NATURAL PRODUCTS

BIOC-342

LAB MANUAL

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Contents

Testing Leaves for Starch

Demonstrating That Light is Dissipated as Chlorophyll a Fluoresces

Introduction:

Leaves come in different shapes, sizes, colors, thicknesses and different types. The plant where the leaf is usually obtained is a geranium.

Photosynthesis happens in the mesophyll cells of leaves. The mesophyll cells contain tiny bodies called chloroplasts, which contain chlorophyll, which is used to catch the light energy needed in photosynthesis. Glucose can be converted into starch and stored. Both starch and sucrose can be turned back into glucose and used in respiration. Most plants store starch. They can turn starch back into glucose when they need it for respiration.

Exp. 1/ Testing Leaves for Starch

Aim of the Experiment:

The aim of the experiment is to see if a green leaf that had been left in the light for 48 hours would have the same amount of starch as a leaf that had no light for 48 hours.

Hypothesis:

The prediction is that both the green leaf that has been in the light will have starch present. The one left in the dark will not have starch present. The leaf will turn brown/black if starch is present.

Equipment:

- Beaker
- Test tube
- Water
- Boiling water
- Ethanol
- Gauze
- White tile
- Iodine solution
- Bunsen burner
- Safety Goggles
- Heatproof mat
- Leaves
    - one that has been in light for 48 hours
    - one that has had no light for 48 hours

Safety procedures:

- Wear goggles
- Keep ethanol away from Bunsen burner; ethanol is flammable

- Tie Hair back

Method:

1. Set up the equipment.
2. Light the bunsen and boil the water. When the water had boiled add the first geranium leaf (the one that had been the light for 48 hours).
3. Wait one minute for the leaf to boil (this is to get rid of the waterproof layer and break the open cells and make it soft).
4. Turn off the Bunsen burner (for safety reasons, we are going to use ethanol), and take out the leaf.
5. Put the leaf in a boiling tube and cover with ethanol.
6. Put the tube of ethanol plus leaf into the beaker of hot water. Ethanol boiles at 80° so it should come to boil even though the bunsen is off.
7. Dip it back into the hot water so it can get the ethanol off.
8. Spread the leaf out on a tile. Add about five drops of iodine on to the leaf and observe. After about two minutes the iodine had soaked in.
9. Repeat using a leaf that had been in the dark for 48 hours.

References:

www.wacklepedia.com

Exp.2 / Demonstrating That Light is Dissipated as Chlorophyll a Fluoresces:

Introduction:

When a molecule of chlorophyll a absorbs a photon of light, an electron is excited from the ground state to a higher energy state. In an acetone extract, the light energy is not used in the photochemical processes of photosynthesis. Therefore, electrons are returned to the ground state and results in energy being re-emitted as fluorescent light. This light can be seen in the red region of the spectrum and is generally at higher wavelengths than the absorption maximum of chlorophyll a.

Aim of the Experiment:

-To demonstrate that light is dissipated as chlorophyll a fluorescences. This is depicted as a red ring on the very top of the test tube.

-Fluorescence represents a means by which a molecule dissipates excess energy. It represents excess energy in a molecule releasing light.

Materials:

· Acetone

· Spinach

· Test tube with cap/cover

· Flashlight

· Gloves

· Sand

Procedure:

Preparation of spinach:

- The solution should be stored in a tightly capped bottle.
- To prepare a saturated solution of spinach, add 50 ml acetone or nail polish remover in a fairly large test tube to 5 leaves of spinach (as needed) and 1 tablespoon of sand (if available). Mix well and allow the solution to chill in the freezer overnight.
- A dark free liquid on the top of the test tube indicates that the chlorophyll is successfully extracted from the membrane of the chloroplast.

1. Remove test tube of solution from the refrigerator (very carefully).
2. Turn the lights off. Room should be dark to get the full effect!!!
3. While one student is holding the test tube of saturated spinach another should turn the flashlight on directly towards the test tube.

4. Observe. The spinach/acetone solution will have a red ring on the top of the solution, indicating that the molecule of chlorophyll absorbs light and then sends the ray of light seen as the red ring.

Note: Fluorescent light is not used in the process of photosynthesis and therefore to prevent it from accumulating, a photon is emitted (not absorbed).

References:

www.books.nap.edu

Result Sheet

Experiment-2

Isolation of Chloroplast and assay of Chlorophyll

Introduction:

Chlorophyll:

- the most abundant pigment in plants
- the principal light-absorbing pigment in photosynthesis
- from Greek ***chloros*** "yellowish green"
- porphyrine ring similar to heme (of hemoglobin), but magnesium (not iron) central atom
- not water soluble (grass stain)
- forms tight molecular complexes with some carcinogens: aflatoxin-B1, polyaromatic hydrocarbons (tobacco smoke) & heterocyclic amines (cooked meat)
- chlorophylll absorbs red & violet light strongly
- chlorophyll reflects green light (making leaves green)
- chlorophyll in leaves decays in autumn, leaving carotenoid colors
- **chlorophyll a** has a $-CH_3$ side-chain
- **chlorophyll b** has a −CHO side-chain
- plants contain both chlorophyll a and chlorophyll b
- chlorophyll b is missing from cyanobacteria
- (**cyanobacteria** are the toxin-producing pond scum bacteria known as "blue-green algae")
- chlorophyll a absorbs red light more strongly
- chlorophyll b absorbs violet light more strongly

A-Isolation of Chloroplast:

Material:

Spinach leaves

Buffered sucrose

Isolation medium

Reaction medium

Method:

1- Wash the spinach leaves, remove the midribs

2- Weigh 100gm leaves, add 100ml buffered sucrose isolation medium in blender for 2min.

3- Filter the mixture by muslin

4- Centrifuge the filtrate at 1000 r.p.m for 2min in cold centrifuge

5- Take the supernatant and centrifuge for 5min at 6000 r.p.m.

6- Remove the supernatant, wash the sediment with the isolation medium 3ml and centrifuge for 5min at 6000 r.p.m.

