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RATHINAM COLLEGE OF PHARMACY

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PRACTICAL MANUAL

SECOND YEAR B.PHARM (III-SEMESTER)

SUBJECTS

PHARMACEUTIAL ORGANIC CHEMISTRY-II

PHYSICAL PHARMACEUTICS-I

PHARMACEUTICAL MICROBIOLOGY

PHARMACEUTICAL ENGINEERING

Name :

Course : Bachelor of Pharmacy

Semester: III semester

INDEX

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PRACTICAL MANUAL

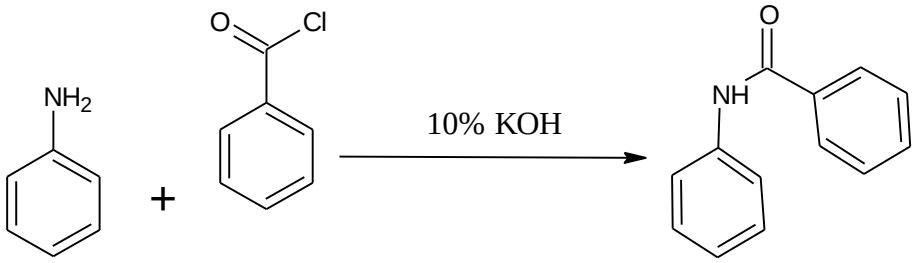
SECOND YEAR B.PHARM (III-SEMESTER)

SUBJECT: PHARMACEUTIAL ORGANIC CHEMISTRY - II (BP305P)

PREPARED BY
Dr.C.BUVANA M. Pharm,PhD.,
Professor
Department of Pharmaceutical Chemistry

CONTENTS

REACTION



aniline benzoyl chloride

Benzanilide

CALCULATION

Molecular weight of

Molecular weight of

Theoretical yield =

Practical yield =

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Expt No:

Date:

PREPARATION OF BENZANILIDE FROM ANILINE

AIM

To prepare and submit recrystallised product of benzanilide and find its percentage yield.

REFERENCE

1. Advanced practical organic chemistry by O.P.Agarwal, page no. 305.
2. Vogel's text book of practical organic chemistry 2nd edition, page.no. 1049

REQUIREMENTS

Aniline, Benzoyl chloride, 10% sodium hydroxide, conical flask, beaker, round bottomed flask, funnel, tripot stand

PRINCIPLE

Benzanilide or N-phenylbenzamide is a simple aromatic amide chemical formula ($C_{13}H_{11}NO$) It is prepared by schotten bauman reaction. In the Schotten-Baumann method of benzoylation, the hydroxyl or amino compound (or a salt of the latter) is either suspended or dissolved in an excess of freshly prepared 10% (w/v) aqueous sodium hydroxide solution, together with a small excess of benzoyl chloride (i.e., nearly 10% more than the theoretical quantity), and the resulting mixture is shaken vigorously in ambient conditions. It has been observed that under these experimental parameters 'benzoylation' proceeds smoothly. Thus, the solid benzoylated product, which being insoluble in the aqueous medium, gets separated briskly. Simultaneously, the NaOH solution hydrolyses the excess of benzoyl chloride present in reaction mixture, thereby resulting into the formation of sodium chloride and sodium benzoate, which being watersoluble remain in solution.

PROCEDURE

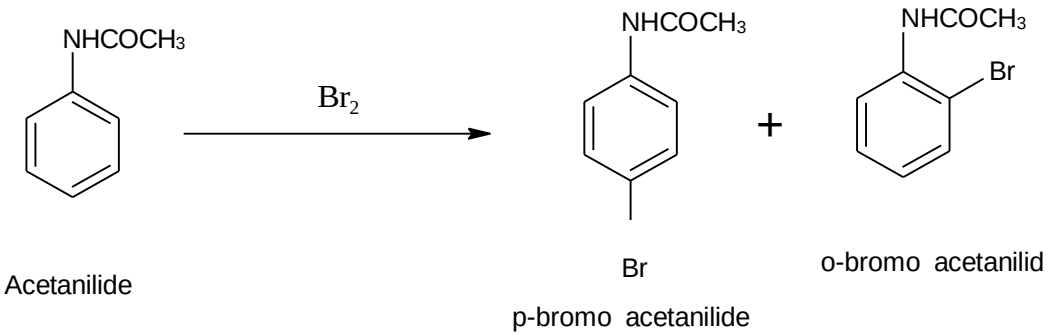
1. In a clean & dry conical flask dissolve (2ml) of aniline in (20ml) of (10% NaOH).
2. Add (3ml) of benzoyl chloride to the solution.
3. Enclose the conical flask then shake the solution to about (12) minutes.
4. Separate the produced benzanilide from the solution by filtration process.
5. Wash the precipitate with distilled water several times, each time (10ml) D.W.

REPORT

Benzanilide was prepared from aniline and submitted

1. The theoretical yield of benzanilide =
2. The Practical yield of benzanilide =
3. The yield of benzanilide was found to be _____

REACTION



Acetanilide

p-bromo acetanilide

o-bromo acetanilid

CALCULATION

Molecular weight of

Molecular weight of

Theoretical yield =

Practical yield =

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Expt.No:

Date:

PREPARATION OF P-BROMO ACETANILIDE FROM ACETANILIDE

AIM

To prepare and submit the recrystallised product of p-bromo acetanilide from acetanilide and find its percentage yield.

REFERENCE

1. Practical Pharmaceutical Chemistry Anees Ahamed Siddiqui and Mohammed Ali. Page no.:137

REQUIREMENTS

Acetanilide , Bromine , Glacial Acetic acid, Sodium bisulphite, Rectified spirit, conical flask, beaker, round bottomed flask, funnel, tripot stand.

PRINCIPLE

Acetanilide (i.e., the acetyl derivative of aniline) on being subjected to bromination (with Br2) in a medium of glacial acetic acid gives rise to the para-bromoacetanilide with the liberation of a mole of HBr. The acetamido (—$NHCOCH_3$) is an ortho, para director function ; hence, the incoming bromo moiety shall yield both ortho- and para-isomers. The para-isomers is produced predominantly (upto 90%) as a white solid.

PROCEDURE

1. Dissolve acetanilide (13.5g) in glacial acetic acid (45ml) in 250 ml conical flask.
2. Add dropwise by burette, bromine (5.5ml) dissolved in glacial acetic acid (25ml) with constant shaking. (Precaution: reaction should be carried out in fuming cupboard)
3. Allow to stand the orange coloured reaction mixture at room temperature for half an hour and then pour the contents into cold water.
4. Stir well and add sufficient sodium bisulphite to discharge orange colour which is due to slight brown colour of bromine.
5. Filter the crude product, wash with cold water and recrystallise with dilute alcohol to obtain white crystalline compound, m.p.167^o, yield 18g.

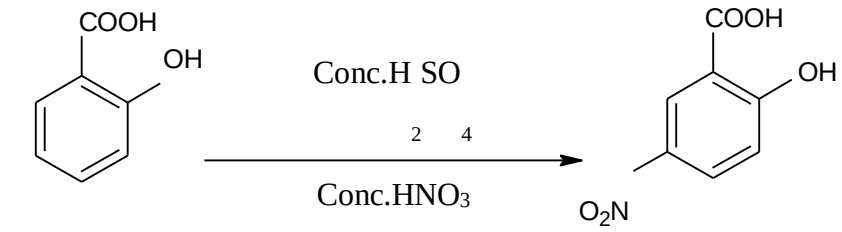
   Note: o-bromoacetanilide remains in the alcoholic solution during crystallisation due to its high solubility.

REPORT

p-bromo acetanilide was prepared from acetanilide and submitted

1. The theoretical yield of p-bromo acetanilide =
2. The Practical yield of p-bromo acetanilide =
3. The yield of p-bromo acetanilide was found to be _____

REACTION



Salicylic acid 5-nitro salicylic aci

CALCULATION

Molecular weight of Salicylic acid

Molecular weight of 5-nitro salicylic acid

Theoretical yield =

Practical yield =

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Expt No:

Date:

PREPARATION OF 5-NITROSALICYLIC ACID

AIM

To prepare and submit the recrystallised product of 5-nitro salicylic acid from salicylic acid.

REQUIREMENTS

Salicylic acid, glacial acetic acid or acetic acid, Conc.HNO_3, Conc.H_2SO_4, conical flask, beaker, round bottomed flask, funnel, tripot stand.

PRINCIPLE

Salicylic acid undergoes nitration reaction gives 5 nitro salicylic acid. The nitrating reagent used in Conc. Nitric acid and Conc.Sulphuric acid. The reaction is an example of electrophilic aromatic substitution reaction. There are a lot of acids in this reaction, which means that each one acid must act as the "base." Sulfuric acid is the stronger acid, so it gets to act as the "acid" and therefore, nitric acid will acid as the "base" because it is the weaker of the two acids. The nitration reaction has a rate determining step that is the attack of the electrophile.

In this experiment, electrophilic aromatic substitution was used. This reaction involved an electrophile, which is low in electrons seeking an electron rich compound (which in this case is an aromatic compound) to add to. The electrophile was the Nitronium Ion (Nitric acid + sulfuric acid).

PROCEDURE

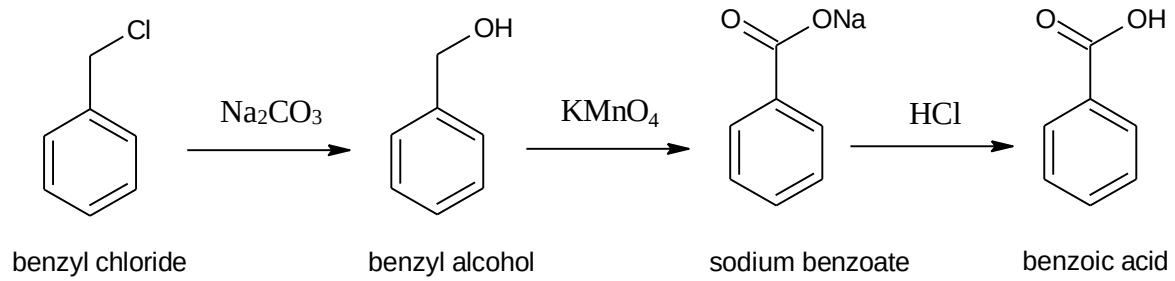
1. Take 2.5g of salicylic acid in conical flask and 2.5ml of glacial acetic acid.
2. To this 5ml of Conc.H_2SO_4 is added and the mixture kept in ice bath and the temperature is maintained at 10ºC 1.5ml of Conc.HNO_3 is added drop wise with constant stirring.
3. The reaction mixture is kept at room temperature at 30min.
4. Then it is poured into ice water mixture.
5. The separated product is filtered and dried.

REPORT

5-nitro salicylic acid was prepared from nitrobenzene and submitted

1. The theoretical yield of 5-nitro salicylic acid =
2. The Practical yield of 5-nitro salicylic acid =
3. The yield of 5-nitro salicylic acid was found to be _____

REACTION



benzyl chloride benzyl alcohol sodium benzoate benzoic acid

CALCULATION

Molecular weight of

Molecular weight of

Theoretical yield =

Practical yield =

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield=}} \times 100$$

Expt. No:

Date:

PREPARATION OF BENZOIC ACID FROM BENZYL CHLORIDE

AIM

To prepare and submit recrystallised product of benzoic acid from benzyl chloride and find out the percentage yield.

REFERENCE

Practical organic chemistry by F.G Mann and B.C Saunders page No:239

REQUIREMENTS

Benzyl chloride, Anhydrous sodium carbonate, Potassium permanganate, Sodium sulphite, Round bottom flask (RBF), beaker, Measuring cylinder, conical flask, funnel, tripot stand.

PRINCIPLE

Benzoic acid having the molecular formula $C_7H_6O_2$. It is an oxidation reaction. If oxidation occurs to an aromatic compound having an aliphatic sie chain then, fission of the side chain occurs between first and second carbon atom from the benzene ring and first carbon atom thus becoming part of a carboxyl (-COOH) group.

The oxidation process is carried out with a mixture of potassium permanganate and sodium carbonate in aqueous solution or with dilute nitric acid. The reaction is quite slow if the side chain is a simply alkyl group. The side chain containing chlorinated alkyl group is more susceptible to oxidation. Hence in comparison to toluene, benzyl chloride more rapidly oxidizes in presence of an aqueous oxidising agent. Here benzyl chloride is first hydrolysed to benzyl alcohol and then undergoes oxidation of a primary alcohol to the corresponding carboxylic acid.

PROCEDURE

1. About 2 ml of benzyl chloride is added to a solution of about 2 grams of anhydrous sodium carbonate dissolved in 20 ml of distilled water. The mixture is taken in a round bottom flask.
2. The round bottom flask is fitted with a water reflux condenser and heated.
3. 4 grams of potassium permanganate in 80 ml of water is added in small quantities through the water condenser until a permanent pink color persists even after continuous boiling.

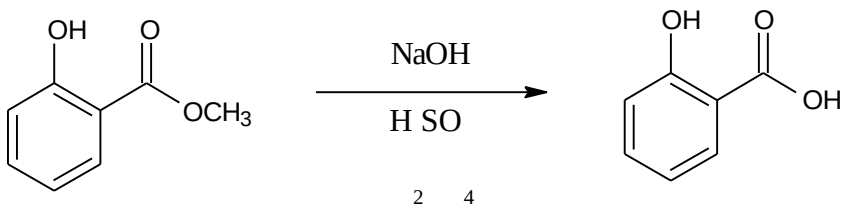
4. boiled for about 1 hour.
5. The mixture is transferred to a beaker.
6. About 4 grams of sodium sulfite are added to this mixture.
7. Now add concentrated hydrochloric acid to this solution until the solution is acidic.
8. The solution is cooled, precipitated benzoic acid is filtered and washed.
9. The acid is recrystallized from boiling water.

REPORT

Benzoic acid was prepared from benzyl chloride and submitted

1. The theoretical yield of benzoic acid =
2. The Practical yield of benzoic acid =
3. The yield of benzoic acid was found to be _____

REACTION



methyl salicylate Salicylic acid

CALCULATION

Molecular weight of methyl salicylate

Molecular weight of salicylic acid

Theoretical yield =

Practical yield =

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Expt. No:

Date:

PREPARATION OF SALICYLIC ACID FROM METHYL SALICYLATE

AIM

To prepare and submit the recrystallised product of salicylic acid from methyl salicylate.

REQUIREMENTS

Methyl salicylate, 10% of sodium hydroxide,dilute sulphuric acid and porcelain pieces. Funnel, measuring cylinder, beaker, iodine flask, glass rod.

PRINCIPLE

Oil of wintergreen (methyl salicylate), is organic ester. Salicylic acid is prepared from methyl salicylate. It is a hydrolysis reaction. Methyl salicylate is an ester easily recognized by its odor and is known as oil of wintergreen because of its natural source. This ester will be treated with aqueous base. The preparation involves the initial hydrolysis of methyl salicylate to give sodium salt of salicylic acid. This sodium salt of salicylic acid is acidified with dilute sulphuric acid gives salicylic acid.

PROCEDURE

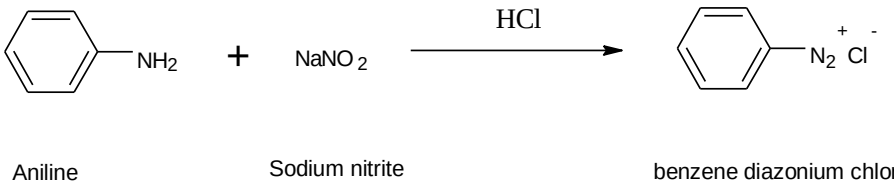
1. Take 5ml of methyl salicylate in an iodine flask
2. Add 4ml of 10% NaOH solution to the above iodine flask.
3. Add some porcelain pieces to it.
4. Heat gently for 40 minutes over water bath.
5. Cool the above solution
6. Add dilute sulphuric acid till it is acidic
7. Crude salicylic acid gets precipitated.
8. Collect the crude product by filtration and
9. Recrystallised from hot water.

REPORT

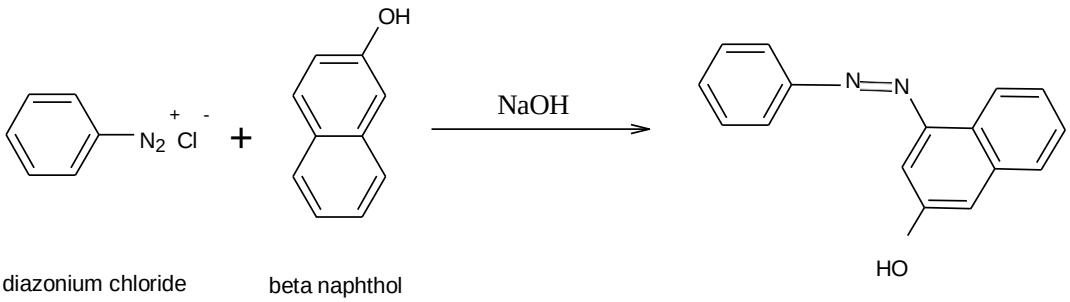
Salicylic acid was prepared from methyl salicylate and submitted

1. The Theoretical yield of salicylic acid =
2. The Practical yield of salicylic acid =
3. The yield of salicylic acid was found to be _____

REACTION



Aniline Sodium nitrite benzene diazonium chloride



benzene diazonium chloride beta naphthol

phenyl azo beta naphol

CALCULATION

Molecular weight of

Molecular weight of

Theoretical yield =

Practical yield =

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Expt.No:

Date:

PREPARATION OF PHENYL AZO β NAPTHOL FROM ANILINE

AIM

To prepare and submit the recrystallised product of phenyl azo β napthol from Aniline and find its percentage yield.

REFERENCE

Vogel's text book of practical organic chemistry 2nd edition, page.no. 948.

REQUIREMENTS

Aniline, Con.HCl, sodium nitrite solution, alkaline beta napthol, funnel, measuring cylinder, beaker, iodine flask, glass rod.

PRINCIPLE

Azo compounds are prepared by the interaction of the diazonium salt with phenol in the presence of NaOH or with 1^0 amine in the presence of sodium acetate. The coupling reaction is an electrophilic substitution react ion involved in the diazonium ion which react at the position of greater electron availability i.e, the position of ortho or para to electron releasing phenoxy or amino groups. 2-napthol couples in the more reactive at the 1st position as in the mode of synthesis of phenyl azo beta napthol. The diazotization reaction is bringing into the solution by introducing the required quantity of sodium nitrate and pouring onto a mixture of Hcl and ice. Nitrous acid is liberated, immediately react and separates the internal disodium salts from solution. This disodium salt coupling with 2-napthol in presence of NaOH solution yields the dye stuff.

PROCEDURE

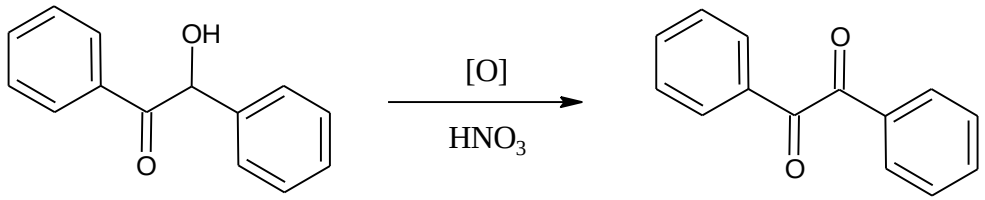
1. Dissolve 2ml of aniline in a mixture of 5ml of HCl and 10ml of H_2O in beaker.
2. Cool the solution at 5^0c
3. Diazotized by the addition of 10ml of 20% sodium nitrite solution.
4. To the nitrite solution slowly add 30 ml, 12% alkaline beta naphthol solution maintained at 0^0c by the direct addition of crushed ice.
5. Allow the mixture to stand in an ice bath for 20 minutes. Filter the above solution and wash thoroughly with water,
6. Dry and weigh the product.
7. Recrystallised from Ethanol

REPORT

Salicylic acid was prepared from methyl salicylate and submitted

1. The Theoretical yield of salicylic acid =
2. The Practical yield of salicylic acid =
3. The yield of salicylic acid was found to be _____

REACTION



Benzoin Benzil

CALCULATION

Molecular weight of

Molecular weight of

Theoretical yield =

Practical yield =

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Expt.No:

Date:

PREPARATION OF BENZIL FROM BENZOIN

AIM

To prepare and submit the rerystallised product of benzyl from benzoin and find out the percentage yield.

REFERENCE

Vogels textbook of Practical organic chemistry, V edition, Page No.:1046. Round bottom flask, water bath and measuring cylinder.

REQUIREMENTS:

Benzoin, Concentrated nitric acid, ethanol, rectified spirit, o-phenylene diamine, Round bottom flask, water bath and measuring cylinder.

PRINCIPLE

Benzoin (organic compound), an organic compound with the formula PhCH(OH)C(O)Ph. It appears as off-white crystals, with a light camphor-like odor. Benzoin is synthesized from benzaldehyde in the benzoin condensation. Benzoin on oxidation gives diaryl ketone called benzil. The Secondary alcoholic group of benzoin on treatment with concentrated nitric acid to form a diketone molecule. Nitration of aromatic ring is not occurring as sulphuric acid is totally absent in the whole process.

PROCEDURE:

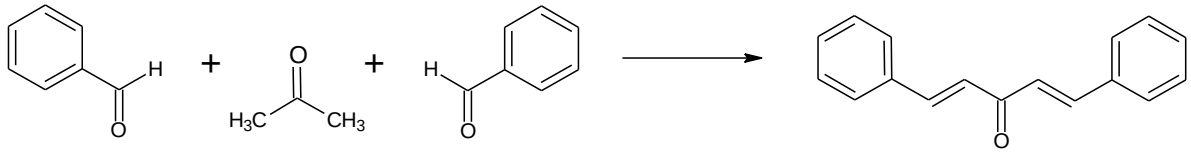
1. Place 5gm of crude benzoin and 25ml of concentrated nitric acid in a 250 ml round bottom flask
2. Heated on a boiling water bath with occasional shaking until the evolution of nitrogen leased (about 1.5 hours).
3. Pour the reaction mixture into 300-400ml of cold water containing in the beaker.
4. Stir well until the oil is crystallized as a yellow solid.
5. Filter the crude benzil and wash thoroughly with water to remove nitric acid.
6. Recrystallized from ethanol or rectified spirit.
7. Melting point – 94-96^{0}C.

REPORT

Benzil was prepared from benzoin and submitted

1. The Theoretical yield of Benzil =
2. The Practical yield of Benzil =
3. The yield of Benzil was found to be _____

REACTION



benzaldehyde acetone benzaldehyde Dibenzal acetone / Dibenzilidine acetone

CALCULATION

Molecular weight of

Molecular weight of

Theoretical yield =

Practical yield =

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Expt. No:

Date:

PREPARATION OF DIBENZYL ACETONE

AIM

To prepare and submit the recristallised product of dibenzilidine acetone from benzaldehyde.

REFERENCE

Practical organic chemistry 4th edition by F. G. Mahn and B. C. Sounders page no 231.

REQUIREMENTS

Benzoin, Concentrated nitric acid, ethanol, rectified spirit, o-phenylene diamine, Round bottom flask, water bath and measuring cylinder.

PRINCIPLE

This synthesis in an example of condensation reactions when an ethanolic solution containing both acetone and two equivalent of benzaldehyde is made alkaline with sodium hydroxide rapid condensation occur with the formation of dibenzyl acetone or dibenzilidine acetone. This is a particularly example of claisen reactions. In this reaction it is probably that an intermediate hydroxide compound is formed and water is lost from the unstable group. Claisen shown that aldehyde under the influence of sodium hydroxide will condense with another aldehyde or a ketone with the elimination of water.

PROCEDURE

1. Dissolve 1ml of benzaldehyde and 0.4ml of pure acetone in 10ml of methylated spirit contained in a conical flask or wide mouthed bottle.
2. Dilute 2ml of 10% aqueous sodium hydroxide solution with 8ml of water and add this dilute alkali solution to the former solution.
3. Shake the mixture vigorously for about 10min (releasing the pressure from time to time).

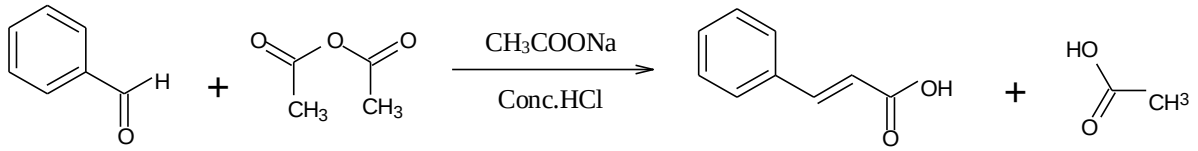
4. Allow to stand for 30mnts.
5. Finally cool in ice water for a few minutes.
6. During shaking the dibenzyl acetone.
7. Separates at first as a fine emulsion which rapidly from pale yellow crystals. Recrystallized from hot methylated spirit or rectified spirit.

REPORT

Dibenzyl acetone was prepared and submitted from benzaldehyde

1. The theoretical yield of dibenzyl acetone =
2. The practical yield of dibenzyl acetone =
3. The yield of dibenzyl acetone was found to be_____

REACTION



benzaldehyde acetic anhydride Cinnamic acid acetic acid

CALCULATION

Molecular weight of

Molecular weight of

Theoretical yield =

Practical yield =

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Expt. No:

Date:

PREPARATION OF CINNAMIC ACID FROM BENZALDEHYDE

AIM

To prepare and submit the recrystallised product of cinnamic acid from benzaldehyde

REFERENCE

Practical organic chemistry 4th edition by F. G. Mahn and B. C. Sounders page no 231.

REQUIREMENTS

Benzoin, Concentrated nitric acid, ethanol, rectified spirit, o-phenylene diamine, benzaldehyde, acetic anhydride, sodium acetate , Round bottom flask, water bath and measuring cylinder.

