

## Experiment (7)

### Extraction and Identification of volatile oils by TLC

Essential oils contain some highly volatile organic substances that can be isolated from odoriferous plants by various physical processes. The oils are usually concentrated in the seeds or flowers but may exist in other parts of the plants as well. Such oils were called essential because they were thought to represent the very essence of odor and flavor. Most essential oils are primarily composed of terpenes and their oxygenated derivatives (terpenoids).

Chemical constituents of volatile oils can be divided into two broad classes: 1.) Terpene derivatives formed via acetate mevalonic acid pathway and 2.) Aromatic compounds formed via shikimic acid phenyl propanoid route.

Several points of differentiation exist between volatile oils and fixed oils. Volatile oils can be distilled from their natural sources; they do not consist of glyceryl ester of fatty acids. Hence, they do not leave a permanent grease spot on paper and cannot be saponified with alkalies. Volatile oils do not become rancid, as do fixed oils, but instead, on exposure to light and air, they oxidize and resinify. Unlike essential oil, fixed oils are not volatile. Meaning, they do not evaporate rapidly even when under normal temperature or pressure. It acts as a carrier oil and diluent for essential oil to be easily absorbed.

Essential oils can be obtained from plants by a number of processes such as mechanical pressing and grinding, maceration, solvent extraction, distillation and concentration. In many cases a combination of processes are required for an efficient and effective isolation. The specific extraction method employed is dependent upon the plant material to be distilled and the desired end-product. Most essential oils are extracted by steam distillation.

| Volatile oils                                                                            | Fixed oils                           |
|------------------------------------------------------------------------------------------|--------------------------------------|
| Volatile                                                                                 | Non-volatile                         |
| Can be distilled                                                                         | Cannot be distilled as volatile oils |
| No glyceryl esters of fatty acids                                                        | Glyceryl esters of fatty acids       |
| No permanent grease spot                                                                 | Leave greasy spot                    |
| Cannot be saponified                                                                     | Can be saponified                    |
| Oxidize + resinify                                                                       | Become rancid                        |
| ★ أهم نقطة للحفظ:<br>Volatile oil → evaporates<br>Fixed oil → does NOT evaporate rapidly |                                      |

## Distillation:

Distillation is a method of separating liquids in a solution by using the differences in their boiling points. Several laboratory scale techniques for distillation exist, steam distillation is preferred for essential oils extraction.

### ➤ Steam distillation:

Steam distillation is a method for distilling compounds which are heat-sensitive. This process involves bubbling steam through a heated mixture of the raw material. Many organic compounds tend to decompose at high sustained temperatures. Separation by normal distillation would then not be an option, so water or steam is introduced into the distillation apparatus. By adding water or steam, the boiling points of the compounds are depressed, allowing them to evaporate at lower temperatures, preferably below the temperatures at which the deterioration of the material becomes appreciable.

الخلاصة للحفظ:   
 Steam distillation يستخدم خصوصاً للمركبات heat-sensitive.   
 Water/steam → ↓ boiling point → evaporation at lower temperature → prevents decomposition.

## Chromatographic Techniques:

One of the problems continually facing biochemists is the separation and purification of one or more biological compounds from a mixture of such compounds. One of the most convenient methods for achieving such separation is the use of chromatographic techniques. The selection of a particular form of chromatography to achieve a separation is dependent on the material to be isolated, and often several chromatographic methods may be used sequentially to achieve the purification of a compound.

The term partition or distribution coefficient is normally used to describe the way in which a compound distributes itself between two immiscible phases. The distribution of compound can be described in term of its distribution between any two phases, such as liquid/liquid, solid/liquid, or gas/liquid phases. This concept of the distribution coefficient is the basic principle of chromatography.

All chromatographic system consists of two phases. One is the stationary phase (SP), which may be solid, liquid or mixture. The second is the mobile phase (MP) may be liquid or gaseous and flows through the stationary phase. The choice of stationary or

| Stationary Phase (SP)      | Mobile Phase (MP) |
|----------------------------|-------------------|
| ثابت                       | متحرك             |
| (Solid / Liquid / Mixture) | (Liquid / Gas)    |
| لا يتحرك                   | يتحرك خلال SP     |

mobile phases is made so that the compounds to be separated have different distribution coefficients. This can be achieved by setting up an equilibrium between SP and MP.

