East Point College of Pharmacy

East Point Campus, Jnana Prabha, Virgo Nagar Post
Bengaluru – 560049, Karnataka

Approved
by
Pharmacy Council of India, New Delhi

Affiliated
to
Rajiv Gandhi University of Health Sciences
Karnataka
Bengaluru – 560 041
India

LAB MANUAL

PHARMACEUTICAL ANALYSIS
PHARM D
3rd Year

EAST POINT COLLEGE OF PHARMACY
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Bengaluru – 560049, Karnataka

PROGRAM SPECIFIC OUTCOMES	
DOCTOR OF PHARMACY	
PSO1	Acquire a thorough foundational knowledge in pharmaceutical sciences, including pharmacology, pharmaceutics, pharmaceutical chemistry, pharmacognosy and pharmaceutical analysis to excel in further academic pursuits
PSO2	Acquire and apply the pharmacotherapeutic concepts for better patient care enhancing employability across various sectors including clinical research organizations, academic and hospitals
PSO3	Equip with entrepreneurial skills and knowledge of pharmacoepidemiological studies and regulatory aspects to initiate and run successful ventures in the healthcare sector

Course:	**Code: 3.2P** **Pharmaceutical Analysis**
CO1	To recall the principle involved in spectroscopy and importance of absorption maximum in the estimation of organic compounds
CO2	To experiment with selected drugs by UV, Visible spectroscopy and fluorimetry
CO3	To characterize and quantify the organic compounds/amino acids/plant pigments by using various chromatographic and spectroscopic techniques
CO4	To maximize the knowledge on integration and interpretation of chromatograms and spectra and viva- voce

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PRACTICAL INSTRUCTION

Practical: Students are expected to perform the number of experiments listed in the respective syllabus. Marks shall be awarded out of a maximum of 10 to each of the practical exercise and an average of those shall be computed out of maximum of 10 each marks. In addition, three practical sessional examinations evenly spread during academic year shall be conducted. The average marks of the best two practical examinations shall be computed out of a maximum of 20 marks. A total of 30 marks shall constitute the sessional award in practical. While awarding the sessional marks of practical experiments, the following considerations should be taken into account.

1. Preparation of the candidate.
2. Manipulative skills.
3. Results of the experiment.
4. Knowledge of the experiment
5. Viva voce pertaining to the experiments only.

The College shall maintain the sessional books of the students and the record of sessional marks of thestudents.

A regular record of both theory and practical class work and sessional examinations conducted in aninstitution imparting the course shall be maintained for each student in the institution.

Examination for Pharm D

(1) Every year there shall be an examination to examine the students.

(2) Each examination may be held twice every year. The first examination in a year shall be the annualexamination and the second examination shall be supplementary examination.

(3) The examinations shall be of written and practical (including oral nature) carrying maximum marks foreach part of a subject.

Attendance and Progress

A candidate is required to put in at least 80% attendance in theory and practical subjects separately in a recognized institution approved by Pharmacy council of India and affiliated to Rajiv Gandhi University of Health Sciences, Karnataka. The candidate shall complete prescribed course satisfactorily to be eligible to appear for the respective examination.

Medium of Instruction

Medium of Instruction and Examination Shall be English.

LABORATORY SAFETY GUIDELINES

Laboratory safety

When working in a chemical laboratory we handle several chemicals with more or less adverse effects to human health, and we perform experiments that have a number of potential hazards associated with them. Thus, a chemical laboratory can be a dangerous place to work in. With proper care, strictly following all precautionary measures, however, practically all accidents can be prevented.

It is the prevention of accidents and damages posed by the specialty of the chemical laboratory experiments that requires you to follow the instructor's advice as well as keep the laboratory order during work in the laboratory. You should never forget that your carelessness or negligence can threaten not only your own safety but that of your classmates working around you.

This section has guidelines that are essential to perform your experiments safely, without accidents.

Preparation in advance

- Read through the descriptions of the experiments carefully. If necessary, do study the theoretical background of the experiments from your textbook(s). After understanding, write down the outline of the experiments to be performed in your laboratory notebook. If any item is still unclear, do ask your instructor before starting the work.
- Prepare your notebook before the laboratory practice. Besides description of the outline of the experiments, preliminary preparation should also include a list of the work you did prior to the start ofpractical work.

Laboratory rules

- Always wear laboratory coat and shoes in the laboratory. Sandals and open-toed shoes offer inadequateprotection against spilled chemicals or broken glass.
- Always maintain a disciplined attitude in the laboratory. Careless acts are strictly prohibited. Mostof the serious accidents are due to carelessness and negligence.
- Never undertake any unauthorised experiment or variations of those described in the laboratory manual.
- Maintain an orderly, clean laboratory desk and cabinet. Immediately clean up all chemical spills fromthe bench and wipe them off the outer surface of the reagent bottles with a dry cloth.
- Drinking, or eating is not permitted during the laboratory practice. Do not bring other belongings than your notebook, stationery, and laboratory manual into the laboratory. Other properties should beplaced into the cupboard at the corridor.
- Be aware of your bench neighbours' activities. If necessary, warn them of improper techniques or unsafe manipulation.

Laboratory safety and accident protection

At the end of the laboratory session, completely clean all glassware and equipment, and clean them with a dry cloth. After putting back all your personal labware into your cabinet,lock it carefully.

- Always wash your hands with soap before leaving the laboratory.

Handling chemicals and glassware

- At the beginning of the laboratory practices the instructor holds a short introduction when all questions related to the experimental procedures can be discussed.
- Perform each experiment alone. During your work always keep your laboratory notebook at hand in order to record the results of the experiments you actually perform.

- Handle all chemicals used in the experiments with great care. Never taste, smell, or touch a chemicalor solution unless specifically directed to do so.
- Avoid direct contact with all chemicals. Hands contaminated with potentially harmful chemicals may cause severe eye or skin irritations.
- Reactions involving strong acids, strong bases, or chemicals with unpleasant odour should be performed under the ventilating hood. If necessary, safety glasses or goggles should be worn.
- When checking the odour of a substance, be careful not to inhale very much of the material. Never hold your nose directly over the container and inhale deeply.
- When performing an experiment, check the label on the bottle twice to make sure that you use the correct reagent. The wrong reagent can lead to accidents or "inexplicable" results in your experiments.
- Do not use a larger amount of reagents than the experiment calls for. Do not return any reagent to a reagent bottle! There is always the chance that you accidentally pour back some foreign substance which may react with the chemical in the bottle in an explosive manner.
- Do not insert your own pipette, glass rod, or spatula into the reagent bottles; you may introduce impurities which could spoil the experiment for the person using the stock reagent after you.
- Always mix reagents slowly. Pour concentrated solutions slowly and stirring it continuously into water or into a less concentrated solution. This is especially important when diluting concentrated sulphuric acid.
- Discard waste or excess chemicals as directed by your laboratory instructor.
- Using clean glassware is the basic requirement of any laboratory work. Clean all glassware with a test- tube brush and a detergent, using tap water. Rinse first with tap water and then with distilled water. If dry glassware is needed, dry the wet one in drying oven, or rinse with acetone and air dry it.

CHROMATOGRAPHY.

