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RATHINAM COLLEGE OF PHARMACY

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PRACTICAL MANUAL

SECOND YEAR B.PHARM (IV-SEMESTER)

SUBJECTS: MEDICINAL CHEMISTRY-I {BP406P}

PHYSICAL PHARMACEUTICS-II {BP407P}

PHARAMACOLOGY-I {BP408P}

PHARMACOGNOSY AND PHYTOCHEMISTRY-I {BP409P}

Name :

Course : Bachelor of Pharmacy

Semester : 4 th semester

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MEDICINAL CHEMISTRY-I

B. PHARM 4th SEMESTER PREPARED

BY
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LIST OF EXPERIMENTS: (BP406P)

I Preparation of drugs/ intermediates

1 1,3-pyrazole

2 1,3-oxazole

3 Benzimidazole

4 Benzotriazole

5 2,3- diphenyl quinoxaline

6 Benzocaine

7 Phenytoin

8 Phenothiazine

9 Barbiturate

II Assay of drugs

1 Chlorpromazine

2 Phenobarbitone

3Atropine

4 Ibuprofen

5 Aspirin

6 Furosemide

III Determination of Partition coefficient for any two drugs

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Experiment no: 1
Date:

PREPARATION OF BENZIMIDAZOLE

AIM:

To prepare and submit Benzimidazole from o-phenylenediamine and formic acid. Calculate

- (i) Percentage yield
- (ii) Melting point

REFERENCE:

1. Vogels Textbook Of Practical Organic Chemistry,5th edition, pg no: 1162

2. Practical in organic chemistry, by Hitesh G. Raval, Sunil L. Baldania and Dimal A. Shah, nirav Prakashan, Page No.-301.

CHEMICALS REQUIRED:

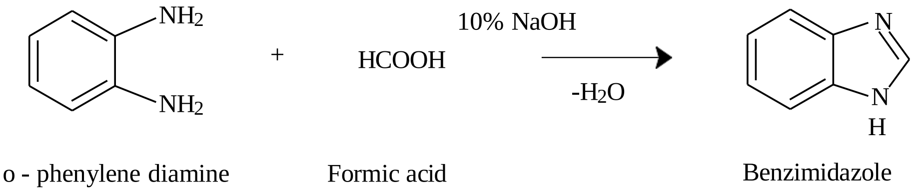
o-phenylenediamine, formic acid and NaOH (10%).

APPARATUS REQUIRED:

Conical flask, Beakers, Funnel, Spatula, Glass rod, Filter paper, Water bath.

PRINCIPLE:

The two Carbon-nitrogen bonds in benzimidazole when disconnected give o-phenylenediamine and formic acid. Therefore, synthesis of benzimidazole is affected by simply heating the o-phenylenediamine and formic acid together by condensation type of reaction.



PROCEDURE

Place 27g (0.25mol) of o-phenylenediamine in a round bottom flask and add 17.5g (16ml, 0.34mol) of 90% formic acid. Heat the mixture on a water bath at 100°C for 2 hours. Cool and add 10% of NaOH slowly with constant rotation of the flask until the mixture is alkaline to litmus. Filter off the crude benzimidazole at the pump, wash with ice-cold water, drain well and wash again with 25ml of cold water. Dissolve the crude product in 400ml of boiling water, add 2g of decolorizing carbon and digest for 15 minutes. Filter rapidly at the pump through a preheated Buchner funnel and cool the filtrate to about 10°C, filter off the benzimidazole, wash with 25ml of cold water and dry at 100°C . The yield of pure Benzimidazole, M.P 171-172°C is 25g (85%).

.

IDENTIFICATION:

Experiment	Observation	Inference
Melting point	Melts at 171-172°C	Presence of benzimidazole

REPORT:

Experiment no: 2
Date:

SYNTHESIS OF BENZTRIAZOLE

AIM:

To synthesize Benztrizole from o-phenylenediamine and acetic acid. Calculate

(i) Percentage yield
(ii) Melting point

REFERENCE

1. Vogels Textbook Of Practical Organic Chemistry,5th edition, pg no: 1163
2. Practical in organic chemistry, by Hitesh G. Raval, Sunil L. Baldania and Dimal A. Shah, nirav Prakashan, Page No.-301.Advanced practical organic chemistry. N. K Vishnoipages 303

CHEMICALS REQUIRED:

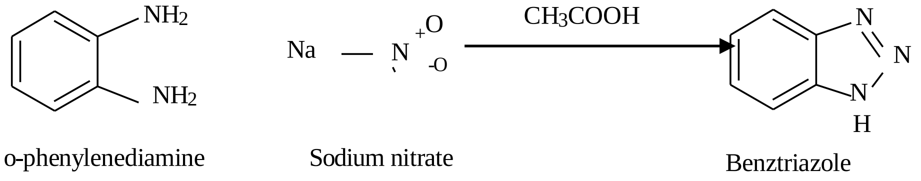
o-phenylenediamine, Glacial acetic acid, Sodium nitrite.

APPARATUS REQUIRED:

Round bottom flask, beaker, condenser, funnel, filter paper.

PRINCIPLE:

Benzotriazole can be prepared by treating o-phenylenediamine with nitrous acid (liberated during the reaction between sodium nitrite and acetic acid) to form monodiazonium salt that follows spontaneous intramolecular cyclization reaction to produce benzotriazole.



PROCEDURE

5.48 gm o-phenylene diamine is dissolved in a mixture of 5.7ml glacial acetic acid and 15ml water by slight warming if necessary. The mixture is cooled to 15°C stirred, and a solution of 3.8gm sodium nitrate in 8 ml water is added in one lot. The reaction mixture becomes warm and the temperature reaches to about 80°C. The colour changes from deep red to pale brown. The stirring is continued for 15 min and the mixture is cooled in ice bath. The separated benzotriazole is filtered, washed with ice cold water and crystallised from hot water. It melts at 99-100°C

IDENTIFICATION

Experiment	**Observation**	**Inference**
Melting point	99-100°C	Presence of benztriazole

REPORT:

Experiment no: 3
Date:

PREPARATION OF 2, 3-DIPHENYL QUINOXALINE

AIM:

To prepare and submit recrystallized dried product of phenytoin and calculate

(i) Percentage yield
(ii) Melting point

REFERENCE:

1. Vogels Textbook Of Practical Organic Chemistry,5th edition, pg no: 1190

CHEMICALS REQUIRED:

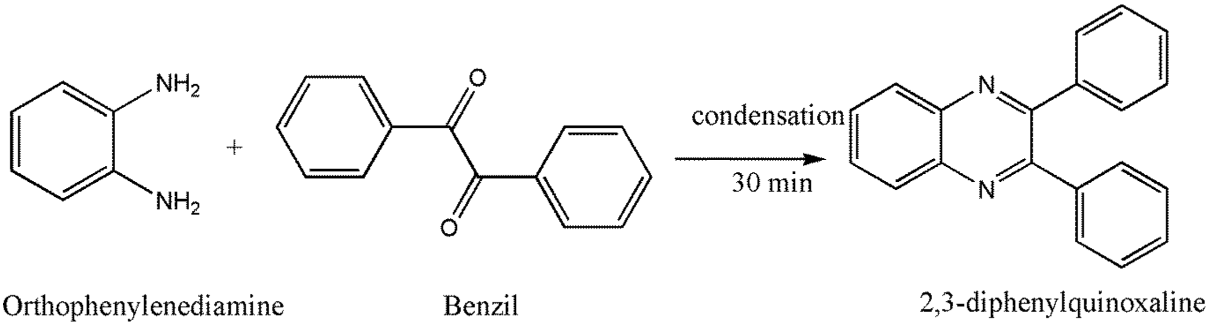
Benzil, Ethanol, Orthophenylene diamine.

APPARATUS REQUIRED:

Beaker, funnel, beaker, filter paper, glass rod.

PRINCIPLE:

2,3-Diphenyl Quinoxaline is prepared by condensation reaction. Benzyl reflexed with Orthophenylenediamine in presence of ethanol. 2,3-Diphenyl Quinoxaline is synthesised by removal of water molecule.



PROCEDURE:

To a warm solution of 2.1g (0.01mol) of Benzil ($C_{14}H_{10}O_2$) in 8 ml of rectified spirit a solution of 1.1 g (0.01mol) of orthophenylene diamine($C_6H_8N_2$) in 8 ml of rectified spirit was added, warmed in water bath for 30 minutes, water was added until slight cloudiness persists and allowed to cool. Filtered and recrystallized from absolute alcohol.

IDENTIFICATION:

Experiment	Observation	Inference
Melting point	125-126^{O}C	Presence of 2,3-Diphenyl quinoxaline

REPORT:-

Experiment no: 4
Date:

SYNTHESIS OF BENZOCAINE

AIM:

To synthesis recrystallized product of benzocaine from para amino benzoic acid and calculate

(i) Percentage yield
(ii) Melting point

REFERENCE:

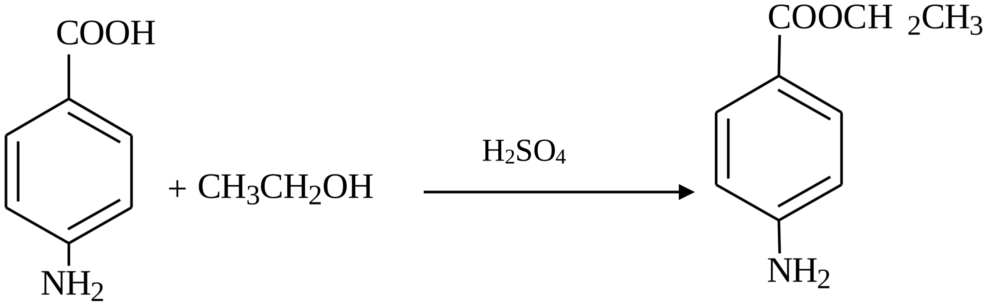
2. Vogels Textbook Of Practical Organic Chemistry,5th edition, pg no: 896

REQUIREMENTS:

PABA, Conc:sulphuric acid, ethanol, reflux condenser, RB flask , beaker

PRINCIPLE:

Benzocaine is the ethyl ester of para amino benzoic acid (PABA).It can be prepared from PABA and ethanol by Fischer esterification.



PABA Ethyl para amino benzoate

PROCEDURE:

To a 100ml RB flask, add 8ml of ethanol, 4.12g of para amino benzoic acid(PABA) and 1.2ml of conc:H_2SO_4 keep the mixture under reflux for 1hour up on cooling reaction mixture sets to a solid mass of hydrochloride of ethyl para amino benzoate. Pour the hot solution in to excess of water (no hydrochloride) add Na_2CO_3 to the clear solution until it is neutral to litmus. Filter wash and dry the product.

IDENTIFICATION

Experiment	**Observation**	**Inference**
Melting point	88-90^{O}C	Presence Benzocaine confirmed

REPORT:-

Experiment no: 5
Date:

PREPARATION OF PHENYTOIN

AIM:

To prepare and submit recrystallized dried product of phenytoin and calculate

(iii) Percentage yield
(iv) Melting point

REFERENCE:

Practical Medicinal Chemistry By Dr.G.Devala Rao , Page no:12-13

CHEMICALS REQUIRED:

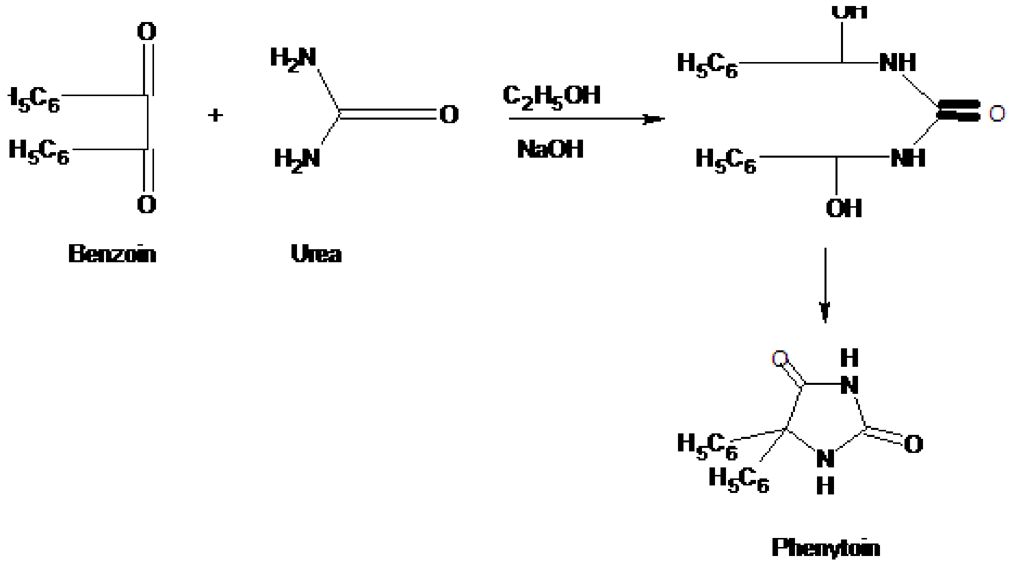
Urea, Nitric acid, Benzoin, Sodium hydroxide, ethanol, Conc:HCl

APPARATUS REQUIRED:

Round bottom flask, reflux condenser, funnel, beaker, filter paper, glass rod.

PRINCIPLE:

Phenytoin is 5,5-diphenyl imidazoline 2,4-dione.Benzil react with urea in the presence of alkali and alcohol to give phenytoin by pinacolone rearrangement.



PROCEDURE:

a) Preparation of Benzil from Benzoin:

Place 2g of benzoin and 5ml of concentrated HNO_3 in a round bottom flask and heat on a boiling water bath till crystalline benzoin is replaced by oily benzil.Pour the mixture in to beaker of cold water with stirring the oily benzil crystallize in to yellow salt.

b) Preparation of phenytoin from benzil:

Place 1g benzil, 1g urea ,5ml 30% aqueous sodium hydroxide and 20ml ethanol in a round bottom flask which is attached to reflux condenser and boil for 2hours.Cool the mixture to attain room temperature. Pour the mixture to 100ml water and, mix and allow to stand for 15minutes.Filter to remove insoluble biproducts. Render the filtrate strongly acidic with concentrated HCl .Cool the filtrate in ice cold H_2O.Filter the precipitate product dry and submit.

IDENTIFICATION:

Experiment	**Observation**	**Inference**
To the sample solution add hydrochloric acid	White precipitate	Presence of phenytoin
To the sample add pyridine and copper sulphate solution	Blue colour	Presence of phenytoin
Melting point	286-290^{O}C	Presence of phenytoin

REPORT:

Experiment no: 6
Date:

PREPARATION OF PHENOTHIAZINE

AIM:

To synthesis phenothiazine from Diphenylamine, sulphar and anhydrous aluminium chloride and report

(i) Percentage yield
(ii) Melting point

REFERENCE:

1. Systematic organic chemistry, by W. M. Cumming, 325-326, 1937.

CHEMICALS REQUIRED:

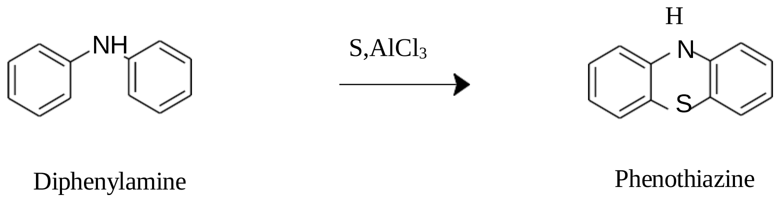
Diphenylamine, sulfur, anhydrous aluminum chloride

APPARATUS REQUIRED:

Beaker, funnel, filter paper, measuring cylinder.

PRINCIPLE

Phenothiazine is synthesized by condensation of diphenylamine and sulphur using aluminium chloride as catalyst.



PROCEDURE

22 gm of diphenylamine, 8.2 g of sulfur, and 3.2 gms. of anhydrous aluminum chloride are melted together. The reaction sets 140-150° C with the rapid evolution of hydrogen sulfide; by lowering the temperature, a few degrees the reaction can be slackened. When the reaction has moderated, the temperature is raised to 160° C for a time. The melt, when cool, is ground up and extracted, first with water and then with dilute alcohol. The residue consists of almost pure phenothiazine. It can be recrystallised from alcohol. Yield 93%, yellowish leaflets; m.p. 180° C.

IDENTIFICATION:

Experiment	**Observation**	**Inference**
Melting point	Melts at 180° C	Presence of phenothiazine

REPORT

Experiment no: 7
Date:

PREPARATION OF BARBITURATE (BARBITURIC ACID)

AIM:

To synthesis barbituric acid from diethyl malonate and urea. report

(i) Percentage yield
(ii) Melting point

REFERENCE:

1. Practical Medicinal Chemistry by Dr. G. Devala Rao Pg. No. 35.

CHEMICALS REQUIRED:

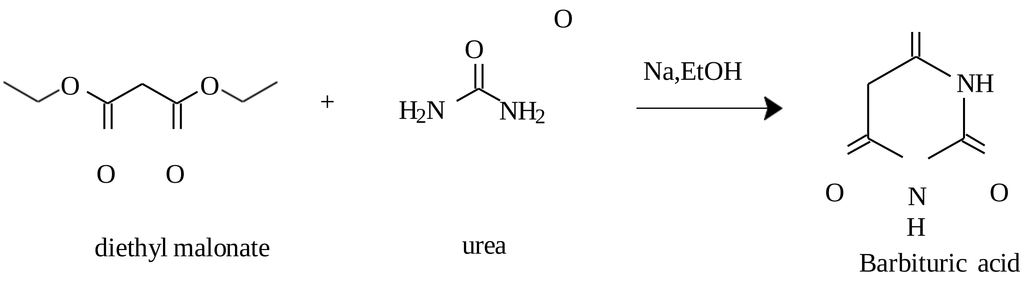
Diethyl malonate Urea, Ethanol, sodium metal, Con.H Cl. Calcium chloride

APPARATUS REQUIRED:

Round bottom flask, reflex condenser, beaker, funnel, filter paper

PRINCIPLE

Barbituric acid is readily prepared by the condensation of diethyl malonate with urea in the presence of sodium ethoxide. The reaction involved are as follows.



PROCEDURE

Dissolve 1.3 gm of sodium in 30ml of absolute ethanol in 200ml flask containing a reflux condenser, then add 10 gm of redistilled ethyl malonate, and place the flask on a boiling water-bath. Without any delay, add a solution of 5.3 gm of urea in 100ml of absolute ethanol. The sodium salt of barbituric acid rapidly begins to separate. Fit the water condenser with a calcium chloride guard tube and boil the mixture on the water-bath for 1 hour. Cool the mixture, filter

off the sodium salt at the pump and wash it with a small quantity of cold acetone dissolve the sodium barbiturate in warm water and liberate the free barbituric acid by addition of hydrochloric acid (a mixture of 30 ml conc. HCl and 30 ml water).
Now cool the reaction mixture, filter the barbituric acid and recrystallize the compound from hot water. The yield of barbituric acid is 35 gm and the melting point is 245 °C.

IDENTIFICATION:

Experiment	**Observation**	**Inference**
Melting point	Melts at 245° C	Presence of barbiutric acid

REPORT:-

Experiment no: 8

Date:

SYNTHESIS OF 3-METHYL-1-PHENYL PYRAZOLE-5-ONE

Aim:

To perform synthesis of 3-methyl-1-phenyl pyrazole-5-one

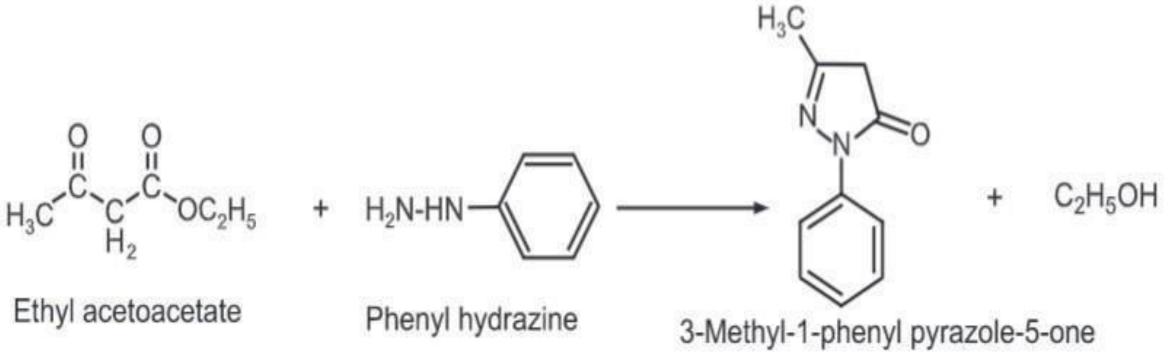
Reference:

A. Tiwari, R. Kumar, A practical book of Medicinal Chemistry, Nirali Prakashan, Pune, 2019, 1.

Principle:

3-methyl-1-phenyl pyrazole-5-one is synthesized by condensation of phenyl hydrazine and ethyl acetoacetate. It is an example of intermediate used in bulk drug industry. In thissynthesis ethylacetoacetate is used along with hydrazine. Ethylacetoacetate is heated with an equal quantity of phenyl hydrazine. On further heating ring formation occurs with loss of ethanol. Resultant compound is methyl phenyl pyrazolone.

