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).

other name to volatile oils

- **Chemical constituents of volatile oils can be divided into two broad classes:**

two pathway :

1. Terpene derivatives formed via acetate mevalonic acid pathway.
2. Aromatic compounds formed via shikimic acid phenyl propanoid route.

phenyl propanoid derivatives

Shikmate

Several points of differentiation exist between volatile oils and fixed oils.

acetate malonate pathway.

Difference between fixed oils and volatile oils :

① 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.

③ 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

④ when under normal temperature or pressure. It acts as a carrier oil and

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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 is required for an efficient and effective isolation. The specific extraction method employed is dependentupon the plant material to be distilled and the desired end-product. Most essential oils are extracted by steam distillation.

processes for Extraction ?

تفريغ

differences between fixed oils and volatile oil

* لذا وضعنا fixed oil على ورقة ما رح تتبخر على عكس volatile oil إلى رح يتبخر ويختفي.

* fixed oils → esterification بصيرلها
fatty acid + glycerol → esters

fatty acid → (salt) ممكن نحولها لصابون
↳ by treatment with strong alkali

* Saponification فقط في fixedoils غير موجود بالـ volatileoil.

* volatile oil ما بتصير Rancid (فاسدة)
الـ Rancidity ← fixed oil

* ممكن بـ volatileoil ← and light when exposure to ~~oxygen~~
air → oxidation → (they resinify)
يعني يصيروا زي المادة الصمغية

* Volatile oil evaporate on room tempreture
rapidly

unlike fixed oil ~~a~~ that do not evaporate even in high tempreture.

* كيف بتصير steam distillation (هيك زي ما عملنا بالاب الدوز انيل بتجربة استخلاص الـ anise oil)
بنسخن مي والبخار تاعها بيتعرض إلى النبتة إلي طحنتها (grinding)
بيحمل الـ steam الـ volatile oil من النبتة وبروح بينتقل للـ Condenser
إلي بكون معه ماء باردة لحتى ينزل درجة حرارة الـ steam فبيتحول
الـ steams ← volatileoil + liquid water

الـ boiling point بهذه الخطوة للماء أقل لأنه خالطينه مع oil آخر
فرح يتبخر على درجة حرارة أقل، لو أحنا خالطين الـ seeds
لحالهم وبس يتعرض الـ steam فرح تتبخر الماء على درجة حرارة
طبيعية وليست أقل، وبعدها الـ steam بينتقل للـ seed وبحمل volatile oil للـ Condenser

وبتكتشف مرة أخرى حامل معه volatile oil وبنجمل collection ٥ هاي العملية

زي التجربة عملناها ب organic lab : Anise extraction

* بنجمله ب collection beaker وبنحطه ب flask وبنجمل separation

لل aqueous compound organic لحال ، فممكن اني اضيف ال organic solvent

بحيث احسن extraction of volatile oil from water وبهيك نجمع ال aliquotes من ال

organic solvent إلي حامل معه volatile oil

بس مفتاحيه distillate من steam distillation

* احنا بس نضيف organic solvent لل distillate ↑ رح يسحب

معظم ال oil إلي استخلصنا من anise وبعدهيك ممكن نتخلص من

هاد ال solvent

واحنا عملنا أكثر من aliquotes من organic solvent وعملنا

shaking ب separatory funnel وبعدها اضفنا عملنا مرة ثانية

اضفنا كمان aliquote من organic solvent وحطيناها ب separating funnel

ومرة ثالثة لحد ما جمعنا كمية جيدة من organic phase سحبنا فيها

كل ال volatile oil من aquous layer • ~~وبنتخلص من~~

* بنقدر نتخلص من organic phase ← heating لإذا المادة
not heat labile

heat labile كانت لإذا evaporator ← اوسكين

• pure oil صيك بنقدر نحصل على organic solvents ما نتخلص من

* احنا بتجربتنا بدنا نشتغل على انه إلى استخلاصناه بالطب النورمانيك
لحتى نشوف ~~[illegible]~~ هايد داته.

chromotographic technique is for separation mixture of compounds

the way in which a "compound" distributes itself between the two 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.

1. **The stationary phase (SP),** which may be solid, liquid or mixture.
2. **The mobile phase (MP),** which may be liquid or gaseous and flows through the stationary phase.

The choice of stationary or mobile phases is made so that the compounds to be separated have different distribution coefficients. This can be [57]

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 theplate more quickly. The speed at which the molecules will move up the plate thusdepends 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 (Rf)** 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*

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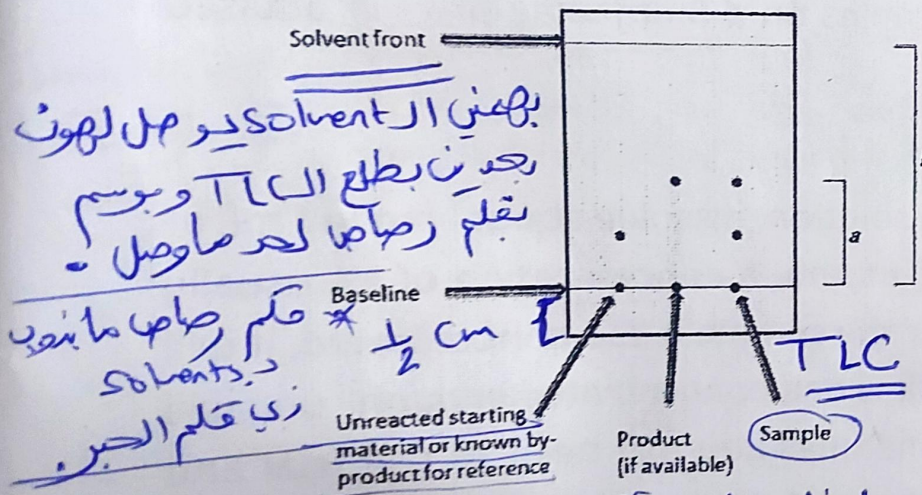
- *TLC is a simple, quick, and inexpensive procedure that commonly used in the separation of substances from mixture, identification of a compound by comparingit with known substances, checking the purity, and in following the progress of a chemical reaction.*



$$R_f = \frac{\text{Distance from baseline traveled by solute}}{\text{Distance from baseline traveled by solvent (solvent front)}}$$

$$R_f = \frac{a}{b}$$

TLC

✓ *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

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

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.

Step 4: Develop 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 arenot colored and need to be visualized with a UV lamp after spraying.

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 arenot colored and need to be visualized with a UV lamp / sparing reagent after spraying.

Notes:

- ✓ *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.*

*Visualize system in this lab is iodine champer.

2. TLC:

- **Solvent system:**

  (Should be place in the chamber before plate preparation to allow the chamber tobecome saturated)

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

non polar

* mobile phase is nonpolar system
* Stationary phase is polar system

- **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 Rf values.