### ➤ Thin layer chromatography (TLC)

It is an adsorption chromatographic technique in which the two phases are: a solid stationary phase and a liquid mobile phase. The solid stationary phase is a thin layer of finely divided solid, such as silica gel or alumina, supported on glass, plastic or aluminum, and the mobile phase is a solvent.

As the solvent moves past the sample spot that was applied on the solid phase, equilibrium is established for each component of the mixture. The more polar molecules, the higher the affinity it will have for the more polar silica plate and will therefore spend less time in the mobile phase.

As a result, it will move up the plate more slowly. Conversely, a less polar molecule will spend more time in the mobile phase and will therefore move up the plate more quickly. The speed at which the molecules will move up the plate thus depends on the polarity of the solute, solvent, and adsorbent.

The difference each molecule travels along the adsorbent in relation to how far the mobile phase has traveled is called the Retention factor (R<sub>f</sub>) and can be used to identify molecules, as the value is molecule specific. The retention factor for any given molecule will vary depending on the mobile and stationary phases used.

➤ TLC is a simple, quick, and inexpensive procedure that commonly used in the separation of substances from mixture, identification of a compound by comparing it with known substances, checking the purity, and in following the progress of a chemical reaction.



## ✓ How to Run a TLC Plate:

### Step 1: Prepare the developing container ✓



The developing container for TLC can be a specially designed chamber, a jar with a lid, or a beaker with a watch glass on the top (the latter is used in the undergrad labs at CU). Pour solvent into the chamber to a depth of just less than 0.5 cm. To aid in the saturation of the TLC chamber with solvent vapors, you can line part of the inside of the beaker with filter paper. Cover the beaker with a watch glass, swirl it gently, and allow it to stand while you prepare your TLC plate.

### Step 2: Prepare the TLC plate ✓

كلما كان عددك عينات، احمى لنتية إلى لوحة أوسع.

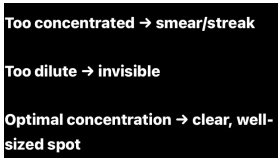
TLC plates are usually 5 cm x 20 cm sheets. Each large sheet is cut horizontally into plates which are 5 cm tall by various widths; the more samples you plan to run on a plate, the wider it needs to be.



Handle the plates carefully so that you do not disturb the coating of adsorbent or get them dirty. Measure 0.5 cm from the bottom of the plate. Using a pencil, draw a line across the plate at the

0.5 cm mark. This is the origin: the line on which you will spot the plate. Take care not to press so hard with the pencil that you disturb the adsorbent. Under the line, mark lightly the name of the samples you will spot on the plate, or mark numbers for time points. Leave enough space between the samples so that they do not run together; about 4 samples on a 5 cm wide plate is advised.

### Step 3: Spot the TLC plate



If the sample is not already in solution, dissolve about 1 mg in 1 mL of a volatile solvent. As a rule of thumb, a concentration of 1% usually works well for TLC analysis. If the sample is too concentrated, it will run as a smear or streak; if it is not concentrated enough, you will see nothing on the plate. Sometimes you will need to use trial and error to get well-sized, easy to read spots.

Capillary → Origin → small spot → dry → repeat x2  
يعني بالنهاية:  
Each sample = spotted 3 times. ✓

Dip the capillary tube into the solution and then **gently** touch the end of it onto the origin line location on the TLC plate. Don't allow the spot to become too large - if necessary, you can touch it to the plate, lift it off and blow on the spot. After each spot is dry, you will repeat the entire process 2 more time, so that each solution has been spotted 3 times.

كل عينة يتم وضعها 3 مرات

#### Step 4: Develop the plate

● **Step 4** للتحفظ:  
Place plate → Cover → Leave undisturbed  
↓  
Solvent rises by capillary action  
↓  
⚠ Solvent must NOT cover the spots  
↓  
Stop when solvent is 0.5 cm below top  
↓  
Immediately mark solvent front with pencil  
↓  
Dry the plate

Place the prepared TLC plate in the developing beaker, cover the beaker with the watch glass, and leave it undisturbed on your bench top. The solvent will rise up the TLC plate by capillary action. Make sure the solvent does not cover the spot. Allow the plate to develop until the solvent is about half a centimeter below the top of the plate. Remove the plate from the beaker and immediately mark the solvent front with a pencil. Allow the plate to dry.