Reaction:



Requirements:

Chemicals: Phenyl hydrazine, Ethyl acetoacetate, Ether, Ethanol

Apparatus: Round bottom flask, Reflux condenser, Beaker, Glass rod, Funnel, Measuring cylinder

Procedure:

A mixture of phenyl hydrazine (3.65 ml) and ethyl acetoacetate (4.9 ml) were heated in a round bottom flask on a boiling water bath for 2 hours. The reaction mixture was stirred with the help of a glass rod. Then, the reaction mixture was cooled and to it was added 20 ml ether with stirring. The separated product was filtered, washed with ether and recrystallized with dilute ethanol.

CALCULATION:

REPORT:

Experiment no: 9

Date:

SYNTHESIS OF 4-BENZYLIDENE-2-PHENYL OXAZOLE-5-ONE

Aim: To perform synthesis of 4-benzylidene-2-phenyl oxazole-5-one

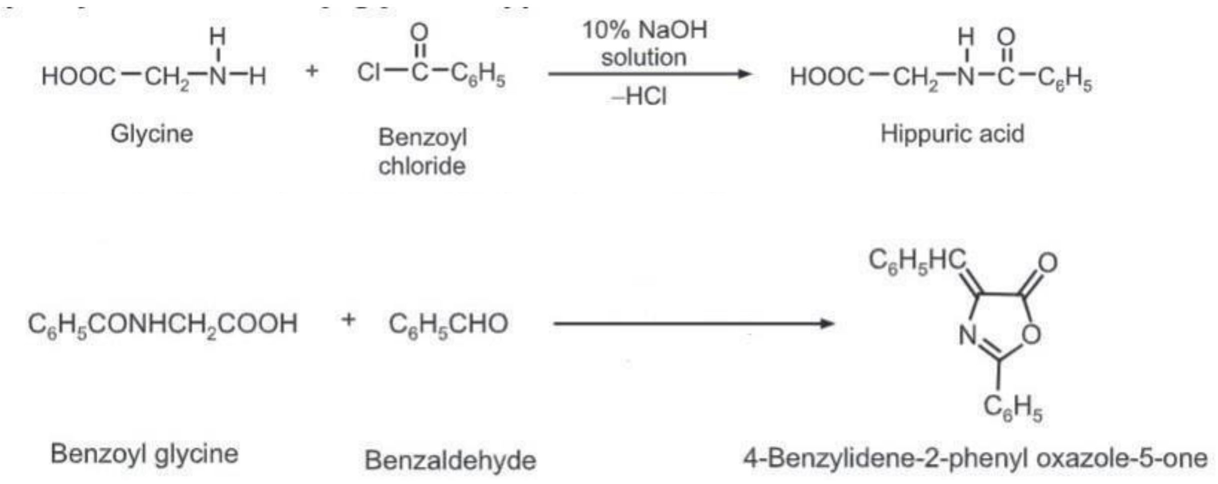
Reference:

A. Tiwari, R. Kumar, A practical book of Medicinal Chemistry, Nirali Prakashan, Pune, 2019, 5-6.

Principle:

The principle involved in the preparation of 4-benzylidene-2-phenyl oxazole-5-one is dehydration followed by cyclization method. The active methylene group react with aromatic aldehydes. Benzoyl glycine reacts with benzaldehyde followed by dehydration gives 4-benzylidene-2-phenyl oxazole-5-one. The hippuric acid is formed by reacting glycine with benzoyl chloride.

Reaction:



Requirements:

Chemicals: Glycine, Sodium hydroxide, Benzoyl chloride, Concentrated hydrochloric acid, Benzaldehyde, Acetic anhydride, Anhydrous sodium acetate, Ethanol

Apparatus: Round bottom flask, Reflux condenser, Beaker, Glass rod, Funnel, Measuring cylinder

Procedure

About 1 gm of glycine was dissolved in aqueous sodium hydroxide solution (10 ml) in a flask and to it 1.5 ml of benzoyl chloride was added. The mouth of the flask was plugged with cotton and was shaken vigorously. Then 1-2 drops of conc. HCl was added. The product was filtered, washed with water and recrystallized.

A mixture of benzaldehyde, benzoyl glycine, acetic anhydride and anhydrous sodium acetate was taken in a conical flask and the contents were heated on sand/oil bath till the mixture had liquified completely. Now, the contents were heated on a water bath for two hours. Then it was cooled, and to it 25 ml ethanol was added slowly. The product was filtered, washed with hot water, dried, and recrystallized.

CALCULATION:

REPORT:

Experiment no: 10

Date:

MONOGRAPHIC ANALYSIS OF IBUPROFEN TABLET I.P

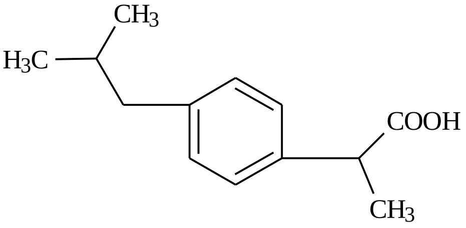
AIM:

To carry out the monographic analysis of ibuprofen tablet I.P

REFERENCE:

Indian pharmacopoeia 1996, Vol I page No. 387

Name of the drug : Ibuprofen
Molecular formula : $C_{13}H_{18}O_2$
Molecular weight : 206.28
Molecular structure:



Description: White or almost white crystalline powder or colourless crystals, odour slight.

Storage: Store in a well closed container

Standard: Ibuprofen contains not less than 98.5% and not more than 101%

Category: Anti-inflammatory, analgesic

Dose: 600 mg to 1.2 gm daily in divided doses after food

Solubility: Feely soluble in acetone, chloroform, 95% ethanol and in ether practically insoluble in water.

Identification test	**Observation**	**Inference**
Extract a quantity of the powdered tablets equivalent to 0.5 g of Ibuprofen with 20 ml of *acetone*, filter and evaporate the filtrate to dryness in a current of air without heating. Recrystallized with light petroleum (boiling range 40° to 60°).	Melts at about 75°C	Presence of Ibuprofen

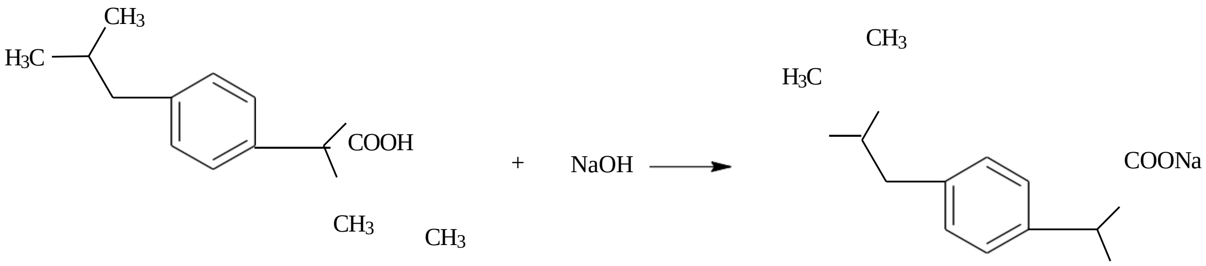
ASSAY OF IBUPROFEN TABLET IP

Aim: To carry out the assay of ibuprofen tablet
Reference: Practical medicinal chemistry, Dr. G. Devala Rao, Pg. No. 79 Indian
pharmacopoeia 1996, Vol I page No. 388

Reagents: Ibuprofen tablet, chloroform, sodium hydroxide
Apparatus: Burette, Pipette, conical flask, weighing bottle

PRINCIPLE :

Ibuprofen is an example of non-steroidal anti inflammatory drugs. It is also having analgesic and antipyretic activity. It is widely used in the management of pain and fever. Ibuprofen is aryl acetic acid derivative and is weakly acidic in nature. It is estimated by alkalimeter. In this method, the alcoholic solution of ibuprofen is titrated against a standard solution of sodium hydroxide (0.1N) using phenolphthalein as indicator. The reaction evolved are as follows:



PROCEDURE:
Standardization of 0.1M Sodium hydroxide

Dissolve 0.5 gm of potassium hydrogen phthalate in 75ml of water and add 0.1ml phenolphthalein indicator and titrate using 0.1M sodium hydroxide. Each ml of 0.1 M sodium hydroxide = 0.02042 gm of potassium hydrogen phthalate

Assay of Ibuprofen tablet IP

Weigh and powder 20 tablets. Weigh accurately a quantity of powder equivalent to 0.5 g of Ibuprofen, extract with 60 ml of chloroform for 15 minutes and filter. Wash the residue with 3 quantities each of 10ml of chloroform and gently evaporate the filtrate just to dryness in a current of air. Dissolve the residue in 100 ml of ethanol previously neutralized to phenolphthalein solution and titrate with 0.1M sodium hydroxide using phenolphthalein as indicator. Each ml of 0.1M sodium hydroxide = 0.02063 g of ibuprofen

REPORT:

Experiment no: 11
Date:

MONOGRAPHIC ANALYSIS OF ASPIRIN TABLET I.P

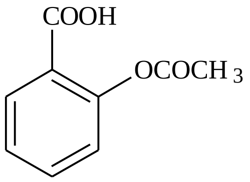
AIM:

To carry out the monographic analysis of aspirin tablet IP

REFERENCE:

Indian Pharmacopoeia 1996, Vol-I, Page No: 69-70.

Name of the drug: Aspirin (acetyl salicylic acid)
Molecular formula : $C_4H_8O_4$
Molecular structure



Description: Colourless crystals or white crystalline powder almost odourless

Storage: Protected from moisture at temperature not exceeding 30^0C

Standard: Aspirin contains not less than 94.5% and not more than 100.5%

Category: Analgesic, Antipyretic, anti-thrombotic, anti-rheumatic

Dose: As analgesic and anti pyretic 300 – 600 mg 4 – 6 times

As anti rheumatic: 1-2 g, 4-6 time a day

As anti thrombotic: 75 mg daily

Solubility: Freely soluble in ethanol, soluble in chloroform and other and slightly soluble in water.

Identification test	**Observation**	**Inference**
Boil a quantity of powdered tablet containing 0.55g of aspirin with 10 ml of sodium hydroxide solution for 3 min, cool & add 10 ml of sulphuric acid	A white crystalline precipitate is produced with odor of acetic acid	Presence of salicylic aid
Filter and dissolve the precipitate in 2 ml water and added ferric chloride	A deep violet color is produced	Presence of salicylic acid
To the filtrate add ethanol and sulphuric acid and warm	Odor of ethyl acetate	Presence of salicylic acid is confirmed

ASSAY OF ASPIRIN TABLETS I.P

Aim: To carry out the assay of aspirin tablets.

Reference:

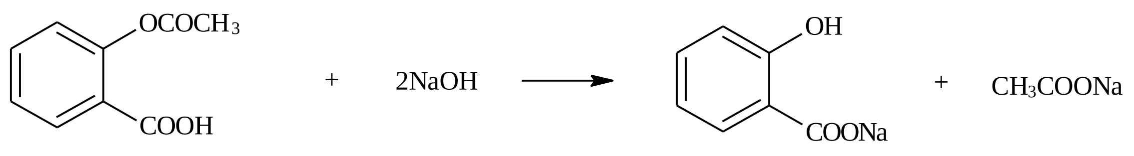
1. Practical medicinal chemistry by Dr, G Devala Rao, Pg No. 71
2. Indian Pharmacopocia1996, Vol-I Page No. 70.

Reagents: Aspirin, sodium carbonate, sodium hydroxide, hydrochloric acid

Apparatus: Burette, weighing bottle, conical flask etc

PRINCIPLE:

Aspirin is an example of analgesic, antipyretic which is widely used in the treatment of pain. It is estimated by acidimetric and alkalimetry. It's determination depends upon the alkaline hydrolysis of aspirin to acetic acid and salicylic acid (Sodium salts are formed immediately) followed by back titration of the excess alkali using phenol red as indicator. A blank determination is needed in the assay the reactions involved are as follows:



$NaOH + HCl \longrightarrow NaCl + H_2O$

PROCEDURE:

Standardization of 0.5M hydrochloric acid

Weigh accurately 0.75 g anhydrous sodium carbonate heated at 210^0C for 1 hour. Dissolve in 100 ml of water. Add 0.1 ml methyl red. Titrate with 0.5m hydrochloric acid till solution becomes pink. Heat to boiling, cool, continue the titration. Heat again to boiling. Titrate till pink color is no longer dissolved.

Each ml of 0.5M hydrochloride acid = 0.026495 gm of Na_2CO_3

Assay of Aspirin tablets

Weigh and powder 20 tablets, weight accurate quantity equivalent to about 0.5 g of aspirin. Add 30 ml 0.5 M sodium hydroxide. Boil gently for 10 minutes. Cool titrate in excess of alkali with 0.5M hydrochloric acid using phenol red as indicator. Also perform a blank determination. Difference between titration represents the amount of sodium hydroxide required.

Each ml of 0.5 M HCl – 0.04504 gm of aspirin.

REPORT:

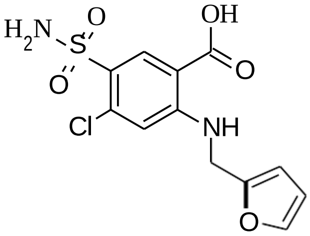
Experiment no: 12

Date:

MONOGRAPHIC ANALYSIS OF FUROSEMIDE TABLET I.P

AIM:
To carry out the monographic analysis of furosemide tablet IP

REFERENCE:
Indian Pharmacopoeia 1996, Vol-I, Page No: 332.
Name of the drug: Furosemide
Molecular formula : $C_{12}H_{11}ClN_2O_2S$
Molecular structure



Description: White or almost white crystalline powder.

Storage: Store in well-closed, light-resistant containers.

Standard: Frusemide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of frusemide, $C_{12}H_{11}ClN_2O_5S$.

Category: Diuretic

Dose: Orally, in oedema, 20 to 40 mg daily; in oliguvria, 250 mg 4 to 6 times daily; by intramuscular or slow intravenous injection, 20 to 50 mg (rate not exceeding 4 mg per minute)

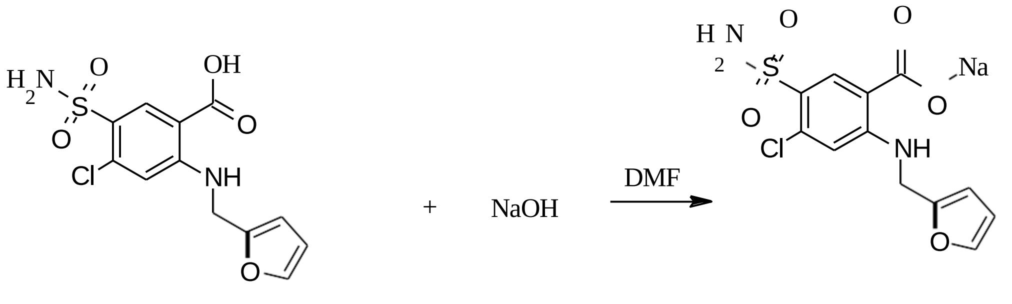
Solubility: Soluble in acetone; sparingly soluble in ethanol (95%); slightly soluble in ether; practically insoluble in water. It dissolves in dilute aqueous solutions of alkali hydroxides.

Identification test	**Observation**	**Inference**
The light absorption in the range 220 to 360 nm of the final solution obtained in the Assay.	Exhibits three maxima at about 228, 271 and 333 nm.	Presence of furosemide.
Shake a quantity of the powdered tablets equivalent to 80 mg of Frusemide with 10 ml of ethanol (95%), filter and evaporate the filtrate to dryness. Dissolve 25 mg of the residue obtained in 2.5 ml of ethanol (95%) and add 2 mlof 4-dimethylaminobenzaldehyde solution;	A green colour is produced which changes to deep red.	Presence of furosemide.

ASSAY OF FUROSEMIDE TABLETS I.P

Aim: To carry out the assay of furosemide tablets.
Reference:
1. Practical medicinal chemistry by Dr, G Devalo Rao, Pg No. 101-102
2. Indian Pharmacopocia1996, Vol-I Page No. 333-334
Reagents: Furosemide, sodium carbonate, sodium hydroxide, hydrochloric acid
Apparatus: Burette, weighing bottle, conical flask etc.

PRINCIPLE:
Frusemide is an example of diuretic. It is also known as furosemide. It is widely used in management of oedema. It is official in 1985 and estimated by alkalimetry. In this method, the solution of frusemide in DMF is titrated against 0.1 N sodium hydroxide using bromothymol blue as indicator. The reactions involved are as follows:



Furosemide sodium salt of furosemide

PROCEDURE:
Standardization of 0.1N sodium hydroxide solution

Dissolve 0.5 gm of potassium hydrogen phthalate in 75ml of water and add 0.1ml phenolphthalein indicator and titrate using 0.1M sodium hydroxide. Each ml of 0.1 M sodium hydroxide = 0.02042 gm of potassium hydrogen phthalate

Assay of furosemide tablets

Weigh and powder 20 tablets, weight accurate quantity equivalent to about 0.5 g of furosemide and dissolved in 40ml of dimethyl formamide and titrate with 0.1N sodium hydroxide solution using bromothymol blue solution as indicator. Repeat the operation without the substance being examined. The difference in titration represents the amount of sodium hydroxide required by the furosemide.

Each ml of 0.1 N sodium hydroxide solution – 0.03308 gm of frusemide.

REPORT:

Experiment no: 13

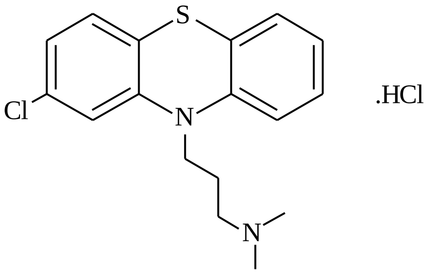
Date:

MONOGRAPHIC ANALYSIS OF CHLORPROMAZINE TABLET I.P

AIM:
To carry out the monographic analysis of chlorpromazine tablet IP

REFERENCE:
Indian Pharmacopoeia 1996, Vol-I, Page No: 178.

Name of the drug: Chlorpromazine
Molecular formula : $C_{17}H_{19}ClN_2S,HCl$
Molecular structure



Description: White or creamy-white, crystalline powder; odourless.
Storage: Store in tightly-closed, light-resistant containers.
Standard: Chlorpromazine Hydrochloride Tablets contain not less than 92.5% per cent and not more than 107.5 %
Category: Antipsychotic agent with Anti-Emetic activity
Dose: As Antipsychotic agent with Anti-Emetic activity **As anti rheumatic:** 10-200 mg daily
Solubility: Very soluble in water; freely soluble in ethanol (95%); soluble in chloroform; practically insoluble in ether.

Identification:

Identification test	Observation	Inference
Digest a quantity of the powdered tablets equivalent to 25 mg of Chlorpromazine Hydrochloride with 25 ml of *water* and filter. A: (To the filtrate) The *light absorption* in the range 230 to 360 nm.	Exhibits two maxima at about 254 nm and 306 nm; absorbance at about 254 nm, 0.45 to 0.48.	Presence of chlorpromazine hydrochloride
(To the filtrate 2 ml) . Acidify with dilute nitric acid, add 0.5 ml of silver nitrate solution, shake and allow to stand.	A curdy white precipitate is formed, which is insoluble in nitric acid but soluble, after being well washed with water, in dilute ammonia solution, from from which it is reprecipitated by the addition of dilute nitric acid.	Presence of chlorpromazine hydrochloride
Introduce into a test-tube a quantity of the substance being examined equivalent to about 10 mg of chloride ion, add 0.2 g of potassium dichromate and 1 ml of sulphuric acid. Place a filter-paper strip moistened with 0.1 ml of diphenylcarbazide solution over the mouth of the test-tube;	paper turns violet-red	Presence of chlorpromazine hydrochloride.

ASSAY OF CHLORPROMAZINE TABLETS I.P

Aim: To carry out the assay of chlorpromazine tablets.

Reference:

1. Indian Pharmacopocia1996, Vol-I Page No. 178.

Reagents: Hydrochloric acid,

Apparatus: Centrifuge, weighing bottle, conical flask, beaker etc.

PRINCIPLE:

The IUPAC name of chlorpromazine hydrochloride (Cpn- HCl) is 3-(2-chlorophenothiazine-10-yl) propyl dimethyl-amine. It is an important compound in the group of phenothiazine and is used for psychoses control, including schizophrenia, mania, and severely disturbed or agitated behavior. It is also used for the relief of nausea and vomiting, pre-operative anxiety. Quantitative analysis, using UV-visible absorption spectroscopy, is one of the most widely used methods.

PROCEDURE:

Assay of Chloropromazine tablets

Weigh 20 tablets and reduce to a fine powder. Weigh accurately a quantity of the powdered tablets equivalent to about 0.1 g of Chlorpromazine Hydrochloride, add 5 ml of dilute hydrochloric acid and 200 ml of water. Shake for 15 minutes, add sufficient water to produce 500.0 ml. Centrifuge about 15 ml and to 5.0 ml of the clear, supernatant liquid add 10 ml of dilute hydrochloric acid and sufficient water to produce 200.0 ml. Measure the absorbance of the resulting solution at the maximum at about 254 nm. Calculate the content of $C_{17}H_{19}ClN_2S$, HCl taking 915 as the value of A(1%, 1 cm) at the maximum at about 254 nm.