PRINCIPLE

Cinnamic acid together with cinnamaldehyde are natural products from cinnamon oil. In this experiment, cinnamic acid is synthesized by the Perkin condensation reaction, which involves the reaction between an acid anhydride (acetic anhydride) and an aromatic aldehyde (benzaldehyde) catalyzed by a base (sodium acetate). The anhydride generates a carbanion due to the influence of the base (sodium acetate), which attacks the carbonyl group of the aldehyde. Then the anhydride group undergoes a process of dehydration and hydrolysis.

PROCEDURE

1. In a 250 ml round-bottom flask provided with a reflux condenser and a drying tube, put 5 g of benzaldehyde 7.5 g of acetic anhydride and 2.5 g of sodium acetate (anhydride), and heat to reflux for 3 h.
2. Allow to cool to r.t. Cool the reaction crude, and add 100 ml of water.
3. Then perform a steam distillation (internal vapor source) until all the unreacted benzaldehyde (one volume of approximately 75 ml of distillate) separates; then discard.
4. Vacuum filter the remaining residue to remove resinous solids that have been formed.
5. The filtrate is acidified by slowly adding concentrated HCl.

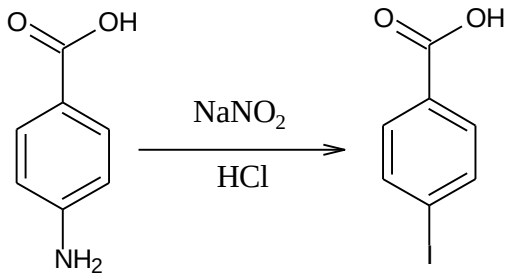
6. Cool in an ice bath and isolate the resulting solid by filtration.
7. Recrystallize from water; then dry, weigh, and determine the yield

REPORT

Cinnamic acid was prepared and submitted from benzaldehyde

1. The theoretical yield of Cinnamic acid =
2. The practical yield of Cinnamic acid =
3. The yield of Cinnamic acid was found to be_____

REACTION



4-aminobenzoic acid 4-iodobenzoic acid

CALCULATION

Molecular weight of

Molecular weight of

Theoretical yield =

Practical yield =

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Expt No:

Date:

PREPARATION OF P-IODO BENZOIC ACID FROM P-AMINO BENZOIC ACID

AIM

To prepare and submit the recrystallised product of p-iodo benzoic acid from p-amino benzoic acid and find out the percentage yield.

MATERIALS REQUIRED

p-amino benzoic acid, sodium nitrite, potassium iodide, conc.HCl, testube, beaker, glass rod.

PRINCIPLE

p-iodo benzoic acid is prepared from p-amino benzoic acid in presence of KI and solution of Sodium nitrite in HCl. 4-Iodobenzoic acid, or *p*-iodobenzoic acid, is an organic compound with the formula IC_6H_4COOH. The synthesis of 4-iodobenzoic acid *via* the diazotization of p-amino benzoic acid. 4-Iodobenzoic acid can be synthesized *via* a Sandmeyer reaction consisting of the diazotization of p-amino benzoic acid followed by a diazo replacement. **Sandmeyer reaction** is an organic reaction used to convert an aryl diazonium salt to an aryl halide. First p-amino benzoic acid is treated with nitrous acid in order to convert the amino group into the diazo group. The diazo group is ejected, yielding a carbocation which is then attacked by highly nucleophilic I^- anion.

PROCEDURE

1) Quantitatively transfer the given sample of solid 4-aminobenzoic acid into a 100 mL beaker placed in the ice-bath.
2) Add 7.2 mL of Conc.HCl and mix the contents thoroughly for 1 minute with the help of a glass rod. Cool the solution for 5 minutes.
3) Add 4.4 mL of cooled $NaNO_2$ solution taken in a test tube to the acid solution with constant gentle stirring using a glass rod to obtain an almost clear solution (3-5 minutes).

4) Remove the beaker from the ice bath and then slowly add 9.4 mL of KI with stirring.

5) Keep the beaker in hot water for 5 minutes.

6) Filter the crude product and wash it thoroughly with distilled water (10 mL). Collect the washings along with the main filtrate.

REPORT

p-iodo benzoic acid was prepared and submitted from p-amino benzoic acid

1. The theoretical yield of p-iodo benzoic acid =
2. The practical yield of p-iodo benzoic acid =
3. The yield of p-iodo benzoic acid was found to be_____

CALCULATION (STANDARDIZATION)

Weighing details of oxalic acid

Weight of bottle	Weight in (gm)	Weight in (mg)	Rider position	Rider value	Total weight (gm)
Weight of empty weighing bottle (W_1)					
Total					
Weight of bottle + substance (W_2)					
Total					

Actual weight of oxalicacid =…….

Standardization of 0.1N potassium hydroxide

Oxalic acid vs 0.1N potassium hydroxide

Contents of conical flask	Burette reading (ml)		Volume of 0.1 N KOH consumed (ml)	Indicator	End point
	Initial	Final			

Normality $= N_1V_1 = N_2V_2$

Normality of .....N_1=

Volume of .....,V1=

Volume of ……..., V_2=

Normality of ....., $N_2 = \frac{N_1V_1}{V_2}$

Expt No:

Date:

DETERMINATION OF ACID VALUE

AIM

To determine the acid value of the given oil.

REFERENCE

1. Pharmaceutical titrimetric analysis theory and practical by A.A.Napolean, page no. 10.9.
2. Text book of organic chemistry, natural products OP Agarval, volume II, page number 334-338.

REQUIREMENTS

Oil (Cator oil), 0.1N KOH solution, Phenolphthalein, Iodine flask, Burette, Pipette, beaker.

PRINCIPLE

Acid value is the number of milligrams of potassium hydroxide required to neutralize the free fatty acid present in 1gm of fat or oil. Oils are made of esters of fatty acid with glycerol, called triglyceride, along with free fatty acid. Acid value determines the free fatty acid present in the sample. Acid value determines the freshness of oil. Fresh oil constitutes less acid value compared to rancid oil. Rancidity liberates more fatty acid from triglycerides, which constitutes higher acid value. The standard acid value of castor oil was found to be not more than two.

RCOOR' + KOH RCOOK +R'OH

In this method a known amount of oil is dissolved in a mixture of previously neutralized ether and alcohol and titrated against standard potassium hydroxide using phenolphthalein as indicator. The end point is the colour change is from colorless to pale permanent pink colour.

Acid value can be calculate from the equation,

$$\text{Acid value} = \frac{\text{N* 5.61*Actual normality of KOH}}{\text{Weight taken * given normality of KOH}}$$

N is the number of ml of potassium hydroxide consumed during the titration

Weighing details of oil

Weight of bottle	Weight in (gm)	Weight in (mg)	Rider position	Rider value	Total weight (gm)
Weight of empty weighing bottle (W_1)					
Total					
Weight of bottle + substance (W_2)					
Total					

Actual weight of oil =

Determination of Acid value

Oil vs 0.1N potassium hydroxide

Contents of conical flask	Burette reading (ml) Initial	Final	Volume of 0.1 N KOH consumed (ml)	Indicator	End point

Acid value = $\frac{\text{N x 5.61 x Actual normality of KOH}}{\text{Weight taken x given normality of KOH}}$

PROCEDURE

Standardization of 0.1N potassium hydroxide

1) Weigh accurately about 0.63gm of oxalic acid, dissolve and make up to 100 ml with distilled water
2) Pipette out 20 ml of the above solution into a conical flask
3) Titrate against standard 0.1N potassium hydroxide using phenolphthalein as indicator.
4) The end point is the colour change is from colorless to permanent pale pink colour.

Determination of acid value

1) Weigh 1 gm of oil and transfer it into 250 ml iodine flask.
2) Add a 5ml mixture of equal quantities of alcohol and ether previously neutralized with 0.1N potassium hydroxide using phenolphthalein as indicator.
3) If the sample is not dissolved in cold solvent connect the flask with reflux condenser and warm slowly until sample dissolve.
4) Add 1 or 2 drops of phenolphthalein indicator.
5) Titrate this against 0.1N KOH solution.
6) The appearance of pale pink colour indicates the end point.

REPORT

1. The actual normality of KOH was found to be ----------
2. The acid value of given sample of oil was found to be ----------

CALCULATION

Weighing details of Sodium carbonate

Weight of bottle	**Weight in (gm)**	**Weight in (mg)**	**Rider position**	**Rider value**	**Total weight (gm)**
Weight of empty weighing bottle (W_1)					
Total					
Weight of bottle + substance (W_2)					
Total					

Actual weight of substance =

Standardization of 0.5 N Hydrochloric acid (back titration)

Sodium carbonate Vs 0.5 N HCl

Contents of conical flask	**Burette reading (ml)**		**Volume of 0.5 N HCl consumed (ml)**	**Indicator**	**End point**
	Initial	**Final**			

Normality of sodium carbonate, N_1=weight taken*expected normality

Weight to be taken

V1, volume of sodium carbonate =

V_2, volume of HCl=

Normality of KOH, $N_2 = \frac{N_1V_1}{V_2}$

Expt No:

Date:

DETERMINATION OF SAPONIFICATION VALUE

AIM

To determine the Saponification value of the given sample of oil.

REFERENCE

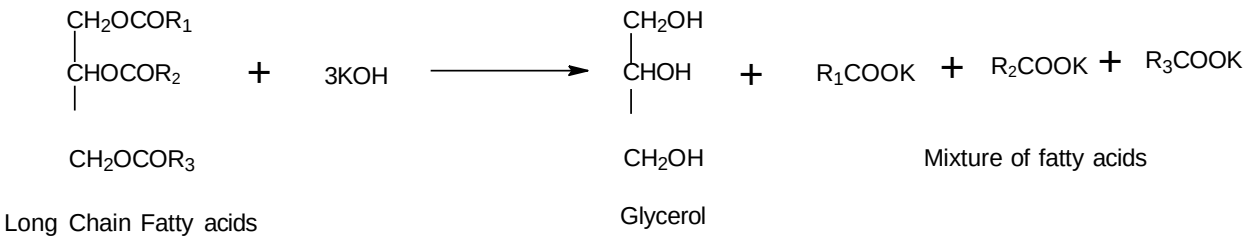
1. Pharmaceutical titrimetric analysis theory and practical by A.A.Napolean, page no. 10.14-10.15.
2. Text book of Organic Chemistry, Natural Products O P Agarval, volume II, page number 336-338.

PRINCIPLE

Saponification value is the value is the number of milligrams of potassium hydroxide required to neutralize the fatty acid and to saponify the ester present in 1gm of fat or oil . The known amount of sample is refluxed with excess of alcoholic potassium hydroxide, during the ester get hydrolyzed to glycerol and esters of higher fatty acids (SOAP).The unreacted potassium hydroxide is back titrated using a standard acid.. A blank titration should be done to know the amount of alkali reacted with oil. Since each molecule of fat requires three molecules of KOH to saponify it, it is evident that the saponification value indicates the number of fat molecule per gram of fat, i.e. a low saponification value indicates the oil or fat is made of higher fatty acidsand is having high molecular weight.

It is used to detect the adulteration of fat or oil by one of the higher or lower saponification value.

$$Na_2CO_3 + 2HCl \rightarrow H_2CO_3 + 2NaCl$$



Weighing details of Oil

Weight of bottle	**Weight in (gm)**	**Weight in (mg)**	**Rider position**	**Rider value**	**Total weight (gm)**
Weight of empty weighing bottle (W_1)					
Total					
Weight of bottle + substance (W_2)					
Total					

Actual weight of oil =

Oil vs 0.5 N Hydrochloric acid

Contents of conical flask	**Burette reading (ml)**		**Volume of 0.5 N HCl consumed (ml)**	**Indicator**	**End point**
	Initial	**Final**			

Saponification value = $\frac{28.05 * (b-a) * \text{actual normality of HCl}}{\text{Weight taken} * \text{given normality of HCl}}$

PROCEDURE

Standardization of 0.5N Hydrochloric acid

1. Weigh accurately about 2.65gm of anhydrous sodium carbonate, dissolve and made up to 100 ml with distilled water.
2. Pipette out 20 ml of the above solution in to a conical flask
3. Titrate against standard 0.5N hydrochloric acid using methyl red as indicator.
4. The end point is the colour change is from yellow to pale permanent pink colour.
5. Calculate the normality of hydrochloric acid.
6. Using the blank reading calculate the normality of given potassium hydroxide solution.

Determination of saponification value

1. Accurately weigh about 2gms of oil in a 250 ml round bottom flask fitted with a reflux condenser
2. Add 25 ml of 0.5N alcoholic potassium hydroxide
3. Boil under reflux in a water bath for 30 minutes, frequently rotating the condenser.
4. Add 1ml of phenolphthalein and titrate with standard 0.5N hydrochloric acid.
5. Note the volume of hydrochloric acid 'a' ml.
6. Carry out a blank titration under the same condition omitting the sample note the volume as 'b' ml.
7. Calculate the saponification value using the formula

$$\text{Saponification value} = \frac{28.05 * (b-a) * \text{actual normality of HCl}}{\text{Weight taken} * \text{given normality of HCl}}$$

REPORT

1. The actual normality of KOH was found to be ----------
2. The saponification value of given sample of oil was found to be ----------

CALCULATION (STANDARDIZATION)

Weighing details of anhydrous sodium carbonate

Weight of bottle	Weight in (gm)	Weight in (mg)	Rider position	Rider value	Total weight (gm)
Weight of empty weighing bottle (W_1)					
Total					
Weight of bottle + substance (W_2)					
Total					

Actual weight of oxalic acid =…….

Standardization of 0.1N sodium thiosulphate

Potassium bromate Vs 0.1N sodium thiosulphate

Contents of conical flask	Burette reading (ml) Initial	Final	Volume of 0.1 N sodium thiosulphate consumed (ml)	Indicator	End point

Normality $= N_1V_1 = N_2V_2$

Normality of …….. ,N_1 =

V1, volume of …… =

V_2, volume of ……. =

Normality of ……., $N_2 = \frac{N_1V_1}{V_2}$

Expt No:

Date:

DETERMINATION OF IODINE VALUE

AIM

To determine the iodine value of the given oil.

REFERENCE

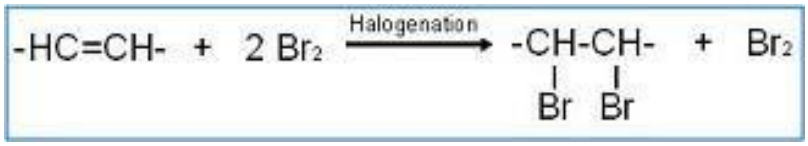
1. Text book of Pharmaceutical Analysis by A.A Nepolian, page number 10.10-10.14.
2. Text book of Organic Chemistry, Natural Products O P Agarval, volume II, page number 335-338.
3. Indian Pharmacopoeia, volume, edition, page number

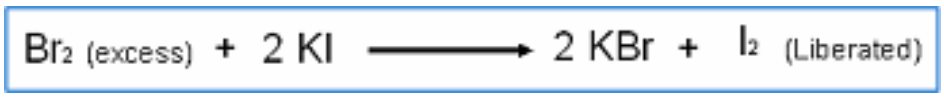
PRINCIPLE

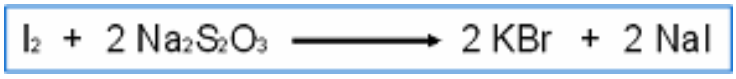
Iodine value is the value is the number which expressed in grams, the quantity if iodine which absorbed by 100gm of fat or oil under prescribed condition. The absorption is due to direct addition of iodine to the double bonds of unsaturated fat or oil. Thus iodine value gives an indication of the extent of unsaturation of a compound. Higher the iodine value, greater the unsaturation. Iodine value allows the classification of fat or oil in to non-drying, semi-drying and drying oil. Non-drying oils have iodine value less than 100 (e.g. olive oil), drying oil have values over 130 (e.g. linseed oil) and semidrying oil have value in between 100-130.

Iodine value can be determined by iodine mono chloride method and pyridine bromide method. The pyridine bromide method for determining iodine values make use of an additive compound of pyridine, bromide and sulphuric acid (C_5H_5N, Br_2, H_2SO_4).

This reagent forms additive compounds with unsaturated glycerides without any substitution or oxidation, and the excess of pyridine bromide can subsequently be detrmined by addition of potassium iodide and titration with sodium thiosulphate.







Determination of Iodine value

Weighing details of oil

Weight of bottle	Weight in (gm)	Weight in (mg)	Rider position	Rider value	Total weight (gm)
Weight of empty weighing bottle (W_1)					
Total					
Weight of bottle + substance (W_2)					
Total					

Actual weight of oil =…….

Determination of iodine value

Oil VS 0.1N Sodium thiosulpahte

Contents of conical flask	Burette reading (ml) Initial	Final	Volume of 0.1 N sodium thiosulpahte consumed (ml)	Indicator	End point

Iodine value = 1.269 *(b-a) *actual normality of sodium thiosulphate / Weight taken * expected normality of sodium thiosulphate

PROCEDURE:

Standardization of 0.1 N Sodium thiosulphate

Weigh accurately about 0.2g of potassium bromate, dissolve and made upto 250ml with distilled water and pipette out 50ml of the above solution, add 2gm of potassium iodide and 5ml 2N Sulphuric acid. Titrate against sodium thiosulphate using starch mucilage as indicator. The end point is the appearance of blue colour.

Each ml of 0.1N $Na_2S_2O_3$ is equivalent to 0.002784g of KBrO3

Determination of Iodine Value

- Weigh about 1g oil in 250ml of iodine flask and dissolved in 10 ml of carbon tetrachloride.
- Add 25 ml of pyridine-bromide solution, stopper and set aside for ten minutes.
- Place 15ml of KI solution in the cup top, carefully remove the stopper, rinse the stopper and the sides of the flask with 100ml of water
- Shake and titrate with 0.1M sodium thiosulpahte using starch solution as indicator

$$\text{Iodine value} = \frac{1.269 * (b-a) * \text{actual normality of sodium thiosulphate}}{\text{Weight taken} * \text{expected normality of sodium thiosulphate}}$$

REPORT:

1. The actual normality of KOH was found to be ----------
2. The iodine value of given sample of oil was found to be ----------

Expt No.

Date:

RECRYSTALLISATION

INTRODUCTION

Solid organic compounds when isolated from their organic reactions are seldom pure, they are usually contaminated with small amount of impurities. Purification is usually affected by crystallisation a suitable solvent or mixture of solvents.

Purification of solids by crystallisation is based upon differences in their solubility in a given solvent or mixture of solvents. In a simplest form the crystallisation process consist of

a) Dissolving the impure substances in some suitable solvents at or near the boiling point.
b) Filtering the hot solution from particles of insoluble materials and dust
c) Allow the hot solution to cool thus causing the dissolved substances to crystallise out and
d) Separating the crystals from the supernatant solution.

The resulting solid after drying is tested for purity.

AIM

To purify the given samples by recrystallisation technique.

REFERENCE

Vogel's text book of practical organic chemistry 5th edition Page no: 135-138.

REQUIREMENTS

Iodine flask, tripot stand, beaker, funnel, filter paper, samples.

PRINCIPLE

Recrystallisation is a technique used to purify chemicals. By dissolving both impurities and a compound in an appropriate solvent, either the desired compound or impurities can be

removed from the solution, leaving the other behind. It is named for the crystals often formed when the compound precipitates out. The process of recrystallization involves dissolution of the solid in an appropriate solvent at an elevated temperature and the subsequent re-formation of the crystals upon cooling, so that any impurities remain in solution. This technique, called solution re crystallization.

The technique of solution recrystallization involves the following steps:

1. Selection of an appropriate solvent.
2. Dissolution of the solid to be purified in the solvent near or at its boiling point.
3. Decolouration with an activated form of carbon, if necessary, to remove coloured impurities and filtration of the hot solution to remove insoluble impurities and the decolorizing carbon.
4. Formation of crystalline solid from the solution as it cools.
5. Isolation of the purified solid by filtration.
6. Drying the crystals.

PROCEDURE

***Dissolving the Sample*:**

- Weigh about 4gm of commercial acetanilide into a 250 ml conical flask.
- Add 80ml of water and heat nearly to the boiling point on an electric hotplate.
- The acetanilide will appear to melt and form an oil in the solution.
- Add small portions of hot water, while stirring the mixture and boiling gently until the solid has dissolved (or almost completely dissolved).
- If the solution not colourless, allow to cool slightly
- Add about .1g of decolourisng carbon and continue the boiling for a few minutes in order to remove the coloured impurities.

Hot Filtration:

- Filter the boiling solution through a fluted filter paper supported in a short necked funnel.
- If the solution cannot be filtered in a single operation, keep the unfiltered portion hot by returning conical flask to the hotplate.
- Alternatively, the solution may be filtered through a hot water funnel.
- Collect the filtrate in a 250ml conical flask.

Cooling Down:

- When all the solution has been filtered, cover the flask containing the hot filtrate with a clock glass and cool rapidly with swirling.
- Allow to stand for about 30 minutes to complete the separation of the solid.

Cold Filtration:

- Filter with suction through a small Buchner funnel, wash the crystals twice with 5 ml portions of cold water (to remove the adhering mother liquor)
- Press them in the funnel with a spatula or the back of a flat glass stopper.

Washing and Drying the Solid

- Remove the funnel from the filter flask, invert it on two thickness of filter or absorbent paper resting upon a pad of news paper and if necessary dislodge the pad of crystals by tapping the funnel
- Allow the crystals the crystals to dry in the air.
- It is advisable in air-drying to cover the crystal with a large clock glass resting upon corks, or the crystals may be covered with a large filter paper perforated with a number of holes in order to allow the solvent to evaporate.
- For more rapid drying, the crystals may be placed on a clock glass or in an evaporating basin in an oven held at a temperature of about 80ºC.

Weigh the yield of recrystallised product is not sufficiently pure (melting point low or melting over a range of several degrees), repeat the recrystallisation. Pure acetanilide has m.p.114 ºC.

$$\% \text{ Recovery} = \frac{m_{\text{recrys}}}{m_{crude}} \times 100$$

REPORT

Recrystallisation of crude acetanilide was performed.

Percentage recovery =

Expt No.

Date:

STEAM DISTILLATION

INTRODUCTION

Distillation is a technique of purifying liquids by converting them into their vapour state by heating and carrying over the vapours through a cooling device, thus condensing the vapours back to the liquid state. If the liquid contains non-volatile impurities they remain in the distillation flask.

AIM

To purify the given sample by steam distillation.

REFERENCE

1. Practical pharmaceutical organic chemistry by Harikishan singh and V.K Kapoor.

REQUIREMENTS

Round bottom flask, bend tube, separating funnel, reflux condenser, beaker, iodine flask, connectors, sample solvent.

PRINCIPLE

Steam distillation is a technique of separating and purifying organic compounds which are water-insoluble or are sparingly soluble in water. The techniques essentially consist of passing steam into a mixture of the compound and water. If the compound has an appreciable vapour pressure (at least 5-10 mm) at 100ºC, it will distill with steam. Thereafter the compound can be readily separated from the distillate as it is immiscible with water. Steam distillation is a useful purification technique provided that the compound is stable and the impurities non-volatile under the condition.

PROCEDURE

Assemble the apparatus. Place the material to be steam distilled in the round bottom

lask, clamp a inclined at an angle; it prevents the material from splashing and being blown over into the condenser. Connect the flask through a glass bend of wide bore (5-8 mm) with a Liebig condenser. Fit the other end of the condenser with an adapter and place a conical flask to collect the distillate. Fit a glass inlet tube which should reach almost to the base of the round bottom flak. Connect this tube to the steam generator. The steam generating can should be fitted with a long glass tube dipping well below the surface of water. It serves as a safety device. Heat the can to generate the steam. Pass steam into the round bottom flask. Heat the flask with a small flame to prevent too much water to condense in it. Continue the passage of steam into the flask until there is no appreciable amount of water-insoluble material in the distillate. Disconnect the rubber tube joining into a separating funnel. Separate the layer of sample material.

REPORT

Steam distillation was performed.

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RATHINAM COLLEGE OF PHARMACY

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PRACTICAL MANUAL

SECOND YEAR B.PHARM (III-SEMESTER)

SUBJECT: PHYSICAL PHARMACEUTICS - I (BP306P)

PREPARED BY
Mr.Ch.Venkat, M. Pharm,
Assistant Professor
Department of Pharmaceutics

CONTENTS

Experiment No.01

Date:

DETERMINATION OF SOLUBILITY OF DRUG AT ROOM TEMPERATURE

Aim:

To determine the solubility of given drug (solid substance) at room temperature.

Requirements:

Beaker, China dish, Analytical balance, porcelain dish, hot plate, pipette, thermometer, sodium chloride and water.

Principle:

Solubility is defined as the number of grams of solute required to produce 100ml of saturated solution in a given solvent at a room temperature or given temperature.

Quantitatively solubility is expressed in molarity, Molarity and percentage (w/v (or) w/w). a saturated solution is defined as the one in which the solute is in equilibrium in with the solid phase (solute) at a certain temperature.

A solution may sometimes contain more amount of solute than in saturated one, such a solution is known as super saturated solution The solubility of the solid depends on temperature, melting point of the solid and molar heat of fusion.

Procedure:

1. Note down the temperature. Take approximately 100ml of water in a clean beaker.
2. In beaker add sodium chloride little by little with shaking until a portion of the salt remains undissolved.
3. After ensuring the saturated solution has been formed, filter the solution through a filter paper over a funnel into a clean and dry vessel.
4. Discard approximately 20ml of the filtrate. Collect the subsequent filtrate.
5. From this, pipette out accurately 10ml of the solution after rinsing the pipette with the same solution into a tarred china dish.

