H. R. Patel Institute of Pharmaceutical Education and Research, Shirpur

Lab Manual

Subject- Instrumental Methods of Analysis

By

Dr. Zamir G. Khan (M.Pharm)

Assistant Professor

Department of Pharmaceutical Chemistry

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BP705P. INSTRUMENTAL METHODS OF ANALYSIS

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Experiment No. 1

AIM

Determination of absorption maxima and effect of solvents on absorption maxima of organic compounds.

PRINCIPLE

The solvent dissolves the drug substance and exerts an intense influence on the quality and shape of the UV-visible spectrum. Hence the absorption spectrum of drug substance changes mostly as per the change of solvent that has been used to dissolve the drug substance. Here, the change in either wavelength (absorption maxima) or absorption intensity is monitored by changing the different solvents.

THEORY

Ultraviolet–visible spectroscopy or ultraviolet–visible spectrophotometry (UV–Vis or UV/Vis) refers to absorption spectroscopy or reflectance spectroscopy in part of the ultraviolet and the full, adjacent visible regions of the electromagnetic spectrum.

Absorption spectroscopy deals with the spectroscopic techniques that measure the absorption of radiation, as a function of frequency or wavelength, due to its interaction with a sample. The sample absorbs energy, i.e., photons, from the radiating field. The intensity of the absorption varies as a function of frequency, and this variation is the absorption spectrum. Absorption spectroscopy is employed as an analytical chemistry tool to determine the presence of a particular substance in a sample and, in many cases, to quantify the amount of the material present. Infrared and ultraviolet–visible spectroscopies are particularly common in analytical applications. Absorption spectroscopy is also employed in studies of molecular and atomic physics, astronomical spectroscopy and remote sensing.

There are a wide range of experimental approaches for measuring absorption spectra. The most common arrangement is to direct a generated beam of radiation at a sample and

detect the intensity of the radiation that passes through it. The transmitted energy can be used to calculate the absorption. The source, sample arrangement and detection technique vary significantly depending on the frequency range and the purpose of the experiment.

Lambda max or absorption maxima (λmax)

Lambda max refers to the wavelength along the absorption spectrum where a substance has its strongest photon absorption. Simply, the wavelength at which a substance displays maximum absorption is called as lambda max (figure 1). Different compounds may have very different absorption maxima and absorbances. Intensely absorbing compounds must be examined in dilute solution (absorbance value less than 1), so that significant light energy is received by the detector, and this requires the use of completely transparent (non-absorbing) solvents. The most commonly used solvents are water, ethanol, hexane and cyclohexane. Solvents having double or triple bonds, or heavy atoms (e.g. S, Br & I) are generally avoided.

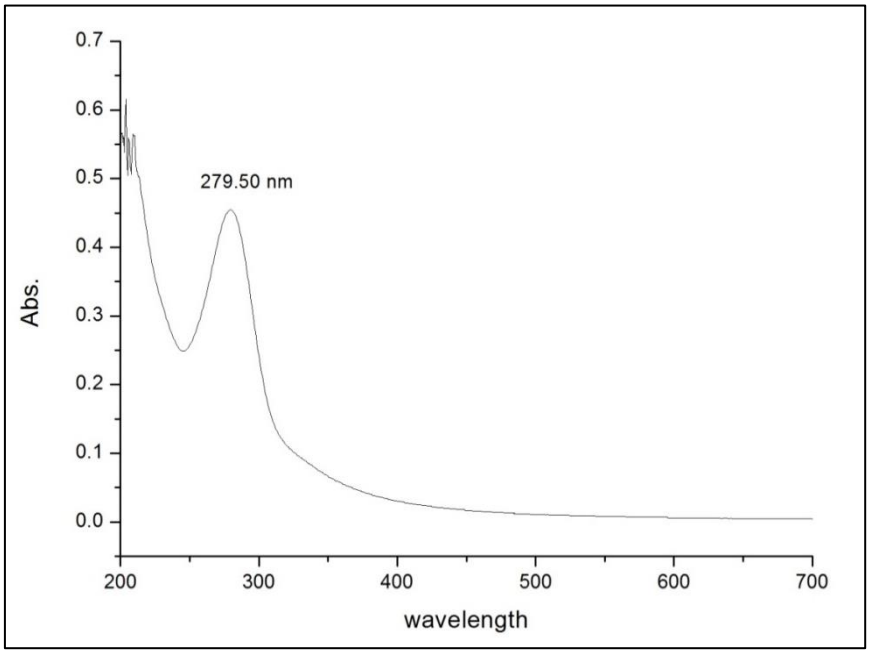


Figure 1: Typical example of unknown sample depicting absorption maxima at 279.50 nm

The Beer–Lambert:

The Beer–Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length.[3] Thus, for a fixed

path length, UV/Vis spectroscopy can be used to determine the concentration of the absorber in a solution.

Ultraviolet–visible spectrophotometer

The instrument used in ultraviolet–visible spectroscopy is called a UV/Vis spectrophotometer. It measures the intensity of light after passing through a sample and compares it to the intensity of light before it passes through the sample.

Choice of solvents

Every solvent is supposed to exhibit UV-vis absorbance cut-off wavelength. The solvent cut-off is the wavelength below which the solvent itself absorbs all of the light. So when choosing a solvent student has to be careful of its absorbance cut-off. If the solvent is showing cut-off near the absorption maxima of the substance under examination, another solvent is to be chosen.

Solvent	UV Absorbance Cut-off (nm)
Water	180
Ethanol	205
Toluene	285
Dimethyl formamide	267
Acetone	329
Benzene	278

Applications:

1. UV/Vis spectroscopy is routinely used in analytical chemistry for the quantitative determination of different analytes, such as transition metal ions, highly conjugated organic compounds, and biological macromolecules.

2. Solutions of transition metal ions can be colored (i.e., absorb visible light) because d electrons within the metal atoms can be excited from one electronic state to another. The colour of metal ion solutions is strongly affected by the presence of other species, such as certain anions or ligands. For instance, the colour of a dilute solution of copper sulfate is a very light blue; adding ammonia intensifies the colour and changes the wavelength of maximum absorption (λmax).
3. Organic compounds, especially those with a high degree of conjugation, also absorb light in the UV or visible regions of the electromagnetic spectrum.
4. UV/Vis can be applied to determine the kinetics or rate constant of a chemical reaction.
5. Spectroscopic analysis is commonly carried out in solutions but solids and gases may also be studied.

REQUIREMENTS

Apparatus: Glass beakers, Measuring flasks, Whatmann filter paper, Measuring cylinder, etc.

Chemicals: Paracetamol, Distilled water, Ethanol, 0.1 N NaOH, 0.1N HCl etc.

PROCEDURE

1. Weigh accurately about 10 mg of Paracetamol and dissolve in sufficient quantity of solvent (2/3 volume), in 100mL of measuring flask, shake well to dissolve completely and makeup the volume upto mark to prepare 100 ppm of stock solution.
2. Pipette out 1 mL of solution from stock solution and add to 10 mL measuring flask and make up the volume with fresh solvent to prepare 10 ppm of solution.
3. Scan the solution in UV visible spectrophotometer to obtain the absorption maxima.

Observation

Solvent	λmax (nm)
Distilled water	
Ethanol	
0.1 N NaOH	
0.1N HCl	

RESULTS

The absorption maxima (λmax) of paracetamol in various solvents like distilled water, ethanol, 0.1 N NaOH, and 0.1N HCl was observed and compiled.

MCQ's

1. When absorption intensity of compound is decreased it is called

   A. Red shift (Bathochromic shift)

   B. Blue shift (Hypsochromic shift)

   C. Hypochromic shift

   D. Hyperchromic shift

2. When absorption maximum of a compound shift to longer wavelength, it is known as

   A. Red shift (Bathochromic shift)

   B. Blue shift (Hypsochromic shift)

   C. Hypochromic shift

   D. Hyperchromic shift

3. If solvent is polar and electron transition is $n \rightarrow \pi*$ which type of shift are seen in graph?

   A. Hypsochromic shift

   B. Red shift

C. Blue shift

D. A and C

4. If solvent is non-polar and electron transition is $n \rightarrow \pi *$ which type of shift are seen in graph?

A. Hypsochromic shift

B. Red shift

C. Blue shift

D. A and C

5. Which of the following compounds does not absorb light in the UV/visible spectrum?

A. Aspirin

B. Paracetamol

C. Phenobarbitone

D. Chloral hydrate

Questions

1. What is principle of UV visible spectroscopy?

   Ans: UV Spectroscopy uses ultraviolet light to determine the absorbency of a substance. In simple terms, the technique maps the interaction between light and matter and measures. As matter absorbs light it undergoes either excitation or de-excitation, which generates what is known as a spectrum. This allows scientists to measure the rate at which a beam of light weakens after passing through a substance. Absorption spectroscopy is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions of electrons from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state.

2. Enlist factors affecting UV visible spectroscopy.

Ans:

i. Solvent

ii. sample PH

iii. sample concentration

iv. sample temperature

v. effect of conjugation

3. Define chromophore and auxochrome?

Ans:

(a) **CHROMOPHORE**: The term chromophore was previously used to denote a functional group of some other structural feature of which gives a color to compound. For example- Nitro group is a chromophore because its presence in a compound gives yellow color to the compound. But these days the term chromophore is used in a much broader sense which may be defined as "any group which exhibit absorption of electromagnetic radiation in a visible or ultra-visible region "It may or may not impart any color to the compound. Some of the important chromophores are: ethylene, acetylene, carbonyls, acids, esters and nitrile groups etc.

Types of chromophores: Two types of chromophores are known.

1. Chromophores in which the groups have π electrons undergo π-π* transitions. For examples:-ethylenes, acetylenes etc.

2. Chromophores having both π- electrons and n (non-bonding) electrons undergo two types of transitions. i.e., π-π* and n-π*, for examples: - carbonyls, nitriles, azo compounds and nitro compounds etc.

(b) AUXOCHROMES: It is a group which itself does not act as a chromophore but when attached to a chromophore, it shifts the adsorption towards longer wavelength along with an increase in the intensity of absorption. Some commonly known auxochromic

groups are: -OH, -NH2, -OR, -NHR, and –NR2. For example: When the auxochrome –NH2 group is attached to benzene ring. Its absorption change from λ max 225 (εmax 203) to λmax 280 (εmax1430)

All auxochromes have one or more non-bonding pairs of electrons. If an auxochromes is attached to a chromophore, it helps is extending the conjugation by sharing of non-bonding pair of electrons

4. What is Bathochromic shift?

   Ans: It is a change of spectral band position in the absorption, reflectance, transmittance, or emission spectrum of a molecule to a longer wavelength (lower frequency). Because the red color in the visible spectrum has a longer wavelength than most other colors, the effect is also commonly called a red shift.

5. What is Hypsochromic shift?

   Ans: It is a change of spectral band position in the absorption, reflectance, transmittance, or emission spectrum of a molecule to a shorter wavelength (higher frequency). Because the blue color in the visible spectrum has a shorter wavelength than most other colors, this effect is also commonly called a blue shift.

Experiment No. 2

AIM: To perform estimation of dextrose by colorimetry

PRINCIPLE

P-hydroxybenzoic acid hydrazide (PHBAH) reacts with dextrose in alkaline medium, which gives colour products, these colored products can be analysed by analytical technique of colorimetry. Dextrose reacts with PHBAH reagent and produces colored product; the absorbance of which is proportional to concentration of dextrose being analysed.

THEORY

Colorimetry or (colourimetry) is a technique used to determine the concentration of colored compounds in solution. Colorimetry is the measurement of concentration of analyte in a solution by determining absorption of a particular wavelength. A colorimeter is a device used to test the concentration of a solution by measuring its absorbance of a specific wavelength of light. To use this device, different solutions must be made, and a control (usually a mixture of distilled water and another solution) is first filled into a cuvette and placed inside a colorimeter to calibrate the machine. Only after the device has been calibrated you can use it to find the densities and/or concentrations of the other solutions.

The technique is based upon Beer-Lambert's law. Overall the process is accomplished with the measurement of analyte in a colored solution or development of color that appears in the solution following reaction with specific reagent. In alkaline medium p-hydroxybenzoic acid hydrazide (PHBAH) reacts with dextrose that give products which can be assayed by colorimetry. Dextrose reacts with p-hydroxybenzoic acid hydrazide (PHBAH) reagent to give coloured product; the absorbance noted is proportional to concentration of sugar.

Applications of colorimeters

1. Besides being valuable for basic research in chemistry laboratories, colorimeters have many practical applications. For instance, they are used to test for water quality, by

screening for chemicals such as: chlorine, fluoride, cyanide, dissolved oxygen, iron, molybdenum, zinc hydrazine etc.

2. They are also used to determine the concentrations of plant nutrients (such as phosphorus, nitrate and ammonia) in the soil or haemoglobin in the blood and to identify substandard and counterfeit drugs.
3. In addition, they are used by the food industry and by manufacturers of paints and textiles. In these disciplines, a colorimeter checks the quality and consistency of colors in paints and fabrics, to ensure that every batch comes out looking the same.

REQUIREMENTS

Apparatus: Test tubes, Burette, Colorimeter, and Water bath, etc.