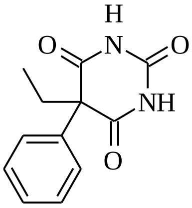
REPORT:

Experiment no: 14
Date:

MONOGRAPHIC ANALYSIS OF PHENOBARBITONE TABLET I.P

Aim:
To carry out the monographic analysis of phenobarbitone tablet IP
Reference:
Indian Pharmacopoeia, 1996, Vol II pg No.579

Name of the drug: Phenobarbitone
Molecular formula: $C_{12}H_{12}N_2O_3$
Molecular weight: 232.24
Molecular structure:



Description: Colourless crystals or white, crystalline powder; odourless.
Standard: Phenobarbitone contain not less than 99% and not more than 101%
Category: Sedative; anticonvulsant.
Dose: 60 to 300 mg at night.

Identification test	**Observation**	**Inference**
. 1) Dissolve 50 mg (assay) in 2 ml of a 0.2% w/v solution of *cobaltous acetate* in *methanol*, warm, add 50 mg of powdered borax and heat to boiling;	A bluish violet colour is produced.	Presence of phenobarbitone
2) Triturate a quantity of the powdered tablets equivalent to 0.2 g of Phenobarbitone Sodium with 5 ml of water and filter; the filtrate is alkaline to litmus solution.	It yields a white precipitate on the addition of dilute hydrochloric acid.	Presence of phenobarbitone
3) Melting point	Melts at about 175^o,	Presence of phenobarbitone

ASSAY OF PHENOBARBITONE

Aim:

To perform the assay of phenobarbitone tablet and find out the percentage purity

Reference:

i) IndianPharmacopoeia vol –III page no.2900

Reagents: Phenobarbitone, sodium methoxide, dimethyl formamide, thymol blue.

Apparatus: Extraction apparatus, Weighing bottle, conical flask, beaker etc.

PRINCIPLE:

Phenobarbitone tablet is assayed by Non-aqueous titrimetry. Since it is weak acid the non aqueous solvent dimethyl formamide is added as leveling solvent as it is titrated a strong base like sodium hydroxide solution using a suitable indicator such as thymol blue.

PROCEDURE

Standardization of 0.1M NaOH

Dissolve 0.5 gm of potassium hydrogen phthalate in 75ml of water and add 0.1ml phenolphthalein indicator and titrate using 0.1M sodium hydroxide.

Each ml of 0.1M NaOH = 0.02042 gm of potassium hydrogen phthalate

Assay of phenobarbitone tablet

Weigh and powder 20 tablets, weigh accurately quantity of powder equivalent to 0.2g of phenobarbitone. Dissolve in5 ml of pyridine, add 0.25 ml of thymolphthalein solution and10 ml of silver nitrate-pyridine reagent and titrate with 0.1 M ethanolic sodiumhydroxide until a pure blue color is obtained. Repeat the operation without the substance under examination. The difference between the titration represents the amount of sodium hydroxide required.

Each ml of .0.1 M ethanolic sodium hydroxide is equivalent to 0.01161 g of Phenobarbitone

REPORT :

Experiment no: 15
Date:

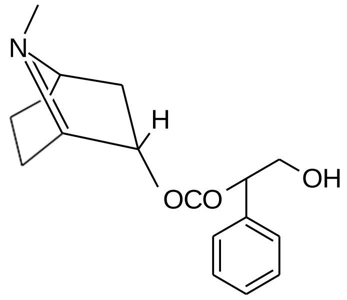
MONOGRAPHIC ANALYSIS OF ATROPINE TABLET I.P

AIM:

To carry out the monographic analysis of atropine tablet tablet I.P

REFERENCE:

Indian Pharmacopoeia, 1996, Vol- 1, pg. No. 76
Name of the drug : atropine
Molecular formula : $(C_{17}H_{23}NO_3)_2$, H_2SO_4,H_2O
Molecular weight : 694.84
Molecular structure :



$.H_2SO_4\,H_2O$

Description: Colorless crystals or white, crystalline powder; odorless.

Storage: Store in a well closed, , light-resistant containers.

Standard: Atropine Sulphate contains not less than 90.0 per cent and not more than 110.0 per cent of $(C_{17}H_{23}NO_3)_2$,H_2SO_4.

Category: Anticholinergic; antidote to cholinesterase inhibitors.

Dose: As Anticholinergic, orally, 250 m g to 2 mg daily in single or divided doses; by subcutaneous, intramuscular, or by intravenous injection, 400 m g to 600 m g four to six times a day; as antidote to cholinesterase inhibitors, by intravenous injection, 2 to 4 mg initially, followed by intramuscular injection, 2 mg repeated every 5 to 10 minutes.

Solubility: Very soluble in water; freely soluble in ethanol (95%) and in glycerin; practically insoluble in chloroform and in ether.

Identification test	Observation	Inference
Triturate a quantity of the powdered tablets equivalent to 1 mg of Atropine Sulphate with 1 drop of *strong ammonia solution*, add 2 ml of *chloroform*, triturate thoroughly, decant the chloroform solution and evaporate the chloroform. To the residue add 4 drops of *fuming nitric acid* and evaporate to dryness on water-bath; a yellow residue is obtained. Cool the residue, add 2 ml of *acetone* and 4 drops of a 3% w/v solution of *potassium hydroxide* in *methanol*;	A deep violet colour is produced.	Presence of atropine sulphate.
Test for sulphate: A: Dissolve about 50 mg of the substance being examined in 5 ml of water or use 5 ml of the prescribed solution. Add 1 ml of dilute hydrochloric acid and 1 ml of barium chloride solution.	A white precipitate is formed.	Presence of sulphate
B: Add 0.1 ml of iodine solution to the suspension obtained in test A; the suspension remains yellow (distinction from sulphites and dithionites) but is decolorised by adding, dropwise, stannous chloride solution (distinction from iodates). Boil the mixture;	No coloured precipitate is formed (distinction from selenates and tungstates).	Presence of sulphate
C: Dissolve about 50 mg of the substance being examined in 5 ml of water or use 5 ml of the prescribed solution. Add 2 ml of lead acetate solution.	A white precipitate, soluble in ammonium acetate solution and in sodium hydroxide solution, is produced.	

ASSAY OF ATROPINE TABLETS IP

Aim: To carry out the assay of atropine sulphate tablets IP
Reference: Indian Pharmacopoeia, 1996, Vol- 1, pg. No.78-79
Reagents: Atropine, hydrochloric acid, methanol, chloroform, ammonia, sodium sulphate, dichloromethane, trimethyl acetamide, trimethyl chlorosilane.

Apparatus: Burette, pipette, weighing bottle, conical flask, volumetric flask.

PRINCIPLE:

Atropine Sulfate is the sulfate salt of atropine, a naturally-occurring alkaloid isolated from the plant Atropa belladonna. Atropine functions as a sympathetic, competitive antagonist of muscarinic cholinergic receptors, thereby abolishing the effects of parasympathetic stimulation. This agent may induce tachycardia, inhibit secretions, and relax smooth muscles.

PROCEDURE:

Assay of atropine sulphate tablet

Weigh 0.5 g, dissolve in 30 ml of anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically. Carry out a blanktitration.1ml of 0.1M perchloric acidisequivalentto0.06768 g of atropine sulphate

REPORT:

Experiment no: 16

Date:

DETERMINATION OF PARTITION COEFFICIENT OF BENZOIC ACID

AIM:

To determine the partition coefficient of benzoic acid

REFERENCE:

.

PRINCIPLE:

When a particular solute soluble in 2 immiscible solvent is allowed to distribute between this two solvents the ratio of the concentration of that particular solute in this 2 immiscible solvent remain constant. Thus if C_1 and C_2 are the concentration of solvents, their ratio C_1/C_2 is a constant. However the formula may be changed depending up on the nature of 2 solvents. If both exist as monomer, then C_1/C_2 ratio is a constant, but if exist as dimer in solvents, then the factor is n C_1/C_2 constant. But not C_1/C_2. These constants are known as partition coefficient. Here benzoic acid is distributed in between water and ether. It remains as monomer in water and dimer in ether. The partition co efficient = C_1/C_2 which is a constant quantity.

PROCEDURE:

In two stoppered bottle; following mixture were prepared.

Bottle No.	**Volume of ether**	**Vol. of water**	**Weight of benzoic acid**
1	20 ml	30 ml	1.0 g
2	20 ml	30 ml	1.5 g

a) **Standardization of 0.1N sodium hydroxide.**
Dissolve 0.5g of potassium hydrogen phthalate in 75ml of water and add 0.1ml phenolphthalein as indicator and titrate using 0.1N sodium hydroxide
Each ml of 0.1N sodium hydroxide = 0.02042g of potassium hydrogen phthalate.

b) **Determination of partition coefficient**
The bottles are well stoppered and shaken for 20 minutes. After completion of shaking the mixture is allowed to stand 20-30 minutes. So that the two layer separate completely. Ether is lighter than water and exist at top while water remains at lower portion (bottom) .Ether layer is evaporated to dryness and residue was dissolved in 10ml ethanol and titrate with 0.1N sodium hydroxide using phenolphthalein as indicator similarly 10ml of water layer is pipetted out and titrate with 0.1N NaOH Each ml of 0.1N sodium hydroxide = 0.01221g of benzoic acid.

REPORT:-

1. The partition coefficient of benzoic acid in bottle 1 =
2. The partition coefficient of benzoic acid in bottle 2 =

Experiment no: 17

Date:

PARTITION CO-EFFICIENT OF SALICYLIC ACID

AIM

To determine the partition co-efficient of benzoic acid in chloroform water system.

PRINCIPLE

Partition co-efficient represent the ratio of drug distribution in two phase i.e aqueous phase and organic phase and water as an aqueous phase.Diffusion of drug across the membrane depends on drug lipid solubility. The rate of diffusion of a neutral molecule depends not only on the concentration gradient on either side of membrane but also related to lipid – water partition coefficient of the drug.In general higher the partition co-efficient, greater the diffusion of drug in to the membrane. The partition co-efficient is defined as the equilibrium constant P of the drug concentration in lipid phase and water phase. As it is difficult to measure partition co-efficient in biological system it is usually determined invitro.The partition co-efficient P represents the ratio of drug distribution in a two phase system of organic solvent and aqueous phase.

P = concentration of drug in organic layer / concentration of drug in aqueous layer

P is dependent to drug concentration, if only drug molecule have a tendency to dissociation in solution, for an ionisable drug the equation are expressed as

P = concentration of drug in organic phase / (1-α) concentration of drug in water.

If the solute undergo association in solvent;

Then $K_D = C_1/n\sqrt{C_2}$

If the substance is added to immiscible solvents in an amount in sufficient to saturate the solution it will become distributed between the two layers in a definite concentration.

The equilibrium is expressed as

$\mathbf{K = C_1 / C_2}$.

C_1 and C_2 are equilibrium concentrations of substance in organic solvent and aqueous solvent. K is the partition coefficient constant.

Partition coefficient P is an additive property of a molecule. On each structural component has its contribution towards lipophilic and hydrophilic character of molecule. Partition coefficient is an important biochemical parameter which greatly influences transport and diffusion characteristics of drug on the basis of concepts, derived from partition coefficient. Mode of action of certain non Specific general anesthesia has been explained. Action of certain barbiturates has hypnotic and disinfectant action of certain compounds is concept of partition coefficient.

PROCEDURE

50 ml of chloroform and 50 ml of water is taken in a separating funnel and add 1 g of benzoic acid to it and shake well for about one hour. Allow the mixture to separate into two layer pipette out 5 ml of organic layer and 10ml of aqueous layer, add 25 ml of 0.1M NaOH and reflux for 30 minutes. Titrate the content of flasks against 0.1M HCl using red as indicator. Repeat the same without the sample.

REPORT

www.rathinamcollege.edu.in/pharmacy

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PHYSICAL PHARMACEUTICS-II

B. PHARM 4th SEMESTER PREPARED

BY

Mr. KARTHIKEYAN J .M. Pharm.,

ASSOCIATE PROFESSOR

DEPARTMENT OF PHARMACEUTICS

CONTENTS

PHYSICALPHARMACEUTICS-II

(PRACTICAL MANUAL) SECOND YEAR (IV- SEMESTER)

EXP.NO:1

DATE:

DETERMINE THE ANGLE OF REPOSE AND INFLUENCEOF LUBRICANT ON ANGLE OF REPOSE

AIM

To determine the effect of Glidants on lubricants of angle of repose

REQUIREMENTS

- Lactose powder
- Starch paste
- Talc
- Mortar and pestle
- Funnel, stand

PRINCIPLE

Lubricants are glidants of friction during tablet, ejection between the starch of the tablet and the walls of the die cavity. The most widely used lubricants have been stearic acid and stearic acid derivatives such as calcium and magnesium stearate and talc. Glidants are intended to produce flow of the tablet granulation of powder materials by reducing friction between the particles. The most widely used glidants have been derivation of talc and corn starch.

PROCEDURE

- Select a glass funnel which has a round shape of 15-30mm of diameter with flat edge
- Fix the funnel with a clamp (on the ring)
- Place the glass plate on the ring and arrange it below the glass funnel
- Keep on graph paper on the glass funnel
- Weigh approximately 100gm of granules
- Pour the granules while blocking the orifice of the funnel be thumb
- Remove the thumb the granules load at flow down into the graph paper and form a cone shaped

- Adjust the thumb the funnel clamp so that the gap between the bottom of the funnel peak of the powder pile is about3mm
- Repeat the 5-7 steps and approximate graph is maintained
- Finally pour the granules back into funnel and allow to flow
- Mark four points which are opposite to each other on the circular base on the graph paper
- Record the readings in table, this value is the diameter calculate the radius in®
- Measure the height of the pile using two rulers
- Keep one ruler vertically and another horizontally to touch the peak of the pile, then read the value for the vertical scale.
- Substitutes the value in equation to obtained the angle of repose, generally the (h/r) measure is the angle of repose data were plotted semi-long paper and copies of curves made available for the purpose of calculating angle
- Repeat the procedure 2 more time and take on average

REPORT

The angle of repose of the given granules (without glidant) = ___________________________

The concentration is _______________

The effect of glidants of lubricants of angle of repose is = ______________________________

Inference is that the flow of granules _________

TRIAL	HEIGHT (cm)	RADIUS (r) (cm)	h/r	Angle of Repose $\theta = \tan^{-1} h/r$
I				
II				
Average angle of Repose = _____				

EXP.NO:2

DATE:

DETERMINATION OF BULK DENSITY, TRUE DENSITYAND PERCENTAGE POROSITY

AIM

To determine the bulk density, true density and percentage porosity of the given granules

PRINCIPLE

- It is defined mathematically as

  Bulk density = Mass of the Powder⁄Bulk Volume

- When particles are packed loosely lots of gaps between the particles are observed. Hence the bulk volume increases by making the powder light based on bulk volume powder are classified as light and heavy
- light powder have high bulk volumes on the other hand smaller particles the powder assume low bulk volume or high bulk volume density such powder are called heavy powder. The bulk density depends on particle size distribution, shape, and cohesiveness ofparticles.
- True density is the density of the powder itself True density=Mass of the Powder⁄Bulk Volume
- The density depend on the type of atom in a molecular rearrangement of atoms in a molecule and arrangement of molecule in the sample volume occupied by voids and the intra particle pores are not included in the most common method used in the determination of true density or gas displacement or liquid displacement method.
- This method is used to select a solvent in which the powder is insoluble

PROCEDURE:

- ✓ Approximately 20gm of powder is transformed to a 500ml cylinder and tap mechanically or by tapping device until a constant volume is obtained thus volume is bulk volume and the void space among powde rparticle

True density:

Determination of true density of the material by solvent displacement method

- ➢ Weigh accurately a clean and dry density bottle
- ➢ Take the weight of density bottle with small quantity of powder sample
- ➢ Now fill the density bottle by solvent without removing the powder material
- ➢ Calculate the true density of given powder sample

Determination of percentage porosity

- ➢ Porosity is defined as the void volume to the bulk volume of the granules

$$\text{Porosity}=1- V_p/V_b$$

REPORT

- ✓ The bulk density of the given sample of granules was found to be = ____ g/cm^3
- ✓ The true density of a given powder was found to be =_________ g/cm^3
- ✓ The percentage porosity of the a given powder is= __________%

EXP.NO;3

DATE:

DETERMINATION OF VISCOSITY OF LIQUIDUSING OSTWALD'S VISCOMETER

AIM

To determine the viscosity of the unknown liquid by using Ostwald's viscometer

REQUIREMENTS:

- Ostwald's viscometer
- Stop clock
- Specific gravity bottle
- Sample
- Distilled water

PRINCIPLE:

The force of friction with one part of a liquid offers to another part of the liquid is called viscosity. For measuring the viscosity coefficient Ostwald's viscometer method is used which is based on poiseuilles's law. According to this law, the rate of flow of liquid through a capillary tube having viscosity coefficient (n)

ɳ= ———

Where

V= volume of liquid (ml)

T= flow of time in seconds through capillary (in second)

R= radius of capillary (cm)

ɳ= viscosity coefficient (poise)
p= hydrostatic pressure
Since the hydrostatic pressure (driving force) of the liquid is given by

- ɳ=dgh

Where,
h= height of the column

d= density of the liquid

PROCEDURE:

- Wash the relative density bottle with distilled water and dried.
- Take the weight of empty bottle and filled given liquid
- Clean and rinse the viscometer properly with distilled water
- Fix the viscometer vertically in the stand and filled the specific amount of given unknown liquid in viscometer
- Time of flow recorded when the liquid starts to flow from the mark c and d above and below the bulb a. the experiment repeated 3-4 times to get viscosity of the given unknown liquid.

LIQUID	**FLOW TIME IN (SEC)**			**AVERAGE (SEC)**	**DENSITY (g/ml)**
	1	2	3		
distilled water					
given sample					

REPORT:

The viscosity of the unknown liquid is = ______________

EXP.NO:4

DATE:

DETERMINATION OF SEDIMENTATION VOULME WITHEFFECT OF DIFFERENT SUSPENDINGAGENT

AIM:

To determine the sedimentation volume with effect of different suspending agent.

PRINCIPLE:

SUSPENSION

Pharmaceutical suspension may be defined as a coarse dispersion in which insoluble solids are suspended in liquid medium. It is also known as heterogeneous system (or) more precisely biphasic system. The insoluble solids may have size range from 10-10000µm and liquid medium is normally water or a water based vehicle.

SUSPENDING AGENT

Suspending agent is defined as physiologically inert substance which increases the viscosity when added to suspensions. It helps in the keeping the dispersed particles. Suspended thus there enhanced the physical stability and re-dispersion of the sediment or shaking.

PHYSICAL STABILITY

Physical stability may be defined as a condition in which particles remain uniformly distributed throughout the dispersion with any signs of sedimentation. In practice Physical stability may be defined as a condition in which particles should be easily re-suspended by a moderate shaking. If they settle suspensions when kept aside. The solid tends to settle at the bottom of the container due to gravitational pull on the particles of higher size. It is not possible to prevent the sedimentation volume and its case of re-dispersion is the common evaluation procedure for assessing the physical stability. The two sedimentation parameters are employed such as

1. Sedimentation volume
2. Degree of flocculation

SEDIMENTATION VOLUME:

- Sedimentation volume is defined as F=
- When a suspension is taken in a measuring cylinder volume and height is proportional and height can be conveniently measured through the term volume is included in the terminology sedimentation volume “F” is a dimension less number. Most pharmaceutical suspension has an “F” value less than one. If f=1 the product has no sediment and no clear supernatant on standing which is an ideal condition. Normally “F” value lies between 0 and 1. Sometimes the network of flow is loose and fluffy and ultimate volume of sediment increase. In this situation “F” value will be greater than one.

PROCEDURE:

1. Weigh 5gm of calcium carbonate and place in a mortar and add small quantity of water and triturate the sample. After suspending the powder uniformly transfer the suspension into a 100ml measuring cylinder makeup the volume to 100ml with distilled water.
2. Separately prepare 5% w/v of calcium carbonate suspension with 1% of different suspending agent such as bentonite, methyl cellulose, respectively in different vessel add small quantity of water and triturate well. After powder is uniformly suspended transfer the suspension into separate 100ml measuring cylinder.
3. Makeup the volume to 100ml with distilled water
4. Shake the suspension simultaneously and kept aside
5. Note the volume of sediment at time periods 0,10,20,30 …60 minutes. Calculate the sedimentation volume
6. Draw the plot by taking “F” values on “Y” axis and the time on “X” axis.

REPORT:

5% calcium carbonate suspension 1% carboxyl methyl cellulose as suspending was found to be more physically stable compared with other suspending agent.