Modern pharmaceutical formulations are complex mixtures including one or more medicinally active ingredients, a no. of inert materials such as diluents, disintegrating agents, coloring agents and flavoring agents.

To ensure the quality and stability of the final product, the pharmaceutical analyst must be able to separate these mixtures into individual components prior to quantitative analysis.

Among the most powerful techniques available to the analyst for the resolution of these mixtures are a group of highly efficient methods collectively called **"chromatography" (color writing).**

Because this technique is involved so intimately in all aspects of pharmaceutical research and development, the pharmacist should possess a working knowledge of chromatographic principles and technique.

Chromatography comprises a group of methods for separating molecular mixtures that depends on the differential affinities of the solutes between two immiscible phase .one of the phases is a fixed be of large surface area, where as the other is a fluid that moves through, or over the surface of the fixed phase. The components of the mixture must be of molecular a dimension, which requires that they be in solution of the vapor state. The relative affinity of the solutes for each of the phases must be reversible to ensure that mass transfer occurs during the chromatographic separation.

The fixed phase is called the **"Stationary Phase"** and the other is termed the **"Mobile Phase".** The stationary phase may be a porous or finely divided solid or a liquid that has been coated in a thin layer on an inert supporting material. It is necessary that the stationary phase particles be as small and homogeneous a s possible to provide a large surface area so that sorption and desorption of the solutes will occur frequently. Depending on the type of chromatography

employed, the mobile phase may be pure liquid or a mixture of solutions (Eg. Buffers) or it may be a gas (pure or a homogeneous mixture).

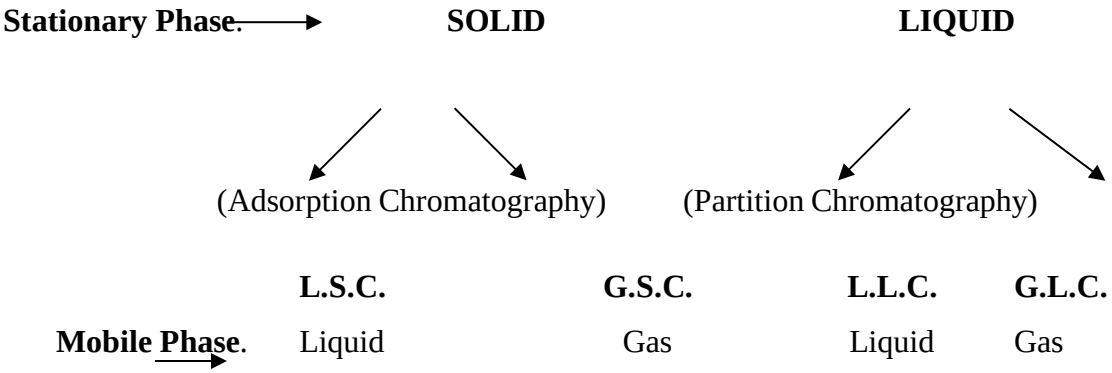
Chromatography methods can be classified according to the nature of the stationary and mobile phases. If the stationary phase is solid, the process is called **"adsorption chromatography",** where as if the stationary phase is liquid it is termed **"partition chromatography".** The difference between adsorption and partition can be ascribed to the nature of the forces that influence the distribution of the solutes between the two phases.

In **adsorption chromatography** the mobile phase containing the dissolve solutes passes over the surface of the stationary phase. Retention of the components and their consequent separation depends on the ability of the atoms on the surface to remove the solutes from the mobile phase and adsorb them temporarily by means of electrostatic forces. If the mobile phase is liquid, the process is called **liquid -solid chromatography (LSC)** but when the mobile phase is a gas the method is called **gas-solid chromatography (GSC).**

In **partition chromatography** an inert solid material such as silica gel, diatomaceous earth or even the walls of the column itself serves to support a thin layer of liquid which is the effective stationary phase. As the mobile phase containing the solutes passes in close proximity this liquid phase, retention and separation occur due to the relative solubility of the analytes. If the mobile phase is liquid, this is called **liquid-liquid chromatography (LLC)** and, if the mobile phase is a gas the process is termed as **gas-liquid chromatography (GLC).**

Three other modes of chromatography in which the stationary phase is a solid are classified differently from LSC and GSC because of the unique nature of their separation processes. These are ion-exchange, size-exclusion and affinity chromatography.

CHROMATOGRAPHY



Ion-exchange chromatography: the stationary phase consists of a polymeric matrix onto the surface of which ionic functional groups, such as carboxylic acids or quaternary amines, have been bonded chemically. As the mobile phase passes over this surface, ionic solutes are retained by forming electrostatic chemical bonds with the functional groups. The mobile phases used in this type are always liquid.

Size-exclusion chromatography: the stationary phase is a polymeric substance e containing numerous pores of molecular dimensions. Solutes whose molecular size is suffiencently small leave the mobile phase to diffuse into the pores. Larger molecules that will not fit into the pores remain in the mobile phase and are not retained. This method is most suited to the separation of mixtures in which the solutes vary considerably in molecular size. The mobile phase in this type may be either liquid or gaseous.

Affinity chromatography: a specific ligand such as an antibody, is bound to the inert stationary phase to achieve a highly selective separation. When a mixture of solutes containing a molecule that preferentially binds to the ligand, such as an antigen, is passed through the system, the antigen binds strongly to the ligand antibody and is retained, while the other solutes elute. The antigen then can be displaced and eluted in a purified state.

Experiment. No:1

Separation And Identification Of Given Sample Of Amino acids ByAscending Paper Chromatography

AIM: To separate the given mixture of amino acid using ascending paper chromatography

REFERENCE: Instrumental method of chemical analysis. Gurudeep R chatwal, Sham K. Anand Page No:588-592.

REQUIREMENTS: TLC Chamber, whatmann filter paper, distilled water, alanine, tryptophan, leucine, n-butanol, glacial acetic and ninhydrin reagent.

Principle:

Chromatography is defined as a method of separating a mixture of compound into individual components through equilibrium distribution between two phases. Chromatographic techniques are classified according to the mobile phase and stationary phase used. Paper chromatography is one of the most valuable tools for the separation of amino acids. This technique is an example of partition chromatography in which the substance are distributed between two liquids i.e., one is the stationary liquid (usually water) which is held in the fibers of filter paper and called stationary phase, the other is the moving liquid or developing solvent and called mobile phase. The components of the mixture to be separated migrate at different rates and appear as spots at different point on the paper.

R_f = Distance travelled by the solute / Distance travelled by the solvent

PROCEDURE:

Prepare mobile phase (n-butanol, acetic acid, water in the ratio of 4:1:5), pour in chromatographic chamber for saturation. Place filter paper strip attaching to wall of chamber. When mobile phase run more than 75% height of paper, confirms saturation. Prepare standard amino acids solutions and0.1% of ninhydrin solution.