Chemicals: Dextrose: 5mg, Sodium hydroxide: 3-5 pellets, p- hydroxybenzoic acid hydrazide (PHBAH): 50 ml

PROCEDURE

Alkaline solution

Put 3-5 pellets of sodium hydroxide in about 50 ml of water, stir to mix well.

Diluent

Add 10.0 ml of p-hydroxybenzoic acid hydrazide (PHBAH) solution with 30.0ml of water along with few drops of Sodium hydroxide.

Standard stock solution preparation

Weigh 5.0 mg of dextrose; add in a test tube containing 15.0 ml of water, 2-3 drops of sodium hydroxide and 5.0 ml of p-hydroxybenzoic acid hydrazide (PHBAH) solution, and mix well.

Working standard solution preparation

Dilute 5.0ml of stock solution with 15.0 ml of diluent.

Sample solution preparation

Take a test tube, add 1.0 ml of sample with burette, add 4.0 ml of water to it, and shake gently to mix. Keep the above sample solution for 30 -35 minutes at normal temperature. Again shake gently, then add 5.0 ml of p-hydroxybenzoic acid hydrazide (PHBAH) coloured solution and few drops of sodium hydroxide, mix well and keep the test tube in water bath in boiling mode. After about 5 minutes withdraw test tube from water bath, allow to cool, then add 10 ml of water and mix well.

Instrumentation

Before measuring absorbance by colorimeter clean the glass tube with water, and ensure zero absorbance with diluent.

To finalize the light source, take sample solution in glass tube and keep first in blue light source and repeat with the available light source simultaneously recording absorbance.

Use the light source with highest absorbance and record the absorbance for the followings.

1. Standard stock
2. Working standard solution
3. Sample of unknown concentration.

Note the above three different absorbance. Now plot the graph of two known solution (1 is standard stock, 2 is working standard) i.e. absorbance (Y-axis) against concentration (X-axis), plot a straight line in between two points and find the concentration of unknown sample with the sample absorbance.

CONCLUSION

The chelate formed from the reaction of sucrose and p-hydroxybenzoic acid hydrazide (PHBAH) in alkaline medium is measured by colorimeter which is the basis of quantifying the amount of sucrose present in sample.

MCQs

1. What is the name of an instrument used to measure the absorbance of a coloured compound in solution?

A. Colorimeter.

B. Colourymeter

C. Coulometer.

D. Calorimeter.

2. The dichromate ion absorbs light of wavelength close to 500 nm. Based on this information, what can you conclude?

A. The dichromate ion absorbs outside the visible region.

B. Solutions of the dichromate ion are colourless

C. The dichromate ion absorbs in the ultraviolet region.

D. The dichromate ion absorbs within the visible region.

3. The wavelength of absorption is 495 nm. In what part of the electromagnetic spectrum does this lie?

A. Ultraviolet-visible.

B. Microwave.

C. Infrared.

D. Radiowave.

4. Which part of the spectrophotometer is adjusted to select the desired wavelength?

A. Light source.

B. Filter.

C. Sample.

D. Photo detector.

5. The plot of absorption verses wavelength for a particular compound is referred to as the

A. Path length.

B. Emission spectrum.

C. Absorption spectrum.

D. None of these.

Questions

1. What is principle of colorimetry?

   Ans: The working principle of the colorimeter is based on Beer-Lambert's law, which states that the amount of light absorbed by a color solution is directly proportional to the concentration of the solution and the length of a light path through the solution.

2. What do you mean by Colorimeter?

   i. **Ans**: The colorimeter instrument is very simple, consisting merely of a light source (lamp), filter, cuvettes, and photosensitive detector to collect the transmitted light. Another detector is required to measure the incident light; or a single detector may be used to measure incident and transmitted light, alternately. The latter design is both cheaper and analytically better, because it eliminates variations between detectors. The filter is used here to obtain an appropriate range of wavelengths within the bands, which it is capable of selecting.

3. What are the components of colorimetry?

   Ans: The components of colorimetry are;

   i. a light source (often an ordinary low-voltage filament lamp);
   ii. an adjustable aperture;
   iii. a set of colored filters;
   iv. a cuvette to hold the working solution;
   v. a detector (usually a photoresistor) to measure the transmitted light;
   vi. a meter to display the output from the detector.

4. What are the advantages of colorimetry?

Ans: It is fast, economical, and has a simple operation of a spectrometer. To handle the Colorimeter, experienced person is not the requirement. The method is fast and convenient to that of the gravimetric or volumetric processes, and they are easily optimized for automation.

5. What are applications of colorimetry?

Ans: Colorimeters are widely used to monitor the growth of a bacterial or yeast culture. They provide reliable and highly accurate results when used for the assessment of color in bird plumage. They are used to measure and monitor the color in various foods and beverages, including vegetable products and sugar. Certain colorimeters can measure the colors that are used in copy machines, fax machines, and printers. Besides being used for basic research in chemistry laboratories, colorimeters have many practical applications such as testing water quality by screening chemicals such as chlorine, fluoride, cyanide, dissolved oxygen, iron, molybdenum, zinc, and hydrazine.

Experiment No. 3

AIM

Estimation of sulfanilamide by colorimetry

PRINCIPLE

Principle involved in the estimation of sulphanilamide is based on formation of derivative of para-amino benzoic acid analogue. This derivative can be analysed calorimetrically at wavelength of 510 nm.

THEORY

Colorimetry or (colourimetry) is a technique used to determine the concentration of colored compounds in solution. Colorimetry is the measurement of concentration of analyte in a solution by determining absorption of a particular wavelength. A colorimeter is a device used to test the concentration of a solution by measuring its absorbance of a specific wavelength of light. To use this device, different solutions must be made, and a control (usually a mixture of distilled water and another solution) is first filled into a cuvette and placed inside a colorimeter to calibrate the machine. Only after the device has been calibrated you can use it to find the densities and/or concentrations of the other solutions.

The technique is based upon Beer-Lambert's law. Overall the process is accomplished with the measurement of analyte in a colored solution or development of color that appears in the solution following reaction with specific reagent.

Applications of colorimeters

1. Besides being valuable for basic research in chemistry laboratories, colorimeters have many practical applications. For instance, they are used to test for water quality, by screening for chemicals such as: chlorine, fluoride, cyanide, dissolved oxygen, iron, molybdenum, zinc hydrazine etc.

2. They are also used to determine the concentrations of plant nutrients (such as phosphorus, nitrate and ammonia) in the soil or haemoglobin in the blood and to identify substandard and counterfeit drugs.
3. In addition, they are used by the food industry and by manufacturers of paints and textiles. In these disciplines, a colorimeter checks the quality and consistency of colors in paints and fabrics, to ensure that every batch comes out looking the same.

Requirements:

Chemicals: Sulphanilamide, 0.1 N NaOH solution, Conc. H_2SO_4, Ethanol Solution (95%v/) Distilled water.

Apparatus: Beaker, Volumetric flask, Pipette, Spectrophotometer.

Theory: Sulphanilamide is a para-amino benzoic acid analogue. It is estimated calorimetrically after derivatization at 510 nm. Visible spectrophotometry is one of the most employed methods of pharmaceutical analysis. It involves the amount of radiation absorbed by substance in a solution. The instrument that measures the ratio or the function of the ratio of the intensity of the two beams of light in the visible region is called as colorimeter.

Single Point Standardization:

The single point procedure involves the measurement of the absorbance of a sample solution and of a standard solution of the reference substance.

The standard and sample solutions are prepared in a similar manner; ideally the concentration of the standard solution should be close to that of the sample solution.

The concentration of the substance in the sample is calculated from the proportional relationship that exists between absorbance and concentration.

Procedure:

1. Weigh accurately about 0.15 gm of Sulphanilamide and add 1 ml of conc. sulphuric acid in 100 mL measuring flask. Now add 2 ml of 0.1 N NaOH solutions and make the volume up to 100 ml with distilled water and shake for 15 to 20 minutes.
2. Pipette out 10 ml of this solution and make the volume up to 100 ml with distilled water.
3. Again, pipette out 10 ml of this solution and add 10 ml of ethanol solution 95% v/v and make the volume up to 100 ml with distilled water.
4. Analyse calorimetrically by setting the filter to 510 nm.

Observation Table:

Sr. no.	Name of sample	Wavelength (λ)	Absorbance
1.			
2.			

Calculation: $A = a \times b \times c$

A = Absorbance

a = Absorptivity

b = Path length (1cm)

c = Concentration of absorbing substance

Result

Observed % purity of given sample was found to be = _____ %.

Conclusion:

The quantitative spectrophotometric analysis and the estimation of impurity level in the drug are determined by colorimetric technique.

Precautions: Wait to initialize the spectrophotometer. Handle the cuvettes carefully. Handle apparatus with care. Instruments must be used carefully.

MCQs

1. Colorimeter is applied only in relation to_?

A. X rays.

B. Visible light

C. IR Rays.

D. UV light.

2. Which of the following is not an application of colorimeter?

A. Analysis of paint.

B. Analysis of ink.

C. Analysis of cosmetic.

D. Composition detection.

3. Colorimeters are used in applications where great accuracy is required.

A. True

B. False

4. Colorimeters are used to determine the concentration of solutions.

A. True

B. False

5. Which of the following is the purpose of balance indicator in double beam photometer or colorimeter?

A. Selects a particular wavelength

B. Splits the wavelength selected into two equal beams

C. Detects and indicates the amount of light falling on it

D. Indicates the difference between the output of two photometers

Questions

1. Describe laws governing the absorption of radiation in colorimetry.

Ans: The absorption of radiation in colorimetry is based on Beer-Lambert's law, which states that the amount of light absorbed by a color solution is directly proportional to the concentration of the solution and the length of a light path through the solution.

Beer law: The intensity of beam of monochromatic light decreases exponentially with increase in the concentration of absorbing species.

Lambert's law: The intensity of beam of monochromatic light decreases exponentially with increase in the thickness of the medium.

2. Explain the properties of sources of light used in colorimetry.

   Ans: **SOURCE OF LIGHT**: The visible spectrum ranges from 400nm to 800 nm. Hence, any lamp source, which gives adequate intensity of radiation over the entire wavelength region, can be used. The requirements of a source of light for colorimeter are:

   i. It should not observe fluctuations.
   ii. It should offer radiation in between 400nm -800nm.
   iii. It should provide adequate intensity.

3. What are sources of light used commonly in colorimetry?

Ans: The following are the sources of light used commonly.

   i. **Tungsten lamp**: As it satisfies the above criteria, this lamp finds its place in most of colorimeter spectrophotometer. The lamp consists of a tungsten filament in a vacuum bulb similar to the ones used domestically. However, it offers sufficient intensity.
   ii. **Carbon arc lamp**: For a source of very high intensity, carbon arc lamp can be used. It also provides a whole range of visible spectrum.

4. Describe the filters used in colorimetry?

Ans: Two types of filters are used in colorimetry.

i. **Absorption filters**: These filters are made up of glass, coated with pigments or they are made up of dyed gelatin. They absorb the unwanted radiation and transmit the rest of the radiation, necessary for colorimetry.

ii. **Interference Filters**: This filter is known as Fabry – Perot filter. The features include (1): It has dielectric spacer film made up of CaF_2, MgF_2 or SiO, between two parallel reflecting silver films.

5. What do you mean by monochromators?

Ans: Monochromators are better and more efficient than filters in converting a polychromatic light or heterochromatic light into monochromatic light.

A monochromator has the following components

1. Entrance slit (to get narrow source).

2. Collimator (to render light parallel).

3. Grating or prism (to disperse radiation).

4. Collimator (to reform the images of entrance slit).

5. Exit slit (to fall on sample cell).

Experiment No. 4

AIM: Simultaneous estimation of ibuprofen and paracetamol by UV spectroscopy.

Ibuprofen (Ibu) (400mg) + Paracetamol (Para) (325mg) tablet

PRINCPLE:

Estimation of combined dosage form such as ibuprofen and paracetamol tablet can be done using the Vierodt's method. Here two absorbing drugs, display absorption at the wavelength of each other, and the concentrations of both the drugs can be analysed by technique of simultaneous equation method called as Vierodt's method.

THEORY

Simultaneous equation method

If a sample contains two absorbing drugs (X and Y) each of which absorb at the Lambda Max of the other, it may be possible to determine both drugs by the technique of simultaneous equation method (Vierodt's method) Provided that certain criteria apply.

The Information required is;

a) Absorptivities of X at lambda 1 and lambda 2, ax1 and ax2 respectively.
b) Absorptivities of Y at lambda 1 and lambda 2, ay1 and ay2 respectively.
c) The absorbances of diluted sample at lambda 1 and lambda 2, A1 and A2 respectively.

Let Cx and Cy be the concentrations of X and Y respectively in a diluted sample.

Two equations are constructed based upon the fact that at lambda 1 and lambda 2 the absorbance of the mixture is the sum of the individual absorbances of X and Y.

1. **At lambda 1**

   A1= ax1 b cx + ay1 b cy---------- (1)

2. **At lambda 2**

   A2= ax2 b cx + ay2 b cy---------- (2)

For measurements in 1 cm cell, b =1

Rearrange equation (2)

Cy = A2- ax2 cx/ay2

Substituting for Cy in equation (1) and rearranging

Cx = A2 ay1-A1ay2 / ax2 ay1- ax1 ay2 ---------- (3)

Cy = A1 ax2-A2ax1 / ax2 ay1- ax1ay2---------- (4)

Absorptivity: E 1%, 1 cm

A = abc---------- (5).

