MARRI LAXMAN REDDY INSTITUTE OF PHARMACY
(Approved by AICTE & PCI, New Delhi and Affiliated to JNTUH)
Dundigal - Gandimaisamma (V) &(M), Medchal (Dt), Hyderabad, Telangana - 500 043.

PHYSICAL PHARMACEUTICS – I

LAB MANUAL

B. PHARMACY II-I

About MLRIP

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

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

Program Educational Objectives

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

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

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

PROGRAM OUTCOMES

1. **Pharmacy Knowledge:** Possess knowledge and comprehension of the core and basic knowledge associated with the profession of pharmacy, including biomedical sciences; pharmaceutical sciences; behavioral, social, and administrative pharmacy sciences; and manufacturing practices.
2. **Planning Abilities:** Demonstrate effective planning abilities including time management, resource management, delegation skills and organizational skills. Develop and implement plans and organize work to meet deadlines.
3. **Problem analysis:** Utilize the principles of scientific enquiry, thinking analytically, clearly and critically, while solving problems and making decisions during daily practice. Find, analyze, evaluate and apply information systematically and shall make defensible decisions.
4. **Modern tool usage:** Learn, select, and apply appropriate methods and procedures, resources, and modern pharmacy-related computing tools with an understanding of the limitations.
5. **Leadership skills:** Understand and consider the human reaction to change, motivation issues, leadership and team-building when planning changes required for fulfillment of practice, professional and societal responsibilities. Assume participatory roles as responsible citizens or leadership roles when appropriate to facilitate improvement in health and well-being.
6. **Professional Identity:** Understand, analyze and communicate the value of their professional roles in society (e.g. health care professionals, promoters of health, educators, managers, employers, employees).
7. **Pharmaceutical Ethics:** Honour personal values and apply ethical principles in professional and social contexts. Demonstrate behavior that recognizes cultural and personal variability in values, communication and lifestyles. Use ethical frameworks; apply ethical principles while making decisions and take responsibility for the outcomes associated with the decisions.
8. **Communication:** Communicate effectively with the pharmacy community and with society at large, such as, being able to comprehend and write effective reports, make effective presentations and documentation, and give and receive clear instructions.
9. **The Pharmacist and society:** Apply reasoning informed by the contextual knowledge to assess societal, health, safety and legal issues and the consequent responsibilities relevant to the professional pharmacy practice.
10. **Environment and sustainability:** Understand the impact of the professional pharmacy solutions in societal and environmental contexts, and demonstrate the knowledge of, and need for sustainable development.
11. **Life-long learning:** Recognize the need for and have the preparation and ability to engage in independent and life-long learning in the broadest context of technological change. Self-assess and use feedback effectively from others to identify learning needs and to satisfy these needs on an ongoing basis.

PS306: PHYSICAL PHARMACEUTICS – I LAB

B. Pharm II Year I Sem

L	T	P	C
0	0	4	2

List of Experiments

1. Determination the solubility of drug at room temperature
2. Determination of pKa value by Half Neutralization/ Henderson Hassel Balch equation
3. Determination of Partition co- efficient of benzoic acid in benzene and water
4. Determination of Partition co- efficient of Iodine in Carbon tetra chloride and water
5. Determination of % composition of sodium chloride in a solution using phenol-water system by CST method
6. Determination of particle size, particle size distribution using sieving method
7. Determination of particle size, particle size distribution using Microscopic method
8. Determination of bulk density, true density and porosity
9. Determine the angle of repose and influence of lubricant on angle of repose
10. Determination of stability constant and donor acceptor ratio of PABA-Caffeine complex by solubility method
11. Determination of stability constant and donor acceptor ratio of Cupric-Glycine complex by pH titration method

Recommended Books: (Latest Editions)

1. Physical pharmacy by Alfred Martin
2. Experimental pharmaceutics by Eugene, Parrott.
3. Tutorial pharmacy by Cooper and Gunn.
4. Stocklosam J. Pharmaceutical calculations, Lea & Fibiger, Philadelphia.
5. Liberman H.A, Lachman C., Pharmaceutical Dosage forms, Tablets, Volume-1 to 3, Marcel Dekker Inc.
6. Liberman H.A, Lachman C, Pharmaceutical dosage forms. Disperse systems, volume 1, 2, 3. Marcel Dekker Inc.
7. Physical pharmaceutics by Ramasamy C and Manavalan R.
8. Laboratory manual of physical pharmaceutics, C.V.S. Subramanyam, J. Thimma settee

1. DETERMINATION OF SOLUBILITY OF DRUG AT ROOM TEMPERATURE

Aim: To determine the solubility of benzoic acid at different temperatures.

Requirements: benzoic acid, distilled water, 0.1N Sodium hydroxide, phenolphthalein indicator, and filter paper, Measuring cylinder, funnel, beaker, conical flask, 10 ml bulb pipette, rubber bulb, burette, burette stand.

Principle:

The amount of drug dissolved in solution at a particular temperature is called solubility. Example: The solubility of paracetamol is 1 g in 70 ml water at 20 ^{0}C. The solubility of a drug is determined by preparing a saturated solution of the drug. A saturated solution is prepared by shaking excess quantity of the drug with the solvent for a long time (48 hours). This system is filtered and the saturated solution is analyzed for drug content by titration or suitable analytic method. In this experiment solubility of benzoic acid is determined by using distilled water. The amount of benzoic acid dissolved in the solvent is analyzed by titrating with 0.1 N Sodium hydroxide solution using phenolphthalein as indicator. When a drug (benzoic acid) has poor solubility in water, then the solubility of benzoic acid is improved by rise of temperature.

