion to react OH^- , now further addition of NaOH increases the conductance of solution due increasing no. of OH^- ions and a V shaped graph is obtained.

PROCEDURE:

Conductometric Titration:

  i.   Switch on the coductometer and allow to stabilize for 30 min.
 ii.   Rinse the conductivity cell a number of times with double distilled water.
iii.   Take 20 mL of 0.1 N HCl in a beaker and dip the conductivity cell in it, so that the cell should dip completely in solution.
 iv.   Note the temperature of the sample solution and accordingly set the temperature control or keep the cell in   a thermostat at room temperature.
  v.   Determine the initial conductance of acid.
 vi.   Add small amount of 0.1N NaOH solution (few drops) from burette, stir it and measure the conductance after each addition.
vii.   Several readings has to note before and after the approximate end point
viii.   Plot a graph between conductance and volume of titrant (NaOH solution) and determine the end point
 ix.   Calculate the normality of acid

Observation Table

S.NO	Vol. of NaOH Added in ml	Conductivity (mho)
1	0	
2	1	
3	2	
4	3	
5	4	
6	5	
7	6	
8	7	
9	8	

10	9	
11	10	
12	11	
13	12	
14	13	

REPORT: The end point of conductometric titration of strong acid V_s strong base was found to be -----

at ------- conductance and normality of acid was found to be----------