



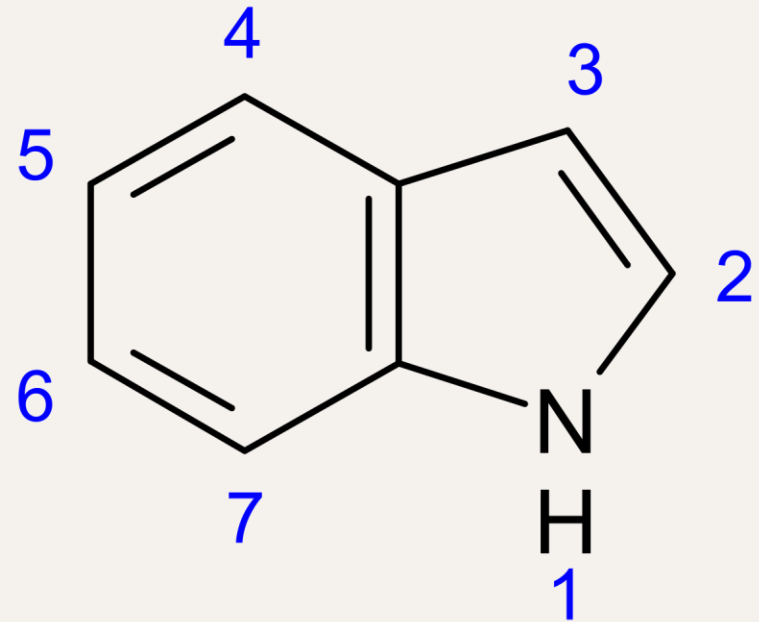
Pharmacognosy
3rd class , 2nd semester
By MSc. Aya Alashqar

Indole alkaloids

Harmala alkaloids

Introduction about indole alkaloids

- Indole alkaloids are a class of alkaloids containing a structural moiety of indole.
- Indole is an aromatic heterocyclic organic compound. It has a bicyclic structure



HARMALA ALKALOIDS

Botanical name:

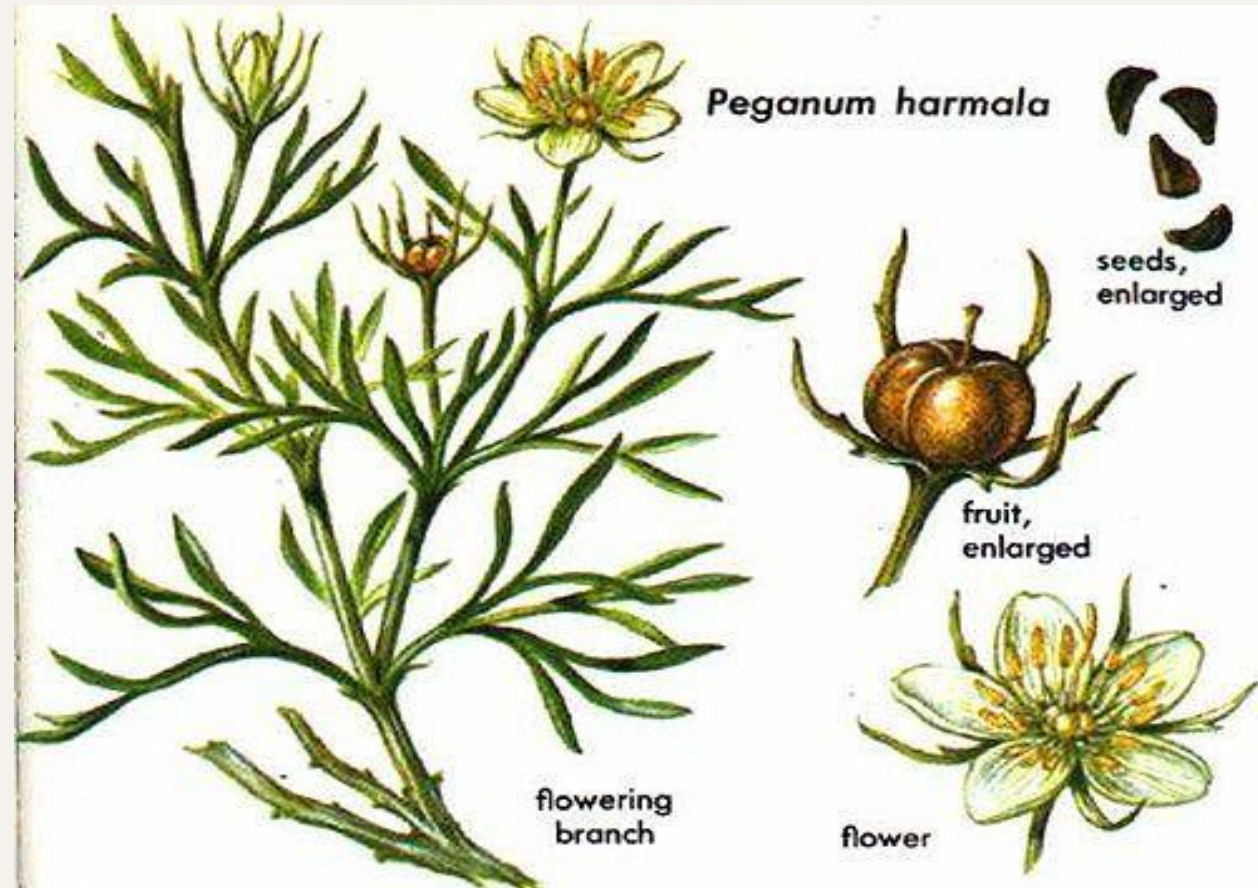
Peganum harmala

Family name:

Nitrariaceae

Part used: seed

Local name: الحرمل



Introduction about harmala alkaloid

- *Peganum harmala* of the family Nitrariaceae
 - It is a woody, perennial, succulent shrub, native to arid regions.
 - The leaves are bright green, finely divided and about 1cm long.
 - Both the roots and seeds contain significant quantities of beta-carbolines (indole) alkaloids, which are absent from the rest of the plant.
-

Introduction about harmala alkaloid

- The round seed capsules measure about 1–1.5 cm in diameter, have three chambers and carry more than 50 seeds.



c



The Traditional uses of harmala

- As the dye "turkey red" is a color that was widely used to dye cotton in the 18th and 19th century
- As incense from ancient times.



The medicinal uses of harmala

- *Peganum harmala* was claimed to be an important medical plant. Its seeds were known to possess hypothermic and essentially hallucinogenic properties since it is MAO inhibitor agent .
- Many studies were conducted to antibacterial, antifungal and antiviral effects of *Peganum harmala* seeds.
- In Moroccan traditional medicine , seed powder is sometimes used on skin and subcutaneous tumors.
- Many studies were designed to investigate some aspects of the anti neoplastic properties of *Peganum harmala*, and found that their alkaloids have significant anti tumor activity

The active alkaloids of Harmal seeds

1-Beta-carbolines

Harmane

Harmine

Harmaline

Harmalol

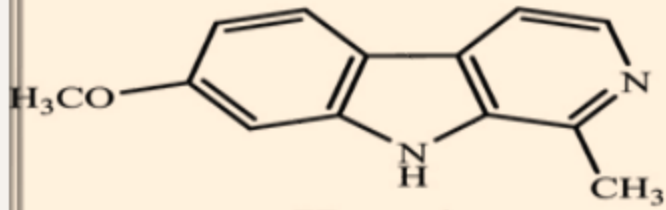
2- Quinazoline derivatives

Vasicine

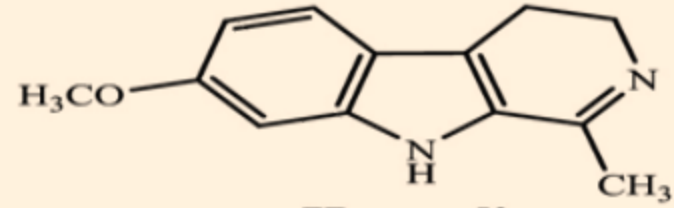
Vasicinone

3- Fluorescent indole alkaloid

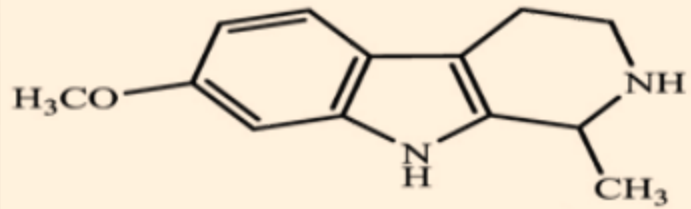
Tetrahydroharmine



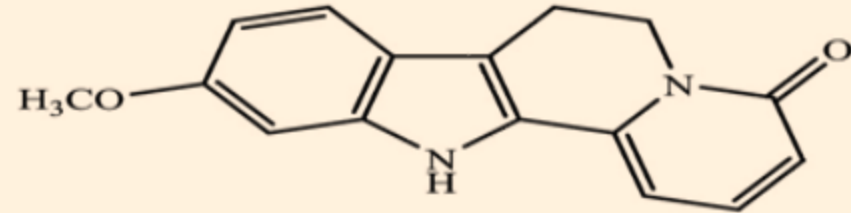
Harmine



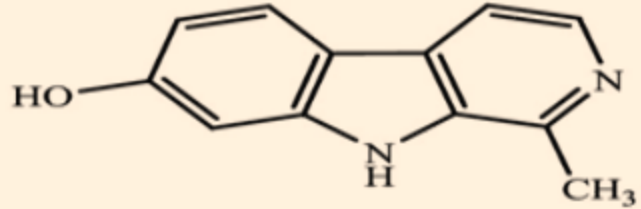
Harmaline



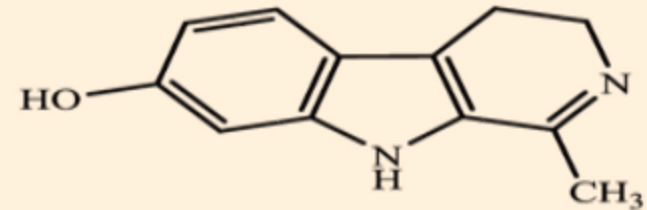
Tetrahydroharmine



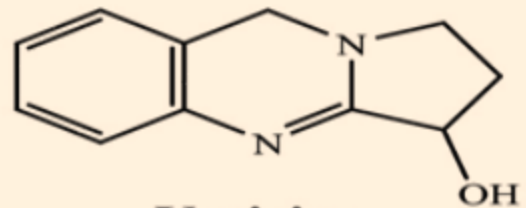
Harmalanine



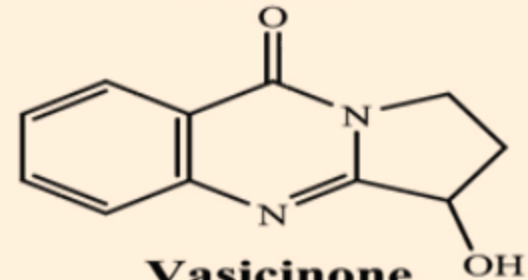
Harmol



Harmalol



Vasicine



Vasicinone

- These quinazoline alkaloids are responsible for the abortifacient activity of *Peganum harmala* extracts.
- It has been reported that these chemicals have a uterine stimulatory effect, apparently through the release of prostaglandin.
- *Peganum harmala* alkaloids are characterized by the fluorescence property.

ISOLATION OF THE HARMALA ALKALOIDS

- Aim: - To isolation the harmala alkaloids
- Equipment's and reagents:-
 - 1) Large beaker
 - 2) Reflux instrument
 - 3) Water bath
 - 4) Large evaporating dish
 - 5) Separatory funnel
 - 6) Litmus paper
 - 7) Petroleum ether
 - 8) 90 % ethanol
 - 9) 2% HCL
 - 10) Ammonium hydroxide solution
 - 11) Chloroform
 - 12) Methanol

ISOLATION OF THE HARMALA ALKALOIDS

- **Procedure:**

- Method of extraction: Reflux.

Plant used: *Peganum harmala*.

- Part used: Seeds.

Maceration 50 gm of the harmala seeds in 500 ml of petroleum ether for 24 hrs (over night)

**Maceration process
is for defatting
process**

Filter

Reflux with 500ml of 90% ethanol for 1 hr

**Use of reflux
condenser since the
plant is not affected
by heat**

Cool & Filter

ISOLATION OF THE HARMALA ALKALOIDS

Take 20 ml of Extract in conical flask



Evaporate the filtrate on water bath to about 2 ml (syrup liquid)



Add

5ml of 2% HCl (Filter if necessary)



Partition with Chloroform (10 ml x 2), take the acidic layer (upper layer)

ISOLATION OF THE HARMALA ALKALOIDS

↓
Add

Ammonium hydroxide solution (check by litmus paper)

↓

Place the basic solution in the separatory funnel

↓
Add

[10 ml of Chloroform] two times

ISOLATION OF THE HARMALA ALKALOIDS

(Shake & stand)



Take the organic lower layer and put it in the conical flask

Add



Small amount of Anhydrous sodium Sulphate & allow standing for few minutes until get a clear solution , decant and concentrate by evaporation to give the product crude alkaloids (fraction A)

ISOLATION OF THE HARMALA ALKALOIDS

↓
Add 10 ml methanol to fraction A, then filter, the result
is two fractions

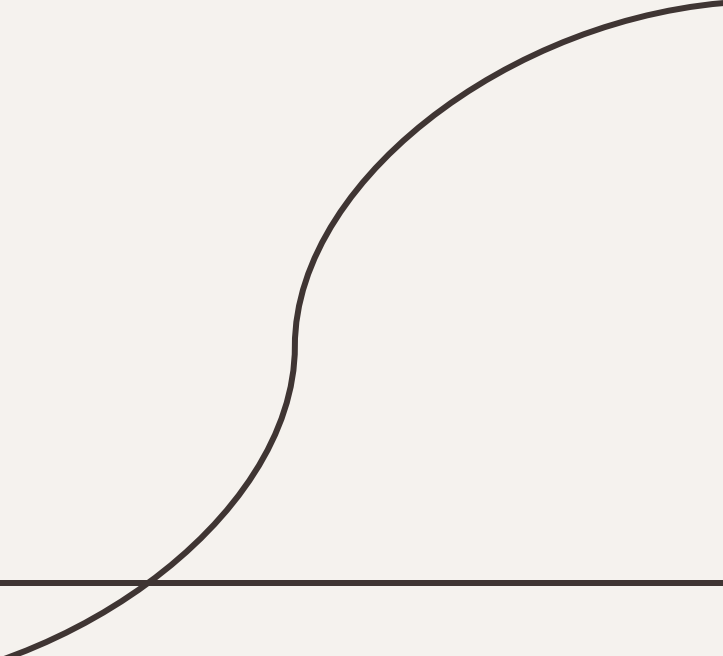
↙
Fraction B (filtrate)
(vasicine and deoxyvasicine
alkaloids)

↘
Fraction C (precipitate)
(harmine and harmaline)

**Use of methanol is to separate
some of harmala alkaloids, since
some are soluble in methanol
and others are not**

Study problems

Give the reason of:

- Maceration process for *Peganum harmala*
 - Using the reflux in extraction the indole alkaloids from *Peganum harmala*
 - Making the evaporation step after filtration?
 - Addition of 2% HCl to the alcoholic extract?
 - Addition of methanol to fraction A
- 



Citric acid



Pharmacognosy 3rd class , 2nd
semester

By MSc. Aya Alashqar



Introduction about Citric acid



- Botanical Name: *Citrus limonum*
- Synonyms and local names: Citrus Limonum, Leemoo, Limoun, Limone.
- Family Name: Rutaceae
- Genus: Citrus




Introduction of Citric acid

- The lemon is a small evergreen tree native to Asia, and produces a yellow fruit.
 - The fruit is used primarily for its juice, though the pulp and rind (zest) are also used in cooking and baking.
 - The juice of the lemon is citric acid, which gives lemons a sour taste.
 - The distinctive sour taste of lemon juice makes it a key ingredient in drinks and foods such as lemonade.
- 
- 



Therapeutic uses of Citric acid

- 1- Aromatherapy, as it boosts the immune system and enhances the mood.
 - 2-Strong antibacterial and antiviral agent
 - 3-Aids in digestion and liver cleanser: As it eliminate waste more quickly from your body.
 - 4-Anticancer properties.
 - 5-A rich source for many vitamins and minerals mainly (5-6) % citric acid and offers potassium, calcium, vitamin C, bioflavonoids, pectin and limonene which promotes immunity and fights infections.
- 

Extraction of Citric acid



Measure 90 ML of lemon juice concentrate it to 250 ml in a beaker



Add a sufficient quantity of 10% NaOH solution



Filtrate by using filter paper to remove all the excess large particles and pulp



Measure the filtrate place in a beaker and add 5 ml of 10% Calcium Chloride solution for each 10 ml of the filtrate





Wash twice with small quantities of boiling water



Resuspend in a minimum quantity of cold water, heat to boiling and once again collect the insoluble calcium citrate by filtration



Allow The salt to dry, weight calculate the yield and turn in the product to the laboratory instructor the result is citric salt



Citric acid may be prepared from the citrate salt as in the following:

- Weight the air-dried salt, place in a beaker
- Add the calculated amount of 1N sulphuric acid required to convert the salt to acid.
- Allow the mixture to stand for few minutes, filter off the insoluble calcium sulphate and concentrate the filtrate to a small volume by steam bath.
- Citric acid crystallization out, because of its relatively great water solubility citric acid is somewhat difficult to recrystallize in small amounts.
- Collect the crystals, dry and calculate the final product what is citric acid.



Thank you!