A - Absorbance

a = absorptivity (Extinction coefficient)

b = path length

c = concentration

Rearranging equation (5)

a = A/bc---------- (6).

b=1cm

c=1% (1gm/100mL)

a = A/c (1gm/100mL) ---------- (7).

Procedure:

Selection of common solvent

After studying solubility of both the drugs in various solvents, select a common solvent in which both drugs (Ibuprofen and Paracetamol) are soluble.

Preparation of stock standard solutions of Ibu and Para

Ibu 40 and Para 32.5 mg were transferred into two separate 100 mL volumetric flasks to prepare (400 and 325 µg/mL). Withdraw 0.1mL and dilute upto 10 mL with fresh solvent to prepare (4 and 3.25 µg/mL).

Selection of analytical wavelengths

Above two solutions were scanned separately between 400-200 nm. The overlain spectra of both drugs were recorded. λmax of both the drugs were recorded (λ1 and λ2).

Linearity study of Ibu and Para

Linearity of Ibu and Para was recorded as function of absorbance (absorbance should be less than 1). Calibration curve was constructed by plotting absorbance versus concentration.

Concentration of Ibu (µg/mL)	**Absorbance**	**Concentration of Para (µg/mL)**	**Absorbance**

Determination of E (1%, 1cm) values of both drugs at selected wavelengths

E (1%, 1cm) values of these drugs were calculated using following formula;

$$\text{E (1\%, 1cm)} = \frac{Absorbance}{Concentration\ (g/100mL)}$$

Sr no.	**Absorptivity at λ1**		**Absorptivity λ2**	
1.	Ibu	Para	Ibu	Para

2.				
3.				
4.				
5.				
6.				
Mean	ax1 =_____	ay1 =_____	ax2 =_____	ay2 =_____

Formula:

$$\mathbf{Cx\ (Ibu)} = \frac{\mathbf{A2\ ay1 - A1ay2}}{\mathbf{ax2\ ay1 - \ ax1\ ay2}}$$

$$\mathbf{Cy\ (Para)} = \frac{\mathbf{A1\ ax2 - A2ax1}}{\mathbf{ax2\ ay1 - \ ax1ay2}}$$

Where,

A_1 and A_2 are absorbance of mixture at λ1 and λ2;

ax1 and ax2, are E(1%, 1cm) of Ibu at λ1and λ2, respectively;

ay_1 and ay_2, E (1%, 1cm) of Para at λ1and λ2, respectively;

Cx and Cy are concentrations of Ibu and Para in mixture.

Result:

The percentage purity of Ibuprofen and Paracetamol in combination form by Spectrophotometric method was found to be _____%w/w and _____%w/w respectively.

MCQs

1. Which of the following statement is incorrect regarding UV-visible spectrophotometry?

A. It gives a measure of the amount of light absorbed by a sample.

B. The sample is exposed to UV-visible light of different wavelengths.

C. It is used to determine the concentration of a compound in solution.

D. The amount of UV-visible light absorbed by a sample during UV-visible spectrophotometry is called its absorbance.

2. The photomultiplier tube is a widely used ----------in UV-Vis spectroscopy.

A. Wavelength selector

B. Detector

C. Signal processors

D. Cuvette

3. Select the wavelength range corresponding to UV-visible region.

A. 400-800 nm

B. 200-800 nm

C. 25 μm-2.5 μm

D. 2.5 μm – 1mm

4. The spectrophotometer is made up of-

A. Spectrometer

B. Photometer

C. both of these

D. none of these

5. Spectrophotometers measure intensity as a function of the wavelength of the

A. Light source

B. Colour

C. Sound

D. none of these

Questions:

1. What is simultaneous equation method of analysis?

   Ans: If a sample contains two absorbing drugs (X and Y) each of which absorb at the Lambda Max of the other, it may be possible to determine both drugs by the technique of simultaneous equation method (Vierodt's method). Vierodt's method is typically applied to estimate drug combinations that contain two drugs or more than two drugs in combined dosage form.

2. What is multicomponent analysis?

   Ans: In multicomponent systems, specific analytical methods are required to determine the concentrations of individual components in the presence of interfering substances. Ultraviolet and visible spectrometric methods have widely been developed for the analysis of drugs in mixtures and pharmaceutical preparations.

3. What are the three main components of a spectrophotometer?

   Ans: A spectrophotometer consists of three primary components: a light source, optics to deliver and collect the light, and a detector.

4. Which detector is used in UV Visible Spectroscopy?

   Ans: photomultiplier tube: The photomultiplier tube is the most popular detector used in UV- Visible spectroscopy. It comprises of a photosensitive cathode, anode and several dynodes. Photons entering the tube strike the cathode resulting in emission of electrons.

5. What is the difference between colorimeter and spectrophotometer

   Ans: **A colorimeter** is generally any tool that characterizes color samples to provide an objective measure of color characteristics. In chemistry, the colorimeter is an apparatus that allows the absorbance of a solution at a particular frequency (color) of visual light to be determined. Colorimeters hence make it possible to ascertain the concentration of a known solute, since it is proportional to the absorbance.

A spectrophotometer is a photometer (a device for measuring light intensity) that can measure intensity as a function of the color, or more specifically, the wavelength of light. There are many kinds of spectrophotometers. Among the most important distinctions used to classify them are the wavelengths they work with, the measurement techniques they use, how they acquire a spectrum, and the sources of intensity variation they are designed to measure. Other important features of spectrophotometers include the spectral bandwidth and linear range. The most common application of spectrophotometers is the measurement of light absorption.

Experiment No. 5

AIM: To perform assay of paracetamol by UV- Spectrophotometry.

PRINCIPLE

Assay of paracetamol tablet is based on determination of absorption intensity of the solution prepared as per procedure of Indian Pharmacopeia. Absorption of radiation, as a function of frequency or wavelength, is measured, this absorption value is proportion to the concentration of the drug substance (paracetamol) being analysed.

THEORY

Ultraviolet–visible spectroscopy or ultraviolet–visible spectrophotometry (UV–Vis or UV/Vis) refers to absorption spectroscopy or reflectance spectroscopy in part of the ultraviolet and the full, adjacent visible regions of the electromagnetic spectrum.

Absorption spectroscopy deals with the spectroscopic techniques that measure the absorption of radiation, as a function of frequency or wavelength, due to its interaction with a sample. The sample absorbs energy, i.e., photons, from the radiating field. The intensity of the absorption varies as a function of frequency, and this variation is the absorption spectrum. Absorption spectroscopy is employed as an analytical chemistry tool to determine the presence of a particular substance in a sample and, in many cases, to quantify the amount of the material present. Infrared and ultraviolet–visible spectroscopies are particularly common in analytical applications. Absorption spectroscopy is also employed in studies of molecular and atomic physics, astronomical spectroscopy and remote sensing.

REQUIREMENTS

Apparatus: Beaker, Volumetric flask, Pipette etc.

Chemicals: Paracetamol tablets, 0.1N Sodium hydroxide, distilled water etc.

PROCEDURE:

Assay: Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.15 g of Paracetamol, add 50 ml of *0.1 M sodium hydroxide*, dilute with 100 ml of *water*, shake for 15 minutes and add sufficient *water* to produce 200.0 ml. Mix, filter and dilute 10.0 ml of the filtrate to 100.0 ml with *water*. To 10.0 ml of the resulting solution add 10 ml of *0.1 M sodium hydroxide*, dilute to 100.0 ml with *water* and mix. Measure the absorbance of the resulting solution at the maximum at about 257 nm (2.4.7). Calculate the content of $C_8H_9NO_2$ taking 715 as the specific absorbance at 257 nm.

Observations

S. no.	Weight of tablet (gm)	S. no.	Weight of tablet
1.		11.	
2.		12.	
3.		13.	
4.		14.	
5.		15.	
6.		16.	
7.		17.	
8.		18.	
9.		19.	
10.		20.	
		Average weight (gm)	

Label claim of given tablet = _____ gm

Weight of twenty tablets = _____ gm

Average weight of each tablet = _____ gm-------- (A)

Average weight of each tablet (A) contains = 0.500 gm of paracetamol

x gm of powder contains = 0.15gm of paracetamol

x = $\frac{0.15 \times (A)}{0.500}$ = _____ gm --------- (B)

Wavelength	Absorbance
257 nm	

% Dilution factor= $\frac{(B)}{200} \times \frac{10}{100} \times \frac{10}{100} \times 100$ = _____%w/v----------(C)

Formula

% Purity = $\frac{Absorbance}{E\ 1\%\ 1\ cm} \times \frac{Average\ weight}{Label\ claim} \times \frac{100}{(C)}$

% Purity = _____% w/w

Result: The Percent purity of the paracetamol tablet was found to be_____% w/w.

MCQ's

1. Beer Lambert's law gives the relation between which of the following?

A. Reflected radiation and concentration

B. Scattered radiation and concentration

C. Energy absorption and concentration

D. Energy absorption and reflected radiation

2. In which of the following ways, absorption is related to transmittance?

A. Absorption is the logarithm of transmittance

B. Absorption is the reciprocal of transmittance

C. Absorption is the negative logarithm of transmittance

D. Absorption is a multiple of transmittance

3. Beer's law states that the intensity of light decreases with respect to _____

A. Distance

B. Concentration

C. Volume

D. Composition

4. Beer's law states that the intensity of light decreases with respect to _____

A. Distance

B. Concentration

C. Volume

D. Composition

5. What is the unit of absorbance, which can be derived from Beer Lambert's law?

A. L mol-1 cm-1

B. L gm-1 cm-1

C. cm

D. No unit

Questions

1. What is principle of Ultraviolet-visible spectroscopy?

   Ans: Ultraviolet-visible spectroscopy (UV-Vis spectroscopy) makes use of absorption spectroscopy in ultraviolet and visible wavelength ranges—180–380 nm and 380–750 nm, respectively—for characterizing molecules. This is one of the most basic techniques that need to be conducted while characterizing an analyte. All the major classes of biomolecules contain certain light absorbing functional groups known as chromophores. Upon absorbing UV/Vis light, these chromophores get excited from

ground state to a higher energy level, thus giving out characteristic spectra, aiding in the identification of specific biomolecules.

2. Which lamp is used in UV spectroscopy?

   Ans: Deuterium lamps are always used with a Tungsten halogen lamp to allow measurements to be performed in both the UV and visible regions. Also known as quartz Iodine lamps, this measure most effectively in the visible region from 320 - 1100 nm.

3. What are the components of UV Visible Spectrophotometer?

   Ans: There are four basic components to a simple single beam UV/Vis spectrophotometer; a light source, a monochromator, a sample, and a detector.

4. What are the 7 visible spectrum colors?

   Ans: A commonly taught acronym that helps people remember the colors in the visible spectrum is (ROY G BIV) which stands for Red, Orange, Yellow, Green, Blue, Indigo, and Violet.

5. Does cuvette size affect absorbance?

   Ans: Yes, The absorbance is directly proportional to the concentration (c) of the solution of the sample used in the experiment. The absorbance is directly proportional to the length of the light path (l), which is equal to the width of the cuvette.

Experiment No. 6

Aim:

Estimation of Quinine sulfate by Fluorimetry

Principle:

Fluorescence is the phenomenon of emission of radiation when there is transition from singlet excited state to singlet ground state. The wavelength of absorbed radiation is called as excitation wavelength and that of emitted radiation is called as emission wavelength. Fluorescent intensity is directly proportional to concentration of substance. Fluorometer is the instrument used to measure fluorescence with a wavelength varying between 240 nm to 800 nm.

Requirement:

Apparatus: Volumetric flask, Beaker, Pipette

Chemicals: Quinine sulphate, 0.1N H_2SO_4, Distilled water

Instrument: Fluorometer

Preparation of Standard Solution:

1) Weigh accurately 100 mg of Quinine sulphate powdered drug
2) Dissolve in 100 mL of 0.1 N H_2SO_4 to get Stock solution (1000 µg/mL)
3) Take 10 mL of stock solution and dilute to 100 mL with 0.1 N H_2SO_4 (100 µg/mL)
4) Again add 1 mL of above solution and dilute to 100 mL with 0.1 N H_2SO_4 (10 µg/mL)
5) Prepare concentration of 0.5, 1, 1.5, 2, and 2.5 µg/mL from above solution and dilute to 10 mL with 0.1 N H_2SO_4.

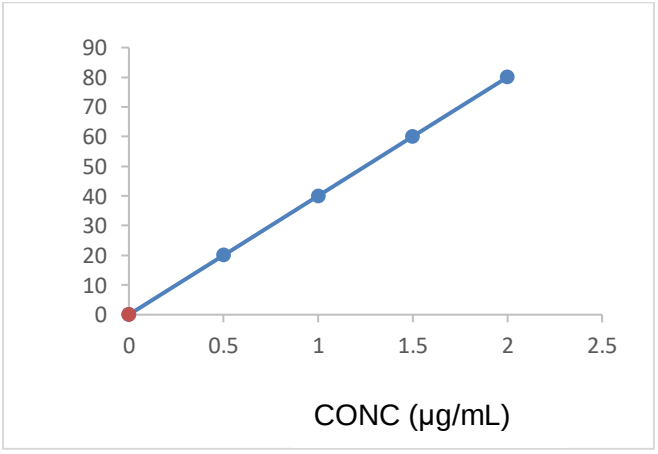
Preparation of Sample Solution:

1) Pipette out 1 mL of given sample solution and make up the volume to 10 mL with with 0.1 N H_2SO_4

2) Switch on the instrument, set the excitation and emission filters at the wavelength 360 to 460 nm respectively
3) Set the Fluorescent Intensity (FI) to 0 % by using 0.1 N H_2SO_4 as blank and 100 % by using highest concentration of the standard solution 2.5 µg/mL.
4) Repeat the same at least for two or more time to avoid instrumental error
5) Measure the % FI of different standard and sample solutions
6) Plot the graph between the concentration vs FI and determine the concentration of unknown sample by extrapolating the found FI.