7- Discard the supernatant and repeat again under the same conditions

8- Suspend the chloroplast in 20ml ice-cold reaction medium

(Store at -20 ^{0}C if the assay step will carry on the next lab)

B- Assay of chlorophyll content:

(a) Add 1ml of the suspension to 10ml acetone (80% v/v acetone in water)

(b) Shake.

(c) Filter with filter paper into 25ml volumetric flask.

(d) Wash with the acetone to complete the volume to 25ml.

(e) Read the extinction of green solution at 652nm against a solvent blank acetone.

Calculations:

Chlorophyll concentration (mg/ml) = Absorbance X **5.8**

Results Sheet

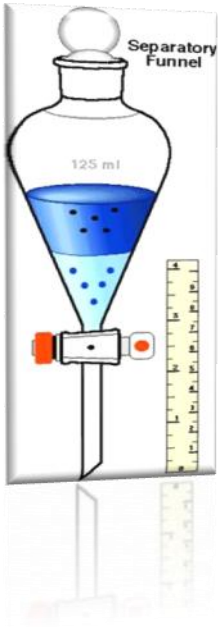
Extraction of Caffeine from Coffee and Tea

Introduction:

- Caffeine is a natural product found in the fruit and bark of certain plants.
- It is well known that caffeine is a stimulant, a diuretic and is addictive. The stimulant action is why a lot of us drink so much tea, coffee and certain soft drinks, but it is also used in some medications to reverse drowsiness.
- Caffeine has been described as the "most abused drug in the US": a single serving of coffee may contain 125mg, tea 75mg, Cocoa 40mg, Coca-Cola 46mg. A regular 4-cups-a-day coffee drinker on withdrawal can experience headaches, insomnia, even nausea!

Extraction Theory:

Solvent extraction is a process of purification involving the use of two solvents or solutions that are immiscible with one another.



- The main use is to employ extraction as a means of isolating chemicals that are present in plant or animal tissue (natural products).

- It is extensively used in organic chemistry as a way purifying reaction mixtures after a laboratory experiment. It is based on the differential solubility of compounds in water and an immiscible organic solvent (typically diethyl ether, chloroform, dichloromethane, ethyl acetate). This allows the desired

organic product to be separated from inorganic or very other polar by-products. This is the basis of an older industrial method for "decaffeinating" coffee.

- A conically shaped piece of glassware called the separatory funnel is the laboratory tool used for most types of extraction. Be careful when using them, they are not cheap!!! The general process involves adding the two immiscible solutions to the separatory and shaking with occasional opening of the stopcock.

(1) The Basic Process of Extraction:

(a) First make sure that the stopcock is secured and CLOSED.

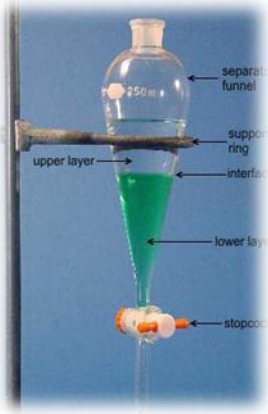
(b) Place a collection flask underneath the funnel.

(c) Use an iron ring to support the separatory funnel.

(d) Two heterogeneous liquids/solutions are added to the separatory funnel.

(e) The mixture of solutions is shaken with occasional venting to relieve any pressure buildup.

(f) The lower layer is drawn off through the stopcock.



(2) The Difference between Extraction and Washing:

- Extraction is the removal of a desired compound from one phase (usually the aqueous phase) into another (usually the organic phase).

- Washing involves the removal of impurities by shaking the solution with an aqueous solvent that will dissolve only the impurities and leave the desired compound behind in the organic phase.

(3) Identifying the Aqueous Phase and the Organic Phase:

Keeping track of which layer is the organic layer and which is the aqueous layer can be frustrating. However, if you remember one simple rule, then you troubles will be few. The rule here is that the more dense liquid will be on the bottom. If you are in doubt, there are two things you should do:

1. Remove a drop of one layer and place it in a small test tube. Add a drop of water. If it dissolves, the layer is aqueous. If it doesn't, then the layer is organic.

2. Save ALL of your discarded layers until the end of the experiment.

(4) How much solvent/ how many extractions?

The general rule is to perform multiple extractions with smaller amounts of extracting solvent rather than one extraction with a large amount of solvent. Remember, we are dealing with the partitioning of a solute equally between two phases. By performing the extraction a number of times, we can effectively pull out more material than if doing it once with a large amount.

Aim of the Experiment:

-In this experiment you will extract caffeine from tea leaves with dichloromethane (caffeine is about 9 times more soluble in dichloromethane than in water) using a separatory funnel. Be careful not to shake too vigorously or you will get an emulsion that is difficult to separate. You will also earn how to dry an organic solution (removing trace quantities of water) using a chemical drying agent.

Materials:

1- Tea, coffee.
2- Dichloromethane (DCM).
3- 6M NaOH
4- Anhydrous $MgSo_4$
5- DW, Ice

NOTE: Wear gloves when handling DCM. It also has harmful vapours. Avoid skin contact and avoid breathing the vapor. Keep in the fume-hood whenever possible.

Procedure:

1- Weigh tea from 20 tea bags, 3 spoons Arabic coffee.
2- Add 100ml boiling DW and stir for 7 min.
3- Filter by filter paper- cool the filtrate in an ice bath.

Extraction:

1- Pour the cold tea filtrate into a separatory funnel.
2- Slowly add 20ml DCM and gently stir the 2 layers for about 5 min.
3- Replace the SF into the stand, remove the stopper and allow separating for about 5min.

4- Collect the organic phase into conical flask (Don't allow any of the darker material to escape through the stopcock).
5- Repeat the extraction twice more with fresh 20ml of DCM.
6- Add these 2 DCM layers to the first (If an emulsion remains in the DCM, filter through a Buchner funnel).
7- Return the organic phase to the SF and extract with 20ml 6M NaOH (twice) and once with 20ml DW.
8- Collect the DCM layer into conical flask.
9- Dry the solution by adding 1 teaspoonful of anhydrous $MgSO_4$, and then allow standing 5min.
10- Filter
11- Weigh empty flask.
12- Transfer the filtrate into flask and evaporate DCM on the water bath.
13- Weigh the flask containing the crude product.