6. Evaporate the solution (i.e. the solvent) by heating the china dish over flame.
7. At the final stage of evaporation, reduce the flame and continue heating until the residue is obtained.
8. Wipe out the outer surface of the china dish to get rid of any soot formed.
9. Cool the china dish to room temperature and weigh the china dish with the residue and find out the weight of the residue.

Calculation:

Then the solubility of sodium chloride is given as:

$$= \text{Residue} \frac{\text{Weight} \times 100}{10}$$

Observation:

Temperature ^{o}C	**Weight of the empty dish (W_1) gm**	**Weight of the dish with residue (W_2) gm**	**Weight of the dish with residue (W_2 - W_1) gm**

Report:

The solubility of sodium chloride at room temperature is equal to _____gm / 100ml of solution.

Experiment No.02

Date:

DETERMINATION OF pKa VALUE BY HALF NEUTRALIZATION / HENDERSON HASSEL BALCH EQUATION

Aim:

To determine the $_{P}$Ka value by Half Neutralization/Henderson Hassel Balch equation.

Chemicals and Apparatus required:

Distilled water, buffer solution of known pH, 0.04 N sodium hydroxide solution (standard), and 0.04N acetic acid solution.

pH meter, magnetic stirrer, burette, pipette, beakers, tissue paper, electronic weighing balance.

Principle:

When acetic acid is titrated against strong base it forms a mixture of acid and its salt. The mixture act as a buffer solution. During the course of titration acid concentration decreases and salt concentration goes on increasing. During any stage of titration, the pH of buffer solution is explained by the Henderson Hasselbalch equation.

pH = Pka + log (salt)/(acid)

pKa= dissociation constant of the acid (-log Ka)

[Salt]= concentration of salt CHCOONa

[Acid]= concentration of the acid CHCOOH

At the half neutralization point [Salt]=[Acid]

And equation (1) above gives pH =pKa

Procedure:

1. Thoroughly clean all the required glassware with acid and wash them 2 to 3 times with fresh distilled water.

2. Carefully read manual of pH meter and understand the method of operation.
3. Switch on pH meter and allow it to stabilize for 15 minutes.
4. Wash electrode by dipping them in distilled water. Wipe and dry them from outside using tissue paper.
5. Take 25 ml of buffer solution in a beaker and dip electrode in it. (Standardize pH meter by rotating respective knobs on pH meter to show exact pH of the buffer solution).
6. Wash the electrode again by dipping them in distilled water. Wipe and dry them as mentioned earlier.
7. In another dry beaker take 25 ml 0.04 N acetic acid solution and dip electrode in it, place the magnetic stirrer in the beaker containing solution and operate it. Read the value displayed on digital display of the pH meter.
8. Add 2 ml 0.04 N sodium hydroxide (V_1 ml) to beaker containing 25ml 0.04 M acetic acid.
9. Stir the mixture of acetic acid and sodium hydroxide and record the pH and when you get stable value note the total volume (V_2 ml).
10. Continue addition of 2ml of 0.04 N sodium hydroxide each time and record the pH till there is sudden increase in pH.

Observations:

Temperature: Room temperature = ---------- ^{o}C

Observations Table:

Volume of 0.04N NaoH (V_1 ml)	**pH**	**Total Volume (V_2 ml)**	**ΔpH**	**$\Delta B = \frac{2}{(25 \times V_2)}$**	**$\beta = \frac{\Delta B}{\Delta pH}$**
0					
2					
4					
6					
8					

Where,

β - buffer capacity

V_2 - total volume of acetic acid and sodium hydroxide solution

ΔpH - change in pH (difference in pH between two consecutive readings)

ΔB - equivalent of NaOH added per litre of solution

Calculation:

The ΔB is calculated as

Since, 1000ml 1 N NaOH = 1 equivalent of NaOH

$$2 \text{ ml } 0.04 \text{ N NaOH} = \frac{1 \text{ x } 2}{25 \text{ x } 1000} \text{ equivalent of NaOH}$$

If 'x' ml 0.04 N NaOH is added to 25 ml 0.04 N acetic acid solution, the volume of the solution is (25 + x) ml. The gram equivalent of NaOH per litre of solution added per 2 ml increment in NaOH is,

$$\Delta B = \frac{2 \text{ x } 1000}{(25 \text{ x } 1000)(25+X)}$$

$$\Delta B = \frac{2 \text{ x } 1000}{[25(25+X)]}$$

Plot the graph of β vs. pH and find the pH at which β is maximum.

Report:

The maximum buffer capacity (β_{max}) of the solution was found to be _____ and since it is at half neutralization stage, the dissociation constant

pKa = pH = _____

Experiment No.03

Date:

DETERMINATION OF PARTITION CO-EFFICIENT OF BENZOIC ACID IN BENZENE AND WATER

Aim:

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

Apparatus required:

Separating funnel, burette, beaker, conical flask, volumetric flask and tripod stand.

Chemicals Required:

Benzoic acid, Oxalic acid, Benzene, Phenolphthalein, Sodium hydroxide.

Principle:

Nernst distribution states that if a system contains two immiscible (or) slightly miscible solvents and if a third substance more or less soluble in both solvents is added then third substance will distribute itself between two liquids in definite manner depending upon its solubility.

$$K = \frac{Concentration\ of\ solute\ in\ organic\ phase}{Concentration\ of\ solute\ in\ aqueous\ phase} = \frac{Co}{Cw}$$

Where K is distribution co-efficient of solute or Partition Co-efficient Benzoic acid when added to mixture of water and benzene it distributes between benzene and water. It exists as associated (dimer) molecule in benzene and as individual or simple (un associated) molecule in aqueous phase, partition law applies only to the solute species common in both the solvents. Hence the above equation is modified as:

$$K = n\sqrt{Corg}/Caq$$

Preparation of 0.1 N NaOH Solution:

4 gm of NaOH was transferred into 1000ml of volumetric flask and dissolve the NaOH with water makeup of required volume with water allow it to stand overnight and decant the clear liquid into a bottle.

Preparation of 0.1 N Oxalic acid:

Dissolve 0.63g oxalic acid in 1000ml of distilled water.

Procedure:

0.5g of benzoic acid was weighed and transferred into separating funnel and 25ml of benzene and 25ml of water was added. The funnel was shaken for 30minutes and allows them to separate into two layers. Then the separating funnel was kept in constant temperature. The 10ml of aqueous layer was collected and titrated against 0.1N NaOH solution. Similarly, the 10ml of organic layer was collected and titrated against 0.1N NaOH solution. Then calculate the partition coefficient of benzoic acid between benzene and water.

Equivalent factor:

Each ml of 0.1N of NaOH approximately equivalent to 0.0122g of Benzoic acid.

Standardization of 0.1N NaOH Solution

S.No	Contents of conical flask	Burette reading (ml)		Total Volume of NaOH consumed (ml)	Indicator	End Point
		Initial	Final			
1.	10 ml of benzoic acid				2 drops of phenolphthalein	Colorless to pale pink colour
2.						

Average volume of NaOH consumed= ----------=----------= ----------ml

2 2

$N_1V_1 = N_2V_2$

S.No	Contents in conical flask	Burette reading(ml)		Total volume of NaOH consumed (ml)	Average volume of NaOH (ml)	Concentration of Benzoic acid in Solvent phase (g/ml)	Indicator	End point
		Initial	Final					
1	10ml of aqueous layer							
2								
3	10ml of organic layer							
4								

Concentration of Benzoic acid in aqueous layer:

C_w = Eq.factor x Vol. of NaOH consumed by aqu. layer x Act. Normality of NaOH

Weight of Benzoic acid x Theoretical Normality of NaOH

Concentration of Benzoic acid in organic layer:

C_w = Eq.factor x Vol. of NaOH consumed by org. layer x Act. Normality of NaOH

Weight of Benzoic acid x Theoretical Normality of NaOH

K_d = Concentation of solute in organic phase

Concentration of solute in aqueous phase

Observation:

1. The solubility of benzoic acid in aqueous layer was found to be_____g/ml.
2. The solubility of benzoic acid in organic layer was found to be_____g/ml

Report:

Partition coefficient = _____

From the above experiment it was concluded that the solubility of benzoic acid in benzene layer was _____than the solubility of benzoic acid in aqueous layer.

Experiment No.04

Date:

DETERMINATION OF PARTITION CO-EFFICIENT OF IODINE IN CCL_4 AND WATER

Aim:

To determine the partition coefficient of iodine between carbon tetrachloride and water.

Apparatus required:

Separating funnels, reagent bottles, beakers, burettes, pipette, stand conical flask, electronic weighing balance etc.

Chemicals Required:

Distilled water, iodine crystals, carbon tetrachloride, 0.1 N sodium thiosulphate ($Na_2S_2O_3$), 0.01 N sodium thiosulphate, starch indicator etc.

Theory:

Distribution law states that a solute distributes itself between two non-miscible solvents in contact with each other in such a way that at constant temperature the ratio of its concentration in the two layers in constant irrespective of its total amount. If excess of a liquid or solid is added to a mixture of two immiscible liquids, it will distribute itself between the two phases so that each becomes saturated. If the added substance is in sufficient to saturate the liquids, it will be distributed between the two layers in a definite proportion at equilibrium and at constant temperature.

$$K = \frac{\text{Concentation of solute in organic phase}}{\text{Concentration of solute in aqueous phase}} = \frac{C1}{C2} = \frac{C_{org}}{C_{aq}}$$

The equilibrium constant K, is known as Distribution Coefficient or Partition Coefficient. This known as Nernst's law of Distribution law and it is applicable only in dilute solutions to which activity coefficient may be neglected. In this study partition co-efficient will be determined for Iodine between Carbon tetrachloride and water. Added iodine is shared by two solvents and equilibrium is set up.

Procedure:

1. **Iodine in Carbon tetrachloride:**
   Prepare saturated solution of iodine in carbon tetrachloride, i.e., until some solid remains undissolved. Store in amber coloured bottle for use.
2. **0.1N Sodium thiosulphate – IP:**
   Dissolve 26g of sodium thiosulphate Penta Hydrate and 0.2g of Sodium Carbonate in distilled water and dilute to 1000ml with distilled water in 1000ml volumetric flask.
3. **0.005 N Sodium thiosulphate – IP:**
   Dissolve 1.3g of sodium thiosulphate and 0.01g 0f sodium carbonate in distilled water and dilute to 1000 ml with distilled water in 1000 ml volumetric flask.
4. **10% Potassium iodide solution:**
   Add 10g of potassium iodide in 100ml of distilled water.

Determination of partition co-efficient:

1. Take 30 ml of the saturated solution of iodine (in carbon tetrachloride) And 100ml of distilled water in an iodine flask.
2. In another Iodine flask add 15ml of saturated solution of iodine in carbon tetrachloride and 15ml carbon tetrachloride and 100ml distilled water.
3. Shake both the flasks for 20-30 minutes while keeping in a water bath at room temperature.
4. Using two separating funnels allow them to separate into two layers.
5. Collect the organic layer from first flask and immediately pipette out 5 ml of organic layer into a conical flask. Add 10ml of 10% potassium iodide solution. Titrate the solution against 0.1 N sodium thiosulphate solution using starch mucilage as indicator. Add the indicator just before end-point.
6. Similarly collect the organic layer from second flask and titrate following the same procedure.
7. Collect the aqueous layer from first flask. Immediately pipette out 10 ml of aqueous layer into a conical flask. Add 10ml 10% potassium iodide solution to titrate the solution against 0.005 N sodium thiosulphate using starch mucilage as indicator. In this case, indicator can be added at the beginning of the titration. Note the readings.

8. Collect the aqueous layer from the second flask and titrate following the same procedure.
9. Calculate the distribution coefficient of iodine between carbon tetrachloride and water.

Observation:

Organic layer

Normality of Sodium Thiosulphate (N_1 = 0.1 N)

Volume of organic layer pipetted (V_2) = 5ml

N_1V_1 (Sodium thiosulphate) = N_2V_2 (Organic layer)

Sets	**Volume of Sodium thiosulphate Consumed in ml (V_1)**	**Conc. of iodine in organic layer $N_2 = N_1V_1/V_2$**
Flask – 1		
Flask – 2		

Aqueous layer

Normality of Sodium Thiosulphate (N_1 = 0.005 N)

Volume of layer pipette (V_2) = 10ml

N_1V_1 (Sodium thiosulphate) = N_2V_2 (aqueous layer)

Sets	**Volume of Sodium thiosulphate Consumed in ml (V_1)**	Conc. of iodine in organic layer $N_2 = N_1V_1/V_2$
Flask – 1		
Flask – 2		

$$K = \frac{\text{Concentration of solute in organic phase}}{\text{Concentration of solute in aqueous phase}} = \frac{C1}{C2} = \frac{C_{ccI4}}{C_{Water}}$$

Flask 1, K =

Flask 2, K =

Report:

The distribution coefficient of iodine between carbon tetra chloride and water is -----

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Experiment No. 05

Date:

DETERMINATION OF CRITICAL SOLUTION TEMPERATURE OF PHENOL –WATER SYSTEM

Aim:

To determine the critical solution temperature of phenol –water system.

Requirements:

Chemicals: • Phenol in liquid state. • Distilled water.

Apparatus: • Analytical balance • Hard glass tube • Thermometer • Beakers • Stirrer • Water-bath

Principle:

Critical solution temperature is defined as the temperature at which two conjugate solution merge into one another to form one layer. This is characterized of a particular system. When small quantity of phenol is mixed with water and the mixture is shaken. Phenol dissolves forming a single layer and adding layer quantities of phenol however two kinds of composition of liquid are formed. The lower layer consists of small amount of water dissolves in phenol and the upper layer of phenol dissolves in water.

Procedure:

• Weigh accurately 4 gm of phenol in a glass tube

Introduce glass rods and thermometer into it, fix the tube in a vertical position is a water bath setup the apparatus.

• Add 2ml of distilled water in a phenol and keep the test tube in a water bath whose temperature is initially raised to about 30 $\mathring{C}$ on low flame with constant stirring. Note the temperature (T1) of solution is obtained.

• Remove the tube from water bath and allow to cooling. It forms a turbid solution as temperature decreases. Note this temperature as (T2). That mean of T and T is the transition temperature (T).

• Add 2ml more of distilled water to a same tube and determine the transition temperature as in previous continue the addition of 2ml distilled water at the time to the same tube and record the transition temperature each time till the maximum transition. Temperature is reached and comes down to about 40˚C.

Calculation

% by weight of phenol = $\frac{W1}{W2+W1} \times 100$

Weight of phenol (W1) =

Weight of water (W2) =

Density of distilled water =

Density of phenol = 1.071g/ml

Water added = V ml

Volume (V) of water added (ml)	**Weight of water (W2=V×d)**	**% w/w of phenol**	**Transition Temperature (°c)**		
			While heating (T1)	While cooling (T2)	Mean (T)

Report:

The critical solution temperature for phenol – water system is 40 C and its occurs at a concentration of 33 % percentage by weight of phenol.

Experiment No. 06

Date:

Determination of surface tension of given liquids by drop count and drop weight method

Aim:

To determine the surface tension of given liquids by drop count and drop weight method

Apparatus required:

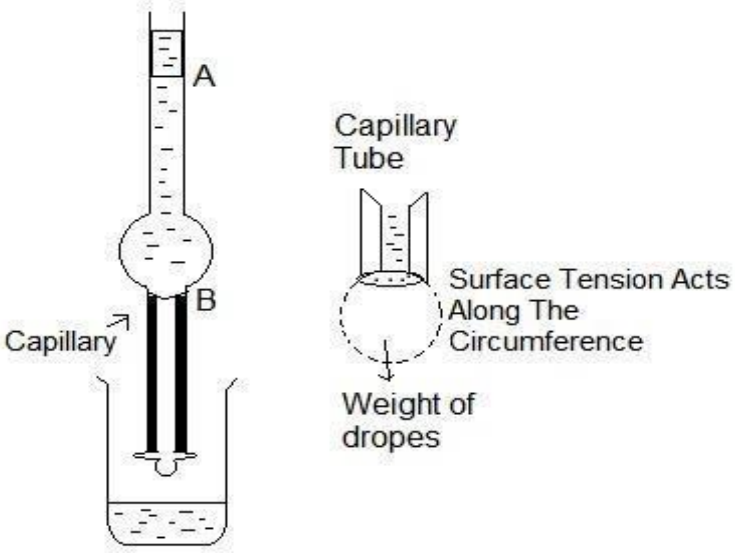
Ostwald's stalagmometer, density bottle, graduated pipette, beakers, electronic weighing balance, thermometer.

Chemicals Required: Distilled water, benzene and toluene.

Theory:

Number Drop Method:

In this method number of drops of some fixed volume of reference liquid and test liquid are determined by using Ostwald's stalagmometer.



Ostwald's stalagmometer

Upward force = Downward force

Procedure:

a) Drop count method:

1. Clean thoroughly all glassware.
2. Determine densities of all liquids using density bottle.
3. Mount Ostwald's stalagmometer on suitable stand in vertical position to avoid unnatural fall of drop.
4. Fill water in dry stalagmometer up to mark A and count total number of drops formed from mark A to mark B. (Use fixed volume for all liquids).
5. Repeat step 3 for three times to obtain correct reading.
6. Before start of counting number of drops of benzene, rinse stalagmometer with it then fill it with fresh benzene and count number of drops formed from mark A to mark B.
7. Similarly obtain number of drops of other liquids, if any.

b) Weight Drop Method:

1. Clean thoroughly all glassware.
2. Mount Ostwald's stalagmometer on suitable stand in vertical position to avoid unnatural fall of drop.
3. Fill water in dry stalagmometer up to mark A and allow drops to fall.
4. Collect twenty drops of test liquid from mark A of a stalagmometer in a weighing bottle and weigh them to determine average weight of drops.
5. Similar type of determination is carried out for the reference liquid upon properly cleaning the apparatus.

Precautions:

1. Use clean and dry stalagmometer.
2. Hold stalagmometer exactly in vertical position to form proper drops.
3. Allow drops to grow slowly by controlling air pressure above liquid in stalagmometer by holding it with pinch cork or thumb.
4. Use distilled water as a reference liquid.
5. Determine densities accurately.
6. Use calibrated and sensitive weighing balance.

Observations:

1. Room temperature = _____ ^{o}C
2. Surface tension of water(γ_1)= _____dynes/cm
3. One drop of water = _____divisions from mark A on capillary tube of stalagmometer
4. One drop of benzene = _____divisions from mark A on capillary tube of stalagmometer
5. One drop of toluene = _____divisions from mark A on capillary tube of stalagmometer

Observations Tables:

a) Drop Count Method:

Liquids	Density (ρ) g/ml	Number of drops				Surface tension (dynes/cm)
		(i)	(ii)	(iii)	Mean	
Water						
Ethanol						
Toluene						
Acetone						

b) Weight Drop Method:

Liquids	Weight of 20 drops of liquid (g)			Average weight of liquid (g)	Surface tension (dynes/cm)
	1	2	3		
Water					
Ethanol					
Toluene					
Acetone					

Calculation:

a) Drop Count Method:

Surface tension of liquids can be calculated as $\gamma_1 = \frac{\rho 1 n 2}{\rho 2 n 1} \times \gamma_2$

Where ρ_1 and ρ_2 are the densities, n_1 and n_2 are number of drops and γ_1 and γ_2 are surface tension of unknown liquids and water, respectively.

(b) Weight Drop Method:

Surface tension of liquids can be calculated as $\gamma_1 = \left[\frac{m1}{m2}\right] \times \gamma_2$

Where γ_1 and γ_2 are surface tensions and m_1 and m_2 are weights of sample and reference liquids, respectively.

Report:

The surface tension of given liquid by number drop method was found to be dyne/cm and by drop weight method it was ____dynes/cm determined at room temperature.

Experiment No. 07

Date:

DETERMINATION OF HLB NUMBER OF A SURFACTANT BY SAPONIFICATION METHOD

Aim:

To determine the HLB of a surfactant by Saponification method.

Apparatus required:

Round bottom flask, reflux condenser, conical flask, beakers, burettes, graduated pipette, electronic weighing balance, thermometer etc.

Chemicals Required: Distilled water, Glyceryl mono stearate (GMS Surfactant), 0.5 N alcoholic potassium hydroxide solution, stearic acid, ether, 0.5 N hydrochloric acid, 0.1 N sodium hydroxide solution, phenolphthalein as indicator.

Procedure:

a) Preparation of 0.5N alcoholic KOH:

1. Dissolve around 4 grams of KOH in 3 to 5 ml distilled water in a volumetric flask and make-up total volume to 100ml with ethyl alcohol.
2. Allow it to stand for about 24 hours and separate out clear liquid by decantation. Use this clear solution for experiment. Alcoholic KOH is used because surfactants are freely soluble in alcohol than in water. The solubility improved by alcohol hydrolysis is effective one.

b) Saponification Number:

1. Weigh accurately 0.5 gram of GMS and transfer into round bottom flask, add 15 ml alcoholic KOH to it and reflux on boiling water bath for about an hour.
2. Reflux separately 15 ml alcoholic KOH (without GMS) on boiling water bath for about an hour as blank.
3. Cool both the solutions to room temperature and titrate separately against 0.5N hydrochloric acid using phenolphthalein as an indicator. The end point is pink to colorless or slightly yellowish.
4. Let the titre reading of sample be V_1 and blank as V_2.

c) Acid Number:

1. Weigh accurately 0.5gram stearic acid; add it to a mixture of 10 ml alcohol and 10ml ether. If stearic acid does not dissolve in the solvent mixture, warm it on water bath until it dissolves. (Note: Take care while warming, since both ether and alcohol are highly inflammable solvents).
2. Titrate stearic acid solution against 0.1 N sodium hydroxide using phenolphthalein as the indicator.
3. Let the titre reading be V_3.

Precautions:

1. Prepare exact normality solutions.
2. Determine correct normality's of the solutions.
3. Weigh accurate amounts of ester and acid.
4. Warm alcohol and ether carefully as they are inflammable solvents.

Observations:

1. **Saponification Number:**
   (i) Volume of 0.5 N HCL consumed by sample (V_1) = _____ml.
   (ii) Volume of 0.5 N HCL consumed by blank (V_2) = _____ml.
2. **Acid Number**
   (i) Volume of 0.5 N NaOH consumed by (V_3) = _____ml.

Calculation:

1. **Saponification Number:**

1000ml 1 N KOH = 56000mg KOH

$(V_2 - V_1)$ ml 0.5 N KOH = $\frac{(V2 - V1) \times 0.5 \times 56000}{1000}$ mg KOH / 0.5g GMS

Substitute values of $V_1 - V_2$ to determine Saponification number.

$S = \frac{2(V2 - V1) \times 0.5 \times 56000}{1000}$ mg KOH / g GMS =

Saponification Number = _____

2. Acid Number:

V_3 ml 0.1 N NaOH = 0.5g stearic acid

$2V_3$ ml 0.1 N NaOH = 1g stearic acid

(Because 1000ml 1 N NaOH = 1000 ml 1N KOH = 56000 mg KOH)

$2V_3$ ml 0.1 N KOH = [56000/1000] x 0.1 x V_3

Substitute value of V_3 and calculate acid number.

3. HLB Calculation:

$$HLB = 20\ (1 - \frac{S}{A})$$

Where, S is Saponification Number of ester and A is Acid Number of fatty Acid.

Result:

The HLB of (GMS) Glyceryl mono stearate was found to be_____

Experiment No. 08

Date:

DETERMINATION OF FREUNDLICH AND LANGMUIR CONSTANTS USING ACTIVATED CHARCOAL

Aim:

To determine Freundlich and Langmuir constants using activated charcoal.

Apparatus required:

Analytical balance, reagent bottle, beakers, burette, graduated pipette, stand, conical flask, thermometer, constant temperature bath.

Chemicals Required:

Distilled water, activated charcoal, 0.5 N acetic acid, 0.1 N sodium hydroxide and phenolphthalein as indicator.

Theory:

Adsorption is a surface phenomenon and is defined as a process in which adsorbate gets adhered to the surface of adsorbent. Adsorption is of two types namely physical and chemical.

The quantitative relationship between the amount of adsorbate adsorbed by an adsorbant at equilibrium pressure and at constant temperature is referred as isotherm.

Procedure:

1. Prepare 0.5 N acetic acid solution and determine its exact normality (NA) by titrating against 0.1 N sodium hydroxide using phenolphthalein as an indicator. The end point is pink colour.
2. Prepare following mixtures of acetic acid and distilled water in five separate reagent bottles and label them as A, B, C, D and E.

Bottle No.	Distilled water (ml)	Acetic acid (ml)	Effective normality of acetic acid solution (N_1)
A	00	50	5/5 = 1 x NA=____
B	10	40	4/5 = 0.8 x NA=____
C	20	30	3/5 = 0.6 x NA=____
D	30	20	2/5 = 0.4 x NA=____
E	40	10	1/5 = 0.2 x NA=____

3. Add one gram of activated charcoal to each of the five reagent bottles and keep them in constant temperature water bath for one hour to attend adsorption equilibrium.
4. Shake bottles occasionally during the hour.
5. Using dry glassware filter all the mixtures separately.
6. Titrate 10 ml filtrate from each bottle against 0.1 N NaOH using phenolphthalein as an indicator. The end point is pink color.
7. Determine corresponding effective normality's of each of the solutions (N_2).

Precautions:

1. Determine exact normality of acetic acid solution.
2. Use activated charcoal only.
3. Allow adsorption equilibrium to attain before filtration.
4. Use dry filter papers and funnels for filtration.
5. Collect filtrate in clean and dry beakers.

Observations:

1. Temperature of water bath = _____ºC.
2. Mass of activated charcoal (m) = _____g.
3. Exact normality of acetic acid solution (NA) = _____N

Bottle No.	N_1	$C_e=N_2$	N_1-N_2	$C_e = 3N2$	Log C_e	$W = 3(N_1-N_2)$	$\frac{w}{m} = w$	w/m	$\frac{Ce}{w/m}$
A									
B									
C									
D									
E									

Where, N_1 = Initial (before adsorption)

= Initial concentration of acetic acid in gram equivalent / L

$C_e=N_2$ = Equilibrium normality (at adsorption equilibrium).