Observation:

Sr No.	Conc. (µg/mL)	% FI
1	0	
2	0.5	
3	1	
4	1.5	
5	2	
6	2.5	
7	unknown	



Concentration of unknown sample =…… µg/mL

Sample solution dilutes by 10 times, so the conc. = ……. × 10 = ……. µg/mL

Result:

The concentration of quinine sulphate in the given sample was found to be ……. mg/mL

MCQ`s

1) The process of raising electrons in the atoms or molecules to higher energy level is called?
    a) Partition
    b) Transition
    c) **Excitation**
    d) Derivatization
2) …….. is the general term applied to the absorption and emission of radiant energy
    a) Fluorescence
    b) Phosphorescence
    c) **Luminescence**
    d) Affinity Chromatography
3) Re-emission as radiation of all or a part of radiant energy is known as ……...
    a) Phosphorescence
    b) **Fluorescence**
    c) Adsorption
    d) Absorption
4) An increase in the intensity of the light incident on the sample produces proportional …… in the fluorescence intensity
    a) Decrease
    b) **Increase**

c) Alternation

d) Conjugation

5) Electron donating groups like -NH_2, -OH, etc. often ……. Fluorescence.

a) **Increase**

b) Decrease

c) Nullify

d) Withdraw

Questions

1. Define Fluorescence. Enlist the factor affecting it.

   Ans: 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.

2. Enlist types of Luminescence?

   Ans:

   i. Photoluminescence
      a. Fluorescence
      b. Phosphorescence
   ii. Chemiluminescence
   iii. Bioluminescence
   iv. Triboluminescnce
   v. Cathodluminescence
   vi. Thermoluminescence

3. Define Phosphorescence?

   Ans: It is related to fluorescence in emitting a photon; however a phosphorescent material does not immediately re-emit the radiation it absorbs.

4. Enlist the factor affecting Fluorescence.

   Ans:

   i. Temperature

   ii. pH

   iii. Dissolved Oxygen

   iv. Solvent

   v. Adsorption

   vi. Conjugation

5. Define Photoluminescence

   Ans: The absorption of light is due to the absorption of photon (light) this luminescence is called as Photoluminescence.

Experiment No. 7

Aim:

Study of quenching of fluorescence

Requirements:

Apparatus: Volumetric flask, Beaker, Pipette

Chemicals: Quinine sulphate, 0.1N H_2SO_4, Distilled water, Potassium Iodide

Instrument: fluorometric

Principle:

Quenching is the decrease in fluorescence intensity due to specific effects or presence of certain ions, constituents of the solution itself. These effects may be due to various factors like Concentration, pH, presence of specific chemical substances, temperature, viscosity, etc.

Types of Quenching

Self-quenching

Chemical quenching

Static quenching

Collisional quenching

Procedure:

Preparation of Potassium Iodide solution:

Dissolve 100 mg KI in 100 mL 0.1N H_2SO_4

Take 10 mL of above solution and dilute to 100 mL with 0.1 N H_2SO_4 (100 µg/mL).

For final conc. Again add 1 mL of above solution and dilute to 100 mL with 0.1 N H_2SO_4 (10 µg/mL)

Preparation of Standard Quinine sulphate solution

1. Weigh accurately 100 mg of Quinine sulphate powdered drug
2. Dissolve in 100 mL of 0.1 N H_2SO_4 to get Stock solution (1000 µg/mL)

3. Take 10 mL of stock solution and dilute to 100 mL with 0.1 N H_2SO_4 (100 µg/mL).
4. For final conc. Again add 1 mL of above solution and dilute to 100 mL with 0.1 N H_2SO_4 (10 µg/mL)
5. In six 10 mL volumetric flask take 1 mL of standard quinine solution.
6. Add 1,2,3,4 and 5 mL of KI solution in each flask and make up the volume to 10 mL.
7. Set the FI to 0% by using 0.1 N H_2SO_4 as blank and 100 % by using standard solution (without KI)

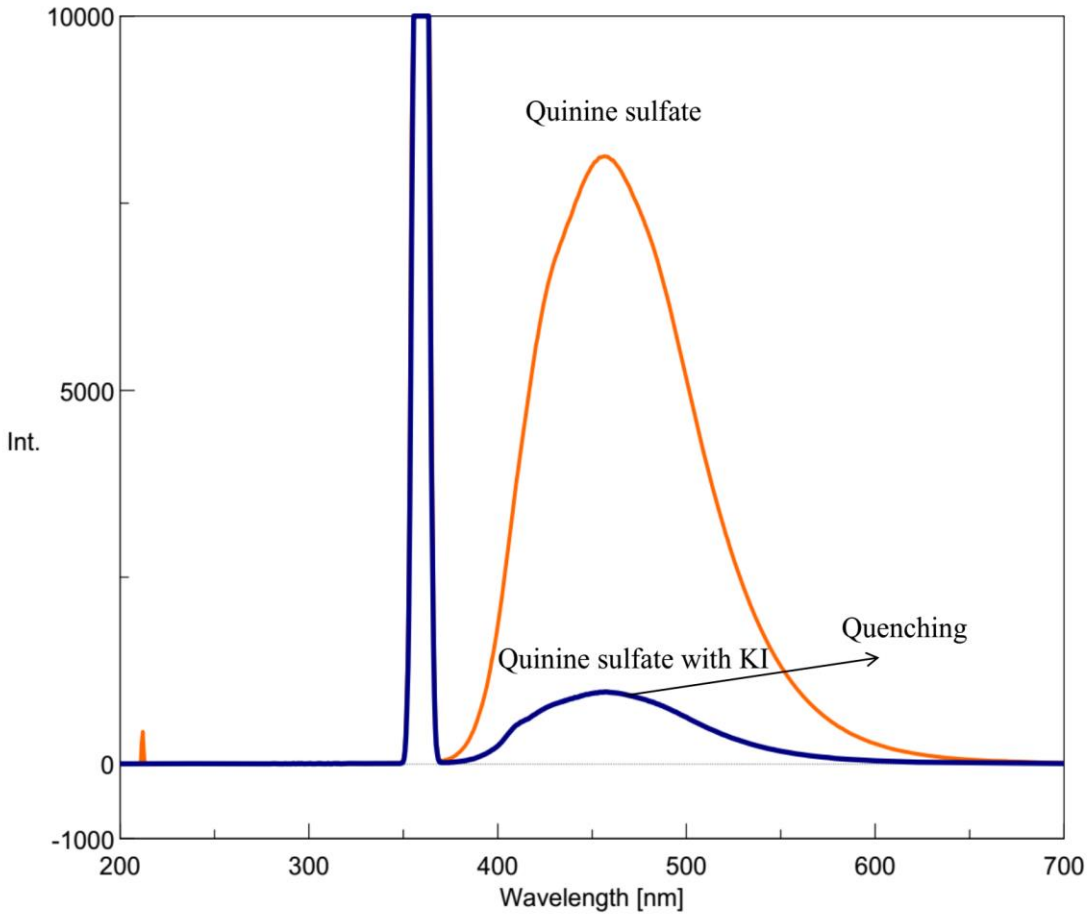


Figure 3 : Typical spectrum depicting quenching of fluorescence intensity of quinine sulfate by potassium iodide

Observation Table:

Sr No.	Volume of KI (mL)	% F I
1	0	
2	1	
3	2	
4	3	
5	4	
6	5	

Result:

A decrease in fluorescence intensity shall be observed for quinine solution with the addition of potassium iodide solution.

MCQ`s

1) …… is reduction of fluorescence intensity by the presence of substances in the sample other than the analyte
    a) **Quenching**
    b) Filtering
    c) Excitation
    d) Derivatization
2) Fluorescent compounds have characteristic …….
    a) Excitation
    b) Emission
    c) **A and B**
    d) None of above
3) Fluorescence is ……. In molecules that possess rigid structure

a) **Increases**

b) Decreases

c) Remain unaffected

d) None of above

4) Static quenching occurs mainly because of …….

a) Colloidal effect

b) Collisional effect

c) **Complex formation**

d) Chemical changes

5) When conc. increases, fluorescence intensity does not increase proportionally. This phenomenon is called as ……

a) **Self-Quenching**

b) Chemical Quenching

c) Static Quenching

d) Collisional Quenching

Questions

1. Define Quenching.

   Ans: In materials science, quenching is the rapid cooling of a work piece in water, oil or air to obtain certain material properties. A type of heat treating, quenching prevents undesired low-temperature processes, such as phase transformations, from occurring.

2. Enlist types of Quenching

   Ans:

   i. Self or Concentration Quenching
   ii. Collisional

iii. Static
iv. Chemical

3. Enlist Detectors used in Fluorimetry.

Ans:

i. Photovoltaic Cell
ii. Phototube

4. What is the effect of viscosity in Fluorescent intensity?

Ans: Increase in viscosity leads to decreased collisions of molecules which lead to enhancement of fluorescence intensity & vice versa.

5. Give the instruments that measure fluorescence?

Ans:

i. Filter monochromator
ii. Spectrofluorometer

Experiment No. 8

Aim:

Determination of sodium by flame photometry

Requirement:

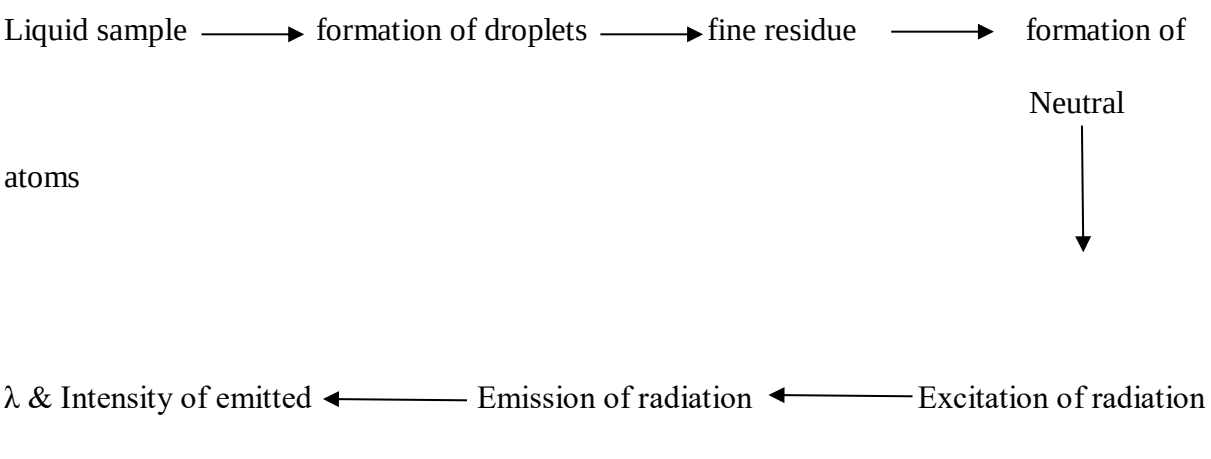
Apparatus: Volumetric flask, beaker, pipette

Chemicals: Sodium Chloride, Distilled water

Instrument: Flame Photometer

Principle:

When a solution of metallic salt is sprayed on the flame, fine droplets are formed. Due to the thermal energy of the flame, the solvent in the droplets evaporate, leaving behind fine residue, which are converted to neutral atoms. These neutral atoms are converted to excited state atoms by the thermal energy of the flame. As the excited state is not stable, these excited atoms return to ground state, with the emission of radiation of specific wavelength. The wavelength of the radiation emitted is characteristic of the element and is used to identify the element. The intensity of the radiation emitted depends upon the conc. of the element analysed.



Procedure:

1) Weigh accurately 100 mg of NaCl and dissolve in 100 mL of distilled water
2) Take 10 mL of the solution and dilute to 100 mL with distilled water
3) Prepare a series of standard solutions of 10, 20, 30, 40 and 50 µg/mL concentrations.
4) Switch On the Instrument and select the sodium filter
5) Set the gas in the flame in order to get non-luminous flame and air pressure at 0.4 to 0.5 kg/cm^3
6) Atomize the Flame intensity to 0 % using distilled water with the knob.
7) Atomize the Flame intensity to 100 % using 50 µg/mL standard solutions (highest conc.).
8) Measure the percent flame intensity of all the standard solutions (10, 20, 30, 40 and 50 µg/mL concentrations)
9) Plot the graph of conc. of solution and % Flame intensity
10) From the graph, mark the % flame intensity of the unknown sample and by extrapolating, determine the corresponding conc.