Calculation:

Determine the weight of pure caffeine in each of your sample and calculate the weight % of caffeine.

Wt (g) of caffeine →→→→→→→→Wt (g) of sample

Microscopic examination:

- Caffeine + drops of mercuric chloride $HgCl_2$ ----------→ examine under microscope------→ needle-shape

References:

L. F. Fieser and K. L. Williamson, Organic Experiments, 6/e, D. C. Heath and Co., Lexington, MA, 1987.
2. D. L. Pavia, G. M. Lampman, G. S. Kriz, R. G. Engel, Introduction to Organic Laboratory Techniques, A Microscale Approach, Saunders College Publications, San Francisco, 1990.

Results Sheet

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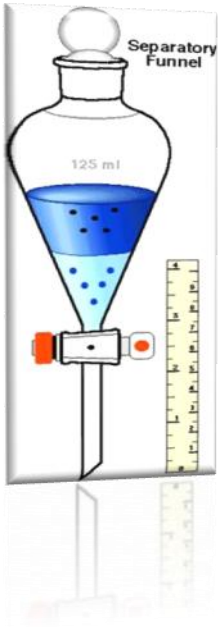

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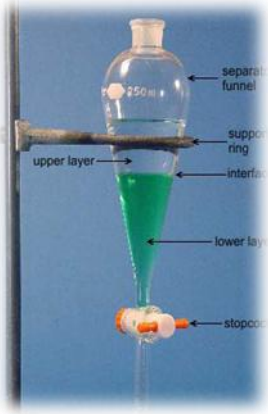

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Results Sheet

Extraction of Nicotine from Cigarettes

Introduction:

- Nicotine is an alkaloid found in the nightshade family of plants (*Solanaceae*), predominantly in tobacco, and in lower quantities in tomato, eggplant, and in green pepper.
- Nicotine alkaloids are also found in the leaves of the coca plant.
- Nicotine was first isolated from the tobacco plant in 1828 by German chemists, Posselt & Reimann.

- Nicotine constituents 0.3 to 5% of the tobacco plant by dry weight, with biosynthesis taking place in the roots, and accumulate in the leaves.
- It is a potent neurotoxin and is included in many insecticides.
- In lower concentrations, the substance acts as a stimulant and is one of the main factors responsible for the dependence-forming properties of tobacco smoking.

Chemistry:

- Nicotine is hygroscopic, oily liquid that is miscible with water in its base form.

- As a nitrogenous base, nicotine forms salts with acids that are usually solid and water soluble.

- Nicotine easily penetrates the skin.

Toxicology:

- The LD_{50} of nicotine is 50mg/kg for rats and 3mg/kg for mice. 40-60mg/kg can be lethal dosage for adult human beings. This makes it an extremely deadly poison.

- It is more toxic than many other alkaloids such as cocaine which has a lethal dose of 1000 mg.

Aim of the experiment:

-In this experiment you will extract nicotine from cigarettes and muassil with ether and precipitate it as nicotine di-picrate salt.

Principle:

- The extraction depends on isolation of base by dissolving the cigarettes in NaOH. Then extract nicotine from the filtrate by ether. After evaporation of ether you will get nicotine oil.

- The factories of cigarettes remove large quantities of nicotine from cigarette leaves because of high toxicity. This is why the produced oil is very little. To get nicotine crystals, saturated solution of picric acid is added to form nicotine di picrate yellow crystals.

Materials:

1- Cigarettes, cigar, and muassil.

2- Ether.

3- NaOH solution (5%)

4- Saturated picric acid solution in methanol.

5- Beaker 250ml

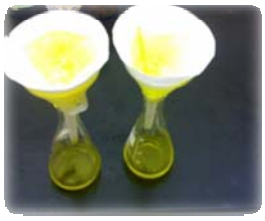
6- Separating funnel.

7- Conical flasks.

8- Buchner glass wool.

Procedure:

1- Weigh 10 g of cigarettes leaves in beaker.
2- Add 100ml NaOH solution and stir very well for 15 min.
3- Filter in Buchner using glass wool and press the cigarettes very well by using other beaker.
4- Transfer the cigarettes again to beaker.
5- Add 30ml DW and stir and filter again.
6- Collect the filtrate together. (If there is any impurities re-filter).
7- Transfer the filtrate to the SF and extract by 25ml ether.
8- Repeat the extraction 3times.
9- Gather the 4 filtrates in conical flask.
10- Dry by using 1teaspoon anhydrous potassium carbonate.
11- Filter.
12- Evaporate ether on water bath. (Avoid extra heat because nicotine is hydrolyzed by extreme heating).
13- After evaporation of ether add 4ml methanol to dissolve the resulted oil.
14- Add 10ml saturated picric acid solution.
15- Cool in an ice bath to precipitate the nicotine di picrate crystals.
16- Filter; allow drying and weighing the product.



Microscopic examination:

- Nicotine + mercuric chloride $HgCl_2$ ----------→ examine under microscope------→ flowery-shape

References:

- Pavia, D. L., Lampman, G. M. and Kriz, G. S. Jr., Introduction to organic laboratory technique, W. B. Saunders Co., Philadelphia, 1976, p. 50-54.

Results Sheet

Thin Layer Chromatography Characterization of Flavonoids

Introduction:

- Flavonoid is a class of plant secondary metabolites based around phenylbenzopyrone structure.
- Flavonoids are most commonly known for their antioxidant activity.
- Flavonoids are also commonly referred to as bioflavonoids because all Flavonoids are biological in origin.
- They have been referred to as nature's biological response modifiers because of strong experimental evidence of their ability to modify the body's reaction to allergens, viruses and carcinogens.
- They show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity. In addition, flavonoids act as powerful antioxidants, protecting against oxidative and free radical damage.
- The beneficial effects of fruits, vegetables and tea have been attributed to flavonoid compounds rather than to known nutrients and vitamins.