= Equilibrium concentration of acetic acid in gram equivalent / L

N_1-N_2 = Gram equivalent of acetic acid adsorbed per litre of solution.

Calculations:

1. Calculation of exact normality (NA) of acetic acid solution.

   10 ml acetic acid required _____ml (V) 0.1 N NaOH

   Thus, exact normality (NA) of acetic acid solution = $\frac{V \times 0.1}{10}$

   = _____

2. Calculation of amount of acetic acid adsorbed.

   Amount of acetic acid (W) adsorbed per 50 ml of solution per gram of charcoal is calculated as;

$$W = \frac{(N1-N2)\text{x Equivalent } Weight}{1000} \; x\; 50$$

$$= \frac{(N1-N2)\text{x } 60}{1000} \; x\; 50$$

$$= \frac{3000(N1-N2)}{1000}$$

$$= 3(N_1 - N_2)$$

3. Calculation of Freundlich constant k and b

   Plot the graph of log (w/m) vs. log C

Take any point P on straight line as shown in fig and determine its corresponding x and y co – ordinates. Also determine slope (m_1) of the line. Substitute values in equation to calculate value of k.

$$\log \left[\frac{w}{m}\right] = \frac{1}{b} \log \mathbf{Ce} + \mathbf{log\ k}$$

4. Calculation of Langmuir isotherm constants b.

   Since amount of charcoal used is one gram (m=1), $\frac{W}{m} = w$

   Plot the graph of $\frac{\mathbf{Ce}}{w}$ $vs.$ $\mathbf{Ce}$

   $\left[\frac{-}{m}\right]$

   Slope the graph (m_1) $= \frac{1}{ym} =$ _____

   $y_m = \frac{1}{\text{slope}}$

   Take any point P on straight line as in fig and determine its corresponding x and y co-ordinates. Also determine slope (m_1) of the the line. Substitute values in equation to calculate the value b.

   $$\frac{\mathbf{Ce}}{w} = \left[\frac{1}{(ym \times b)}\right] + \frac{\mathbf{Ce}}{ym}$$

   $\left[\frac{-}{m}\right]$

Result:

The constants of Freundlich adsorption isotherm were found to be k = _____ and b = _____and Langmuir adsorption isotherm was found to be b = _____

Experiment No. 09

Date:

DETERMINATION OF CRITICAL MICELLAR CONCENTRATION OF SURFACTANTS.

Aim:

To determine the critical micellar concentration of surfactants.

Apparatus required:

Stalagmometer, density bottle, analytical balance, beakers, graduated pipette, stand, conical flask, thermometer, electronic weighing balance.

Chemicals Required:

Distilled water, sodium lauryl sulphate (surfactant).

Theory:

As the surface tension is the characteristic property of material it varies with certain conditions. The factors that affect surface tension as well as interfacial tension includes surfactant concentration, temperature, polarity of liquid, hydrogen bonding, solute concentration etc. Surfactant molecules display distinct behavior when interacts with water. The polar part of the molecule seeks to interact with water while the non-polar part shuns interaction with water. There are two ways in which surfactant molecules achieve both these states. Surfactants attach to newly created surfaces being surface-active and reduction of surface tension is one of its most important qualities. The resulting change depends upon concentration of the surfactant. When the surface of liquid in which surfactants are added is saturated with surfactant they enter into the bulk of liquid and start forming aggregate in particular form. This aggregate is called as micelle. The concentration of surfactant at which micelle formation takes place is called critical micelle concentration (CMC). The surface tension is reduced by a surfactant up to a limiting value.

The characteristic discontinuity in the plots of surface tension against surfactant concentration is CMC and can be determined experimentally. Above limiting value (CMC), surfactant does not show any effect on the surface tension as their molecules are loosely integrated into the water structure.

Procedure:

1. Prepare 250 ml stock solution by dissolving 250mg SLS in distilled water (concentration 1mg/ml).
2. Prepare solutions of different strengths in distilled water with 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 mg SLS per 50 ml distilled water, from the stock solution.
3. Determine the surface tensions of all the solutions of SLS prepared in step 2.
4. Plot the graph of surface tension vs. concentration of SLS.

Precautions:

1. Rinse stalagmometer with respective solutions before determination of number of drops.
2. Use clean and dry stalagmometer each time and count number of drops formed accurately.
3. Prevent foaming to obtain accurate readings.
4. Use distilled water to avoid clogging of stalagmometer.

Observations:

1. Temperature = Room temperature = _____ ^{o}C

Observation table:

Concentration(C_e) of SLS (mg/50ml)	**Number of drops of water**				**Density (ρ) g/ml**	**Surface tension (dynes/cm)**
	(i)	**(ii)**	**(iii)**	**Mean**		
0.0						
5.0						
10.0						
15.0						
20.0						
25.0						
30.0						
35.0						
40.0						
45.0						
50.0						

Calculations:

1. **Density:**
   Calculate densities of different solutions using mass/volume relation.
2. **Surface Tension:**
   Calculate surface tensions of all solutions using following equation.

$$\gamma_2 \frac{n1\rho2}{n2\rho1} \text{ x } \gamma_1$$

Where, ρ_1 and ρ_2 are the densities, n_1 and n_2 are number of drops and γ_1 and γ_2 are surface tensions of distilled water and unknown liquids, respectively.

3. **Critical Micelle Concentration:**
   Plot the graph of surface tension vs. concentration of surfactant and identify the CMC by extrapolating lines. The extrapolated lines meet at a point. Extrapolate that point to x-axis to determine CMC.

Graph:

The CMC is calculated using equation

$$\text{CMC} = \frac{20\ x\ C}{1000\ M}$$

= _____moles/L

Where, M is Molecular weight of surfactant and C is CMC in mg/50ml.

Result:

The critical micelle concentration of given surfactant = _____mg/50ml by graph and _____moles/L by calculation at _____ºC.

Experiment No. 10

Date:

DETERMINATION OF STABILITY CONSTANT AND DONOR ACCEPTOR RATIO OF PABA-CAFFEINE COMPLEX BY SOLUBILITY METHOD

Aim:

To evaluate the Complexation behavior of Caffeine and Para Amino benzoic acid (PABA). The parameters are:

a) Donor Acceptor Ratio
b) Complex Stability Constant

Apparatus required:

Volumetric flask, conical flask, burette, beakers, graduated pipette, iron stand with clamp, thermometer, electronic weighing balance.

Chemicals Required:

Distilled water, p-amino benzoic acid, caffeine, sodium hydroxide, phenolphthalein as indicator, Whatman filter paper.

Theory:

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, but one of these bonds is somewhat anomalous.

Among complexes, organic molecular complexes have innumerable applications in pharmacy. These are as follows:

Physical state:

It is possible to convert liquid medications into solid complexes, which have improved processing characteristics. For example, nitroglycerin is liquid and explosive proof.

Chemical stability:

Complexation alters chemical reactivity. For example, the rate of hydrolysis of benzocaine can be reduced by complexing it with caffeine.

Volatility:

Complexation reduces the volatility and unpleasant odours. For example, iodine is sublimable (volatile). Volatility can be reduced by preparing a complex of iodine with PVP (Povidone ointment).

Dissolution:

Complexation enhances or reduces solubility, which subsequently improves the dissolution of drugs. For example, the dissolution rate of phenobarbitone is enhanced when complexed with cyclodextrin.

Bioavailability:

Complexation alters the bioavailability of drugs. Either inhibitory or enhancement effect may be observed. For example, bioavailability of tetracyclines is reduced, when co-administered with antacids such as aluminium, magnesium and calcium. This behavior is attributed to the formation of insoluble complexes. On the other hand, improved bioavailability has been observed in case of indomethacin, when complexed with β cyclodextrin.

Principle:

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, p- amino benzoic acid (PABA) 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:

PABA + Caffeine ----------> 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 non-polar parts of the molecules. The phase solubility diagram of the caffeine and PABA complex is shown in below.

The analysis of complexes generally involves the estimation of two parameters.

These are:

1. $$\textbf{Stoichiometric Ratio} = \frac{(\textbf{Caffeine in complex})}{(\textbf{PABA in complex})} \qquad \textbf{(01)}$$

2. $$\textbf{Complex stability constant} = \frac{(\textbf{PABA Caffeine})}{(\textbf{PABA})\,(\textbf{Caffeine})} \qquad \textbf{(02)}$$

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; saturated solution is collected and analyzed for drug content. The corresponding concentrations are substituted in equations (01) and (02).

Graph:

Preparation of sodium hydroxide solution (0.025N) IP:

Solution of any Normality x N may be prepared by dissolving 40x g of sodium hydroxide in water and diluting to 1000ml. Dissolve one gram of sodium hydroxide in water and dilute to 1000 ml.

Method:

1. Prepare various concentrations of caffeine (use 100ml conical flasks or beakers). The concentrations of caffeine are given in below table.
2. Transfer the samples of PABA into each flask containing the above caffeine solutions.
3. Fix the flasks in a constant temperature bath (either reciprocating or rotary shaker can be used). Manual shaking of flasks can also be done in a water-bath.

Concentration of caffeine solutions to be Prepared for complexation method

S.No	Caffeine stock solution in ml	Distilled water in ml	Concentration of Caffeine C x 10^2, mol/1
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	7

4. Shake the flasks until equilibrium is attained (Normally it may take 24 hours to 72 hours for equilibrium). For laboratory purposes, about 30 min may be sufficient.
5. Filter the above solutions. Good quality filter paper (Whattman) should be used.
6. Take 10 ml of the filtrate and titrate against 0.025 N sodium hydroxide solution using phenolphthalein as indicator.
7. Complete the titration for all samples. Report the date in table below.
8. Process data as per the columns in below table.

9. Draw the phase solubility diagram of PABA. Take concentration of caffeine on x-axis and concentration of PABA on y-axis.
10. Calculate the stoichiometric ratio of caffeine to PABA using equation (01).
11. Calculate the stability constant for the complex using equation (02).

Observations:

1. Temperature = Room Temperature = _____ ^{o}C
2. Molecular weight of PABA = 137.140g/mol
3. Molecular weight of Caffeine = 194.194g/mol

Observations table:

Data Processing for the Analysis of Complexes

Conc., mol/litre x 10^2	**Burette reading of NaOH**		**Volume of NaOH, ml**	**Conc. Of PABA mol/1**
	Initial reading, ml	**Final reading, ml**		
0				
1				
2				
3				
4				
5				
6				
7				
8				

Calculations: Estimation of parameters of Complexes

Consider the example as shown in figure – (01) Stoichiometric ratio: it is defined as follows:

$$\textbf{Stoichiometric Ratio} = \frac{(\textbf{Caffeine in complex})}{(\textbf{PABA in complex})}$$

Consider the concentration of caffeine and PABA entering complexation during plateau region,

$$\textbf{Stoichiometric Ratio} = \frac{(\textbf{Caffeine entering into complex})}{(\textbf{PABA entering into complex})}$$

Caffeine entering into plateau = (Caffeine) at point C – (Caffeine) at point B

$$= (3.6 \times 10^{-2}) - (1.8 \times 10^{-2})$$

$$= 1.8 \times 10^{-2} \text{ mol/1}$$

PABA entering into complex = (PABA) taken at Point C–(PABA) taken at Point B

$$= (7.3 \times 10^{-2}) - (5.5 \times 10^{-2})$$

$$= 1.8 \times 10^{-2} \text{ mol/1}$$

$$\textbf{Stoichiometric Ratio} = \frac{(\textbf{Caffeine in complex})}{(\textbf{PABA in complex})} = \frac{(1.8 \times 10{-}2)}{(1.8 \times 10{-}2)}$$

Therefore, Donor: Acceptor = 1:1

Stability Constant:

The initial linear portion (AB) should be considered for the purpose of calculation. Let the concentration of caffeine selected on x-axis be 1.0×10^{2} mol/1

$$\textbf{Stability Constant K} = \frac{(\textbf{PABA} - \textbf{Caffeine})}{(\textbf{PABA})\ (\textbf{complex})}$$

(PABA – Caffeine) complex = (PABA) at saturation B – (PABA) solubility A

$= (5.3 \times 10^{-2}) - (4.58 \times 10^{-2})$

$= 0.73 \times 10^{-2}$ mol/1

(Caffeine) complexed = (PABA – Caffeine)

$= 0.73 \times 10^{-2}$ mol/1

(Caffeine) uncomplexed = (Caffeine) total - (Caffeine) already complexed

= (Caffeine) chosen on x-axis – (PABA – Caffeine)

$= (1.0 \times 10^{-2}) - (0.73 \times 10^{-2})$

$= 0.27 \times 10^{-2}$ mol/1

$$\textbf{Stability Constant K} = \frac{(0.73 \times 10-2)}{(4.58 \times 10-2)\,(0.17 \times 10-2)}$$

The equilibrium stability constant for the complex of caffeine and PABA is 59mol/l

Graph:

Report:

The stability constant and donor acceptor ratio of PABA- Caffeine complex by solubility method were found to be _____and _____respectively.

Experiment No. 11

Date:

DETERMINATION OF STABILITY CONSTANT AND DONOR ACCEPTOR OF CUPRIC-GLYCINE COMPLEX BY P^H TITRATION METHOD

Aim:

To analyze Cupric-Glycine Complex by pH titration method and to calculate the formation curve and stability constants.

Apparatus required:

Volumetric flask, conical flask, burette, beakers, graduated pipette, stand, thermometer, electronic weighing balance.

Chemicals Required:

Distilled water, p-amino benzoic acid, caffeine, sodium hydroxide, phenolphthalein as indicator.

Theory:

Complexes results from a Lewis acid-base reaction between two or more different, chemical constituents forming co-ordination compounds. Vanderwaals forces, dipolar and induced dipolar types, hydrogen bonds are involved.

The complexation of cupric ion with Glycine molecules can be represented by the following equation.

$$Cu^{++} + 2NH_3 + CH_2COO \text{----------} > Cu(NH_2COO)_2 + 2H+$$

Since two protons are formed in the above equation, the addition of Glycine to a solution containing cupric ions should result in decrease in P^H. Titration curves can be obtained to analyze the complexation.

Titration curves can be obtained by adding Sodium Hydroxide solution (0.25N) to the solution of Glycine and complex solution and plotting the pH against equivalent of base added. 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.

'n' is the number of ligand molecules bound to a metal ion. It can be calculated as:

$$= \frac{\text{total concentration of ligand bond}}{\text{total concentration of metal ion}}$$

The concentration of ligand bound can be calculated as follows. The horizontal distances represented by the lines in the graph between the titration curves for Glycine and that of complex solution give the amount of alkali used up in the reaction. This quantity is exactly equal to the concentration of ligand bound at any pH.

$-\log (A) = pA$

$= pKa - pH - \log [(HA_{intial}) - NaOH]$

These values and n will help to calculate the complex formation constants and overall stability constant of the complex. pKa of Glycine is 9.69.

The two successive equilibrium between the copper ion or metal M and Glycine or the ligand A in stepwise form can be written as.

$M + A = MA$

$K_1 = (MA) / (WA)$ and

$MA + A = MA_2$

$K_2 = (MA_2) / (MA) (A)$

So, one can write the overall reaction as:

$M + 2A = MA_2$

$\beta = K_1K_2 = (MA_2) / (M) (A)^2$

Therefore, $n = \frac{(MA) + 2\ (MA2)}{(A) + (MA) + (MA2)}$

'n' has a definite value for each species of complex (1 or 2 in this case), for n=1 the above equation become (MA_2) (M)

So, $\beta = K_1K_2 = 1 / \log (A)^2$ or $\log \beta = 2 \log (A)$

i.e, $pA_2 = \frac{1}{2} \log \beta$ at n = 1

and $pA = \log K_1$ at $n = \frac{1}{2}$

and $pA = \log K_2$ at $n \frac{3}{2}$

Procedure:

1. **Preparation of Glycine solution (3.34×10^{-2} mole / litre:**

Weigh 250 mg of Glycine and transfer into a 100 ml volumetric flask. Add distilled water and make up the volume upto mark.

2. **Preparing of complex solution: (Glycine 3.34×10^{-2} mole / litre, Cupric chloride 9.45×10^{-2} mole / litre).**
   (i) Accurately weigh 250 mg Glycine and 160 mg cupric chloride and transfer into a volumetric flask (100 ml).
   (ii) Add distilled water to dissolve solids and make-up the volume upto mark.
3. **Preparing sodium hydroxide solution (0.25N):**
   (i) Accurately weigh 1g sodium hydroxide and transfer into a volumetric flask (100 ml).
   (ii) Add about 50 ml distilled water in flask to dissolve flakes.
   (iii) Make final volume by adding distilled water up to the mark to get 0.25 N sodium hydroxide solution.

4. **Kinetic Method:**
   (i) Transfer 75 ml Glycine solution into a 250 ml beaker and measure the pH of the solution using pH meter.
   (ii) Gradually add 0.25 N sodium hydroxide solution to the Glycine solution and periodically measure the pH.
   (iii) Plot a graph by taking pH on y-axis and volume of NaOH consumed

on ml on x-axis.

(iv) Transfer 75 ml of complex solution into a 250 ml beaker and measure pH of the solution.

(v) Repeat the titration as done in earlier step

(vi) Calculate n at different pH.

(vii) Calculate PA.

(viii) Then plot the values. Plot a graph of pH vs Volume of NaOH and 'n' vs pA.

The curve obtained is known as a formation curve. It is observed to reach a limiting value at n = 2, signifying that the maximum number of Glycine molecules that can combine with one atom of copper is two.

From the obtained curve at n = 0.5, at n = $\frac{3}{2}$ and at n = 1.0 the approximate values for log k_1, log k_2 and log β respectively are obtained.

Observations:

1. Temperature = Room Temperature = _____ ^{o}C.
2. Molecular weight of Glycine = 75g / mole.
3. Molecular weight of Cupric Chloride = 134.45g / mole.

Observation table:

Glycine Solution		**Complex Solution**	
Volume of 0.25 N NaOH in ml	pH	Volume of 0.25 N NaOH in ml	
0		0	
1		1	
2		2	
3		3	
4		4	
5		5	
6		6	
7		7	
8		8	
9		9	

Graph:

Calculations: The analysis of data obtained by pH titration method is carried out using following equations.

$$\text{Moles of NaOH} = a \times 2.5 \times 10^{-4}$$

$$\text{Concentration of NaOH} = \frac{X \times 1000}{75}$$

$$n = \frac{Y}{9.45 \times 10-3}$$

$$pA = pKa - pH - \log [Glycine_{intial} - NaOH]$$

Analysis – pH Titration Method

pH	Volume (ml) of NaOH obtained from graph	Moles of NaOH	Concentration of NaOH	n	pA
	A	x	y		

Report:

The stability constant of Glycine-Cupric complex by pH titration method were found to be log K_1 = _____

log K_2 =_____

log β = _____

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PRACTICAL MANUAL

SECOND YEAR B.PHARM (III-SEMESTER)

SUBJECT: PHARMACEUTICAL MICROBIOLOGY (BP307P)

PREPARED BY
Dr.G.Vinotha pooshan,M.Pharm,Ph.D.,
Professor
Department of Pharmaceutics

INDEX

Expt No : 01
Date:

INTRODUCTION AND STUDY OF DIFFERENT EQUIPMENTS AND PROCESSING

Aim:

To study different equipment and processing used in Pharmaceutical microbiology lab

1.BOD INCUBATOR:

It is often called low temperature incubators are one of the most important lab equipment in many research centers, hospitals and other pharmaceutical labs. BOD incubators is the most versatile and reliable low temperature incubator which is designed to maintain at 20'C , necessary for biological oxygen demand/ biochemical oxygen demand determination. BOD incubators provide controlled temperature conditions accelerated tests and exposures.

Applications

It measuring waste loadings to treatment plants and in evaluating the BOD removal efficiency of such treatment systems

It measures the molecular oxygen utilized during a specified incubation period for the biochemical degradation of organic materials and the oxygen used to oxidized in organic materials such as sulfides and ferrous iron.

2. ASEPTIC HOOD

The air of laboratory is full of microorganism. Air borne micro organism may contaminate sterile medium while handling pure culture during inoculation. Thus , an inoculation procedure is usually carried out under a hood or special cabinet. To have aseptic environment, two types of the devices are placed in practice – laminar air flow aseptic cabinets.

The inoculation hood is used to create bacteria, fungal free atmosphere in the chamber with ultra violet germicidal light and it is used in biological culture studies.

3. LAMINAR FLOW CABINET

Laminar hood sometimes also known as laminar air flow is an enclosed bench designed to prevent contamination like biological particles or any particle sensitive device

A laminar flow hood consist of a fan, HEPA filter. The fan sucks the air through the filter pad where dust is trapped , after that the pre filtered air has to pass the HEPA filter where contaminating fungi, bacteria, dust etc are removed. Now the sterile air flows into the working areas where you can do all your flasking work without risk of contamination.

4. AUTOCLAVE

It is laboratory equipment used to sterilize the equipment culture medium, aprons, rubber tubing's etc. by steam under pressure technique. It is also used to sterilize infected materials like contaminated dressings, used culture medium etc., a process known as decontamination.

Types of autoclave:

1. Stove top autoclaves: they are considered to be modified pressure cookers and require outside heat source.
2. Front load auto claves: they are most widely used for sterilization being more convenient as compared to stove top[autoclaves. They are box shaped and self contained, equipped with heating unit to turn water into steam sterilization . the various controls allow the operator to set the desired temperature. They are also equipped with temperature and pressure gauges to note the respective parameters.

5. HOT AIR OVEN

It is electrically operated equipment used for the sterilization of glass ware such as syringes, pipettes, Petri dishes, test tubes, liquid paraffin, oils, powders and flasks.

Precautions:

- The glass ware should be clean and dry before loading the articles for sterilization .
- The glass ware like test tube , flasks and pipettes must be wrapped in a paper or aluminum foil before sterilization.
- The temperature should not be allowed to increase above 180'C

- All sterilized articles must be removed only after they have cooled down to room temperature.

6. DEEP FREEZERS FOR MEDICAL LABORATORIES

Deep freezers are the testing equipment that are used in scientific laboratories to preserve and store medical equipment, blood samples, medicines and injections, etc for a long period of time. There are numerous types of deep freezers such as blood bank refrigerators, freezer drier, ultra low deep freezer. These devices are available indifferent sizes and shapes sometimes it is designed with compact designs and sometimes with regular designs. The specifications and functions of the instruments varies as per the requirements of the test application.

7. COMPOUND MICROSCOPE:

The compound microscope is a basic tool for scientific education and research without which world of micro organism would have remained un explored. A compound microscope helps in magnification an image in two stages. It uses an objective lens that has many different powers and eye piece that helps in magnifying the image formed by the objective lens. A typical compound microscope consist of certain mechanical and optical parts.

A. MECHANICAL PARTS

1. Foot or base: It is a horseshoe shaped solid part at the bottom of microscope on which the most of microscope stands
2. Pillar: it is the small projection from the foot or base with inclination joint at top
3. Inclination joint: it is a hinge joint by which the upper part of microscope can be tilted in upwards and downward direction
4. Stage: it is a metallic platform with two clips and a hole in the centre. The clips holds the glass slide and the hole allows the light from the mirror to reflect on the object.
5. Arm: it is a curved handle used to carry the microscope. It supports the tube and connects it to the base
6. Body tube: it holds the optical parts like eye piece and objectives at proper working distance from each other.
7. Nose piece: it is fitted at the base of the body tube and holds different objectives with different magnifying powers (10×, 40× 100×)
8. Coarse adjustment knob: it is used to move the body tube up and down to focus the image on the object.
9. Fine adjustment: it is used to view finer details of the objects or specimen. **B.** OPTICAL PARTS:

theses parts consists of glass lenses that produce a magnified image and include:

1. Mirror: it is fixed below the stage and used to regulate the light to illuminate the object through the hole on stage. It has two faces; a plane and a concave. When

Expt No: 2
DATE:

STERILIZATION OF GLASSWARE, PREPARATION AND STERILIZATION OF MEDIA

Aim: To perform sterilization of glassware, preparation and sterilization of media

Sterilizing methods for glassware in a laboratory hot air oven

Sterilizing glassware such as bottles, Petri dishes and test tubes, dry heat is required and this is carried out in a hot air oven. The ideal temperature of the oven needs to reach at least 160'c and the content need to regulated at this 45 to 50 min . the content must not be removed from oven immediately as a slow cooling period is necessary when the temperature has reduced down 50'c.

AUTOCLAVING LAB GLASSWARE

Autoclave are widely used to sterilize instruments, glassware and plastic ware, solutions and media and to decontaminate biological wastes. Because of the physical hazards associated with autoclaving, extra care must be taken to ensure their safe use. The following safety practices are followed when autoclaving laboratory glassware:

- Never autoclave items containing corrosives or radio active materials
- Use only borosilicates, glassware's, which can be better withstand the stresses of high autoclave temperatures and pressures.
- Load the auto clave properly as per the manufacturers recommendations.
- Individual glassware vessels should be placed within a heat resistant plastic or metal tray on a shelf or rack and never placed directly on the autoclave bottom or floor.
- Add ¼ to ½ inch of water to the tray so the glassware will heat more evenly
- Check any plastic caps, tubing or other items to ensure they can be safely autoclaved with the glassware
- Fill glassware only half full with liquids to be sterilized. Take into account the volume of liquid to be autoclaved.