Dilutions:

100 mg dissolve in 100 mL water = 1000 µg/mL

10 mL diluted to100 mL water = 100 µg/mL

1 mL of 100 µg/mL diluted to 100 mL =10 µg/mL

2 mL of 100 µg/mL diluted to 100 mL = 20 µg/mL

3 mL of 100 µg/mL diluted to 100 mL = 30 µg/mL

4 mL of 100 µg/mL diluted to 100 mL = 40 µg/mL

5 mL of 100 µg/mL diluted to 100 mL = 50 µg/mL

Observation Table:

Sr No.	Conc (µg/mL)	FI (%)
1	0	

2	10	
3	20	
4	30	
5	40	
6	50	
7	unknown	

Result:

The conc. of sodium ion in the given sample of NaCl solution was found to be ……. µg/mL by Flame photometry

MCQ`s

1) To get higher temperature in the burner, in flame photometer, the following combination is used …….

   Hydrogen/Air

   Hydrogen/Oxygen

   Acetylene/Air

   Acetylene/Oxygen

2) Which of the following element is most easily detected by flame photometry?

   Lithium

   Calcium

   Sodium

   Beryllium

3) Which of the following is not the requirement of a good flame in flame photometer?

   Liquid sample must be evaporated to form solid residue

   Solid residue must decompose to form atoms

Atoms must be produced such that they have the ability to get excited to higher states

Atoms must be produced such that they are in stable states

4) Which of the following is the principle of flame emission photometers?

Radiation is absorbed by non-excited atoms in vapour state and are excited to higher states

Medium absorbs radiation and transmitted radiation is measured

Colour and wavelength of the flame is measured

Only wavelength of the flame is measured

5) Which is application of flame photometry?

To determine functional group

To study of chemical structure

To assay of drug

To estimate metallic ions like sodium, potassium, etc.

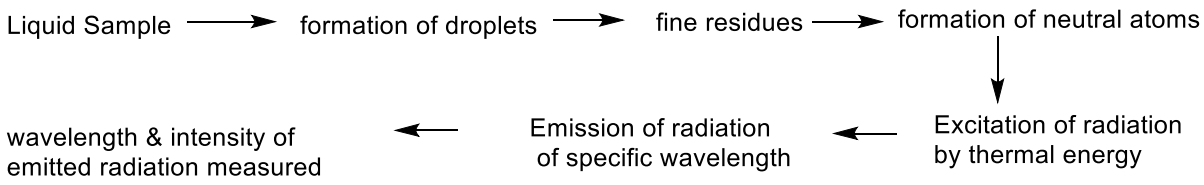
Questions

1. Define Flame Photometry.

   Ans: It is the measurement of the intensity of light emitted at a particular wavelength from the atoms that are excited thermally in flame.

2. Describe the steps involved in Flame Photometry.

   Ans:



3. Enlist the components of Flame Photometer.

   Ans:

   i. Fuel gas Pressure Regulator

ii. The atomizer

iii. Sample cell

iv. The Nebulizer-burner System

v. Optical System

vi. Photo sensitive detector

vii. Recording or read out device

4. Describe the function of flame in Flame Photometer.

Ans:

To convert the constituent of liquid sample into vapour state

To decompose the constituent into atoms or simple molecules:

$$M^{+} + e^{-} \xrightarrow{\text{From flame}} M + hv$$

To electronically excite a fraction of the resulting atomic or molecular species.

$$M \longrightarrow M^{*}$$

5. Enlist methods of Atomization

Ans:

i. Flame atomization

ii. Electro Thermal Atomization.

Experiment No. 9

Aim:

Determination of potassium by flame photometry

Requirement:

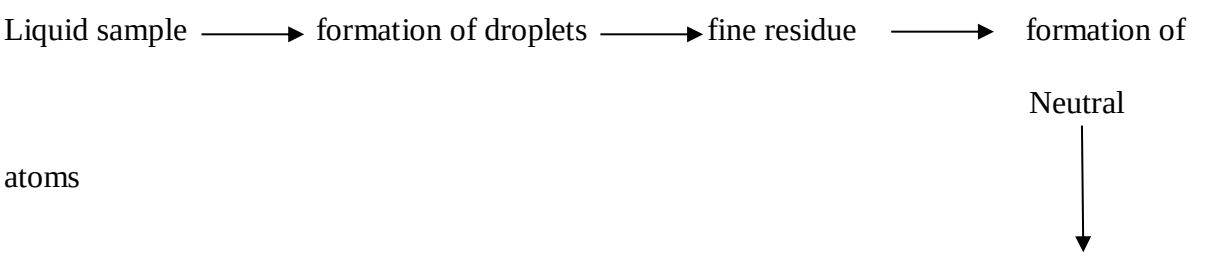
Apparatus: Volumetric flask, beaker, pipette

Chemicals: Potassium Chloride, Distilled water

Instrument: Flame Photometer

Principle:

When a solution of metallic salt is sprayed on the flame, fine droplets are formed. Due to the thermal energy of the flame, the solvent in the droplets evaporate, leaving behind fine residue, which are converted to neutral atoms. These neutral atoms are converted to excited state atoms by the thermal energy of the flame. As the excited state is not stable, these excited atoms return to ground state, with the emission of radiation of specific wavelength. The wavelength of the radiation emitted is characteristic of the element and is used to identify the element. The intensity of the radiation emitted depends upon the conc. of the element analysed.



Procedure:

1) Weigh accurately 100 mg of KCl and dissolve in 100 mL of distilled water
2) Take 10 mL of the solution and dilute to 100 mL with distilled water
3) Prepare a series of standard solutions of 10, 20, 30, 40 and 50 µg/mL concentrations.
4) Switch On the Instrument and select the potassium filter,
5) Set the gas in the flame in order to get non-luminous flame and air prtessure at 0.4 to 0.5 kg/cm^3
6) Atomize the Flame intensity to 0 % using distilled water with the knob.
7) Atomize the Flame intensity to 100 % using 50 µg/mL standard solution (highest conc.).
8) Measure the percent flame intensity of all the standard solutions (10, 20, 30, 40 and 50 µg/mL concentrations)
9) Plot the graph of conc. of solution and % Flame intensity
10) From the graph, mark the % flame intensity of the unknown sample and by extrapolating, determine the corresponding conc.

Dilutions:

100 mg dissolve in 100 mL water = 1000 µg/mL

10 mL diluted to100 mL water = 100 µg/mL

1 mL of 100 µg/mL diluted to 100 mL =10 µg/mL

2 mL of 100 µg/mL diluted to 100 mL = 20 µg/mL

3 mL of 100 µg/mL diluted to 100 mL = 30 µg/mL

4 mL of 100 µg/mL diluted to 100 mL = 40 µg/mL

5 mL of 100 µg/mL diluted to 100 mL = 50 µg/mL

Observation Table:

Sr No.	Conc (µg/mL)	FI (%)
1	0	

2	10	
3	20	
4	30	
5	40	
6	50	
7	unknown	

Result:

The conc. of potassium ion in the given sample of KCl solution was found to be ……. µg/mL by Flame photometry

MCQ`s

1) Which of the following is the principle of Flame emission photometers?

Radiation is absorbed by non-excited atoms in vapour state and are excited to higher states

Medium absorbs radiation and transmitted radiation is measured

Colour and wavelength of the flame is measured

Only wavelength of the flame is measured

2) Which is not application of flame photometry?

To estimate sodium, magnesium, calcium

Assay of metformin.

Used to determine magnesium and calcium in cement.

To detected metallic ions in sample

3) Choose correct sequence of flame photometry?

Sample residue→excited atoms→Return in ground state→Emission of radiation

Sample residue → ground state → excited state → Emission of radiation

Emission of radiation → excited state →ground state → Sample residue

Sample residue → ground state → excited state → Emission of radiation

4) Which of the following is not a detector used in Flame emission photometers?

Photronic cell

Photovoltaic cell

Photoemissive tube

Chromatogram

5) If Hydrogen and air are used in burner how many °C temp. is produced?

1900°C

2200°C

2300°C

2100°C

Questions

1. Enlist detectors used in Flame Photometry.

   Ans:

   i. Photovoltaic Cells or Barrier-Layer Cell

   ii. Phototubes or Photoemissive tubes

   iii. Photomultiplier tubes.

2. Give advantages of Flame Photometry

   Ans:

   i. Very simple method of analysis

   ii. Quick

   iii. Convenient

   iv. Selective & Sensitive for analysis

v. Low Concentrations of metals can be determined.

3. Enlist the fuel gas used in Flame Emission Spectroscopy

Ans:

i. Propane

ii. Hydrogen

iii. Acetylene

4. Define Interferences.

Ans:

Differences between the sample & the standard solutions can lead to systemic errors are called as interferences.

5. Enlist Interferences in Flame Emission Spectroscopy

Ans:

i. Spectral Interferences

ii. Background Interferences

iii. Chemical Interferences

iv. Cation Interferences

v. Anion Interferences

vi. Oxide Formation Interferences

vii. Foreign Elements Interferences

viii. Salts & Acids Interferences

ix. Matrix Interferences

Experiment No. 10

Aim:

Determination of Chlorides and Sulphated by Nepheloturbidimtery

Requirement:

Chloride standard solution, 0.1N Silver Nitrate, Sulphate Standard solution, Dilute nitric acid, Barium Chloride Solution, Dilute acetic acid, Distilled water.

Principle:

Nephelometry: It is the measurement of scattered light as function of concentration of suspended particles. The intensity of scattered light is proportional to the concentration. The intensity of scattered light is normally measured at 90º. It can also be measured at any convenient angle like 45º, 60º,135º etc.

Turbidimetry: It is measurement of transmitted light as function of concentration of suspended particles. The intensity of transmitted light is measured at 180º. The intensity of transmitted light as a function of concentration i.e. when concentration is more, it is less and when concentration is less, It is more.

Procedure:

Determination of Chloride:

Preparation of Standard Opalescence:

1) Take 10 mL of Chloride standard solution (25 ppm) into Nessler cylinder.
2) Add 10 mL of Dilute HNO_3 and make up the volume to 50 mL with distilled water.
3) Add 1 mL of 0.1 N $AgNO_3$ solution and stir immediately to produce the turbidity.
4) Allow it to stand for 5 minutes protected from light.

Preparation of Sample Opalescence:

1) Pipette out 10 mL of sample solution into Nessler cylinder.
2) Add 10 mL of dilute HNO_3 and make up the volume to 50 mL with distilled water

3) Add 1 mL of 0.1 N $AgNO_3$ and stir immediately.

4) Allow the resulting solution to stand for 5 minutes protected from light.

Measurement of Nepheloturbidimetric unit (NTU)

1) Switch on the instrument

2) Allow it to stabilize for 10 minutes.

3) Place distilled water and adjust reading to 0 %

4) Discard the water and take standard opalescence and adjust the reading to 100 %.

5) Run sample opalescence and compare the % NTU with Standard.

Determination of Sulphate:

Preparation of Standard Opalescence:

1) Pipette out 1.5 mL of ethanolic standard sulphate solution into Nessler cylinder.

2) To this add, 1 mL of 25 % w/v barium chloride solution.

3) Mix and allow to stand for 1 minute.

4) To this add, 15 mL of (10 ppm) standard sulfate solution and 0.15 mL of 5 M acetic acid.

5) Stir immediately with glass rod and allow to stand for 5 minutes.

Preparation of Sample Turbidity:

1) Pipette out 1.5 mL of ethanolic standard sulphate solution into Nessler cylinder.

2) To this add, 1 mL of 25 % w/v barium chloride solution.

3) Mix and allow to stand for 1 minute.

4) To this add, 15 mL of sample solution.

5) Add 0.15 mL of 5 M acetic acid.

6) Make up the volume to 50 mL with distilled water.

7) Stir immediately with glass rod and allow to stand for 5 minutes.

Measurement of Nepheloturbidimetric Unit (NTU):

1) Switch on the instrument

2) Allow it to stabilize for 10 minutes.

3) Place distilled water and adjust reading to 0 %

4) Discard the water and take standard opalescence and adjust the reading to 100 %.

5) Run sample opalescence and compare the % NTU with Standard.

Observation Table:

For Chloride:

Sr. No.	**Preparation**	**NTU Range**	**Meter Reading**	**Total NTU x Meter Reading**
1.	**Standard**	**1000**		
2.	**Standard**			

For Sulphates:

Sr. No.	**Preparation**	**NTU Range**	**Meter Reading**	**Total NTU x Meter Reading**
1.	**Standard**	**1000**		
2.	**Standard**			

Result:

As the NTU range x meter reading of the sample found less than that of the standard solution, therefore the given sample passes/fails the limit test for sulphate. Similarly, as the NTU range x meter reading of the sample found less than that of the standard solution, therefore the given sample passes/fails the limit test for chloride.

MCQ`s

1) Smaller particles undergo scattering to give rise a symmetrical pattern of secondary rays in space having maximum intensity of ……

1) **90**

2) 180

3) 60

4) 45

2) Phosphorous can be determined by turbidimetry using which reagent

1) **Nessler reagent**

2) Strychnine Molybedate reagent

3) Barium sulphate

4) Silver chloride

3) Turbidity is the official assay method for ……

1) **Vitamin B_{12}**

2) Vitamin A

3) Vitamin B_1

4) Vitamin B_6

4) Nepheloturbidimtery method is …… times better than HPLC method

1) 50 times

2) **100 times**

3) 80 times

4) 90 times

5) Turbidity of macromolecules depends on ……

1) Number of molecules

2) Concentration

3) **Molecular weight**

4) Polarity of Molecules

Questions

1. Define Quenching.

   Ans: In materials science, quenching is the rapid cooling of a work piece in water, oil or air to obtain certain material properties. A type of heat treating, quenching prevents undesired low-temperature processes, such as phase transformations, from occurring.