- They are widely distributed in plants producing yellow or red/blue pigmentation in flowers and protection from attack by microbes and insects.

Important Dietary Sources:

- All citrus fruits, berries, onions, green tea and dark chocolate are good sources of flavonoids.

- The citrus bioflavonoids include hesperidine, quercetin, rutin and tangeritin.

Classification:

- Over 5000 naturally occurring flavonoids have been characterized from various plants.

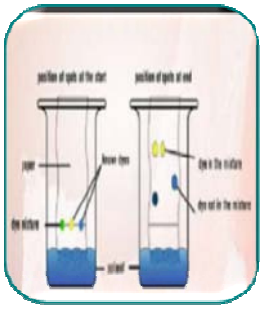
- They have been classified according to their chemical structure and are usually divided into 6 subgroups:

- Flavones
- Flavonols
- Flavanones
- Flavan-3-ols
- Isoflavones
- Anthocyanidins

Rutin (Ruta herb):

- It is a citrus flavonoid glycoside (a sugar of quercetin) found in some plants.
- It combines with cations, supplying nutrients from the soil to the cells in plants.
- In humans, it attaches to the iron ion Fe^{2+}, preventing it from binding to hydrogen peroxide and creating a highly reactive free radical that may damage cells.
- It is an antioxidant and therefore plays an important role in inhibiting some cancer.

Principle of thin layer chromatography:



- TLC is a simple, quick, and inexpensive procedure that indicates how many components are in a mixture.

- A TLC plate is a sheet of glass, metal, or plastic which is coated with a thin layer of a solid adsorbent (usually silica or alumina). A small amount of the mixture to be analyzed is spotted near the bottom of this plate. The TLC plate is then placed in a shallow pool of a solvent in a developing chamber

so that only the very bottom of the plate is in the liquid. This liquid, or the eluent, is the mobile phase, and it slowly rises up the TLC plate by capillary action.

Aim of the experiment:

-In this experiment you will identify flavonoids by TLC in different herbs.

Material:

- Ruta herb, chamomile flowers, caraway.

- Adsorbent: silica gel G.

- Solvent: ethyl acetate: formic acid: water (8:1:1).

Procedure:

1- Preparation of extract:

-Boil 3g of powdered drug + 30ml of methanol for 2min, cool and filter. Use the filtrate for chromatography applying.

2- Reference substance:

- Dissolve 2.5mg of rutin in 10ml of methanol.

3- Development:

- Develop the spotted plate at room temperature to a distance of 12cm.

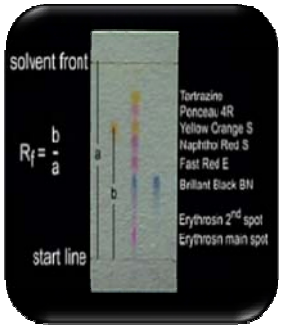
- Mark the front and allow the solvent to evaporate off at room temperature.

4- Detection:

- Spray the plate with the reagent consisting of (15ml 3% boric acid solution and 5ml 10% oxalic acid) heat the plate and examine in UV light.

5- Evaluation of the chromatogram:

- Flavonoids treated with boric and oxalic acids give compounds which after heating fluorescence yellowish green.(see the figure)



6- Results:

- Determine R_f values of reference flavonoids and flavonoids contained in the tested extracts.

References:

- www.wikipedia.org

- El-Olemy, M. Al-Muhtadi, F. Afifi, A. Experimental Phytochemistry. A Laboratory Manual. Riyadh: College of Pharmacy, King Saud University; 1994.

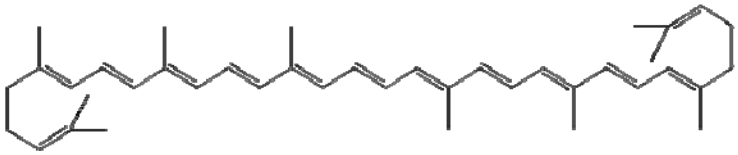
Results Sheet

Isolation of Lycopene and β-Carotene from Tomato

Introduction:

- Lycopene ($C_{40}H_{56}$) is a bright red crotenoid pigment that found in tomato and other red fruits.

- Lycopene is a terpene assembled from 8 isoprene units.



- It is not water soluble and stains any porous material, including most plastics.

Dietary Sources:

Fruits and vegetables that are high in lycopene include tomatoes, watermelon, pink grapefruit, pink guava, papaya, and rosehip.

- Unlike other fruits and vegetables, where nutritional content such as vitamin C is diminished upon cooking, processing of tomatoes increase the concentration of bioavailable lycopene. Lycopene in tomato paste is four times more available than in fresh tomatoes. The processed tomato products such as pasteurized tomato juice, soup, sauce and ketchup contain the highest concentrations of bioavailable lycopene. This is because lycopene is so insoluble in water and is so tightly bound to vegetable fiber; therefore its bioavailability is increased by food processing.

Nutritional benefits:

Lycopene is the most powerful quencher of singlet oxygen. Singlet oxygen from ultraviolet is a primary cause of skin aging.

- There is evidence that frequent intake of such product is associated with reduced risk of cardiovascular disease, cancer (especially prostate cancer), diabetes, osteoporosis, and even male fertility.

Extraction of Lycopene:

- Lycopene can be extracted by reflux condensation.

- The term reflux is very widely used in distillation columns. The laboratory reflux apparatus add energy to chemical reaction.

- A liquid reaction mixture is placed in a vessel open only at the top.

- The vessel is connected to Liebig condenser, such that any vapors given off are cooled back to liquid and fall back into the reaction vessel.

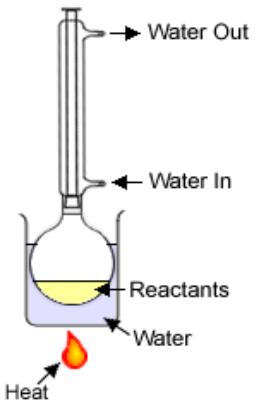
- The vessel is then heated vigorously for the course of reaction.