EXP.NO:5

DATE:

DETERMINATION OF SEDIMENTATION VOLUME WITH EFFECT OF DIFFERENT CONCENTRATION OF SINGLE SUSPENDINGAGENT

AIM:

Determination of Sedimentation volume With Effect of Different Concentration of Single Suspending Agent

PRINCIPLE:

SUSPENSION

Pharmaceutical suspension may be defined as a coarse dispersion in which insoluble solids are suspended in liquid medium. It is also known as heterogeneous system (or) more precisely biphasic system. The insoluble solids may have size range from 10-10000µm and liquid medium is normally water or a water based vehicle.

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2. Degree of flocculation

SEDIMENTATION VOLUME:

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PROCEDURE:

1. Separately prepare 5% w/v of calcium carbonate suspension with0.5%, 1%, 1.5%, 2 % of single suspending agent such as (bentonite) in different vessel. Add small quantity of water and triturate well. After powder is uniformly suspended transfer the suspension into separate 100ml measuring cylinder.
2. Makeup the volume to 100ml with distilled water
3. Shake the suspension simultaneously and keptaside
4. Note the volume of sediment at time periods 0, 10, 20, 30,…60 minutes. Calculate the sedimentation volume
5. Draw the plot by taking “F” values on “Y” axis and the time on “X”axis.

REPORT:

- Increase the concentration of suspending agent and also increase

  the viscosity so lower the sedimentation volume
- The concentration of single suspending agent bentonite has more physical stable.

Sedimentation volume of 5% calcium carbonate suspension with 5% bentonite

S.NO	TIME (MIN)	$F= V_{U/}V_0$
1	0	
2	10	
3	20	
4	30	
5	40	
6	50	
7	60	

EXP.NO:6

DATE:

DETERMINATION OF PARTICLE SIZE DISTRIBUTION BY SIEVING METHOD

AIM:

To determine the average particle size and find out their distribution pattern for the Given granules by sieve analysis method.

PRINCIPLE:

Sieve method gives sieve diameter, sieve diameter is defined as the diameter of the sphere that possess through the sieve aperture as the asymmetric particle sieve method directly give weight distribution. Particles having size range from 50 and 1500µm are estimated by sieving method. In this method, the size is expressed as d_{sieve}. The sieving method finds application in dosage and development of tablets and capsules. Normally 15 percent of fine powder (passed through mesh 100) should be present in granulated material to get a proper flow of material and achieve good compaction in table ting. Therefore, percent of coarse and fine can be quickly estimated. Sieves for pharmaceutical testing are constructed from wire cloth with square meshes, woven from wire of brass, bronze, stainless steel or any other suitable material.

Designations and Dimensions of I.P specification sieves

Sieve Number	Aperture Size Micrometer	Sieve Number	Aperture Size Micrometer
10	1700	44	325
12	1400	60	250
16	1000	85	35
22	710	100	36
25	600	120	34
30	500	150	36
36	425	170	35

Advantages of sieving method

1. It is in expensive, sample and rapid with reproducible results.
2. Sieving method is useful when particles are having size range between 50 and1500µm.

Disadvantage of sieving method

1. Lower limit of the particle size is 50µm.
2. If the powder is not dry, apertures become clogged with particles leading to improper sieving.
3. During shaking, attrition occurs causing size reduction of particles. This leads to errors in estimation.

Factors influencing the sieving method

Factors influencing sieving are weight of sample, duration of shaking and type of motion. The types of motion influencing sieving are vibratory motion, (most efficient), side tap motion, bottom pat motion, rotary motion with tap and rotary motion. The type of motion standardized. Care should be taken in order to get reproducible results.

PROCEDURE:

1. Standard sieves set is selected (sieve no: 10, 22, 36, 44, 65, 80, 100,120) arrange them in such manner that the coarsest remains at the top and finest at the bottom.

2. Weigh approximately 50g of sample place the sample on the coarsest sieveno.10.

3. Fix the above sieves set on hand sieve shaker and shaken for 20minutes.

4. Collect the Sample retained on each sieve into a paper, weigh all the Sample.

5. Report the weights retained on each sieve in the table against corresponding sieve number.

REPORT:

The average diameter of the given granules was found to be ________µm.

EXP.NO:7

DATE:

CALIBRATION OF EYE PIECE MICROMETR

STANDARD STAGE MICROMETER:

- ✓ Standard stage micrometer is used to calibration of eye piece micrometer. Eye piece micrometer is a glass slide (7.5cm into 2.5cm) which has the scales engraved in the scale usefully 0.1mm is length. 1mm divided into 100 divisions. Thus smallest division least count of the stage micrometer represents 0.01mm or 10µmlength.
- ✓ In this experiment in the optical combination of 10x eye piece and 45x objective is used
- ✓ The stage micrometer is least on the stage of the microscope. The objective is position to the centre of objective
- ✓ Initially disc focus low power the scale of stage micro meter observed (100divisions)
- ✓ Now the objective is focus to high power(45x)
- ✓ Two points were selected one point on the left side where divisions both scales coincide and another point on the right side
- ✓ The number of small division that is eye piece were counted and big division stage micrometer were counted and recorded

$$1 \text{ eye piece} = y/x \times 0.01\text{mm} \quad 1 \text{ eye piece} = y/x \times 10\ \mu\text{m}$$

PROCEDURE:

Counting of the sample

1. A small portion of given sample transfer to a clean slide
2. One (or) two drops of liquid paraffin is added to the slide
3. The sample is dispersed uniformly with help of brush and particles should be in depended and distribution should be uniform
4. The cover slip is placed carefully entrancement of air bubbles is avoided
5. The slide is placed the stage of microscope

Measurement of particle size:

- The slide is focus in low power (10x) the presence of individual particle is absorb (if aggregation or lumps are present the sample should be mounted again)
- The size of the each particles measure is terms of eye piece division
- A total 300 particles should be considered for size distribution analysis. Ideally 625 particles measure according to BPC.

REPORT:

From the graph it was found the particles were distributed uniformly from size range of 0-400µm.

Size Range (µm)	Mean Size (D) µm	Number Of Globules In Each Size Range	% Number Of Globules	Cumulative % Number Of Globules	Number Size
0-50					
50-100					
100-150					
150-200					
200-250					

EXP.NO:8

DATE:

DETERMINATION OF REACTION RATE CONSTANT FIRST ORDER

AIM:

To determine the reactant rate constant and half-life and the ester (methyl acetate or number of globules) at 0.5M HCL at room temperature.

APPARATUS AND CHEMICALS REQUIRED:

- Conical flask (250ml)
- 10 ml volumetric pipette
- Burette
- Ethyl acetate or methyl acetate
- Test tube
- 0.5 N HCL solution
- 0.25 N NAOH solution
- Phenolphthalein indicator
- Ice coldwater

PROCEDURE:

Preparation of hydrochloric acid solution (0.5N) IP:

Solutions of any normality XN may be prepared by diluting 85 x ml of HCL to 1000ml with water. Measure 850 ml of distilled water into a 1000ml volumetric flask. Add 42.5 ml of conc.HCL and slowly added. Finally make up the water in 1000ml.

Preparation of NAOH solution (0.25 N) IP:

Solutions of any normally XN may be prepared by dissolving 40 x gm of NAOH in water and diluting to 1000ml. weigh 10gm of NAOH and transferred into 1000ml volumetric flask. Add water slowly with stirring finally makeup the water into 1000ml.

PROCEDURE:

KINETIC METHOD

- 100ml of 0.5N HCL solution is measured and transferred into a 250mlconical flask
- It should be kept in the water bath for equilibrium (do not heat)
- 10ml of the given ester it transfer into the test tube and kept in the water bath for equilibrium. Normally it takes10minutes
- The acid solution its mix ester sample thoroughly and kept in water bath
- Immediately after mixing 5ml of the mixer is withdrawn using the pipette and transferred in to a conical flask containing 10mlicewater (0time)
- The few drops of phenolphthalein indicator is added to the mixture
- The reaction mixer is titrated against 0.25N NAOH solution. This value of alkali consumed represents V_0.
- 5ml samples by periodically at 10, 20, 30, 40, 50, 60, 75minutes. The volume consumed at each time interval represent V_t
- The reaction mixture is heated at water bath at the 60^0c at 20minutes
- The mixture cooled to room temperature
- 5ml of sample by withdrawn at transfer into the conical flask containing 10ml ice cold water. The titration is repeated and this value represents $V\alpha$.

REPORT:

The reaction rate constant (k) of the given data (methyl acetate or ethyl acetate) in 0.5N HCL acid is

From graphical method= _________minutes

From substitution method=_________minutes

The half -life ($t_{1/2}$) of the given ester (methyl acetate or ethyl

acetate) in 0.5N HCL acid is From graphical method= ____

From substitution method=___________

EXP.NO:9

DATE:

DETERMINATION OF VISCOSITY OF SEMISOILD BYUSING BROOKEFIELD VISCOMETER

AIM:

To determine the viscosity of semisolid by using Brooke field viscometer

PRINCIPLE:

Newton was the first to study the flow properties of liquids in quantitative terms liquids that obey Newton’s law of flow are called as Newtonian fluids

$$F= \eta G$$

Shear stress- shear rate

Relationship is normally in the form of a curve Rheogram or consistency curve. When data are plotted by taking “F” on x-axis and “G” on y-axis, a flow curve is obtained. The rheogram passes through the origin and the slope given the coefficient of viscosity system that follow this linear relationship are called as Newtonian fluids. This class includes liquids such as water, glycerine, chloroform, solutions of syrups, very dilute colloidal solution. Simple liquids exhibit Newtonian flow. Rheological properties of heterogeneous dispersions such as emulsions, suspensions and semisolid are more complex and do not obey Newton’s equation of flow based on the pattern of consistency curve, Non-Newtonian fluids are categorized as

- Plastic flow
- Pseudo plastic flow
- Dilatant flow

PROCEDURE:

- Prepare bentonite magma (5%w/v), methyl cellulose (2%w/v) and mineral oil. They show Non-Newtonian rheological profile
- Measure the viscosity of these liquids using a Brookfield viscometer and observe the thixotrophy phenomenon

- Place the spindle with the correct number listed in the data sheet in each liquid and rotate the spindle at the speeds indicated. Once the dial reading has stabilized, record the values of viscosity in(cps).

CALCULATION:

Viscosity in cps= dial reading x factor

S. No	**Spindle Speed**	**Factors**	**Dial Reading**	**Viscosity (F × Dr)**
1.	6	1000		
2.	12	500		
3.	30	200		
4.	60	100		
5.	30	200		
6.	12	500		
7.	6	1000		

REPORT:

The viscosity of the given sample was found to be= ________centipoise (cps)

EXP.NO:10

DATE:

DETERMINATION OF REACTION RATE CONSTANTSECOND ORDER

AIM:

To determine the reaction rate constant and half-life period of ethyl acetate in 0.025N sodium hydroxide solution at room temperature.

PRINCIPLE:

The alkaline hydrolysis of an ester (ethyl acetate) is irreversible and follows the second order kinetics.

$$CH3COOC2H5+NaOH \longrightarrow CH3COONa + C2H5OH$$

Ethyl acetate sodium Sodium
acetate ethyl
alcohol hydroxide

The molecularity of the reaction is two and the order is also two. Second order reaction is defined as the reaction in which the rate of reaction depends upon the concentration of two reactants with each term raised to the first power.

Apparatus and chemicals:

- Conical flask (250ml)
- Water bath
- Pipette
- Burette (50ml)
- Ethyl acetate solution(0.05N)
- Hydrochloric acid solution
- Ice coldwater
- Phenolphthalein indicator

PROCEDURE:

Preparation of ethyl acetate solution (0.05N)

The molecularity weight of ethyl acetate is 88.10 density is 0.90g/ml. percentage purity is 99%. Measure 50 ml of Ethyl acetate and transfer into 1000ml volumetric flask dilute to 1000ml with distilled water.

Preparation of hydrochloric acid solution (0.02N)

Solutions of any normality XN may be prepared by diluting 85 xml of hydrochloric acid to 1000ml with water. Measure 850 ml of distilled water into 1000ml volumetric flask. Add 1.7ml of concentrated hydrochloric acid slowly and shake. Finally make up the volume to the mark.

Preparation of sodium hydroxide solution (0.05N)

Weigh 2.0gm of sodium hydroxide in water and transfer into 1000ml volumetric flask. Add water slowly with continuous stirring, while cooling the flask under running tap water. Add sufficient water to make 1000ml. allow it to stand overnight and pour off the clear liquid into a bottle. This clear solution is used.

Kinetic method:

- Measure50mlof0.05Nsodiumhydroxidesolutionandtransferintoaconicalflask. Keep it in a water bath for equilibrium at room temperature.
- Measure 50ml of 0.05N of the given ester and transfer into a conical flask. Keep it in above water bath for equilibrium. Normally it takes about 10minutes.
- Mix the alkali and ester solution thoroughly and keep in same water bath.
- Immediately after mixing, withdrawn a 10ml sample of the mixture with pipette and transfer into a conical flask containing 10ml ice coldwater
- Add few drops of Phenolphthalein indicator
- Titrate against 0.02N hydrochloric acid. This titter value times t=0 corresponds to the original concentration “a” report the results

- ✓ Periodically withdrawn samples at 5, 10, 15, 20, 25, 30 minutes time periods. Repeat the steps 4 to 6. These titter values denote the amount of sodium hydroxide or ethyl acetate remain unreacted ie, (a-x) at time. Record the results
- ✓ Substitute the values in integral equation and calculate the reaction rate constant (K_2).These values will more or less constant
- ✓ Calculate the average of the reaction rate constant(K_2)
- ✓ Draw a plot by taking x/a (a-x) on y-axis and time on x-axis
- ✓ Estimate the slope. This slope same as K_2value.

Calculation:

Time (min)	**volume of HCL consumed**		**volume of HCL (ml)**	**concentration in mol/liter a or (a-x)**	**x= a(a-x)**	**x/a(a-x)**	**K_2= x/at (a-x) liter/mol**
	Initial (ml)	final (ml)					
00							
5							
10							
15							
20							
25							
30							

REPORT:

The reaction rate constant (K_2) of the given ester (methyl acetate or ethyl acetate) in 0.025N NAOH at room temperature

From graphical method= ______________________minutes From substitution method= ________________________________minutes

The half -life ($t_{1/2}$) of the given ester (methyl acetate or ethyl acetate) in 0.025N NAOH at room temperature

From graphical method= ___________

From substitution method=___________

EXP.NO:11

DATE :

ACCELERATED STABILITY STUDIES

AIM:

To determine the shelf-life of the product. If stored at 25^0C from the given data.

- A pharmaceutical product needs to be physically, chemically, therapeutically, toxicologically and microbiologically stable throughout its shelf-life. The pharmaceutical companies do stability testing for estimating the shelf-life and based on this the expiry date is given for the product.
- The real time studies at recommended condition are ideal method for predicting shelf- life often the studies are designed to increase the rate of chemical degradation or physical change of pharmaceutical products by using exaggerated storage conditions. This is known as accelerated stability testing. The pharmaceutical products are subjected to higher temperature and humidity conditions for accelerating the degradation. However the results of accelerated testing are not always predictive of physical changes and potency.
- The pharmacopoeia specifies certain storage conditions. The following table gives the details as specified in Indian Pharmacopoeia.

Storage Condition	Meaning
Cold	any temperature not exceeding 8^0C (2-8^0C)
Cool	any temperature between 8-25^0C
Warm	any temperature between 30-40^0C
Excessive Heat	any temperature above 40^0C

PRINCIPLE:

Though the medicinal products needs to be physically, chemically, therapeutically, toxicologically and microbiologically stable. The chemical instability is most often the main consideration for determining the shelf-life or expiry date. The medicinal products are stored at higher temperature conditions to accelerate the degradation rate. This is known as accelerated stability testing. The rate of chemical reaction increases by 2-3 folds for every rises in 10^0C at room temperature. The Arrhenius equation plot (log k vs 1/t) from the equation

$$\text{Log } k = \log A - K/2.303\ RT$$

Where,

K= Rate constant

R= gas constant

T= absolute temperature

E= energy of activation is used to find out the reaction rate constant at 25^0C.

PROCEDURE:

- The order of drug decomposition reaction is determined first by plotting curve. Percent potency retained VS time. Here it is first order
- The k value is determined for each temperature curve.
- The Arrhenius plot is drawn log k vs1/t
- The value at desired temperature is determined by extrapolating Arrhenius equation
- The value of k is placed in the first order rate equation and is calculated. Three drug products were kept at 4^0C ± 2^0C/ 75% RH ± 5%RH

Storage PeriodIn Months	**Potency Retained Product-I**	**Potency Retained Product-II**	**Potency Retained Product-III**
0			
3			
6			

REPORT:

The shelf-life of the medicinal product is

Product Ik=

Product IIk=

Product k

The best product is

RATHINAM
RATHINAM COLLEGE OF PHARMACY

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PHARMACOLOGY-I

B. PHARM 4th SEMESTER PREPARED

BY

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SCIENTISTS WHO SIGNIFICANTLY CONTRIBUTED TO THE EXPERIMENTAL PHARMACOLOGY

François Magendie
(6 October 1783 – 7 October 1855)
French Physiologist

Heinrich Gustav Magnus
(2 May 1802 – 4 April 1870)
German Scientist

Claude Bernard
(12 July 1813 – 10 February 1878)
French Physiologist

Carl Friedrich Wilhelm Ludwig
(29 December 1816 – 23 April 1895)
German Physician and Physiologist

Rudolf Buchheim
(1 March 1820 – 25 December 1879)
German Pharmacologist

Oswald Schmiedeberg
(10 October 1838 – 12 July 1921)
Baltic German Pharmacologist

John Jacob Abel
(19 May 1857 – 26 May 1938)
American Biochemist
and Pharmacologist

Jan Frans Heymans
(25 December 1859 – 10 April 1932)
Belgian Pharmacologist
and Physiologist

Reid Hunt
(20 April 1870 – 10 March 1948)
American Pharmacologist

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EXP. NO:1
DATE:

INTRODUCTION TO EXPERIMENTAL PHARMACOLOGY

Pharmacology is the science which deals with the study of drugs. The word '**pharmacology**' is derived from the Greek words 'Pharmakon'(a drug or poison) and logos(discourse).

It broadly covers the information about the history, source, Physio chemical properties, Physiological properties, mechanism of action, absorption, distribution, metabolism and excretion of drugs. Drugs are chemical agents used for the purpose of diagnosis, prevention relief or cure of a disease in man or animals. The word drug is derived from the French word 'drogue' meaning herb.

Experimental pharmacology is relatively the youngest branch of basic medical sciences. Pharmacological research started in Europe in the second half of the 19th century when their founders, e.g., Rudolf Buchheim and Oswald Schmiedeberg, investigated the action of existing drugs in animal experiments (Kochweser and Schechter 1978).It has been developed to its present status only during the first part of 20th century.

Today the experimental pharmacology has drifted from the conventional approach to molecular and biochemical aspects. The advancement in the field of electrophysiology, biochemistry, molecular biology and electronic or digital recording systems and software's have enriched and broadened the horizons of experimental pharmacology.

The main aims of the experimental pharmacology are to

1. Find out a therapeutic agent suitable for human use
2. Study the toxicity of a drug
3. Study the mechanism of action of drugs

Since experimental pharmacology involves the discovery of new drugs or to study the action of existing drugs it is done in **two main** stages

- Preclinical experimental pharmacology which involves the identification and optimization of novel chemical lead structures and testing them on animals and animal tissues or organs for their biological actions.
- Clinical pharmacology where testing of drugs is done on human volunteers and patients for assessing the pharmacokinetics, safety and efficacy in humans

EXP NO:3
DATE:

COMMONLY USED INSTRUMENTS IN EXPERIMENTAL PHARMACOLOGY

1. ACTOPHOTOMETER

The locomotors activity can be measured using an actophotometer (30cm long and 30 cm deep box with iron rod at the bottom) which operates on photoelectric cell that are connected in a circuit with the counter. A photocell is activated when the rays of light falling on the photo cells are cut off by animal during crossing the beam of light and the counter counts the number of cut-offs. More the locomotor activity more is the number of cut-offs. An actophotometer can have either a circular or a square area in which the animal moves. Both rats and mice can be used in this experiment.

2. EDDY'S HOT PLATE METHOD

In this equipment heat is used as source of pain to screen centrally acting analgesics. Animals are individually placed on the hot plate, maintained at constant temperatureof 55^0C. The animals are placed on the heated surface and reactions are observed. Reaction of animals such as jumping, paw licking or jumping response is taken as endpoint. Analgesics increases pain threshold. A cut-off period of 12-15sec is observed to prevent damage.

3. TAIL FLICK ANALGESIOMETER

Tail flick apparatus contains a nicrome wire, which can be heated by switching ONthe electric current. The heated nicrome wire acts as noxious stimulus. The reaction time (i.e flicking the tail) to radiant heat is measured by placing the mouse in the restrainer and keeping the tip of the tail on the analgesiometer (above the nicrome wire) and switching ON the apparatus for heating. Flicking response of tail is taken asthe end point. A cut off period of 12-15 sec is taken to avoid damage to the tail. It is used for screening the central analgesics in rats and mice.