Procedure:

1. Take 50 ml of distilled water into a 100 ml beaker. Add required quantity of benzoic acid and shake vigorously for 30 minutes. If the added benzoic acid has dissolved, add further some amount of benzoic acid and continue shaking to obtain a saturated solution.
2. Heat the benzoic acid on the water bath up to 85^{0}C.
3. Allow the temperature to fall gradually to 80^{0}C.
4. Filter the contents into a clean dry beaker.
5. Titrate 10 ml of the filtrate with 0.1 N sodium hydroxide solution using phenolphthalein as indicator.
6. Continue the procedure and obtain data of solubility at 70,60,50,40 and 30^{0}C temperatures.
7. Draw a plot by taking solubility of benzoic acid on y-axis and temperature on x-axis.
8. Calculate the solubility of benzoic acid in water.

Observations and Calculations:

S.NO	Temperature (^{0}C)	Volume of sodium hydroxide consumed (ml) (V_1)	Normality of benzoic acid (N_2)	Solubility of benzoic acid (gm/ml)
1.	80			
2.	70			
3.	60			
4.	50			
5.	40			
6.	30			

Equivalent weight of benzoic acid is 122 gm

Normality of sodium hydroxide (N_1) is 0.1N

Volume of sodium hydroxide consumed is (V_1)

Volume of benzoic acid (V_2) is 10 ml sample taken at different temperatures.

Normality of benzoic acid (N_2) = $\frac{N_1V_1}{V_2}$

Solubility of benzoic acid = $N_2 \times \frac{122}{10}$

Solubility of the drugs is expressed in various units in Merk Index

Term	Parts of solvent required for 1 part of solute
Very soluble	Less than 1part
Freely soluble	1 to 10 parts
Soluble	10 to 30 parts
Sparingly soluble	30 to 100 parts
Slightly soluble	100to 1000 parts
Very slightly soluble	1000 to 10,000 parts
Practically insoluble	More than 10,000 parts

Report: The solubility of benzoic acid in water_____gm/ml at 80^0C.As the temperature increases the solubility of benzoic acid is increased.

2. DETERMINATION OF P^{Ka} VALUE BY HALF NEUTRALISATION/ HENDERSON HASSEL BALCH EQUATION.

Aim: To determine the P^{Ka} value of the weak acid (acetic acid) by Henderson Hassel Balch equation.

Requirements: Acetic acid, distilled water, 0.1N Sodium hydroxide, 0.1N Oxalic acid, Measuring cylinder, funnel, beaker, conical flask, pH meter.

Principle:

pH is a measure of hydrogen ion concentration, a measure of the acidity or alkalinity of a solution. The pH scale usually ranges from 0 to 14. Aqueous solutions at 25°C with a pH less than seven are acidic, while those with a pH greater than seven are basic or alkaline. A pH level of is 7.0 at 25°C is defined as 'neutral' because the concentration of H_3O^+ equals the concentration of OH^- in pure water. pH is given by equation as

$$pH = -\log[H^+]$$

where log is the base-10 logarithm and $[H^+]$ stands for the hydrogen ion concentration in units of moles per liter solution. pH can be measured by using Henderson Hassel Balch equation which is given by

$$pH = pK_a + \log \frac{(salt)}{(acid)}$$

To derive the dissociation constant (pKa). Consider that the weak acid under gone partial dissociation.

$$CH_3COOH + H_2O \leftrightarrow CH_3COO^- + H_3O^+$$

At equilibrium $K = \frac{K1}{K2} = \frac{(CH_3COO^-)\ (H_3O^+)}{(CH_3COOH)\ (H_2O)}$

where K1 and K2 are the rate constants of forward and backward reactions respectively. (H_2O) is a constant at about55.3 moles/liter.

$$K_a = K \times 55.3 = \frac{(CH_3COO^-)\ (H_3O^+)}{(CH_3COOH)}$$

where K_a is the dissociation constant. $(H_3O^+) = \frac{K_a\ (CH_3COOH)}{(CH_3COO^-)}$

Take –log on both sides, it becomes as

$$-\log (H_3O^+) = -\log K_a - \log (CH_3COOH) + \log (CH_3COO^-)$$

$$pH = pK_a + \log \frac{(CH_3COO^-)}{(CH_3COOH)}$$

$$pH = pK_a + \log \frac{(salt)}{(acid)}$$

In this study pH, pK_a and K_a will be determined for acetic acid.

Procedure:

Prepare the buffer solutions using standard buffer tablets 4, 7 and 9.4. Calibrate the pH meter by using the buffer solutions. Take 0.05 ml of acetic acid in volumetric flask having capacity of 100 ml and make up the volume. This solution was taken in beaker and measures the pH of the solution. Finally calculate pK_a of acetic acid by using the equation.

Observations and Calculations:

Molecular weight of acetic acid = 60.05

Weight per ml of solution = 1.0495

$pH = -\log[H^+]$

$$K_a = \frac{(CH_3COO^-)\ (H^+)}{(CH_3COOH)} = \frac{(H^+)^2}{(CH_3COOH)}$$

60.05 gm of acetic acid in 1000 ml = 1M

6.005 gm of acetic acid in 100 ml = 1M

$(CH_3COOH) = \frac{\text{Volume of acetic acid x weight per ml}}{\text{Weight of acetic acid for 1M}} = \frac{0.05 \times 1.0495}{6.005} =$ 0.0087 moles per liter.

$$K_a = \frac{(10^{-pH})^2}{(CH_3COOH)}$$

Therefore $pK_a = pH = -\log [H^+]$

Report:

The pH of the acetic acid solution is ----------

The dissociation constant (K_a) of acetic acid is ----------

The pK_a of the acetic acid solution is----------

3. DETERMINATION OF PARTITION COEFFICIENT OF BENZOIC ACID BETWEEN BENZENE AND WATER

Aim: To determine the partition coefficient of benzoic acid between benzene and water.

Requirements: Benzene, benzoic acid, 0.1N sodium hydroxide solution, phenolphthalein indicator, separating funnel, tripod stand, reagent bottles, two small beakers, measuring cylinder, conical flask, burette, burette stand, tile and digital balance.