- The advantage of this technique is that it can be left for a long period of time without the need to add more solvent or fear of the reaction vessel boiling dry as any vapor is immediately condensed in the condenser.

- In addition, as a given solvent will always boil at a certain temperature, the reaction will proceed at the same temperature.

- This diagram shows a reflux apparatus for adding energy to chemical reactions.

- Determine each part of this apparatus??



Aim of the experiment:

-In this experiment you will isolate lycopene by reflux condensation.

Material:

- Tomato paste (0.12g/Kg).

- Ethanol (95%).

- Dichloromethane.

- Anhydrous sodium sulphate (Na_2SO_4).

- Saturated NaCl solution.

- Condenser.

- Buchner filter.

Procedure:

1. Weigh 5g tomato paste in a flask

2. Add 10ml ethanol and heat for 5 minutes.

3. Filter with filter paper and press to take off all the filtrate

4. Keep the filtrate in conical flask.

5. Put the crude in round-bottom bottle and add 10ml DCM, start condensation

6. Boil the solution for 4min. then separate the supernatant and add it to the first filtrate.

7. Repeat this step 3 times.

8. Collect all the filtrate in separatory funnel

9. Add 10ml saturated NaCl solution, shake gently and allow separating into 2 layers.

10. Collect the lower layer.

11. Add 1 teaspoon anhydrous Na_2SO_4 and allow to stand for 5 minutes.

12. Filter with filter paper.
13. Keep the filtrate in dark bottle away from light otherwise the color of lycopene will disappear.

References:

- www.wikipedia.com

- I.D.L. Pavia, G. M. Lampman and G.S. Kriz, JR. Introduction to Organic Laboratory Techniques, W.B. Saunders Company, 1976.

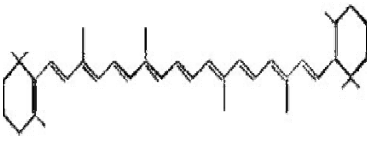
Results Sheet

Identification of Plant Pigments (Carotenoids) by Thin Layer Chromatography

Introduction:

- Carotenoids are a class of natural fat-soluble pigments found principally in plants, algae, and photosynthetic bacteria, where they play a critical role in the photosynthetic process. They also occur in some non-photosynthetic bacteria, yeasts, and molds, where they may carry out a protective function against damage by light and oxygen. Although animals appear to be incapable of synthesizing carotenoids, many animals incorporate carotenoids from their diet. Within animals, carotenoids serve as antioxidants, and can be a source for vitamin A activity (Ong and Tee 1992; Britton *et al.* 1995).

- Carotenoids are responsible for many of the red, orange, and yellow colors of plant leaves, fruits, and flowers, as well as the colors of some birds, insects, fish, and crustaceans. Some familiar examples of carotenoid coloration are the oranges of carrots and citrus fruits, the reds of peppers and tomatoes, and the pinks of flamingoes and salmon (Pfander 1992). Some 600 different carotenoids are known to occur naturally (Ong and Tee 1992), and new carotenoids continue to be identified (Mercadante 1999).



Beta-carotene

Aim of the experiment:

-In this experiment you will extract plant pigments and then identify these pigments by chromatography .

Material:

- Petroleum ether
- Acetone
- NaCl (10%)
- $CaCO_3$
- Anhydrous Na_2SO_4
- Fresh leaves

- TLC chamber 22 × 22 × 10
- TLC silica gel plate
- Mortar & pestle
- Separating funnel 100 mL
- Measuring cylinder 100 mL
- 5 measuring cylinder 25 mL
- Erlenmeyer flask 100 mL
- Round bottom flask 100 mL

Procedure:

Developing solvent (mobile phase):

100 mL of petroleum ether, 11 mL of acetone and 5 drops of dist. water

Preparation of the TLC chamber:

The developing solvent is placed into a TLC chamber. The solvent should completely cover the bottom of the chamber to a depth of approximately 0.5 cm. The chamber is closed and shaken. It is kept covered so that evaporation doesn't change the composition of the developing solvent mixture. After 15 minutes the chamber will be saturated with the solvent vapor.

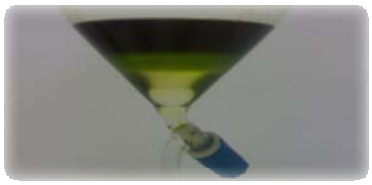
Extraction of the leaf pigments:

1- Using a pestle fresh leaves are grinded in a mortar containing 22 mL of acetone, 3 mL of petrol ether and a spatula tip-full of $CaCO_3$.

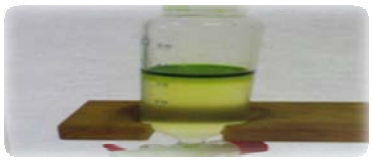
2- The pigment extract is filtered.

3- The filtrate is put into a separating funnel and is mixed with 20 mL of petrol ether and 20 mL of 10% aqueous NaCl solution.

The separating funnel is shaken carefully.



4- When the layers have separated the lower layer is allowed to drain into a beaker. This phase is thrown away.



5- The upper layer is washed 3-4 times with 5 mL of DW.

6- Afterwards the extract is placed in an Erlenmeyer flask and is dried with about 4 spatula tips of Na_2SO_4.

Filter and evaporate the solvent on water-bath until the final volume become of about 3 mL.

Application of the extract to the TLC plate:

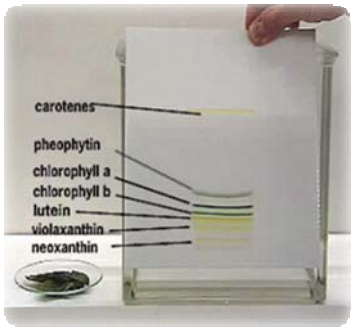
- With a pencil a line is drawn approximately 1,5 cm from the bottom of the plate. The procedure is repeated until the line is very dark green. The transferred extract is allowed to dry thoroughly after each addition. The line is kept as thin and straight as possible.