4. PLETHISMOGRAPH

It is a simple apparatus containing mercury used to screen the compounds for anti-inflammatory activity. The mercury displacement due to dipping the paw can be directly read from the scale attached to the mercury column and adjusting the mercurylevel by moving arm B. Various devices have been developed for plethysmography ofthe paw, like mercury for immersion of the paw, more sophisticated apparatus based on the principle of transforming the volume being increased by immersion of the paw into a proportional voltage using a pressure transducer, sensitive method of measuringmouse paw volume by interfacing a Mettler Delta Range top-loading balance with a microcomputer.

5. ELECTRO CONVULSIOMETER

In Maximal electro shock induced convulsion, electric shock is applied through corneal electrode through optic stimulation. The control excitations the MES convulsions are divided into 5 phase: Tonic flexion (sustained contraction of one or more muscle group independent of position (i.e can be flexed, extended), Tonic extension, Clonic convulsion (sustained rhythmical jerking of whole body or parts of body), Stupor, Recovery or death. The duration of each phase of seizure is recorded and the effect of drug on each phases of convulsions is studied. This procedure may be used to produce convulsions both in rats and mice.

6. ROTAROD APPARATUS

The loss of muscle grip along with disturbances in the maintenance of tone and posture is an indication of centrally mediated skeletal muscle relaxation. The rota rod test is used to evaluate fore and hind limb motor coordination of rodents. The apparatus consists of a horizontal metal rod (coated with rubber) of 3cm diameter attached to a motor with speed adjusted to 2 rotations/minute to 6 rotations/min. The rod is 75cm in length and is divided into 4 or 6 sections by plastic discs, thereby allowing the simultaneous testing of 6mice. The cut off time for the test is 2 min. The retention time (sec) for each mouse/rat is recorded. The difference in the fall of time isnoticed between control and drug related animal. The length of time that a given animal stays on the rod is a measure of their balance, co-ordination, physical condition and motor-planning

7. COOK’S POLE CLIMBING APPARATUS

It is used for screening antipsychotics on condition avoidance response. It consists of transparent chamber with electrified floor and a lid which is attached to pole. Whole chamber is surrounded by wooden box. Rats are trained to climb a pole within 30s when shock is given. In a classical conditioning method, animals trained to act in a certain way (climbing a pole) in response to a signal (buzzer) to avoid noxious stimulus. Response to the signal is conditioned response while response to noxious stimulus is unconditioned response. The shock is then preceded by a buzzer for 15sec.This is done for 2-3 times a day for 8 days till rats are trained to climb the pole at the sound of the buzzer.

EXP NO:3
DATE:

STUDY OF COMMON LABORATORY ANIMALS

Parameters	**Rat**	**mouse**	**rabbit**	**Guniea pig**	**dog**
Body weight (g)	200-300	25-30	2000-3000	500-800	5000-8000
Life span(yrs)	2-3	1-2	4-5	2	Upto10
Body temperature(·c)	37.5	37.4	38.3	38.9	37.7
Blood volume(ml/kg)	50-65	70-80	45-70	65-90	75-100
Oestrus cycle	4-5	4-5	Spontaneous ovulator	15-19	180
Gestation period(d)	21	19	32	67	62
Breeding age(d)	60-80	42-56	90-120	365	90
Breeding life(yrs)	1-1.25	0.75-1.5	3	1	6-7
Litter size	10-12	10-12	4-8	2-4	2-6

Selection of animal model is one of the most important steps in any of the experimental pharmacology study. Animal model preferred for the study must be producing similar disease profile as in human. Hence, suitable animal model shouldbe selected which follows three main objectives;

1. Use of an animal phylogenetically closer to man
2. Use of an animal in which the process under investigation is as close as possible to that in man

3. The anatomy physiology and biochemistry are considered to be similar

Broadly experimental animals are divided into three categories; Rodents (Mouse, Rat, Guinea pig, Gerbil, Hamster) Non rodents (Rabbit, Monkey, Dog, Cat, Pig etc), miscellaneous (Frog, Pigeon, Zebra fish, Chicken etc)

There are selection criteria as follows:

- **Size**: Smaller animals are performed because they are easy to handle and less quantity of drug is required.
- **Availability**: Animals which are commonly available should be selected forexperiments. eg: Rat, mice and rabbit.
- **Sensitivity**: Animals which are sensitive to drugs for some sensitive trials.eg:guinea pig is more sensitive to histamine.
- **Species**: in rabbits the intra cerebro-ventricular injection of 5HT have tends tolower the temperature; but in cat it induce fever.

1. **RAT-** Biological name: *Rattus norvegicus*

Albino rat (officially known as the Pink-Eyed White or PEW) is one of the most common laboratory animal suitable for experimental work because it is smaller in sizeand greater sensitivity to most of the drugs. It can be used to obtain pure and uniform strains and it is found to be very sensitive to withstand longer period of experimentation under anaesthesia. Nude rats resemble nude mice in their lack of thymus and functionally mature T cells and are phenotypically hairless with possible fine-sparse hair growth and most preferred model in immunological research. There are two original strains of albino rat; Wistar and Sprague Dawley rat. These are been widely used throughout the world. Further development and genetic characterisation of inbred, congenic, and recombinant strains took place in the United States, Japan and other European countries.

2. **MICE (Mouse)-** Biological name: *Mus musculus*.

Albino mice are the smallest laboratory animals that can breed uniformly and are cheap, easy to handle and require small place for housing. It is sensitive to small doses of substances. Swiss Albino mice are the most widely used strain for lab investigation. Due to the large similarity in mice and human genome (>99% conserved) it provides good model. Inbred and mutant mice are ubiquitously accepted as the preferred models for identifying and understanding inherited human diseases.

Most importantly, selection as the first model animal to has its genome sequenced in the human genome. Knockout and Knock in mice have been developed for the selective assessment at the genetic level. In the knockout mice, selective genes are taken out where as in knock in mice gene of interest is introduced into the mice.

The development of nude mice, a hairless genetic mutant that lacks thymus which leads to deficient in T-cells has been of fundamental interest in the fields of tissue immunity and transplantation research. Biege mice lack natural killer cells and are susceptible to cancer.

3. **GUINEA PIG-** Biological name: *Cavia porcellus*.

It is a docile animal, highly susceptible to TB and anaphylaxis and also highly sensitive to histamine and penicillin. It requires exogenous ascorbic acid in diet. Serum contains an enzyme asparaginase, which shows antileukemic action.

4. **RABBIT**- Biological name: *Oryctolagus cuniculus*

Rabbit is also a docile animal with large ears. Usually New Zealand white rabbit are used. It has huge cecum and large appendix. They are very sensitive to histamine. It cannot vomit like the rat and horse. Cytochrome 3A4 is absent which is corresponding to cytochrome 3A6. The enzyme atropine esterase is present in rabbit lever and plasma which degrade atropine. So it can tolerate large dose of belladonna (atropine). Carotid nerve forms a separate depressive nerve. It lacks vasomotor reversal phenomena (absence of adrenergic vasodilator nerve). Ovulation is related to the release of luteinising hormone and occurs 10hrs after coitus. Only known mammal from which tubules of the kidney can be dissected with basement membrane intact.

5. **HAMSTER-** Biological name: *Mesocricetus auratus*

They are the third most commonly used animals in biomedial research. Different strains are Syrian hamsters (golden), Chinese hamsters (striped back), European hamsters and Armenian hamsters. Among these Syrian hamsters are widely used for laboratory purpose because of availability and easy production. They are relatively free from spontaneous disease and susceptible to many introduced pathogenic agents. Their anatomical and physiological features are unique for the experimental study and have rapid development with short life cycles.

6. **FROG-** Biological name: *Rana tigrina*

One of the most commonly used experimental animal in physiology, pharmacology and toxicology. It has been used for experimental purpose for past 200 years. It is easily available during rainy season. It is the only tail less amphibian used in biomedical research and safe to handle. Anatomically their heart contains three chambers which are different from the other mammals used in the experiment. There are several important substances like epibatidine, painkiller 200 times more potent than morphine and other toxins like irritants, hallucinogens, convulsants, nerve poisons and vasoconstrictors which are obtained from different species of frog

7. **CAT-** Biological name: *Felis catus*

It is a carnivorous animal relatively easy to obtain and to use for experimental purpose. The physiology of circulatory and neuromuscular system is very much similar to that of man. It has highly developed nictitating membrane which is contracted by sympathetic nerve. Morphine produces excitation of CNS in cat.

8. **DOG-** Biological name: *Canis familiaris*

Commonly Mongrel or Beagle dogs are used due to manageable size, moderate length of hair cot, docile nature and ease to handle. It is easily available and large sized animal. Dogs hs small alimentary tract and easily get trained.

9. **MONKEY-***Biological name: Macaca mulatta*

These are the primates belonging to the highest order of the mammals. The anatomy and physiology of these animals are closely related to that of man. The studies done (majorly drug metabolism) in monkeys are directly translated to man. Considering thehuman respect, tests in primates should be done only in last stage of evaluation of drugs before clinical trials.

10. **PIGEONS**

They are mainly preferred in screening antiemetic activity, cardiovascular diseases such as spontaneous atherosclerosis in pigeons and standardisation of cardiac glycosides, CNS such as anxiety pigeon method, apomorphine induces stereotypic behaviour in pigeons and screening of intravenous anaesthetics in pigeon. Bioassay ofprolactin through the pigeon crop method is one of the important methods in prolactinassessment.

EXP NO:4

DATE:

MAINTENANCE OF LABORATORY ANIMALS AS PER CPCSEA GUIDELINES

AIM

1. Realize importance of ethical requirements for preclinical students.
2. Understand the principle of different guidelines and their importance in animalexperiments.
3. Justify the need for proper standards of maintenance and care in the use ofanimals for research and teaching.

OBJECTIVE

To study the maintenance of laboratory animals as per CPCSEA guidelines.

Maintenance of laboratory animals as per CPCSEA Guidelines:

CPCSEA (**Committee for Purpose of Control and Supervision Experiments on Animals)** has set guidelines for laboratory animal facilities. The goal of these guidelines is to promote the humane are of animals used in biomedical and behavioural research and testing with the basic objective of providing specifications that will enhance animal well-being quality in the pursuit of advancement of biological knowledge that is relevant to humans and animals. The CPCSEA provides the below mentioned guidelines to be followed by all research institutions in the country.

1. Adequate veterinary care must be provided by a veterinarian or a person who has training or experience in laboratory animal sciences and medicine. Institutions should employ people trained in laboratory animal science or provide for both formal and on-the-job training for the care of animals. It is essential that the animal care staff maintain a high standard of personal cleanliness.
2. The transport of animals from one place to another is very important and must be undertaken with care. The main considerations for transport of animals are the mode of transport, the containers, and the animal density in cages, food and water during transit, protection from transit infections, injuries and stress. Quarantine is the separation of newly received animals from those already in the facility. An effective quarantine minimizes the chance for introduction of pathogens into an established colony.

3. All animals should be observed for signs of illness, injury, or abnormal behaviour, health and nutritional status of the animals should be properly maintained.
4. Animals should be fed palatable, non-contaminated and nutritionally adequate food daily unless the experimental protocol requires otherwise. Ordinary animals should have continuous access to fresh, potable, uncontaminated drinking water, according to their particular requirements.
5. Laboratory animals are very sensitive to their living conditions. It is important thatthey shall be housed in an isolated building located as far away from human habitations as possible and not exposed to dust, smoke, noise, wild rodents, insects and birds. The location, building, cage , material and environment of animal rooms are the major factors, which affects the quality of animals.
6. Housing, care, breeding and maintenance of experimental animals are necessary tokeep them in physical comfort, good health and behave normally. Bedding should be removed and replaced with fresh materials as often as necessary to keep the animals clean and dry.
7. Sanitation is essential in an animal facility. Animal rooms, corridors, storage spaces, and other areas should be cleaned with appropriate detergents and disinfectants as often as necessary.
8. The institute shall maintain SOPs describing procedure/methods adapted with regard to animal husbandry, maintenance, breeding animal house microbial the different records of animals as instructed by CPCSEA.
9. Acceptable experimental techniques and procedures for anaesthesia and euthanasia are applied during the study.
10. Transgenic animals are used to study the biological functions of specific genes, to develop animal models for diseases of human or animals, to produce therapeutic products, sanitation and routine management practices for such animals are similar to those for the other animals of the species as given in guidelines. However, special care has to be taken with transgenic/ gene knockout animals where the animals can become susceptible to diseases.
11. All scientists working with laboratory animals must have a deep ethical consideration for the animals they are dealing with. From the ethical point of view it is important that such considerations are taken care at the individual level, at institutional level and finally at the national level.

EXP.NO:5

DATE:

COMMON LABORATORY TECHNIQUES: BLOOD WITHDRAWAL, SERUM AND PLASMA SEPERATION, ANESTHETICS AND ETHANASIAUSED FOR ANIMAL STUDIES

General information on blood withdrawal in animals (Objective 1)

Animals	Site for blood withdrawal	Needle	No. of sample/24 hr	Sample Volume
Mouse/Rat (M/R)	Tail vein	25-27G(M) 21-23G(R)	1or2(M) <8(R)	50µl-0.2ml(M) 0.1-2ml(R)
	Tail snipping	Sterile scalpel blade(M)	≤4(M)	10µl(M)
	Saphenous vein	27G or 25G(M) 23G(R)	≤4(M/R)	Up to 0.15ml(M) Up to 0.2ml(R)
	Sublingual vein	23G(R)	1(R)	Up to 0.2ml(R)
	Retro-orbital	Pasteur pipette or Glass capillary tube(M/R)	Only 1 (M/R)	0.2ml with recovery 0.5ml without recovery (M);Up to 4ml recovery, 4-10ml non-recovery(R)
	Jugular vein	23 G(R)	<8(R)	0.1-2ml (R)
	Cardiac puncture	23-25G (M) 19-21G (R)	1	Up to 1ml (M) Up to 15ml (R)
	Abdominal/thoracic blood vessel (ATBV)	25 G(M) 19-21G(R)	1(M/R)	Up to 1 ml (M);10ml Hepatic Portal vein/15 ml from other ATBV
Guinea pig	Cannulation	23G-25G cannula	Up to 6 sample/2hr	0.1-0.5 ml
	Tarsal vein	23G	Up to 6 samples	0.1-0.3ml
	Saphenous vein	23G	Up to 4 samples	0.5% of BW/sample
	Abdominal/thoracic	19-21G	1	Up to 15 ml
	Cardiac puncture	20-21G	1	1-25ml
	Decapitation	---	1	10-20ml

Rabbit	Marginal ear vein/artery	19-23G	Up to 8	0.5-10ml
	Cardiac puncture	19-21G	1	60-200 ml

Objective2

AIM

To study the techniques for separation of serum and plasma from collected blood sample

DESCRIPTION

Blood is a fluid connective tissue, provides one of the means of communication between the cells of different parts of the body and the external environment. By homeostatic mechanisms blood volume and the concentration of its constituents are maintained within narrow limits.

Plasma: Blood is composed of a straw-coloured transparent fluid, plasma, in which different cells (RBCs, WBC and platelets) are suspended. Plasma constitutes about 55% and cells about 45% of blood volume. The constituents of plasma are water (90 to 92%) and dissolved substances, including plasma proteins (e.g. albumins, globulins, fibrinogen, clotting factors etc.), inorganic salts or minerals salts (e.g. sodium chloride, sodium bicarbonate, potassium, magnesium, phosphate, iron, calcium, copper, iodine, cobalt, etc.), nutrients principally from digested foods (e.g. monosaccharides, amino acids, fatty acids, glycerol and vitamins), organic waste materials (e.g. urea, uric acid, creatinine etc.), hormones, enzymes gases (e.g. oxygen, carbon dioxide, nitrogen etc.).

Serum: In blood, the serum is similar in composition with plasma but exclude clotting factors (Fibrinogen). Fibrinogen is a protein that is involved in blood coagulation by converting into fibrin. Serum includes all proteins not used in blood clotting (coagulation) and all the electrolytes, antibodies, antigens, hormones and other exogenous substances.

SERUM = PLASMA – FIBRINOGEN

Blood is centrifuged to remove cellular components. Anti-coagulated blood yields plasma containing fibrinogen and clotting factors. Coagulated blood (Clotted blood) yields serum without fibrinogen, although some clotting factor remains.

REQUIREMENTS

Animals: Laboratory animals.

Drug: Anaesthetic agents, anticoagulants.

Equipments: Cotton, syringe, needle, eppendorf tubes, centrifuge, micro pipette.

PROCEDURE

From estimation of different biochemical parameters blood may be drawn from the animals (fasted for 12 hours) and the serum or plasma is prepared as described below.

Serum preparation:

Collect whole blood in a cleaned and covered test tube. Allow the blood to clot for 30 minutes by leaving it undistributed at room temperature. Remove the clot by centrifuging for 10 minutes at 3000 rpm in a cooling centrifuge. Following centrifugation, it is important to immediately transfer the liquid component (serum) into a clean eppendorf tube using a micro pipette or Pasteur pipette. The serum used as specimen, should be free from haemolysis and hence separate it from the clot promptly.

Plasma preparation:

Collect whole blood into anticoagulant treated (e.g., EDTA or citrate-treated) test tubes. Heparinised tubes are specified for some applications; however, heparin can often be contaminated with endotoxin, which can stimulate white blood cells to release cytokines. Remove the cells from plasma by centrifugation for 10 minutes at 2000 rpm using a cooling centrifuge. Following centrifugation, it is important to immediately transfer the liquid component (plasma) into a clean eppendorf tube using a micro pipette or Pasteur pipette

Anesthetic agent (OBJECTIVE 3)	**Dose (mg/kg), Route of administration**						
	Mouse	Rat	Hamster	Guinea pig	Rabbit	Frog	Zebra fish
α-Chloralose Halothane, Isofurane, Enflurane	— 3-4%Induction 1-2% Maintenance	— 3-4%induction; 1-2% maintenance	— 3-4%induction; 1-2% maintenance	— 3-4%induction; 1-2% maintenance	80-100, iv	— 1-5%	— 1-5%
Hexobarbital Ketamine HCL Pentobarbitone sodium	60, iv or ip 22-24, im 35-50, iv or ip	60, iv or ip 22-24,im 25-50, iv or ip	— — 35, iv	— 22-24, im 30-40, iv or ip	— 22-24, im/iv 30-40, iv or ip	— 50-150 60, dorsal Lymph sac	— 50-150
Thiopentone sodium	25-50,iv or ip	20-40, iv or ip	20-40, iv or ip	20-55, iv or ip	20, iv	—	—
Urethane Tricaine and benzocaine	— —	0.75-1.5g/kg, ip	— —	1.25-1.5g/kg, ip	1g/kg ,iv or ip	— 25-100 mg/L	— 25-100 mg/L
Ketamine (K) + Xylazine	100 (K) + (5-10)(X)i.p	(60-90) (K) + (6-9)(X), i.p	(80-100) (K) + (7-10)(X), i.p	40 (K) + 5 (X) , i.p	(25-50) (K) + (6-10) (X), i.m	—	—
Ketamine(K)+ Medetomidine (M)	75 (K) + 1 (M), i.p	75 (K) + 0.5(M), i.p, s.c	100 (K) + 0.25 (M) , i.p	40(K) + 0.5 (M), s.c	25 (K) + 0.5(M), i.m.	—	—

Different methods of euthanasia (OBJECTIVE 4)

Euthanasia means the humane killing (sacrifice) of an animal which produces rapid unconsciousness and subsequent death without or minimal pain or distress to animal.

Preferred euthanasia	**Animal**
Cervical dislocation	Mice, rat, guinea pig and gerbil
Decapitation	Mice, rat, guinea pig, gerbil, hamster
70-80% CO_2	Mice, rat, guinea pig, gerbil, hamster, rabbit, cat, dog
Sodium	Mice, rat and guinea pig (150mg/
Pentobarbitone	kg;i.p.);Hamster (300mg/kg;i.p.);rabbit (120mg/kg;i.v)
Inhalation	Nearly all laboratory animals including amphibians
Pithing (single or double)	Amphibian

EX NO:6

DATE:

STUDY OF DIFFERENT ROUTES OF DRUGS ADMINISTRATION IN MICE/RATS

AIM

To study the onset and duration of action of pentobarbital sodium in rat when given by different routes of administration.

PRINCIPLE

The beginning of pharmacological response is called as onset of action**.** It occurs when the plasma drug concentration just exceeds the required MEC (Minimum effective concentration). The time required to start producing pharmacological response is known as onset time. The time period up to which pharmacological response is observed or the time period for which plasma concentration of drug remains above the MEC level is called as duration of action.

The onset and duration of action of a drug depends on the route of administration, for example, when a drug is given any IV the effect is seen by immediately and produce 100% bioavailability as compared to oral administration of drug where it takes up to 1hour to show the effect. Pentobarbital sodium is chosen for the present experiment because it shows a clear cut onset of action (animal goes to sleep by muscle tone and righting reflex, ability to maintain upright posture) and endpoint is also clear, when animal recover from sleep. Moreover its effect can be seen when administers by all routes of administration; intraperitoneal, intravenous, oral and Subcutaneous.