2. Enlist types of Quenching

   Ans:

   i. Self or Concentration Quenching

   ii. Collisional

   iii. Static

   iv. Chemical

3. Enlist Detectors used in Fluorimetry.

   Ans:

   i. Photovoltaic Cell

   ii. Phototube

4. What is the effect of viscosity in Fluorescent intensity?

   Ans: Increase in viscosity leads to decreased collisions of molecules which lead to enhancement of fluorescence intensity & vice versa.

5. Give the instruments that measure fluorescence?

   Ans:

   i. Filter monochromator

ii. Spectrofluorometer

Experiment No. 11

AIM- To perform the paper chromatography for the separation of amino acids present in the given sample.

THEORY

The chromatographic techniques used to separate out mixtures of different substances into their discrete components. All forms of chromatographic techniques work on the same principle. They all have basic requirements of stationary phase (a solid or a liquid supported on a solid) and a mobile phase (a liquid or a gas). The mobile phase tides through the stationary phase and carries the components of the mixture with it. Different components travel at different rates based on their affinities toward stationary phase and mobile phase. In paper chromatography, the stationary phase is a very uniform adsorbent paper and the mobile phase is a suitable liquid solvent or mixture of solvents.

Retention (or) retardation factor (Rf)

Retention factor value (Rf Value) is defined the ratio of the distance travelled by the solute to the distance travelled by solvent. The distance travelled relative to the solvent is called the Rf value.

Rf = Distance travelled by solute / Distance travelled by solvents

REQUIREMENTS

Apparatus: Glass beakers, Whatmann filter paper, Petridishes, Measuring cylinder, developing chamber and Capillary tubes etc.

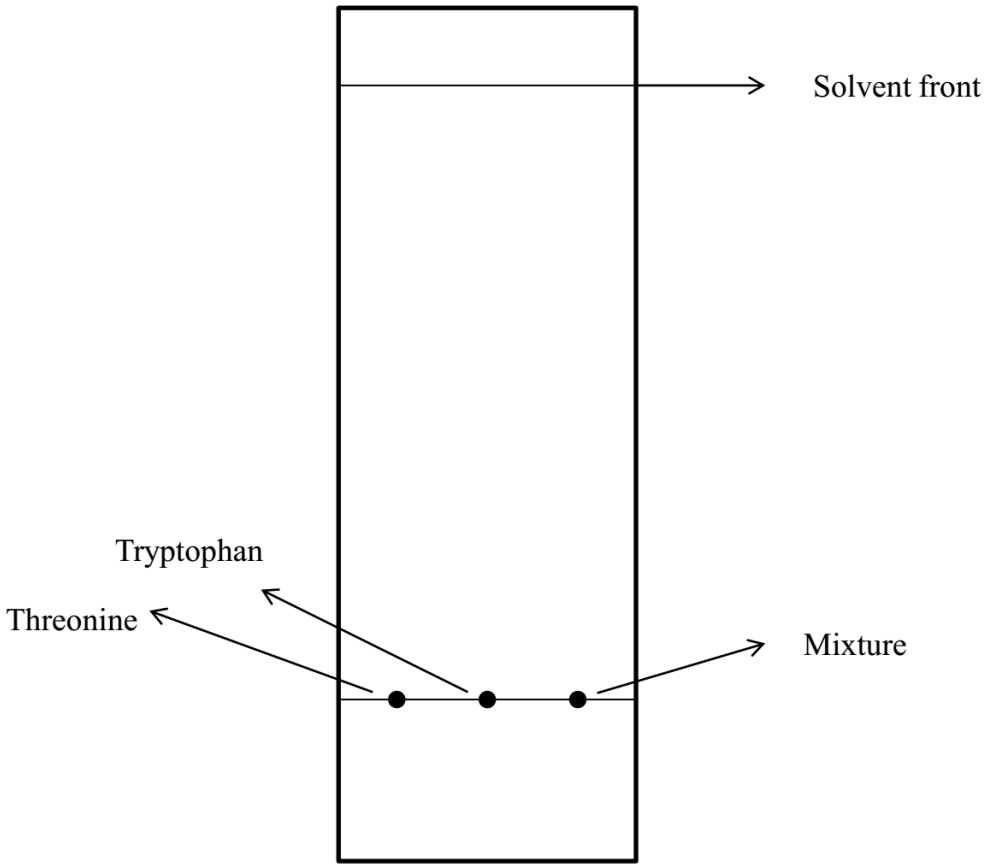
Chemicals: n-butanol, Glacial acetic acid, Distilled water (4:1:5), Amino acids (Tryptophan and threonine), Ninhydrin reagents etc.

PROCEDURE

Solvents system and methods preparation – For solvents system and methods preparation n-butanol and water are taken in ratios of 4:5 in a flask and allow it to saturate for 24 hours,

by using the separating funnel separate out the n - butanol and water. The saturated n - butanol and glacial acetic acid both are taken in the 4:1 ratio which can be used as a solvent system (or) mobile phase.

Paper chromatography - For paper chromatography method is quite simple as compared to other methods of chromatography. The chromatography paper is cut into rectangular strips and marks a line on the paper with pencil at about 2 cm from the bottom. With the help of capillary tube, the samples are applied at different points on the starting line and place the chromatography paper in the developing chamber, which contains the mobile phase. While placing the paper, it is important that the solvent level should not reach the starting line or the sample spots and paper shouldn't touch the walls of the developing chamber. After sometime the solvent rises up the paper or the stationary phase by capillary action and dissolves the sample. The components of the sample move along with the solvent in upward direction. Checkered if the solvent has reached near the top level of chromatography paper, then the paper is removed when it reaches the top and marked the level with pencil. This level (or) height is called the "solvent front". By using UV light, Ninhydrin or iodine vapors examined the different spots of varied colors.



RESULTS

For paper chromatography

The distance moved by tryptophan and threonine is …… cm and ….. cm respectively, and the solvent is ……cm

Rf value of tryptophan is ……

Rf value of threonine is ……..

Rf value of unknown mixture is ….. & …..

MCQ's

1. Which is not development technique of paper chromatography?

    a. HPLC

    b. Ascending

c. Descending

d. Two dimensional

2. Which type of mobile phase (MP) is used in paper chromatography?

a. The MP which gives Rf value range between 0.8-1

b. The MP which gives Rf value range between 0.6.

c. The MP which gives Rf value range between 0.1-0.3

d. The MP which gives Rf value range between 0.2-0.8

3. When there are too complex mixture are separated which development technique of paper Chromatography is used?

a. Ascending

b. Two dimensional

c. Descending

d. Radiation

4. How much time is essential to saturate of chamber?

a. 24 hour

b. 6 hour

c. 12 hour

d. 10 hour

5. Which force is control for the separation of the components in descending paper chromatography?

a. Partition

b. Adsorption

c. Gravity

d. All of the above

Questions

1. **What do you mean by paper chromatography?**

   Ans. Paper chromatography is applicable as a separation technique where separation of a mixture of compound is mainly achieved by a flow of solvent on a chromatographic paper and stationary phase has bound or absorbed water present with the cellulose of the chromatographic paper and the mobile phase is an organic solvent which is immiscible with stationary phase.

2. **What is a principle of paper chromatography**?

   Ans. Paper chromatography is mainly work on principal of the adsorption & partition. The mechanism of separation in paper chromatography is mainly partition in Type. So, when a drop of the solute is treating with the solvent on the paper, the more strong components back while less strong components are move forward.

3. **Define Rf value with their formula.**

   Ans. The relative rate of the movement of solvent and solute is expressed by a term Rf. It is defined as the ratio of the distance travelled by the compound at its maximum.

   Rf = Distance travelled by solute / Distance travelled by solvents

   In many cases it has been observed that the solvent front os run off the end of the chromatogram. Rx value is the ratio of the distance travelled by a substance to the distance travelled by a reference standard.

4. **Enlist factors, which effects on RF value.**

   Ans. Following factors are generally effect on Rf Value - The temperature of environment, Purity of solvent used viscosity of solvent, The type of paper, speed of paper, ash content, pH of the mobile phase, Chemical reaction if any during development.

5. **Give chromatographic development stages.**

Ans. There are mainly three technique are developed

- Ascending
- Descending
- Radial
- Two dimensions

Experiment No. 12

AIM: To perform the separation and analysis of some sugars by using thin layer chromatography (TLC)

THEORY

The inordinate consideration has been given just too some comparatively rapid techniques of analysis. Thin layer chromatography (T.L.C.) is previously accepted and established as a laboratory tool for routine analytical work. Its low cost effective, ease, and rapidity along with its capacity for separating and identifying small quantities of compound mixtures make the technique important tool for research. The components with less affinity towards the stationary phases are travels rapidly. The major objective of this investigation was to adapt a method for separation, identification, and approximation of different sugars in beet processing liquors, thick juice from storage, and beet storage samples.

REQUIREMENTS

Apparatus: Glass beakers, measuring cylinder, developing chamber glass plates etc.

Chemicals: Chloroform, acetic acid, and water, 85% Orthophospheric acid, diphenylamine, aniline acetone etc.

PROCEDURE

For the preformation of TLC practical be prepared following things firstly as Glass plates are precoated with 0.25 mm dry silica gel. Solvent system which consists of a mixture of chloroform, acetic acid, and water (3:3.5:0.5) by volume, respectively and spraying agent is

made from 1 gram diphenylamine and 1 ml of aniline in 100 ml acetone. This mixture is further mixed with 85% orthophosphoric acid prior to use (10: 1 v/v, respectively).

Sample is applied and then the plate is dried in air for approximately 30 minutes. The solvent system irrigated within the ascending direction in a tight container. The solvent is allowed to move upward about 12.5 cm, this usually requires 90 minutes. The plate is removed from the tank. Then it is kept for drying in air for about 30 minutes. The plate is placed back in the same developing solvent and let the solvent to move in the same direction to the same distance of 12.5 cm. It takes 45minutes and plates should then be dried for approximately 30 minutes.

RESULTS

The distance moved by glucose …. and …… is …… cm and ….. cm respectively and the solvent is ……cm.

Rf value of glucose is ……

Rf value of fructose is ……..

MCQ's

1. The components with less affinity towards the stationary phases is

    a. Travels slowly

    b. Travels rapidly

    c. A and B

    d. None of the above

2. The mobile phase is flow by

    a. Partition coefficient

    b. Temperature

c. Capillary action

d. All of the above

3. Which verdict is false about

   a. It is less time consuming.

   b. It is very sample method

   c. It is very costly.

   d. Applied to allow type of sample.

4. What is the principle of Thin Layer Chromatography?

   a. Absorption

   b. Adsorption

   c. Partition

   d. A and B

5. In TLC following which is not factor for choosing adsorbent?

   a. Temperature.

   b. Nature of the adsorbent

   c. Solubility of the adsorbent

   d. Chemical nature

Questions

1. **What is a principle of thin layer Chromatography (TLC)**

   Ans. The principle of TLC is separation is adsorption. When the mixture contains one or more components is spotted on a TLC of absorbent coated on a chromatographic plate and introduce into mobile phase, which are continuing distributing. The mobile phase is flow by capillary action. The components with more affinity travel slow and that of lesser affinity travel faster. TLC is method of analysis in which the stationary phase is spread as a thin layer on a rigid supporting plate. The mobile phase a liquid is allowed to migrate across the stationary of plate. TLC is a solid- liquid form of Chromatography, where the stationary phase is polar and mobile phase is non polar.

2. **Give characteristics of thin layer Chromatography (TLC)**

Ans.
- It's a simple and rapid technique
- Low cost technique and takes less time
- Applied for all types of mixture samples
- Detection is easy and not tedious
- More efficiency of separation
- Corrosive spray reagents can be used without any damage to plate

3. Why activation of TLC plate is necessary?

Ans. Plate is also allowed to saturate with these solvent vapour if required by exposing the plate to solvent vapour is closed chamber. Then the plate is dipped in developing to effect separation of analytical mixture. Horizontal, sandwich, two-dimensional techniques are also use in TLC.

4. What is a purpose of TLC?

Ans. Thin - layer chromatography (TLC) is a chromatography technique used to separate non-volatile mixtures. Thin-layer chromatography can be used to monitor the progress of a reaction, identify compounds present in a given mixture, and determine the purity of a substance.

5. Why silica gel is used in TLC?

Ans. Silica gel is by far the most widely used adsorbent and remains the dominant stationary phase for TLC. The surface of silica gel with the highest concentration of geminal and associated silanols is favored most for the chromatography of basic compounds because these silanols are less acidic.

Experiment No. 13

AIM - To separate the plant pigments by using column chromatography.