Experimental procedure:

- The loaded TLC plate is carefully placed in the TLC chamber with the sample line toward the bottom. The plate whose top is leaned against the jar wall should sit on the bottom of the chamber and be in contact with the developing solvent (solvent surface must be below the extract line). The TLC chamber is covered. The TLC plate is allowed to remain undisturbed. When the solvent front has reached three quarters of the length of the plate, the plate is removed from the developing chamber and the position of the solvent front is immediately marked.

Results and discussion:

- As the solvent rises by capillary action up through the TLC plate, the components of the pigment mixture are partitioned between the mobile phase (solvent) and the stationary phase (silica gel) due to their different adsorption and solubility strength. The more strongly a given component is adsorbed to the stationary phase, the less easily it is removed by mobile phase. The more weakly a component is adsorbed the faster it will migrate up the TLC plate. On the other hand, the running distance depends on the solubility of the pigment in the solvent. Since the experiment employs a high non-polar solvent (petroleum ether), the pigments that are least polar (carotenes) will be best solved in the non-polar solvent and will thus have the largest running distance.



R_f	leaf pigments	color
0.95	β-carotenes	golden
0.83	pheophytin	Olive-green
0.65	chlorophyll a	blue green
0.45	chlorophyll b	yellow green
	lutein	yellow
	violaxanthin	yellow
	neoxanthin	yellow
0.71	xanthophyll	Yellow-brown

References:

Britton, G. (1995). Structure and properties of carotenoids in relation to function. F*ASEB J.*, 9:1551-1558.

Britton, G., S. Liaaen-Jensen, and H. Pfander. (1995). Carotenoids today and challenges for the future. In: Britton, G., S. Liaaen-Jensen, and H. Pfander [eds], Carotenoids vol. 1A: Isolation and Analysis. Basel: Birkhنuser.

Mercadante, A. (1999) New carotenoids: recent progress. Invited Lecture 2. Abstracts of the 12th International Carotenoid Symposium, Cairns, Australia, July 1999.

Ong, A.S.H., and E.S. Tee. (1992) Natural sources of carotenoids from plants and oils. *Meth. Enzymol.*, 213: 142-167.

Pfander, H. (1992) Carotenoids: an overview. *Meth. Enzymol.*, 213: 3-13.

Results Sheet

Extraction of Essential Oils from Cinnamon, Clove and Nigella Sativa by Distillation

Introduction:

- Essential oil plants include a broad range of plant species that are buted worldwide.

- Essential oils are concentrated natural plant products which accumulate in specialized structures such as oil cells.

- An essential oil is a hydrophobic liquid, volatile aromatic compound responsible for the aromas commonly associated with many plants.

- Essential oil is also known as volatile oil and ethereal oil. It may also be referred to as "oil of" the raw plant material from which it was extracted, such as *oil of clove*.

- The term essential is intended to indicate that the oil is the fragrant essence of the plant from which it is extracted and not in the more common sense of being indispensable. It is not to be confused with essential fatty acids.

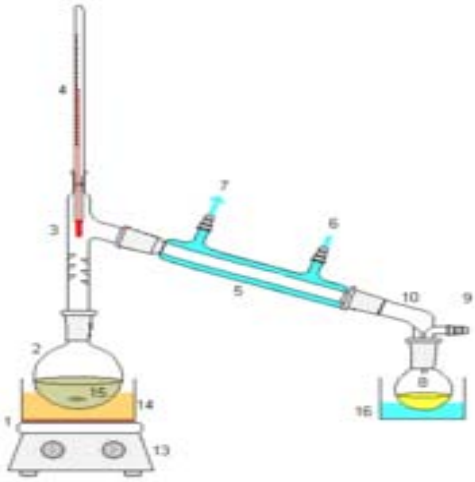
Principle of essential oil extraction:

- The specific extraction method employed is dependent upon the plant material to be distilled and the desired end-product.

- The essential oils which impart the distinctive aromas are complex mixtures of organic constituents, some of which being less stable, may undergo chemical alterations when subjected to high temperatures. In this case, organic solvent extraction is required to ensure no decomposition or changes have occurred which would alter the aroma and fragrance of the end-product.

- Most essential oils are extracted by distillation except in case of flowers.

Principle of Distillation:



- Distillation is a method of separation of substances based on differences in their volatilities.

- The technique of steam distillation allows the separation of volatile components from non-volatile materials without the need for raising the temperature of the distillation above 100 ^{o}C. It provides a method for the isolation of natural products such as essential oils, which tend to be prone to decomposition at elevated temperatures.

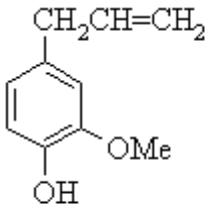
- Steam distillation is a method for distilling compounds which are heat-sensitive. This process involves using bubbling steam through a heated mixture of the raw material. Therefore, some of the target compound will vaporize. The vapor mixture is cooled and condensed, usually yielding a layer of oil and a layer of water.

- Steam distillation of various aromatic herbs can result in two products; an essential oil as well as a watery herbal distillate. The essential oils are often used in perfumery and aromatherapy while the watery distillates have many applications in aromatherapy, food processing and skin care.

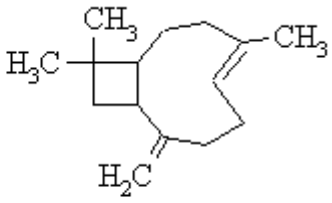
Essential Oils from Spices: Oil of Cloves or Cinnamon:

The chief constituents of the essential oils from cloves and cinnamon are volatile *aromatic* compounds - organic molecules containing one or more benzene rings. Oil of cloves (from

Eugenia caryophyllata) is rich in eugenol (4-allyl-2-methoxyphenol; bp 250 ^{o}C), which is one of a class of compounds known as *phenols*, which are compounds containing a hydroxy-substituted benzene ring. Caryophyllene is also present in relatively small amounts, along with other terpenes.