Calculation of the onset of action (time taken to loss of righting reflex) and duration of action (regains consciousness) in different group of animals will give an idea of the effect of various routes of administration

REQUIREMENTS

Animal : Mice (20-25g)
Drug : Pentobarbital sodium (50mg/kg)
Equipment : 1ml syringe hypodermic (no:20) oral feeding needle, Stop watch

PROCEDURE

- Weigh the animals and number them by marking.
- Divide them into 4 groups, each group 3 animals.
- Prepare a fresh stock solution containing 5mg/ml of Pentobarbital sodium

- Administer the Phenobarbital sodium (50mg/kg) to the all the animals via different routes such as IV, SC, IP, Oral respectively and place into individual cages for observation.
- Note the time of injection and time of onset of sleep, time of awake
- Animal should be placed apart so that no animal disturb other one when it recovers from the barbiturate induced sleep. Note the onset of action and time of recovery. Calculate the duration of action.

Compare the onset of action and duration of action of sleep by different route of administration of pentobarbital sodium

EXP NO:7
DATE:

STUDY OF EFFECT OF HEPATIC MICROSOMAL ENZYME INDUCERSON THE PENTOBARBITAL INDUCED SLEEPING TIME IN MICE

AIM

To study the effect of hepatic microsomal enzyme induction on the pentobarbital induced sleeping time in mice.

PRINCIPLE

The hepatic microsomal enzyme inducers enhance the rate of metabolism of other drugs. Hence, the duration of action of other drug will be reduced. Phenobarbitoneand rifampin like inducers of CYP3A and CYP2D6 affect the metabolism of a large number of drugs, because these isoenzymes act on metabolism of many drugs. Co- administration of either of these drugs with any other drug may affect the biotransformation and also the desired pharmacological effect of the drug.

REQUIREMENTS

Animals: Mice (20-25 g)

Drug: Phenobarbitone sodium (50mg/kg), Pentobarbital sodium (50mg/kg)

Equipments: Stop-watch, syringes, needles.

PROCEDURE

- Use mice of either sex with a body weight between 20-25 g for the study. Weigh and number the animals.
- Divide them randomly into two groups (each consists of 6 animals); which servesas control and test group.
- Prepare a fresh stock solution containing 5.0 mg/ml of the phenobarbitone sodium and 5.0 mg/ml of the pentobarbital sodium.
- To the control group mice inject saline (equivalent quantity of drug) once daily for 5 days and to the test group mice inject phenobarbitone (50mg/kg, i.p.) once daily for 5 days.
- Inject pentobarbital sodium (50 mg/kg, i.p.) to all the animals on next day and place into an individual plastic cage fir observation. Record the time of injection, time of sleep and time of wake, after injecting the pentobarbital sodium.
- Calculate the onset of action, duration of action in all the animals. Compare these

parameters in between different group of animals and also calculate the percentage reduction in sleeping time in test group animals as compared to control group animals.

- Differences among test and control group means values should be significantly different, by using statistical methods.

EXP NO: 8

DATE:

EFFECT OF DRUGS ON CILIARY MOTILITY OF FROG ESOPHAGUS

AIM:

To study the effect of cholinergic and anticholinergic on ciliary movement in frog buccal esophagus.

PRINCIPLE:

Cilia in the buccal cavity and in the oesophagus help in the movement of food particles. Similarly, the importance of mucociliary function has been established in respiratory tract and of pulmonary disease such as chronic bronchitis, asthma and in cystic fibrosis. The integrity of mucociliary function is very important in these air waydiseases. Cilia exhibit a great degree in that they are capable of functioning in the absence of nervous innervations. It has also been of trachea and buccal cavity which helps in the ciliary movement. The ciliary motility in buccal cavity and esophagus are stimulated by acetylcholine and other cholinergic drugs. Anticholinergics like atropine paralyze the cilia and decreases ciliary motility

REQUIREMENTS

Animal : Frog

Drugs : Physostigmine stock (1.0 µg/ml), atropine stock (1.0 µg/ml), Acetyl choline(1.0 µg/ml), Frog ringer solution.

Physiological solution : Normal saline.

Equipment: Frog board, Poppy seeds, Stop watch and Surgical instruments.

PROCEDURE

- Decapitate the frog and pin the frog to the frog board on its back.
- Pin the lower jaw to the abdomen cutting sufficiently the buccal cavity and exposing the oesophagus. Keep the buccal cavity and the opening of the oesophagus wet by irrigating it with normal saline.

Fix two parts i.e. from a point in the lower jaw (cephalic end) to the beginning of the oesophagus (caudal end). Keep this distance constant to measure the time taken by the particles to move from a point in the lower jaw to the beginning of the oesophagus.

- Remove the blood gently and moisten the esophagus surface with the help of cotton swab wetted with frog ringer solution.
- Place a poppy seed at the premarked spot in the jaw. Turn on the stop watch and note the time taken by the object to reach the beginning of the oesophagus. Repeat this for 5 times
- Wet the oesophageal membrane with frog ringer solution and then treat with few drops of physostigmine on the buccal cavity and repeat the procedure for 5 times.Note the time.
- Remove the effect of the drug by repeatedly washing the oesophageal membrane with frog ringer solution.
- Repeat the procedure with other drugs
- Find out the difference in the time taken by the object to move between the pre-marked distance in the buccal cavity in presence of saline, physostigmine, atropine and acetyl choline.

EXP NO:9

DATE:

EFFECT OF DRUGS ON RABBIT EYE

AIM

To study the effect of topically applied drugs on rabbit eye

PRINCIPLE

The eye is responsible for the vision which is supplied by both sympathetic and parasympathetic nervous system. The iris of the eye is controlled by the sphincter papillae muscle (circular fibres) and dilator papillae muscle (radial fibres). The circular muscle is innervated by parasympathetic nerves where acetylcholine acts on M_3 cholinergic receptors. Radial fibres are innervated by sympathetic nerves where the adrenaline acts on $\alpha 1$ adrenergic receptors. Contraction of circular muscle of iris leads to miosis and contraction of radial muscle of iris leads to mydriasis. Pupil of the eye is dilated with paralysis of the parasympathetic or stimulation of sympathetic system. Local action of large number of drugs in the eye can be achieved without systemic effect by application of drug or eye drops or eye ointments.

The drugs like miotics (constrict pupil/decrease pupil size) and mydriatics (dilate pupil/increases pupil size) are generally screened by this method. The size of the pupil is measured by pupilometer with varying size of round holes i.e 0.5mm, 1.0mm, 1.5mm, 2mm, 2.5mm etc. When cornea is touched rabbit closes the eyes, but when desensitized with local anaesthetics, there will be no corneal reflex. Light reflex is elicited by pointing the light source on the pupil with the help of torch. There is constriction of pupil due to light reflex.

REQUIREMEMTS

Drugs: Miotics: Physostigmine(1%), Mydriatics: Ephedrine(3-5%), Atropine(1%), Adrenaline(0.1%) Anestheics- Lignocaine(4%)

Animal: Rabbit

Equipment: Dropper, rabbit holder, cotton swab, distilled water, torch light, Pupilometer

PROCEDURE

- Put the rabbit in a suitable rabbit holder keeping the head outside
- Accommodate and acclimatize the rabbit into the rabbit holder for 5min

- Measure the size of the pupil of both the eyes by placing pupilometer/transparent plastic scale in front of the eyes
- Examine the effect of light reflex by pointing the torch light beam on the pupil
- Examine the effect of corneal reflex with a fine cotton swab
- Measure the intraocular tension
- Select the control and test eye according to your preference (right or left). Eye lashes of both eyes is trimmed out with scissors and normal saline is put in both the eyes to clean
- Instill the drug/saline with the help of dropper to the eyes (not more than 2-3 drops). Drug can be administered 3-4 times over a period of 5-8min (volume to be instilled in rabbit eye sac should be 40-60µl). After 10 minutes, study the effect of drug.
- Note the size of the pupil, light and corneal relex. Do the study until animal get normal size pupil.
- Repeat the experiment with other drugs.

EXP NO:10

DATE:

STUDY OF SKELETAL MUSCLE RELAXANT PROPERTY USING ROTAROD APPARATUS IN MICE

AIM

To study of muscle relaxant property of Diazepam in mice using Rota rod apparatus.

PRINCIPLE

The loss of muscle grip along with disturbances in the maintenance of tone and posture is an indication of centrally mediated skeletal muscle relaxation. The rota rod test is used to evaluate fore and hind limb motor coordination of rodents. The apparatus consists of a horizontal metal rod (coated with rubber) of 3cm diameter attached to a motor with speed adjusted to 2 rotations/minute to 6 rotations/min. The rod is 75cm in length and is divided into 4 or 6 sections by plastic discs, thereby allowing the simultaneous testing of 6mice. The rod is at a height of about 50cm above the tabletop in order to discourage the animals to escape from the instrument. The cut off time for the test is 2 min. The retention time (sec) for each mouse/rat is recorded.

REQUIREMENTS

Animal : Mice/Swiss albino/Either sex/20-
30gDrug : Diazepam (4mg/kg i.p)
Apparatus : Rota rod, 1ml Syringe preferably 24G onwards

PROCEDURE

- Animals are weighed and marked
- The rotarod apparatus was turned on and an appropriate speed of 20-25 rpm wasselected.
- The fall off time was noted if the mice fall from the rotarod. A normal untreated mice (before treatment) generally falls within 3-5 minutes.
- Divide the animals into two groups; Control and Test receiving saline and Diazepam respectively and administer the saline and drug to the respective groups
- After 30 minutes, the procedure was repeated and note the animal fall off time.
- The fall off time before and after treatment was compared.

EXP.NO:11

DATE:

STUDY OF DRUGS ON LOCOMOTOR ACTIVITY USING ACTOPHOTOMETER IN MICE

AIM

To study the CNS depressant and stimulant activity of drugs on locomotors activity of mice using the actophotometer.

PRINCIPLE

Most of the central nervous system acting drugs influence the locomotors activity in man and animals. The CNS depressants drugs such as barbiturate and alcohol reduce the motor activity where as stimulants such as caffeine and amphetamine increases it. In other word the locomotors activity can be an index of alertness (wakefulness) of mental activity. CNS stimulation and depression occurs due to imbalance between inhibitory neurotransmitters (GABA, Glycine) and excitatory neurotransmitters (Glutamate, Aspartate) in the brain.

The locomotors activity can be measured using an actophotometer (30cm long and 30 cm deep box with iron rod at the bottom) which operates on photoelectric cell that are connected in a circuit with the counter. A photocell is activated when the rays of light falling on the photo cells are cut off by animal during crossing the beam of light and the counter counts the number of cut-offs. More the locomotor activity more is the number of cut-offs. An actophotometer can have either a circular or a square area in which the animal moves. Both rats and mice can be used in this experiment.

REQUIREMENTS

Animal	-	Mice/Swiss albino/20-25g
Drug	-	Diazepam (3mg/Kg) and Caffeine(8mg/Kg)
Equipment	-	Actophotometer

PROCEDURE

- The animals are weighed and marked
- The equipment is turned on (check and make sure that all the photo cells are working for accurate reading)
- Individually each mouse was placed in the activity cage for 10minutes. The basal activity score of all animals were noticed for 10min.
- Give Caffeine or Diazepam and 30 minutes after repeat the procedure and note the

number of cut-offs made by each animals. Reset each mouse for activity before and after administration of drug.

- Calculate percentage decrease/increase in motor activity and report.

EXP NO:12

DATE:

STUDY OF ANTICONVULSANT ACTIVITY USING MAXIMAL ELECTRO SHOCK AND PENTYLENETETRAZOLE INDUCED CONVULSIONS IN RAT/MICE

Objective 1

AIM

To study the anticonvulsant activity using maximal electro shock induced convulsion in rats.

PRINCIPLE

Epilepsy is a clinical paroxysmal disorder of recurring seizures, with or without characteristic body movements (convulsions). It is characterised by seizure which is transient dysfunction of brain due to an abnormal firing of cerebral neurons. The maximal electroshock (MES) model is a model for grandma epilepsy (generalised tonic-clonic seizures lasts for 1-2min sequenced by aura, cry, unconsciousness, tonic spasm of all body muscles, clonic jerking followed by prolonged sleep and CNS depression) and the end point considered as tonic hind limb extention (THLE) which are evoked by electric stimuli.

The agents screened through this model considered an anti epileptic drug if it corrects or suppress THLE in rats. The maximal electroshock is induced through corneal electrode and ear electrode. The electric stimuli produce cortical excitation and induce characteristic seizures in the animals.

The MES convulsions are divided into 5 phases.

a) Tonic flexion (sustained contraction of one or more muscle group independent of position(i.e can be flexed, extended)
b) Tonic extension
c) Clonic convulsion(sustained rhythmical jerking of whole body or parts of body)
d) Stupor
e) Recovery or death

The duration of each phase of seizure is recorded and the effect of drug on each phases of convulsions is studied. This procedure may be used to produce convulsions both in rats and mice.

REQUIREMENTS

Animal	-	Rat/Wistar/150-200g
Drug	-	Phenytoin (25mg/kg i.p or po)
Equipments	-	Electro-convulsiometer (For rat: Intensity of stimulus:150 mA,50Hz for 0.2sec), corneal /ear electrodes, stopwatch, 1ml syringe, preferably 23G

PROCEDURE

- Weigh and mark the animals, divide into two groups each with 3 animals.
- One group is served as control (saline solution equivalent to drug volume) andthe other as test (Phenytoin 25mg/kg, i.p) treatment.
- Thirteen minutes after administration of the above drugs, apply prescribed maximal electroconvulsive shock of 150mA for 0.2sec to all the rats through the electrodes placed on ear pinna.
- Record the time spent by the animals in each phase of convulsions. And also note the presence or absence of Straub's tail (tail becoming rigid and erected across the back of the animal in an S-shaped curve).
- Note the reduction in time or abolition of different phases of MES convulsions.

Objective 2

AIM

To demonstrate the anticonvulsant activity using pentyleneterazole (PTZ) induced convulsion in mice.

PRINCIPLE

Seizures are finite episodes because of abnormal discharge of cerebral neurons and are broadly divided into 2 groups: 1. Epileptic seizure: seizures which are periodic and unpredictable associated with disorder of brain function and 2. Non epileptic: seizures which are evoked in a normal brain by treatment with electroshock or chemicals. That means animal model which is developed to assess the effect of the drug is a non- epileptic seizure model. Most animal models cannot show all the patho-physiological, behavioral, electrophysiological and neurochemical alteration of the spontaneous human epileptic syndrome. However, PTZ induced seizures represent theabsence seizures (clonic convulsions/jerky movements) which resemble petit mal convulsions in man and regarded as a good chemical model (others are strychnine, picrotoxin, isoniazid and yohimbine). The seizures induced are characterized by generalized spike and wave discharges on the EEG. PTZ mainly acts through inhibition on GABA coupled chloride channel.

MATERIALS REQUIRED

Animal : Mice/Swiss albino/Male/20-30g
Drug : Pentylenetetrazole (PTZ: 80-85 mg/kg i.p.), Diazepam (4mg/kg i.p)
Equipments: Plastic cage, stop watch, 1mlsyringe preferably 24G onwards

PROCEDURE

- Weigh the animal and mark properly
- Divide animal into two groups (n=6 in each group)
- Group 1: Control group (n=6) mice are given saline at the equivalent dose of drug
- Group 2 : Treatment group (n=6); mice are given diazepam at the dose of 3 mg/kg, ip
- After 15-30 min inject PTZ in a dose of 30-40 mg/kg, ip
- Observe the animal for 1 hour
- Assessment : (1) onset of seizure (2) severity of seizure (score) (3) No, of seizures in an hour (4) percentage of positive responder (if seizures score >3) and (5) total duration of seizure
- Seizures are recorded in a seven point score according to the following scale

Score:

0 = no behavioral changes; 0.5 = atypical behavior (e.g. intestine grooming, sniffing, and moving arrests) 1= isolated myoclonic jerks, ear and facial twitching; 2= atypical minimal seizures, convulsive wave through the body 3= fully developed minimal seizures, clonus of the head muscles and forelimbs, rightling reflex presnt; 4 = major seizures (generalized without the tonic phase) 5 = generalized tonic – clonic seizures beginning with running.

The number of protected animals in the diazepam treated groups is calculated as percentage against the affected animals in the control group. Furthermore the percentage delay of onset is calculated in comparison with the control group.

EXP NO:13

DATE:

STUDY OF STEREOTYPE AND ANTI-CATATONIC ACTIVITY OF DRUGS ON RATS/MICE

AIM:

To study drug (phenothiazines)- induced catatonia (extrapyramidal side effect in rats.

To study the anticatatonic (antiparkinsonian) effect of scopolamine.

PRINCIPLE:

Phenothiazine and butyrophenone type antipsychotic drugs are known to produce extrapyramidal side-effects in man these effects, such as akinesia, rigidity and tremors, are called Parkinson's- like because in Parkinson's disease the major clinical symptoms include difficulty to move and change posture (akinesia and rigidity) and tremors.

These effects of antipsychotic drugs are due to excessive blockade of dopamine receptors in the extrapyramidal motor system.Therefore, phenothiazines (chlorpromazine or perphenazine) are commonly used to produce Parkinson's-like extrapyramidal symptoms in laboratory animals and to study anti-parkinsonism drugs. The students are advised to know the pharmacology of anti-parkinsonism drugs before performing this experiment.

REQUIREMENTS

Animal:Rats(150-200g)

Drugs: Perphenazine (dose: 5 mg/kg IP; prepare a stock solution containing 1 mg/ml of the drug and inject 0.5 ml/100g bodyweight of the animal). Scopolamine (dose: 2mg/kg, IP prepare a stock solution containing 0.4 mg/ml of the drug and inject 0.5 ml/100 g body weight of the animal)

Equipment: two wooden blocks, one being 3 cm high and the other 9 cm high

PROCEDURE:

1. Weigh and number the animal. Divide the animals into two groups, one for control (for study of perphenazine effect) and the other for studying the effects of scopolamine. Each group should consist of at least 5 animals.
2. Inject perphenazine to control animals. observe severity of catatonic response as follows:
3. Observe the severity of catatonia at 5,15,30,45,60,90 and 120 min after perphenazine .
4. To the second group inject scopolamine and after 30 minute inject perphenazine to these animals already treated with scopolamine. Observe and score the severity of catatonic as in step 2.
5. Compare the onset and severity of catatonic response in the both groups. Plot a graph, time along the x-axis. Note the difference in the onset and severity of catatonic response in both the groups.

EXP NO: 14

DATE:

STUDY OF ANXIOLYTIC EFFECT OF DIAZEPAM USING ELEVATED PLUS MAZE IN RAT

AIM

To demonstrate anti-anxiety effect of diazepam in rat using elevated plus maze apparatus.

PRINCIPLE

It is a novel test for the selective identification of ‘anxiogenic and anxiolytic’ drug effects in rodents. The plus maze apparatus consists of two open (16 x 5 cm for mouse and 50 x 10 x cm for the rats) and the two closed arms (16 x 5 x 12 cm for mouse and 50 x 10 x 40 cm for rats), and an open roof of the entire maze elevated (25 cm for mouse and 50 cm for rats) from the floor. The animals are placed individually at the centre of the elevated plus maze with their head facing towards the open arm during the 90 sec-5 min test. The preference of the animal for the first entry, the number of entries into the open and closed arms reflects the relative safety of closed arms as compared with the relative fearfulness of open arms. Rat/mouse are rodents and feel safe in dark, hence normal rodents prefer dark arm first. Anxiolytics would be expected to increase the proportion of entries into and time spent in open arms.

REQUIREMENTS

Animal/species	: Rat/ Wistar/Either sex/150-250 g
Syringe/needle	: 1 ml/preferably 23G
Drug	: Diazepam (1 mg/kg ,i.p)
Equipment	: Elevated Plus Maze

PROCEDURE

- Weigh the animals and mark properly
- Divide animals into two groups (n=6 in each group)
- Group 1: Control group (n=6); rats are given saline at the equivalent dose ofdrug
- Group 2: Treatment group (n=6); rats are given Diazeparm at the dose of 1mg/kg ,ip
- Observe the animals for 5 minutes (cut off time)
- Observation parameters: (1) First arm preference, (2) No. of entries into the open and closed arm, (3) Time spent in the open and closed arm and (4) Total no. of entries in the arm.

EXP NO:14
DATE:

STUDY OF LOCAL ANESTHETICS BY DIFFERENT ROUTES

AIM

To study the local anaesthetic effects by intradermal injection of lignocaine in guinea pig.

PRINCIPLE

Local anaesthetics are agents which block conduction of Na+ by decreasing or preventing the large transient increase in the permeability of excitable membranes andat higher concentration they also block K+ channels. This is based on the principle of loss of the sensation, even after given external stimuli. Local infiltration occurs when the nerve endings in the skin and subcutaneous tissue are blocked by direct contact with a local anaesthetic which is injected into the tissue. Local infiltration is used primarily for surgical procedures involving a small area of tissue (e.g. suturing a cut). Pricking on the skin produces a pain along with squeak response (twitch or movementof that part). Intra dermal injection inhibits the pain and squeak response when prick to injectable area of skin. This activity is better studied in guinea pig flanks.