Preparation of paper: Cut whatmann paper into suitable dimensions so that it fixed well in a chamber. The edges of the paper should not touch the sides of the chamber. At a suitable distance from the bottom edge of the paper, draw a line by pencil where standard and sample solutions are placed. At specific distances from each other, spot 3 amino acids and sample. Dry the spots and hang the paper in the chamber such that lower edge of the paper dipped into the solvent. Close the chamber and allow for development. Remove the paper when mobile phase run more than 75% of the total height of paper. remove the paper from the chamber, dry and spray with ninhydrin reagent. Mark the spots and solvent front using a pencil and calculate Rf values of different amino acids and components of the amino acids in mixture.

REPORT: The given sample mixture contains ----------and ----------

Experiment. No: 02

Separation And Identification Of Given Sample Of Amino acids By CircularPaper Chromatography

AIM: To separate the given mixture of amino acid using radial paper chromatography

REFERENCE: Gurudeep R chatwal, Sham K. Anand. Instrumental method of chemical analysis.2013. PgNo:588-592.

REQUIREMENTS: Petridish, whatmann filter paper, distilled water, Alanine, tryptophan, leucine, n- butanol, glacial acetic and ninhydrin reagent

PRINCIPLE:

Chromatography is defined as a method of separating a mixture of compound into individual components through equilibrium distribution between two phases. Chromatographic techniques are classified according to the mobile phase and stationary phase used. Paper chromatography is one of the most valuable tools for the separation of amino acids. This technique is an example of partition chromatography in which the substance is distributed between two liquids i.e., one is the stationary liquid (usually water) which is held in the fibers of filter paper and called stationary phase, the other is the moving liquid or developing solvent and called mobile phase. The components of the mixture to be separated migrate at different rates and appear as spots at different point on the paper.

R_f = Distance travelled by the solute / Distance travelled by the solvent

PROCEDURE:

A drop of each amino acid is spotted on filter paper and it is allowed to dry. 10-20ml of mobile phase is prepared and poured into petridish and covered with a lid and keep it for saturation for 20min.Wick of paper is attached to the centre of filter paper then paper is placed on the petridish in such a way that the wickshould immersed in a mobile phase. Then it is covered with lid. The

solvent is allowed to run once it is reaching 3/4th it is removed and dried. Visualizing agent (ninhydrin reagent) is added and spots are visualized. R_f value is calculated to identify the compound.

REPORT: The given sample mixture contains --------- and ----------

Experiment. No:03

Separation And Identification Of Given Sample Of Aminoacids By 2-Dimensional Paper Chromatography

AIM: To separate the given mixture of amino acid by ascending paper chromatography.

REFERENCE: Gurudeep R chatwal, Sham K. Anand. Instrumental method of chemical analysis.2013. PgNo:588-592.

REQUIREMENTS: TLC chamber, Whatmann filter paper, amino acids, n-butanol, glacial acetic, water andninhydrin reagent.

PRINCIPLE:

Paper chromatography is one of the most valuable tools for the separation of amino acids. this technique is an example of partition chromatography in which the substance are distributed between two liquids i.e., one is the stationary liquid (usually water) which is held in the fibers of filter paper and called stationary phase, the other is the moving liquid or developing solvent and called mobile phase. The components of themixture to be separated migrate at different rates and appear as spots at different point on the paper

PROCEDURE:

1. A drop of each amino acid spotted on Whatman filter paper and allow to dry.
2. Add 10 ml of mobile phase in development chamber and keep it for saturation.
3. Keep this paper in chamber for development. Once the mobile phase is reaching 3/4th of the paper it is removed and dried. Once again turn the paper to an angle of 90 ºC and allow it torun. Once after reaching 3/4th of the paper it is removed and dried.
4. Spots are visualized using Ninhydrin reagent.
5. Mobile phase is n-butanol: glacial acetic acid: water (4:1:5)

REPORT: The given sample mixture contains ____

Experiment. No:4

Thin Layer Chromatography Of Sulpha Drugs

AIM: To identify the given sulpha drugs by thin layer chromatography

REFERENCE: A Practical Approach to Pharmaceutical Analysis by Rajesh Kumar Nema and S.N.Meyyanthan. 1st ed: CBS Publishers;2008: pg.no.155.

REQUIREMENTS: TLC chamber, Silica gel, Chloroform and Methanol.

PRINCIPLE: Principle involved in thin layer chromatography is adsorption chromatography. Samples move along with mobile phase over thin layer of inert substance. The distance travelled is according to the affinity of sample applied. The components with more affinity travel slower and less affinity towards the stationary phase travels faster.

PROCEDURE

Make suspension of silica gel G in a volatile organic solvent such as methanol or in water. Pour this suspension on the plate, spread uniformly. Allow it for drying in air and then in oven for 1hr at 100 ºC when silica gel slurry is prepared in water (10 mins for slurry prepared in methanol). Prepare mobile phase by mixing the given solvent in the given proportion. Pour in TLC chamber; keep it for saturation.

Use a sharp hard pencil or a pin for marking on the layers. Spot the sample and standard on the line,allow it to dry. Develop the plate in TLC chamber. Remove the plate after 3/4th of total plate run, mark solvent front and dry it. If required spray detecting regents, mark the spot and calculate the $R_f$value.

Mobile Phase: Chloroform: Methanol (90:9.5)

Standards: Sulphadiazine, Sulphamethoxazole and Trimethoprim Detecting regent: UV or Iodine chamber.

REPORT: The given unknown sample was found to be---------- --.

SPECTROSCOPY

Spectroscopy is the measurement and interpretation of electromagnetic radiation absorbed or emitted when the molecules or atoms or ions of a sample move from one energy state to another state. This change may be from ground state to exited state or converse. Electromagnetic radiation is made up of discrete particles called photons. EMR has got both wave characteristic as well as particle characteristics. This means that it can travel in vacuum also.

The different types of EMR are visible radiation, UV radiation, Irradiation; Microwaves, Radio waves, X-rays, gamma rays or cosmic ray.

The energy of an electromagnetic radiation can be given by the following equation$E = h \upsilon$
Where E = Energy of radiation
h = Planks constant ($6.624*10^{-34}$)υ = frequency of radiation

Frequency = c / λ or velocity of light in vacuum/wavelengthHence $E = h \upsilon = h c / \lambda = h c \bar{v}$
$\bar{v}$ = wave number

Therefore the energy of a radiation depends upon frequency and wavelength of the radiation.

Colorimetry: Colorimetry is concerned with the study of absorption of visible radiation whose wavelength ranges from 400nm-800nm.any coloured substance will absorb radiation in this wavelength region. Coloured substances absorb light of different wavelength in different manner and hence we get absorption curve (absorbance Vs wavelength) in a unique pattern for every colored solution. In this absorption curve, the wavelength at which maximum absorption of radiation takes place is called as **λmax**. This λmax is characteristic or unique for every colored substance and is a qualitative aspect, useful in identifying the substance. λmax is not usually affected by concentration of the substance. The absorbance of a solution increases with concentration of a substance. But there is no change in λmax when concentration changes. When we plot a graph of concentration Vs absorbance, we get a calibration curve or standard

curve. This calibration curve is useful in the given sample solution or a formulation, by extrapolation or interpolation and calculation.

UV SPECTROSCOPY:

Ultraviolet spectroscopy is concerned with the study of absorption of UV radiations. The wavelength of ultraviolet region ranges from 200nm-400nm.