Principle:

When a substance is added to a system containing two immiscible liquids, it distributes between the two liquids in a definite ratio." This is called Nernst distribution law. The added substance should have solubility in the two liquids for distribution to occur. This is known as the partition coefficient **K** of a substance between two liquids is given by the formula

$$\mathbf{K} = \frac{\text{Concentration of substance in organic layer}}{\text{Concentration of substance in aqueous layer}} = \frac{C_1}{C_2}$$

In the present experiment, distribution of benzoic acid between benzene and water is studied. Benzoic acid is an organic substance and has high solubility in benzene. It has less solubility in water. As a result, benzoic acid will partition preferably into benzene layer. The formula used for calculating partition coefficient of benzoic acid between benzene and water is given below. C_1 and C_2 are concentration of benzoic acid in organic and aqueous layer. In the present experiment, benzoic acid is shaken with benzene and water for 30 minutes to achieve distribution. Shaking is required to achieve distribution equilibrium. At equilibrium the speed of forward process is equal to the speed of backward process.

Benzoic acid is distributed as associated molecules in benzene layer and un associated molecules in aqueous layer. Hence the equation is given as follows.

$$\mathbf{K} = \frac{\sqrt{\text{Concentration of substance in organic layer}}}{\text{Concentration of substance in aqueous layer}} = \frac{\sqrt{C1}}{C2}$$

The partition coefficient K will be remains constant only if there is neither association nor dissociation of solute molecules in both the phases.

Procedure:

Preparation of 0.1N Sodium hydroxide: 4 gm of sodium hydroxide was dissolved in 1000 ml of distilled water and make up the final volume in volumetric flask.

1. Weigh the samples (250 mg, 500 mg and 750 mg) of benzoic acid into three reagent bottles and add 50 ml of benzene and 50 ml of water to all the three reagent bottles.
2. Keep the bottles on constant temperature water bath and Shake the bottles for 30 minutes.
3. Transfer the contents into a separating funnel and allow them to separate as two layers.
4. Collect the aqueous layer and titrate 10ml of sample with 0.1N sodium hydroxide solution using phenolphthalein as indicator.
5. Similarly collect the organic layer (benzene) and titrate 10ml of sample with 0.1N sodium hydroxide solution using phenolphthalein as indicator.
6. Calculate the partition coefficient of benzoic acid between benzene and water.

Observations and Calculations:

Equivalent factor: Each ml of 0.1N sodium hydroxide = 0.0122 gm of benzoic acid

Concentration of benzoic acid = Volume of sodium hydroxide consumed x 0.0122

S.NO	Volume of aqueous / benzene layer taken	Volume of sodium hydroxide consumed in ml	Concentration of benzoic acid	$\sqrt{C1}$	Partition coefficient = $\frac{\sqrt{C1}}{C2}$
1	10 ml organic		C1=		
2	10 ml organic		C1 =		
3	10 ml aqueous		C2 =		
4	10 ml aqueous		C2 =		

Report: The partition coefficient of benzoic acid between benzene and water is _____.

4. DETERMINATION OF PARTITION COEFFICIENT OF IODINE BETWEEN CARBON TETRA CHLORIDE AND WATER

Aim: To determine the partition coefficient of iodine between carbon tetra chloride and distilled water.

Requirements: Iodine, carbon tetra chloride, 0.1N sodium thiosulphate solution, 0.005N sodium thiosulphate solution, starch mucilage as indicator, separating funnel, tripod stand, reagent bottles, two small beakers, measuring cylinder, conical flask, burette, burette stand, tile and digital balance.

Principle:

When a substance is added to a system containing two immiscible liquids, it distributes between the two liquids in a definite ratio." This is called Nernst distribution law. The added substance should have solubility in the two liquids for distribution to occur. This is known as the partition coefficient **K** of a substance between two liquids is given by the formula

$$\mathbf{K} = \frac{\text{Concentration of substance in organic layer}}{\text{Concentration of substance in aqueous layer}} = \frac{C_1}{C_2}$$

Where K is known as partition coefficient or distribution coefficient, C_1 and C_2 are the total concentrations of the solute in the two layers of organic and aqueous phases.

Procedure:

Preparation of saturated solution of Iodine: Dissolve the sufficient amount of iodine in carbon tetra chloride until some solid remains undissolved.

Preparation of 0.1N sodium thiosulphate solution: 26 gm of sodium thiosulphate and 0.2 gm of sodium carbonate was dissolved in 1000 ml of distilled water and make up the final volume in volumetric flask.

Preparation of 0.005N sodium thiosulphate solution: 1.3 gm of sodium thiosulphate and 0.01 gm of sodium carbonate was dissolved in 1000 ml of distilled water and make up the final volume in volumetric flask.

1. By means of a graduated pipette place about 30ml and 15ml of a saturated solution of iodine in carbon tetra chloride was prepared (stock solution) and properly labeled for glass stoppered bottles.
2. To these bottles add 100 ml of distilled water and shake the bottles for 20 minutes while keeping in water bath at room temperature. Keep it aside and allow them to separate as two phases of solution.
3. Withdraw 10 ml of the organic layer from first bottle carefully and titrate against 0.1 N sodium thiosulphate using starch solution as indicator.
4. Withdraw 10 ml of the organic layer from second bottle carefully and titrate against 0.1 N sodium thiosulphate using starch solution as indicator.
5. Similarly withdraw 10 ml of the aqueous layer from first bottle carefully and titrate against 0.005 N sodium thiosulphate using starch solution as indicator.
6. Similarly withdraw 10 ml of the aqueous layer from second bottle carefully and titrate against 0.005 N sodium thiosulphate using starch solution as indicator.
7. Calculate the partition coefficient of Iodine between carbon tetra chloride and water.

Observations and Calculations:

Titration of organic layer

S. No	Container	Volume of 0.1N sodium thiosulphate consumed in ml V1	Concentration of iodine in organic layer N2= N1V1/ V2
1	Bottle 1		
2	Bottle 2		

N1= Normality of the sodium thiosulfate = 0.1N

V1= volume of the sodium thiosulfate consumed =?

V2= Volume of the organic layer = 10 ml

N2= Normality (concentration) of the iodine =?