REQUIREMENTS

Animal/species : Guinea pig
Sex/Body weight : Either sex/250-350 gm
Drug : Lignocaine (2% gel)
Instruments : Scissor, razor

PROCEDURE

- Weigh the animals and mark properly to distinguish from one another
- Remove the hair on its flanks on the both sides
- Control side: (Mark ‘C’): Saline or control jelly is applied at ‘C’ site
- Treatment side: (Mark ‘T’): drug is applied locally at the ‘T’ site
- Anesthetic activity is checked by giving the small pinch with forceps on the control and treatment side and then check the animal response (squeak response).
- Take the responses at the 0, 5,10, 20,30,45 and 60 min after application of gel
- The effect of the local anaesthetic is expressed as ‘Yes’ for the LA effect and ‘No’ for the absence of the effect.

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PHARMACOGNOSY AND PHYTOCHEMISTRY-I

B. PHARM 4th SEMESTER PREPARED
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INTRODUCTION

Pharmacognosy, known initially as materia medica, may be defined as the study of crude drugs obtained from plants, animals and mineral kingdom and their constituents. There is a historical misinformation about who created the term pharmacognosy.

According to some sources, it was C. A. Seydler, a medical student at Halle, Germany, in 1815; he wrote his doctoral thesis titled Analectica Pharmacognostica. However, The physician J. A. Schmidt (Vienna) used that one in his Lehrbuch der materia medica in 1811, to describe the study of medicinal plants and their properties. The word pharmacognosy is derived from two Latin words pharmakon, ‘a drug,’ and gignoso, ‘to acquire knowledge of’. It means ‘knowledge or science of drugs’.

Crude drugs are plants or animals, or their parts which after collection are subjected only to drying or making them into transverse or longitudinal slices or peeling them in some cases. Most of the crude drugs used in medicine are obtained from plants, and only a small number comes from animal and mineral kingdoms.

Drugs obtained from plants consist of entire plants, whereas senna leaves and pods, nux vomica seeds, ginger rhizome and cinchona bark are parts of plants. Though in a few cases, as in lemon and orange peels and in colchicum corm, drugs are used in fresh condition, and most of the drugs are dried after collections.

Crude drugs may also be obtained by simple physical processes like drying or extraction with water. Therefore, aloe is the dried juice of leaves of Aloe species, opium is the dried latex from poppy capsules and black catechu is the dried aqueous extract from the wood of Acacia catechu.

Plant exudates such as gums, resins and balsams, volatile oils and fixed oils are also considered as crude drugs. Further drugs used by physicians and surgeons or pharmacists, directly or indirectly, like cotton, silk, jute and nylon in surgical dressing or kaolin; diatomite used in filtration of turbid liquid or gums; wax, gelatin, agar used as pharmaceutical auxiliaries of flavouring or sweetening agents or drugs used as vehicles or insecticides are used in pharmacognosy.

Drugs obtained from animals are entire animals, as cantharides; glandular products, like thyroid organ or extracts like liver extracts. Similarly, fish liver oils, musk, bees wax, certain hormones, enzymes and antitoxins are products obtained from animal sources. Drugs are organized or unorganized. Organized drugs are direct parts of plants and consist of cellular tissues. Unorganized drugs, even though prepared from plants are not the direct parts of plants and are prepared by some intermediary physical processes, such as incision, drying or extraction with water and do not contain cellular tissue. Thus aloe, opium, catechu, gums, resins and other plant exudates are unorganized drugs. Drugs from mineral sources are kaolin, chalk, diatomite and other bhasmas of Ayurveda.

ORIGIN OF PHARMACOGNOSY

Views on the beginning of life on planet Earth have forever remained controversial and an unending subject of debate.

Nevertheless, we can say with certainty that the vegetable kingdom was already there when man made his appearance on Earth.

As man began to acquire closure acquaintance with his environment, he began to know more about plants, as these were the only curative agents he had.

As he progressed and evolved, he was not only able to sort on as to which plant served for eating and which did not, but he went beyond and began to associate curative characteristics with certain plants, classifying them as painkillers, febrifuge, antiphlogistics, soporific and so on. This must have involved no doubt, a good deal of trial and error, and possibly some deaths in the beginning also, but as it happened antidotes against poisons were also discovered.

As we shall see later, drug substitutes were also forthcoming. All these states of affairs indicate that the origin of pharmacognosy, i.e. the study of natural curative agents points towards the accent of human beings on mother earth, and its historical account makes it clear that pharmacognosy in its totality is not the work of just one or two continental areas but the overall outcome of the steadfast work of many of the bygone civilizations like the Chinese, Egyptian, Indian, Persian, Babylonian, Assyrian and many more.

Many of today’s wonderful modern drugs find their roots in the medicines developed by the tribal traditions in the various parts of the world.

SCOPE OF PHARMACOGNOSY

Crude drugs of natural origin that is obtained from plants, animals and mineral sources and their active chemical constituents are the core subject matter of pharmacognosy.

These are also used for the treatment of various diseases besides being used in cosmetic, textile and food industries.

During the first half of the nineteenth century apothecaries stocked the crude drugs for the preparation of herbal tea mixtures, all kinds of tinctures, extracts and juices which in turn were employed in preparing medicinal drops, syrups, infusions, ointments and liniments.

The second half of the nineteenth century brought with it a number of important discoveries in the newly developing fields of chemistry and witnessed the rapid progress of this science. Medicinal plants became one of its major objects of interest and in time, phytochemists succeeded in isolating the pure active constituents.

These active constituents replaced the crude drugs, with the development of semisynthetic and synthetic medicine, they became predominant and gradually pushed the herbal drugs, which had formerly been used, into the background.

It was a belief that the medicinal plants are of no importance and can be replaced by man-made synthetic drugs, which in today's scenario is no longer tenable. The drug plants, which were rapidly falling into disuse a century ago, are regaining their rightful place in medicine. Today applied science of pharmacognosy has a far better knowledge of the active constituents and their prominent therapeutic activity on the human beings.

Researchers are exploiting not only the classical plants but also related species all over the world that may contain similar types of constituents. Just like terrestrial germplasm, investigators had also diverted their attention to marine flora and fauna, and wonderful marine natural products and their activities have been studied.

Genetic engineering and tissue culture biotechnology have already been successful for the production of genetically engineered molecules and bio transformed natural products, respectively. Lastly, crude drugs and their products are of economical importance and profitable commercial products. When these were collected from wild sources, the amount collected could only be small, and the price commanded was exorbitantly high.

All this has now changed. Many of the industrially important species which produced equally large economic profits are cultivated for large-scale crop production. Drug plants, standardized extracts and the therapeutically active pure constituents have become a significant market commodity in the international trade.

In the light of these glorious facts, scope of pharmacognosy seems to be enormous in the field of medicine, bulk drugs, food supplements, pharmaceutical necessities, pesticides, dyes, tissue culture biotechnology, engineering and so on.

EXP.NO:1
DATE:

TRACAGANTH

SYNONYMS: Tragacanth, gum tracaganth

BIO.SOURCE: Dried gummy exudate obtained from the stem of *Astragalus gummifer*

FAMILY: Leguminosceae

MORPHOLOGICAL CHARACTERS

Odour and taste: No odour and no taste

Colour: white or pale yellowish white

Fracture: breaks with a short fracture

Form: Flat, lamellated or ribbon shaped flakes or filaments

ACTIVE CONSTITUENTS

Tragacanthin, bassorin

USES

- Demulcent and emollient
- Suspending agent for insoluble substances.
- Binding agent for pills and tablets
- Laxative because of swelling

CHEMICAL TEST FOR TRACAGANTH

REQUIREMENTS

Required chemical

α-napthol, sulphuric acid, ferric chloride, alcoholic KOH, HCl, NaoH, fehling A&B, Barium chloride, ruthenium red, n/50 Iodine, Lead acetate, Ammonium hydroxide

Required apparatus

Microscope, microscope slide, test tubes, test tube holder, water bath

REPORT

S.no	Chemical test	Observation	Inference
1.	**Molisch's test** In a tube, add 2ml of the test carbohydrate solution and 2 drops of α-napthol solution. Carefully incline the tube and pour drop wise conc. H_2SO_4 ,using a dropper, along the sides of tube		
2.	Boil with freshly prepared 10% aqueous ferric chloride solution		
3.	If powder is warmed with 5% alcoholic KOH solution		
4.	To 5ml of 0.5 % w/v aqueous solution of tragacanth, add 0.5ml of HCL on water bath Divide the hydrolysed product in 2 parts: a) In the first part add 1.5ml of NaOH solution to neutralize and add Fehling's solution A and B in equal quantities (mixed before) and warm over water bath. b) In the second part, add barium chloride solution		
5.	Dissolve tragacanth and precipitated cone. Ammonium hydroxide.		
6.	To 0.1gm of powder add N/50 iodine		
7.	Add Ruthenim RED solution (0.1% in H20) to powdered gum tragacanth and examine microscopically.		
8.	To 0.5% w/v solution add 20% w/v solution of lead acetate		

EXP.NO:2
DATE:

ACACIA

SYNONYMS

Acacia, gum acacia, gum Arabic

BIOLOGICAL SOURCE

Dried gummy exudate obtained from the stem and branches of *Acacia Senegal*

FAMILY

Leguminoseae

MORPHOLOGICAL CHARACTER

Odour and taste: No odour, taste is bland and mucilaginous.

Solubility: soluble in water and insoluble in alcohol

Colour: white, yellow, pale, amber or opaque

Fracture: Brittle and breakes easily

ACTIVE CONSTITUENTS

Enzyme: oxidase, peroxidase

Arabin, Arabic acid

USES

- Suspending agent in mixture
- Emulsifying agent in emulsion
- Binding agent in pills and tablet
- Demulcent in cough remedies

CHEMICAL TEST FOR ACACIA

REQUIREMENTS

Required chemicals

α-Napthol, sulphuric acid, borax, alcohol, Lead sub acetate, Hcl, NaoH, Fehling’s A&B, Lead acetate, N/50 Iodine

Required apparatus

Test tube,test tube holder, water bath, Filter paper

S.no	Chemical test	Observation	Inference
1.	**Molisch’s Test** In a tube, add 2ml of the test carbohydrate solution and 2 drops of α-		

	napthol solution. Carefully incline the tube and pour drop wise conc.H_2SO_4 ,using a dropper, along the sides of tube.		
2.	**Solubility** a) Water b) alcohol		
3.	To 2 ml solution add 500mg borax,boil and cool.		
4.	To the aqueous solutions add few drops of dilute solution of lead sub acetate.		
5.	To 5ml of 0.5 % w/v aqueous solution of tragacanth, add 0.5ml of HCL on water bath Divide the hydrolysed product in 2 parts: a) In the first part add 1.5ml of NaOH solution to neutralize and add Fehling's solution A and B in equal quantities (mixed before) and warm over water bath. b) In the second part, add barium chloride solution		
6.	1ml of gum + 4 ml of water + lead acetate solution		
7.	To 0.1 g of powder, add 1 ml of N/50 iodine		

REPORT

EXP.NO:3
DATE:

AGAR

SYNONYMS

Agar-agar, japanese insinglass

BIOLOGICAL SOURCES

It is the dried colloidal concentrate from a decoction of various red algae particularly *Gelidium amasii*(Japan), *Gelidium corneum*(spain)

FAMILY

Gelidiaceae

MORPHOLOGICAL CHARACTER

Odour and taste: odourless and mucilaginous

Colour: yellowish white or greyish white

Form: strips, Flakes or coarse powder

ACTIVE CONSTITUENTS

Agarose, agropectin

USES

- ♣ To prepare nutrient media in bacteriological culture
- ♣ In the preparation of capsules and suppositories
- ♣ As a bulk laxative and in chronic constipation
- ♣ In industrial application like emulsion, sizing silk textiles, adhesives and thickening ice cream

CHEMICAL TEST FOR AGAR

REQUIREMENTS

Required chemical

Hcl, NaoH, Fehling’s A and B, Barium chloride, iodine, ruthenium red, potassium hydroxide, tannic acid

Required apparatus

Test tubes, test tube holder, microscope, microscope slide.

S.no	Chemical test	Observation	Inference
1.	Incinerate agar to ash, add a drop of conc. HCI, observe under microscope		

2.	0.5% solution of agar. Heat it on water bath for 30minutes, cool at room temperature and divide into two portions. a) Add 3ml of 10% NaOH solution and Fehling's solution A and B in equal quantities and warm over water bath. Add 10% of barium chloride solution		
3.	Add 1 drop of N/10 solution of iodine to 10ml of decoction of agar. Rapidly cool under tap water to room temperature.		
4.	Mount a pinch of agar in the solution of ruthenium red and examine microscopically		
5.	Warm little sample in alcoholic solution of Potassium Hydroxide		
6.	0.2% solution of agar + aqueous solution of tannic acid		
7.	Boil 0.5 gm of agar with 10ml of water until solution is affected, cool to room temperature		
8.	Agar with cold water		

REPORT

EXP.NO:4
DATE:

GELATIN

SYNONYMS

Gelatinum

BIOLOGICAL SOURCE

Bos tourus, ovis aries

FAMILY

Bovidea

MORPHOLOGICAL CHARACTER

Colourless or pale yellowish translucent sheets, strips,

Odour and taste: slight bland

ACTIVE CONSTITUENTS

Lysine, gluten protein

USES

- In the manufacture of capsules and suppositories, also for coating pills and tablets.
- As a culture medium for growing micro-organisms.
- As a substitute for blood plasma

CHEMICAL TEST FOR GELATIN

REQUIREMENTS

Required chemicals

Soda lime, tannic acid, Millon’s reagent, picric acid, sodium hydroxide, cuso4.ninhydrin reagent, nitric acid

Required apparatus

Test tubes, test tube holder, water bath

S.no	Chemical test	Observation	Inference
1.	**Biuret test**: Take 3 ml test solution, add 4% NaOH and few drop of 1% $CuSO_4$		
2.	**Millon’s test:** Take 3 ml test solution, add 5 ml Millions reagent		
3.	When sample is heated with soda lime in dry test tube		
4.	Sample treated with tannic acid solution.		

5.	Sample solution treated with picric acid		
6.	**Xanthoprotein test:** Sample is treated with nitric acid		
7.	**Ninhydrin test:** Sample is treated with ninhydrin solution		

REPORT

EXP.NO:5

DATE

STARCH

SYNONYMS

Amylum

BIOLOGICAL SOURCE

Oryza *sativa* - rice

Zea mays - maize

FAMILY

Gramineae

MORPHOLOGICAL CHARACTER

Colour-rice and maize are white

Odour-odourless

Taste -Mucilaginous

ACTIVE CONSTITUENTS

Amylopectin, amylose

USES

- Nutritive, demulcent, protective and absorbent
- Antidote for iodine poisoning

CHEMICAL TEST FOR STARCH

REQUIREMENTS

Required chemical

Alpha- naphthol, sulphuric acid, iodine

Required apparatus

Waterbath, test tube, testtube stand.

S.no	Chemical test	Observation	Inference
1.	**Molisch's test:** In a test tube, add 2 ml of the test carbohydrate solution and 2 drops of a-naphthol solution. Carefully incline the tube and pour drop wise conc. H_2SO_4 using a dropper, along the sides of the tube.		

2.	**Iodine Test:** Add 2 drops of iodine solution to about 2 mL of the carbohydrate containing test solution.		
3.	Boil 1 gm starch with 15 ml water and cool it.		
4.	In above form of translucent jelly add few drop of iodine solution		

REPORT

EXP.NO:6

DATE

HONEY

SYNONYMS

Madhu, mel

BIOLOGICAL SOURCE

Apis mellifera

FAMILY

Apidea

MORPHOLOGICAL CHARACTER

Colour-pale yellow to yellowish-brown liquid

Odour-characteristic, pleasant

Taste-sweet and faintly acid

ACTIVE CONSTITUENTS

Enzyme: invertase, diastase, inulase

Glucose, sucrose, fructose

USES

- Demulcent and sweetening agent
- it is antiseptic and applied burns and wounds.

CHEMICAL TEST FOR HONEY

REQUIREMENTS

Required chemicals

Alpha napthol. Sulphuric acid, Fehling’s solution A & B, Petroleum ether, resorcinol, HCL

Required apparatus

Test tube, testtube holder, waterbath

S.no	Chemical test	Observation	Inference
1.	**Molisch's test** In a test tube, add 2 ml of the test carbohydrate solution and 2 drops of a-naphthol solution. Carefully incline the tube and pour drop wise conc. H_2SO_4 using a dropper, along the sides of the tube.		
2.	**Reducing Sugar Test**		

	Dissolve 0.5 ml of honey in 10ml of water add 2 ml of Fehling’s A&B and boil		
3.	**Fiehe's test** Take about 3ml of honey + 2ml of ether and shake thoroughly and allow the 2 layers to separate and evaporate to dryness. The upper ethereal layer is separated and put in a evaporating dish and evaporate, to the residue add 1% resorcinol and HCL		
4.	**Seliwanoff’s test** To the test solution, add a crystal of resorcinol. To this mixture add equal volume of con.Hcl , warm the test on water bath		

REPORT

EXP.NO:7
DATE:

CASTOR OIL

SYNONYMS

Oleum ricini

BIOLOGICAL SOURCE

Ricinis communis

FAMILY

Euphoribiaceae

MORPHOLOGICAL CHARACTER

Colour: colourless or pale-yellow viscous liquid.

Odour and taste: slightly nauseating and acrid

Solubility: Soluble in alcohol and miscible with all organic solvents

ACTIVE CONSTITUENTS

Ricinoleic acid, heptaldehyde, undecanoic acid, sebaic acid, linoleic acid, iso ricinoleic acid, stearic acid, isostearic acid.

USES

- Used as a purgative
- Hydrogenated castor oil as an ointment base
- In hair oil and hair fixers
- In soap industry

CHEMICAL TEST FOR CASTOR OIL

REQUIREMENTS

Required chemicals

Petroleum ether, alcohol

Required apparatus

Test tube, testtube stand, test tube holder

S.no	Chemical test	Observation	Inference
1.	Add 5ml of light petroleum ether (40 ^{o} - 60^{o}) to 10ml of castor oil		
2.	Oil + equal volume of alcohol and cool to 0^{o}C for 3hrs		

REPORT

EXP.NO:8
DATE:

DETERMINATION OF STOMATAL NUMBER AND STOMATAL INDEX

AIM

To determine the stomatal number and stomatal index of the given fresh leaves

PRINCIPLE

Stoma (plural-stomata) is a minute epidermal opening covered by two kidneys shaped guard cells in dicot leaves. These guard cells, in turn, are surrounded by epidermal (subsidiary) cells. Stomata perform the functions of gaseous exchange and transpiration in plants. The nature of the stomata, as well as, the stomatal index and stomatal number are important diagnostic characteristics of dicot leaves. Stomatal number is defined as the average number of stomata per sq mm of epidermis of the leaf. The actual number of stomata per sq mm may vary for the leaves of the same plant grown in different environment or under different climatic conditions. It is, however shown that the ratio of the number of stomata to the total number of epidermal cells in a given area of epidermis is fairly constant for any age of the plant and under different climatic conditions. Stomatal index is the percentage which the number of stomata form to the total number of epidermal cells, each stoma being counted as one cell. Stomatal index can be calculated by using the following equation:

$$\textbf{Stomatal Index} = S \times 100/E+S$$

Where,

S= Number of stomata per unit area

E= Number of epidermal cells in the same unit area.

Whilst stomatal number varies considerably with the age of the leaf and due to changes in environmental conditions, stomatal index is relatively constant and therefore, of diagnostic significance for a given species

REQUIREMENTS

Required chemicals

Chloral hydrates, glycerin water

Required apparatus

Compound microscope Camera lucida Drawing board Micro slides Cover glasses Forceps Spirit lamp Small watch glass Blade Cello tape Drawing sheet, Dark coloured pencil

PROCEDURE

1. Preparation of lamina Take a mature leaf. If the leaf is small, the whole leaf may be taken and if the leaf is large cut 5 mm square pieces from the middle portion between the lamina and midrib.

Fresh leaf

1. Sometimes the epidermis can be easily peeled off in thick leaves by breaking it into pieces by sheering action. Separate the epidermis and treat with chloral hydrate.

2. Cut a number of 5mm pieces from the middle portion between the lamina and midrib.

3. Boil with chloral hydrate in a test tube placed in a water bath. The epidermis separates out. Carefully place the epidermis on a slide with the help of a brush along with 1-2 drops of chloral hydrate; cool and then place a cover glass. OR

4. Prepare an imprint of the epidermis: Take a little piece of gelatin gel (50%) with the help of a needle. Smear it on a hot slide, place a fresh leaf and slightly press the leaf. Invert the slide and cool it under a water tap till the gel is solidified. Then the leaf is removed. This leaves an imprint of the stomata and epidermal cells on the gel.

5. Trace the epidermal cells and stomata with the help of camera lucida.

Tracing of cells

1. Draw a square of about 8-10 cm square on a drawing sheet or any unit area.

2. Place the prepared slide on the stage of the microscope.

3. Focus epidermal cells and the stomata first with 10×10 and later focus with 10×40 or 10×20.

4. With the help of camera lucida, trace the stomata and the epidermal cells in the square.

5. Trace epidermal cells and the stomata outside the square to completion on two adjacent sides of the square, for counting purpose.