Principle: Any molecule has either n, π, or σ or a combination of these electrons. These binding (σ and π) and non binding (n) electrons absorb the characteristic radiation and undergo transition from ground state to exited state. By the characteristics absorption peaks the nature of theelectrons present and hence the molecular structure can be elucidated.

It was stated earlier that σ, n and π electrons are present in a molecule and can be excited from the ground state by the absorption of UV radiation. **The various transitions are n → π*, π→ π*, n→ σ* and σ→ σ*.**

The energy required for excitation for transitions are: n → π* < π→ π* < n→ σ* < σ→ σ*. Of these transitions n → π* required lowest energy and σ→ σ* requires the highest energy for excitation in the UV region.

1) **Solvents** - the commonly used solvents in this region are water, cyclohexane,methanol, and ethanol.
2) **Detectors** - Photo multiplier tubes are mainly used

Applications-

1) Qualitative analysis-detection of impurities
2) Determination of dissociation constant of acids and bases

Laws Governing Absorption of Radiation

1. Beer's law (related to concentration of absorbing species)

2. Lambert's law (related to thickness/path length of absorbing species)These two laws are applicable under the following condition:

$I = I_a + I_t$

I = intensity of incident light. I_a = intensity of absorbed light.

I_t = intensity of transmitted light and

No reflection / scattering of light take place.

Beer's law: it states that the intensity of beam of monochromatic light decreases exponentially with increase in the concentration of absorbing species arithmetically.

Accordingly: $-dI/dC \alpha I$

$-dI/dC = KI$

Lambert's law: The rate of decrease of intensity (monochromatic light) with the thickness of the medium is directly proportional to the intensity of incident light.

i.e., $-dI/dt \alpha I$

$I = I_o e^{-kt}$

Parts of colorimetry:

Source of light: a) Tungsten lamp b) Carbon arc lam

Filters: a) Absorption filters b) Interference filters

Prisms: a) Refractive type b) Reflective type

Gratings: a) Diffraction grating b)Transmission grating

Detectors: a) Barrier layer cell or photo voltaic cell b) Photo tubes or photo emissive cell c) Photo multiplier tubes

Applications:

1) Determination of ligand / metal ratio in metallic complexes.
2) Structure elucidation of organic compounds.
3) Determination of pKa value of indicators.
4) Determination of Molecular weight of amines.

Experiment. No: 5

Determination of Λ_{max} Of Potassium Permanganate

AIM: To determine the absorption maxima of given solution of potassium permanganate and to checkthe linearity of the same.

REFERENCE: B K Sharma. Instrumental method of chemical analysis. 2005. Pg. No. 68-192.

REQUIREMENTS: UV-Visible spectrophotometer, Potassium permanganate, distilled water, standard volumetric flask and pipette.

PRINCIPLE:

Spectroscopy is the measurement and interpretation of electromagnetic radiation absorbed, transmitted or emitted when the molecule of atom of sample move from one energy state to another. The technique used here is a colorimetry includes the study of absorption of visible radiation whose wavelength range is from 400-800nm. Wavelength is the distance between two successive crest ortrough. The UV-Visible spectrometer is constructed according the Beer's Lambert's law. It states that the absorbance is directly proportional to the path length and the concentration of the absorbing species.

The wavelength at which maximum absorption is recorded for a given substance is called Absorbance maxima or λ_{max}. The absorption maxima is recorded by plotting a spectrum of absorbance on y-axis and wavelength on x-axis. λ_{max} can be used for the identification of compound and most of the estimations are done at λ_{max} of particular substance, in order to increase the accuracyof the result.

PROCEDURE:

1. Weigh accurately about 100mg of $KMnO_4$ in a 100ml volumetric flask, dissolve it indistilled water. Make up the volume to 100ml with distilled water (I stock solution).
2. Take 20 ml from first stock solution and dilute to 100ml with distilled water (II stocksolution).
3. Take 1, 2, 3, 4 and 5ml of II stock solution and dilute to 10ml.
4. Record the absorption spectrum on a double beam spectrophotometer in the visible rangeof400-800nm
5. A graph of absorbance Vs concentration is plotted to obtain the calibration curve andabsorbance Vs wavelength is plotted to determine the λ_{max}

REPORT: λ_{max} of Potassium Permanganate was found to be_____nm.

Linearity of Potassium Permanganate was found to be in the range of_____µg/ml.

Experiment. No: 06

THE EFFECT OF PH ON λmax OF SULPHANILAMIDE

AIM: To study the effect of pH λmax of sulphonamide.

REFERENCE: A.H. Beckett, J. B. Stenlake; Practical Pharmaceutical Chemistry; 4th Edition;2004; CBS Publications and Distrtibutors; p. 328-330.

REQUIREMENTS: Beaker, volumetric flask, pipette, funnel, measuring cylinder, sulphanilamide, 1N Hcl,1N NaoH, Distilled water

PRINCIPLE: Sulphailamide are derivatives of Para amino benzene sulphanilamide. These are bacteriostatic agents but may act as a bactericidal. The presence of free amino group is essential for antibacterial activity.

In the alkaline solution the primary amino group is retained as the auxochrome, but in acidic solution quaternisation occurs to a coordinatively saturated auxochrome i.e. the amino is replaced by NH_2- NH_2 which is considerably less efficient has on auxochrome.

This is much less effective in modifying adsorption and the characteristic benzoid absorption is obtained at 265nm. Note that solutions of sodium hydroxide absorb radiation below about 230 nm, so that, unless the concentration of alkali is very small, the readings will be unreliable. Absorption of light in ultraviolet region of the spectrum is due to the presence of chromophores in theabsorbing molecule. The term chromophore was originally used for unsaturated compound or groups of atom. The absorption of 2 or more chromophores which are separated by more than 1 bond are usually additive but when such chromophores are conjugated i.e. separated by a single bond, protonated effect are produced. The maximum absorption shifting to longer wavelength is called bathochromic shift. The reverse change is as Hypsochromic shift.

PROCEDURE:

Preparation of 1N NaoH solution: Dissolve 40 gms of NaoH in 100 ml of distilled water, then make up the volume to 1000ml

Preparation of 1N HcL solution: Dissolve 85ml of concentrated Hcl in 100 ml of distilled water, then make up the volume to 1000ml

Prepare 100ml of a 0.1% w/v aqueous solution of sulphanilamide. Dilute 1ml of this solution to 100ml using 1M hydrochloric acid and also dilute 1ml to 100ml using 1M sodium hydroxide. Record the absorption spectrum of each solution over the range 210-300 nm for acid solution and 230-300 nm for the alkaline solution, using 1cm cells and appropriate reference solutions.

Report:

1) **λmax** of sulpanilamide in 1N HCL =
2) **λmax** of sulpanilamide in 1N NaoH =

Experiment. No: 7

The Acid **Dissociation Constant of Methyl Red**

AIM: To determination the acid dissociation constant of methyl red (MR) in the acidic(HMR) and basic (MR^-) forms of methyl red.