Titration of aqueous layer

S. No	Container	Volume of 0.005N sodium thiosulphate consumed in ml V1	Concentration of iodine in aqueous layer N2= N1V1/ V2
1	Bottle 1		
2	Bottle 2		

N1= Normality of the sodium thiosulphate = 0.005N

V1= volume of the sodium thiosulphate consumed =?

V2= Volume of the aqueous layer = 10 ml

N2= Normality (concentration) of the iodine =?

For bottle 1: K = $\frac{\text{Concentration of substance in organic layer}}{\text{Concentration of substance in aqueous layer}} = \frac{C_1}{C_2}$

For bottle 2: K = $\frac{\text{Concentration of substance in organic layer}}{\text{Concentration of substance in aqueous layer}} = \frac{C_1}{C_2}$

Report: The partition coefficient of iodine between carbon tetra chloride and distilled water was found to be ---------- --.

5. DETERMINATION OF % COMPOSITION OF SODIUM CHLORIDE IN SOLUTION USING PHENOL WATER SYSTEM BY CST METHOD

Aim: To determine the % composition of sodium chloride in a solution using phenol water system by CST method.

Requirements: Phenol, distilled water, sodium chloride, thermometer, pipette, beaker, water bath and funnel.

Principle:

The temperature at which complete miscibility is reached as the temperature is raised or in some cases lowered used of two liquids that are partially miscible under ordinary conditions called also consulate temperature. The lower critical solution temperature (CST) or lower consulate temperature is the critical temperature below which the components of a mixture are miscible for all compositions. The word lower indicates that the LCST is a lower bound to a temperature interval of partial miscibility, immiscibility for certain compositions only. For example, the system triethylamine water has an LCST of 19^0 C, but not at higher temperatures. The Upper critical solution temperature or upper consulate temperature is the critical temperature above which the components of a mixture are miscible in all proportions. The word upper indicates that the UCST is an upper bound to a temperature range of partial miscibility, or miscibility for certain compositions only. For example, hexane nitrobenzene mixtures have a UCST of 19^0C, so that these two substances are miscible in all proportions above 19^0C but not at lower temperatures. When water and phenol are mixed together two layers are formed. The upper layer is solution of phenol in water. At a given temperature, composition of each solution is fixed and both solutions are in equilibrium. The two solution of different composition are existing in equilibrium with one another are known as conjugate solution. As the temperature increases, mutual solubility increases at a particular temperature this conjugate solution becomes completely miscible with one another. A temperature of which two conjugate solution are mutually soluble is called miscibility temperature. The miscibility temperature can be identifying as the disappearance of turbidity and reappearance of turbidity.

Procedure:

1. Prepare 50 ml of 1% w/v of sodium chloride in water and this stock solution is used for the preparation of different concentrations such as 0.1, 0.2, 0.4, 0.6, 0.8 and 1% v/v in the experiment.
2. Take 10 ml of stock solution each in boiling tubes and add 2 ml of phenol to each sample of stock solution.
3. Heat the mixture on water bath and note the temperature at which mixture becomes one layer in all the tubes (turbidity disappears). Note this miscibility temperature as $T_1{}^0C$.
4. Stirrer and thermometer are introduced in the sample tube. Continuously stir and observe the reappearance of turbidity of the mixture after cooling. Note this temperature at which turbidity reappears as $T_2{}^0C$.
5. Take the average of the temperature values that gives the CST of the solution. Similarly take 10 ml of the given unknown sample and add 2 ml of phenol to the sample and determine the CST of the sample.
6. Draw mutual solubility curve by plotting average miscibility temperature on Y-axis and percent composition of sodium chloride on X-axis. It will give the straight line. Using the graph read the percentage composition of unknown sample.

Observations and Calculations:

0.5 gm of sodium chloride in 50 ml gives 1% sodium chloride solution.

S. No	Sodium chloride solution [ml]	Distilled water [ml]	Percentage composition of sodium chloride	Turbidity disappears temperature [T1]	Turbidity reappears temperature [T2]	Average of temperature
1	1	9	0.1			
2	2	8	0.2			
3	4	6	0.4			
4	6	4	0.6			
5	8	2	0.8			
6	10	0	1.0			
7	unknown	Up to 10	unknown			

Report: The CST of unknown sample was found to be ----------^{0}C and the percent composition of sodium chloride in a solution (from graph) is----------%.

6. DETERMINATION OF PARTICLE SIZE AND SIZE DISTRIBUTION USING SIEVING METHOD

Aim: To determine the average particle size and size distribution using sieving method.

Requirements: Granular sample, series of sieves (No: 20, 40, 60, 80, 100 and 120), mechanical sieve shaker.

Principle:

A sieve, or sifter, is a device for separating wanted elements from unwanted material or for characterizing the particle size distribution of a sample, typically using a woven screen such as a perforated mesh or metal. The particles sufficiently small will pass through and those that are over size retained on the sieve. A sieve is will classify the particles as less than dimension of mesh (under size) and more than the dimension of mesh (over size) A Sieving method (or gradation test) is a procedure used to assess the particle size distribution (also called *gradation*) of a granular material by allowing the material to pass through a series of sieves of progressively smaller mesh size and weighing the amount of material that is stopped by each sieve as a fraction of the whole mass. In this experiment powder sample is passed through a set of sieves arranged with descending aperture size (coarsest sieve at the top) the weight remained on each sieve quantifies the particle size.

Procedure:

1. Arrange the sieves on the sieve shaker as larger aperture size on the top followed by smaller aperture size at the bottom.
2. Weigh accurately 100 g of the supplied powder, then place on the top sieve of the stack of sieves, cover and shake (mechanically) for 20 minutes.
3. Weigh the remaining powder on each sieve.
4. Enter the data of results in the table
5. Plot a graph between mean size of aperture on x-axis and percent weight retained on y-axis that gives the size distribution of particles.