THEORY

The plants leaves contain a different colored pigments generally falling into two categories, carotenoids and chlorophylls. Carotenoid is a part of a larger collection of plant - derived compounds called terpenes and they are tetraterpenes (eight isoprene units). Chlorophylls A and B are the pigments that make plants look green. Lycopene, the compound responsible for the red coloring of watermelon, tomatoes and β -carotene, the compound that causes carrots and apricots to be orange, are examples of carotenoids. Spinach leaves contain chlorophyll a and b and β -carotene as major pigments as well as smaller amounts of other pigments. Chlorophyll A and B are same in structure and may not be able to be resolved in this process.

REQUIREMENTS

Chemicals: Petroleum ether (hexane) and acetone etc.

Apparatus: Round bottom flask, chromatography column pipettes, glass beakers, measuring cylinder etc.

PROCEDURE

Extraction of the pigments – For Extraction of the pigments takes about 5 grams of leaves and dried and placed it in a mortar and then pigments are extracted by grinding the leaves with a pestle with about 5-10 ml of in the ratio 80:20 mixture (v/v), petroleum ether (hexane) and acetone after it liquid decanted into a 50 ml round bottom flask. A quick filtration is must require.

Preparation of the column – For Preparation of the column wet pack method is used. The chromatography column made with plastic tip with frit, the one-way stopcock, and the plastic

funnel. The column is filled with enough alumina to get the required height. The dry alumina is poured into a beaker and hexane (pet ether) is added. The mixture is swirled and then poured into the column. The column is tapped gently, so air is not trapped as the alumina settles. Then it is added with a small amount of sand after the alumina has been settled. The column should not contain air bubbles and should be homogeneous. Then the solvent level is allowed to drop to the level of the alumina sand intersection.

Running the column - Using a long pipette, some of the pigment mixture is added directly onto the sand. Then it is added enough to fill the sand layer with color. Then the stopcock is opened and let the liquid level falls to the top of the alumina. Gently add petroleum ether to fill the sand layer. Then the stopcock is opened and let the liquid level fall to the top of the alumina. These steps are repeated at least three times or until all the colored compounds are in the alumina. Now the column is filled with petroleum ether.

Kindly do not ever let the column run dry, then the open stopcock to allow a drip rate of around 1 drop per second. First the yellow-orange β-carotene is eluted. As the yellow-orange colored product is eluted, it was collected in a test tube. When the β-carotene has been eluted, the elutions of the chlorophylls are eluted by using a more polar solvent. Let the solvent level fall to the top of the alumina. Gently the column was filled with either pure acetone or a petroleum ether acetone combination by using 70% petroleum ether: 30 % acetone combination, it might be able to separate chlorophylls A and B and then the chlorophylls collected in the separate test tube.

RESULTS

The Rf values of the above plant pigments are _____.

MCQ's

1. . What do you mean eluent?

    a. is a liquid solution that is a result from Elution

    b. is a liquid solution

    c. is a solvent that used for separation of absorbed material from stationary phase

    d. None of the above

2. In column chromatography which compound are hold by stationary phase?

    a. Nom polar compound

    b. Polar compound

    c. Both A and B

    d. None of the above

3. Chromatogram is a _____

    a. Solute concentration vs. Elution time

    b. Solute concentration vs Elution volume

    c. Both A and B

    d. None of the above

4. Which force is involved in the column chromatography?

    a. Hydrogen bonding

    b. London force

    c. Electric static force

    d. All of the above

5. In which Chromatography stationary phase is more polar than mobile phase?

    a. Reversed chromatography

    b. Ion exchange

c. Liquid Chromatography

d. None of the above

Questions

1. What is principle of column chromatography?

Ans. The principle behind column chromatography is adsorption, in which a mixture of components dissolved in the mobile phase is introduced in to the column and the components move depending on their relative affinities. The choice of the solvent depends on the solubility characteristics of the mixture.

2. Enlist steps involved in column chromatography?

Ans. - Preparation / Packing of an adsorbent column

- Selection of Solvent system / Mobile phase
- Application of samples
- Sample elution
- Collection and analysis of fraction

3. What are the kinds of column chromatography?

Ans. Five distinct chromatographic methods that are use columns are gas chromatography (GC), liquid chromatography (LC), Ion exchange chromatography (SEC) and Chiral Chromatography.

4. Why is R_f useful?

Ans. R_f values can be used to identify unknown chemicals if they can be compared to a range of reference substances. The R_f value for a particular substance is always the same if the same solvent and stationary phase are used.

5. What do you mean by chromatography?

Ans. Chromatography is based on the main principle where molecules applied in mixture onto the surface or into the solid, and fluid stationary phase is separating from each other while moving with the aid of a mobile phase.

Experiment No. 14

AIM - To perform assay of Azithromycin by HPLC in tablet dosage form.

THEORY

As an antibiotic azithromycin is administered in different dosage forms for the treatment infections. Few of them infection symptoms includes as intestinal infections, middle ear infections, throat infection, pneumonia, traveler's diarrhea, and overall for respiratory tract infections. The molecular weight of azithromycin is 748.996 g/mol and molecular formula is C_{38} H_{72} N_2 O_{12}.

REQUIREMENTS

Chemicals: Azithromycin tablets, dipotassium hydrogen phosphate, phosphoric acid, acetonitrile, water etc.

Apparatus: Volumetric flask, mortar pestle, weighing balance, filter paper, HPLC instrument

HPLC column [C18 column (15 mc x 4.6 mm, 5 μm)] etc.

PROCEDURE

Buffer solution - Dissolve 1.16 gm of dipotassium hydrogen phosphate in water and dilute to 100 ml with water. Adjust pH to 6.5 with phosphoric acid.

Mobile phase - Mix buffer: acetonitrile: water in the ratio of 10:35:55

Diluent - Mix acetonitrile: water in the ratio of 40:60

Standard solution - Weigh 20 mg Azithromycin (API) in 100 ml volumetric flask containing about 50 ml of diluent, mix well and make up the volume up to 100 ml by diluent.

Sample solution - Triturate azithromycin tablets in a mortar pestle. Weight 20 mg of powdered sample in a 100 ml volumetric flask containing about 50 ml of diluent, shake strongly to dissolve complete for about 10 -15 minutes, make up the volume up to 100 ml by diluent. Filter sample solution by 0.45 µm filter paper.

Chromatographic conditions – Kindly maintain the chromatographic conditions are as - Column size - 18 column (15 mc x 4.6 mm, 5 µm), Column temperature - 70°C, Mobile phase flow rate - 1 ml/min, Detection wavelength - 215 nm, Injection volume - 100 µl, Run the HPLC only with mobile phase at least for 10 minutes for conditioning of column.

At the present inject standard solution six times by taking solution from volumetric flask each time and record the response/area. Similarly inject filtered sample solution six times by taking solution from volumetric flask each time and record the response/area.

Observation table -

Sr. No.	Standard Area	Sample Area
1		
2		
3		
4		
5		
6		
Avg.		

Calculation –

$$\text{Content (mg/tab)} = \frac{\text{Avg. sample area} \times 20\text{ mg (Std. Wt.)} \times 100\text{ ml (sample dilution)} \times 99.8\text{ (Potency of API)} \times \text{Avg. Wt. of Tab}}{\text{Avg. standard area} \times 100\text{ ml (Std. dilution)} \times \text{Wt. of sample powder equivalent to } 20\text{ mg} \times 100}$$

RESULTS

The content (mg/tab) of market brand tablet of Azithromycin was found to be ____.

MCQ's

1. Which of the following sentence is not applicable of HPLC?

    a. Ion-exchange chromatography of protein

    b. Pre-concentration of trace components

    c. Identification of polysaccharide

    d. Ligand-exchange chromatography

2. Which of the following types of liquid chromatography uses immobilized biochemical as a stationary phase?

    a. Affinity chromatography

    b. Gel permeation chromatography

    c. Exclusion chromatography

    d. Ion exchange chromatography

3. In reversed phase HPLC, we used _____.

    a. Non-polar solvent / polar column

    b. Polar solvent / polar column

    c. Polar solvent/ Non polar column

    d. All of the above

4. The main purpose of the derivatization in HPLC is _____.

    a. To decrease the retention time

    b. To increase the resolution

    c. To increase the detectability of a compound

    d. All of the above

5. In HPLC degassing of the mobile phase can be done by all of the following except

   a. Reverse Osmosis

   b. Vacuum Pumping

   c. Distillation

   d. Sparging

Questions

1. What is principle of HPLC?

Ans. High performance liquid chromatography (HPLC) is an analytical technique which useful for separation, identification or quantification each component in a mixture. The mixture is separated using the basic principle of column chromatography and then identified and quantified.

2. Which types detectors is used in HPLC?

Ans. Following common detectors used in HPLC are as UV-Vis Detectors, Refractive Index Detector, Fluorescence Detectors, Evaporative Light Scattering Detector, and Conductivity Detector.

3. Which typescolumn employed in HPLC?

Ans. Normal Phase HPLC Columns

Reverse Phase HPLC Columns

Ion Exchange HPLC Columns

Size Exclusion HPLC Columns

4. What are troubleshooting errors in HPLC?

Ans. Pumping system is common problem occurs and some of the more common indications are erratic retention times, noisy baselines, or spikes in the chromatogram. Leaks at pump fittings or seals will result in poor chromatogram.

5. Gives process for preparation of standard and sample solution in HPLC

Ans. Standard solution - Weigh 20 mg sample in 100 ml volumetric flask containing about 50 ml of diluent, mix well and make up the volume up to 100 ml by diluent.

Sample solution - Triturate the sample in a mortar pestle and weight 20 mg of powdered sample in a 100 ml volumetric flask containing about 50 ml of diluent, shake strongly to dissolve complete for about 10 -15 minutes, make up the volume up to 100 ml by diluent. Filter sample solution by 0.45 μm filter paper.

Experiment No. 15

AIM – Introduction of Gas Chromatography

THEORY

This technique is generally used to separate the gases in gaseous solutions. The more mutual technique is gas - liquid chromatography in which the stationary phase is a porous solid covered with an absorbing the liquid. Gas-liquid chromatography (GLC) is commonly used for separation of wide variety of organic compounds. The basic requirements for GLC are that the sample may be volatile and that it not decomposes in all vaporization process. Meanwhile the vaporization occurs in an inert atmosphere and decomposition of sample is generally not a problem in which.

A basic chromatograph consists of the following:

- An injector for introduction and vaporization of the sample.
- A separating column consisting with metal tubing packed with a solid material coated with the stationary absorbing liquid.
- A carrier gas, N_2 or He to sweep of sample throughout column.
- A flow control equipment for the maintenance a constant flow of carrier gas throughout column.
- A detector for the measurement of quantity of a separated component.
- A hot air ovens and heaters for the column, detector and injector temperature control.
- An integrator and strip chart recorder combination to provide the maintain record of these analysis.

The separation of different mixtures into its components depends upon the solubility differences of sample vapour in a liquid (stationary phase), and generally stationary phase is coated in a thin layer on the solid particles of large surface area and then packed uniformly into the column. A constant flow of an inert carrier gas badges through the column and transports solute molecules in the gas phase. The column is enclosed by an oven for precise temperature control.

A sample of the analyte is introduced by syringe injection into the heated injector tube, where it is vaporized and mixed with the carrier gas. As the sample vapor/carrier gas mixture flows onto the column, the analyte partitions between the gas and liquid phases according to the analyte component's solubility in the liquid at the column operating temperature. This equilibrium partitioning continues as the sample is moved through the column by the carrier gas. The rate at which the sample travels through the column is determined by the sample solubility in the stationary phase, the carrier gas flow rate, and the temperature gradient (temperature program) applied. Each component travels at a characteristic rate, and if the column has sufficient length and resolving power, the sample will be completely separated by the time it reaches the detector and identified by comparing the "retention time".