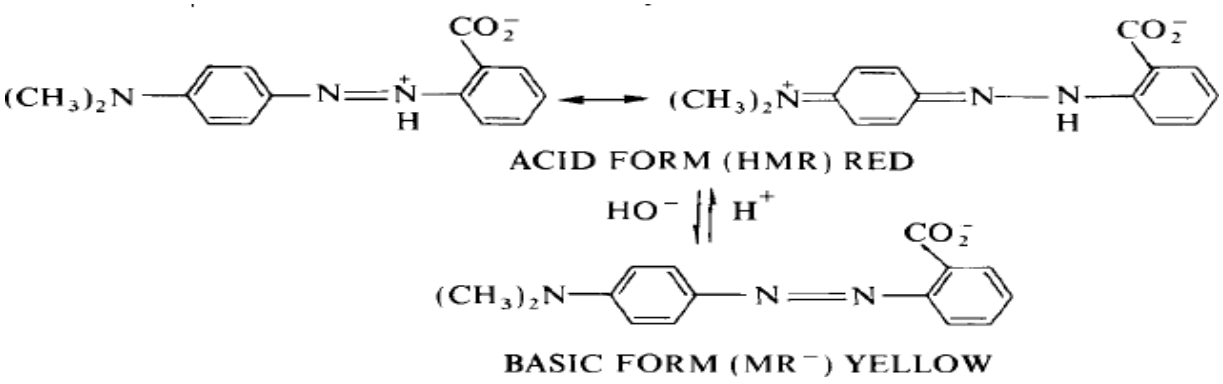
REFERENCE: Vogels Text book of quantitative chemical analysis G.H.jeffery J. bassett J. mendham RC. denney. 5th ed: 1989.Pg.718-21.

REQUIREMENTS: UV-Visible spectrophotometer, methyl red, HCl, sodium acetate, acetica acid, distilled water, standard volumetric flask and pipette.

REAGENTS: Methyl red solution: Dissolve 0.10g pure crystalline methyl red in 30 mL 95 percent ethanol and dilute to 50 mL with water. The solution required in the experiment (standard solution) is prepared by transferring 5.0 mL of the above stock solution to 50mL of 95 per cent ethanol contained in a 100mL graduated flask and diluting to 100 mL with water.

Sodium acetate: 0.04 M and 0.01 M.Acetic acid: 0.02M.

Hydrochloric acid: 0.1 M and 0.01 M. The exact concentrations of these two solutions are not critical.PRINCIPLE:



Both HMR and MR- have strong absorption peaks in the visible portion of the spectrum; the colour change interval from pH 4 to pH 6 can be conveniently obtained with a sodium acetate-aceticacid buffer system.

The determination of pK involves three steps:

(a) Evaluation of the wavelengths at which HMR (A,) and MR^- (A,) exhibit maximum absorption.

(b) Verification of Beer's Law for both HMR and MR- at wavelengths A, and AB.

Determination of the relative amounts of HMR and MR- present in solution as a function of pH. By using the same concentration of indicator in each of the measurements at different values of pHand measuring the absorbance for each solution at A, and at AB, the relative amounts of HMR andMR- in solution can be calculated from the equations.

PROCEDURE: The study can be carried out using either a manually operated single-beam spectrophotometer, or an automatic recording double-beam spectrophotometer. In both cases the wavelengths at which HMR and MRexhibit absorption maxima are readily obtained from the spectra.

(a) Prepare solution **A** by diluting a mixture of 10.0 mL of the standard solution of the indicator (MR) and 1.0 mL of 0.1 M hydrochloric acid to 100 mL; the pH of this solution is about 2, so that the indicator MR is present entirely as HMR. Using 1 cm cells, determine the absorption spectrum of this solution over the range 350-600 nm against a blank of distilled water. For manual plotting cover the range in increments of 25 nm except for the portion between 500 and 550 nm which should be coveredin l0 nm increments. From the spectrum of absorbance against wavelength determine the wavelength **1,** at which the maximum absorbance occurs: this is about 520 nm.

(b) Prepare solution B by diluting a mixture of 10.0mL of the standard solution of the indicator and

25.0 mL of 0.04M sodium acetate to 100 mL. The pH of this solution is about 8, so that the indicator MR is present entirely as MR-. Measure the absorbance of solution B over the range 350-600 nm as detailed for solution A: with a manual spectrophotometer use 25 nm steps except for 400-450 nm, where 10 nm steps are recommended. Determine the wavelength **1,** of maximum absorbance as above: this is about 430 nm. The type of plots obtained for solutions A and B. The absorption peaks are not completely separated, but cross at a wavelength of about 460nm. This point is known as the 'isobestic point'. If the absorbance of a solution containing

both HMR and MR- is measured at this wavelength, the observed absorbance is independent of the relative amounts of HMR and MR- present and depends only on the total amount of the indicator MR in the solution.

Prepare solutions in four 100 mL graduated flasks as listed in Table. Determine the pH values of each of the solutions (typical values are incorporated in the table) and measure the absorbance of each solution at wavelengths ***A,*** and ***AB.*** Al1 these solutions contain the same concentration of indicator as solutions A and B used in (a). For each prepared solution, obtain the values of the absorbances $d_{A\ HMR}$, $d_{A\ MR^-}$, $d_{B\ HMR}$, and $d_{BA\ MR^-}$ from the plots in, at relative concentrations of 1.0, and solve the simultaneous equations and in order to evaluate the relative amounts of HMR and MR- in solution. From the relative amounts of HMR and MR- present as a function of pH, calculate the value of pK for methyl red using equation.

REPORT: The dissociation constant of methyl red (MR) is

Experiment. No: 8

Simultaneous Estimation Of Paracetamol And Ibuprofen

AIM: To carry out the simultaneous estimation of paracetamol and ibuprofen byUV Spectrophotometric method.

REFERENCE: A.H. Beckett, J. B. Stenlake; Practical Pharmaceutical Chemistry; 4th Edition;2004;CBS Publications and Distrtibutors; Pg;285-287.

REQUIREMENT: Paracetamol, Ibuprofen, 0.1N Hcl, Distill water, 0.1N NaOH, methanol, chloroform.

PRINCIPLE:

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

Two equations are constructed based upon the fact that at λ_1 and λ_2 the absorbance of the mixture is thesum of the individual absorbances of X and Y.

At λ_1 $A_1 = ax_1\, bcx + ay_1\, bcy$ ---------- 1

At λ_2 $A_2 = ax_2\, bcx + ay_2\, bcy$ ---------- 2

ax_1 and ax_2 = Absorptivities of X at λ_1 and λ_2 ay_1 and ay_2 = Absorptivities of Y at λ_1 and λ_2

A_1 and A_2 = Absorbances of the duluted samples at λ_1 and λ_2Rearranging the equation 2 we get,

$$cx = \frac{A_2\, ay_1 - A_1\, ay_2}{ax_2\, ay_1 - ax_1\, ay_2}$$

$$cy = \frac{A_1\, ax_2 - A_2\, ax_1}{ax_2\, ay_1 - ax_1\, ay_2}$$

PROCEDURE:

Estimation of Paracetamol: Triturate the tablet of label containg as follows.

Each tablet contains.Ibuprofen I.P 200mgParacetamol 400mg

Preparation of sample solution:

Weigh accuretly100mg tablet powder and dissolve in 100ml of methanol solution. This gives 1000mg/ml stock now take 0.1ml from stock and dilute in to 10ml methanol this gives 10 µg/ml.