#### Step 5: Visualize the spots

If there are any colored spots, circle them lightly with a pencil. Most samples are not colored and need to be visualized with a UV lamp after spraying.

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- ✓ *If the TLC plate runs samples, which are too concentrated, the spots will be streaked and/or run together.*
  - ✓ *If this happens, you will have to start over with a more dilute sample to spot and run on a TLC plate.*
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# Experimental

|                                |                                                                                                                                                                                                                                                                                              |
|--------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Materials and Equipment</b> | Glassware: Round-bottomed flasks (250 mL), distillation head, adapter, condenser, Erlenmeyer flask (100 & 250 mL), beaker (100 mL), 2 stands, heat mantle, 2 clamps, 2 clamp holders, graduated cylinder (10 mL), separatory funnel (100 mL), water bath. TLC paper, pencil, capillary tubes |
|                                | Chemicals, sodium chloride, anhydrous sodium sulfate, dichloromethane, anethole, eugenol and one of the following: anise seeds or cloves.                                                                                                                                                    |

## Isolation of essential oil:

### Procedure:

1. Place 15g of the ground spice and 200 mL of water in a 250 mL round-bottomed flask. Add some glass beads.
2. Connect the flask to a distillation apparatus and heat the mixture to boiling. Collect the distillate until droplets of oil come over; a minimum of 75 mL should be collected.
3. Add 5-7 g of sodium chloride and stir until completely dissolved. Cool the distillate to room temperature, transfer to a 150 mL separatory funnel, and extract with 10 mL of dichloromethane.
4. Separate the layers and collect the organic layer in a small flask.
5. Repeat the extraction twice with another 10 mL each of dichloromethane and combine the organic extracts.
6. Dry the organic phase with anhydrous sodium sulfate until the solution is clear. Decant the dichloromethane solution into a small flask.
7. Evaporate the solvent on the steam bath, in the fume hood, until the solution has been concentrated to an oily residue.

## TLC:

### Solvent system:

(should be placed in the chamber before plate preparation to allow the chamber to become saturated)

- Toluene: Ethyl acetate (93:7), prepare 100ml.

### Spraying reagent:

- Vanillin / Sulfuric acid

### Procedure:

1. Prepare your TLC plate as instructed and develop in the solvent system.
2. After the solvent had reached 10 cm, dry your TLC and spray it with the spraying reagent.
3. Analyse your results and calculate the Rf values.

$$R_F = \frac{\text{Distance traveled by compound}}{\text{Distance traveled by solvent front}}$$

## ***Practical work:***

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### **1. Volatile oil extraction:**

➤ Plants to be used and their suitable reference:

- Clove flower: Eugenol as a ref.
- Anise fruit: Anethole as ref.
- Thyme leaves: Thymol as ref.
- Coriander: Linalol as a ref.

➤ Procedure:

1. Weigh 1.0 g of powdered plant and put it with 10 ml distilled water in 50 ml Erlenmeyer flask, add few glass beads.
2. Connect the Erlenmeyer flask to a bent tube through rubber stopper into a receiving test tube.
3. Heat slowly on direct flame with caution until you collect in the tube about 2 ml distillate.
4. Add the distillate to a small test tube, then add a small amount of petroleum ether and shake to extract the oil.

### **2. TLC:**

➤ Solvent system:

(should be place in the chamber before plate preparation to allow the chamber to become saturated)

- Toluene: Ethylacetate (93:7), prepare 100ml.

➤ Spraying reagent:

- Vanillin / Sulfuric acid

➤ Procedure:

1. Prepare your TLC plate as instructed and develop in the solvent system.
2. After the solvent had reached 10 cm, dry your TLC and spray it with the spraying reagent.
3. Analyze your results and calculate the R<sub>f</sub> values.