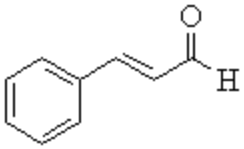


Eugenol



Caryophyllene

The principal component of cinnamon oil (from *Cinnamomum zelyanicum*) is cinnamaldehyde (*trans*-3-phenylpropenal; bp 252 ^{o}C), one of a class of natural products known as *phenylpropanoids*.



Cinnamaldehyde

Aim of the experiment:

-In this experiment you will extract essential oils of Cloves, Cinnamon and Nigella Sativa by steam distillation.

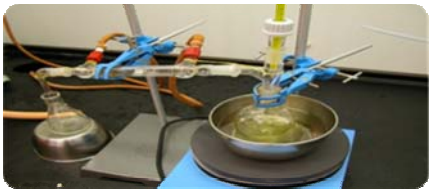
Material:

- Cloves, Cinnamon and Nigella Sativa.
- Anhydrous Na_2SO_4
- Dichloromethane.
- Separating funnel 100 mL.
- Distillation apparatus.

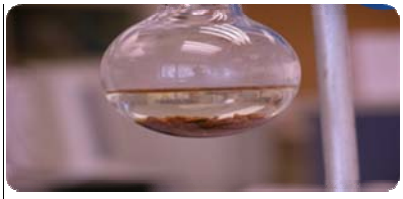
Procedure:

1- Weigh 10g of clove, cinnamon and nigella sativa.

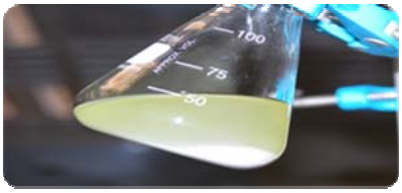
2- Add 100ml DW and allow standing for 10min with stirring.



3- Start the distillation and collect the distillate. Pour the distillate in separatory funnel.



5- Extract the essential oil by 20ml DCM.Repeat the extraction twice



6- Collect all the extracts in conical flask.Dry with 1spoon anhydrous Na_2SO_4, stir for 15min and then filter in a weighed flask.

7- Evaporate the solvent completely and dry the surface of the conical and weigh the conical again.

Calculations:

- Calculate the weight of essential oil in each item in g/10g and g%.

References:

www.wikipedia.org

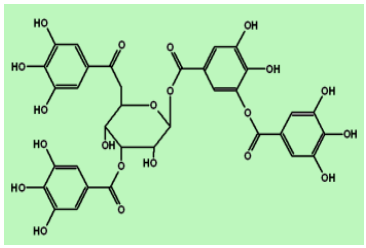
www.chemistry.mcmaster.ca

Results Sheet

Experiments-10

DETERMINATION OF TANNINS IN TEA

Introduction:



The word tannin is very old and reflects a traditional technology. "Tanning" (waterproofing and preserving) was the word used to describe the process of transforming animal hides into leather by using plant extracts from different plant parts of different plant species.

Tannins are complex phenolic compounds responsible for the sensation of astringency and are active in tanning of hide. Many food products contain tannins in their consumable forms e.g.:

- Tea, Cocoa.
- Unripe fruits (apples, cherries, strawberries, bananas)
- Walnuts
- Plant parts containing tannins include bark, wood, fruit, fruit pods, leaves, roots, and plant galls.
- Examples of plant species used to obtain tannins for tanning purposes are wattle (Acacia sp.), oak (Quercus sp.), eucalyptus

(Eucalyptus sp.), birch (Betula sp.), willow (Salix caprea), pine (Pinus sp.), quebracho (Scinopsis balansae).

One of the most satisfactory definitions of tannins was given by Horvath (1981):

"Any phenolic compound of sufficiently high molecular weight containing sufficient hydroxyls and other suitable groups (i.e. carboxyl's) to form effectively strong complexes with protein and other macromolecules under the particular environmental conditions being studied".

Tannins can complex with:

- Proteins.
- Starch.
- Cellulose.
- Minerals.

Tannins are phenolic compounds that precipitate proteins. They are composed of a very diverse group of oligomers and polymer. There is some confusion about the terminology used to identify or classify a substance as tannin, in fact.

Astringency is the contracting or drying taste, which results from coagulation of the proteins of saliva and the mucous epithelium of the mouth, causing a reduced lubricant action.

Tannins are water-soluble so they are extracted from tea by boiling with water.

Principle:

Ferric chloride reagent gives a color with tannins under acidic conditions. The color is measured spectrophotometrically and compared with the color obtained with a standard tannins solution

Procedure:

1- Weigh accurately 0.5g of tea.
2- Add 75ml of water and boil for 30 min.
3- Filter in a 100ml-measuring flask and complete to volume with water.
4- Take 1ml of solution; add 1ml of ferric chloride reagent and 8ml of water.
5- The standard is prepared by adding in a tube 1ml of standard, 1ml of reagent and 8ml of water.
6- Read the absorbance of unknown and standard against blank at 540 nm.
7- Calculate the concentration of tannin

$C_{unknown} = A_{unknown} \times C_{std}/A_{std}$

Results Sheet

Experiments-11

Isolation of an Alkaloid :Isolation of Piperine from Black Pepper.

Introduction:

Piperine is an alkaloid found naturally in plants belonging to the *Piperaceae* family, such as *Piper nigrum* L, commonly known as black pepper, and *Piper longum* L, commonly known as long pepper. Piperine is the major pungent substance in these plants and is isolated from the fruit of the black pepper and long pepper plants. Piperine comprises 1 to 99% of these plants. The term black pepper is used both for the plant *Piper nigrum* and the spice that is mainly in the fruit of the plant.