Observe the absorbance of tablet concentration (10 µg/ml) at absorbance at 222nm and at 244nm Similarly take the absorbance of standard paracetamol (10 µg/ml) at 244nm also take the absorbanceof standard ibuprofen at 222nm.

REPORT: The amount of paracetamol and ibuprofen present in the tablet is …….

Experiment. No:9

CONDUCTOMETRIC TITRATION

AIM: To perform Conductometric titration of strong acid Vs strong base.

REFERENCE: BK Sharma. Instrumental method of chemical analysis. 2005. Page No 3-6.

REQUIREMENTS: Conductometer, 0.1N HCl, 0.5N NaOH

PRINCIPLE:

Determination of conductance of electrolyte solution by conductometer is called conductometry. Solution of electrolyte conducts electricity by migration of ions under the influence of an electric field.This conductivity of electrolyte solution at any temperature depends upon the concentration of ions present. Conduction is defined as reciprocal of resistance and it is expressed in form of mho. Conductometric titration is widely used for acid base precipitation, complexometric titration, redox titration and displacement titration.

Titration of strong acid Vs strong base

Here in this titration, there will be initial fall in conductance due to replacement of hydrogen ion of high ionic mobility with slow mobility sodium ions. After the end point conductance increases because of excess of hydroxyl ion being added.

PROCEDURE:

Standardization:
Standardisation of NaOH: Take 0.2 gm of oxalic acid. Dissolve in 10ml of water. Add phenolphthalein indicator. Titrate against 0.5 N NaOH.

Sample Titration:
Take 50 ml of HCl in a beaker and note the conductance at each addition of 1 ml of NaOH. Stop the titration when the conductance increases after initial decrease. Plot the graph of conductance v/s volume of NaOH. Determine the end point and calculate the amount of HCl.

REPORT: The normality of Hydrochloric acid was found to be_____.

Experiment. No:10

Potentiometric Titration

AIM: To determine end point of 0.1 N HCl using potentiometric titration

REFERENCE: BK Sharma. 2005. Instrumental method of chemical analysis. Page No 3-6.

REQUIREMENTS: Potentiometer, 0.1N HCl, 0.5N NaOH.

PRINCIPLE:

Potentiometric titration is the one in which end point of titration can be determined by measuring the potential (EMF) or changing potential due to addition of titration. Alternating pH can also be monitored during titration. In titration, the addition of titration will bring change in concentration of activity of concentration in the solution. End point is determined by plotting the graphs

1) Normal curve, i.e., emf vs volume of NaOH.

2) 1^{st} derivation curve, i.e., $\Delta E/\Delta V$ Vs. Volume of NaOH.

3) 2^{nd} derivation curve: $\Delta^2E/\Delta V^2$ Vs. Volume of NaOH

PROCEDURE:

1) In the beaker, transfer 20 ml of given acid and 30 ml water.

2) Insert the electrode and measure potential.

3) Start addition of NaOH in 1ml interval and measure potential.

4) Draw normal curve and 1^{st} derivation curve and find the end point from graph.

REPORT: The normality of Sodium Hydroxide was found to be _____ The end point was found to be ----------

Experiment. No:11

Estimation Of Quinine Sulphate By Flourimetry

AIM: To estimate the concentration of Quinine sulphate in the given sample by Flourimetry.

REFERENCE: Beckett and Stenlake's. Practical Pharmaceutical Chemistry. 2001.Pg.No. 365 and 372.

REQUIREMENTS: Spectroflourimetry, Quinine sulphate, 0.1N H_2SO_4, distilled water.

PRINCIPLE:

Flourimeter is an instrument used for measurement of fluorescence. Flourescence is a phenomenon of emission of radiation when there is a transition from singlet excited state to ground state. The wave length of absorbed radiation is called excitation wavelength and the wavelength of emission radiation is called emission wavelength. As those 2 wavelengths are different in most cases, filters of different monochromators are used. The filter used will absorb visible radiation and transmit UV radiation and secondary filter absorb UV and transmit visible radiation. The emitted radiations are measured at angle of 90^oC.

PROCEDURE:

1) Weigh 100mg of Quinine sulphate and make up to 100ml with 0.1NH_2SO_4.
2) Pipette out 10 ml of stock solution and dilute to 100ml with 0.1N H_2SO_4.
3) Again, Pipette out 10 ml and dilute to 100ml with 0.1NH_2SO_4.
4) Pipette out 1 ml of the above solution and make up the volume to 10ml (1 µg/ml).
5) Then prepare various concentrations of 0.02, 0.04, 0.06, 0.08 and 0.1µg/ml and make upthe volume to 10ml with 0.1N H_2SO_4.
6) Measure the fluorescence intensity at 365nm (Excitation) and 455nm (Emission)
7) Plot the calibration curve and find out the unknown concentration from the graph.

REPORT: The amount of Quinine sulphate present in the given sample was found to be

_____µg/ml.

Experiment. No:12

Quenching Effect Of Quinine Sulphate By Fluorimetry

AIM: To study the quenching effect of Quinine sulphate by flourimetry.

REFERENCE: Beckett and Stenlake's. Practical Pharmaceutical Chemistry. 2001.Pg.No. 365and372.

REQUIREMENTS: Spectrofluorimeter, Quinine sulphate, 0.1M Potassium iodide and 0.05MH_2SO_4,

PRINCIPLE:

Quenching is the reduction of fluorescence intensity by the presence of substances in the sample other than the fluorescent analytes. Absorption of incident or emitted radiation quenches fluorescence by the inner filter effect [the fluorescence intensity of a substance is proportional to concentration only when the absorbance in 1cm cell is less than 0.02. With increasing absorbance the factorial term in equation of fluorescence and concentration introduce an increasingly significant error: inner filter effect].

Collisional quenchers reduce fluorescence by dissipating absorbed energy as heat due to collisions with the quenching species. Eg. Quinine is highly fluorescent in 0.05M sulphuric acid but non fluorescent in 0.1M hydrochloric acid due to collisional quenching by the halide ion. Static quenchers form a chemical complex with the fluorescent substance and alter its flourescence characteristics.

PROCEDURE:

1) **Preparation of Quinine Sulphate:**

Weigh 100mg of Quinine Sulphate, transfer into 100ml volumetric flask, dissolve in 0.1NH_2SO_4 and make up the volume with the same. Pipette out 10ml of the above solution and make up the

volume to 100ml. Again Pipette out 10 of the above solution and make up thevolume to 100ml. Then pipette out 10ml and make up the volume to 100ml with 0.1N H_2SO_4.

2) Preparation of Potassium Iodide solution:

Weigh 100mg of Potassium Iodide, transfer to 100ml volumetric flask, dissolve in 0.1N sulphuric acid. Pipette out 10ml of the above solution and make up the volume to 100ml. Pipette out 1ml, 2ml, 3ml, 4ml, 5ml and 6ml and make up the volume to 10ml.

To each flask, add 1ml of 1 µg/ml solution of quinine sulphate and then add 1ml, 2ml, 3ml, 4ml,5ml and 6ml of Potassium iodide. Measure the fluorescence intensity with sulphuric acidas blank. Plot the fluorescence against concentration of potassium iodide.