Expt No: 3
DATE

PREPARATION AND STERILIZATION OF CULTURE MEDIA

AIM

To prepare Nutrient Agar and Nutrient Broth Media

REQUIREMENTS

Nutrient Agar Media , Nutrient Broth Media, Conical Flask, Distilled water, cotton roll,

PROCEDURE

1. Suspend 28g of nutrient agar powder in 1L of distilled water.
2. Mix and dissolve them completely.
3. Sterilize by autoclaving at 121°C for 15 minutes.
4. Pour the liquid into the petri dish and wait for the medium to solidify. Be sure that you are preparing the agar in the clean environment to prevent any contamination.
5. Add 13g of nutrient broth powder in 1L of distilled water.
6. Mix and dissolve them completely.
7. Pour them into the final containers (eg. conical flask)
8. Sterilize by autoclaving at 121°C for 15 minutes.

RESULT

The Nutrient agar and Nutrient Broth Media were prepared.

Expt No -4
DATE:

SUB CULTURING OF BACTERIA & FUNGUS

SUB CULTURING

Aim: To carryout sub-culturing of bacteria and fungus

After incubation has been completed in streak plate , pour plate or spread plate techniques and appearance of the discrete, well separated colonies has been examined, the next step is to subculture some of the cells from one of the colonies to separate agar plates or nutrient agar slants with a sterilized needle or loop for further examination and use. Each of these new cultures represents the growth of a single species called a pure culture or stock culture. Sub culturing term is the term used to describe the procedure of transferring o microorganism from their parent growth source to a fresh one or from one medium to another medium

REQUIREMENTS

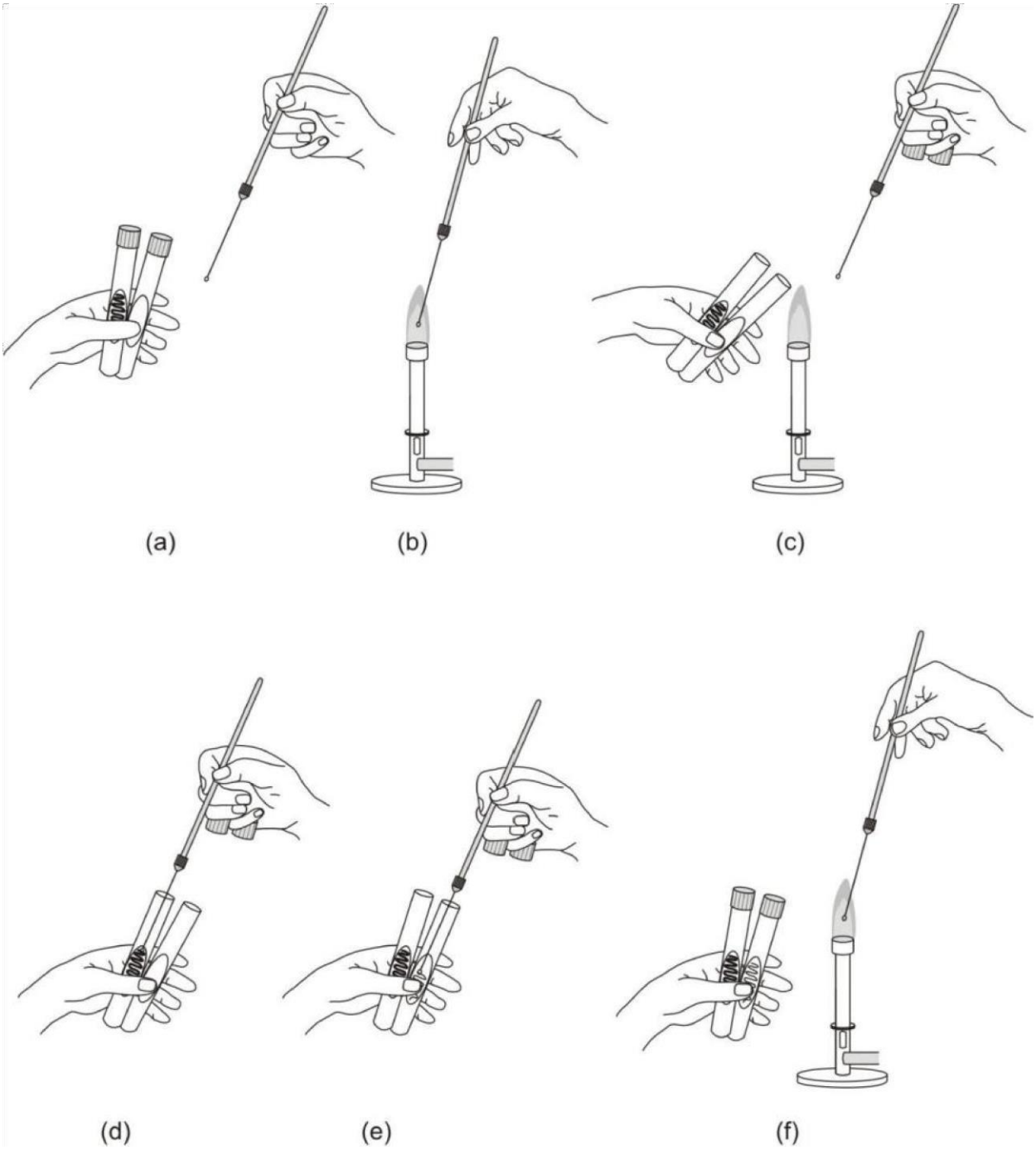
Mixed nutrient agar streaks, pour plate and spread preparations of nay refrences bacteris

Nutrient agar slants or nutrient agar plates

Inoculating loop

Wax marking pencil

Microscope


(a)
(b)
(c)
(d)
(e)
(f)

PROCEDURE:

1. With a wax pencil label the nutrient agar slants and agar plates as bacteria A & B. sterilize the inoculating loop by holding it in the hottest portion of the Bunsen burner flame
2. Flame until entire wire become red hot
3. Allow the loop to cool for a few seconds or cool it by dipping In a fresh agar plate
4. Touch the tip of the loop to the surface of a selected discrete colony or the agar streak plate or the pour plate
5. Remove the plug of the agar slants, grasp the plug with thee little finger of the left hand and pass the neck tube rapidly over the Bunsen burner flame. Inset the loop into the subculture tube rapidly over the Bunsen burner flame. Inset the loop into the subculture tube and inoculate it lightly over the hardened surface in a straight or zig zag line and recap tube.
6. Reflame the inoculating loop/ needle to destroy existing organism
7. Incubate the culture for 48-72hrs.

Observations:

After incubation, observe the slants or plates for the growth of pure colonies.

REPORT

Expt – 05
DATE

ISOLATION OF PURE CULTURE OF MICRO ORGANISM

Aim: To isolate pure culture of micro-organism

A pure culture contains only one kind of micro organism and involves not only the isolation of individual micro organism from a mixed population but also the maintenance of such individuals and their progenies in artificial media. Pure culture are essential in order to study; colony characteristics, biochemical characteristics, morphology, staining reactions and immunological reactions or the susceptibility to antimicrobial agents of a particular strain of bacterium or fungus or actinomycetes

The commonly used methods are.

1.Streak plate method

I. STREAK PLATE METHOD

Principle

The method is based on the principle that by streaking , a dilution gradient gets established across the surface of the Petri plate as bacterial cells are deposited on the agar surface. Because of this dilution gradient, confluent growth takes place on the part of the medium where few bacteria are deposited. Each colony is the progeny of a single microbial cell, thus representing a clone of pure culture. Such colonies may be picked aseptically and re streaked into fresh media
to ensure purity of a particular strain.

Procedure:

1. Place a loopful of the inoculums near the periphery of the petri dish and cover with the close parallel streaks.
2. Turn the plate at right angles and streak approx one half of the remaining portion
3. Without overlapping the previous streaks
4. Turn the plate to 180'c and streak the reminder of the plate, avoiding previous streaked areas.

REPORT:

Expt – 06
DATE

STAINING METHODS – SIMPLE STAINING

Aim: To carryout simple staining Staining methods:

Normally there two staining procedures for light microscopy

a. Simple attaining
b. Differential staining

Simple staining: In positive, the stain is basic having positive charge and attaches to the surface of the object that is negatively charged

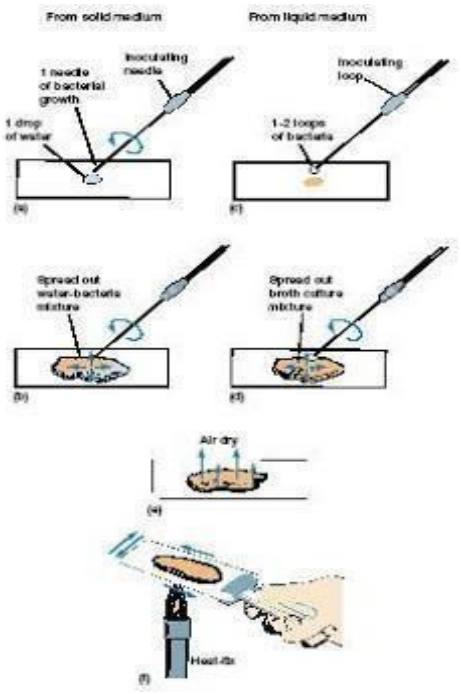
Negative staining: in this procedure, more than one staining reagents are used and specific objects exhibit different staining reactions which are readily distinguishable. Two most widely differential procedures are gram staining an acid fast staining.

SIMPLE STAINING

Principle :

simple staining involves single dye or staining reagents. The purpose of staining is to demonstrate cell size, shape and arrangement of bacterial cells. Since bacterial cells usually have a negative charge on their surface, they are most readily colored by basic stains. These compounds will either give up, which is attracted to the negatively charged cell surface.

Reagents: Loeffler's methylene blue solution



Procedure: prepare a smear of a given culture or materials by spreading a thin film on a clean glass slide

Dry it by waving in air and then heat fix by passing the slides 2 to 3 times through the flame with the smeared slide facing upwards

Stain the smear by flooding it with one of the staining solutions and allowing it to remain covered with the stain for the time designated below.

- Methylene blue – 1 min
- Crystal violet- 30 sec
- Carbol fuchsin – 20 sec

Wash the slide gently with running water to remove the stain Air dry the slide or blot with blotting paper

Apply oil directly to the smear, and focus the smear first under low power objective and then under oil immersion objectives.

REPORT:

Expt – 07
DATE

STAINING METHODS –GRAM STAINING

Aim: To perform gram staining process

The technique was developed by a Danish physician Dr. Hanes Christian gram . this is useful differential staining procedure in bacteriology which besides determining gross morphology, differentiates bacteria into two major distinct groups:

Gram positive bacteria

Gram negative bacteria

The differentiation assists in determining subsequent biochemical tests and the respective media for their culture in laboratory

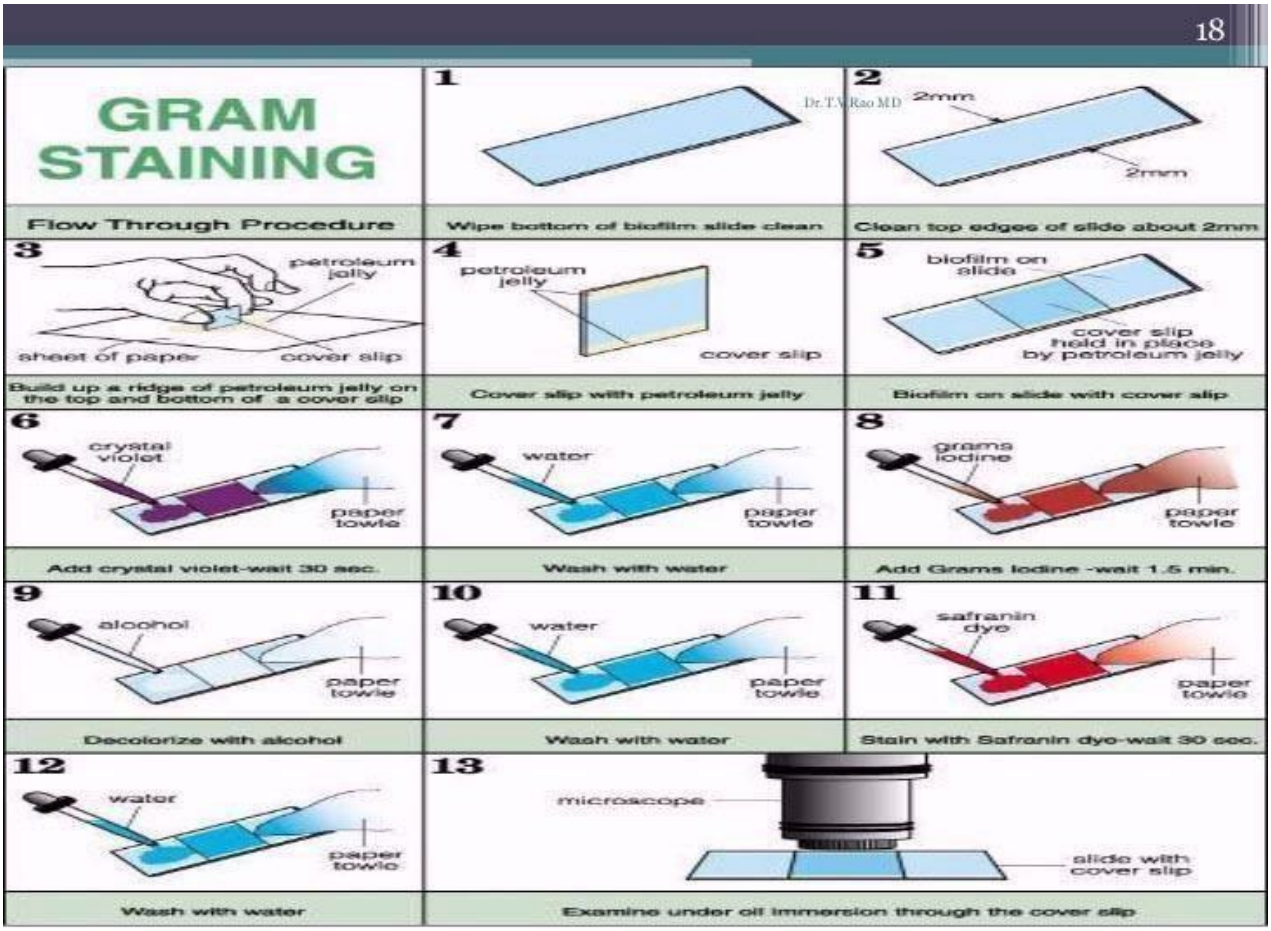
The technique involve 6 basic steps

- Smear preparation
- Heat fixation
- Staining with crystal violet
- Use of iodine/lugol's soln
- Treatment with acetone alcohol mix
- Use of safranin

Principle:

The peculiar response towards the staining is related to physical and chemical difference in the cell walls of the two groups of bacteria . in gram negative bacteria, the cell wall is thin , multi layered containing high lipid contents which are readily dissolved by alcohol , resulting in pore formation in the cell wall facilitating the leakage of the crystal violet iodine complex and resulting in discolouration of gram negative bacteria which takes safranin and appears red. On the other hand cell walls of gram positive bacteria are thick composed mainly proteins and cross linked

mucopeptides. On application of decolorizing agent, dehydration is caused resulting in closure of pores of the cell wall therby retaining the CV-I complex and do appear blue or purple.



Procedure:

1. Make smears of the given culture on a clean glass lside
2. Air dry the smear and heat fix it
3. Cover the smear completely with crystal violet stain and leave the stain on the slide for one min
4. Wash the slide gently with distilled water or tap water
5. Flood the smear with gram iodine solution and wait for one min
6. Wash with tap water gently and drain carefully
7. Add ethyl alcohol or alcohol acetone (1:1) solution drop by drop until the smear becomes free from any colorization

8. Wash the slide gently under running tap water and drain
9. Now counter stain with safranin and wait for 30 sec
10. Wash again and blot dry with blotting paper or simply air dry the slide and observe under oil immersion objectives.

RESULT:

Bacteria that appear blue/ violet/ purple are assigned as gram positive bacteria

Bacteria that appear red/ pink are assigned as gram negative bacteria

Expt. No. 8
DATE: **NEGATIVE STAINING**

AIM

OBJECTIVE

To determine the morphology and arrangement of a bacterium

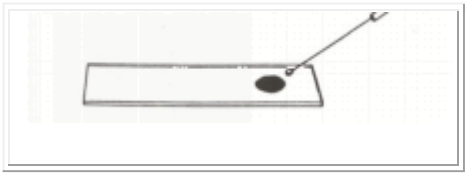
PRINCIPLE

The negative stain is another method of determining morphology of a bacterial cell. As mentioned above, the negative stain uses acidic dyes that are repelled by the bacterial cell wall to stain the background. Because the cells are not stained, they appear as transparent objects against the dark background of the slide. Another key feature of the negative stain is that the cells are not heat fixed. This allows them to maintain their original shape and size without becoming distorted. This makes the negative stain ideal for viewing bacterial capsules.

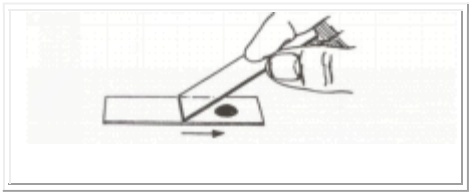
PROCEDURE

1. Place a very small drop (more than a loop full--less than a free falling drop from the dropper) of nigrosin near one end of a well-cleaned and flamed slide.

2. Remove a small amount of the culture from the slant with an inoculating loop and disperse it in the drop of stain without spreading the drop.

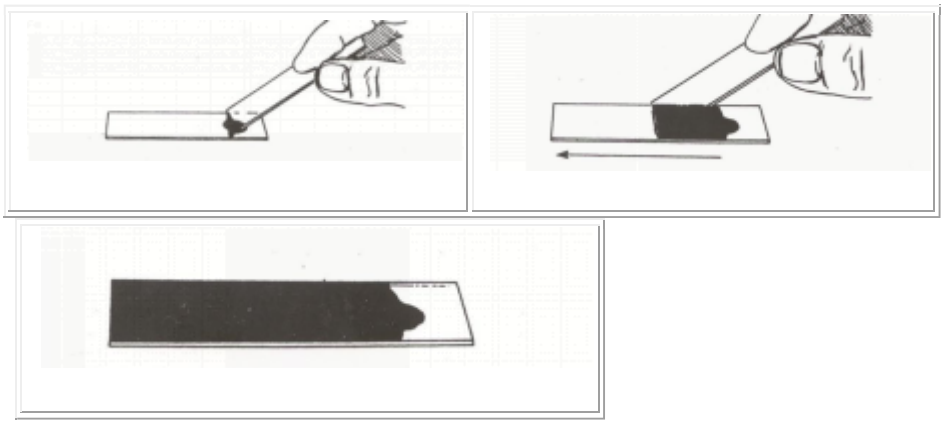


3. Use another clean slide to spread the drop of stain containing the organism using the following technique.



4. Rest one end of the clean slide on the center of the slide with the stain. Tilt the clean slide toward the drop forming an acute angle and draw that slide toward the drop until it touches the drop and

causes it to spread along the edge of the spreader slide. Maintaining a small acute angle between the slides, push the spreader slide toward the clean end of the slide being stained dragging the drop behind the spreader slide and producing a broad, even, thin smear.



5. Allow the smear to dry without heating.

6. Focus a thin area under oil immersion and observe the unstained cells surrounded by the gray stain.

OBSERVATION

Capsules can be seen as clear areas surrounding the cells in a dark background.

REPORT

Expt : 9
DATE:

MOTILITY DETERMINATION BY HANGING DROP METHOD

Aim: To carryout motility differentiation by hanging drop method

Hanging drop method is used to examine the motility of bacteria in a given culture. This method is most frequently used in examination of stool specimen of suspected cholera patients. It is a method in which a drop of bacterial suspension is enclosed in an air tight chamber prepared in a special depression/ concavity slide Method:

1. Hold a clean cover slip by its edges and carefully apply on its corner using a toothpick
2. Place a loopful of the culture to be tested in the centre of the prepared coverslip
3. Turn the clean concavity slide upside down over the drop on the cover slip so that the Vaseline seals the cover slip to the slide around the concavity and the drop remains hanging in the depression of slide.
4. Turn the slide over so the cover slip is on top and the drop can be observed banging from the cover slip over the concavity
5. Place the preparation in the microscope slide holder and align it using the naked eye so an edge of the drop is under the low power objectives
6. Turn the objective to its lowest position using the coarse adjustment and close the diaphragm
7. Look through the eye piece and raise the objectives slowly using the coarse adjustment knob until the edge of the drop is observed as irregular line crossing the field
8. Focus the edge of the drop carefully and look at each side of that line for very small objects that are the bacteria. The cells will look either like dark or slightly greenish , very small rods or spheres.
9. Adjust the light using the diaphragm level to maximize the visibility of the cells.

REPORT:

Expt : 10
DATE

Fungal Staining by Lactophenol Cotton Blue method (LPCB)

Aim

To stain fungal cells by using Lactophenol cotton blue

Principle

The lactophenol cotton blue (LPCB) wet mount preparation is the most widely used method of staining and observing fungi and is simple to prepare. The preparation has three components:

1. **Phenol:** kills any live organisms;

2. **Lactic acid** : It preserves fungal structures, and

3. **Cotton blue** : It **stains the chitin** in the fungal cell walls.

Lactophenol Cotton Blue Solution is a **mounting medium** and **staining agent** used in the preparation of slides for microscopic examination of fungi. Fungal elements are stained intensely blue.

Materials Required

1. Microscope, Glass Slide , Cover Slip ,70 % alcohol , Lactophenol cotton blue (LPCB) Inoculation needle, Fungal culture

Procedure

1. Placed a drop of 70% ethanol on a clean microscopic glass slide

2. The Immersed the specimen in the drop of alcohol

3. Added one or two drops of the LPCB before the alcohol dries out

4. Hold the coverslip between the index finger and thumb, touch one edge of the drop of mountant with a coverslip edge and lower gently avoiding air bubbles

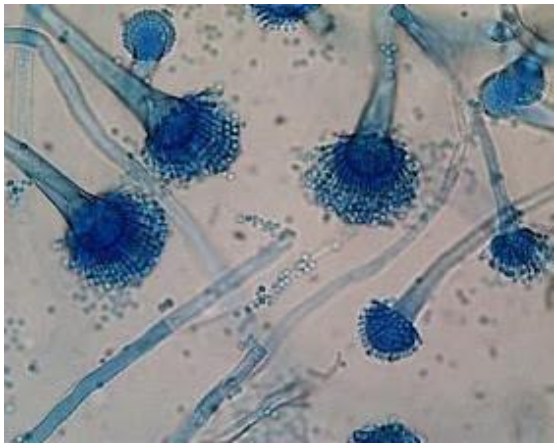
5. Made the initial examination using low power objective then Switched to higher power (40X) objective for more detailed examination of spores and other structures.

Observation

After staining fungus cell observed different parts of a cell like conidia, hypae as well as conidiophore etc.

Result

On basis of observation the type fungal organism identified as Aspergillus sp.,



Expt :11
DATE

IMViC Test

Aim: To carryout IMViC Test

Each of the letters in "IMViC" stands for one of these tests. "I" is for indole; "M" is for methyl red; "V" is for Voges-Proskauer, and "C" is for citrate, lowercase "i" is added for the ease of pronunciation. IMViC is an acronym that stands for four different tests

- Indole test
- Methyl red test
- Voges-Proskauer test
- Citrate utilization test

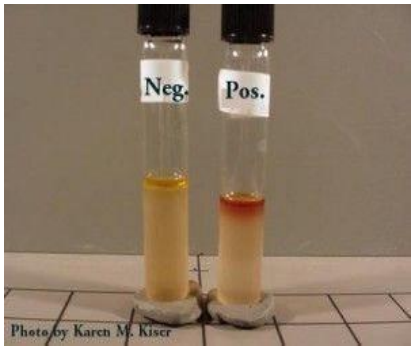
To obtain the results of these four tests, three test tubes are inoculated: tryptone broth (indole test), methyl red – Voges Proskauer broth (MR-VP broth), and citrate. IMViC tests are employed in the identification/differentiation of members of family enterobacteriaceae.

General procedure for performing IMViC Tests and their interpretations: 8 hours at 37°C and the respective tests can be performed:

Indole test

It is performed on sulfide-indole-motility (SIM) medium or in tryptophan broth, or in motility urease indole (MIU) medium. Result is read after adding Kovac's reagent.

1. The positive result is indicated by the red layer at the top of the tube after the addition of Kovács reagent.
2. A negative result is indicated by the lack of color change at the top of the tube after the addition of Kovács reagent.



Indole Test Results: Positive-development of Red-ring

Methyl red test and Voges-Proskauer test both are done in methyl red–Voges-Proskauer (MR-VP) broth, but the reagents that are added varies according to the test **Methyl**

Red (MR)Test:

- Positive methyl red test are indicated by the development of red color after the addition of methyl red reagent.
- A negative methyl red test is indicated by no color change after the addition of methyl red reagent

Voges-Proskauer (VP) test:

1. Negative test is indicated by lack of color change after the addition of Barritt's A and Barritt's B reagents.
2. A positive Voges-Proskauer test is indicated by the development of red-brown color after the addition of Barritt's A and Barritt's B reagents.

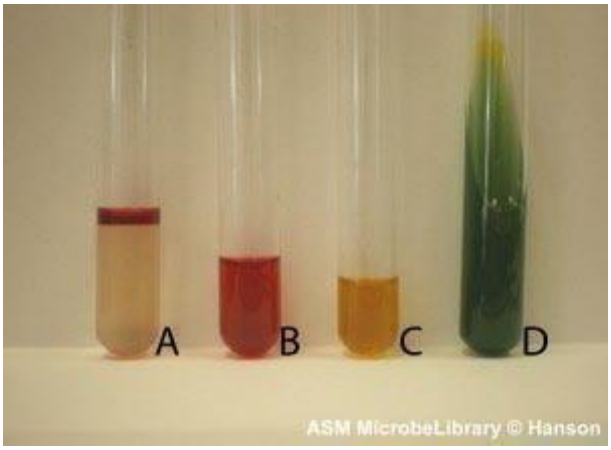
Citrate utilization test

The test is performed on Simmons citrate agar:

1. Negative citrate utilization test is indicated by the lack of growth and color change in the tube
2. A positive citrate result as indicated by growth and a blue color change.