Piperine is a solid substance essentially insoluble in water. It is a weak base that is tasteless at first, but leaves a burning aftertaste. Piperine belongs to the vanilloid family of compounds, a family that also includes capsaicin, the pungent substance in hot chili peppers. Its molecular formula is $C_{17}H_{19}NO_3$, and its molecular weight is 285.34 daltons. Piperine is the trans-trans stereoisomer of 1-piperoylpiperidine. It is represented by the following chemical structure:

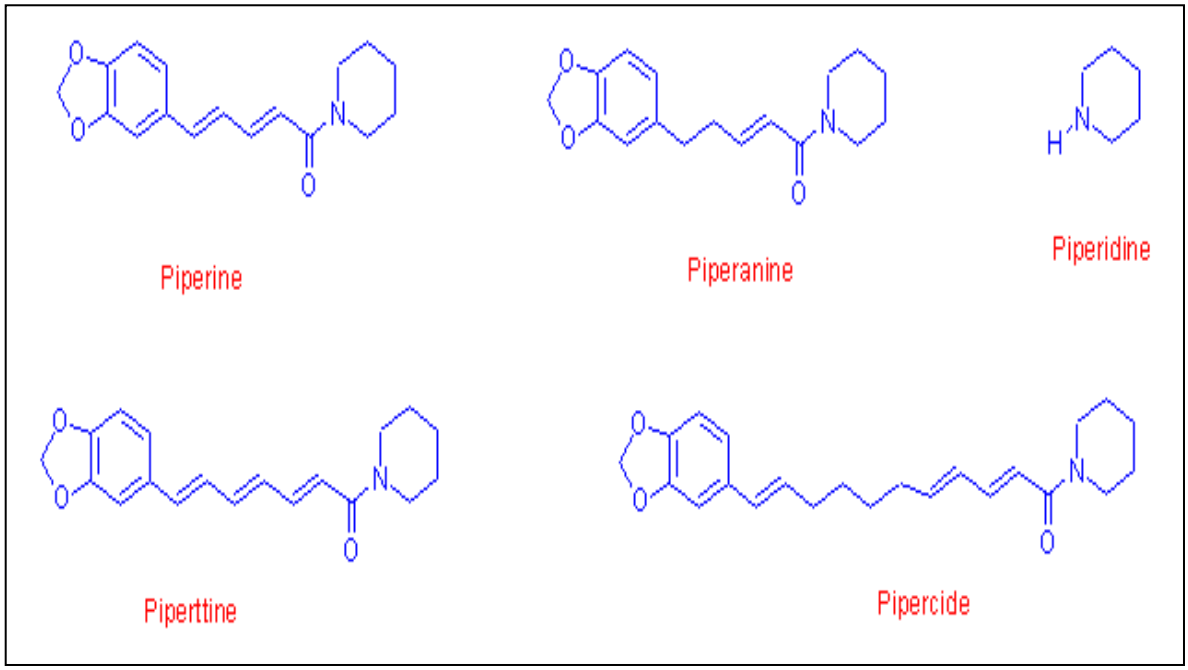
Piperine

Piperine may have bioavailability-enhancing activity for some nutritional substances and for some drugs. It has putative anti-inflammatory activity and may have activity in promoting digestive processes.

In this experiment we will extract the alkaloid components that give black pepper its physiological properties including carminative (relief of intestinal gas), diuretic (increases urine output) as well as the well known "taste bud" effects.

As we know black pepper contains about ;

- -3% of volatile oils(terpenes),
- -10% of the alkaloids piperine, (fig.a. 1).
- -13% starch.
- -the remaining is cellulose & water.
- To get the pure piperine, all other components must be removed, so the starch can be dissolved and volatile oils must soapnification so it can be water soluble.



Among these, piperine is the major component while piperanine and pipercide are only present in minute amounts.

Materials:

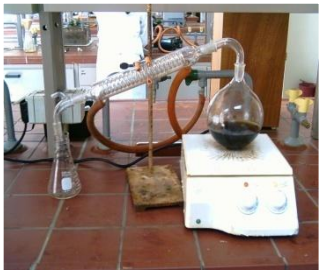
- KOH solved in ethanol (2M).
- Ethanol (95%) 300 ml.
- HCL (6 M).
- RAD condenser
- Buchner Funnel.

Method:

- weight 50gm of black piper. Note; the net result for the piperine extracted should be 5gm. (since it contain 10% of piperine).

- Add 300ml ethanol 95% to the 50gm piper and hold the condenser above the bottle.

- Heat The mixture gradually until it boiled and keeps it on heater for 3 hours then cool it.



- Filtrate the mixture in Buchner funnel.

- Distillate the filtrate in distillation device (fig. 3) to remove the extra ethanol until the volume inside reached 25 ml .

- Then do soapnification by adding 25 ml of KOH solved in ethanol (2 M) to this solvent and then mix it and hold the condencer above the bottle then boil the mixture for exactly 5min.

- after this add 35-40 ml water until the solvent become muddy then put it into a beaker then in Ice bath and scratch the wall with a glass rode.

- Leave it to the next day.

-Filtrate the solution in Buchner Funnel.

- Piperine will expected to be 3gm but if you find it 2.7 gm which is considered to be very close to perfect accuracy.

- finally, record the melting point (……) and comper it with references (129-131 C)(fig 9,10) .

Fig.b.6; the wall was scratched

Note: the final distillated ethanol we got had the odor of black piper , but you can keep this ethanol specially just for this experiment.

Fig.b.7:The soluti was filtrated in Buchner Funnel

Fig . b .9 ; Measuring

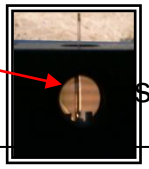
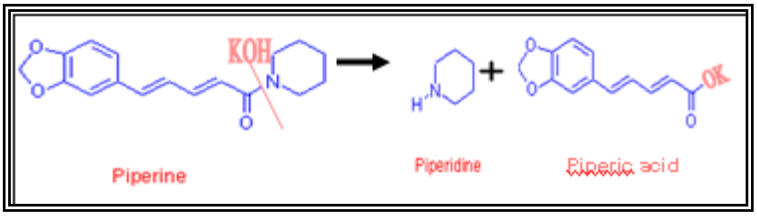


Fig . b .10 ; the sample which was measured.

Warning;

- If boiling period after soapnification still more than 5min, the weak bond between N & C will be broken by the entrance of KoH and so it is hydrolyzed to piperidine + piperic acid which is undesirable.



Results Sheet