6. Number the complete epidermal cells and the stomata within the square.

7. Then continue numbering the cells that are more than half on two adjacent sides.

OBSERVATION &CALCULATION

S.no	Stomatal number		Stomatal index	
	Upper surface	**Lower surface**	**Upper surface**	**Lower surface**

Stomatal index = no. of stomata x 100 No. of stomata + epidermal cells

Stomatal Index = S x 100/E+S

REPORT

EXP.NO:9
DATE:

DETERMINATION OF VEIN-ISLET, VEIN TERMINATION NUMBER AND PALISADE RATIO

AIM

To determine the vein islet, vein termination and palisade ratio of the given fresh leaves.

PROCEDURE

VEIN ISLET NUMBER

Clear a piece of the leaf by boiling in choral hydrate solution for about thirty minutes. Arrange camera Lucida and drawing board for making drawings to scale. Place stage micrometer on the microscope and using 16 mm objectives, draw a line equivalent to 1mm as seen through the microscope. Construct a square on this line. Move the paper so that the squares seen in the eye piece, in the center of the field.

Place the slide with the cleared leaf (epidermis on the stage). Trace off the veins which are included within the square, completing the outlines of those islets which overlap two adjacent sides of the square. Count the number of vein islets in the square millimeter.

Where the islets are intersected by the sides of the square, include those on two adjacent sides and exclude those islets on the other sides. (To obtain a critical result for a leaf, 4 sq. mm. should be used, preferably in one large area of 4 sq. mm). Find the average number of vein islets from the four adjoining squares, to get the values for one sq. mm.

VEIN TERMINATION

Clear a piece of the leaf by boiling in choral hydrate solution for about thirty minutes. Arrange camera Lucida and drawing board for making drawings to scale.

Place stage micro meter on the microscope and using 16 mm objectives, draw a line equivalent to 1mm as seen through the microscope. Construct a square on this line.

Move the paper so that the square is seen in the eye piece, in the center of the field. Place the slide with the cleared leaf (epidermis on the stage). Count the number of veinlet terminations present within the square. Find the average number of vein islet termination number from the four adjoining squares, to get the values for one sq.

PALISADE RATIO

Piece of the leaf is boiled in chloral hydrate and is placed under microscope. Camera Lucida and drawing board were arranged and the outline of four cells of the epidermis was traced using 4 mm objective. Then, palisade layer is focused down and sufficient cells for covering the tracing of the epidermal cells were traced off. The outline of those palisade cells which were intersected by the epidermal walls was completed.

The palisade cells under the four epidermal cells (including cells which are more than half and excluding cells which are less than half within the area of epidermal cells) are counted. The determination for five groups of four epidermal cells from different part of the leaf was repeated. The average number of cells beneath epidermal cells is calculated known as palisade ratio.

OBSERVATION

S.NO	VEINISLET	VEIN TERMINATION	PALISADE RATIO

REPORT

EXP.NO:10

DATE

DETERMINATION OF NUMBER OF STARCH GRAINS BY LYCOPODIUM SPORE METHOD

AIM

To determine the starch grains by lycopodium spore

PRINCIPLE

Lycopodium spores are obtained from club moss, Lycopodium clavatum Linn., belonging to family Lycopodiaceae. The spores are yellow in colour, spheroidol, tetrahedral in shape with reticulate surface. They have uniform average diameter of 25microns. One milligram contains average 94000 spores. They have uniform moisture content, hence the weight remains the same. This is the reason, why these spores are used to evaluate powdered drugs by comparison. The spores are also resistant to pressure.

REQUIREMENTS

Digital balance

Watch glass

Small flexible spatula

Microscope with mechanical stage or a counting square

Suspending agent: Fixed oil or suspending agent; glycerine: tragacanth mucilage: water (2:1:2).

(Note: This keeps the spores and particles in a suspension. Dilution of the suspension should give about 10 to 20 spores in a field.)

PROCEDURE

Determine the loss on drying for the powder at 105^0C. Mix a weighed amount of air-dry powder of the drug and a weighed amount of lycopodium spores in a small watch glass (100 mg drug and 50 mg lycopodium spores). Mix with a small flexible spatula. Add oil or suspending agent. Mix for 10 min till a smooth paste is obtained.

Transfer the suspension to a small glass tube by draining with the help of a glass rod. Add more suspending agent, washing down the mixture into the tube. (about 4 ml of the suspending agent is required for 50 mg of lycopodium spores). This should give about 10 to 20 spores when viewed under 4 mm objective, when a drop of the mixture is mounted under a cover glass.

Slowly oscillate the glass tube between the two palms without any air bubbles, until the suspension is uniform. Take a glasstube with internal diameter of about 2-3 mm and place one drop each on two sides, spread the suspension on the slide lessthan the area of the cover slip. Apply a cover slip and leave the slide on an even surface to settle.

Select 25 fields and count the spores and particles in these fields using 10×40 magnification.

Make a similar suspension as above and count particles in 25 fields on two sides.

Take average of 4 readings.

Calculate the percentage of foreign organic matter from the formula given below:

Percentage of foreign organic matter = 94000 x 100 x n x w / s x m x p

m= weight in mg of the sample, calculated on sample dried at 105 C.

w= weight in mg of the lycopodium spores n= number of particles in 25 fields

p= number of particles in per mg of the pure foreign matter dried at 105 C

94,000= number of spores in one mg of lycopodium

CALCULATIONS

1 mg of lycopodium powder contains spores = 94000

W mg of lycopodium powder powder contains 94000 x w number of spores.

s number of spores in ten fields mix with n number of starch grains in ten fields.

94000 x w number of spores mix with = 94000 x n x w / s (number of starch grain)

1 mg of pure sample of foreign organic matter (F.O.M) contains p number of starch grains

Weight of ginger in the mixture= 94000 x n x w/ s x p (mg of ginger) m (mg) of mixture contain = 94000 x n x w/ s x p

1 mg of mixture contains = 94000 x n x w/ s x p x m

Percentage of F.O.M = 94000 x n x w x 100/ s x p x m

REPORT

EXP.NO:11
DATE:

DETERMINATION OF FIBER LENGTH AND WIDTH

AIM

To determine length and width of fibers of Chinchona Bark Powder

REQUIREMENTS

Required chemicals

Glycerin water, Phloroglucinol, Concentrated hydrochloric acid,Chloral hydrate solution

Required apparatus

Microscope with mechanical stage, Eyepiece and stage micrometer

PROCEDURE

1. Stage micrometer

1. Take the micrometer slide in hand and feel the sides of the slide.

2. The correct sides shows a slight elevation on one side.

3. Place it on the stage of the microscope. View the scale through the eyepiece at 10 x 10 magnification to find the position of the scale on the micrometer.

4. To locate the scale, start viewing from the edge of the cover glass containing the engraved micrometer scale and then move to the centre and locate the scale on the equatorial plane.

2. Eyepiece micrometer

1. Remove the eyepiece and unscrew the part A

2. Place the eyepiece micrometer at level B of the eye piece where there is a hinge or diaphragm (it is the support onwhich the eyepiece micrometer rests).

3. Screw part A back and see whether the numbering on the eyepiece micrometer is in correct position, i,e zero tohundred. If numbers are reverse, remove the scale and place it in a correct manner.

4. The eye-piece can be rotated to make adjustments.

5. Do not introduce the eyepiece micrometer into the draw tube.

3. Calibration of eyepiece micrometer

1. View through the eyepiece with the required optical combinations.

2. Adjust the scales of the two micrometers such that both the scales are superimposed. Rotate the eyepiece to place the scales in a parallel position or remove the stage micrometer till the lines coincide with eyepiece scale.

3. Move the stage micrometer such that the “0” readings of both the micrometer scales coincide or one of the larger division of the stage micrometer coincides with one of the lines of the eyepiece micrometer scale. Note the initial readings.

4. Carefully scan the scales to see, which of the two scale readings exactly coincide on the right side. Note the final readings and calculate the factor for 1 division of the eyepiece micrometer.

Now the microscope is ready for taking measurements.

Using stage micrometer calibrate the eyepiece micrometer. Calculate the factor (average distance between two lines in microns). Take a little quantity of powder drug (Ceylon cinnamon, Cassia bark or Cinchona bark) in a test tube and boil with clearing agent, chloral hydrate solution. Transfer the cleared powder in a watch glass.

Stain the lignified fibers with the staining reagent (phloroglucinol and concentrated hydrochloric acid). Mount this treated powder in glycerin water and observe the slide under low power. (Power should be thinly, uniformly scattered, without overlapping of particles). Focus a stained fiber (intact fiber). By rotating the scale of eyepiece micrometer, note the numbers of divisions of the eye piece micrometer covered by the length of the fiber.

Again rotate the eyepiece micrometer without disturbing the slide and find the numbers of division of the eyepiece micrometer covered by the width of the same fiber. Similarly calculate the length and width of about 25 fibers, and write the readings in two separate columns. Multiply each value by the factor calculated in the first step to get the value in microns. Then calculate the average value and write the range for the length and the width fiber.

REPORT

EXP.NO:12
DATE:

DETERMINATION OF SIZE OF STARCH GRAINS, CALCIUM OXALATE CRYSTALS BY EYE PIECE MICROMETER

AIM

To measurement of starch grains in powdered crude drugs.

REQUIREMENT

Required chemicals

lactophenol, N/50 Iodine

Required apparatus

Compound microscope, eyepiece micrometer, stage micrometer.

PROCEDURE

1. Stage micrometer

1. Take the micrometer slide in hand and feel the sides of the slide.

2. The correct sides shows a slight elevation on one side.

3. Place it on the stage of the microscope. View the scale through the eyepiece at 10 x 10 magnification to find the position of the scale on the micrometer.

4. To locate the scale, start viewing from the edge of the cover glass containing the engraved micrometer scale and then move to the centre and locate the scale on the equatorial plane.

2. Eyepiece micrometer

1. Remove the eyepiece and unscrew the part A

2. Place the eyepiece micrometer at level B of the eye piece where there is a hinge or diaphragm (it is the support onwhich the eyepiece micrometer rests).

3. Screw part A back and see whether the numbering on the eyepiece micrometer is in correct position, i,e zero tohundred. If numbers are reverse, remove the scale and place it in a correct manner.

4. The eye-piece can be rotated to make adjustments.

5. Do not introduce the eyepiece micrometer into the draw tube.

3. Calibration of eyepiece micrometer

1. View through the eyepiece with the required optical combinations.

2. Adjust the scales of the two micrometers such that both the scales are superimposed. Rotate the eyepiece to place the scales in a parallel position or remove the stage micrometer till the lines coincide with eyepiece scale.

3. Move the stage micrometer such that the “0” readings of both the micrometer scales coincide

or one of the larger division of the stage micrometer coincides with one of the lines of the eyepiece micrometer scale. Note the initial readings.

4. Carefully scan the scales to see, which of the two scale readings exactly coincide on the right side. Note the final readings and calculate the factor for 1 division of the eyepiece micrometer.

Now the microscope is ready for taking measurements

- ♣ Compound microscope
- ♣ Eyepiece micrometer
- ♣ Stage micrometer
- ♣ Glycerin water

Calibrate eyepiece micrometer by using stage micrometer and calculate the factor. Mount a little quantity of powdered sample in glycerin water and measure the length and breadth of 25 calcium oxalate crystals. Multiply the values by the factor for the next dimensions of the crystals.

REPORT

EXP.NO:13
DATE:

DETERMINATION OF EXTRACTIVE VALUE

AIM

To determine the alcohol soluble extractive (ASE) value and water-soluble extractive (WSE) value of given sample

REQUIREMENTS

Required chemicals

Alcohol 95%, Powdered drug.

Required apparatus

Stoppered flasks, flat bottomed shallow dish, desicattors,

PRINCIPLE

Extractive values are indicative of approximate measure of chemical constituents of the medicinal plant materials extracted with solvents. Based on the chemical nature and properties of contents of drugs, various solvents like ethyl alcohol, chloroform water or solvent ether are used for the determination of extractives. This method is employed for those materials for which as yet noo suitable chemical or biological assay method exists. The various methods used in the determination of extractives are cold maceration method (ASE&WSE) and hot extraction method applicable only to WSE.

PROCEDURE

A. Determination of alcohol soluble extractive value

1. Weigh accurately about 5 g of coarsely powdered drug into a 250 ml conical flask with stopper.

2. Add 100 ml of alcohol (90%).

3. Shake the flask frequently during first 6 hr.

4. Keep it aside without disturbing for 18 hr. and then filter.

5. Pipette out 25 ml of the filtrate and evaporate to dryness in a weighed shallow flat-bottomed dish on a water bath.

6. Then dry the residue at 105 C to a constant weight.

7. Calculate the percentage of alcohol-soluble extractive.

8. % of alcohol soluble extractive = weight of residue/weight of the drug x 100 It is expressed as percent w/w of the air-dried drug.

B. Determination of water-soluble extractive value

1. Weigh accurately about 5 g of coarsely powdered drug into a 250 ml conical flask with

stopper.

2. Add 100 ml of chloroform water.

3. Shake the flask frequently during first 6 hr.
4. Keep it aside without disturbing for 18 hr. and then filter.

5. Pipette out 25 ml of the filtrate and evaporate to dryness in a weighed shallow flat-bottomed dish on a water bath. Then dry the residue at 105ºC to a constant weight. Calculate the percentage of water-soluble extractive.

CALCULATION

1. **Determination of alcohol soluble extractive value**

Weight of empty chinna dish=

Weight of empty chinna dish with drug=

Weight of residue obtained from 25 ml=

Weight of drug obtained=

B. Determination of water-soluble extractive value

Weight of empty chinna dish=

Weight of empty chinna dish with drug=

Weight of residue obtained from 25 ml=

Weight of drug obtained=

REPORT

EXP.NO:14
DATE:

DETERMINATION OF ASH VALUE

AIM

To determine the total ash value of dried ginger.

PRINCIPLE

Ash values are helpful to determine the quality as well as purity of a crude drug, especially when the drug is present in powdered form. The object of ashing crude drugs is to remove the traces of organic matter which may be interferes in an analytical determination. On incineration, the crude drugs normally produce ash which is usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The total ash of a crude drug reveals the care taken during its preparation. A higher limit of acid- insoluble ash is incorporated especially in cases where silica may be present or when the calcium oxalate content of the drug is very high. Some researchers suggested mixing of acids like sulphuric acid with the powdered crude drug before ashing and making the ash sulphated which is normally less fusible than ordinary ash. The present study is designed to determine the ash value of the supplied sample.

REQUIREMENTS

Required chemicals

Powdered ginger.

Required apparatus

Silica crucible Dessicator, Ash less filter paper Sample and holder

PROCEDURE

Total ash determination

Weigh accurately about 3 gm of the powdered drug in silica crucible.

Incinerate the powdered drug by increasing the heat gradually until the sample was free from carbon and cool it keep it in a desiccators.

Weigh the ash and calculate the percentage of total ash in contrast to the air dried sample.

CALCULATION

TOTAL ASH%=W2-W1/WS×100

W1= weight of the crucible (g)

W2=weight of crucible with ash(g)

WS= weight of the sample(g)

REPORT

EXP.NO:15
DATE:

DETERMINATION OF ACID-INSOLUBLE ASH VALUE

AIM

To determine the acid-insoluble ash value of dried ginger.

REQUIREMENTS

Required chemicals and crude drugs

Powdered ginger, dil.hydrochloric acid

Required apparatus

Silica crucible Dessicator, Ash less filter paper Sample and holder

PROCEDURE

Boil the total ash obtained as above for 5 minutes with 25ml of dilute hydrochloric acid.

Filter and collect the insoluble mattere on ashless filter paper,wash the filter paper with hot water, ignite in crucible, cool and keep in desiccator.

Weight the residue and calculate acid-insoluble ash of ginger with reference to the air-dried drug.

CALCULATION

%ACID-INSOLUBLE ASH=[(W3-W1)/(W2-W1)]×100

W1=Weight of the empty dish

W2=weight of the dish with the sample

W3=weight of the dish with the ash after acid treatment

REPORT

EXP.NO:16
DATE:

DETERMINATION OF WATER-SOLUBLE ASH VALUE

AIM

To determine the water-soluble ash value of dried ginger.

REQUIREMENTS

Required chemicals and crude drugs

Powdered ginger,

Required apparatus

Silica crucible Dessicator, Ash less filter paper Sample and holder

PROCEDURE

Boil the total ash obtained as above for 5 minutes with 25ml of water.

Filter and collect the soluble matter on ashless filter paper, wash the filter paper with hot water, ignite in crucible, cool and keep in desiccator.

Weight the residue and calculate soluble ash of ginger with reference to the air-dried drug.

CALCULATION

%WATER-SOLUBLE ASH=Total ash weight-water insoluble residue in total ash/ weight of the sample×100

REPORT

EXP.NO:17
DATE:

MOISTURE CONTENT OF THE CRUDE DRUGS

AIM

To determine the moisture content of the given crude drugs

PRINCIPLE

Moisture content determination is important, not only to know excess water, but also in conjunction with suitable temperature moisture will lead to the activation of enzymes and gives suitable conditions to the proliferation of living organism. As most vegetable drugs contain all the essential food requirements for mould, insects and mites, deterioration can be very rapid, once infestation has taken place. Various methods for moisture determination are

loss on drying,

separation and measurement of moisture,

chemical methods,

electrometric methods,

spectroscopic methods

REQUIREMENTS

Required chemicals

Ginger

Required apparatus

Petridish, Desiccators, digital balance

PROCEDURE

Accurately weigh 2 grams of the given crude drug in a crucible and keep for one hour at 105^0c cool in a desiccators, and weigh.

Repeat the procedure until there is no further loss in weight and calculate the moisture content of the given drug.

Express the moisture content in percentage with reference of air-dried drug.

CALCULATIONS

Weight of the empty glass dish=… .... (a)

Weight of the sample=.............(b)

Total weight(a+b)=............ (c)

After heating for 1 hours, to a constant weight.

Weight of the dish+drug (together)=................ (d)

Therefore, Loss on drying=(c-d)=....................(e)

Formula,

loss on drying /weight of the initial drug×100

Percentage of moisture content of the given drug is=………%

REPORT

EXP.NO:18
DATE:

DETERMINATION OF SWELLING INDEX

AIM

The main objective of present experiment is to measure the swelling factor of the given sample i.e isapgol seeds.

PRINCIPLE

Many herbal drugs are of specific for the therapeutic or pharmaceutical utility because of their swelling properties − especially gums and drugs those are containing an appreciable amount of constituents like mucilage, pectin or hemicelluloses. The swelling index is defined as the volume in ml taken up by the swelling of 1 g of herbal material under specified conditions. Its determination is based on the addition of water or a swelling agent as specified in the test procedure for each individual herbal material. Using a measuring cylinder with glass-stopper, the material must be shaken repeatedly for 1 hour and then allowed the measuring cylinder to stand for a required period of time. The volume of the mixture (in ml) is then read.

REQUIREMENTS

Required chemicals

Isapgol (Plantagoovata) seed Water (q.s)

Required apparatus:

Stoppered measuring cylinder

PROCEDURE

Determination of swelling index Transfer 1gm of isapgol seed to a 25 ml stoppered measuring cylinder. Fill the cylinder up to 20 ml mark with water. Agitate gently occasionally during 24 hour and allowed to stand. Measure the volume occupied by the swollen. The genuine seed of isapgol occupies a volume of not less than 10 ml.

CALCULATION&OBSERVATION

S.NO	INITIAL VOLUME	FINAL VOLUME	SWELLING INDEX	AVERAGE

REPORT

EXP.NO:19
DATE:

DETERMINATION OF FOAMING INDEX

AIM

The foaming ability of an aqueous decoction of plant materials & their extracts are measured in terms of a foaming index.

REQUIREMENT

Required chemicals

Aqueous decoction

Required apparatus

Stoppered test tube, 500 ml conical flask

PROCEDURE

Test Weigh accurately about 1 g of coarsely powdered drug and transferred to 500 ml conical flask containing 100 ml of boiling water maintain at moderate boiling at 80- 90 C for about 30 min. Then make it cold, filter into a volumetric flask and add sufficient water through the filter to make the volume up to 100 ml (V1).

Cleaned stopper test tubes 10 numbers are taken and marked with 1 to 10. Take the successive portions of 1, 2 ml up to 10 ml drug in separate tubes and adjust remaining volume with the liquid up to 10 ml in each test tube.

After closing the tubes with stoppers, Shake them for 15 seconds and allowed to stand for 15 min. then measure the height. If the height of the foam in each tube is less than 1cm, the foaming index is less than 100 (not significant). Here, if the foam Is more than 1cm height after the dilution of plant material in the sixth tube, then corresponding number of the test tube is the index sought.

If the height of the foam in every tube is more than 1 cm, the foaming index is more than 1000. In this case, 10 ml of the first decoction of the plant material needs to be measured and transferred to a volumetric flask of 100 ml capacity (V2) and volume is to be maintained up to 100 ml and follow the same procedure. Foaming index is calculated by using the following formula

Foaming index = 1000/a in case of V1

Foaming index = 1000 × 10/a in case of V2

Where, a = Volume (ml) of decoction used for preparing the dilution in the tube where exactly 1 cm or more foam is observed.

REPORT