REPORT: A decrease in fluorescent intensity was observed for quinine sulphate with the addition ofpotassium iodide solution.

Experiment. No:13

Estimation Of Sulphanila mide By Colorimetry

AIM :- To carry out the colorimetric estimation of sulphanilamide by colorimetry.

REFERENCE: A.H. Beckett, J. B. Stenlake; Practical Pharmaceutical Chemistry; 4th Edition;2004;CBS Publications and Distrtibutors; Pg; 328-330.

CHEMICALS REQUIRED :- HCl, Sodium nitrite, Ammonium sulphamate, Sulphanilamide, BMreagent (BRATTON MARSHALL reagent), distilled water etc.

APPARATUS REQUIRED :- Conical flask, volumetric flask, measuring Cylinder, Colorimeter etc.

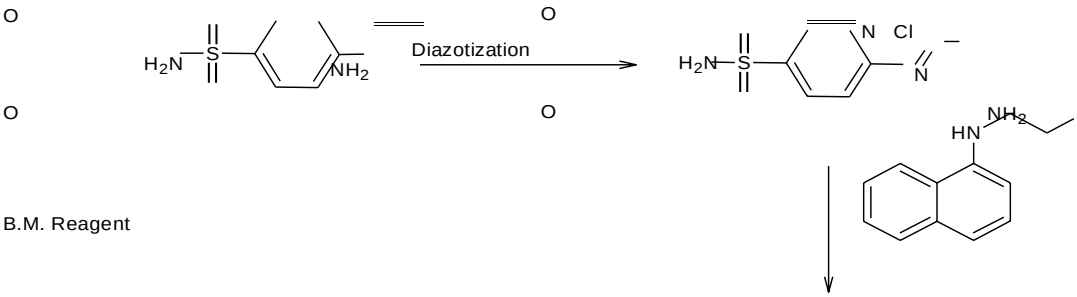
PRINCIPLE: - Drugs containing primary amine group (like sulpha drugs) can be conveniently estimated by diazotization reaction followed by coupling reaction. First the amine group containing drugs are diazotized with the help of nitrous acid (which is produced instantly by the reaction between HCl and sodium nitrite), then the diazotized compound is coupled with BM reagent (N-(1-Napthyl ethylene diamine dihydrochloride), which results in the formation of pink colored dye with λ_{max} of 540 nm.

Since the concentration of the solution is directly proportional to absorbance of solution, series of solutions of varying concentration are prepared and their absorbance is measured at 540 nm. Then the absorbance of the unknown solution is measured. With the help of standard graph, the concentration of unknown sample of sulphanilamide is determined.

If any un utilized nitrous acid remain in the medium, then this unreacted nitrous acid may be decompose the pink color complex which is formed when diazotized compound react with BM reagent. Hence to avoid the effect of unreacted nitrous acid on complex, ammonium sulphamate solution is added.

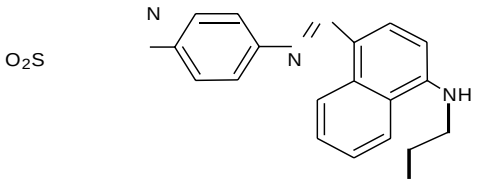
REACTION:-

$HCl + NaNO_2 \longrightarrow HNO_2 + NaCl$



B.M. Reagent

(N-1-napthyl ethylene diamine)



H_2N

Intense orange colour

PROCEDURE:-

Working standard solution: - Prepare 100 µg/ml. solution of sulphanilamide from 1 mg/ml. solutionof sulphanilamide.

1) Take 5 volumetric flask of 100 ml. and label them as S_1 to S_5.

2) Pipette out 2,4,6,8 and 10 ml. of working standard solution to volumetric flask which are labeled as S_1 to S_5.

3) Make the final volume up to 100 ml. with distilled water.

4) Label 5-test tubes as T_1 to T_5.

5) Transfer 2ml. solution from S_1- S_5 to T_1 - T_5 respectively.

6) Add 2 ml. of 0.5N HCl solution to all the test tubes.

7) Add 2 ml. of 0.1% w/v $NaNO_3$ solution to all test tubes.

8) Set a side for 10 min. in ice cold water.

9) Add 2 ml. of 0.5% w/v solution of ammonium sulphamate to all the Test tubes, mix thoroughly

10) Add 2 ml. of BM reagent (N-(1-Napthyl ethylene diamine dihydrochloride) to all the Test tubes and kept aside.

11) Adjust the absorbance of colorimeter to zero at 540 nm. Using reagent blank (2ml. of 0.5N HCl solution + 2ml. of 0.1% w/v $NaNO_3$ solution + 2ml. of 0.5% w/v solution of ammonium sulphamate + 2ml of BM reagent)

12) Prepare the sample in the similar method and measure the absorbance of all the solutions.

13) Plot a graph of concentration v/s absorbance.

14) Determine the absorbance of unknown solution, and from standard graph determine the amountof sulphanilamide present in the given sample.

REPORT:- The amount of sulphanilamide present in given sample is .....µ/ml.

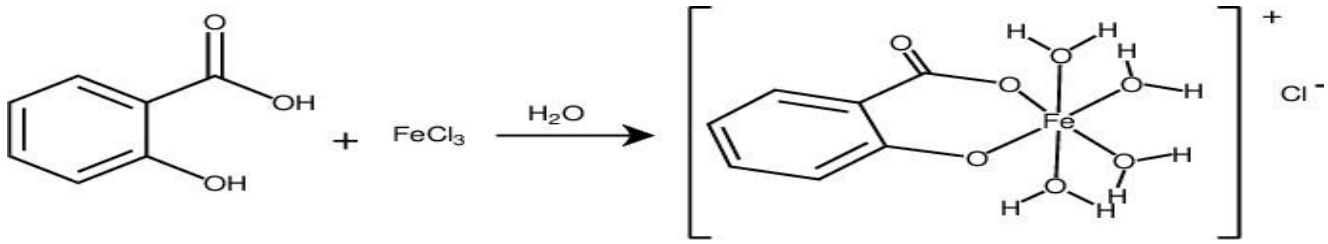
Experiment. No:14

COLORIMETRIC ESTIMATION OF SALICYLIC ACID

AIM: To estimate the amount of salicylic acid present in the given sample by colorimetry.

REQUIREMENTS: Colorimeter, Volumetric flasks, Pipettes, Funnel,Beaker & 1N HCl, Acidicferric ammonium sulphate solution, Salicylic acid

PRINCIPLE: Salicylic acid contains a phenolic hydroxyl groups. This forms a Complex with the ferric ion to give ferric complex, which has violet colour proportional to the Concentration of salicylicacid. The absorbance is maximum at 540 nm. Beers Range is 5 – 45 µ g/ml.



PROCEDURE:

1. Weigh accurately 100 mg of salicylic acid into 100 ml volumetric flask and dissolve with water and make up the volume to 100 ml with distilled water.
2. Pipette out 10ml of the above solution to another 100ml of volumetric flask and make up the volume to 100ml with distilled water.
3. Pipette out 2, 3, 4, 5 and 6 ml of the above solution to separate five 25 ml volumetric flask.
4. To each of the volumetric flask add 10 ml of acidic ferric ammonium sulphate solution and mix thoroughly.
5. Make up the volume to 25 ml with water. Measure the absorbance against water as blank at 540 nm.
6. Plot the graph of concentration Vs absorbance and find the concentration of unknown sample.