LAB (2)



BLACK PEPPER



PHARMACOGNOSY

3RD CLASS

2ND SEMSTER

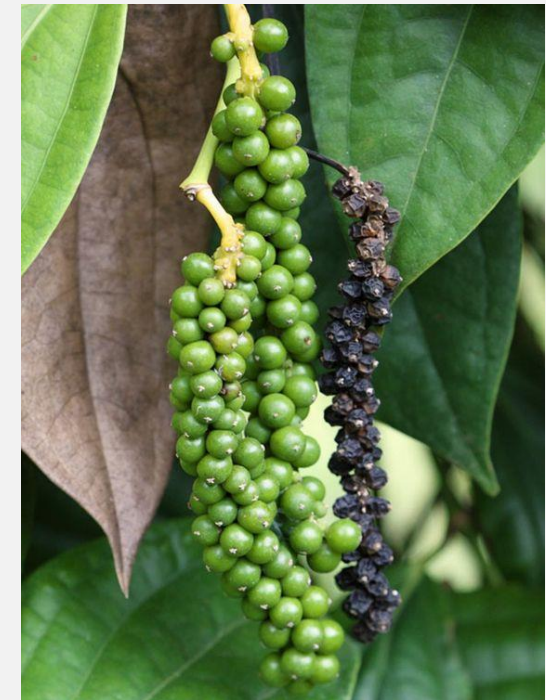
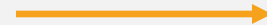
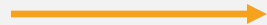
BY: AYA ALASHQAR

INTRODUCTION

- Botanical name *Piper nigrum*
- Family name **Piperaceae**
- Type of alkaloid **Typical alkaloid**
- Group of alkaloid Third group: *Pyridine* and *Piperidine*



- ❖ This plant is a perennial plant producing berry-like and aromatic pungent fruits, that are green when unripe and become red at mature, then the dried berries become black and wrinkled producing black pepper.
- ❖ The pepper yields both, black and white pepper according to the method of drying.
- ❖ In that when the ripe and the unripe fruit are dried directly under the sun, black pepper is the result. While if the fruit is soaked, and then removed the outer skin, before drying and then the result is white pepper.

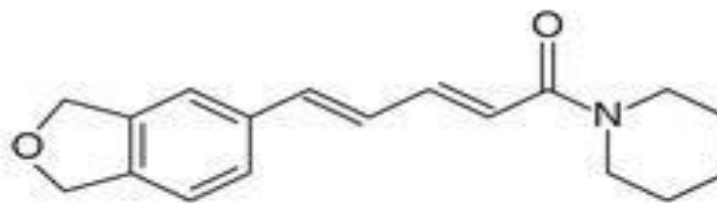


PIPERINE ALKALOID

- The alkaloid extracted from the black pepper is piperine.
- Piperine alkaloid is a solid substance essentially insoluble in water. It is a weak base that is tasteless at first, but leaves a burning after taste.
- The molecular formula is $C_{17}H_{19}O_3$.



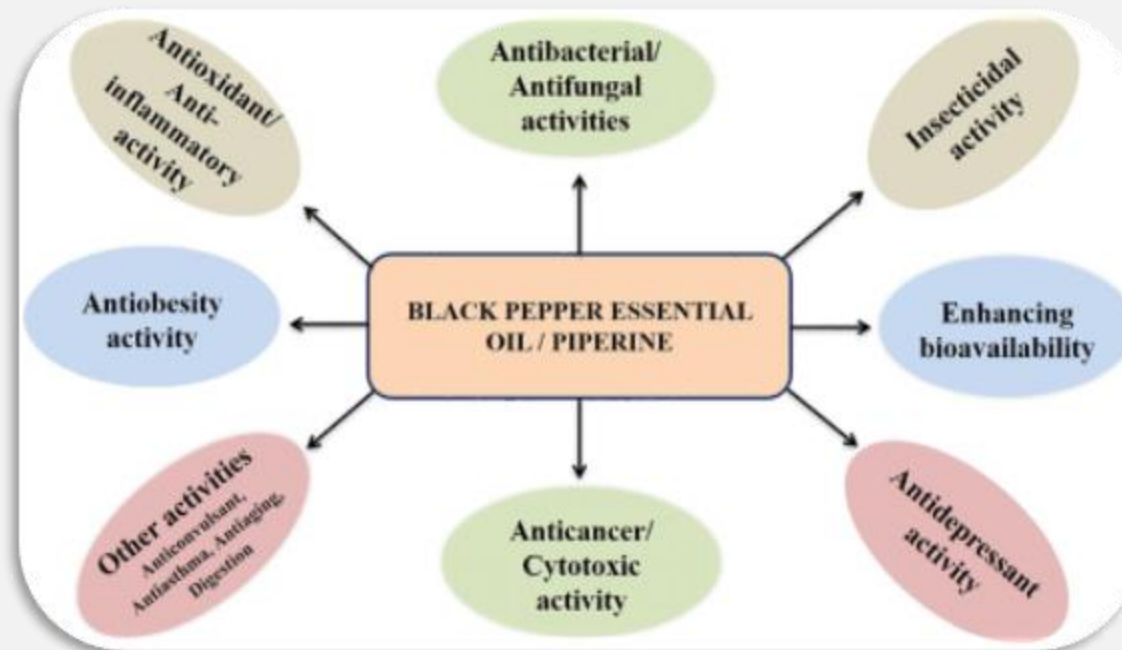
Black pepper



Piperine

THE PHARMACOLOGICAL ACTIVITY OF PIPERINE

- 1) Piperine aid in the digestion of food due to its stimulation to the digestive enzymes.
- 2) There is some evidence that it has an anticonvulsant activity in the treatment of epilepsy.
- 3) There is some evidence that it has an anticancer and anti-inflammatory activity due to its antioxidant property.

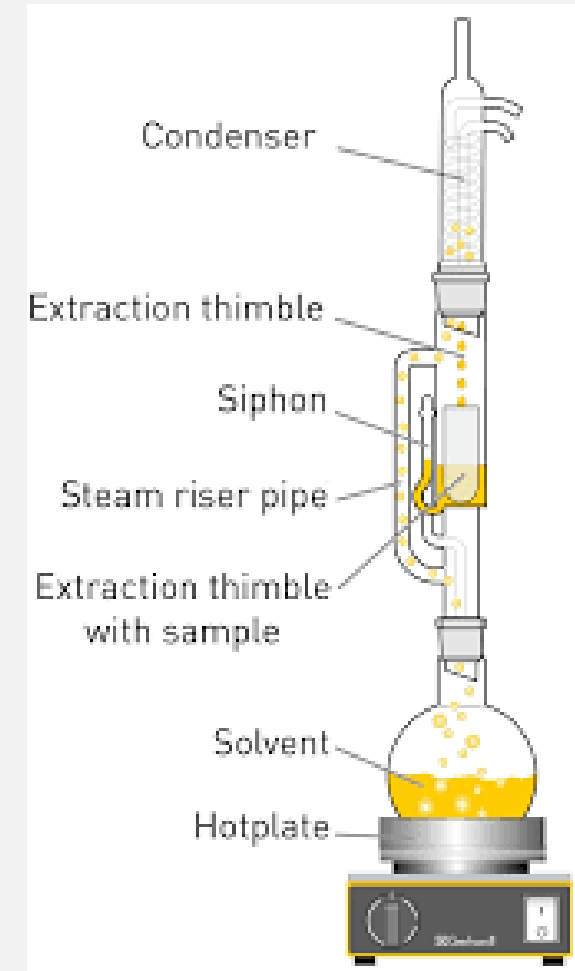


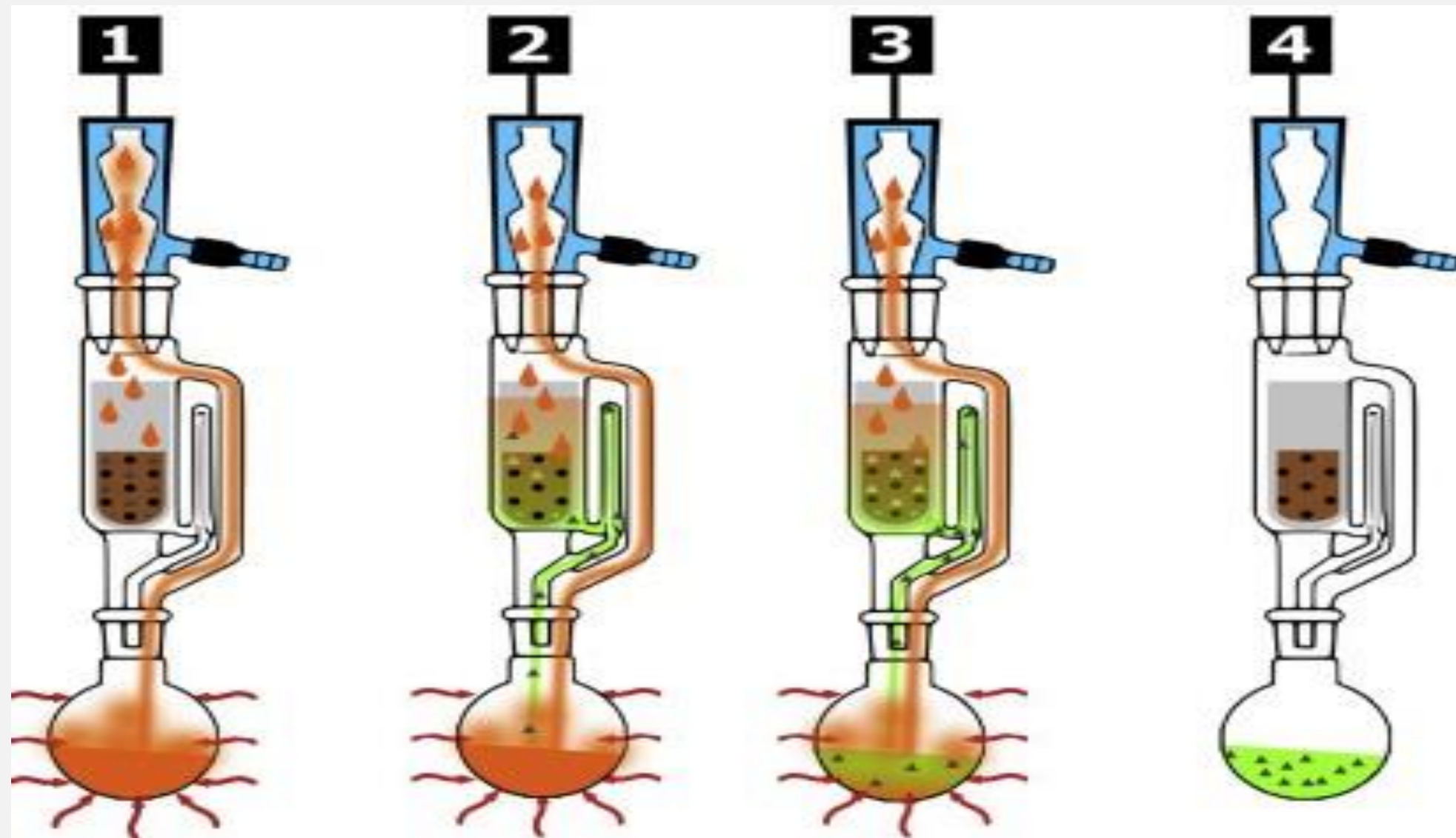
ISOLATION OF PIPERINE FROM BLACK PEPPER

Aim: - Isolation the piperine alkaloid from black pepper

Equipment and reagents:-

- Large beaker and medium size beaker
- Soxhlet instrument
- Water bath
- Filter paper and funnel
- 90% ethanol
- 10% alcoholic potassium hydroxide





ISOLATION OF PIPERINE FROM BLACK PEPPER

Procedure:-

- 1) 10gm of black pepper are ground to a fine powder.
- 2) Extracted with 150 ml of 90% ethanol in Soxhlet extractor for two hours.
- 3) The solution is concentrated .
- 4) Add 10ml of 10% alcoholic potassium hydroxide to the filtrate residue and after a while decanted from the insoluble residue .
- 5) The alcoholic solution is left over night.

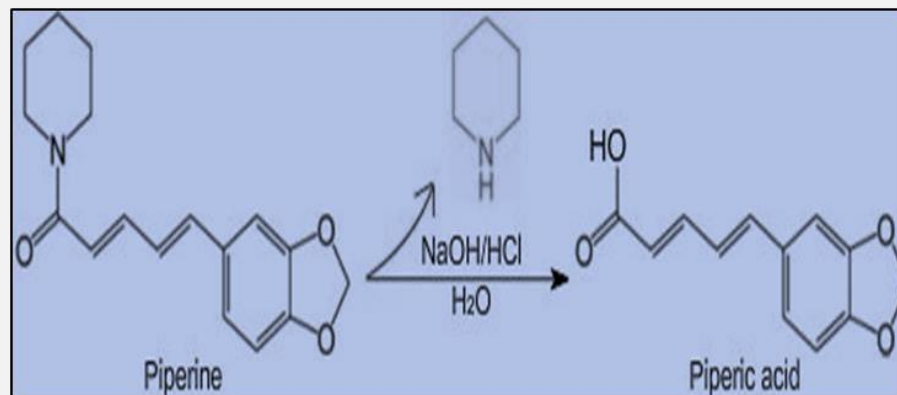
ISOLATION OF PIPERINE FROM BLACK PEPPER

Results:-

- Yellow needles with melting point of 125°C are deposited. Yielding 0.3 gm of piperine alkaloid.

Discussion :-

- 1) The plant is affected by heat therefore Soxhlet apparatus is used in its extraction.
- 2) The use of 90% ethanol is to extract both, the alkaloid and the alkaloidal salt that might be present.
- 3) The use of alcoholic KOH is to precipitate the isomers of piperine that are chuvacine, isochuvacine and piperic acid.
- 4) Alcohol was used in the preparation of KOH instead of water, since water will hydrolyze piperine into piperidine and piperic acid.



IDENTIFICATION OF BLACK PEPPER ALKALOID

ANALYSIS OF PIPERINE ALKALOID

QUALITATIVE ANALYSIS

Chemical general Test
Specific Test

Chromatography
TLC

QUANTITATIVE ANALYSIS

weighing the
crystals

IDENTIFICATION OF BLACK PEPPER ALKALOID

- **Qualitative Analysis:**
- **The General Chemical Tests :**

1. Mayer 's Test:

Aim: to indicate in general the alkaloid as other alkaloids.

Equipment and Reagents:

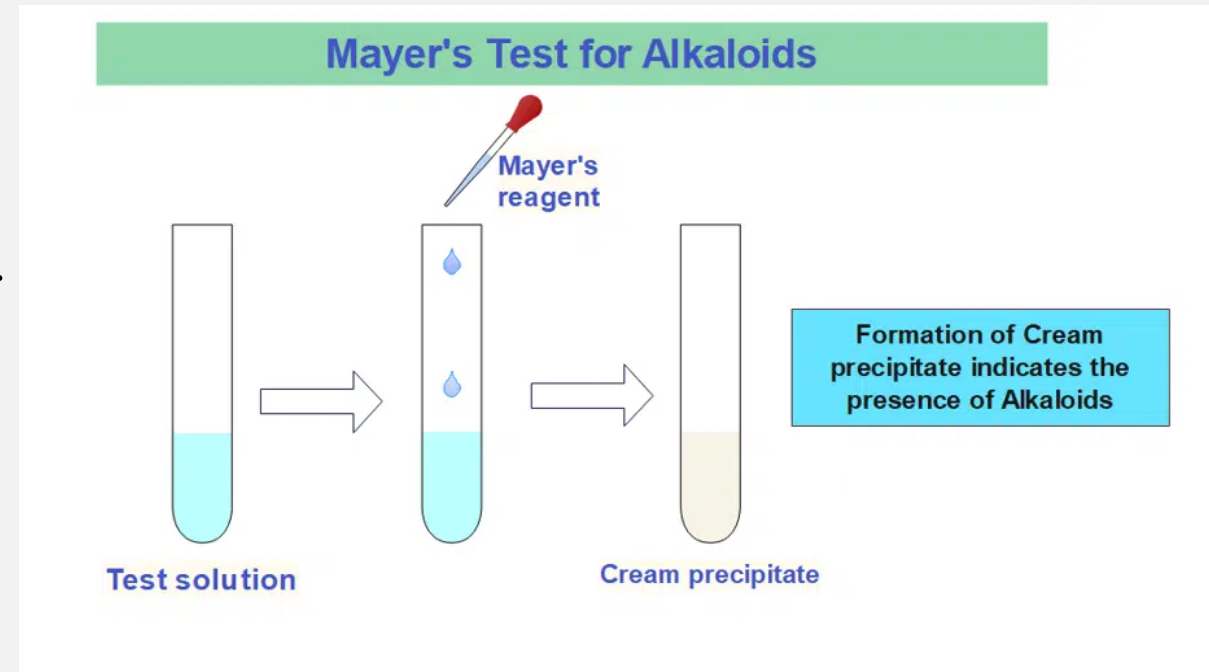
- ✓ Petri dish.
- ✓ Ethanol.
- ✓ HCl.
- ✓ Mayer's reagent.

Procedure:

Take few crystals of piperine alkaloid and dissolve in few mls of ethanol, in Petri dish then add 2 drops of HCl. Then add 2 drops of Mayer's reagent.

Result:

White precipitate will occur.



IDENTIFICATION OF BLACK PEPPER ALKALOID

- **Qualitative Analysis:**

- **The General Chemical Tests :**

2. Wagner 's Test:

Aim: to indicate in general the alkaloid as other alkaloids.

Equipment and Reagents:

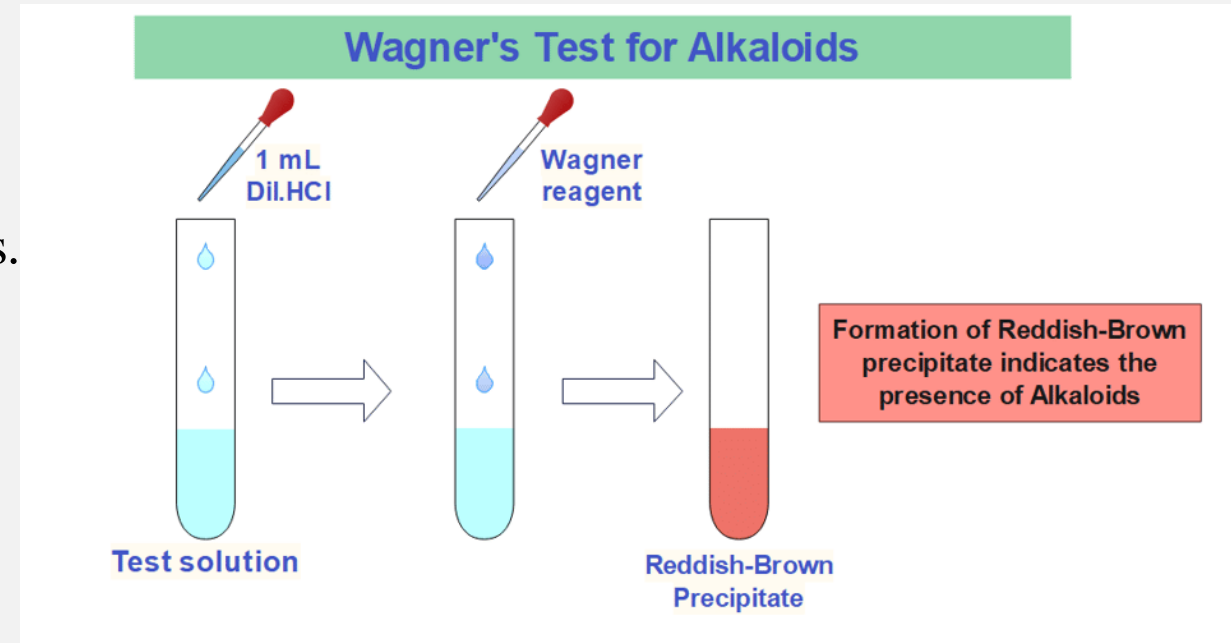
- ✓ Petri dish.
- ✓ Ethanol.
- ✓ HCl.
- ✓ Wagner's reagent.