IMViC Test results of Some Genera of Enterobacteriaceae:

1. IMViC tests of *Escherichia coli*
    1. Indole: Positive
    2. Methyl-Red: Positive
    3. Voges-Proskauer test: Negative
    4. Citrate test: Negative



IMViC Test of Ecoli: ++– (Photo source and copyright: ASM)

2. IMViC tests of *Enterobacter aerogenes*
    1. Indole: Negative
    2. Methyl-Red: Negative
    3. Voges-Proskauer test: Positive
    4. Citrate test: Positive
3. IMViC tests of *Proteus vulgaris*
    1. Indole: Positive
    2. Methyl-Red: Positive
    3. Voges-Proskauer test: Negative
    4. Citrate test: Negative
4. IMViC tests of *Citrobacter freundii*
    1. Indole: Negative
    2. Methyl-Red: Positive
    3. Voges-Proskauer test: Negative
    4. Citrate test: Positive

Expt : 12
DATE:

STERILITY TESTING OF PHARMACEUTICALS

Aim: To carryout sterility testing of different pharmaceutical products

Sterility test can be carried out by using the following two method

MEMBRANE FILTRATION : the method is to be preferred where the substances examined is an oil or ointment that can be put into a solution or non bacteriostatic solid not readily soluble in the culture medium and a soluble powder or a liquid that possesses inherent bacteriostatic and fungi static properties

This method needs goods skill and special knowledge and it also calls for the routine use of positive and negative controls. A positive control is small number of micro organism specified in separate portion of each medium.

Apparatus

The sterility test apparatus consists of a closed reservoir and a container to collect the filtrate , between which a property supported membrane of appropriate porosity is placed. Membrane generally suitable for sterility testing has nominal porosity of 0.45 micrometer , diameter about 50 mm , flow rate 55-75 ml of water . minute at a pressure of 70 mm of mercury . Cellulose nitrate are used for aqueous , oily and weakly alcoholic solutions and cellulose acetate filters for strongly alcoholic soln. complete unit should be free from microganism including the membrane , and operation should be carried out aseptically. Preferably assemble and sterilize the entire with the membrane in place prior to use.

DIRECT INOCULATION

The quantity of the substances or preparation being examined which is to be used for inoculation in the media varies according to the quality in each container.

Method of Test

1. For aqueous and suspension: remove the liquid from the test container with a sterile pipette or syringe . transfer the quality of the preparation under examination directly into the culture medium so that the volume of the preparation under examination is not more than 10 % of the volume of the medium , unless otherwise prescribed. When the quantity in a single container is insufficient to carry out the tests,the inoculated medium is incubated for days at 14 days at 30 to 35'C in the case of fluid thioglycollate medium at 20 to 25'C in the case of soyabean – casein digest medium.

REPORT:

www.rathinamcollege.edu.in/pharmacy

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PRACTICAL MANUAL

SECOND YEAR B.PHARM (III-SEMESTER)

SUBJECT: PHARMACEUTICAL ENGINEERING (BP308P)

PREPARED BY

Mr.J.Karthikeyan, M. Pharm,

Associate Professor

Department of Pharmaceutics

CONTENTS

10		Demonstration of colloid mill, planetary mixer, fluidized bed dryer, freeze dryer and such other major equipment.		
11		Effect of surface area on the rate of filtration		
		Effect of concentration on the rate of filtration		
		Effect of viscosity on the rate of filtration		
		Effect of surface area on the rate of Evaporation		
		Effect of concentration on the rate of Evaporation		
		Effect of viscosity on the rate of Evaporation		
12		To study the effect of time on the Rate of Crystallization.		
13		To calculate the uniformity Index for given sample by using Double Cone Blender.		

GENERAL LABORATORY INSTRUCTIONS

1. Never enter a laboratory without permission.
2. Enter the laboratory wearing a clean white apron and carrying other essential required materials.
3. Before starting experiment clean your work place and keep minimum things with you to avoid congestion.
4. Do not make it a practice to borrow or lend anything to colleagues.
5. Light burners only with matchstick or lighter and never light with adjacent burner or by any other means.
6. Put-off burner by closing gas connection knob and not by blowing by mouth.
7. Keep your belongings and other material properly in safe area.
8. As far as possible, keep important belongings in rack or pocket of apron for maximum utilization of workplace and to avoid spoilage.
9. Maintain silence in laboratory and ask your teacher in case of any query.
10. Use dustbin for throwing waste material and not on floors or platform or in sink.
11. Put-off electrical fans when gas burners are in use.
12. In case of gas leakage, immediately report to lab-assistant or teacher.
13. Places where chemicals, glassware and instruments are stored in laboratory are permanent. You should know all these places.
14. During any injury report immediately to your teacher for first-aid help.
15. Clean your working place after completion of experiment.
16. Always wash your hands with detergent or disinfectant and with fresh water after every experiment.
17. Do not wear aprons in the campus or outside the laboratory.
18. Do not carry costly things in laboratory which may be lost or damaged.
19. Before leaving your table – check gas connection, water supply and electricity plug and ensure they are off.

20. Students will be required to keep a "lab notebook" as a record of all work done in the laboratory.
21. Students are required to read carefully the experimental procedure before coming to the laboratory.
22. Each student must have a clean towel on hand at all times to keep his place and tools clean.
23. All equipment and bottles should be returned to the proper place after use.
24. Assume all chemicals used in the experiment are dangerous.
25. Eating or drinking in the laboratory is prohibited.
26. Do not pipette by mouth or carry reagents around the laboratory.
27. Please note the position of the safety showers.
28. Be careful in removing broken glassware from the sink.
29. Students are supposed to use the nearest reagent shelf and they must bring depleted reagents to the stockroom to be refilled.
30. Do not waste chemicals. Use just the calculated amount of chemicals.
31. Do not contaminate chemicals by using improper techniques or dirty equipment.
32. Never return any excess material from a stock bottle unless advised to do so by the instructor. There is danger of contamination.
33. When pouring from a reagent bottle, the label must be facing-up and be sure to re-stopper each reagent bottle with its own stopper.
34. Only water soluble materials are allowed to be poured into the sink.
35. The balance should be closed when it is not in use.
36. Be sure that pans are clean to avoid contamination.
37. Weighing papers must be used to protect the pans. Never place materials to be weighed directly on the pans.
38. Clean the balance immediately, if you have spilled any substance (Liquid or solid) on it.

39. After the completion of each physical experiment, a full report must be submitted to the instructor on the next day.

40. Work hard for maintaining discipline.

HANDLING OF GLASSWARES

Different types and makes of glassware are used in laboratory for performing experiments. Mostly glassware of borosilicate glass is preferred because of its clarity and heat and chemical resistance. Silica, boric acid, sodium and aluminium oxide are the components of borosilicate glass.

Washing:

1. Before use of any glassware wash them under tap water.
2. Use detergent and scrubber to clean and remove adhesive material.
3. Rinse glassware, before use with material for which it is to be used.
4. If glassware is new before use soak it in 1 percent hydrochloric acid for some time because such glassware are alkaline and may affect the results
5. If glassware contains unwanted sticky material that is difficult to remove then treat it with nitric acid or chromic acid solution.

Drying:

1. After washing keep glassware on stand for draining. Cary out drying at room temperature. Drying by rubbing with dry muslin cloth or napkin can also be suggested.
2. Glassware for volumetric procedures should be dried in hot air oven. For faster drying of glassware, it is advisable to dry them by hot air using a hair dryer.
3. Dried glassware kept covered to protect from dust and any other contaminants
   from the atmosphere.

Heating:

1. While heating any glassware do not leave it unattended because overheating may cause serious problem.
2. Keep hot glassware in right place and handle it carefully to avoid burns.
3. Do not keep hot glassware on damp or wet surface.
4. Use pair of tong or napkin to hold hot glassware.

Cooling:

1. To prevent thermal breakage and damage, cool glassware slowly.
2. Heat glassware slowly and use wire gauze to diffuse excess heat.
3. Use water bath to hold hot glassware for cooling.

Glassware care:

1. Do not leave any sticky material in glassware for long periods.
2. Glassware for reuse must be emptied immediately after use.
3. Label all glassware that contains corrosive or any other chemicals to avoid confusion and accidents.
4. Always clean glassware before and after use.

MATERIALS REQUIRED FOR PRACTICAL

It is necessary that every student must carry a small bag containing materials required to perform experiments. Bag should be checked and maintained regularly. It should contain the following:

1. Clean white apron
2. Practical record book
3. Graph paper sheets
4. Pencil, eraser, ruler, sharpener
5. Glass marker pen
6. Butter paper
7. Napkin (Clean cloth)
8. Calibrated small and big weight boxes
9. Weighing bottle
10. Self-adhesive labels of different size
11. Glass slide
12. Watch Glass
13. Scientific Calculator
14. Match box / Lighter

EX.NO:01

DATE:

DETERMINATION OF RADIATION CONSTANT OF IRON

AIM:

To determine the radiation constant of Iron cylinder

REQUIREMENTS:

Metal cylinder (made up of iron), glass tripod stands, digital thermometer, tongs.

PRINCIPLE:

1. When two objects at different temperature are brought into contact, heat flow occurs from the object having higher temperature to the object having lower temperature.
2. The total heat loss from the body to its surrounding often includes appreciable losses by conduction, convection and radiation.
3. If the body is not in contact with any surfaces the heat transfer by conduction is neglected.
4. So the heat transfer is main convection and radiation.
5. The present experiment demonstrates the pattern of cooling of a hot body by combined convection and radiation.
6. Radioactive cooling is described by Stefan-Boltzmann law which states that the rate of radiation emitted by a body.

$$Q = bAT^4 \quad \text{.....(1)}$$

Where,

Q= energy radiated per second, W (or J/S)

A= area of radiating surface m^2

T= absolute temperature of the radiating surface, K

B= constant, W/m K^4

According to equation (1) the rate of heating depends upon the temperature and surface area of the emitter. At the same time, it also depends upon the absorption capacity of the material to be heated.

The difference in the temperature of hot body and ambient is the temperature gradient for the heat loss by radiation. The radiation constant (a) is calculated using the following equation,

$$Ms\left(\frac{dq}{dt}\right) = \alpha A \left[T_1 \left\{\frac{T1}{100}\right\} T_2 \left\{\frac{\beta A}{100}\right\}\right] (T1\text{-}T_2)^{1.23}$$

Where,

M =mass of the metal cylinder, Wg

S= specific heat of the metal, J/Kg.k

Dq/dt= rate of change of temperature (rate of heat loss by metal cylinder), (K/sec) W/s

T_1 = temperature of the metal body, K

T_2 = temperature of the ambient (room temperature) K

α= radiation constant, $W/m^2 K^4$

β = convection factor

A-surface area for heat transfer, m^2

PROCEDURE:

1. A metal (iron) cylinder is weighed (wg). The average weight is noted.
2. The diameter and height of the cylinder are measured. The radius of the cylinder calculated. The surface area of the cylinder is calculated.
3. Keep it over a metal tripod and heated by using Bunsen burner.
4. After reaching a constant maximum temperature, the hot body (metal cylinder) is transferred to the glass tripod stand using tongs.
5. Insert the digital thermometer to the central hole present in the cylinder and fixed to a stand using a thread (a simple 360°C thermometer can be used).
6. Slowly the temperature of the hot body decreases. The decrease in temperature is noted every 5 minutes interval. The data are recorded.
7. A graph is plotted by taking time (minutes) on x-axis and temperature on y-axis. Normally a curve is observed.
8. Depending on the temperature at which the radiation constant is to be determined, a tangent is drawn at that temperature, the slope of the tangent is calculated, which represents the rate of loss of heat (dq/dt) by the hot body.
9. Radiation constant is determined at that temperature.
10. The same procedure is repeated with cylinders made by different metals.

RESULT:

The radiation constant of Iron cylinder was

EX.NO: 02

DATE:

DETERMINATION OF RADIATION CONSTANT OF BRASS

AIM:

To determine the radiation constant of the given brass

REQUIREMENTS:

Metal cylinder (made up of brass), glass tripod stands, digital thermometer, tongs

PRINCIPLE:

1. When a brass cylinder is heated to a temperature and suspended in air, the cylinder losses its heat by radiation and convection
2. Heat transmission takes place by means of energy transfer using air as medium of transmission as electromagnetic waves
3. A black body is a perfect emitter and absorber for the total art of radiation fall on it
4. The total amount of radiation emitted by a black body may be calculated by using Stephan Boltzmann law

$$Q= bAT^4$$

Q= energy radiated per second, W (or J/S)

A= area of radiating surface m^2

T= absolute temperature of the radiating surface, K

B= constant, $W/m^2 K^4$

Radiation constant is calculated using the following equation

$$Ms(\frac{dq}{dt}) = \alpha A\ [T_1 \{\frac{T1}{100}\} T_2\ \{\frac{\beta A}{100}\}]\ (T1\text{-}T_2)^{1.23}$$

Where,

M =mass of the metal cylinder, wg

S= specific heat of the metal, J/Kg.k

Dq/dt=rate of change of temperature (rate of heat loss by metal cylinder), (K/sec) W/s

T_1 = temperature of the metal body, K

T_2 = temperature of the ambient (room temperature) K

A= radiation constant, $W/m^2 K^4$

β = convection factor

A-surface area for heat transfer, m^2

PROCEDURE:

1. Select a brass cylinder whose surface is smooth to perform the experiment.
2. Weigh it on a weighing balance and record the weight in the observation table.
3. The radius of the cylinder measured and recorded in the observation table.
4. Insert the digital thermometer to the central hole present in the brass cylinder and fixed to a stand using a thread (a simple 360°C thermometer can be used).
5. Place the cylinder on tripod stand and heat the brass cylinder with the help of Bunsen burner or with a heating device to above 300°C
6. Allow the brass cylinder to receive a temperature of above 300°C
7. Keep readily a glass tripod stand.
8. Ensure that the temperature reading of the thermometer is above 300°C.
9. Now hold the brass cylinder with tong and transfer carefully rom the iron tripod stand onto a glass tripod stand without touching any surface and ensure that the brass cylinder is kept properly on the glass tripod stand.
10. Note the temperatures from the thermometer at every 5 minutes interval and record in the observation table.
11. Draw a curve by using data obtained from the experiment, by taking time in minutes on X-axis an temperature on y-axis
12. Draw a tangents at various temperatures and determine its slope dq/dt
13. From the data collected, calculate the radiation constant by using equation (2), draw a graph between arbitrary temperature and corresponding radiations constants and observe the effect of temperature on (a) values

RESULT:

EX.NO:03

DATE:

DETERMINATION OF RADIATION CONSTANT OF GLASS (UNPAINTED AND PAINTED)

AIM:

To determine the radiation constant of glass (unpainted and painted)

REQUIREMENTS:

Round bottom flask with big neck (500m, glass tripod stands, digital thermometer mgs painted (white green collared) round bottom flasks with long neck

PRINCIPLE:

1. Heat transfer by addition involves the transfer of energy in the form of electromagnetic waves
2. This becomes significant at higher temperatures
3. All solid bodies radiated energy when their temperatures are above absolute zero
4. A The radiant energy emitted by a hot body is expressed by Stefan-Boltzmann law which states that the rote of radiation emitted by a body

$$Q= bAT^4$$

Where,

Q= energy radiated per second, W (or J/S)

A= area of radiating surface m^2

T= absolute temperature of the radiating surface, K

B= constant, $W/m^2 K^4$

PROCEDURE:

1. A round bottom flask (unpainted) is cleaned and dried.
2. The weight of the flask is determined (M_2 kg).
3. The diameter (D) of the round bottom flask is determined.
4. The diameter (d) of neck of the flask is determined.
5. Boiled hot water is prepared and a measured volume of hot water is transferred to the flask carefully. The volume of water is recorded (M). The external surface of the round.
6. Thermometer (110'C) s dipped to the centre of the flask and tied at the top to an iron stand.
7. Slowly the temperature of the hot body (water) decreases, the decrease in temperature is noted every minute.

8. A graph is plotted by taking time (minute) on x axis and temperature on y axis. Normally a curve is obtained
9. Depending on the temperature at which radiation constant is determined, a tangent is drawn at that temperature. The slope is calculated. This parameter is related to the rate of heat loss (dg/dt)
10. Radiation constant (a) is determined at the temperature. The same procedure is repeated with round bottom flask painted with silver oxide (white colour) and green colour.

RESULT:

EX.NO:04

DATE:

STEAM DISTILLATION

AIM: To calculate the efficiency of steam distillation

REQUIREMENTS:

Steam generator, distillation flask, condenser, separating funnels, nitrobenzene, double holed rubber cork, measuring cylinder, thermometer, bent tubes, weighing balance and weights.

PRINCIPLE:

Steam distillation is a separation process for temperature sensitive substances. It is a way of separating miscible liquid based on their volatilities. The boiling points of the products are minimized, permitting the constituents to be vaporized. Here no chemical reaction takes place. The fundamental nature of steam distillation is that is enables a compound or mixture of compounds to be distilled (and subsequently recovered) at a temperature substantially below that of the boiling points of the individual constituents. For example: essential oils contain substances with boiling points up to 200°C or higher, including some that are solids at normal temperatures. In the presence of steam or boiling water, however this mixture of hot vapours will, if allowed to pass through a cooling system, condense to form a liquid in which the oil and water comprise two distinct layers.

PROCEDURE:

1. Measure and take 50ml of nitrobenzene in a distillation flask.
2. Close the flask with two holed rubber cork. Insert thermometer through one of the hole sand fix.
3. Through the other hole, fix a bent tube of the steam generator for the steam to pass into the distillation flask.
4. Ensure that a bent tube reaches almost near bottom of the flask but not touching the flask
5. Fix the side tube of the neck of the distillation flask to the condenser.
6. Now allow the steam to pass into the distillation flask continuously from the steam generator.
7. When the thermometer shows a constant temperature at which the mixture of nitrobenzene and steam distils, collect the distillate for 10 minutes into a previously weighed beaker.
8. Determine the weight of the mixture and beaker.
9. Transfer the distillate into a separate funnel and add a few grams of sodium chloride. Shake the funnel vigorously till nitrobenzene and water gets separated.
10. Collect nitrobenzene separately and denotes its weight.
11. Collect water separately and denotes its weight.

RESULT:

Percentage efficiency of steam distillation =

EX.NO:05

DATE:

HEAT TRANSFER COEFFICIENT

AIM:

To determine the overall heat transfer of a heat exchanger (glass cylinder/insulated glass cylinder)

REQUIREMENTS:

Steam generator, water condenser (insulated and non-insulated), thermometer, 110°C, bent tube.

PRINCIPLE:

1. The overall heat transfer coefficient of a glass through one end of the apparatus and the cold fluid is passed through the other end, this arrangement is known as counter-current or counter flow method.

$$U = Q / A \times \Delta tav$$

Where,

Q= amount of heat transferred, w (J/S)

A= surface area of the glass tube, m^2

Δtav = temperature gradient, K

U=overall heat transfer coefficient, w/m^2 K

$$Q = \frac{Q_1 - Q_2}{2}$$

Where, Q_1 = Heat loss by steam W (J, S)

Q2 = Heat gain by cold body W (J, S)

Heat loss by hot body may be expressed as

$$Q1 = M1.L + M1\ S.t1$$

And heat gain by the cold body may be expressed as

$$Q2 = M2.S.t2$$

Where, M1 = Mass of condensed steam, kg

M2 = Mass of circulating water, kg

S = Specific heat of steam, J/ kg.k

L= latent heat of vaporization of water, J/kg

T1 = Temperature drop on steam, k

T2 = temperature rise on the circulatory water side, k

PROCEDURE:

1. Select a steam generator and ensure that is not having any leak and fill sufficient water.
2. Also select a condenser which is having the facility of circulating distillation column.
3. Connect the bent tube of steam generator (through which steam flows after heating) to the inlet of the condenser tube.
4. Arrange for circulation of cold water around the inner tube of condenser by connecting it to a water tap.
5. Also connect the outlet of the inner tube of the condenser to a water collecting drum.
6. Ensure that all the connections and experimental set up is properly made before applying heat to generates steam
7. Now heat the water in the steam generator and ensure that steam passes through the condenser tube.
8. Allow the condensation process to continue for about 5 minutes
9. Stop the water circulation from the inner tube of the condensate by stopping tap water and put off the heating of water by means of putting off steam generator.
10. Collect the circulating water from exit and condensate, the difference gives the temperature drop t_1
11. Note that the temperature of water at exit and entrance points (temperature at inlet and outlet points) and difference gives temperature rise t_2.
12. Measure volume of condensate collected (M_1)
13. Measure volume of water collected (M_2).
14. New change speed of circulating water and repeat the whole experiment.
15. Determine overall heat transfer coefficient for this repeated experiment also.
16. Now calculate the average rate of overall heat transfer coefficient by means of equation.

RESULT:

EX.NO:06

DATE:

CONSTRUCTION OF DRYING CURVES

AIM:

To dry Calcium carbonate and Starch

To plot the rate of drying curve for Calcium Carbonate and Starch

REQUIREMENTS:

Apparatus: tray or Petri dish, hot air oven, thermometer, spatula, weighing balance.

Chemicals: calcium carbonate, starch.

PRINCIPLE:

Drying is defined as the removal of small amounts of water or other liquid from a material by application of heat. Drying involves both heat and mass transfer operation. Heat must be transferred to the material to be dried in order to supply the latent heat required for vaporization of the moisture. Mass transfer involves the diffusion of water through the material to the evaporating surface and subsequent evaporation of the water from the surface.

The behaviour of drying of solids is explained by drying curve. The time required for drying a batch of material in a dryer, can be estimated with the help of drying curve. The difference in two successive weights gives the loss of moisture. The rate of drying can be calculated by the formula.

$$\text{Rate of drying} = (Wn-Wn-1) / \Delta t.A$$

Where

A = area of the plate exposed to drying
Wn = Weights of glass dish + sample slurry
Wn+1 = Weight of glass dish + sample slurry after time t
Δt = time interval

PROCEDURE:

1. Take a clean tray or petridish without lid and determine its weight as W_1 gm.
2. Determine the area of the tray or petridish A and its weight is W_1 gm.
3. Take 10gm of calcium carbonate in the tray or petridish. Let the weight of the tray and powder is W_2 gm.
4. Add water and make slurry, let its weight be W_3 gm.
5. Keep tray or petridish in a hot air oven with temperature maintained at 70°C.
6. Continue drying and determine the weight of the sample at every 15 minutes. Stop drying when there is no change in the weight of the sample.
7. Determine the percentage of moisture content and drying rate for each time interval using the following equation

Percentage moisture content = $(W_3-W_2 / W_2 - W_1) \times 100$

Drying rate = $(W_3-W_2$ / Area of petridish) x Time

8. Plot a graph of drying curve by taking percentage moisture content on x-axis and drying rate on y-axis.

REPORT:

1. Drying rate curve is plotted with average Moisture Content on X- axis and Rate of drying on Y – axis

2. Moisture Content of

    1. Calcium carbonate =

    2. Starch =

EX.NO:07

DATE:

MOISTURE CONTENT AND LOSS ON DRYING

AIM:

To determine the Moisture Content and Loss on Drying

REQUIREMENTS:

Apparatus: tray or petridish, hot air oven, spatula, weighing balance.

Chemicals: calcium carbonate.

PRINCIPLE:

The rate of drying is determined by moisture content and the temperature of the compound and the temperature, the humidity and the velocity of the air in contact with the compound. Equilibrium moisture content (EMC) is the amount of water present in the solid, which exerts a vapour pressure equal to the vapour pressure of the atmosphere surrounding it. When air is continuously passed over the solid containing moisture more than EMC, then solid losses water continuously till EMC is reached. This phenomenon is known as desorption. When air is continuously passed over the solid containing moisture less than EMC, then solid absorb water continuously till EMC is reached. This phenomenon is known as sorption. From this observation, it is clear that material can be dried up to EMC, but not below it.

In this experiment, EMC of calcium carbonate is determined by exposing them, to different humidity condition at room temperature. With the data obtained, a graph is plotted by taking time versus moisture content versus rate of drying.

PROCEDURE:

1. Take empty petridish and measure its weight and diameter.
2. Add 10gm of calcium carbonate powder to it and slowly with stirring to prepare a paste.
3. Spread the prepared paste evenly in the petridish and take its weight.
4. Determine the moisture content (initial) of prepared slurry.
5. Place the petridish in hot air oven at 60°C and determine its weight after 10 minutes.
6. Repeat the procedure after every 10 minutes till weight of petridish become constant.
7. Calculate rate of drying for each time
8. Plot graphs of moisture content vs time, drying rate vs moisture content and rate of drying vs time.
9. Determine EMC and CMC from a graph of moisture content vs time. Also calculate FMC.

RESULT:

The drying parameters for calcium carbonate are:

1. Equilibrium Moisture Content (EMC) =
2. Critical Moisture Content (CMC) =
3. Free Moisture Content (FMC) =

EX.NO:08

DATE:

RELATIVE HUMIDITY OF AIR BY DEW POINT METHOD

AIM:

To determine the Relative Humidity of air by dew point method

REQUIREMENTS:

Thermometer, beaker, absorbent cotton, water, ice, stand, humidity chart.

PRINCIPLE:

1. When the surface temperature reaches the dew point temperature, dew will form.
2. water vapour, like other gases, moves at sonic speeds and continually strikes the surface.
3. When the surface temperature is at the dew point, more water molecules will condense onto the surface than evaporate from the surface.
4. Hence, dew form when the number of water molecules striking the surface and forming hydrogen bonds with other water molecules is bigger than the number of molecules breaking hydrogen bonds and separating off as a gas.