Observations and Calculations:

S. No	Sieve number Passed and retained	Mean of aperture size (µm) d	Weight retained on sieve (gm) (frequency) n	% weight retained	Cumulative percent retained	weight size (n x d)
1	10/ 20					
2	20/40					
3	40/60					
4	60/80					
5	80/100					
6	100/120					
			Σ n =			Σ n d =

The average diameter of the particles is given by $D = \frac{\Sigma n d}{\Sigma n} =$

Sieve no (I P)	Aperture size (µm)
10	1700
20	840
30	500
40	420
50	300
60	250
70	210
80	180
100	150
120	125

Report: The average diameter of the particles of the sample ---------- µm

7. DETERMINATION OF PARTICLE SIZE AND SIZE DISTRIBUTION USING MICROSCOPY METHOD

Aim: To determine the particle size distribution of globule in emulsion by microscopy method.

Requirements: Microscope, glass slide, cover slips, Talc, starch, liquid paraffin

Principle:

The size of globules in an emulsion can be measured by microscopy method using an eyepiece micrometer. Eye piece micrometer has a small scale on it. The scale has to be calibrated using a stage micrometer. Stage micrometer is a glass slide having a scale on it. The scale is **1 mm** in length, and is divided into 100 parts.

The smallest division on the stage micrometer is 0.01 mm or **10 µm** in length. The exact value of each division on the eyepiece micrometer varies with every optical combination. Hence it should be calibrated with the stage micrometer for every optical combination.

If we want to measure the globule size under 45 x magnification, calibration of eyepiece micrometer should be done at 45 x magnification. If globule size is measured under 10 x magnification, calibration is to be done under 10 x. In the present experiment the calibration of eyepiece micrometer is done under 45 x magnification.

Emulsion is a heterogeneous system containing two immiscible liquids one dispersed in another. An ideal emulsion should have small globules, which are almost uniform in size. The smaller the globule size, the better is the stability of the emulsion. One of the methods for evaluating the stability of emulsions is globule size determination by microscopy technique.

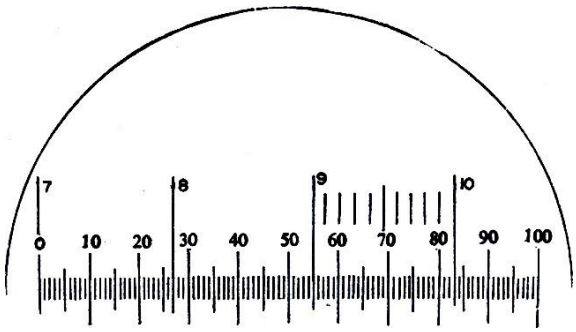
A calibrated eyepiece micrometer is used to measure the globule size in an emulsion. Microscopy method can be used to measure globules in the size range of 0.2 to 100 µm. In the present experiment, Agarose emulsion is suitably diluted with distilled water and the size of 100 globules is measured under 45 x magnification.

Average globule size and standard deviation of the globule size are calculated. A low standard deviation in globule size indicates that the globules are uniform in size.

Procedure:

Calibration of eyepiece micrometer

1. Replace the eyepiece of the microscope with the eyepiece micrometer.
2. Place the stage micrometer on the stage of the microscope and focus the scale under 10 x using coarse adjustment and fine adjustment knobs.
3. Make necessary adjustments so that the two scales are superimposed over one another.
4. Now rotate the nose piece of the microscope and focus the scale under 45 x magnification using fine adjustment knob only. Make necessary adjustments so that the two scales are parallel and superimposed over each other. Adjust the light condenser so that two scales are visible clearly.
5. Search for points of coincidence where the eyepiece division and stage division coincide perfectly. Let them be X and Y.
6. Find the number of divisions between X and Y on the stage and eyepiece micrometer.
7. From the distance between X and Y on stage micrometer and number of divisions between X and Y on eyepiece micrometer find the length of each division on the eyepiece micrometer.
8. The below figure shows the eyepiece micrometer and stage micrometer under 45 x magnification. The shorter lines of the numbered scale are the lines of the eye piece micrometer and the large lines are the lines of the stage micrometer.



9. In the above figure the 55th division of eyepiece micrometer coincides with 9th division of the stage micrometer (point X). Another point of coincidence is the 83rd division of the eyepiece micrometer and 10th division of stage micrometer (point Y). The calculations in calibration are given in the below table.

1.	Point X on eye piece micrometer	55
2.	Point Y on eye piece micrometer	83
3.	No. of eyepiece micrometer divisions between Y and X	83 – 55 = 28
4.	No. of stage micrometer divisions between X and Y	10
5.	Distance between X and Y on stage micrometer	10 X 10 µm = 100 µm
6.	28 eye piece divisions is equal to	100 µm
7.	1 eyepiece division is equal to	100/28 = 3.57 µm

So, each eyepiece division on the eyepiece micrometer is equivalent to 3.57 µm under 45 X magnification.

Measurement of globule size:

1. Dilute Agarose emulsion suitably with distilled water. Spread one or two drops of the diluted emulsion on the glass slide and place a cover slip.
2. Focus the globules under 10x magnification using coarse adjustment and fine adjustment knobs.
3. Now rotate the nose piece of the microscope and focus the globules under 45 X magnification, using fine adjustment knob only. Make adjustments in the light condenser so that the globules and eyepiece micrometer divisions are clearly visible.
4. Measure the size of 100 globules by counting the number of eyepiece divisions occupied by each globule.
5. Calculate the average globule size and standard deviation associated with globule size.

Observations and Calculations:

Each eyepiece division on the eyepiece micrometer is equivalent to 3.57 µm under 45 X magnification.

Table with 100 particles

S. NO	No. of globules on each eye piece	Globule count (Tally marks)	Frequency (n)
1	1		
2	2		
3	3		
4	4		
5	5		

Frequency distribution table:

S. No	Size range (µm)	Mid-Point (µm) d	Frequency n	n x d
1	1-5			
2	5-10			
3	10-15			
4	15-20			
5	20-25			
			Σ n =	Σ n d =

The projected diameter of the globules of the sample is given by formula as

$$D_P = \frac{\Sigma\, n\, d}{\Sigma\, n}$$

Report: The projected diameter of the globules of the sample---------- µm

8. DETERMINATION OF BULK DENSITY, TRUE DENSITY AND POROSITY

Aim: To determine the Bulk Density, True Density and Porosity for the given sample of powders.