Procedure:

Take few crystals of piperine alkaloid and dissolve in few mls of ethanol, in Petri dish then add 2 drops of HCl. Then add 2 drops of Wagner's reagent.

Result:

Brown precipitate will occur.



IDENTIFICATION OF BLACK PEPPER ALKALOID

- **Qualitative Analysis:**

- **The General Chemical Tests :**

3. Dragendorff's Test:

Aim: to indicate in general the alkaloid as other alkaloids.

Equipment and Reagents:

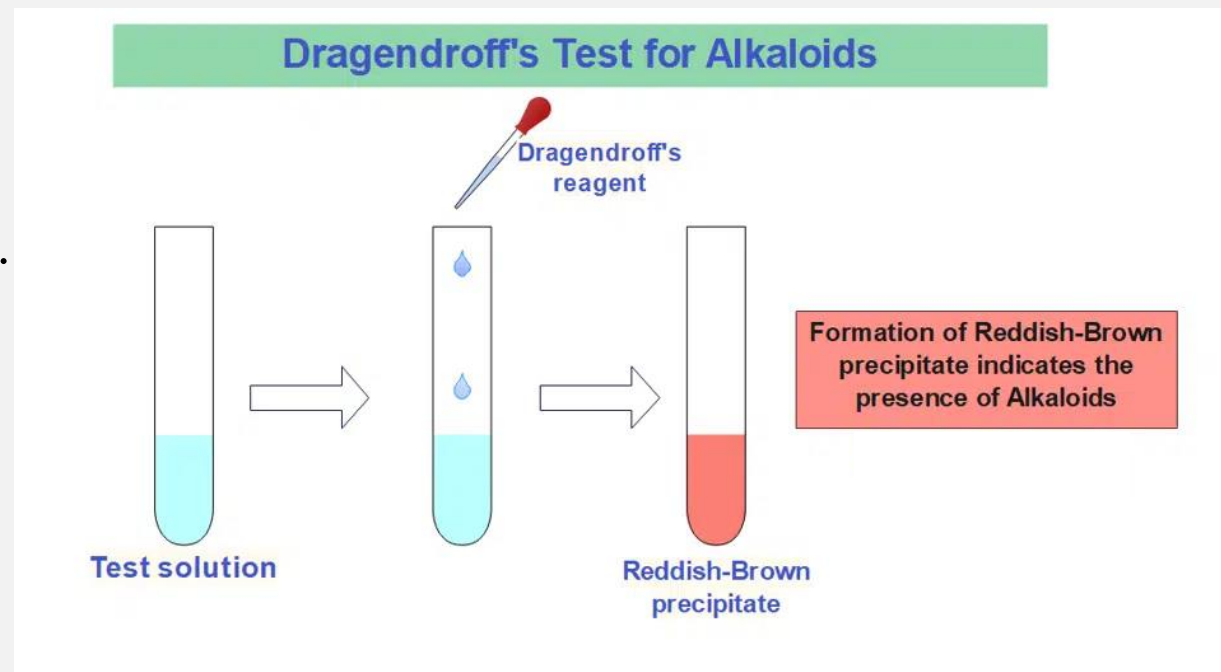
- ✓ Petri dish.
- ✓ Ethanol.
- ✓ HCl.
- ✓ Dragendorff's reagent.

Procedure:

Take few crystals of piperine alkaloid and dissolve in few mls of ethanol, in Petri dish then add 2 drops of HCl. Then add 2 drops of Dragendorff's reagent.

Result:

Orange (reddish-brown) precipitate will occur.



- **Qualitative Analysis:**
- **The Specific Test**

Aim: - to indicate specifically the piperine alkaloid from other alkaloids.

Equipment and reagents:-

- ✓ Petri-dish
- ✓ Ethanol
- ✓ 1,3,5 tri nitrobenzene

Procedure:-

- 1) Take few crystals of piperine alkaloid
- 2) Dissolve in few mls of ethanol in a Petri-dish.
- 3) Add few drops of 1, 3, 5 tri nitrobenzene.

Results:-

Red precipitate occurs with melting point 130°C.

Discussion:-

A complex will be formed between the piperine and 1, 3, 5 tri-nitrobenzene

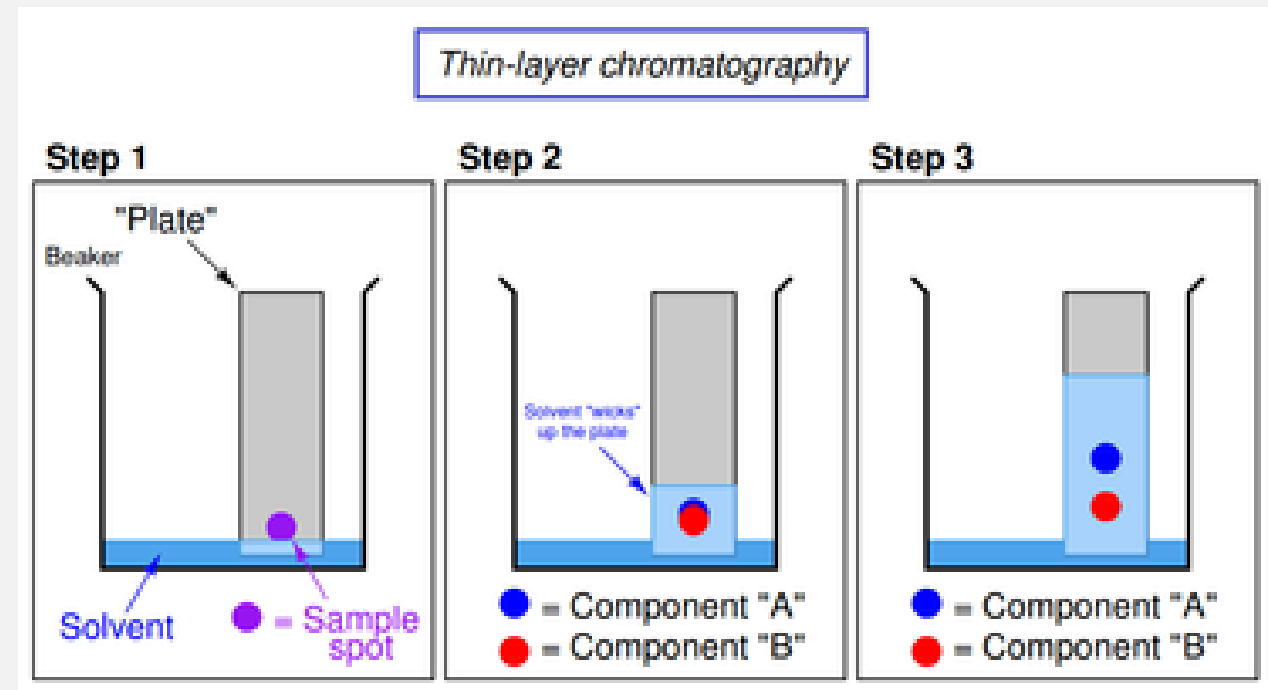
IDENTIFICATION OF BLACK PEPPER ALKALOID

- **The Identification of Piperine Alkaloid By Chromatography (TLC) :**

Aim: - used to identify qualitatively the piperine alkaloids.

Equipment and reagents:-

- Glass jar with its cover
- Silica gel plates
- Standard reagent
- Spray reagent (dragendroffs reagent)
- Mobile phase = (acetone: water: ammonia (90:7:3))
- Capillary tube



IDENTIFICATION OF BLACK PEPPER ALKALOID

- **The Identification of Piperine Alkaloid By Chromatography (TLC) :**

Procedure:-

- 1) Prepare the mobile phase and put in the glass jar, cover the jar and leave it for 45 min.
- 2) Apply the sample and the standard by the use of capillary tube on the silica gel plate.
- 3) Leave the plate in the jar until the solvent reaches 3/4 of the plate. Remove the plate, dry and then spray with spraying reagent.

Results:-

Orange spots appear for both standard and sample.



Study problems:



Q1. Give the botanical name of black pepper and mention its alkaloid?

Q2. What is the pharmacological activity of piperine alkaloids?

Q3. Give the reasons of the use of alcoholic KOH in the extraction procedure of black pepper?

Q4. How can you identify an extract containing piperine alkaloids?



Thank you 😊

LAB (I)

ALKALOIDS



PHARMACOGNOSY

3RD CLASS

2ND SEMSTER

INTRODUCTION

- *Alkaloids* are defined as organic nitrogenous compounds of plant origin that are physiologically active, ending in the suffix "*ine*".
- Alkaloids are natural compounds display an exceptionally wide array of biological activities and have a wide distribution, being present in plants (rich source), fungi, bacteria, insects, animal and marine.
- ▶ Many drugs and poisons are alkaloids and many are well-known, such as:-
 - *Morphine*, *codeine*, *strychnine*, *nicotine*, and *cocaine*.

INTRODUCTION

- The main elements in alkaloid skeleton are **Carbon**, **Hydrogen** and **Nitrogen**.
- In addition alkaloids may also contain **oxygen**, **sulfur** and more rarely other elements such as **chlorine**, **bromine**, and **phosphorus**.
- Alkaloids usually contain one nitrogen atom, but some may contain up to 5.
- The nitrogen may exist as a primary amine (RNH_2), as secondary amine (R_2NH), or as a tertiary amine (R_3N).
- As the nitrogen atom bears an **unshared pair of electrons**, such compounds are **basic** and resemble ammonia's chemical properties.
- The degree of basicity varies greatly, depending on the structure of the molecule and the presence and location of the nitrogen atom and other functional groups.

SOURCES OF ALKALOIDS

☐ Natural sources

- a) Plants (ephedrine) *Ephedra sinica*
- b) Animal (bufotenin) from skin
- c) Bacteria (*Pseudomonas aeruginosa*)
- d) Fungi (psilocybin) from *Psilocybe*

☐ Industrial sources

- Nicotinic acid can be synthesized from tryptophan or aspartic acid.

SOURCES OF ALKALOIDS

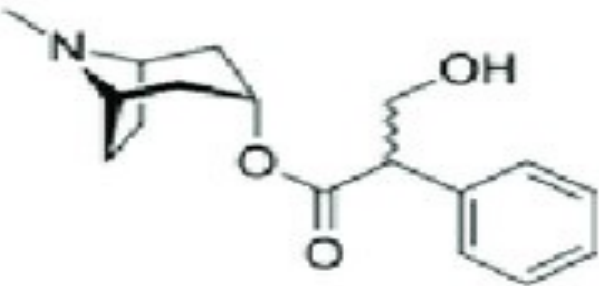
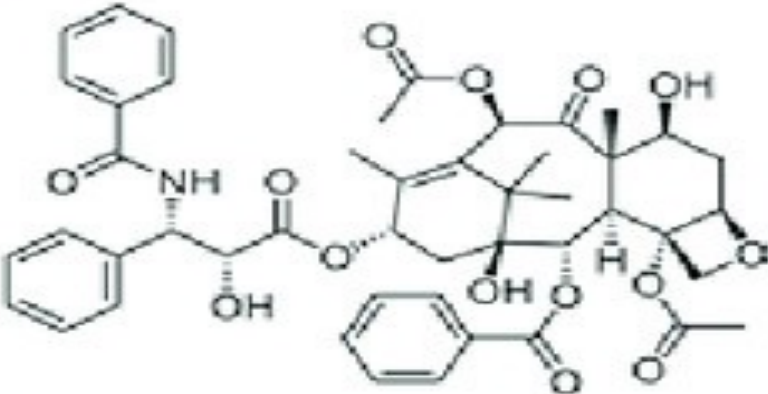
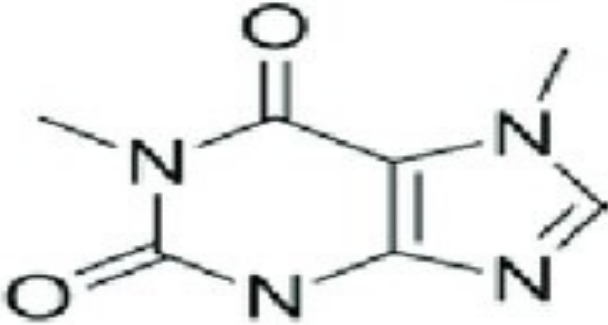
- ❑ Among the plants, the *angiosperms* are rich in alkaloids.
- ❑ The following families represent good examples of plants which contain alkaloids:
 - Leguminosae
 - Papaveraceae
 - Ranunculaceae
 - Rubiaceae
 - Solanaceae
 - Berberidaceae
- ❑ Alkaloids may occur in various parts of the plants (seeds, stems, roots, rhizome and barks).

NAMING OF ALKALOIDS

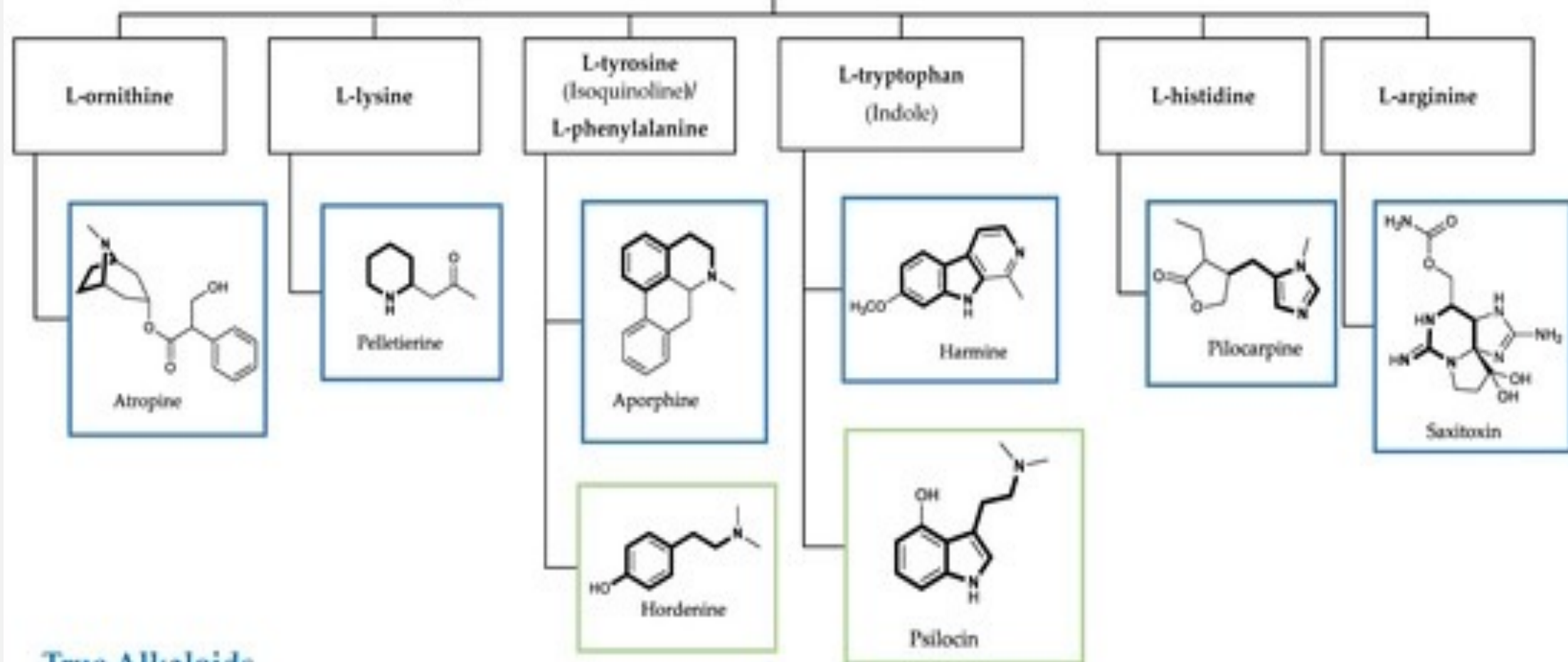
1. From the **generic** name of the plant yielding them as **atropine** (*Atropa belladonna*).
2. From the **specific** name of the plant yielding them as **cocaine** (*Erythroxylum coca*).
3. From the **common** name of the drug yielding them as **ergotamine** (Ergot).
4. From their **physiologic activity** as **emetine** (Ipecac causes emesis).
5. From the **discoverer** as **pelletrine**.
6. Others e.g. **Morphine** derived from **ancient Greek mythology** – Morpheus – god of dreams

CLASSIFICATION OF ALKALOIDS

- **True alkaloids** characterized by a heterocyclic ring with a nitrogen atom and are derived from amino acid e.g. atropine, nicotine, and morphine.
- **Proto alkaloids** characterized by absence of the heterocyclic ring but derived from amino acid e.g. adrenaline and ephedrine.
- **Pseudo alkaloids** characterized by a heterocyclic ring with a nitrogen atom, but are not derived from amino acids (steroidal alkaloids).

Alkaloid	Origin	Example	Biological structure
True alkaloid	Derived from amino acids that have nitrogen in heterocyclic ring	Atropine	 The chemical structure of Atropine is shown. It consists of a tropane bicyclic ring system (8-azabicyclo[3.2.1]octane) with a methyl group on the nitrogen atom. This is linked via an ester bond to a side chain that includes a chiral center with a hydroxyl group and a phenyl ring.
Proto alkaloid	Derived from amino acids that don't have nitrogen in heterocyclic ring	Taxol	 The chemical structure of Taxol (paclitaxel) is shown. It is a complex polycyclic diterpenoid with multiple fused rings, including a phenyl ring and several ester and ether functional groups.
Pseudo alkaloids	Not derived from amino acids but have nitrogen in their heterocyclic ring	Caffeine	 The chemical structure of Caffeine is shown. It is a purine alkaloid, consisting of a fused pyrimidine and imidazole ring system with three methyl groups and two carbonyl groups.