INSTRUMENTATIONS

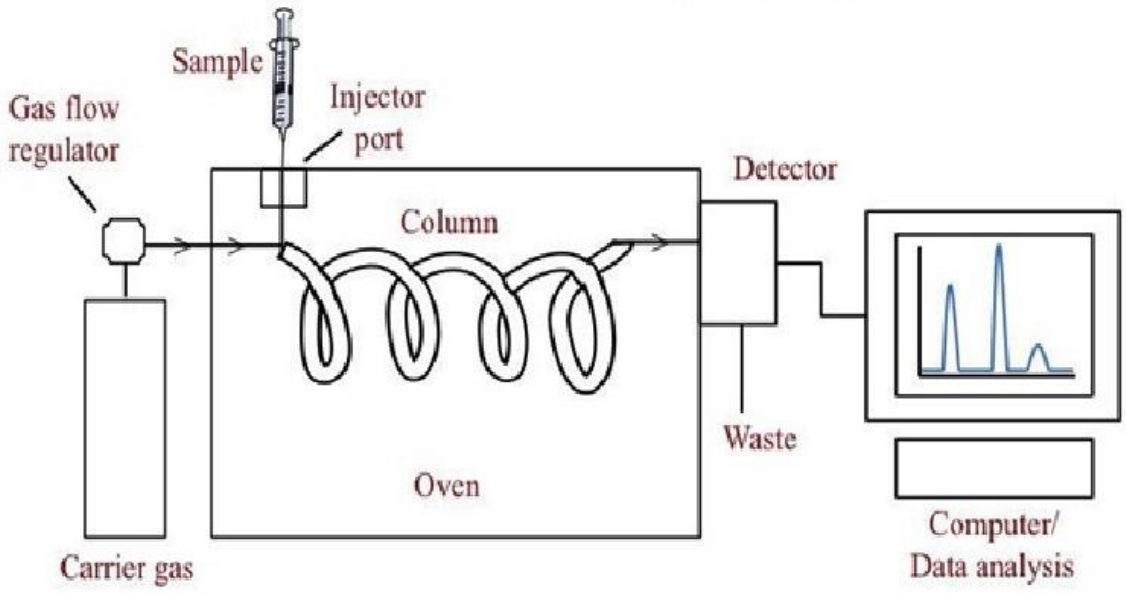


Figure – Instrumentation of Gas Chromatography

1. **Carrier gas in a high-pressure cylinder with attendant pressure regulators and flow meters** - In Gas Chromatography Helium, N_2, H, and Argon are used as carrier gases in high-pressure cylinder. Helium is preferred for thermal conductivity detectors because of its high thermal conductivity relative to that of most organic vapors. N_2 is preferable when a large consumption of carrier gas is employed. Carrier gas from the tank passes through a toggle valve, a flow meter, (1-1000 ml/min), capillary restrictors, and a pressure gauge (1-4 atm). Flow rate is adjusted by means of a needle valve mounted on the base of the flow meter and controlled by capillary restrictors. The operating efficiency of the gas chromatograph is directly dependent on the maintenance of constant gas flow.
2. **Sample injection system –** In sample injection system liquid samples are injected by a micro syringe with a needle inserted through a self-scaling, silicon-rubber septum into a heated metal block by a resistance heater. Gaseous samples are injected by a gas-tight syringe or through a by-pass loop and valves. Typical sample volumes range from 0.1 to 0.2 ml.
3. **The column separation - The** heart of the gas chromatography is the column which is made of metals bent in U shape or coiled into an open spiral or a flat pancake shape and copper is very useful up to 250^0 . Swege lock fittings make column insertion easy. Several sizes of columns are used depending upon the requirements.
4. **Liquid phases - An** infinite variety of liquid phases are available limited only by their volatility, thermal stability and ability to wet the support. No single phase will serve for all separation problems at all temperatures.

- **Non-Polar** – Paraffin, squalane, silicone greases, apiezon L, silicone gum rubber. These materials separate the components in order of their boiling points.
- **Intermediate Polarity** – These materials contain a polar or polarizable group on a long non-polar skeleton which can dissolve both polar and non-polar solutes. For example. Diethyl hexyl phthalate is used for the separation of high boiling alcohols.
- **Polar** – Carbo waxes – Liquid phases with a large proportion of polar groups. Separation of polar and non-polar substances.
- **Hydrogen bonding** – Polar liquid phases with high hydrogen bonding e.g. Glycol.
- **Specific purpose phases** – Relying on a chemical reaction with solute to achieve separations. e.g AgNO3 in glycol separates unsaturated hydrocarbons.

5. **Supports -** The structure and surface characteristics of the support materials they are very important parameters, which determines the efficiency of the support and the degree of separation respectively. The support should be inert but capable of immobilizing a large volume of liquid phase as a thin film over its surface. The surface area should be large to ensure the rapid attainment of equilibrium between stationary and mobile phases. Support should be strong enough to resist breakdown in handling and be capable of packed into a uniform bed. Diatomaceous earth, Kieselguhr treated with Na 2CO 3 for 900^0 C causes the particle fusion into coarser aggregates. Glass beads with a low surface area and low porosity can be used to coat up to 3% stationary phases. Porous polymer beads differing in the degree of cross-linking of styrene with alkyl-vinyl benzene are also used which are stable up to 250^0.

6. **Detector -** Detectors sense the arrival of the separated components and provide a signal. These are either concentration-dependent or mass dependent. The detector should be close to the column exit and the correct temperature to prevent decomposition.
7. **Recorder** - The recorder should be generally 10 mv (full scale) fitted with a fast response pen (1 sec or less). The recorder should be connected with a series of good quality resistances connected across the input to attenuate the large signals. An integrator may be a good addition.

APPLICATIONS

1. Gas Chromatography is useful to compute the content of a chemical product, for example as promising the quality excellence of products in the industry and for measuring and identification of toxic substances in soil, air or water.
2. Gas chromatography is also valuable for analysis of:
    (a) Air borne contaminants
    (b) Recital performance improving drugs in urine samples
    (c) Oil spills
    (d) Essential oils in perfumes
3. Gas chromatography is very precise if used correctly and for measurement pico-moles of a substance or PPB concentrations of gaseous samples.
4. Gas Chromatography also used widely in the forensic science as disciplines as diverse as solid drug dose qualitative and quantitative analysis, paint chip analysis, and toxicology cases, employ gas chromatography to crime-scene evidence.

MCQ's

1. Which method is useful for separation of thermally stable and volatile substance?

a. Gas Chromatography.

b. Nuclear Magnetic Resonance Spectroscopy

c. Mass Spectroscopy

d. High Performance Liquid Chromatography

2. Which one liquid used in GLC?

a. Kieselguhr

b. Diatomaceous earth

c. A and B

d. None of the above

3. . Which one solid used in GSC?

a. Alumina

b. Carbon

c. Granular silica

d. All of the above

4. Following which sentence is true for gas Chromatography?

a. It is work on principal of the adsorption and partitions.

b. When stationary phase is liquid it is called a GSC

c. It has gaseous stationary phase.

d. It is not used to volatile substance.

5. Which of the following is not a feature of carrier gas used in gas chromatography?

a. It must be chemically inert

b. It should be suitable for the detector employed

c. It should not be completely pure

d. It should be cheap

Questions

1. What is principle of Gas Chromatography?

Ans. The principle of gas chromatography is the sample solution injected into the instrument enters a gas (stream) which transport the sample into a separation tube is known as the "column", for that Helium or nitrogen is used as the so-called carrier gas. The various components and mixture of components are separated inside the column.

2. Give applications of Gas Chromatography.

Ans. Gas Chromatography is useful to compute the content of a chemical product; Gas chromatography is also valuable for analysis of: Air borne contaminants, Recital performance improving drugs in urine samples, oil spills and essential oils in perfumes. Gas chromatography also used correctly and for measurement pico-moles of a substance Gas Chromatography widely applicable in the forensic science as disciplines as diverse as solid drug dose qualitative and quantitative analysis, paint chip analysis, and toxicology cases, employ gas chromatography to crime-scene evidence.

3. What are the types of gas chromatography?

Ans. The two types of gas chromatography are encountered: gas solid chromatography (GSC) and gas-liquid chromatography (GLC). The Gas Solid Chromatography is based upon a solid stationary phase on which retention of analytes is the consequence of physical adsorption.

4. What is mobile phase and stationary phase in gas chromatography?

Ans. In gas chromatography (GC), the mobile phase is an inert gas such as helium. The mobile phase carries the sample mixture through what is referred to as a stationary phase. The stationary phase is a usually chemical that can selectively attract components in a sample mixture.

5. What is a purpose of gas chromatography?

Ans. The main purpose of the gas chromatography technique is to separate out the compounds that possess: High volatility, Low molecular weights and Thermal stability.

List of solvents

Solvent	Molecular formula	Polarity index	Boiling point (°C)	Melting point (°C)	density (g/mL)	solubility in H_2O[1] (g/100g)
cyclohexane	C_6H_{12}	**0.006**	80.7	6.6	0.779	0.005
pentane	C_5H_{12}	**0.009**	36.1	-129.7	0.626	0.0039
hexane	C_6H_{14}	**0.009**	69	-95	0.655	0.0014
heptane	C_7H_{16}	**0.012**	98	-90.6	0.684	0.0003
carbon tetrachloride	CCl_4	**0.052**	76.7	-22.4	1.594	0.08
carbon disulfide	CS_2	**0.065**	46.3	-111.6	1.263	0.2
p-xylene	C_8H_{10}	**0.074**	138.3	13.3	0.861	0.02
toluene	C_7H_8	**0.099**	110.6	-93	0.867	0.05
benzene	C_6H_6	**0.111**	80.1	5.5	0.879	0.18
ether	$C_4H_{10}O$	**0.117**	34.6	-116.3	0.713	7.5
methyl *t*-butyl ether (MTBE)	$C_5H_{12}O$	**0.124**	55.2	-109	0.741	4.8
diethylamine	$C_4H_{11}N$	**0.145**	56.3	-48	0.706	M
dioxane	$C_4H_8O_2$	**0.164**	101.1	11.8	1.033	M
N,N-dimethylaniline	$C_8H_{11}N$	**0.179**	194.2	2.4	0.956	0.14
chlorobenzene	C_6H_5Cl	**0.188**	132	-45.6	1.106	0.05
anisole	C_7H_8O	**0.198**	153.7	-37.5	0.996	0.10
tetrahydrofuran (THF)	C_4H_8O	**0.207**	66	-108.4	0.886	30
ethyl acetate	$C_4H_8O_2$	**0.228**	77	-83.6	0.894	8.7
ethyl benzoate	$C_9H_{10}O_2$	**0.228**	213	-34.6	1.047	0.07
dimethoxyethane (glyme)	$C_4H_{10}O_2$	**0.231**	85	-58	0.868	M
diglyme	$C_6H_{14}O_3$	**0.244**	162	-64	0.945	M
methyl acetate	$C_3H_6O_2$	**0.253**	56.9	-98.1	0.933	24.4
chloroform	$CHCl_3$	**0.259**	61.2	-63.5	1.498	0.8
3-pentanone	$C_5H_{12}O$	**0.265**	101.7	-39.8	0.814	3.4
1,1-dichloroethane	$C_2H_4Cl_2$	**0.269**	57.3	-97.0	1.176	0.5
di-n-butyl phthalate	$C_{16}H_{22}O_4$	**0.272**	340	-35	1.049	0.0011
cyclohexanone	$C_6H_{10}O$	**0.281**	155.6	-16.4	0.948	2.3
pyridine	C_5H_5N	**0.302**	115.5	-42	0.982	M
dimethylphthalate	$C_{10}H_{10}O_4$	**0.309**	283.8	1	1.190	0.43
methylene chloride	CH_2Cl_2	**0.309**	39.8	-96.7	1.326	1.32
2-pentanone	$C_5H_{10}O$	**0.321**	102.3	-76.9	0.809	4.3

2-butanone	C_4H_8O	**0.327**	79.6	-86.3	0.805	25.6
1,2-dichloroethane	$C_2H_4Cl_2$	**0.327**	83.5	-35.4	1.235	0.87
benzonitrile	C_7H_5N	**0.333**	205	-13	0.996	0.2
acetone	C_3H_6O	**0.355**	56.2	-94.3	0.786	M
dimethylformamide (DMF)	C_3H_7NO	**0.386**	153	-61	0.944	M
t-butyl alcohol	$C_4H_{10}O$	**0.389**	82.2	25.5	0.786	M
aniline	C_6H_7N	**0.420**	184.4	-6.0	1.022	3.4
dimethylsulfoxide (DMSO)	C_2H_6OS	**0.444**	189	18.4	1.092	M
acetonitrile	C_2H_3N	**0.460**	81.6	-46	0.786	M
3-pentanol	$C_5H_{12}O$	**0.463**	115.3	-8	0.821	5.1
2-pentanol	$C_5H_{12}O$	**0.488**	119.0	-50	0.810	4.5
2-butanol	$C_4H_{10}O$	**0.506**	99.5	-114.7	0.808	18.1
cyclohexanol	$C_6H_{12}O$	**0.509**	161.1	25.2	0.962	4.2
1-octanol	$C_8H_{18}O$	**0.537**	194.4	-15	0.827	0.096
2-propanol	C_3H_8O	**0.546**	82.4	-88.5	0.785	M
1-heptanol	$C_7H_{16}O$	**0.549**	176.4	-35	0.819	0.17
i-butanol	$C_4H_{10}O$	**0.552**	107.9	-108.2	0.803	8.5
1-hexanol	$C_6H_{14}O$	**0.559**	158	-46.7	0.814	0.59
1-pentanol	$C_5H_{12}O$	**0.568**	138.0	-78.2	0.814	2.2
acetyl acetone	$C_5H_8O_2$	**0.571**	140.4	-23	0.975	16
ethyl acetoacetate	$C_6H_{10}O_3$	**0.577**	180.4	-80	1.028	2.9
1-butanol	$C_4H_{10}O$	**0.586**	117.6	-89.5	0.81	7.7
benzyl alcohol	C_7H_8O	**0.608**	205.4	-15.3	1.042	3.5
1-propanol	C_3H_8O	**0.617**	97	-126	0.803	M
acetic acid	$C_2H_4O_2$	**0.648**	118	16.6	1.049	M
2-aminoethanol	C_2H_7NO	**0.651**	170.9	10.5	1.018	M
ethanol	C_2H_6O	**0.654**	78.5	-114.1	0.789	M
diethylene glycol	$C_4H_{10}O_3$	**0.713**	245	-10	1.118	M
methanol	CH_4O	**0.762**	64.6	-98	0.791	M
ethylene glycol	$C_2H_6O_2$	**0.790**	197	-13	1.115	M
glycerin	$C_3H_8O_3$	**0.812**	290	17.8	1.261	M
water, heavy	D_2O	**0.991**	101.3	4	1.107	M
water	H_2O	**1.000**	100.00	0.00	0.998	M