Requirements: Measuring cylinder, Bulk density apparatus, specific gravity bottle, powder sample (magnesium oxide or lactose or talc)

Principle:

The bulk density denotes the total density of the material as it exists. The bulk volume includes the true volume, volume of inter particle spaces and intra particle pores. The packing is mainly responsible for bulk.

Bulk density is defined as:

$$\text{Bulk density, } \rho_b = \frac{weight\ of\ the\ powder}{bulk\ volume\ of\ the\ powder}$$

Since bulk volume includes the true volume, volume of inter particle spaces (voids) and intra particle pores, determining the volume of the powder using a measuring cylinder may be appropriate.

The true density is the density of the powder i.e., material exclusive of voids (inter particle spaces) and intra particle pores. The density is dependent on the type of atoms in a molecule, arrangement of the atoms in a molecule and the arrangement of molecules in a sample. The most common methods used in the determination of the true density are gas (helium or nitrogen) displacement and liquid displacement (mercury, organic liquid) methods. Helium and nitrogen gases obey ideal gas law at ambient temperatures and pressures. Helium penetrates the smallest pores and crevices. Therefore, helium densitometry gives a value closer to its true density. True density is given by the equation as

$$\text{True density, } \rho_t = \frac{\text{weight of the powder}}{\text{mass of displaced liquid}} \text{ x density of liquid}$$

Porosity can provide the information about the nature of powder sample whether the sample of powder is porous or nonporous. Porosity can be expressed in terms of densities as given below

$$\text{Porosity} = \frac{\text{True density - Bulk density}}{\text{True density}}$$

Procedure: For bulk density

1. Pass the required quantity of powder through a sieve no: 20. Weigh the 10 gm of powder and place in 100 ml capacity of measuring cylinder.
2. Fix the measuring cylinder to the bulk density apparatus and note the volume of the powder.
3. Finally determine the bulk density from the formula.

$$\text{Bulk density, } \rho_b = \frac{weight\ of\ the\ powder}{bulk\ volume\ of\ the\ powder}$$

Procedure: For True density

1. Take a clean, dry specific gravity bottle and weigh the empty specific gravity bottle as W1. Fill the bottle with water and keep the stopper and weigh the specific gravity bottle with water as W2. Remove water and wash the bottle with acetone.
2. Dry the bottle with the help of hot-air dryer and fill it with powder weigh it as W3. Now fill the powder in to the bottle and pour liquid to displace the voids in the sample of powder in the bottle and weigh as W4. Use the formula to determine the true density.

$$\text{True density, } \rho_t = \frac{\text{weight of the powder}}{\text{mass of displaced liquid}} \text{ x density of liquid} = \frac{(W3-W1)}{(W2-W1)-(W4-W3)} \text{ x density of liquid}$$

Procedure: For Porosity

Note down the values of true density and bulk density and substitute in the given equation.

$$\text{Porosity} = \frac{\text{True density} - \text{bulk density}}{\text{True density}}$$

Observations and calculations:

Weight the empty specific gravity bottle =W1

Weight the specific gravity bottle +water = W2

Weight of specific gravity bottle + powder = W3

Weight of specific gravity bottle + powder + water =W4

Density of water at room temperature = 0.99 g /cc.

Report: Bulk density of the powder is ---------g/cc. True density of the powder is ----- g/cc. Porosity of the powder is ------.

9. DETERMINE THE ANGLE OF REPOSE AND INFLUENCE OF LUBRICANT ON ANGLE OF REPOSE

Aim: To determine the angle of repose of given powder/granules and influence of lubricant on angle of repose.

Requirements: Powder sample (lactose), funnel, burette stand, talc, magnesium stearate.

Principle:

Angle of repose is defined as the maximum angle possible between the surface of the pile of powder and horizontal plane. The angle of repose is designated by θ and given by equation.

$$\tan \theta = h/r$$

Where h=height of the pile r =radius of the pile

The lower the angle of repose the better the flow properties. When granules are placed in the hopper and allowed to slide down onto the die for compression, it forms as pile. The angle of repose may be calculated by measuring the height of the pile and the radius of the base with ruler. During the flow through the hopper, the granules exhibit internal flow and demixing (i.e. the tendency of the powder to separate into layers of different sizes). Flow of granules is hindered on account of frictional forces. Lubricants are those substances which promote the flow of the granules or powder material by reducing the friction between the particles.

Relationship between the angle of repose and powder flow

Angle of repose	Powder flow
< 25	Excellent
25-30	Good
30-40	Passable
> 40	Very poor

Procedure:

1. Select a glass funnel, which has a round stem of 15 to 30 mm diameter with a flat edge. Fix the funnel with a clamp to the iron stand.

2. Place a 100 gm of granules into funnel while blocking the orifice of the funnel by thumb.

3. Remove the thumb and the granules flow down onto the graph paper and form a cone shaped pile.
4. Adjust the funnel clamp so that the gap between the bottom of the funnel stem and peak of the powder pile is about 3mm.
5. Repeat the steps until appropriate gap is maintained.
6. Finally pour the granules back into funnel and allow to flow. Measure the height of the pile using two rulers. Keep one ruler vertically and another horizontally to touch the peak of the pile. Then read the value on the vertical scale.
7. Record the reading this value represents height and also measure diameter and radius. Substitute the values in equation to obtain the angle of repose.
8. Repeat this procedure for two trials and take an average.
9. Add the lubricant in low concentration (1.0 g of talc or 0.2g of magnesium stearate) to the granules and mix them thoroughly.
10. Add further increments of lubricant and determine the angle of repose.
11. Repeat this procedure with further additions of 1.0 g of talc until optimum concentration is obtained