Alkaloids derived from amino acids

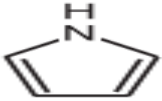

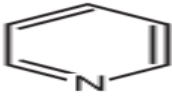
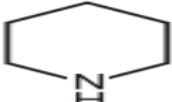
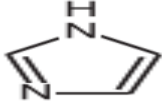
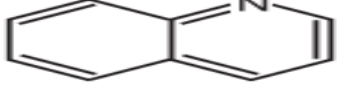
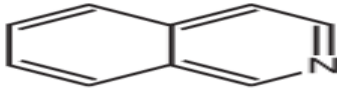
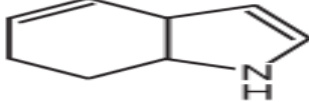
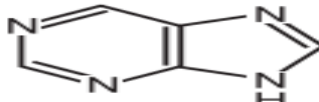


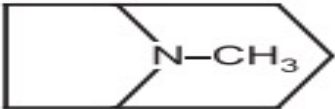
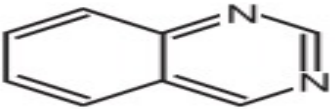
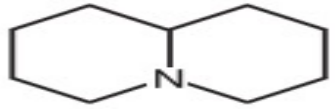
True Alkaloids
Proto Alkaloids

CHEMICAL CLASSIFICATION OF ALKALOIDS

- a. Non-heterocyclic or atypical alkaloids or biological amines
- b. Heterocyclic or typical alkaloids, divided into (14) group according to their structure as follows:

1. Pyrrol and Pyrrolidine.
2. Pyrrolizidine.
3. Pyridine and Piperidine.
4. Tropane.
5. Quinoline.
6. Isoquinoline.
7. Aporphine
8. Norlupinane.
9. Indole.
10. Indolizidine.
11. Imidazole.
12. Purine.
13. Steroid.
14. Terpenoid

Types of alkaloids	Structure	Example	Biological source and Family	Uses
Pyrrole		Hygrine from Coca	<i>Erythroxylum coca</i> Family: Erythroxylaceae	Analgesic
Pyrrolidine		Nicotine from Tobacco	<i>Nicotiana tabacum</i> Family: Solanaceae	Stimulant
Pyridine		Lobeline from Lobelia	<i>Lobelia inflata</i> Family: Campanulaceae	Use in Asthma
Piperidine		Piperine from Black pepper	<i>Piper nigrum</i> Family: Piperaceae	Anti-inflammatory activity
Imidazole		Pilocarpine from Pilocarpus	<i>Pilocarpus microphyllus</i> Family: Rutaceae	Treating glaucoma
Quinoline		Quinine from Cinchona	<i>Cinchona officinalis</i> Family: Rubiaceae	Antimalarial
Isoquinoline		Morphine from Opium	<i>Papaver somniferum</i> Family: Papveraceae	Analgesic
Indole		Reserpine from Rauwolfia	<i>Rauwolfia serpentine</i> Family: Apocyanaceae	Antihypertensive
Purine		Caffeine from Tea	<i>Thea sinensis</i> Family: Theaceae	CNS stimulant

Types of alkaloids	Structure	Example	Biological source and Family	Uses
Tropane		Atropine from Datura	<i>Datura metel</i> Family: Solanaceae	Depressant
Quinazolin		Vasicine from Vasaka	<i>Adhatoda vasica</i> Family: Acanthaceae	Antitussive
Norlupinane		Lupanine from Lupine	<i>Lupinus albus</i> Family: Fabaceae	Carminative, Diuretic

ALKALOIDS DESCRIPTION

- Contains **nitrogen** - usually derived from an amino acid
- Bitter tasting
- Usually **colorless**
- Some alkaloids are colored, **yellow** or **orange**.
- Generally **solids** (exception - nicotine is a brown liquid).
- They give a **precipitate** with heavy metal iodides, except Caffeine.
- Most alkaloids are **weak bases**, but some are amphoteric, for example theobromine and theophyllin.
- Most alkaloids are poorly soluble in water but its **dissolve in organic solvents**, such as diethyl ether, chloroform. However, caffeine dissolves well in boiling water
- Toxic e.g. cyclopamine which is present in the leaves of corn lily.

ALKALOIDS DESCRIPTION

Test	Reagent Composition	Positive Colour Change
Dragendorffs Reagent	Potassium bismuth iodide	Reddish-brown
Mayer's Reagent	Potassio mercuric iodide	White or pale yellow ppt
Hager's Reagent	Picric acid	Yellow
Wagner's Reagent	Solution of iodine in potassium iodide	Yellow or brown ppt.
Murexide Test (for Caffeine and Other Purine Derived Alkaloids)	Potassium chlorate + drops of HCl. Expose the resultant to NH_3	Purple colouration

MEDICINE APPLICATIONS

Alkaloid	Action
Colchicine	Remedy for gout
Morphine	Analgesic
Quinine	Anti malarial
Vinblastine, vincristine	Antitumor
Codeine	Cough medicine





Pharmacognosy
3rd class , 2nd semester
By MSc. Aya Alashqar

IDENTIFICATION OF HARMALA ALKALOIDS



Identification of harmala alkaloids

```
graph TD; A[Identification of harmala alkaloids] --> B[Qualitative analysis]; A --> C[Quantitative analysis]; B --> D[General test]; B --> E[Chromatography TLC]; C --> F[Weighting the crystals];
```

The diagram is a hierarchical flowchart. At the top is a box labeled 'Identification of harmala alkaloids'. A vertical line descends from this box and splits into two horizontal lines, leading to 'Qualitative analysis' on the left and 'Quantitative analysis' on the right. From 'Qualitative analysis', a vertical line descends and splits into two horizontal lines, leading to 'General test' and 'Chromatography TLC'. From 'Quantitative analysis', a vertical line descends to a single box labeled 'Weighting the crystals'. All boxes are light gray with rounded corners and a subtle drop shadow.

Qualitative analysis

Quantitative analysis

General test

Chromatography
TLC

Weighting the
crystals

- 1) QUANTITATIVELY
- By weighting the crystals of each the separated alkaloids.
- 2) QUALITATIVELY
- A) General Tests
- 1- Mayer's test
- Aim:- to indicate in general the alkaloid as other alkaloids.
- Equipment and reagents:-
- Petri dish.
- Ethanol.
- HCL.
- Mayer's reagent.
- Procedure:-
- Take few crystals of Harmala alkaloid.
- Dissolve in few mls of ethanol in a Petri dish.
- Add two drops of HCL.
- Add two drops of Mayer's reagent.
- Results:-
- White precipitation will occur.

- 2- Wagner's reagent —————> Red-brown precipitate
- 3- Dragendorff's reagent —————> Orange precipitate

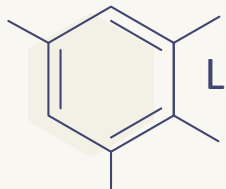
- B) TLC
- It can be considered as specific test due to the fluorescence of these alkaloids.
- Aim: - used to identify qualitatively the harmala alkaloids.
- Equipments and reagents:-
 - Glass jar with its cover
 - Silica gel plates
 - Standard reagent
 - Spray reagent (dragendroffs reagent)
 - Mobile phase =
 - Chloroform : methanol : 10% ammonium hydroxide (80: 20:15).
 - Chloroform : methanol : acetone (35 : 15 :10)
 - Sample F.B, F.C.
 - Capillary tube.
 - U.V apparatus.

- Procedure:-

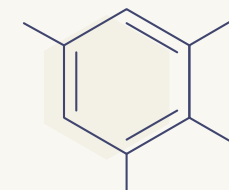
- Prepare the mobile phase and put in the glass jar, cover the jar and leave it for 45 min. for saturation.
- Apply the sample and the standard by the use of capillary tube on the silica gel plate.
- Leave the plate in the jar until the solvent reaches $\frac{3}{4}$ of the plate.
- Remove the plate, dry and identified first by U.V 254 nm. Spraying with dragendroffs reagent .
- Calculate the Rf of each alkaloid.

- Results:-

- Fluorescence spot appear under the U.V while an orange spots are seemed when sprayed with the sprayer.



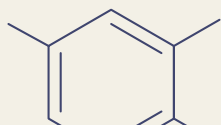
Lab. 8

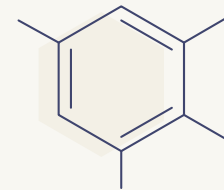
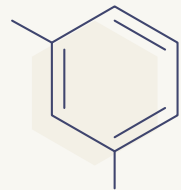


Isoquinoline Alkaloids



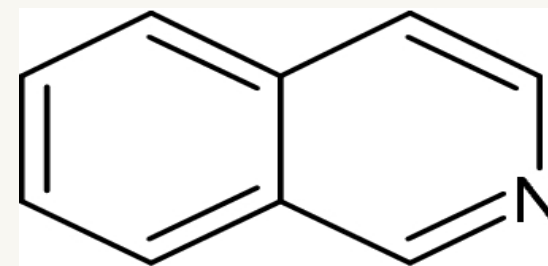
Pharmacognosy
3rd class , 2nd semester
By MSc. Aya Alashqar





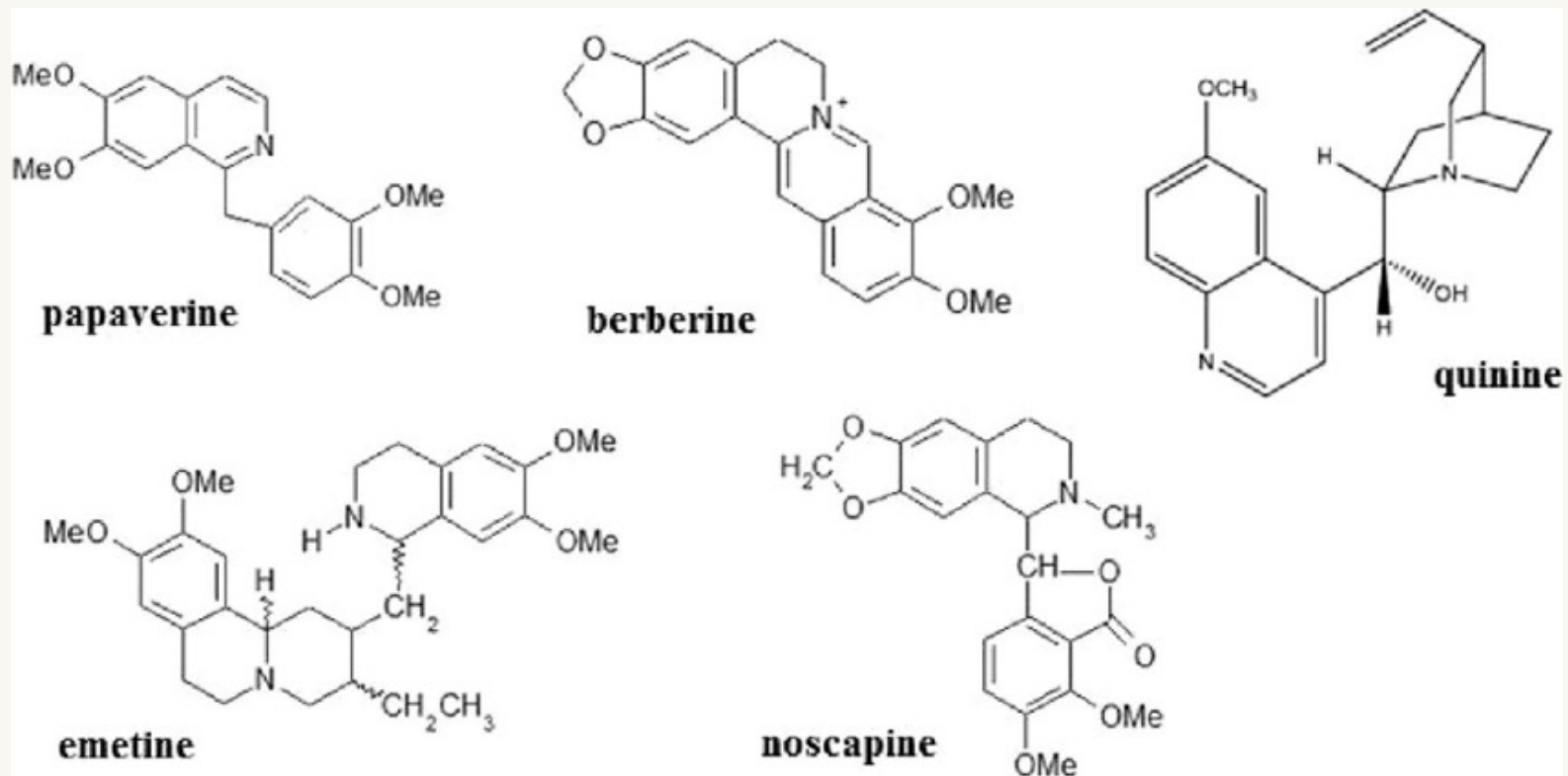
Introduction about isoquinoline alkaloids

- Isoquinoline is a heterocyclic aromatic organic compound.
- It is a structural isomer of quinoline.
- Isoquinoline and quinoline are benzo pyridines derivatives , which are composed of benzene ring fused to a pyridine ring.



Isoquinoline nucleus

Some example of isoquinoline alkaloids



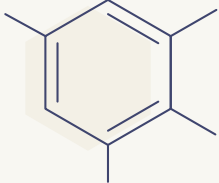




Fumaria densiflora

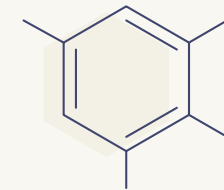
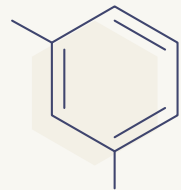
- Botanical Name: *Fumaria densiflora* DC
- Family name: Fumariaceae
- Active components:
 - Protopine (% 0.29-0.27)
 - fumariline (% 0.21-0.20)
 - β -allocryptopine (% 0.32-0.30)
- *Fumaria densiflora* is annual herb that reaches up to 10-25 cm long Leaves are 2-6 cm.
- Flowers are with pink and blackish-red tipped petals.





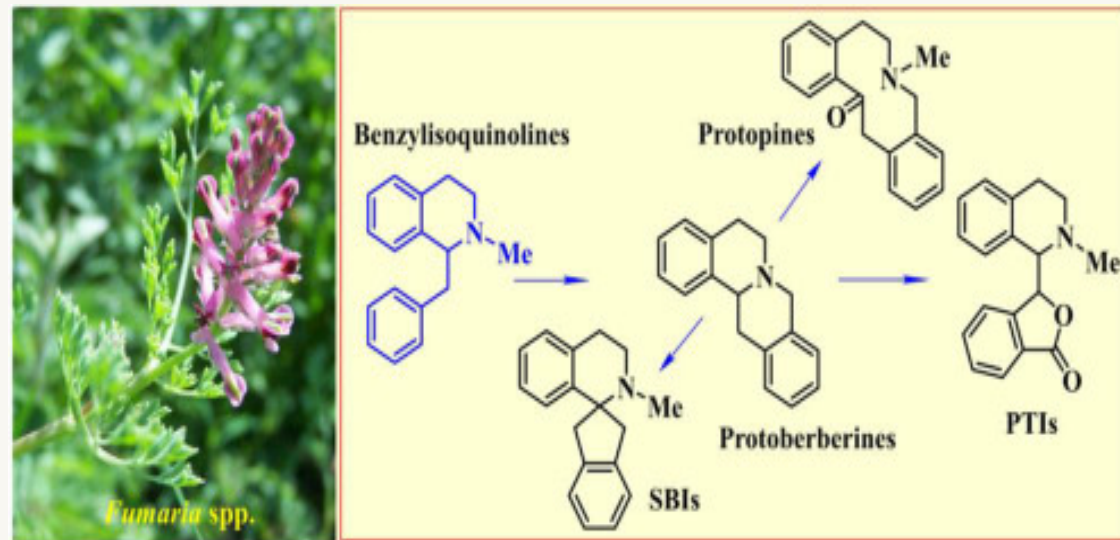
Traditional medicine of Fumaria species

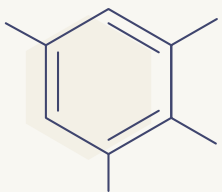
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- Fumaria species have been used in traditional medicine as
 - Antihypertensives
 - Diuretics
 - Hepatoprotectants
 - Laxatives (to treat gastrointestinal disorders)
 - As well as in the treatment of rashes and conjunctivitis
 - The biological activity of Fumaria is mostly associated with the presence of isoquinoline alkaloids in the plant



Phytochemical investigation

- Phytochemical investigation revealed the presence of several alkaloids such as adlumidiceine, copticine, fumariline, perfumine, protopine, fumaranine, fumaritine, paprafumicin and paprarine





Extraction method

Aerial parts of plants (25 g) were dried, powdered and extracted with ethanol in a Soxhlet apparatus until Mayer's test was negative then evaporated in vacuum



The ethanolic residue was taken up in % 1 hydrochloric acid (50 ml), filtered



The aqueous acid solution brought to pH 9-9.5 with % 25 ammonium hydroxide

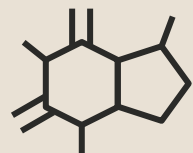


Extracted with chloroform (5 x 150 ml).

The extracts were dried with anhydrous sodium sulphate and the solvent evaporated to afford a crude extract of alkaloids.



Thank *you!*

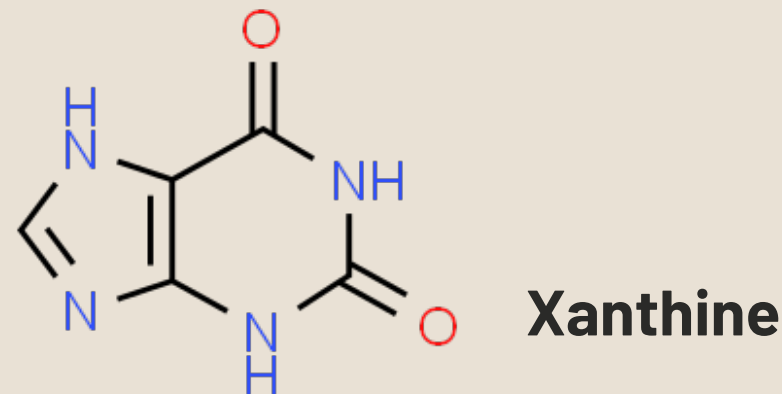


Purine alkaloids

Pharmacognosy
3rd class , 2nd semester
By MSc. Aya Alashqar

Introduction about purine alkaloids

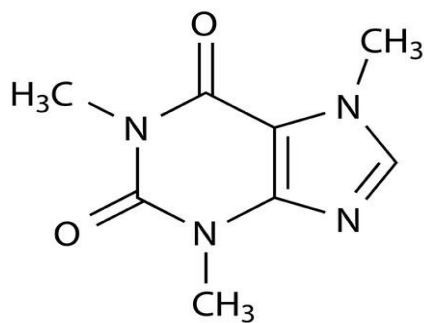
- Purines nucleus is a heterocyclic nucleus consisting of pyrimidine ring fused to 5-membered imidazole ring known as **xanthine**
- Amphoteric Character
- Purines unlike other alkaloids do not give positive results with general tests of alkaloids; instead **murexide test** (Purple Color) is used in its identification



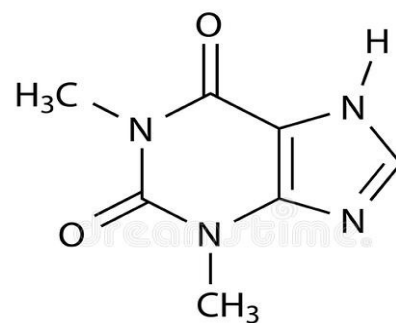
Introduction about purine alkaloids

Purines are present as methylated compounds ,which are:

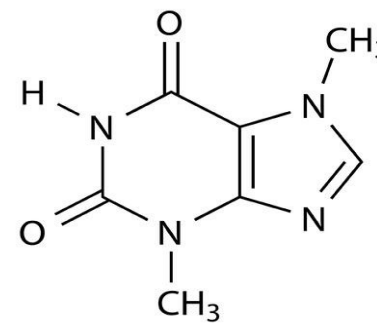
- 1. Caffeine (1, 3,7- tri methyl xanthine).
- 2. Theophylline (1,3-di methyl xanthine).
- 3. Theobromine (3,7- di methyl xanthine).



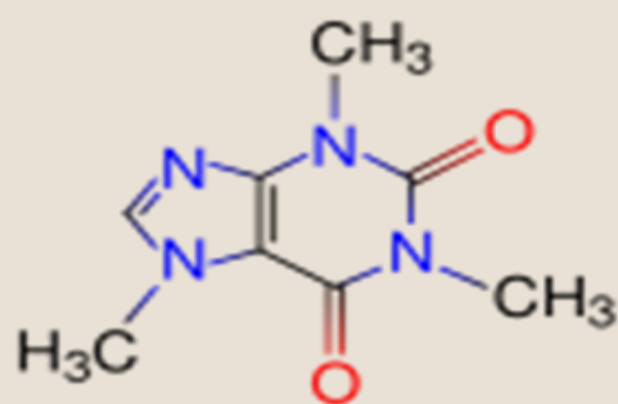
Caffeine



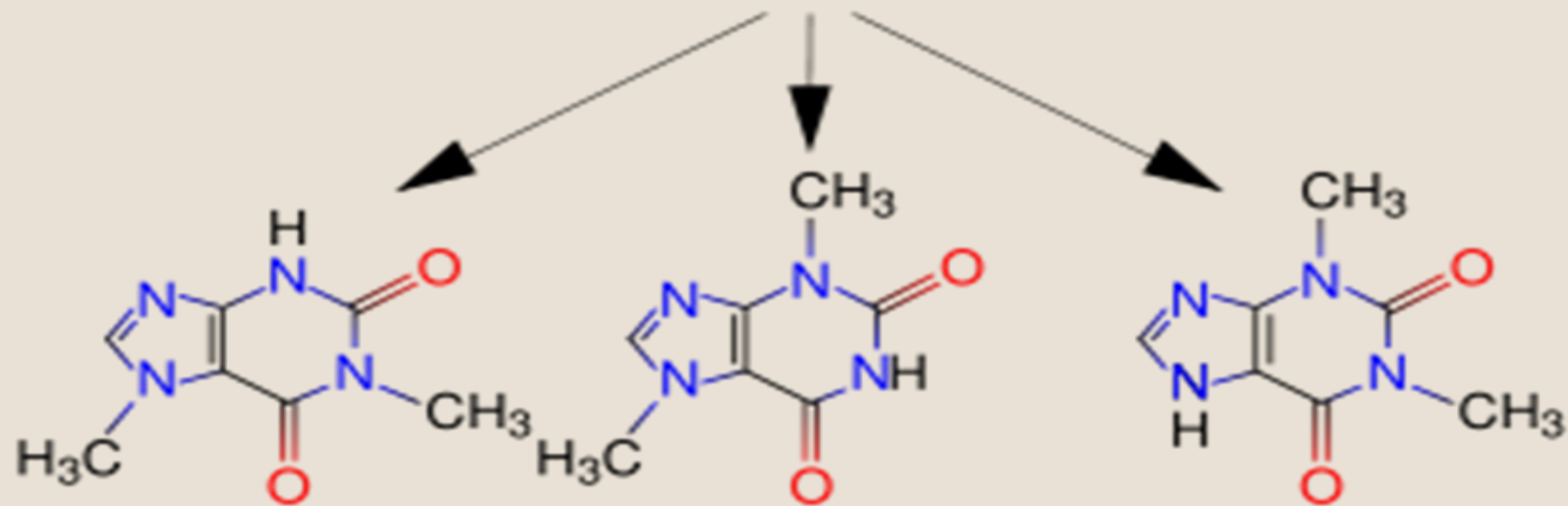
Theophylline



Theobromine



Caffeine



Paraxanthine
(84%)

Theobromine
(12%)

Theophylline
(4%)

Pharmacological activities of the purine alkaloids

- 1. Stimulation of the CNS.
- 2. Diuretic effects.
- 3. Increase gastric acid secretion.
- 4. Relaxation of the bronchial smooth muscle (theophylline).
- 5. Positive inotropic and chronotropic effect on the heart.

The most important plants in this group

- 1. Coffee (*Coffea arabica* of the family Rubiaceae).
Contain about 1-2 % of caffeine.
- 2. Tea (*Camellia sinensis* of the family Theaceae).
Contain about 1-4 % of caffeine.
- 3. Cola (*Cola nitida* of the family Sterculiaceae).
Contain about 3.5 % of caffeine.



Isolation of caffeine from tea

- **AIM:** - To isolate the caffeine alkaloid from tea.
- **Equipment's and reagent:-**
 - Large beaker and small beaker
 - Water bath
 - Muslin
 - Centrifuge test tubes
 - Evaporating dish
 - Boiling water
 - Separatory funnel
 - Basic lead acetate
 - Sulphuric acid
 - Decolorized charcoal
 - Chloroform
 - Hot 60C° ethanol

Isolation of caffeine from tea

- **Procedure :**
- **Method of extraction:** decoction.
- **Plant used:** Camellia sinensis **Part used:** dry leaves.

Place 50 gm of the powdered tea leaf in 250 ml of water

Boil for (15 mins)
(Constant stirring)

Strain the resulting hot extract through muslin, express well

Wash the mass remains on the muslin with 50 ml of boiling water and express again

Add
(carefully)

5 drops of lead sub acetate
(Heat the mixture to boiling)

Centrifuge
(5 mins.)

Decant and take the supernatant (upper layer) and heated to boiling

Add dilute Sulphuric acid until precipitation ceases

1gm of decolorized charcoal is added to the filtrate which is then evaporated over a low Bunsen flame with frequent stirring until a volume of 75-100 ml is reached



Evaporation of the large volume of the liquid may be carried out in portions in a smaller evaporating dish



Evaporation of the large volume of the liquid may be carried out in portions in a smaller evaporating dish and then filtrated hot



Cool, transfer to a separatory funnel and shake out with three successive 10 ml portion of chloroform



Transfer the combined chloroform extract to a small evaporating dish



Carefully evaporate the chloroform on steam bath in the hood



Scrap out the residue, transfer to a small beaker and dissolve in the smallest quantity of hot 60 c ethanol necessary to affect the solution



Allow the solution to stand overnight

Result: Pure crystals with a white color will be obtained.

Isolation of caffeine from tea

- **Discussion:-**
- Purines differ from other alkaloids in that they are soluble in hot water, which is used in it is extraction, and this is the cause why all the time you should heat the mixture (to prevent the precipitation of caffeine in cold water).
- Addition of basic lead acetate is to precipitate the tannins and other unwanted materials.
- Addition of dilute Sulphuric acid is to take the excess of lead acetate.
- The centrifugation is used to isolate the decant containing the unwanted material from the desired supernatant.
- Addition of charcoal is for the decolorization, so that the crystals will take its normal white color.
- Use of chloroform is to extract the caffeine from other component of the mixture.
- Use of hot ethanol is for the recrystallization process.

Study problems

Give the reason of :

Q1. Addition of lead acetate

Q2. Use of the water as solvent in the extraction of caffeine from tea

Q3. Addition of sulphuric acid in the extraction of caffeine from tea

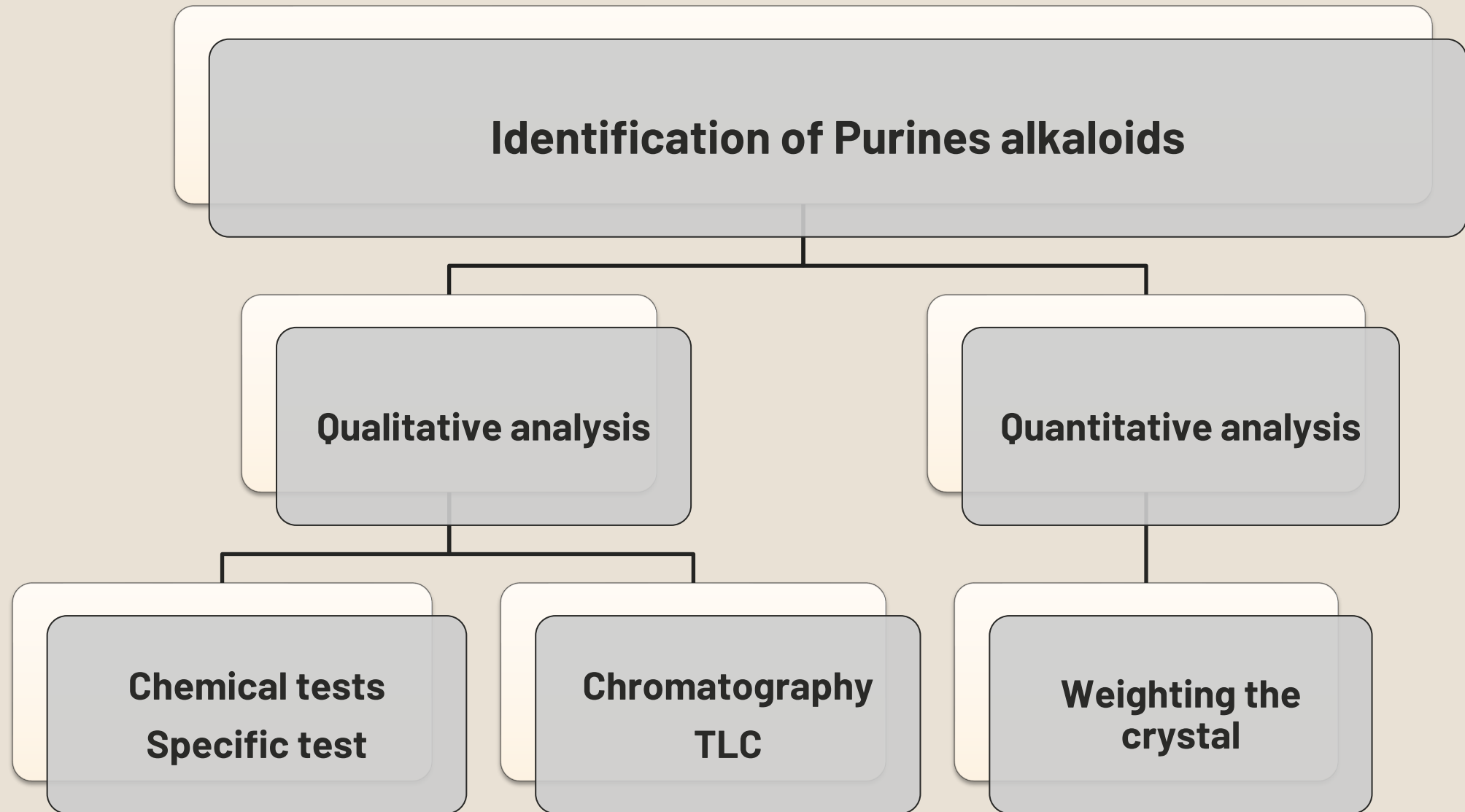
Q4. Using of the centrifuge after the addition of lead sub acetate

Q5. Using the hot ethanol in the last step



Identification Of Caffeine Crystals

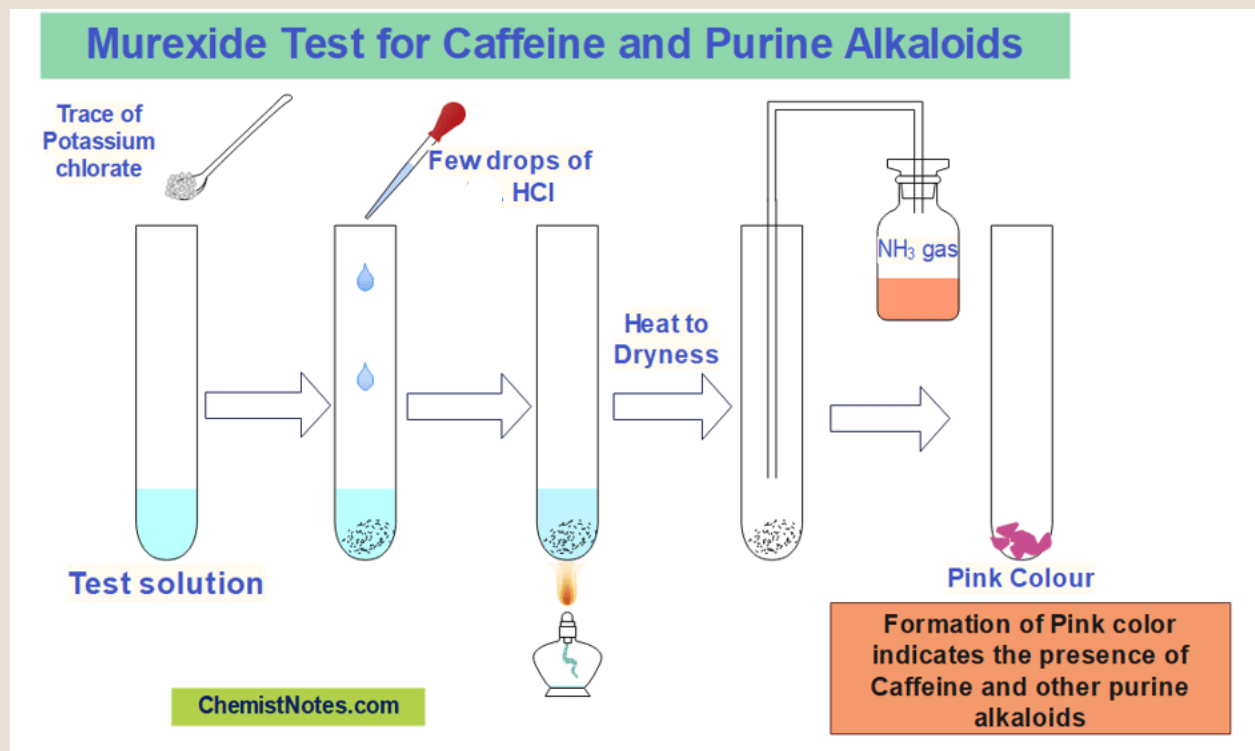
Identification Of Caffeine Crystals



Identification Of Caffeine Crystals

Qualitative analysis

- 1) **Specific Test**
- **The Murexide test**
- **Aim:** - To identify the caffeine alkaloid (Purine alkaloid) from other alkaloids.
- **Equipment's and reagents:-**
 - Porcelain dish or test tube
 - Water bath
 - Potassium chlorate
 - Concentrated HCL
 - Ammonia vapor



Identification Of Caffeine Crystals

- Procedure:-
 - Take few crystals of caffeine in porcelain.
 - Add small amount of potassium chlorate (KClO_3).
 - Add 2 drops of concentrated HCL.
 - Evaporate to dryness.
 - Expose to ammonia vapor.
- Result:-
 - A purple color is produced of caffeine and other Purine derivative.

Identification Of Caffeine Crystals

Qualitative analysis

- 2) **TLC**
- **Aim:** - Used to identify qualitatively the Purine alkaloids.
- **Equipment's and reagents:-**
 - Glass jar with its cover
 - Silica gel plates
 - Standard reagent
 - Mobile phase (acetone; water; ammonia (90; 7; 30)
 - Spray reagent (I/KI)
 - Capillary tube

Identification Of Caffeine Crystals

- Procedure:-
- Prepare the mobile phase and put in the glass jar, cover the jar and leave it for 45 min. for saturation.
- Apply the sample and the standard by the use of capillary tube on the silica gel plate.
- Leave the plate in the jar until the solvent reaches $\frac{3}{4}$ of the plate. Remove the plate, dry, and then spray with the spraying reagent.
- Calculate RF Value.

A photograph of coffee beans and cherries. In the foreground, a pile of dark brown, roasted coffee beans is scattered on a light-colored surface. To the left, a small, dark, glossy cup is tipped over, spilling more beans. In the background, there are green leaves and clusters of coffee cherries in various stages of ripeness, from green to red. The entire image is framed with rounded corners and has a semi-transparent white overlay.

THANKS

Lab. 3

Tropane alkaloids

Pharmacognosy

3rd class , 2nd semester

By MSc. Aya alashqar



Introduction

They are ester alkaloids resulted from the coupling of organic acids with amino alcohol (Base).





Tropane Alkaloids are classified into

Solanaceous Tropane Alkaloids

Occurrence:

Atropa

Datura

Hyoscyamus

Erythroxylon Coca Alkaloids

Occurrence:

Coca leaves contain about 2%
total alkaloids

1- Solanaceous Tropane Alkaloids

Main Alkaloids are:

- 1) Atropine (Strong base)
- 2) Hyoscyamine
- 3) Hyoscine (Scopolamine)

Uses :

The three Alkaloids are anticholinergic agents:

- A. Decrease saliva and GIT secretions so used preoperative.
- B. Decrease motility of smooth muscles so used as antispasmodics.
- C. A mydriatic (causes dilatation of the eye pupil).
- D. Hyoscine has more central effect so it is sedative and hypnotic.
- E. Hyoscine is mainly used as antispasmodic and antiemetic.



2- Erythroxylon Coca Alkaloids

Main Alkaloids are:

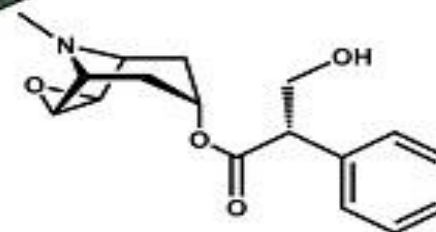
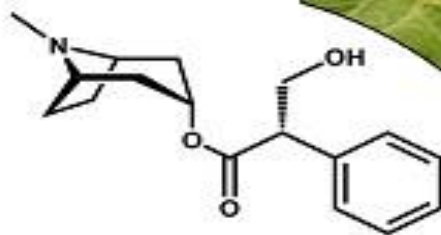
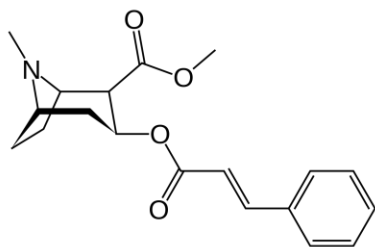
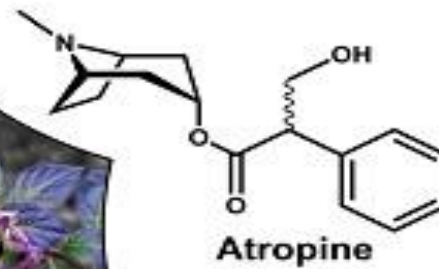
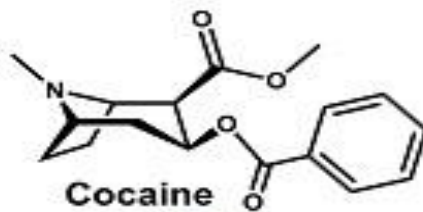
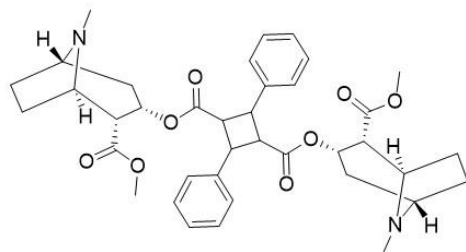
- 1) Cocaine
- 2) Cinnamylcocaine
- 3) α truxilline

Uses :

The three Alkaloids are anticholinergic agents:

- A. Cocaine was used as local anesthetic.
- B. Cocaine has a CNS stimulant activity so is one of the widely abused drugs.





Atropa belladonna

- Family:
Solanaceae





Atropa belladonna

- 1) Belladonna leaf, also known as devils cherries, black cherry.
- 2) The belladonna leaf yield not less than 0.35% of alkaloids.
- 3) Native to Europe, North Africa, and Western Asia
- 4) The name "belladonna" comes from the Italian language, meaning "beautiful lady"



Toxicity of *Atropa belladonna*

1. Belladonna is one of the most toxic plants.
2. All parts of the plant contain atropine alkaloids.
3. The greatest danger to children because they look attractive and have a somewhat sweet taste.
4. The root of the plant is generally the most toxic part.

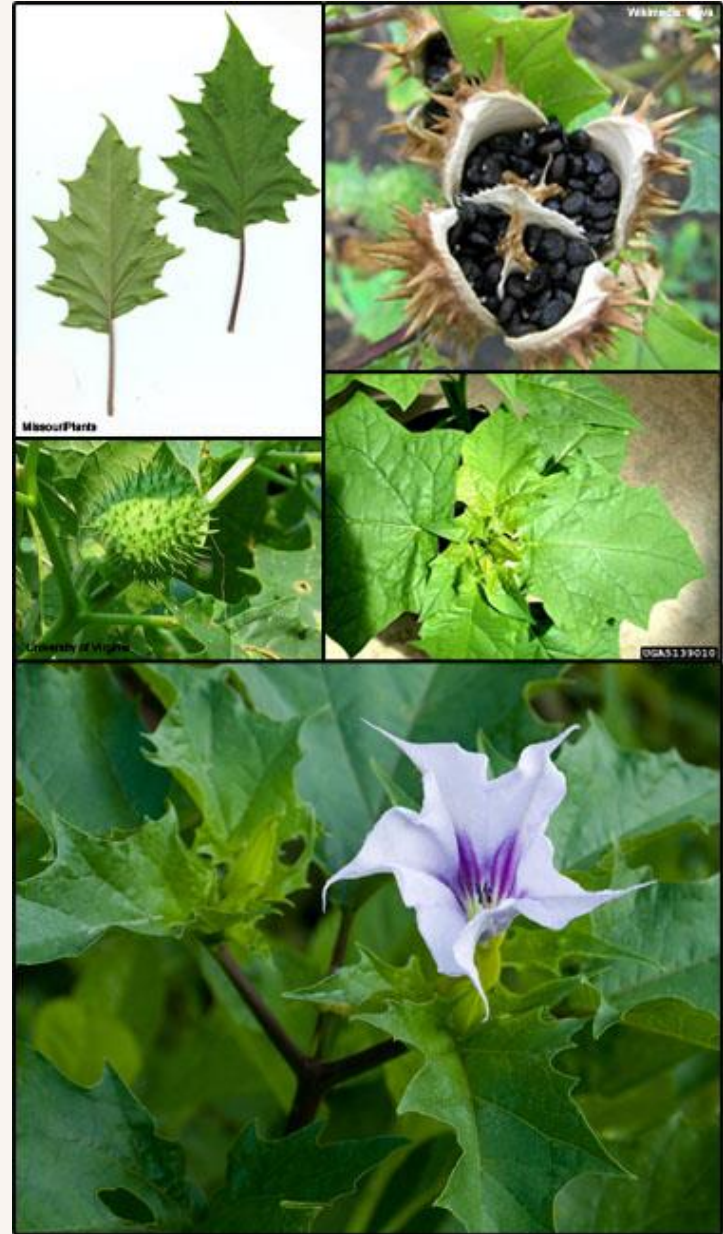


The symptoms of belladonna poisoning

1. Blurred vision.
2. Loss of balance.
3. Headache, rash.
4. Dry mouth.
5. Slurred speech.
6. Confusion, hallucinations.

Datura stramonium

- Family:
Solanaceae





Datura stramonium

1. Known by the common names **Jimson weed** or **datura**
2. Datura has been used as herbal medicine to relieve asthma symptoms
3. And used as an analgesic during surgery or bone setting
4. It is also a powerful hallucinogen and deliriant, which is used spiritually for the intense visions it produces.
5. However, the tropane alkaloids which are responsible for both the medicinal and hallucinogenic properties are fatally toxic in only slightly higher amounts than the medicinal dosage, and careless use often results in hospitalizations and deaths.



Constituents of belladonna and datura are:-

Hyoscyamine and Atropine, which is formed during extraction procedure.

Also, it contains Hyoscine (scopolamine) alkaloid, which is found in trace amounts.

These alkaloids are also present in other plants as:-
Hyoscyamus niger Family Solanaceae.

Isolation of belladonna or datura alkaloids



Extract 50 gm of datura leaves with 150ml alcohol 90% under reflux for one hour (The belladonna alkaloids are not affected by heat therefore the reflux condenser is used in its extraction)



Filter and evaporate the filtrate on a steam bath to a syrup liquid (concentrated) The evaporation step is to get rid of the ethanol solvent (since in the partition step you must have two immiscible layers ,if not this means that the ethanol is still present)




The concentrated filtrate add slowly, with stirring into 10ml of 2%HCL and heat gently for 5 mins (Addition of 2%HCL is to convert the alkaloids present in the extract to alkaloidal salts (Alkaloids are extracted as their salts together with accompanying soluble impurities), the use of 2% not more, is to prevent the destruction of the alkaloid by concentrated HCL)



Cool and filtrate the acidic extract

Washing by adding organic solvent to remove all the non polar impurities such as 5ml of ether, chloroform or hexane & separate two layers by separatory funnel



Take the supernatant (upper layer) and made alkaline by addition of

Made alkaline by adding ammonium hydroxide solution
(according to litmus paper)

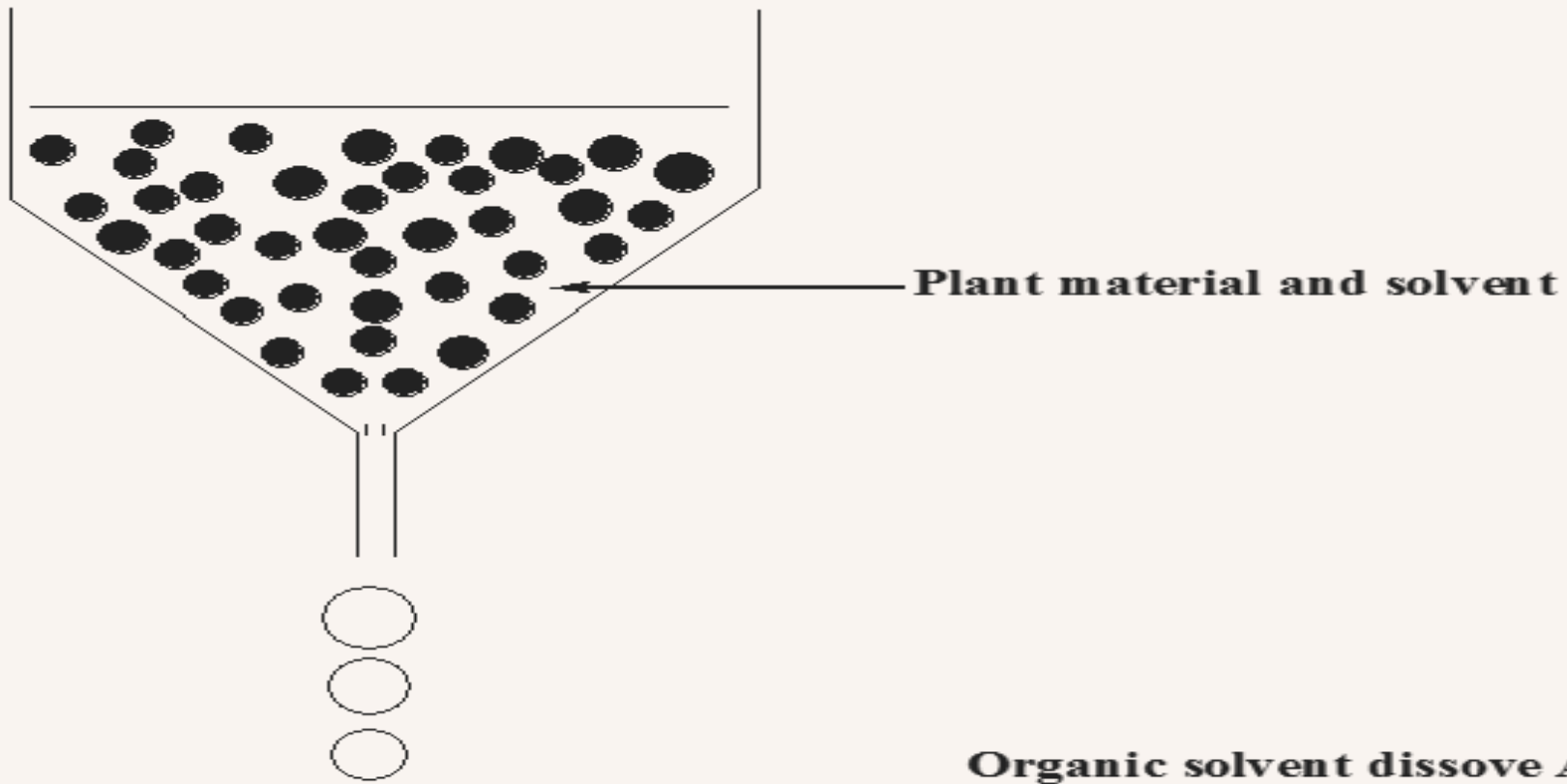
The addition of ammonium hydroxide is used to liberate free base



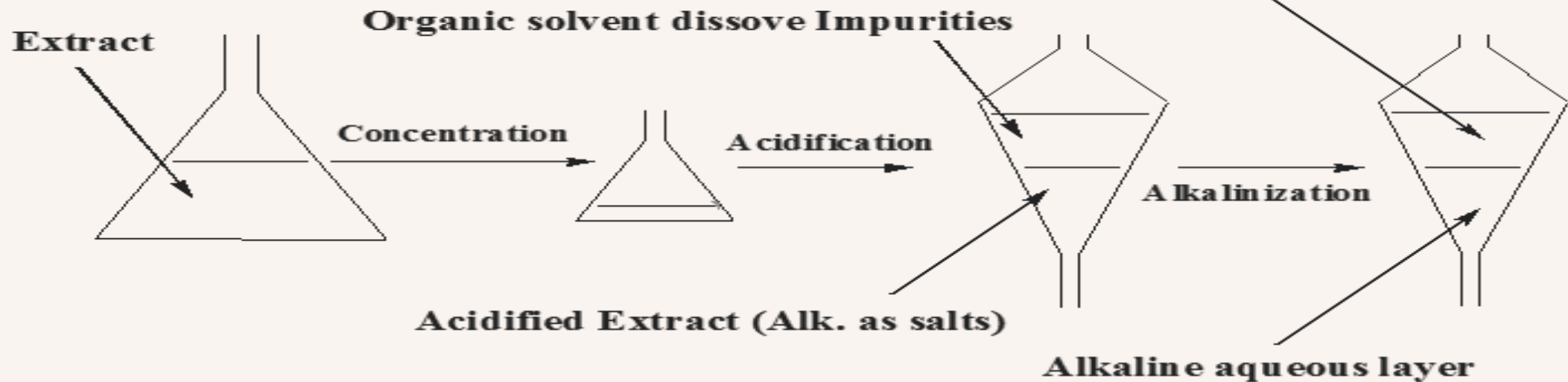
Placed in a separator funnel



Extracted three time with 100 ml of chloroform
(The use of chloroform is to extract the alkaloid base, as it
is soluble in the organic solvent)
dehydrate by adding **anhydrous sod. sulphate** filter (or
decant) , evaporate to dryness



Result:
Product containing the
mixture of the alkaloids.





Study problems:

Give the reason of :

- 1.* Using the reflux in extraction the tropane alkaloid from datura stramonium.
- 2.* Making the evaporation step after filtration?
- 3.* Addition of 2% HCl to the alcoholic extract?
- 4.* Addition of ammonium hydroxide?
- 5.* Using of chloroform in partitioning step?

Thanks!



Lab. 4

Identification of tropane alkaloids

Pharmacognosy

3rd class , 2nd semester

By MSc. Aya alashqar



Identification of tropane alkaloids

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graph TD; A[Identification of tropane alkaloids] --> B[Qualitative analysis]; A --> C[Quantitative analysis]; B --> D["Chemical tests<br/>General test<br/>Specific test"]; B --> E["Chromatography<br/>TLC"]; C --> F["Titration method<br/>(back titration)"]
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The diagram is a hierarchical flowchart. At the top is a box labeled 'Identification of tropane alkaloids'. A line from this box branches into two boxes: 'Qualitative analysis' on the left and 'Quantitative analysis' on the right. From 'Qualitative analysis', a line branches into two boxes: 'Chemical tests' (which contains 'General test' and 'Specific test' on separate lines) and 'Chromatography' (which contains 'TLC' on a separate line). From 'Quantitative analysis', a line leads to a box labeled 'Titration method (back titration)'.

Qualitative analysis

Quantitative analysis

Chemical tests
General test
Specific test

Chromatography
TLC

Titration method
(back titration)

What is the different between direct and back titration?

In the **direct titration**, amount of standard solution, which is chemically equivalent to the analyte amount, is added.

In **back titration**, excess of the standard titrant is added to determine the analyte amount.

Normally, in **direct titration**, only one direct reaction is taking place, which is between the standard titrant and the analyte.

In a **back-titration**, two chemical reactions are taking place, one is with the standard and analyte, and the other is with the excess-standard titrant and a standard solution.

Qualitative analysis

A) The specific test for Tropane alkaloid.

1) Vitali morin test

Aim: - To identify the Tropane alkaloids from other alkaloids.

Equipment's and reagents:-

Small beaker

Fuming nitric acid

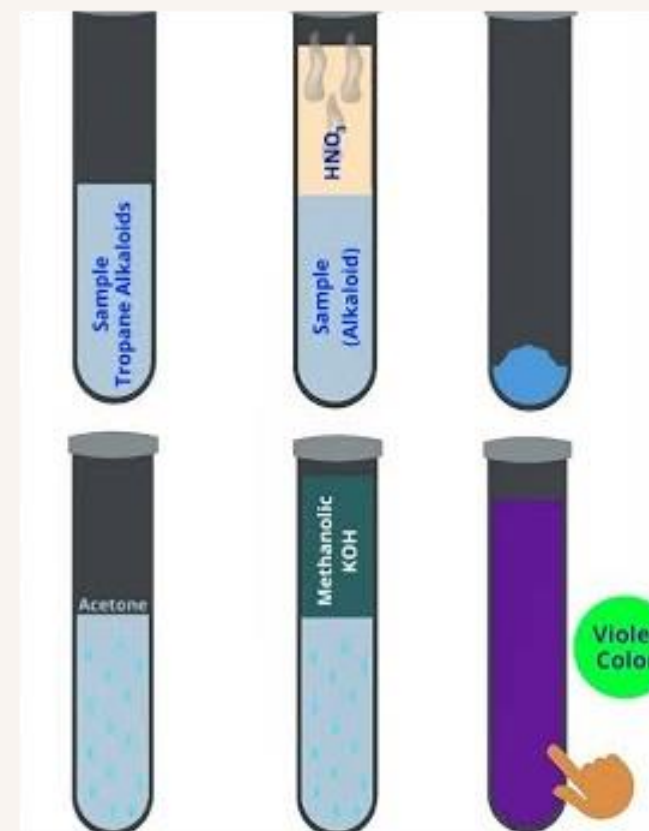
Alcoholic KOH

Procedure:-

Take few mls of the extract.

Add to it drops of fuming nitric acid and evaporate.

Then add 2ml of alcoholic KOH.





Qualitative analysis

A) The specific test for Tropane alkaloid.

1) **Vitali morin test**

Results:-

Violet color will be resulted.

Discussion:-

The nitric acid will nitrate the benzene ring, and the color will be developed after addition of KOH.



Qualitative analysis

A) The specific test for Tropane alkaloid.

2) Gerhard's Test

Aim: - To identify the Tropane alkaloids from other alkaloids.

Equipment's and reagents:-

Small beaker

2% HgCl_2 (Mercuric Chloride) in 50% aqueous ethanol.

Procedure:-

Add 2% HgCl_2 in 50% aqueous ethanol to 0.006g of atropine.

Results:-

A deep red color will be developed.



Qualitative analysis

B) General Tests

1) Mayer's test

Aim:- to indicate in general the alkaloid as other alkaloids.

Equipment and reagents:-

Petri dish, Ethanol, HCL, Mayer's reagent

Procedure:-

Take few crystals of Tropane alkaloid.

Dissolve in few mls of ethanol in a Petri dish.

Add two drops of HCL.

Add two drops of Mayer's reagent.

Results:-

White precipitation will occur.

2) Wagner's reagent \longrightarrow Red-brown precipitate

3) Dragendorff's reagent \longrightarrow Orange precipitate

Qualitative analysis

T.L.C

Aim:- Used to identify qualitatively the belladonna alkaloids

Equipment's and reagents:-

Glass jar with its cover.

Silica gel plates.

Standard reagent.

Mobile phase (acetone: water: ammonia (90:7:3))

Spray reagent (dragendorff's reagent)

Capillary tube.

Qualitative analysis

Procedure:-

Prepare the mobile phase and put in the glass jar, cover the jar and leave it for 45 min. for saturation.

Apply the sample and the standard by the use of capillary tube on the silica gel plate. Leave the plate in the jar until the solvent reaches $\frac{3}{4}$ of the plate. Remove the plate, dry, and then spray with the spraying reagent.

Result

Orange spots appear for both standard and sample.

Note: -

Other used mobile phase =

Chloroform: Acetone: Diethyl amine (50: 40: 10)

Methanol: Benzene (66%: 33%)

Chloroform: Diethyl amine (90:10)



Study problems:

1. How can you identify tropane alkaloids quantitatively?
2. How can you identify belladonna alkaloids specifically?
3. How the positive result will appear in vitali morin test?

2. ASSAY OF HYDROGEN PEROXIDE SOLUTION

Laboratory for the third stage

Organic Pharmaceutical Chemistry

Prepared By Dr. Ali Hussein Mustafa AL-OBAIDI

College of Pharmacy -Tikrit University / 2020

DEFINITION

Chemical name Hydrogen peroxide

C.A.S. number 7722-84-1

Chemical formula H_2O_2

Structural formula H-O-O-H

Formula weight 34.01

ASSAY

1. An odourless, or nearly odourless, transparent and colourless liquid Caution: Powerful oxidizing agent. Avoid contact with eyes and skin.
2. SCOPE This method is designed for the determination of hydrogen peroxide in aqueous solutions containing 20% to 70% hydrogen peroxide.

PRINCIPLE

Hydrogen peroxide in a diluted portion of the sample is quantitatively oxidized by titration with a potassium permanganate solution of known strength. Compounds that are oxidized by potassium permanganate under acidic conditions interfere. (Ref: Solvay & Cie., Method FN 1167/01).

REAGENTS

All reagents should be analytical reagent grade, and only deionized water should be used.

A. Potassium Permanganate (KMnO_4):

0.1N-Potassium permanganate is a strong oxidizer; wear gloves and safety glasses. Weigh 3.2 g of KMnO_4 into a 1-liter beaker. Add 500 mL of water and stir until all the KMnO_4 is in solution. Boil for one hour, cool, and filter through a fritted glass crucible into a 1-liter volumetric flask. Dilute to volume and mix well. Store in a dark-colored bottle. Standardize using the method given in Procedure, Item A. below.

B. Sodium Oxalate ($\text{Na}_2\text{C}_2\text{O}_4$):

Sodium oxalate is toxic; wear gloves and avoid breathing dust. C. Sulfuric Acid (1:3): Wearing gloves and safety goggles, slowly add 50 mL of sulfuric acid (Analytical Reagent Grade 96%) to 150 mL of water in a 250-mL beaker while constantly stirring. Allow the solution to cool to room temperature before using.

PROCEDURE

A. Standardization of Potassium Permanganate (0.1N)

1. Weigh (to the nearest 0.1 mg) about 0.3 g of dry sodium oxalate into a 500-mL Erlenmeyer flask.
2. Add 200 mL of water, 50 mL of H_2SO_4 (1:3), and a few glass beads.
3. Heat the solution to boiling on a hot plate.
4. Remove the flask from heat and add the potassium permanganate solution from a 50-mL Class-A burette until the first appearance of a faint pink color that persists for 30 seconds. Do not let the temperature of the solution in the flask fall below 70°C before the endpoint is reached.

$$\text{Normality of } \text{KMnO}_4 = \frac{(\text{Weight } \text{Na}_2\text{C}_2\text{O}_4)(2)(1000)}{((\text{mL } \text{KMnO}_4)(134))}$$

B. Determination of Hydrogen Peroxide

1. Weigh a 50-mL beaker to the nearest 0.1 mg.
2. Using a Mohr pipette, add to the 50-mL beaker:
 - ± 5 g of sample for 20% to 35% H_2O_2
 - ± 3.5 g of sample for 50% H_2O_2
 - ± 2.5 g of sample for 70% H_2O_2
 - a proportionally larger sample for residual H_2O_2 (e.g., about 20 g for 1% H_2O_2).

PREPARATION METHOD

gloves and safety goggles must be worn when handling concentrated peroxide.

3. Immediately reweigh the beaker to the nearest 0.1 mg. Record the gain in weight as W.
4. Transfer the sample to a 500-mL volumetric flask containing about 250 mL of water and 2 mL of H_2SO_4 (1:3). Thoroughly rinse the beaker into the volumetric flask. Dilute to volume with water and mix well.
5. Pipette 20.0 mL of the solution into a 500-mL Erlenmeyer flask containing 15 mL of H_2SO_4 (1:3) and 60 mL of water.
6. Add the standardized potassium permanganate solution from a 50-mL Class-A burette until the first appearance of a faint pink color that persists for 30 seconds. Record the volume delivered as V.

CALCULATION

$$\% \text{H}_2\text{O}_2 \text{ (w/w)} = \frac{(V)(N)(1.701)(25)}{W}$$

where: V = mL of potassium permanganate used in titration
N = normality of potassium permanganate
W = grams of sample weighed into 50-mL beaker
1.701 = weight per milliequivalent of $\text{H}_2\text{O}_2 \times 100$
25. = dilution factor

NOTE: If the proper equipment is available, this titration can be done potentiometrically.

FIRST AID

In case of product splashing into the eyes and face, treat eyes first. Eye contact: Flush eyes immediately with water for at least 15 minutes. Call a physician. Skin contact: Immediately flush skin with water while removing contaminated clothing and shoes. Call a physician if irritation persists. Inhalation: Remove the victim from the contaminated area to fresh air. Call a physician in case of respiratory symptoms. Ingestion: Consult with a physician immediately in all cases.

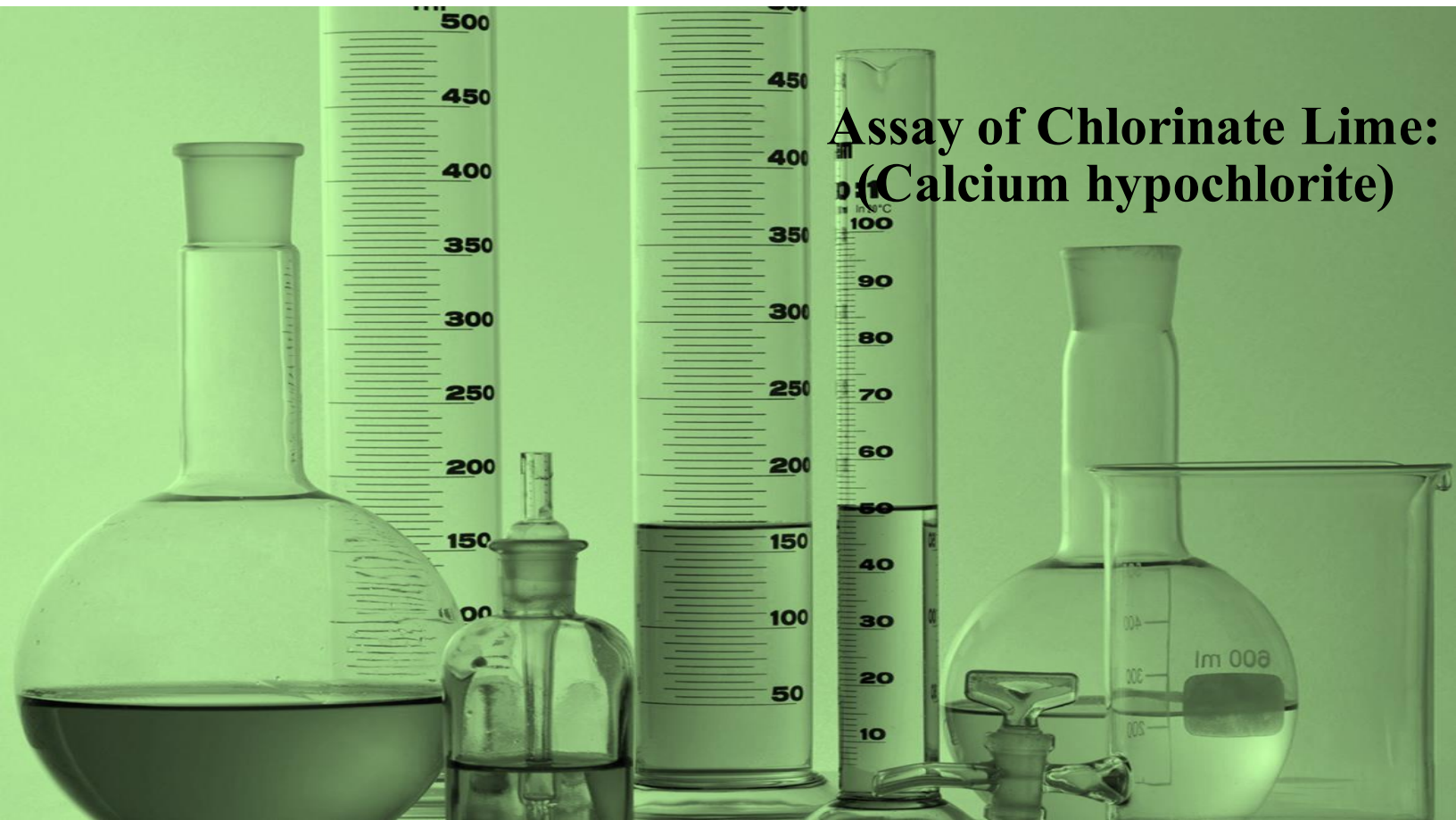
DO NOT

induce vomiting. If victim is conscious, rinse mouth and give fresh water.

DANGER

Hydrogen peroxide solutions are strong oxidizers and corrosive to the eyes, mucous membranes and skin. Consult the SDS for the appropriate Personal Protective Equipment to wear when handling hydrogen peroxide. In case of contact with the eyes, skin or clothing, flush with large amounts of water for 15 minutes. In case of ingestion, sit upright, drink large quantities of water to dilute the stomach contents and seek immediate medical attention. Product in contact with combustible materials may cause fires.

Assay of Chlorinate Lime: (Calcium hypochlorite)



Chlorinated Lime* :

1. It is an inorganic compound with formula $\text{Ca}(\text{OCl})_2$.
2. It is a white solid, although commercial samples appear yellow.
3. It strongly smells of chlorine, owing to its slow decomposition in moist air.

* Citrus plants called "limes"

It is relatively stable as a solid and solution and has [greater](#) available [chlorine](#) than [sodium hypochlorite](#).

5. The pure lime samples have 99.2% [active chlorine](#), but in common industrial purity it has 60-70% .

6. It is the main active ingredient of commercial products called [bleaching powder](#).

7. It is uses:

1. Water treatment : Due to its antimicrobial properties it is used as :

- a. Disinfect drinking water and
- b. Sanitize public swimming pools

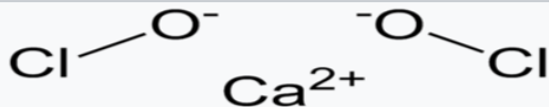
2. Also, In solution, it could be used as a general purpose sanitizer مطهر.

, but due to calcium residue (making the water harder), sodium hypochlorite (bleach) is usually preferred. (Why?)

iii. For removing of earth or military equipment contaminations.

Chemical Structures

1. Calcium hypochlorite



industrial purity



Other names:

2.

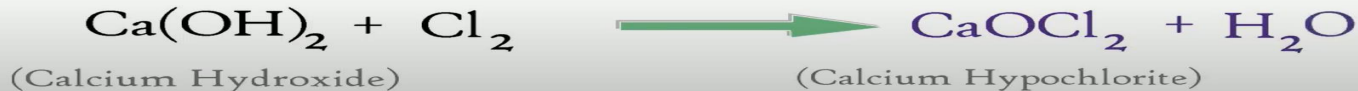
Hypochlorous acid calcium salt, bleaching powder,

3.

4. chloride of lime

Preparation:

Chlorinated lime is prepared by passing chloride gas into calcium hydroxide, until it gets saturated.



Chemical formula

Chemical properties:

1. It is an oxidizing agent
2. It acts as strong base and readily accepts proton (H^+) when dissolve in water.
3. It gives hypochlorite ion on dissociation

as the following equation:

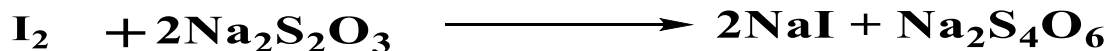
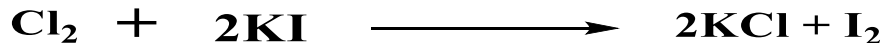
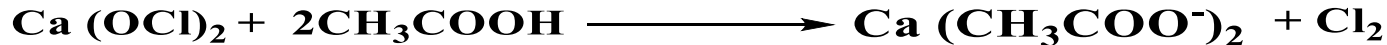


Assay; (Principle):

Redox titration or because it is used the iodine test of the liberated chlorine it is also called **Iodometric titration**

Theory:

1. Chlorinated lime is reacted with acetic acid (or carbon dioxide) to liberate Cl_2 .
2. This chlorine gas is further reacts with potassium iodide (KI) to liberate I_2
3. Iodine is determined by its titration with 0.1 Sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) using Starch as indicator which turns to green as the following equation:



Procedure:

1. Dissolve accurately 4.0 g of chlorinated lime with D.W.
2. Transfer the solution into 1 liter volumetric flask and shake thoroughly.
3. Mix 100 ml of this suspension with a solution of 3 % potassium iodide, acidify with 5ml acetic acid.
4. and titrate the liberated iodine with 0.1N sodium thiosulphate.

Step 1
Liberation
of I_2

Step 2
Assay of
 $Ca(OCl)_2$

Calculation(method A): Equivalent factor:

Each ml. of 0.1 N $\text{Na}_2\text{S}_2\text{O}_3 \equiv$ 0.003545 gm of Chlorine



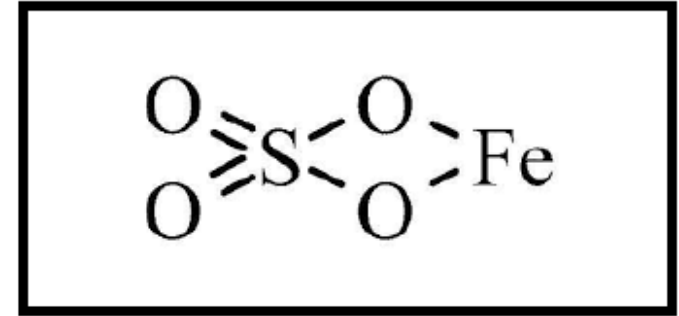
Experiment No.3 Preparation and Assay of Ferrous sulfate

FeSO_4

Aim:

Determine the percentage purity of a given sample of ferrous sulphate (FeSO_4).

Chemical structure of Ferrous Sulphate:



Introduction:

i. Ferrous sulfate is an iron compound, which normally gets by body from the foods, and then iron becomes a part of hemoglobin and myoglobin.

ii. Hemoglobin carries oxygen through blood to tissues and organs.

iii. Myoglobin helps the muscle cells store oxygen.

Ferrous sulphate , It is an official iron product, and oral preparations:

It is the most widely used oral iron preparation and is considered as **the drug of choice** for treating uncomplicated iron deficiency anemia.



Forms:

Oral tablet, oral elixir, oral liquid, oral solution, oral syrup , drops

Overdose:

Accidental overdose of drugs that have iron in them is a leading cause of deadly poisoning in children younger than 6 years of age. **Keep away from children.**

- **Ferrous sulphate**, is transparent green crystals , odourless and has Metallic test, soluble in water and insoluble in alcohol.

Preparation of Ferrous sulphate:

It is obtained by:

1. Adding Metallic iron in con. sulphuric acid.
2. The resulting sol. is i)Stirring ii)Filtered iii) Evaporated and iv) Cooled.
3. The green coloured crystals of ferrous sulfate are obtained
4. The chemical equation represents this reaction is :

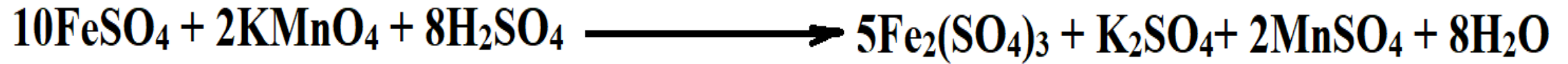


Assay chemical theory: In acidic solution, KMnO_4 rapidly and quantitatively oxidizes iron (II) (Ferrous) to Iron (III) (Ferric), while itself being reduced to manganese (II). The half reactions for the process are:



Oxidation, the loss of electron(s)

The complete molecular equation should be:



Aim of the experiment:

To find out the weight and W% of an unknown sample (solution) of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$

1. Transfer 10ml. from the unknown sol. to a conical flask
2. Add 5 ml. of 6N sulfuric acid, H_2SO_4
3. Titrate with the 0.1 N KMnO_4 sol.
4. Calculate the weight %

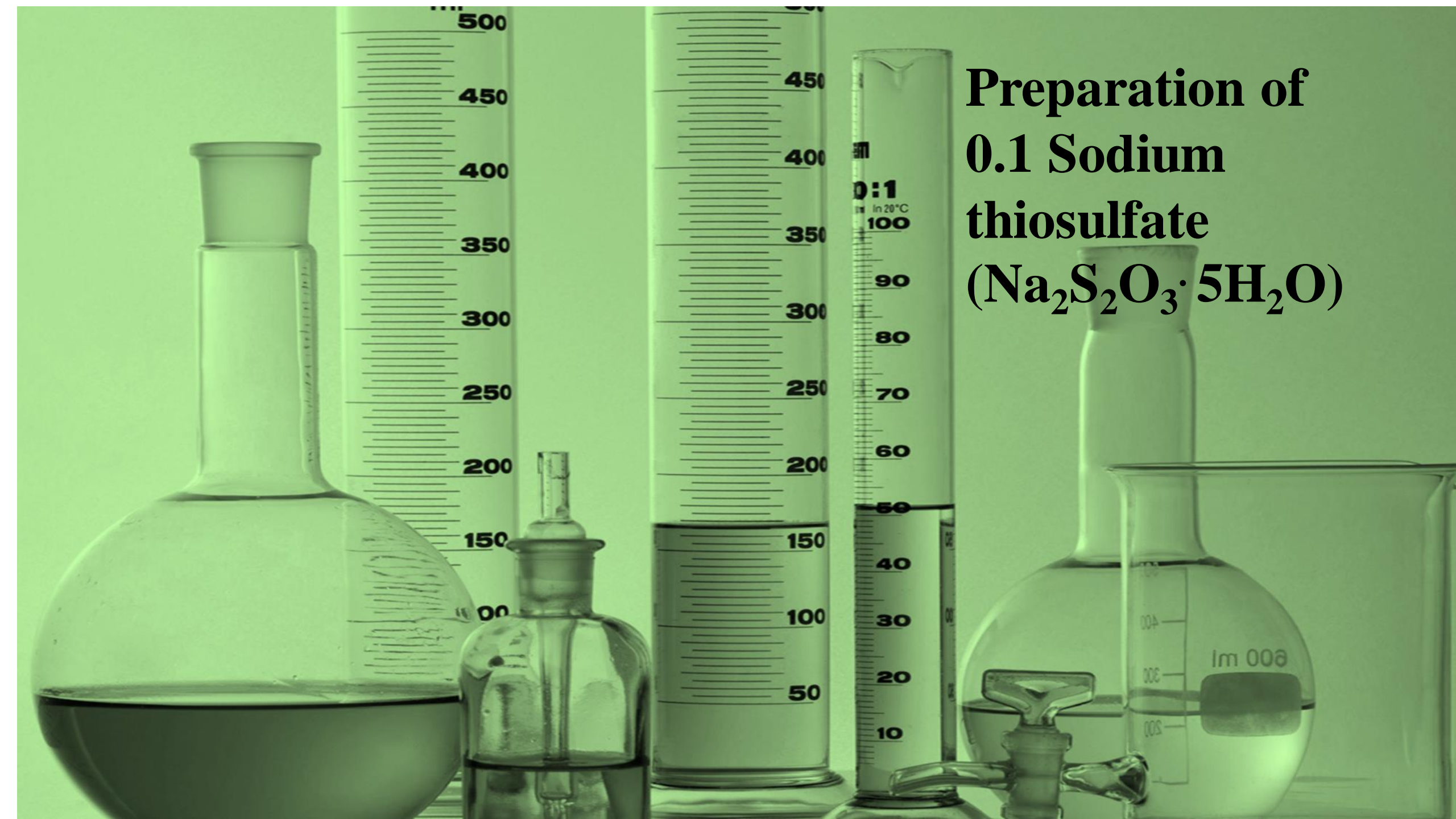
Calculations:

MW of anhydrous = 151.9 g/mol = eq weight (why?)

Each ml. of 0.1 N $\text{KMnO}_4 \equiv 0.01519$ gm of FeSO_4

-- ml. of 0.1 N $\text{KMnO}_4 \equiv 0.0278^*$ ----- g of FeSO_4

$\text{W}\% = \text{---}/10 * 100 = \text{-----}\%$

The image shows a collection of laboratory glassware against a light blue background. On the left is a large round-bottom flask partially filled with a dark liquid. Behind it are two graduated cylinders; the one on the left has a scale from 0 to 500 mL, and the one on the right has a scale from 0 to 500 mL with a 100 mL mark labeled '100'. In the foreground, there is a small glass bottle with a stopper, another round-bottom flask with a stopper, and a beaker. The text 'Preparation of 0.1 Sodium thiosulfate (Na2S2O3·5H2O)' is overlaid on the right side of the image.

Preparation of 0.1 Sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$)

Aim:

To prepare 0.1N sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$)

1. Sodium thiosulfate (or sulphate) is an inorganic compound with different number of water of crystallization $\text{Na}_2\text{S}_2\text{O}_3 \cdot x\text{H}_2\text{O}$.
2. Typically it is available as the white or colorless pentahydrate, $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$.
3. The solid is an efflorescent* crystalline substance that dissolves well in water.

* loses water readily التزهر

Uses:

1. It is used in gold miningتعدين. 2. Water treatment 3. Analytical chemistry

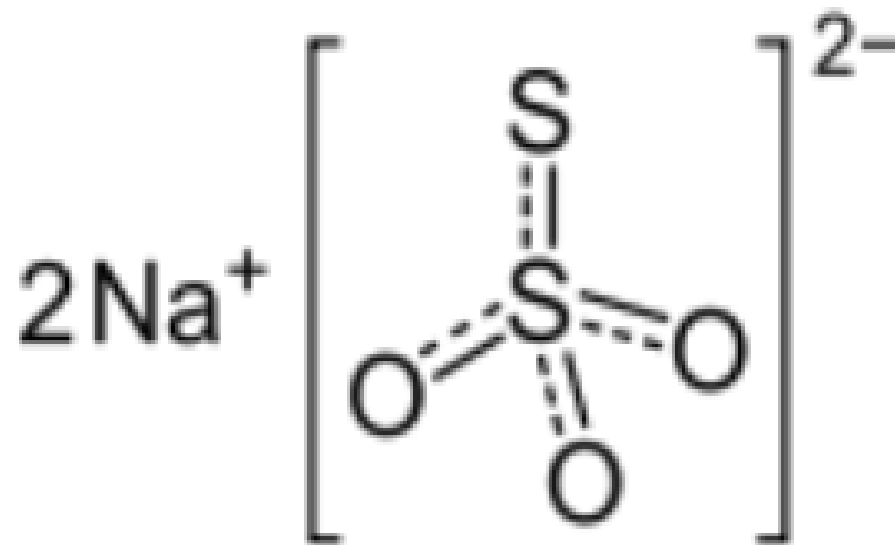
4. The development of silver-based photographic film and prints.

5. In medicine. This include treatment of :

- i. Cyanide poisoning and pityriasis
- ii. Some side effects of hemodialysis غسيل الكلي
- iii. Chemotherapy
- iv. Ringworm and tinea versicolor.



Chemical structure of Sodium thiosulfate of chemical formula ($\text{Na}_2\text{S}_2\text{O}_3$) :



Calculation of molecular weight of
sodium thiosulfate pentahydrate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$)



$$\text{Na} = 23 \Rightarrow (23 * 2 = 46)$$

$$\text{S} = 32 \Rightarrow (32 * 2 = 64)$$

$$\text{O} = 16 \Rightarrow (16 * 3 = 48)$$

$$5\text{H}_2\text{O} = 18 \Rightarrow (18 * 5 = 90)$$

$$248\text{gm}$$

Preparation of 0.1 Starch solution (Indicator):

1. Weight 5gm of starch powder and add it to:
2. Boiled 50 ml. of DW.
3. Filterate the solution.

Preparation of expected 0.1 N Sodium thiosulfate pentahydrate

Dissolve 24.8g of sodium thiosulphate pentahydrate($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$) in 800 ml of freshly boiled and cooled water and mix thoroughly by shaking for approximately 15 minutes. Make up the volume to 1000 ml.



Experiment No.1 Preparation and Standardization of Potassium permanganate

Potassium permanganate Medical uses

It is used for a number of skin conditions, this includes :

- i. Fungal infections of the foot ii. Impetigo القوباء
- iii. Pemphigus
- iv. Superficial wounds v. Dermatitis, and vi. Tropical ulcers.

It is on the World Health Organization's List of Essential Medicines



Impetigo



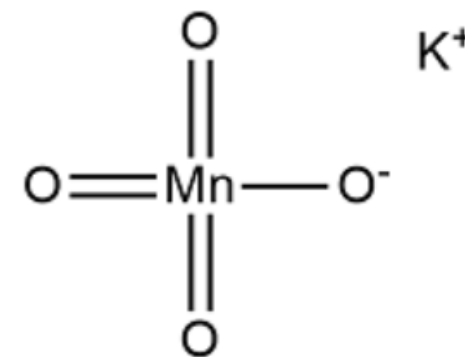
Pemphigus



Tropical ulcers

Potassium permanganate:

1. It is an oxidizing agent.
2. It can retain its concentration over a long period under proper storage conditions.
3. The reactions of permanganate in solution are rapid.
4. It also acts as self-indicator as its slight excess gives a distinct pink color to the solution.
5. It's not a primary standard.
6. Its chemical structure -----



Potassium permanganate chemical structure

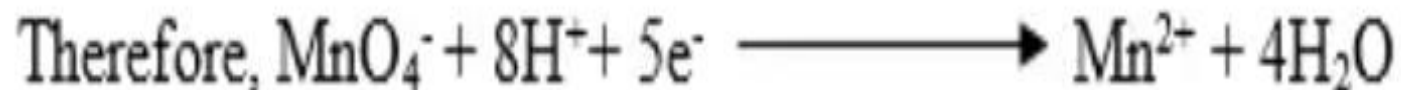
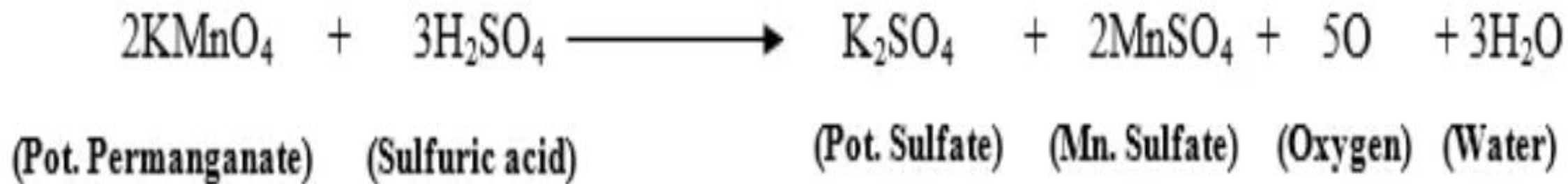
7. Its antibacterial action is dependent on the process of proteins oxidation of bacteria or tissues .

It leaves a stain on skin or tissues.

8. Since it acts by destructive oxidation process on all organic matter, its use is restricted for external purposes only.

9. It acts as an antidote in barbiturates, chloral hydrate, and alkaloidal poisoning. A solution of 1:5000 of permanganate, when used as a gastric wash, oxidizes poison and prevents their absorption.

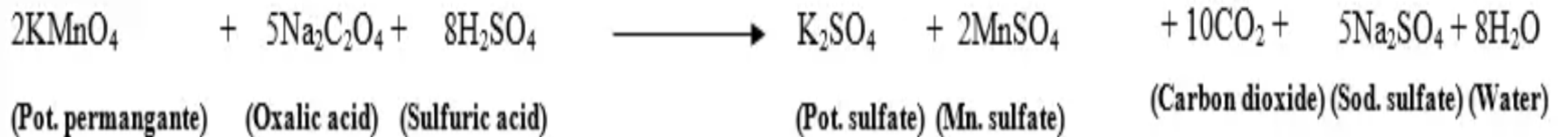
10. The ability of potassium permanganate solution to oxidize is due to the conversion of MnO_4^- [$\text{MnO}_4 = -1$, $\text{Mn} - 8 = -1$; $\text{Mn} = +7$] ion to Mn^{2+} in acidic solution, i.e. **No. of electrons transferred = 5**
11. The MnO_4^- ion is reduced in **acidic medium in** accordance with the following reactions.



Equivalent Weight = Molecular Weight / No. of electrons transferred = $158/5 = 31.6 \approx 32$

- So, 32 gm of KMnO_4 when dissolved in 1000 ml of water = 1N KMnO_4
- Therefore 3.2 gm of KMnO_4 when dissolved in 1000ml of water = 0.1N KMnO_4

As potassium permanganate is not a primary standard it can be standardized by using **sodium oxalate or oxalic acid**. Oxalate is preferred over oxalic acid as available in a higher standard of purity (99.95%). It's available in the anhydrous form.





Equivalent weight of $\text{Na}_2\text{C}_2\text{O}_4 = \text{Molecular weight} / 2 = 134.01 / 2 = 67.01$

67.01 gm of Sodium oxalate when dissolved in 1000ml of water = 1N oxalic acid

6.701 gm of Sodium oxalate when dissolved in 1000ml of water = 0.1N oxalic acid

Apparatus: Watch glass, Beaker – 250 ml, Glass wool, Funnels

Cleaned and calibrated volumetric flask – 1000 ml, Pipette, Burette

Conical flask, Electronic balance

Chemicals: Potassium permanganate AR grade, Oxalic acid AR grade, Conc. sulfuric acid

PROCEDURE

Preparation of Potassium permanganate solution

Weigh 3.2 g of KMnO_4 accurately on a watch glass. Transfer the contents to a 250 ml beaker containing cold distilled water and stirred thoroughly, breaking up the crystals with a glass rod to effect solution. Decant the solution through a small plug of glass wool supported in a funnel into a 1000 ml volumetric flask.

Add more distilled water to the beaker and repeat the above process till all the potassium permanganate gets dissolved. Make up the volume and shake well so as to affect uniform mixing. Keep the flask with stopper for 24h and then filter through asbestos.

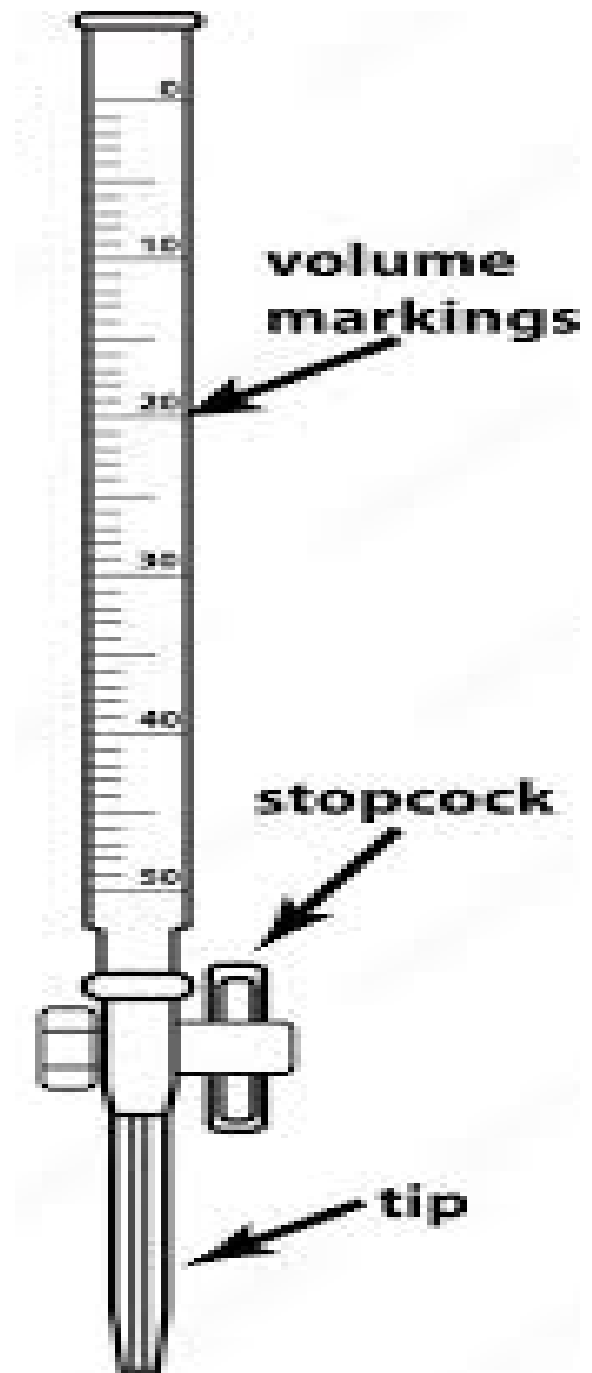
The organic matter present in the distilled water is decomposed by potassium permanganate resulting in the formation of MnO_2 during 24 h. Keep the filtered solution in a dark glass bottle. Standardize the solution 24 h after its preparation.

Standardization of potassium permanganate solution

Dry AR grade* sodium oxalate at 105-110 °C for 2 h. Allow it to cool in a covered vessel in a desiccator. Weigh 6.7 g of pure sodium oxalate accurately (Why ? Mwt = 134) and transfer into a 1000 ml volumetric flask containing 500 ml of water. Make up the volume with distilled water. Draw 20 ml of this solution into a conical flask and add 5 ml of conc. H_2SO_4 along the side of the flask (Why?).

If the reagent also meets the requirements of the American Chemical Society Committee on Analytical Reagent, it will be denoted as an AR (ACS) reagent.

Clean the burette with distilled water, then empty the water, then **rinse** the burette with potassium permanganate solution, and then empty the burette.

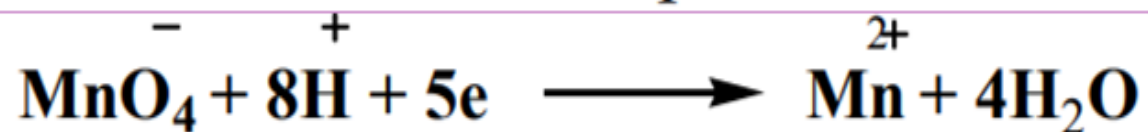


Procedure:

- i. Warm the contents up to 70°C (why?).
- ii. Titrate against potassium permanganate solution from the burette until a faint pink color persists for 30 sec upon shaking the flask. Repeat the process until 3 concordant readings will be obtained.

Sl. No.	Volume of Sodium Oxalate solution taken (ml)	Volume of KMnO_4 consumed(ml)

Sufficient sulphuric acid is added during standardization because potassium permanganate oxidation ability is better in acidic media and to keep hydrogen ion concentration constant thorough out the standardization process.



Keeping the temperature near to 70 °C throughout the standardization process is important because the oxidation of sodium oxalate is rapid enough at such temperature.

Formation of a brown colour during the titration is caused by insufficient acid, applying too high temperature, or the use of a dirty flask.

$$N_1V_1=N_2V_2$$

Where N_1 = Normality of $(\text{COONa})_2$

N_2 = Normality of KMnO_4

V_1 = Volume of $(\text{COONa})_2$

V_2 = Volume of KMnO_4

CONCLUSION

From the above experiment it is evident that potassium permanganate can be effectively standardized by using oxalic acid. After performing the calculations, strength of the prepared potassium permanganate solution was found to be-----N