REPORT:

Experiment. No:15

ESTIMATION OF SULPHATE ION BY NEPHELOMETRY

AIM: - To estimate the amount of sulfate ion in the given sample solution by nephelometry.

REQUIREMENTS: - NaCl-HCl reagent, $BaCl_2$, Glycerol - ethanol reagent, Beaker, Pipettes, volumetric flasks, Nephelometer.

PREPARATION OF REAGENTS: -

1. NaCl – HCl reagent: Dissolve 60g of NaCl in 200ml of distilled water add 5 ml of pure concentrated HCl and make up the volume to 250 ml with distilled water. This reagent is added to inhibit the growth of Micro crystals of $BaCl_2$.
2. $BaCl_2$ reagent: dissolve 3.75g of $BaCl_2$ in 100ml of distilled water, 4 ml of this reagent is equivalent to 0.150g of $BaCl_2$.
3. Glycerol – ethanol reagent: Prepare this solution in the ratio of 1:2 and it is added to stabilize the suspension to be formed.

DISCUSSION: Estimation of sulfate ions by nephelometry method based on reaction that the solution of sulfate when treated with $BaCl_2$, it gives the precipitate of sulfate ion estimated by the nephelometric method, as when the light is scattered and the intensity of scattered light is measured. When this intensity plotted against concentration gives straight line. Now the concentration of unknown is determined by extrapolating the point.

PROCEDURE: -

1. Accurately weigh 0.1814 gm of pure K_2SO_4 and dissolve in distilled water and make up the volume to 100 ml with distilled water.
2. From this standard solution pipette out aliquots of 0.25, 0.5, 0.75, 1.0 and 1.25 ml in to 5 volumetric flask of 50 ml capacity.
3. To each flask add 5 ml of NaCl-HCl reagent, 10 ml of glycerol-ethanol solution and 4 ml $BaCl_2$ reagent make up the volume in each flask to 50 ml with distilled water.
4. Treat the given unknown sample solution similarly and make up the volume to 50 ml with distilled water.

5. Measure the intensity of scattered light of each of these solution using Nephelometer against reagent blank.
6. Plot a calibration curve of concentration Vs Nephelometry turbidimetry units (NTU). Determine the concentration of unknown using calibration curve.

REPORT: The concentration of sulphate ions in the given sample is

Experiment. No:16

Estimation Of Sodium Ion By Flame Photometry

AIM: To estimate the concentration of Sodium ion in the given sample by Flame photometry.

REFERENCE: Instrumental method of chemical analysis. Gurudeep R Chatwal, Sham K. Anand.2016. Page No. 342-345

REQUIREMENTS: Flame Photometer, sodium chloride solution.

PRINCIPLE:

Flame photometry is a type of emission spectroscopy. It is the measurement of light of a particular wavelength by the atoms that are excited thermally in the flame. The intensity of atomic emission depends on number of thermally excited atoms which are highly depends on the temperature. The intensity depends on the energy difference between the excited and ground state. It involved 3 steps:

1) Atomization
2) Excitation
3) Emission

This method is mainly used for estimation of Metals like Sodium, Potassium, Calcium, Lithium etc.

PROCEDURE:

Preparation of sodium:

Dissolve 2.542 g of sodium chloride in 1 L deionized water in a deionized water in a graduated flask. This solution contains the equivalent of 1.000mg Na per mL (i.e. 1000 ppm). Dilute this stock solution to give five solutions containing 10, 8, 6, 4 and 2 ppm of sodium ions.

Preparation of Potassium:

Dissolve 1.909 g Potassium chloride in 1 L deionized water. This solution contains the equivalent of 1.00 mg K per ml (1000 ppm). Dilute this solution to give five solutions containing 20, 16, 12,8 and 4ppm of potassium ions.

Dilute the given sample solution to concentration in between the above range. Measure the emission of allsolutions. Plot the standard curve. Determine the concentration of sample from the graph. Report the concentration of ions in the sample.

REPORT: The concentration of sodium ions in the given sample of Sodium Chloride solution was found to be_.

Experiment. No:17

Demonstration Of High-Performance Liquid Chromatography

AIM: To Demonstrate High performance liquid chromatography.

REFERENCE: A practical Approach to Pharmaceutical Analysis by Rajesh Kumar and S.N. Meyyanathan, first edition, 2008. page no.166-167.

REQUIREMENTS: HPLC-LC20AT, HPLC grade Methanol and HPLC grade water, etc.

PRINCIPLE:

High performance liquid chromatography is based upon the principle of absorption. It is modified technique of column chromatography and also called as high-pressure liquid chromatography. The high performance is because of the superior separation of the components due to the wide surface area provided for absorption. The particle size of the silica gel is about 0.5µ. The use of pump makes the technique faster and various types of detectors can be used to analyze the components.

The different part of high-performance liquid chromatography is:

1. Solvent reservoirs- To store for mobile phase
2. Pumps- To maintain the flow rate of mobile phase.
3. Sample injectors- To inject the sample into the mobile phase.
4. Guard column- To saturate the mobile phase with silica and to guard analytical column from impurities/particles.
5. Analytical column- To carry out the separation of the components from the complex mixtureby absorption technique.
6. Detector- To analyze the components, separate from the columns.
7. Readout- To plot the chromatograms.

PROCEDURE:

1. Switch on the instrument
2. Prepare the mobile phase.
3. Prepare sample solution containing the mixture of components to be separated.
4. Place the mobile phase into the solvent reservoir.
5. Turn on the pumps and set the flow rate as specified in selected method.
6. Maintain the pressure within the limits as per the column used.
7. Turn on the detector and wait for 5 min for stabilization.
8. Set the detection wavelength.
9. Run the mobile phase for at least 10 minutes.
10. Then collect the baseline for 10 min, connect the detector for mobile phase.
11. Inject the sample solution into the injector and allow the sample to run for specified run time.
12. Analyze the chromatogram for different components.

Preparation of standard Paracetamol:

Weigh 10mg of Paracetamol and dissolve in methanol and make up the volume to 100ml with methanol. Make the different dilution containing 10, 20, 30, 40 and 50 µg/ml and then filtered.

Preparation of sample solution:

The unknown concentration of the Paracetamol was prepared with methanol.

REPORT: High performance liquid chromatography was demonstrated

Vision and Mission of the Institution

Vision

The East Point College of Pharmacy aspires to be a globally acclaimed institution, **recognized** for **excellence in** pharmaceutical education, research and nurturing students for **holistic development.**

Mission

M1 Create pharmacy graduates through **quality education**

M2 Promote innovation, **creativity**, and excellence **in teaching**, learning, and **research**

M3 **Inspire** integrity, teamwork, critical thinking**, personal** development, and ethics in **students** and lay **the** foundation for lifelong learning

M4 **Serve** the **healthcare, technological, scientific**, and **economic** needs of then **society**.