Observations and calculations:

Angle of repose without lubricant

Trials	**Height [h] cm**	**Diameter[d] cm**	**Radius[r] cm**	**h/r**	**$\theta = Tan^{-1}$ h/r [^{0}]**
I					
II					

Angle of repose = $Tan\ \theta = h/r = \theta = Tan^{-1}\ h/r$

Angle of repose with lubricant (Talc)

(Talc) gm	Height [h] cm		Diameter[d] cm		Radius[r] cm		h/r	
	Trail I	Trail II	Trail I	Trail II	Trail I	Trail II	Trail I	Trail II
0								
1								
2								
3								
4								
5								
6								

Angle of repose = $\text{Tan } \theta = h/r = \theta = \text{Tan}^{-1}\ h/r$

Report:

The angle of repose of given powder/granules is ----------

When lubricant is added, the angle of repose of the material is ----------.

As the concentration of the lubricant is increased the angle of repose of the material is increased

10. DETERMINATION OF STABILITY CONSTANT AND DONOR ACCEPTOR RATIO OF PABA – CAFFEINE COMPLEX BY SOLUBILITY METHOD

Aim: To determine the complex stability constant and donor acceptor ratio of caffeine and para amino benzoic acid (PABA) by solubility method.

Requirements: Volumetric flask, beakers, conical flasks, pipette, burette, funnel, Para amino benzoic acid, sodium hydroxide (0.025N), caffeine, phenolphthalein indicator, Whatman filter paper.

Principle:

Complex compounds are defined as those molecules in which most of the bonding structures can be described by classical theories of valency between atoms or molecules. Complexes possess some properties, which are different from those of its components. Properties such as solubility, light absorption, conductance, partitioning behavior and chemical reactivity are studied to confirm the formation of complexes. For example, para Amino Benzoic acid and caffeine form complexes in solution. This results in enhanced solubility of PABA at low concentrations of caffeine. Further increase in concentration of caffeine results in decreased solubility of PABA. Therefore, the change in the solubility profile is taken as a criterion to decide the complexation behavior. The equation for the formation of complex is

$$\text{PABA} + Caffeine \rightarrow PABA - caffeine$$

The interaction may be due to dipole-dipole force or hydrogen bonding between the polar carbonyl groups of caffeine and hydrogen atom of the acid. The secondary interaction may probably occur between the nonpolar parts of the molecules. The analysis of complexes generally involves the estimation of two parameters. These are represented by equations as

1. Stoichiometric ratio $= \frac{[\text{caffeine in complex}]}{[\text{PABA in complex}]}$
2. Complex stability constant $= \frac{[\text{PABA}-\text{caffeine }]}{[\text{PABA}][\text{ caffeine}]}$

In this method, caffeine is taken in different concentrations in a series of flasks. Excess quantity of PABA (same quantity) is added to all the flasks. These flasks are corked and agitated at a

constant temperature bath, until equilibrium is attained. The samples are filtered and saturated solution is collected and analyzed for drug content. The corresponding concentrations are substituted in equations 1 and 2.

Procedure:

Caffeine stock solution (0.1N): Weigh 1.949 gm of anhydrous caffeine and transfer into 100 ml of volumetric flask and add distilled water to make up final volume.

Para amino benzoic acid (PABA): Weigh accurately the required number of samples containing 200 mg of Para amino benzoic acid.

Preparation of 0.025 N sodium hydroxide: 1 gm of sodium hydroxide was dissolved in 1000 ml of distilled water and make up the final volume in volumetric flask.

1. Prepare various concentrations of caffeine (use 100ml conical flasks or beaker). The concentrations of caffeine are given in table. Transfer the samples of PABA into each flask containing the above caffeine solutions. Fix the flasks in a constant temperature bath and shake them for 30 minutes to attain equilibrium.
2. Filter the above solutions with Whatman filter paper and 10 ml of filtrate was taken and titrated with 0.025N sodium hydroxide using phenolphthalein indicator. Complete the titration of all samples and process the data in the table.
3. Draw a plot between concentration of caffeine on X-axis and concentration of PABA on Y-axis. Calculate the complex stability constant and donor acceptor ratio of caffeine and para amino benzoic acid (PABA) using the equations.

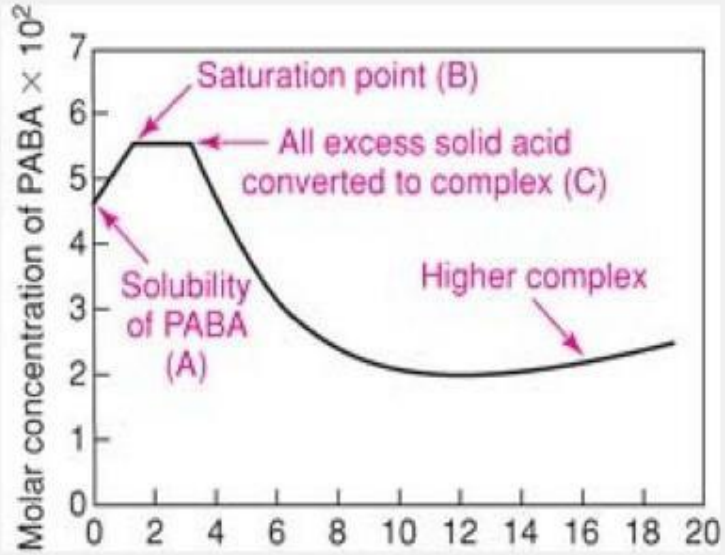
Observations and calculations:

Concentration of caffeine solution

S. No	Caffeine solution (ml)	Distilled water (ml)	Concentration of caffeine mol/liter
1	0	20	0
2	2	18	1
3	4	16	2
4	6	14	3
5	8	12	4
6	10	10	5
7	12	8	6
8	16	4	8

Analysis of complex:

S. No	Concentration of caffeine mol/liter	Volume of sodium hydroxide consumed in ml	Concentration of PABA mol/liter
1	0		
2	1		
3	2		
4	3		
5	4		
6	5		
7	6		
8	8		



The solubility of *para*-aminobenzoic acid (PABA) in the presence of caffeine.

Report: The complex stability constant of caffeine and para amino benzoic acid (PABA) is---- The donor acceptor ratio of caffeine and para amino benzoic acid (PABA) is ----------.

11. DETERMINATION OF STABILITY CONSTANT AND DONOR ACCEPTOR RATIO OF COPPER-GLYCINE COMPLEX BY pH TITRATION METHOD

Aim: To determine the complex stability constant (log β) and donor acceptor ratio (n) of Copper - Glycine complex pH by titration method.

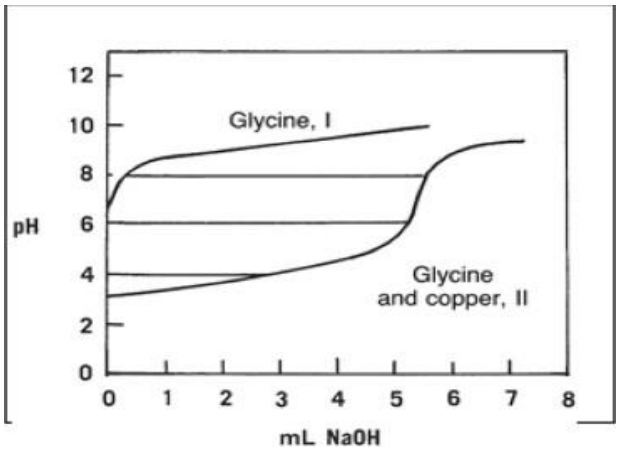
Requirements: Volumetric flask, beakers, conical flasks, pipette, burette, funnel, cupric chloride Glycine, sodium hydroxide (0.25N), phenolphthalein indicator, pH meter, buffer tablets, pH 7, 4 and 9.4.

Principle:

Complexation of copper ions with Glycine can be represented by the following equation.

$$Cu^{+2} + 2NH_3CH_2COO^- \rightarrow Cu^{+2} + (NH_2CH_2COO^{-)}{}_2 + 2H^+$$

Because of two protons are formed in the reaction of equation the addition of glycine to a solution containing cupric ions should result in a decrease in pH. Titration curves can be obtained by adding a strong base to a solution of glycine and to another solution containing glycine and a copper salt and plotting the pH against the equivalents of base added. The results of such a potentiometric titration are shown in the figure. The curve for the metal–glycine mixture is well below that for the glycine alone, and the decrease in pH shows that complexation is occurring throughout most of the neutralization range.



Titration of glycine and cupric glycine complex solution. The difference in pH for a given quantity of base (sodium hydroxide) added indicates the occurrence of a complex.

The average number of ligand groups bound per metal ion can be given by equation as

$$n = \frac{\text{Total concentration of ligands bound}}{\text{Total concentration of metal ion}}$$

The horizontal distance represents the amount of alkali added in the titration. This quantity is equals to the concentration of ligand bound to metal at any pH. The total concentration of metal ion taken initially is known. Thus, n can be calculated. The stability constant (β) and pH of free glycine are related as

$$p(A) = ½ \log \beta \text{ at } n = 1$$

p(A) can be estimated using the equation

$$p(A) = pK_a - pH - \log([HA]_{initial} - [NaOH])$$

Where pK_a is dissociation constant of glycine, (9.69)

[NaOH] is concentration of sodium hydroxide in mol/lit.

Procedure:

Preparation of 0.25N sodium hydroxide: 10 gm of sodium hydroxide was dissolved in 1000 ml of distilled water and make up the final volume in volumetric flask.

Preparation of Glycine solution (3.34×10^{-2}mol/lit): Weigh 250 mg of glycine and transfer into a 100 ml of volumetric flask, add distilled water and make up the volume.

Complex solution (Glycine – 3.34×10^{-2}mol/lit; cupric chloride –9.45×10^{-3}mol/lit): Weigh 250 mg of glycine and160 mg of cupric chloride and transfer into a 100 ml of volumetric flask, add distilled water and make up the volume. Prepare two such samples.

Kinetic method:

1. Transfer the 75 ml of glycine solution into a beaker. Measure the pH of the solution. Gradually add the 0.25N of sodium hydroxide solution to the glycine solution.
2. Transfer the 75 ml of glycine-cupric complex solution into a beaker. Measure the pH of the solution. Gradually add the 0.25N of sodium hydroxide solution to the glycine-cupric complex solution.
3. Identify the range where the sudden increase in pH is obtained in the complex solution.
4. Take another sample of 75 ml of complex solution and add 1 ml increment up to 5 ml to the complex mixture and report the data.

5. Titrate the complex solution further (note: If sudden increase in pH is observed between 5 to 6 ml. Then in the final analysis, increments of 0.2 ml of sodium hydroxide should be added, i.e., 5.0, 5.2, 5.4, 5.6, 5.8 and6.0).
6. After 6.0 ml, add 1 ml increments to the complex mixture and report the data.
7. Draw a graph between volume of sodium hydroxide added on x-axis and pH on the y-axis by using data obtained in the titration of glycine and complex solution.

Observations and calculations:

Data for analysis of complex of cupric-glycine by pH titration method

Glycine solution		**Preliminary study Complex solution**		**Final readings Complex solution**	
Volume of sodium hydroxide solution (ml)	**pH**	Volume of sodium hydroxide solution (ml)	**pH**	Volume of sodium hydroxide solution (ml)	**pH**
0		0		0	
1		1		1	
2		2		2	
3		3		3	
4		4		4	
5		5		5	
6		6		5.2	
		7		5.4	
		8		5.6	
				5.8	
				6.0	
				7.0	
				8.0	

Report: The complex stability constant (log β) of Copper - Glycine complex pH by titration method is----------. The donor acceptor ratio (n) of Copper - Glycine complex pH by titration method is ----------.