PROCEDURE:

1. Note down the atmospheric temperature by using thermometer and record it as dry bulb temperature.
2. Take 100ml beaker containing crushed ice and stir it slowly.
3. Note the temperature at which fog starts appearing as T
4. Note the temperature at which fog starts disappearing as T:
5. Take average of T, and T2 denoted as dew point.

RESULT:

EX.NO:09

DATE:

DETERMINATION OF HUMIDITY OF AIR BY USING WET AND DRY BULB TEMPERATURE OR PSYCHROMETER.

AIM:

REQUIREMENTS:

Thermometer, beaker, absorbent cotton, water, ice, stand, humidity chart, sling psychrometer.

PRINCIPLE:

1. The wet-bulb temperature is the lowest temperature which may be achieved by evaporative cooling of a water wetted (or even ice-covered), ventilated surface.
2. Suppose we gave a thermometer and it is wrapped in a cloth moistened with water that time the faster the water will evaporate.
3. The faster the water evaporates, lower the thermometer will be relative to air temperature.
4. If the relative humidity 100%, no water will evaporate and the thermometer will read the same as an unwrapped thermometer.
5. This principle is why we feel cooler in dry air.
6. The sweat evaporates quicker cooling down the skin in dry air.
7. For air at a known pressure and dry-bulb temperature, the thermodynamic wet-bulb temperature corresponds to unique values of the relative humidity and the dew point temperature.
8. It therefore may be used for practical determination of these values. The relationships between these values are illustrated in a psychometric chart.

PROCEDURE:

1. Note down the atmospheric temperature by using thermometer and record it as dry bulb temperature.
2. Take 100ml beaker which is half filled with water.
3. The absorbent cotton around the bulb of thermometer and keep in a beaker in such a way that only cotton dips in water and not the bulb of thermometer.
4. Allow saturation to achieve and note down minimum temperature observed denoted as the wet bulb temperature.
5. Calculate various parameters using humidity chart.

PROCEDURE USING SLING PSYCHROMETER:

1. Take sling psychrometer and keep the bulb of dry thermometer as such and moist the cloth wick of second thermometer with distilled water.
2. Whirl the sling psychrometer through the air with movable handle so that desired air velocity passes the wet bulb.
3. Note down the dry and wet bulb temperature and find out the difference.

4. By referring slandered table determine the humidity by considering the difference of dry and wet bulb temperature.

RESULT:

EX.NO:10

DATE:

DETERMINATION OF DEW POINT TEMPERATURE BY DEW POINT METHOD

AIM:

REQUIREMENTS:

Thermometer, beaker, absorbent cotton, water, ice, stand, humidity chart.

PRINCIPLE:

1. When the surface temperature reaches the dew point temperature, dew will form.
2. Water vapour, like other gases, moves at sonic speeds and continually strikes the surface.
3. When the surface temperature is at the dew point, more water molecules will condense onto the surface than evaporate from the surface.
4. Hence, dew form when the number of water molecules striking the surface and forming hydrogen bonds with other water molecules is bigger than the number of molecules breaking hydrogen bonds and separating off as a gas.

PROCEDURE:

5. Note down the atmospheric temperature by using thermometer and record it as dry bulb temperature.
6. Take 100ml beaker containing crushed ice and stir it slowly.
7. Note the temperature at which fog starts appearing as T
8. Note the temperature at which fog starts disappearing as T:
9. Take average of T, and T2 denoted as dew point.

RESULT:

EX.NO:11

DATE:

DESCRIPTION OF CONSTRUCTION, WORKING AND APPLICATION OF PHARMACEUTICAL MACHINERY SUCH AS ROTARY TABLET MACHINE, FLUIDIZED BED COATER, FLUID ENERGY MILL AND HUMIDIFIER

AIM:

REQUIREMENTS:

Rotary tablet machine, fluidized bed coater, fluid energy mill and humidifier. Rotary tablet machine

PRINCIPLE:

1. Rotary tablet machine is used to high production of tablets.
2. Tablet press also referred to as tableting machine, pharmaceutical tablet press, tablet comprising machine or tablet punching machine is a mechanical device that compresses powdered into tablets of uniform size, shape and weight containing approximately the same quantity of active pharmaceutical ingredient (API) and excipients.
3. All tablet presses employs the basic principle of compression.

ADVANTAGES OF SINGLE PUNCH MACHINE:

1. The single punch structure is rational and small.
2. Easy to operate and it operates at a high utilization ratio.
3. It can manufacture odd shaped products with a diameter of up to 20mm.
4. It is ideal for development of tablets and small batch production.

CONSTRUCTION:

HOPPER:

The hopper holds the granules/powder mixture (API+ recipients) that is compressed into tablets.

DIE CAVITY:

This is where the powder granules is compressed into tablets and it determines the diameter of the tablets, the size of the tablets and to some extent the thickness of the tablets.

FEED PADDLE:

Helps to force the feed the granules into the dies especially during faster rotation.

Punches:

This comprises the upper and the lower punches. They move within the die bore to compress granules into tablets.

LOWER CAM TRACK:

This guides the lower punches during the filling stage so that the die bore is over filled to allow accurate adjustment.

CAM TRACK:

This guides the movement of both the upper and lower punches.

DEPT OF FILL/CAPACITY CONTROL:

This adjustment the lower punch track during the latter part of the fill stage to ensure that the appropriate quantity of granules remains within the die prior to compression.

PRE-COMPRESSION ROLLERS:

This roller gives the granules an initial compression force to get rid of excess air that might be entrapped in the die.

MAIN COMPRESSION:

This roller applies the final compression force needed for the formation of tablet.

EJECTION CAM:

Guides the lower punch upwards facilitating the ejection of tablet from the die cavity after compression.

TAKE-OFF BLADE:

This is formed in front of the feeder housing and it deflects the tablet down the discharge chute.

DISCHARGE CHUTE:

This is where the tablet passes through for collection after being deflected by the take-off blade

Working:

1. The process is essentially the same as the single press in that the tablets are produced by compression between upper and lower punches within a die.

2. In a multiple station press there are many sets of tools (punches and dies) and these are accurately aligned in such a way that the whole head compressing die table and turrets uniformly rotate in the horizontal plane.

Filling:

Material to be formed into tablets is placed/fed into the fixed hopper which them feeds a fixed frame that fills several dies simultaneously as the die table rotates.

Compression:

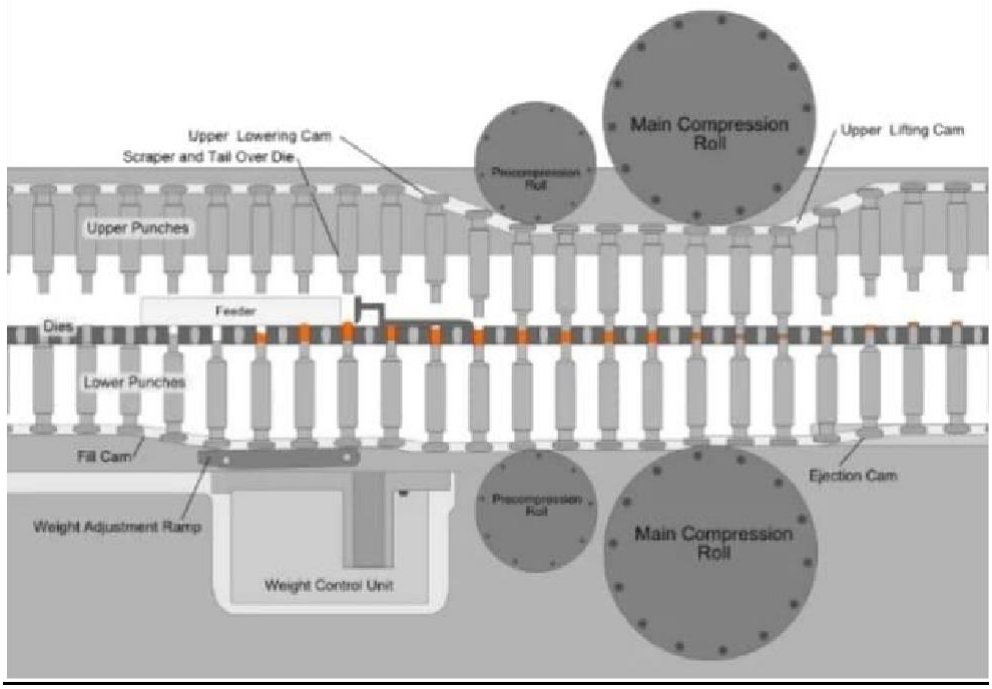
1. Compression, as with the single station press, is performed by the punches which are controlled by cam tracks and rollers that guide the movement of the punches as the table rotates.
2. The compression sequence starts by the lower cam guide pulling a lower punch to the bottom of the dies.
3. This action allows particular die to be over filled with powder.
4. The lower punch will then rise according to a weight control process at which point excess powder is removed by a swipe blade.
5. The blade pushes material into the oncoming die.
6. The lower punch drops slightly allowing the upper punch to penetrate into the die and contact the upper surface of the powder. This is the point at which compression begins
7. In modern presses, there is a pre-compression step in which both punches are forced by pre-compression rollers to squeeze the powder to form a tablet within the die. As the tablet rotates so the punches are engaged by the main compression rollers which are substantial rolls, exerting a massive force on the powder.
8. Both punches have forces applied and both move to compress the tablet (unlike the single table press in which only the upper punch moves during compression step).

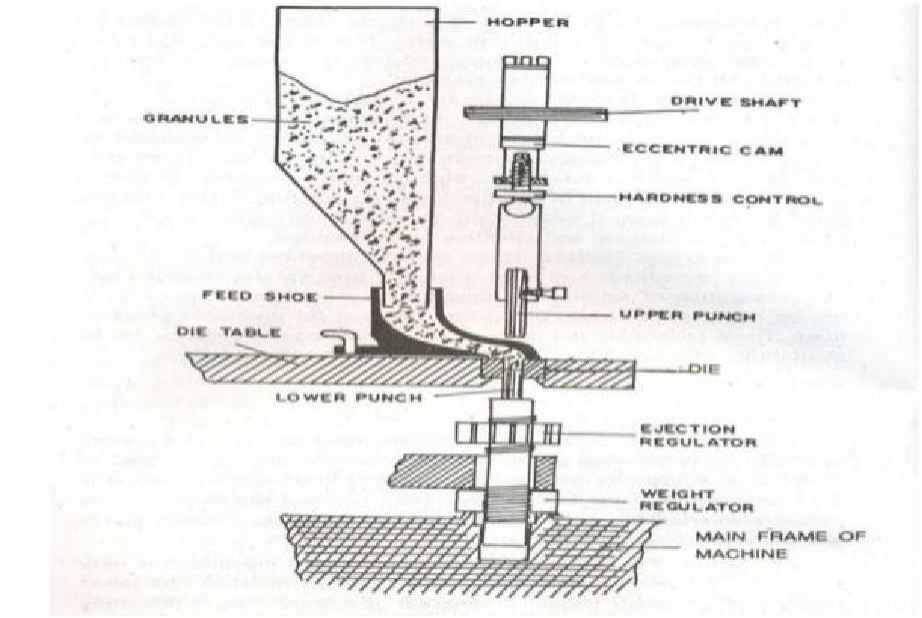
EJECTION:

1. After the full compression force has been applied, the upper punch is withdrawn by the upper raising cam, and the lower punch also rises to bring the tablet above the surface of the die (and die table).
2. At this point the tablet is fully formed and is swept off the die table to catch the tablets.
3. The lower punch will then be engaged by the pull-down cam to be withdrawn to the bottom of the die, and the whole process starts again.

ADVANTAGES OF ROTARY PRESS:

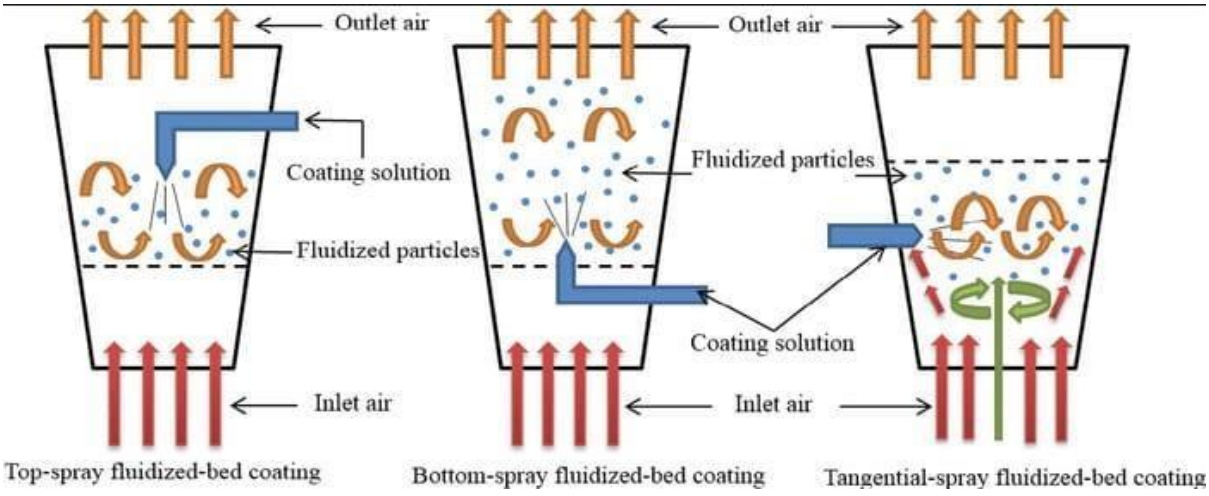
1. High productivity can be gained with a minimal amount of labour while saving money.
2. Rotary press has an output of between 9000-234000 tab/hour thus save time and meets up with the high demand of tablet dosage form.
3. The powder filled cavity can be automatically managed by a moving feeder.
4. Rotary press decreases waste of valuable formulation in non-specific tablets.
5. The machine allows independent control of both weight and hardness.


Upper Lowering Cam
Scraper and Tail Over Die
Precompression Roll
Main Compression Roll
Upper Lifting Cam
Upper Punches
Feeder
Dies
Lower Punches
Fill Cam
Ejection Cam
Weight Adjustment Ramp
Weight Control Unit
Precompression Roll
Main Compression Roll


HOPPER
GRANULES
DRIVE SHAFT
ECCENTRIC CAM
HARDNESS CONTROL
FEED SHOE
UPPER PUNCH
DIE TABLE
DIE
LOWER PUNCH
EJECTION REGULATOR
WEIGHT REGULATOR
MAIN FRAME OF MACHINE

FLUIDIZED BED COATER:

1. The fluidized bed coater used to coat particles, spheres, granules and tablets.
2. Systems have been developed for use with a variety of coating formulations including aqueous/organic solvents, hot saturated solutions and hot metals.



Principle:

In this process the spray is done inside hollow cylinder from the bottom or top or may be from side and pellets are allowed in small quantity to enter the hollow cylindrical tube.

Construction:

Fluid bed processor systems (distinct types of spray systems):

a) Top spray

b) Bottom spray

c) Tangential spray.

TOP SPRAY IN FLUID BED COATING MACHINE:

1. With top spray coating in the fluid bed, particles are fluidized in the flow of heated air, in which is introduced into the product container via a base plate.
2. The coating liquid is sprayed into the fluid bed form above against the air flow (counter current) by means of a nozzle.
3. Drying takes place as the particles continue to move upwards in the air flow.

Small droplets and a low viscosity of the spray medium ensure that the distribution is uniform.

4. The product is continuously fed into one side of the machine and is transported onwards via the sieve bottom by means of the air flow.
5. Depending on the application, the system is sub divided into pre heating zones, spray zones. The dry and coated particles are continuously extracted.

BOTTOM SPRAY IN FLUID BED SYSTEM (FURSTER COATING)

1. In the wurster process, a complete sealing of the surface can be achieved with a low uses of coating substance.
2. The spray nozzle is fitted in the base plate resulting in a spray pattern that is concurrent with the air feed.
3. By using a wurster cylinder and a base plate with different perforations, the particles to be coated are accelerated inside the wurster tube and fed through the spray cone concurrently.

BOTTOM SPRAY COATING (CONTINUOUS FLUID BED):

1. Particularly suitable for protective coating/color coating where the product through put rates are high.
2. The product is continuously fed into one side of the machine and is transported onwards via the sieve bottom by means of the air flow.
3. Depending on the application the system is sub divided into pre heating zones and drying zones whereby spraying can take place from below in the form of a bottom spray.
4. The dry and coated particles are continuously extracted.

TANGENTIAL SPRAY COATING (ROTOR PELLET COATING):

1. Ideal for high solid content.
2. The product is set into a spiral motion by means of a rotating base plate, which has air fed into the powder bed at its edge.
3. The spray nozzle is arranged tangentially to the rotor disc and also spray concurrently into the powder bed.
4. Very thick film layers can be applied by means of the rotor method.

Working of wurster coater:

1. The basic concept of wurster coating is to separate the particles in the fluid bed from one another in an air (gas) stream.
2. 2 While the particles are suspended a coating formulation is sprayed from the bottom of the bed up to onto the particles (bottom up spray).
3. The process takes place inside a specially modified fluid bed that is divided into 2 zones by a partition
4. The inner area is a high velocity zone that separates the particles and pneumatically transports them past the spray nozzle.
5. After passing the nozzle, the particles enter the expanded area of the chamber, slow down
   and fall back in to the outer section of the fluid bed product bowl.
6. The coating dries while the particles are suspended to prevent agglomeration from occurring when they enter tranquil part of the bed.
7. The coated particles in the tranquil storage area remain fluidized just enough to allow them to continue moving towards the bottom of the bowl.
8. When the particles reach the bottom they are draw back into the high velocity air stream and the cycle is repeated.
9. This process continues until the desired level of coating has been achieved.

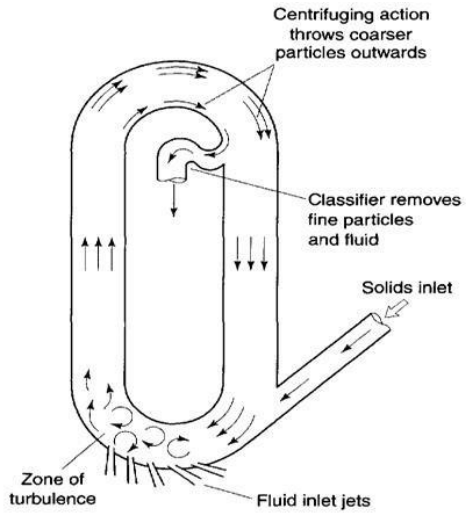
APPLICATIONS:

1. A top coating fluid bed process is common in the fine chemical industry, animal feed and food processing.
2. A bottom spray coating fluid bed process is commonly used in powder and granules coating in the pharmaceutical industry.
3. It provides a uniform and excellent coating on every material.
4. Again in this process, to ensure efficiency and high quality of the final product, it is important to monitor all process variables. This helps to maintain them to a certain level where an optimal result can be realized.
5. The bottom spray in a fluid bed system ensures an even and uniform coating, hence an optimal film quality.

FLUID ENERGY MILL

PRINCIPLE:

Size reduction is effected by combined impact and attrition



FLUID ENERGY MILL

PHOTO CREDIT: AULTON'S PHARMACEUTICS: THE DESIGN AND MANUFACTURE OF MEDICINE

CONSTRUCTION AND WORKING:

1. It consists of a grinding chamber. It is surrounded by a hollow pipe. Solids are introduced into the grinding chamber through the hopper.
2. Air passed at high pressure through the side tube. As the air enters through the nozzles (present at the bottom of the hollow pipe) into the grinding chamber, it carries the solid materials, giving rise to a high velocity circulation in a very turbulence condition.
3. As a result of the high degree of turbulence, impact and attrition occur between rapidly moving particles.
4. The fine particle is passed through the outlet and is collected in a bag collector. The larger particles are carried to the periphery by centrifugal force.
5. The circulating air carries these larger particles and giving rise to a high velocity, again size reduction is effected and the process is repeated.

ADVANTAGES:

1. Contamination of the product is no possible.
2. Heat is not generated during this process.
3. It reduces the particle size to 85 meshes (5µ size or less).

APPLICATIONS:

1. Fluid energy mill is used when fine powders are required.
2. It is used for grinding of heat sensitive substances.

HUMIDIFIER:

A humidifier is a device that increases humidity in a single room or an entire building

PRINCIPLE:

1. In cooling and humidification process the moisture is added to the air by passing it over the stream or spray of water which is at temperature lower than the dry bulb temperature of the air.
2. When the ordinary air passes over the stream of water, the particles of water present within the stream tend to get evaporated by giving up to the heat to the stream.
3. The evaporated water is absorbed by the air so its moisture content increases, thus the humidity increases.
4. At the same time, since the temperature of the absorbed moisture is less than the DB bulb temperature of the air, there is reduction in the overall temperature of the air.
5. Since the heat is releases in the stream or spray of water, its temperature increases.

CONSTRUCTION AND WORKING:

TYPES OF HUMIDIFIER:

Most popular technologies are:

I. Steam
II. Impeller
III. Wick/evaporative system.

Steam:

1. Often referred to as a vaporizer, a steam humidifier boils water and releases the warm steam into the room
2. This is the simplest and therefore the least expensive technology for adding moisture to the air.

Impeller:

1. In this humidifier a rotating disc flings water at a comb-like diffuser.
2. The diffuser breaks the water into the fine droplets that float into the air.

Ultrasonic:

1. An ultrasonic humidifier uses a metal diaphragm vibrating at an ultrasonic frequency, much like the element in a high-frequency to create water droplets.
2. An ultrasonic humidifier is usually silent and also produces a cool fog.

Wick/evaporative system:

1. The wick system uses a paper, cloth or foam wick or sheet to draw water out of the reservoir.
2. The higher the relative humidity, the harder it is to evaporate water from the filter, so this type of humidifier is self-regulating, as humidity increases the humidifier's water-vapour output naturally decreases.

Result:

EX.NO:12

DATE:

EVALUATE SIZE DISTRIBUTION OF TABLET GRANULATIONS (SIZEANALYSIS) BY SIEVING METHOD AND CONSTRUCT SIZE FREQUENCY CURVESINCLUDING ARITHMETIC AND LOGARITHMIC PROBABILITY PLOTS

AIM:

To evaluate size distribution of tablet granulations (size analysis) by sieving method and construct size frequency curves including arithmetic and logarithmic probability plots.

REQUIREMENTS:

Apparatus: sieves of different numbers, weighing balance and required weights, sieve stand with mechanical shaker

Chemicals: powder sample

PRINCIPLE:

1. Sieve analysis consists of determination of particle size distribution of a solid material by determining the amount of powder retained on a series of sieves with different size apertures
2. The weight of the sample of each sieves is then divided by the total weight to give a percentage retained on each sieve.
3. The size of the average particle on each sieve is then analysed to get a cut-off point or specific size range.
4. The results of this test are used to describe the properties of the aggregate.
5. The results of this test are provided in graphical form to identify the types of gradation of the results are presented in a graph of percent passing versus the sieve size
6. On the graph the sieve size scale is logarithmic. To find the percent of aggregate passing through each sieve, first find the percent retained in each sieve. To do so, the following equation is used the aggregate

$$\% \text{ retained} = (W_{sieve} / W_{total}) \times 100\%$$

Where,

$W_{sieve=}$ weight of aggregate in the sieve

W_{total}=weight of the aggregate

7. The next step is find the cumulative percent of aggregate retained in each sieve.
8. Add up the total amount of aggregate that is retained in each sieve and the amount in the previous sieves
9. The cumulative percent passing of the aggregate is found by subtracting the percent retained from 100%.

% cumulative passing = 100%-% cumulative retained.

10. The values are then plotted on a graph with cumulative percent passing on the y-axis and logarithmic sieve size on the x-axis.

PROCEDURE:

1. Arrange set of sieve in descending order of their size. (a top sieve number 10. Below which place sieve no.20, 40, 60, and 80,100 respectively and at bottom 120). Place receiver at the bottom of sieves to collect the sample.
2. Weigh accurately given sample and pour on the top sieve and place the lid to avoid loss during shaking.
3. Operate the sieve-shaking machine for about 1-5min.
4. Collect fractions of sample retained on each sieve and on receiver at the bottom of set.
5. Calculate percent frequency of each size of particles.
6. Determine geometric mean weight diameter and geometric standard deviation.
7. Plot a graph of particle size vs. percentage weight retained and log particle size vs cumulative percentage weight retained.

RESULT:

EX.NO:13

DATE:

VERIFY THE LAWS OF SIZE REDUCTION BY USING BALL MILL AND CALCULATE KICKS, RITTINGER'S BOND'S COEFFICIENT, POWER REQUIREMNTS AND CRITICAL SPEED OF BALL MILL

AIM:

To verify the laws of size reduction by using ball mill and calculate kicks, rittinger's bond's coefficient, power requirements and critical speed of ball mill**.**

REQUIREMENTS:

Apparatus: ball mill, sieve shaker, sieve set, balance.

Chemical: powder sample.

PRINCIPLE:

1. A ball mill consisting hallow cylinder mounted on a metallic frame such that it can be rotated along its longitudinal axis with balls of different diameter which occupy 30-50%of the mill volume and its size depends on the feed and mill size.
2. The large balls tend to break down the coarse feed materials and the smaller balls help to form fine product by reducing void spaces between the balls.
3. Ball mills grind material by impact and attrition.
4. The degree of milling in a ball mill influenced by:
    - Residence time of the material in the mill chamber.
    - The size, density and number of the balls.
    - The nature of the balls (hardness of the grinding material).
    - Feed rate and feed level in the vessel.

PROCEDURE:

1. The initial dial reading of energy matter is noted as N_1
2. The cleaned metal chamber (of ball mill) is taken with sufficient number of balls.
3. The ball mill is operated without load for 30 min.
4. The reading (revolution) in energy meter is noted down as N_2 (The difference ie.$N_3 – N_2$-N_1, gives the energy required for running the ball mill without feed).
5. 100gms of sample is weighed and subjected to sieve analysis. The average particle size of the sample is calculated.
6. 100gms of feed, which is subjected to sieve analysis, is transferred into the ball mill.
7. The ball mill is operated for 30 min.
8. The revolution (reading) is noted down as N_4The difference i.e. $N_5= N_4$-N_2 gives the energy required for running the ball mill and size reduction of material).

9. The difference i.e., $N_6 = N_5 - N_3$ gives the energy actually consumed for the size reduction of material
10. The product is unloaded onto a tray and subjected to sieve analysis.
11. The average particle size of the product after size reduction is determined.
12. The data is substituted kick's constant, rittinger's constant and bond's work index respectively.

RESULT:

EX.NO:14

DATE:

DEMONSTRATION OF COLLOID MILL, PLANETARY MIXER, FLUIDIZED BED DRYER, FREEZE DRYER AND SUCH OTHER MAJOR EQUIPMENT

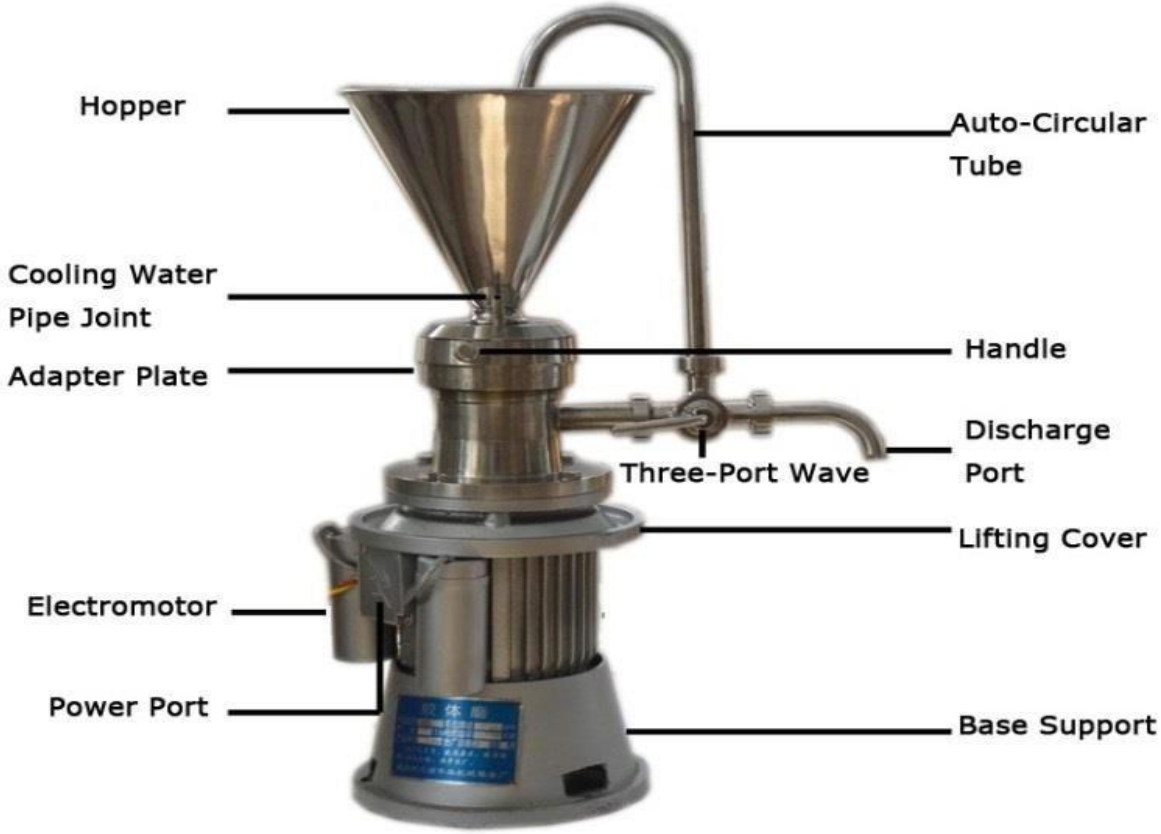
AIM:

To demonstrate the Colloid Mill, Planetary Mixer, Fluidized Bed Dryer, Freeze Dryer

COLLOID MILL

PRINCIPLE:

The size reduction and mixing effected due to shearing when the sample is passed between the narrow gap of milling surfaces of the rotor and stator.



CONSTRUCTION AND WORKING:

1. It consists of a stator and rotor with sharp milling surface. The rotor rotates at 3,000-20,000revolution per minute.
2. Hand-made emulsion or suspension is passed through the hopper or inlet. The instrument is Switched on and the rotor is allowed to rotate at high speed.
3. As the sample (emulsion or suspension) passes through the narrow gap between the rotor and stator, the sample is subjected to a tremendous shearing action (by sharp milling surface)
4. As the result the sample is mixed thoroughly. The final product is collected through the outlet

APPLICATIONS:

1. Colloid mill is used for wet grinding only.
2. It is used for preparing stable emulsion and suspension.
3. By using colloid mill, it is possible to reduce the particle size less than one micron.

PLANETARY MIXER

CONSTRUCTION AND WORKING:

1. It consists of a high speed impeller blades fixed on a vertical cylindrical rod. The samples to be mixed are taken in a mixing tank.
2. The cylindrical rod with impeller blades is allowed to revolve at high speed. When it rotates on its own axis, the planetary movement of the impeller blades within the tank helps uniform mixing of the samples.

ADVANTAGES:

1. It provides uniform mixing.
2. It serves to break down agglomerates rapidly

DISADVANTAGES:

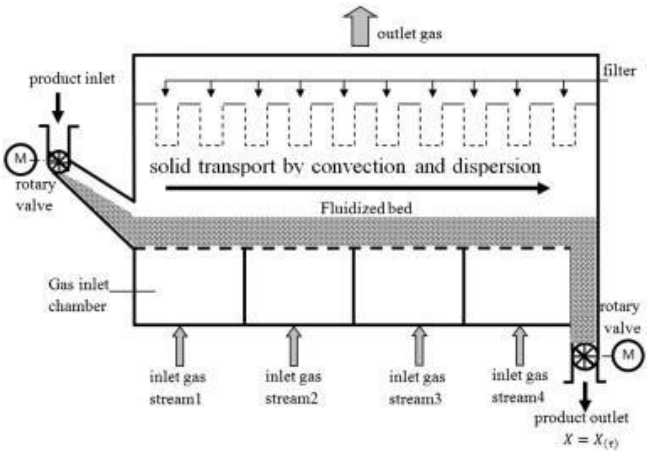
1. It requires high voltage to operate the equipment.
2. Heat is produced during operation.

APPLICATIONS:

1. Planetary mixer is used for mixing semisolids such as ointments, pastes and creams.
2. It is also used for mixing liquids and powders.

FLUIDISED BED DRYER

1. Fluidised bed dryer is known by the name because a gas is allowed to flow upward through a bed of particulate solids at a velocity greater than the settling velocity of the particles, the solids will be blown up and become partially suspended in the stream.
2. The resultant mixture of solids and gas behaves like a liquid and the solids are said to fluidised
3. This technique is very efficient and is used for drying granular solids because each particle is surrounded by the drying gas
4. Two types of fluidized bed dryers are used in pharmaceutical industries,
    - Vertical and
    - Horizontal
5. The vertical type is used for the batch drying whereas horizontal type is used for continuous drying



CONSTRUCTION AND WORKING:

1. It consists of a stainless steel, conical shaped vessel containing drying chamber with a perforated bottom.
2. The powder material to be dried is packed in the drying chamber.
3. Air is passed through the inlet. This air is heated by means of heaters.
4. The dust particles are removed from the hot air by means of pre-filter. The motor is switched on and so the induction fan rotates.
5. As the induction fan rotates, hot air is drawn into the drying chamber through perforations.
6. As the hot air enters into the drying chamber, it carries the powder particles along with in and the particles are suspended in the hot air and thus the powder material is dried.
7. Air filter prevents the escape of powder particles from the drying chamber finally the dried material is removed.
8. With these types of dryers materials ranging from 5kg-200kg can be dried in about 20-40minutes compared with 24hour cycle in conventional tray dryers.

ADVANTAGES:

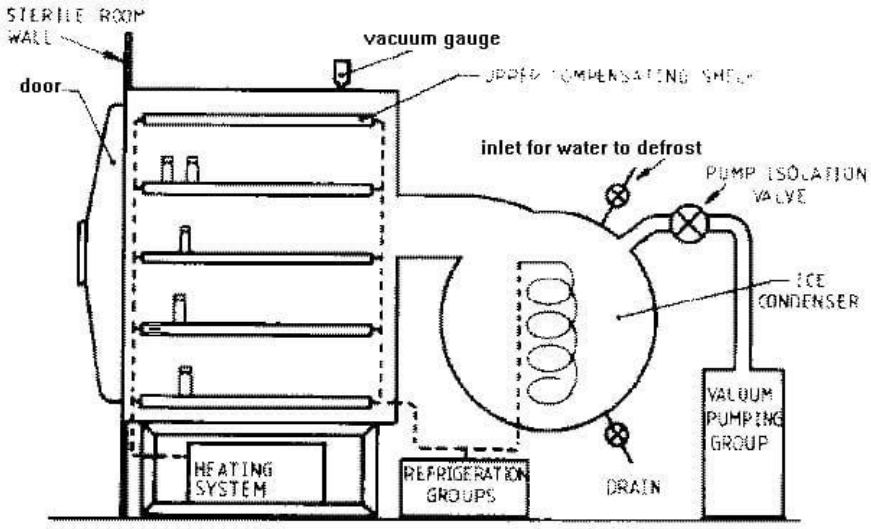
1. Drying time is very shorter.
2. The individual powder particles are dried.
3. The temperature of the fluidized bed is uniform and can be controlled accurately.
4. It produces free flowing powder.
5. Since the containers are mobile which make handling easy thereby labour costs are reduced.
6. Due to short drying time the unit has a high output.
7. It helps in the process of tablet manufacture.
8. Efficient and uniform drying

DISADVANTAGES:

1. The turbulent motion may cause damage to the individual particles
2. The vigorous movement of particles in hot dry air may lead to the generation of electricity and therefore suitable precaution must be taken.
3. Too wet granules stick together on drying therefore too wet granules must not be used.

APPLICATIONS:

1. The fluidized bed dryer can be used for drying any powdered material.
2. A special application is the drying of tablet granules.



FREEZE DRYING OR LYOPHILISATION OR GELSICCATION

PRINCIPLE:

1. Freeze dryer is one of the equipment used for drying liquids. Freeze drying is a process of drying in which water vapour is removed from frozen solution by sublimation.
2. Usually freeze drying is carried out at a temperature of -10°C to 40°C and at a pressure of2000-100microns.
3. The material to be dried is first frozen at a temperature below-40'C.
4. The water vapour is removed from the drying chamber by means of condenser.
5. Then the material is subjected to heat under high vacuum so that frozen liquid sublimes and leaving dry solid.

FREEZE DRYING PROCESS:

1. The aqueous product to be dried is placed on the shelf in the drying chamber. The product is freed at -40'C by circulating refrigerant (usually Freon ammonia or ethylene glycol) from the small compressor through pipes within the shelf.
2. After freezing is complete, the chamber is evacuated by means of vacuum pump.
3. Then water vapour is removed from the drying chamber by means of a condenser.
4. Heat is then introduced from the shelf to the product by means of electric sources or by circulating hot water until the product is dried.
5. During heating, vacuum is applied. The container is then removed and scaled under aseptic conditions

ADVANTAGES:

1. Biological and pharmaceuticals which are unstable when dried by some other methods can be processed.
2. The samples can be dried without elevated temperature.
3. Freeze drying products are more stable and more soluble.

DISADVANTAGES:

1. Cost of equipment is high.
2. It requires more time for processing

APPLICATIONS:

1. Freeze drying is used for preserving human tissues.
2. It is used for drying blood serum, plasma, antibiotics, hormones etc.
3. In food industry, freeze drying is used for preservation.

RESULT:

EX.NO:15

DATE:

EFFECT OF SURFACE AREA ON THE RATE OF FILTRATION

AIM:

To perform the effect of surface area on the rate of filtration**.**

REQUIREMENTS:

Apparatus: measuring cylinder, Buchner funnel (small, medium and large), beaker, glass rod, stop watch, filter paper and weighing balance.

Chemical: calcium carbonate.

PRINCIPLE:

1. Rate of filtration is directly proportional to the surface area of filter medium. Hence the rate of filtration can be increased either using a large filter or connecting a number of small units in parallel.
2. Filter press works on the principle of connecting the units in parallel to increase the filtering surface area.
3. Filtration may be defined as the separation of solids from liquids by means of a porous medium. Filter paper, cotton, sand, talc, sintered glass etc. are used as filter media.

THEORY OF FILTRATION:

1. In the process of filtration, the solid is separated from the liquid by passing through a suitable medium.
2. The medium used for the filtration process is known as filter medium. The liquid to be filtered (contain solids and liquids) is known as slurry. The clear liquid obtained after the process is known as filtrate and the solid retained by the media is known as filter cake.
3. The solid particles which are larger than the pores of the filter medium are retained on the filter
4. The medium (material) used in the filtration process for retaining the solid is known as filter medium.
5. The ability of filter medium to retain solid particles are collected over the filter medium and build up a thick layer called filter bed. The filer bed often acts as a second filter medium

PROCEDURE:

1. Prepare 600ml of 2% of calcium carbonate slurry in a beaker.
2. Take 3 Buchner funnels having 3 different sizes. Measure the area of the funnel and label them as 1, 2 and 3 respectively
3. Cut the filter paper accordingly to fit in a Buchner funnel (one in each funnel) and arrange the filtration assembly for each.
4. Pour 200ml of prepared slurry in each assembly and collect the filtrate at different time intervals (e.g. 5, 10, 15, 20, 25, 30 minute) and measure its volume for each time.
5. Calculate the rate of filtration as given formula.
   Rate of filtration= volume of filtration/time.
   Area of funnel= πr^3
6. Plot a graph of rate of filtration vs. time and volume of filtration vs time.

RESULT:

EX.NO:16

DATE:

EFFECT OF CONCENTRATION OF SLURRY ON THE RATE OF FILTRATION

AIM:

To perform the effect of concentration of slurry on the rate of filtration.

REQUIREMENTS:

Apparatus: measuring cylinder, funnel, beaker and weighing balance.

Chemical: calcium carbonate.

PRINCIPLE:

1. The rate of filtration decreases as thickness of the cake increases.
2. When the rate is uneconomically low the filtration is stopped and the cake is removed mechanically and the filtration is resumed.
3. Thickness of the filter cake increases as the filtration progresses.
4. Highly concentrated slurry is first decanted or strained to reduce the solid content and then it is filtered (this reduces the cake thickness).
5. In a rotary drum, filter cake is removed continuously so that the cake is minimized.

PROCEDURE:

1. The filter paper of appropriate (10cm diameter) is placed in to the Buchner funnel.
2. 100 ml of 5% calcium carbonate suspension is poured over the Buchner funnel.
3. Time required to collect 50ml of the filtrate is recorded.
4. The experiment (steps 1-3) is repeated for the same concentration of calcium carbonate suspension for two more trials.
5. The experiment is repeated for the other concentrations (10 and 15%w/v) also.
6. The reading are recorded and processed.
7. A graph is plotted by taking concentration of calcium carbonate on x-axis and rate of filtration on y-axis.

RESULT:

EX.NO:17

DATE:

EFFECT OF VISCOSITY (THICKNESS) ON THE RATE OF FILTRATION

AIM:

To perform the effect of viscosity (thickness) on the rate of filtration.

REQUIREMENTS:

Apparatus: measuring cylinder, Buchner funnel, beaker, glass rod, stop watch, filter paper, weighing balance.

Chemicals: calcium carbonate, glycerine.

PRINCIPLE:

1. An increase in the viscosity of the liquid will decrease the flow rate.
2. The viscosity of the liquid can be decreased by raising the temperature of the slurry or by dilution with a miscible liquid.
3. Viscosity of the suspension may be due to the presence of solid and viscosity of vehicle.
4. The vehicles containing different concentration of glycerine-water mixtures are used.
5. Viscosity increase with increase in the concentrations of glycerine in the mixture.

PROCEDURE:

1. Prepare 300 ml of 2% of calcium carbonate slurry in a beaker using 3 different vehicles,
   (a) Water (b) 5% glycerine in water (c) 10 % glycerine in water.
2. Take the Buchner funnels and measure the internal diameter and area of the funnel.
3. Cut the filter paper accordingly to fit in the Buchner funnel and arrange the filtration assembly
4. Pour all the prepared slurry in Buchner funnel and collect the filtrate at different time intervals (e.g. 5, 10, 15, 20, 25, 30 min). Measure its volume for each time.
5. Calculate the rate of filtration as given formula. Plot a graph of rate of filtration vs. time and volume of filtration vs. time.
   Rate of filtration = volume of filtration/time
   Area of funnel = πr^2

RESULT:

EX.NO:18

DATE:

EFFECT OF SURFACE AREA ON THE RATE OF EVAPORATION

AIM:

To effect of surface area on the rate of evaporation.

REQUIREMENTS:

Apparatus: beaker 50ml, beaker 100ml, beaker 250ml, water bath, balance, measuring cylinder.

Chemical: purified water

PRINCIPLE:

1. Rate of evaporation is directly proportional to surface area of evaporator.
2. 2 The greater the surface area of the liquid, the greater will be the evaporation.
3. For this reason evaporation is conducted in evaporators with large heating surface area.
4. This is verified by taking beaters of different surface area, Le. 50ml, 100ml, 250mlcapacity
5. When the same quantity of slurry is maintained and exposed to same time and same temperature, the differences in the initial and final weights permit the verification of factor, surface area.

PROCEDURE:

1. Take 3 clean beakers of different capacity (50, 100 and 250 ml) and label them as B_1, B_2 ,B_3
2. Determine radius of the beakers and add 50ml of water to each of it.
3. Weigh the beakers containing water individually and denote it as an initial weight of water (W_1)
4. Heat the beakers on constant temperature water bath for 30min at 60°C5.
5. After half an hour remove the beakers from water bath, take its weight individually and denote it as final weight (W_2).
6. Determine difference in initial and final weight of beakers
7. Plot a graph of rate of evaporation vs. surface area.

RESULT:

EX.NO:19

DATE:

EFFECT OF CONCENTRATION ON THE RATE OF EVAPORATION

AIM:

To perform the effect of concentration on the rate of evaporation.

REQUIREMENTS:

Apparatus: measuring cylinder, beaker, weighing balance, water bath.

Chemical: sodium chloride

PRINCIPLE:

1. Higher the concentration of dissolved solids, the lower the rate of evaporation.
2. This is verified by taking slurries of different concentrations of dissolved solids (NaCI) at constant temperature, constant surface area and constant volume of slurry.
3. The working concentration of sodium chloride does not significantly alter the viscosity factor.

PROCEDURE:

1. 2, 4, 6 and 8 %w/v solutions of sodium chloride are prepared by dissolving 1, 2, 3 and4gm of sodium chloride in 50ml of water in beakers.
2. The beakers containing sodium chloride solutions are weighed (W_1 , g). Weights are recorded.
3. All the beakers are heated in water bath at constant temperature (70°C) for 30min.
4. All the beakers are weighed again after heating (W_6 g). Weights are recorded.
5. The difference between the weights is determined. The difference reflects the amount of water evaporated during 30 min.
6. Rate of evaporation is calculated.
7. A graph is plotted by taking rate of evaporation on y-axis and concentration on x-axis.

RESULT:

EX.NO:20

DATE:

EFFECT OF VISCOSITY (THICKNESS) ON THE RATE OF EVAPORATION

AIM:

To perform the effect of viscosity (thickness) on the rate of evaporation.

REQUIREMENTS:

Apparatus: measuring cylinder, beaker, weighing balance, water bath.

Chemical: glycerine.

PRINCIPLE:

1. 1 Viscosity is a fundamental characteristic property of all liquids
2. When a liquid flows, it has an internal resistance to flow.
3. Viscosity is a measure of this resistance to flow or shear.
4. Viscosity can also be termed as a drag force and is a measure of the friction properties of the fluid
5. Viscosity is a function of temperature and pressure. Although the viscosities of both liquids and gases change with temperature and pressure, they affect the viscosity in a different manner.
6. Viscosity is expressed in two distinct forms: absolute or dynamic viscosity and kinetic viscosity.
7. Rate of evaporation is inversely proportional to viscosity of slurry.
8. Higher the viscosity of the slurry, lower the rate of evaporation.
9. This is verified by taking slurries of different viscosities an subjecting evaporation at constant temperature and constant surface area.
10. For this purpose, glycerine-water mixture of different glycerine concentrations, by maintaining the same volume of slurry.

PROCEDURE:

1. Different concentrations of glycerine and water mixture are prepared in different beakers as shown in following table

Glycerine, ml	Water, ml	Concentration, %V/V
5	45	10
10	40	20
15	35	30
20	30	40

2. The beakers containing glycerine-water mixtures are weighed (w_3 g).
3. All the beakers are heated in a water bath at constant temperature 70°C for 30 min.
4. All the beakers are weighed after heating (w_4 g). Weights are recorded.

5. The difference between the weights is determined. The difference reflects the amount of water evaporated during 30 min.
6. Rate of evaporation is calculated.
7. A graph is plotted by taking rate of evaporation on y-axis and viscosity on x-axis.

RESULT

EX.NO:21

DATE:

EFFECT OF TIME ON THE RATE OF CRYSTALLIZATION

AIM:

To perform the effect of time on the rate of crystallization.

REQUIREMENTS:

Apparatus: measuring cylinder 100ml, beaker 250ml, weighing balance, water bath, hairdryer, microscope, funnel, filter paper and stop watch.

Chemicals: potassium nitrate, ice bath.

PRINCIPLE:

1. The solid is added to a solvent continuously until the solid dissolved. Such a solution is called as saturated solution.
2. The rate of dissolution process is enhanced by increasing the temperature and agitation.
3. Then the un-dissolved solid goes into solution.
4. When some solid remained un-dissolved then such a solution is called as supersaturated solution.
5. When the temperature of supersaturated solution is decreases rapidly (shock cooling), the solubility of solute decreases. As a result, the dissolved solid gets crystallized, through the process of nucleation and crystal growth.
6. The extend of crystallization depends on the time on contact in low temperature.
7. The crystals are collected by filtration and weighed.
8. Yield is expressed as percent weight of crystals obtained.
9. A graph is plotted by taking time vs. weight of crystals

PROCEDURE:

1. 75gm of potassium nitrate is accurately weighed (Wig).
2. 100ml of water is transferred into 250ml beaker
3. Beaker containing water is placed in constant temperature water bath maintained at 50^0 C
4. Potassium nitrate is added into the little by little, the solution is stirred with glass rod to dissolve the solute.
5. This process is continued until saturated solution (with little excess of crystal) is formed.

RESULT:

EX.NO:22

DATE:

CALCULATE THE UNIFORMITY INDEX FOR GIVEN SAMPLE BY USING DOUBLE CONE BLENDER

AIM:

To calculate the uniformity index for given sample by using double cone blender.

REQUIREMENT:

Apparatus: double cone blender, conical flask 250ml, pipette 10ml, burette 50ml, volumetric flask (100ml, 500ml) weighing balance

Chemicals: 0.1N hydrochloric acid solution, 0.1N sodium hydroxide solution, phenolphthalein indicator, calcium carbonate, talk, oxalic acid.

PRINCIPLE:

1. Mixing of calcium carbonate and tale is studied using double cone blender.
2. Blender is allowed to rotate on its own axis
3. During this process, the particles move freely to every spot of the equipment.
4. Time of mixing should be long enough to obtain an acceptable randomization.
5. Samples of the mixed materials are collected at different intervals randomly from the different locations.
6. The components are analysed by method of acid-base titration.
7. Amount of calcium carbonate is determined by treating the sample with known excess of hydrochloric acid.
8. Un-reacted hydrochloric acid solution can be determined by back titrating against sodium hydroxide solution using phenolphthalein as indicator.
9. Degree of uniformity achieved during mixing is calculated by a statistical procedure Uniformity index determined using the following formula

$$Us = \sqrt{\sum(y' - y)^2} / n\,(1- y')y$$

Where,
Us = uniformity index
n = number of samples
y' = true average composition of component A in the mixture
y = actual composition of component A in the mixture

10. Us values are calculated at different time intervals, Based on results, optimum time required for actual mixing is estimated.
11. A graph is plotted by taking time of mixing on x-axis and uniformity index on y-axis.

PROCEDURE:

1. 5 gm of calcium carbonate and 5gm of talc are weighed.
2. These 2 powders are placed in a cylindrical blender.
3. The blender is allowed to rotate for 10min at 25 revolutions per minute.
4. The samples (500mg) are drawn from three different places of the blender and placed in 3different conical flasks. Labelled them as 1A, IB, IC.
5. The blender is again allowed to rotate for another 10min.
6. Again 3 samples are drawn in a similar way as mentioned in step4. They are transferred into 3 different conical flasks and labelled as 2A, 2B and 2C.
7. Repeat the steps 5 and 6 for another 10 min. the samples are labelled as 3A, 3B and 3C.
8. 30ml of standard HCI solution (N_3-------) is placed in each conical flask.
9. The contents of flasks are shaken thoroughly to complete the reaction between HCI and calcium carbonate.
10. Unreacted HCI is determined by titrating against standard sodium hydroxide solution (N_2------).
11. The content of calcium carbonate present in each sample is calculated and reported.
12. A graph is plotted by taking time on x-axis and uniformity index on y-axis.

RESULT: