FE 315 INSTRUMENTAL ANALYSIS
TLC/FID
(Thin Layer Chromatography/Flame Ionization Detector

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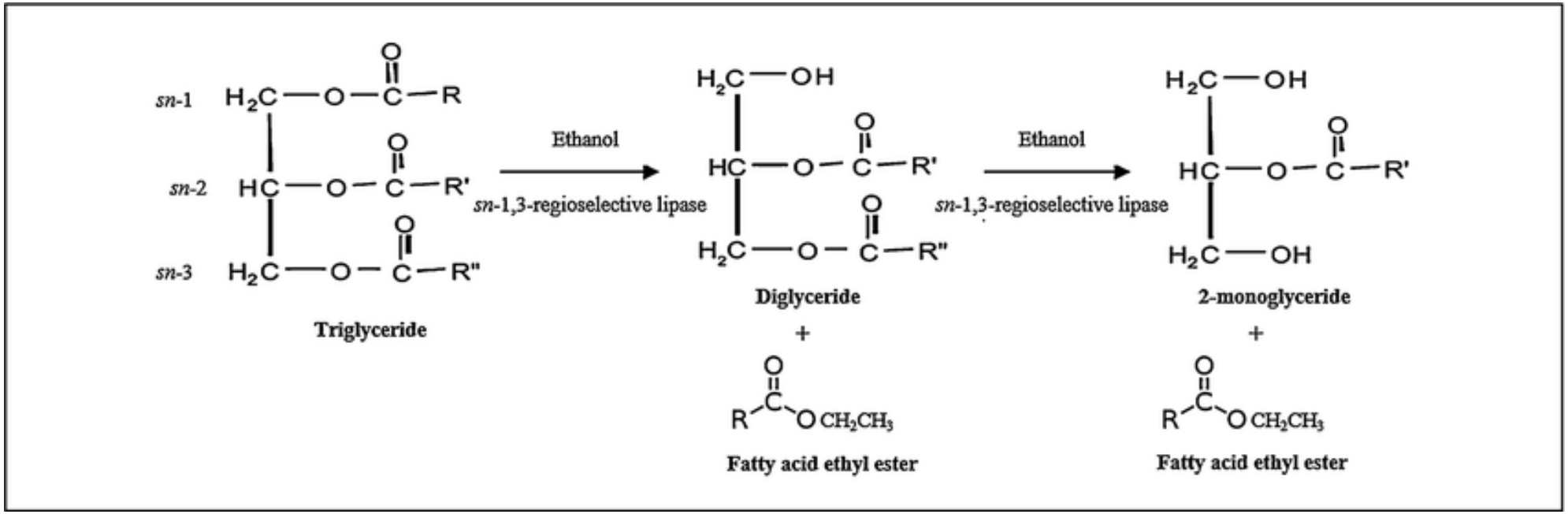
Experimental Section



Experimental Procedure

1. Preparation of sample solution (1% (v/v)).

2. Execute blank scan for activation of the rods.

3. Spot 1 µL of sample on chromarod.

4. First development: Development distance: 8 cm

   Development solvent: 100% chloroform.

5. Dry the sample at room temperature for 5 min.

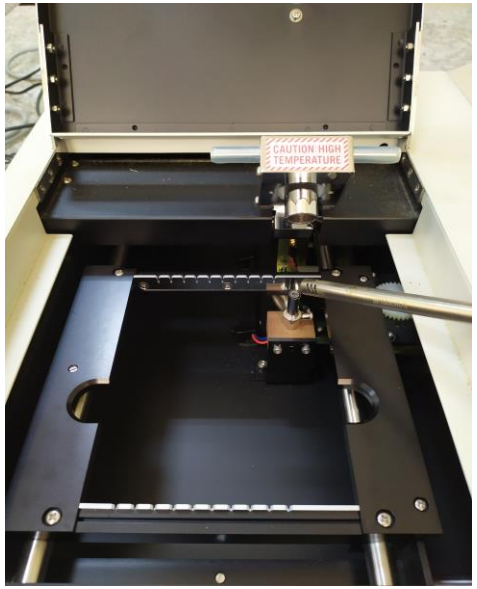
6. Second development: Development distance: 10 cm

   Development solvent: chloroform:(methanol: NH_4OH, 8: 2, v:v), (70:0.05, v:v).

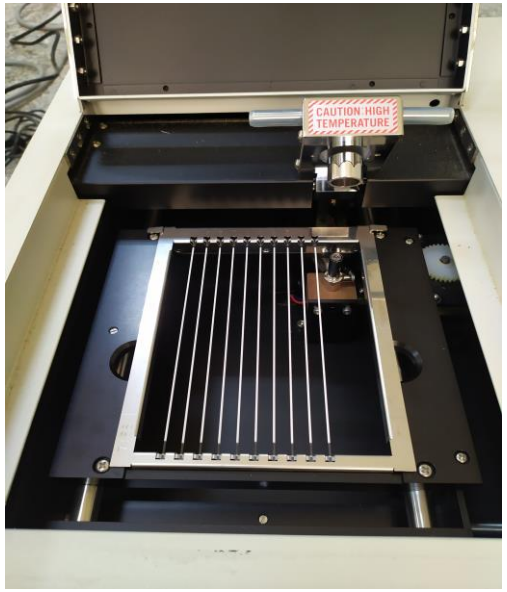
7. Dry the sample in dryer at 120°C for 5 min.

8. Perform scanning under following conditions: gas flow rate 160 mL/min, air flow rate 2.0 L/min, scanning speed 30 s/scan.

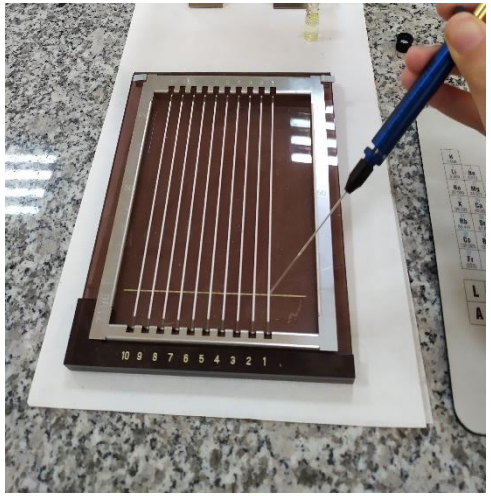
TLC/FID Instrument



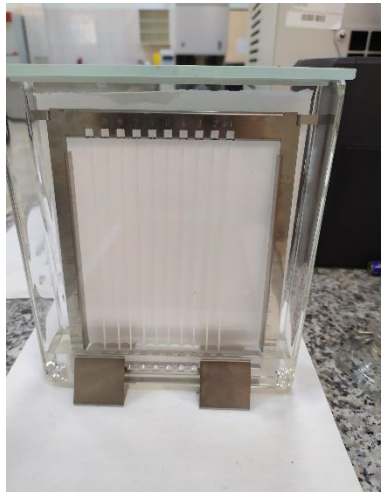
Flaming using hydrogen



Blank scan for activation

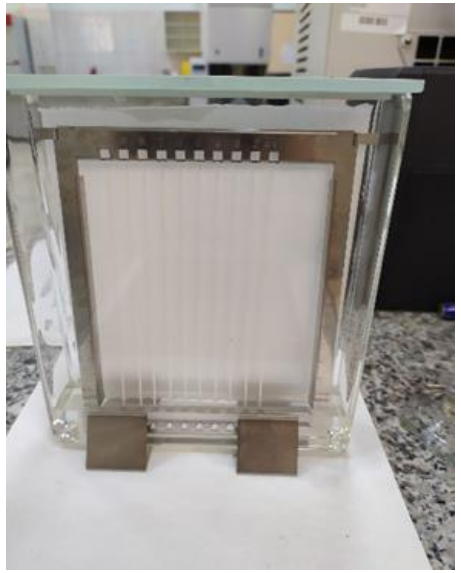


Spotting of the sample



First development

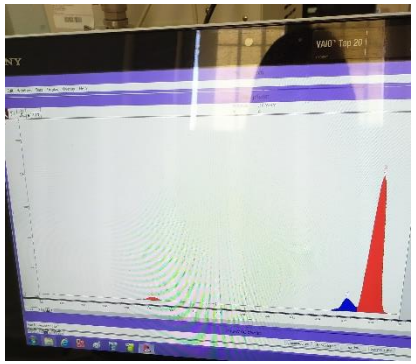
Drying of the chromarod



Second development

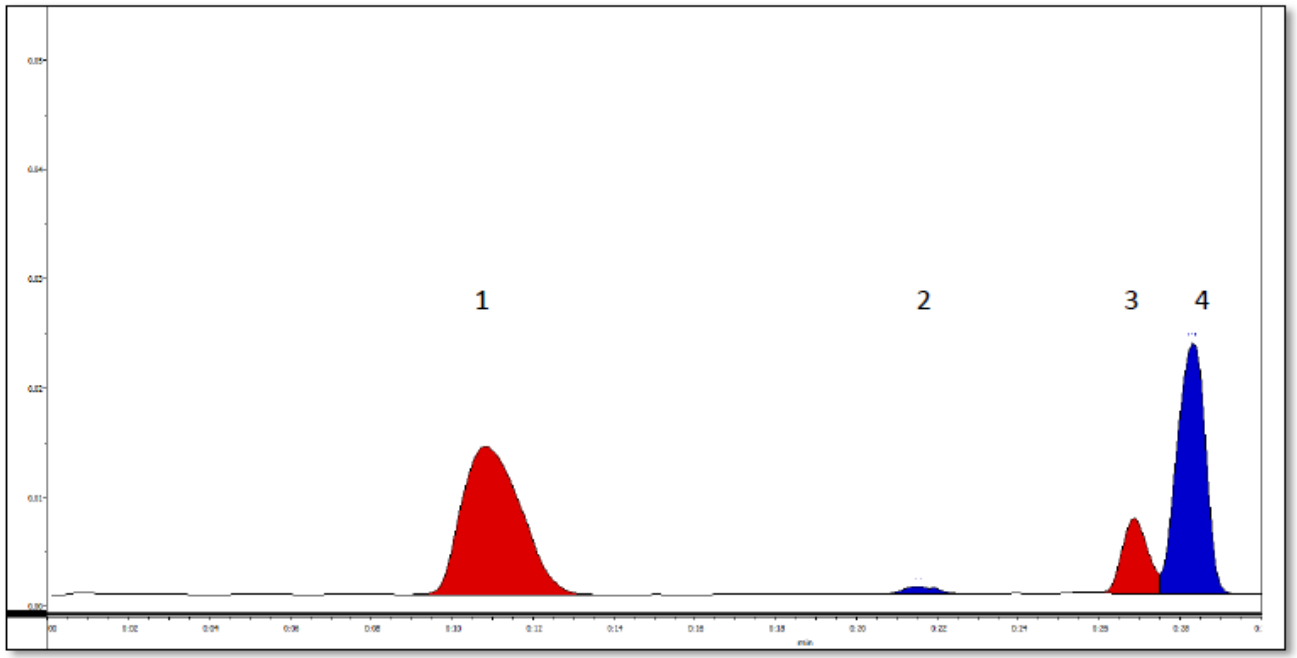
Drying at 120 C

Scanning



Monitorizing

DATA



Identify the peaks and their percentages that were obtained after the experiment considering the chromatographic separation principle.

Peak No:Peak Area

1:481.50

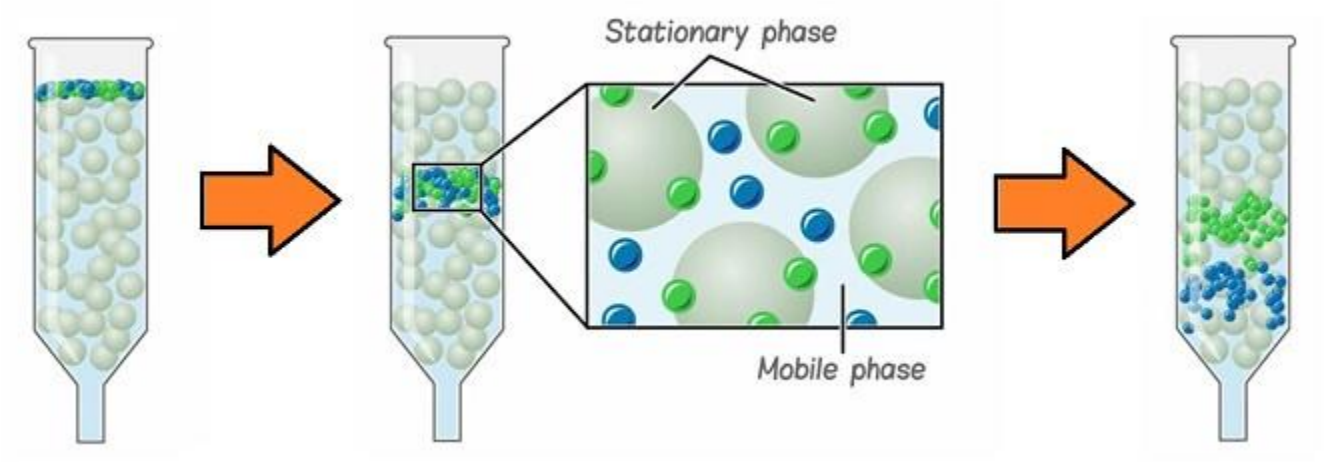
2:10.23

3:130.00

4:365.25

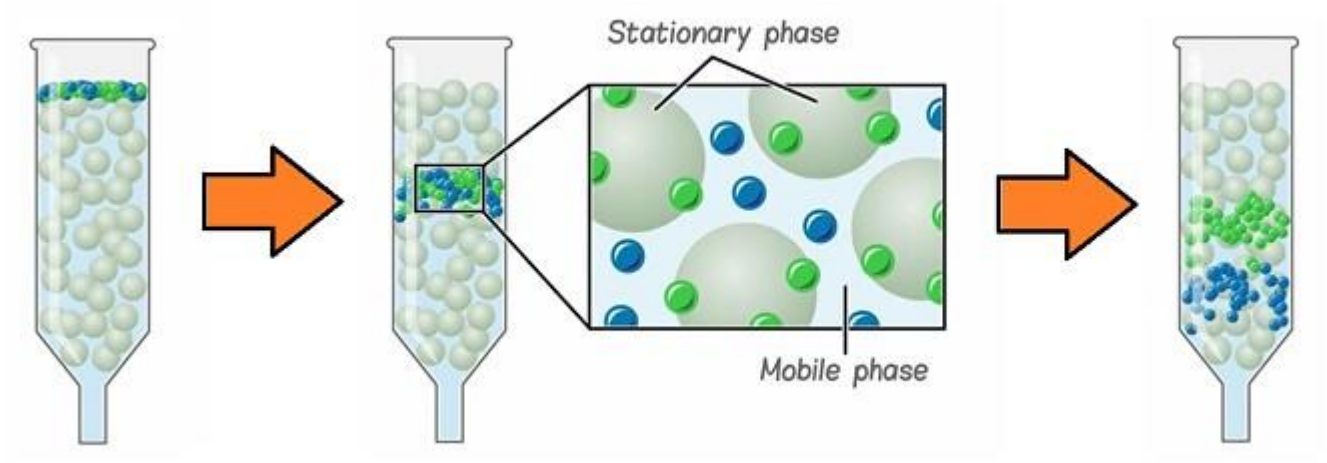
Chromatography

- Chromatography is a technique for the analysis and separation of chemical mixtures.
- Individual components of a chemical mixture can be analyzed by chromatographic methods.
- The separation principle is based on the polarity difference between the sample components, stationary phase and the mobile phase.
- *Different affinities of the different components of the sample towards the stationary and mobile phases results in the differential separation of the components*.



Stationary Phase and Mobile Phase

- Stationary phase is solid and mobile phase is liquid or gas.
- Mobile phase (carrier) is the solvent moving through the stationary phase.
- Stationary phase (adsorbent) is the substance that stays fixed inside the column
- Stationary phase does not move, while the mobile phase flows across, or through the solid phase



Examples for Chromatographic methods

- Thin Layer Chromatography (TLC)
- High performance liquid chromatography (HPLC)
- Gas chromatography (GC)
- Ion-Exchange chromatography (IEC)
- Affinity chromatography (AC)

- Each chromatographic metod differs in the kind of stationary and mobile phase they use.

General Information about TLC/FID

TLC can be used for:

- Determining the number of components in a mixture
- Determining the identity of compounds
- Determining the purity of a compound
- Monitoring the progress of a reaction

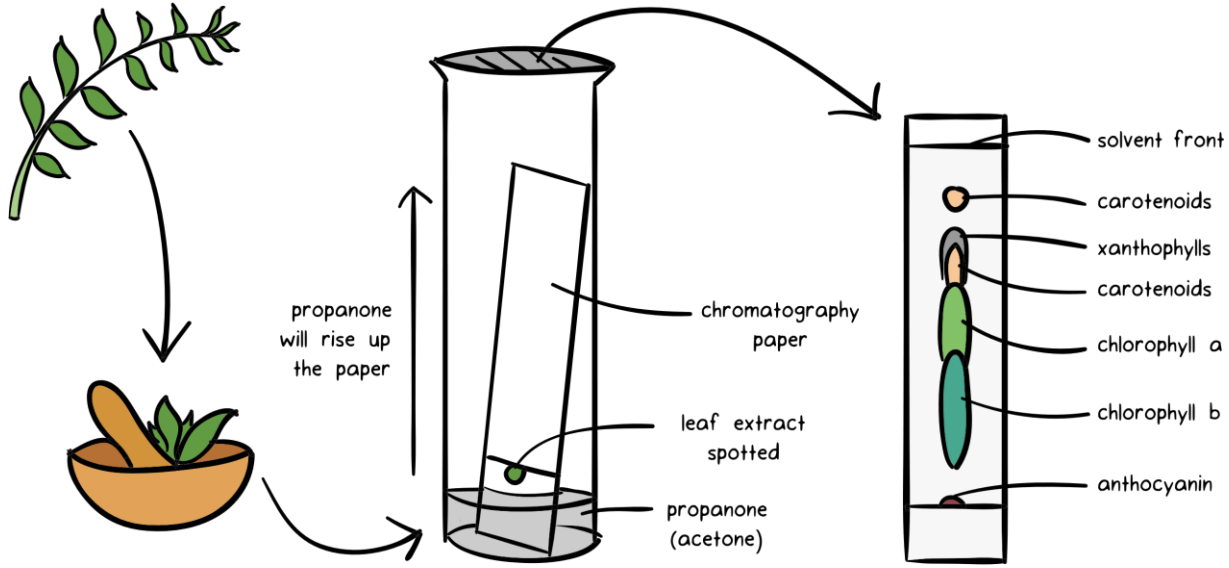


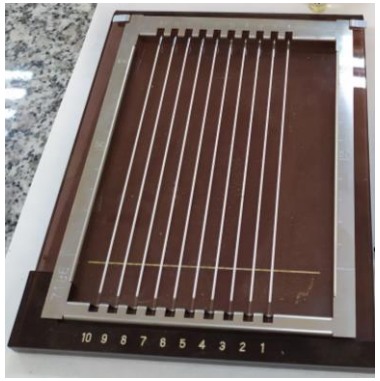
Figure 1. khanacemy.org/18.10.2020

General Information for TLC/FID

- Stationary phase: Chromarods are thin quartz rods coated with an adsorbent such as silica gel or aluminum oxide embedded in porous sintered glass can be prepared by coating the rods with a suspension of the adsorbent

- Mobile phase: The solvent in which the rod is dipped and that runs up the plate by capillary action is the *mobile phase*.

- The stationary phase (i.e. Silica) is very polar in nature, while the solvent is less polar compared to silica.



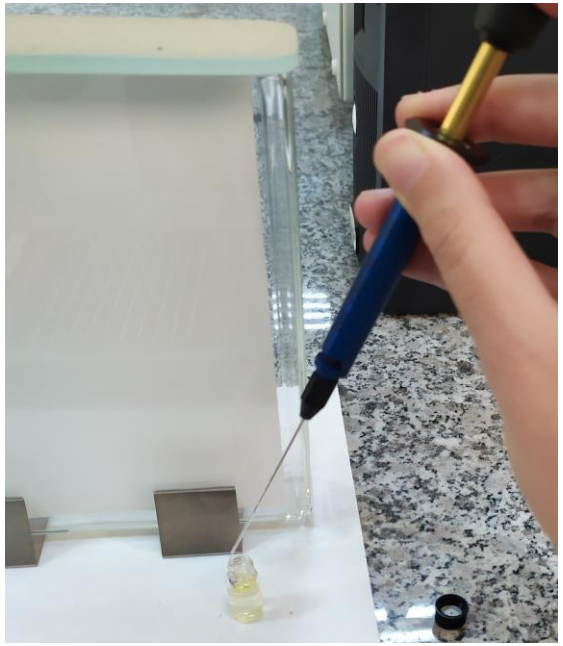
General Information about TLC/FID

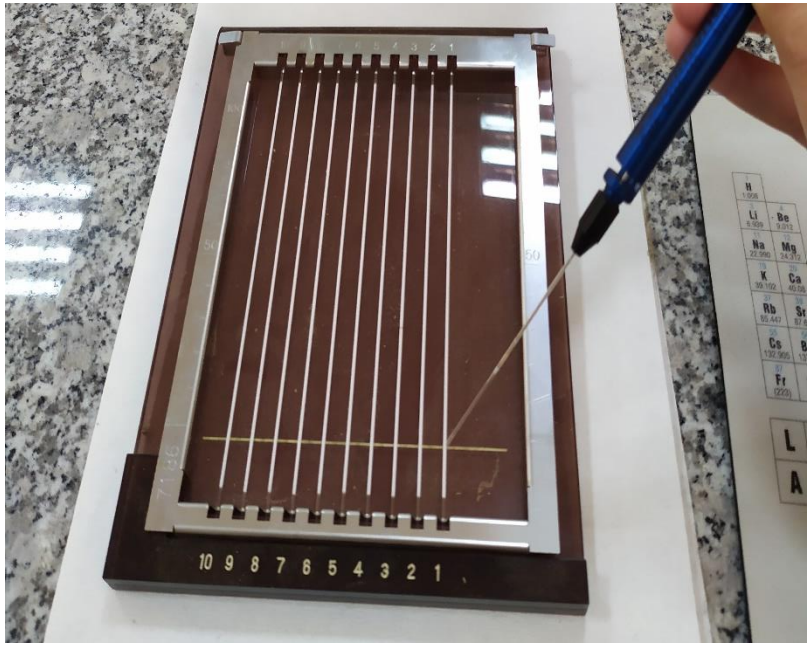
- TLC/FID analysis consists of three steps:
  - spotting,
  - development
  - monitorizing and quantification

1- Spotting

Spotting of the sample on chromarod:

- Sample is dissolved in a solvent to produce a dilute (1%) solution.
- Spotting is done using a micro pipet to transfer a small amount of this dilute solution to chromarod (stationary phase)

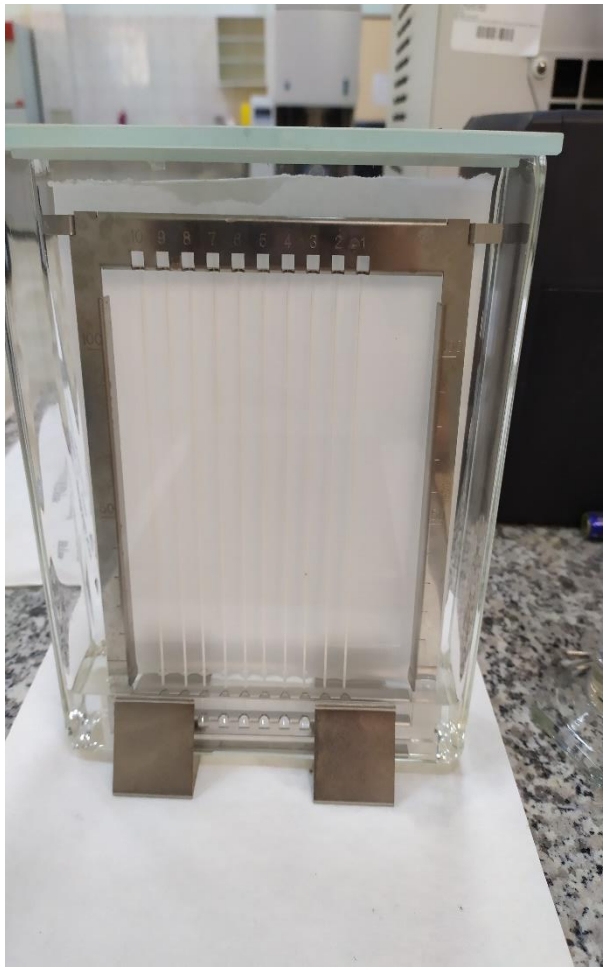


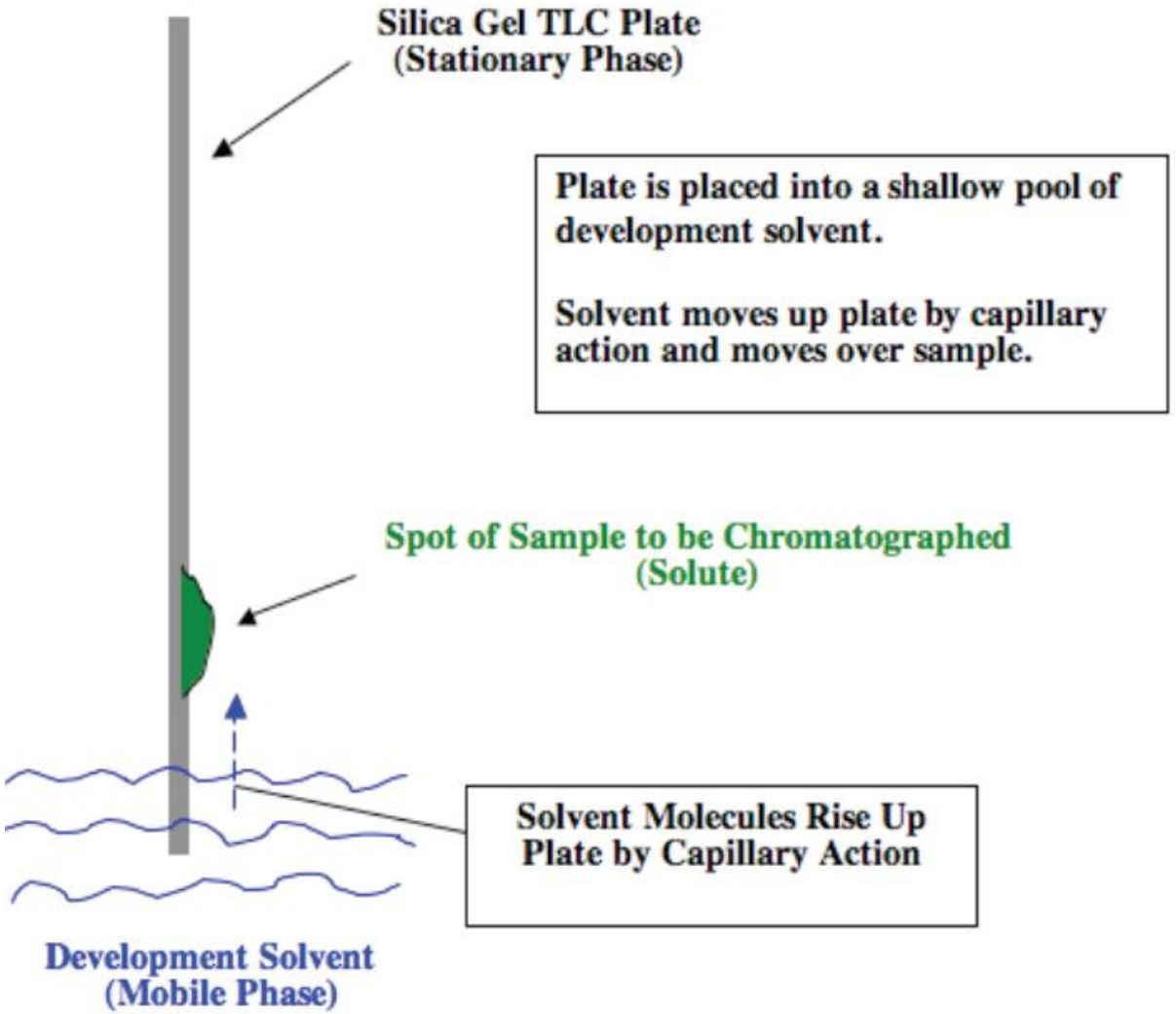


2. Development

- Step 1: Chromarods are placed in a development tank containing solvent (mobile phase).
- Step 2: The mobile phase is adsorbed by silica of the chromarods and travels up the rod by capillary action.
- Step 3: When the mobile phase is traveling up the chromarod, it moves over the original spot sample.
- A competition starts between the silica gel rod and the development solvent for the spotted material. (silica gel: polar, development solvent: non-polar)
- Different components in the sample, having different polarities, will move different distances from the original spot location and show up as separate spots.

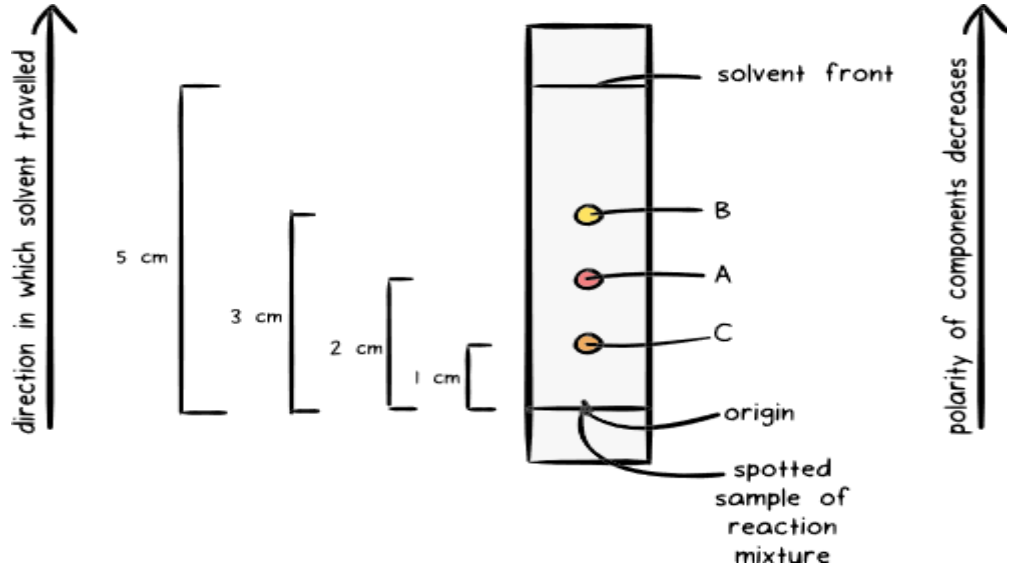
- When the solvent goes up to nearly top of the silica, the rod is removed the solvent is allowed to evaporate.




Silica Gel TLC Plate
(Stationary Phase)
Plate is placed into a shallow pool of development solvent.
Solvent moves up plate by capillary action and moves over sample.
Spot of Sample to be Chromatographed
(Solute)
Solvent Molecules Rise Up Plate by Capillary Action
Development Solvent
(Mobile Phase)

Separation Principle of TLC/FID

- The polar components of the sample has high affinity to silica gel and adhere to the silica strongly and travel up the rod slowly.
- The less polar or non-polar components has low affinity to the silica and do not adhere that strongly to the silica and travel up the rod relatively fast with the mobile phase.

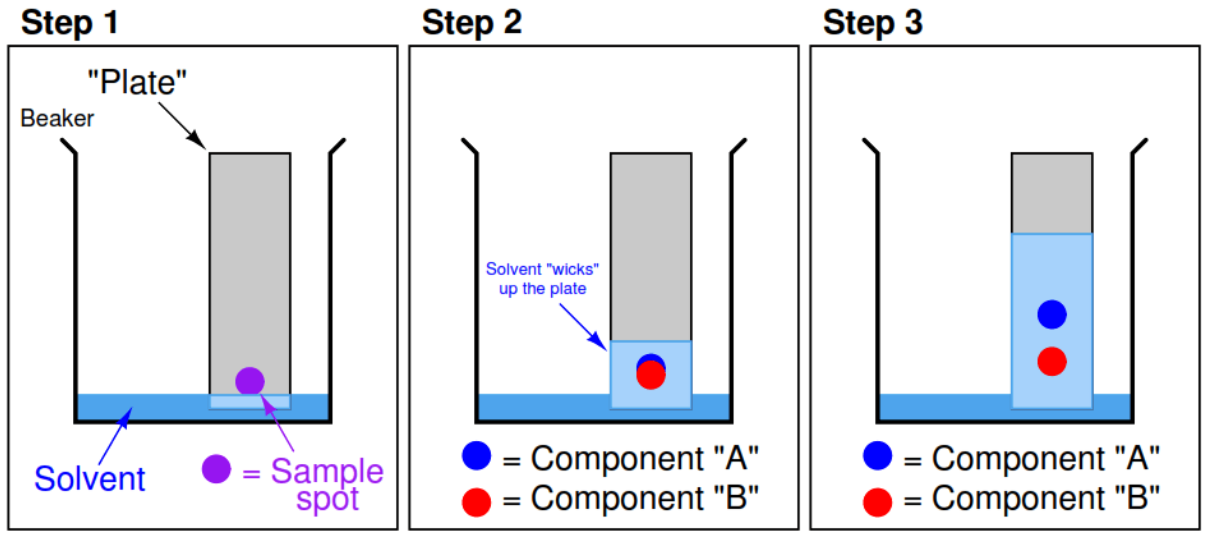


Separation Principle of TLC/FID (Continued)

- What is adsorption and solubility?
- Adsorption shows how well a component of the mixture sticks to the stationary phase, while solubility is the property of how well a component of the mixture dissolves in the mobile phase.
- Higher the adsorption to the stationary phase, the slower the molecule will move through the chromarod.
- Higher the solubility in the mobile phase, the faster the molecule will move through the chromarod.

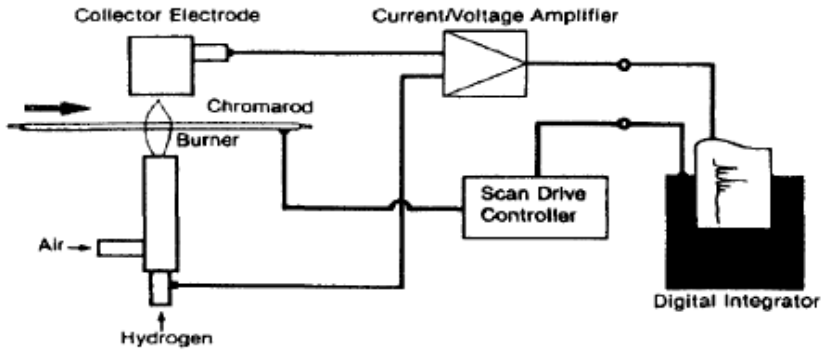
Separation Principle of TLC/FID (Continued)

- Question: Why do different compounds have different affinities on the stationary and mobile phases?
- Answer: *“Polarity” of the compounds dictates their affinities towards the stationary and mobile phases*.



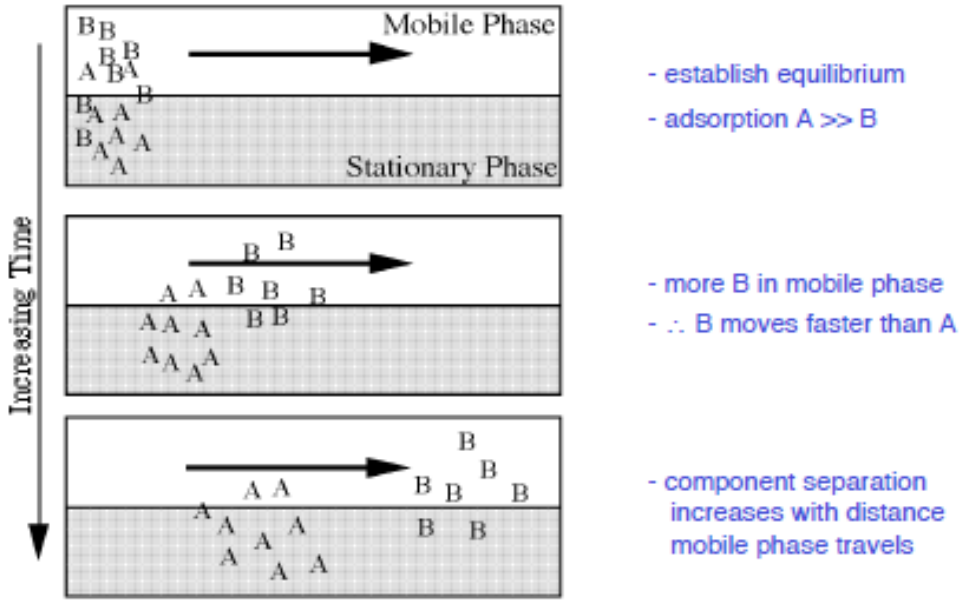
3. Monitorizing and Quantification

- After development the rods are burned and ionized in a hydrogen flame. An electrode in the detector is disposed above the hydrogen flame and the ionized gas. Between this electrode and the gas burner there is a high voltage and thus negative and positive ions migrates respectively to the burner and collector electrode. An electric current, proportional to the amount of each separated substance, is detected quantitatively and amplified by a detector electrode surrounding the negative electrode.
- A data processing unit converts the amplified signal and calculates the percentage area of each peak in the run as a percentage of total area of all peaks, which in turn corresponds to the relative amount of each components.
- The peaks are identified as the different fractions based on a retention time specified for each reference peak in the reference method



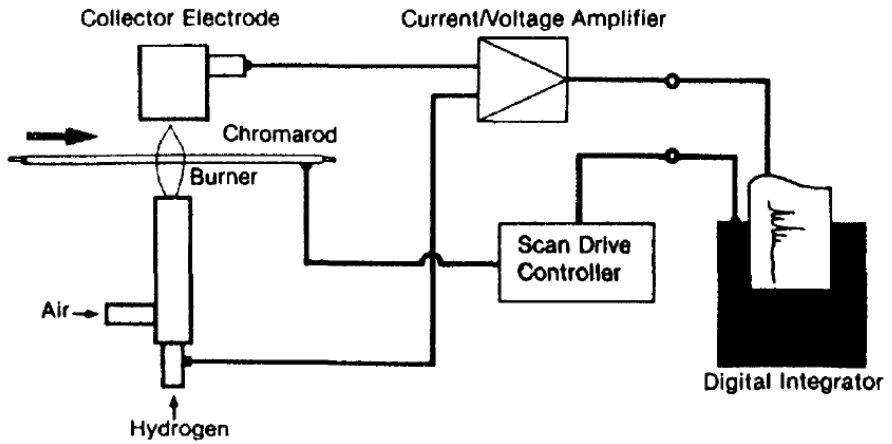
Chromatographic separation

The different compounds in the sample mixture move through the stationary phase at different rates, due to different attractions for the mobile and stationary phases. Separation occurs because each component of a mixture has a different affinity for the stationary phase, and thus will be adsorbed to a greater or lesser extent than the other components. A component which is quite soluble in the stationary phase will take longer to travel through it than a component which is not very soluble in the stationary phase but very soluble in the mobile phase. As a result of these differences in mobilities, sample components will become separated from each other as they travel through the stationary phase

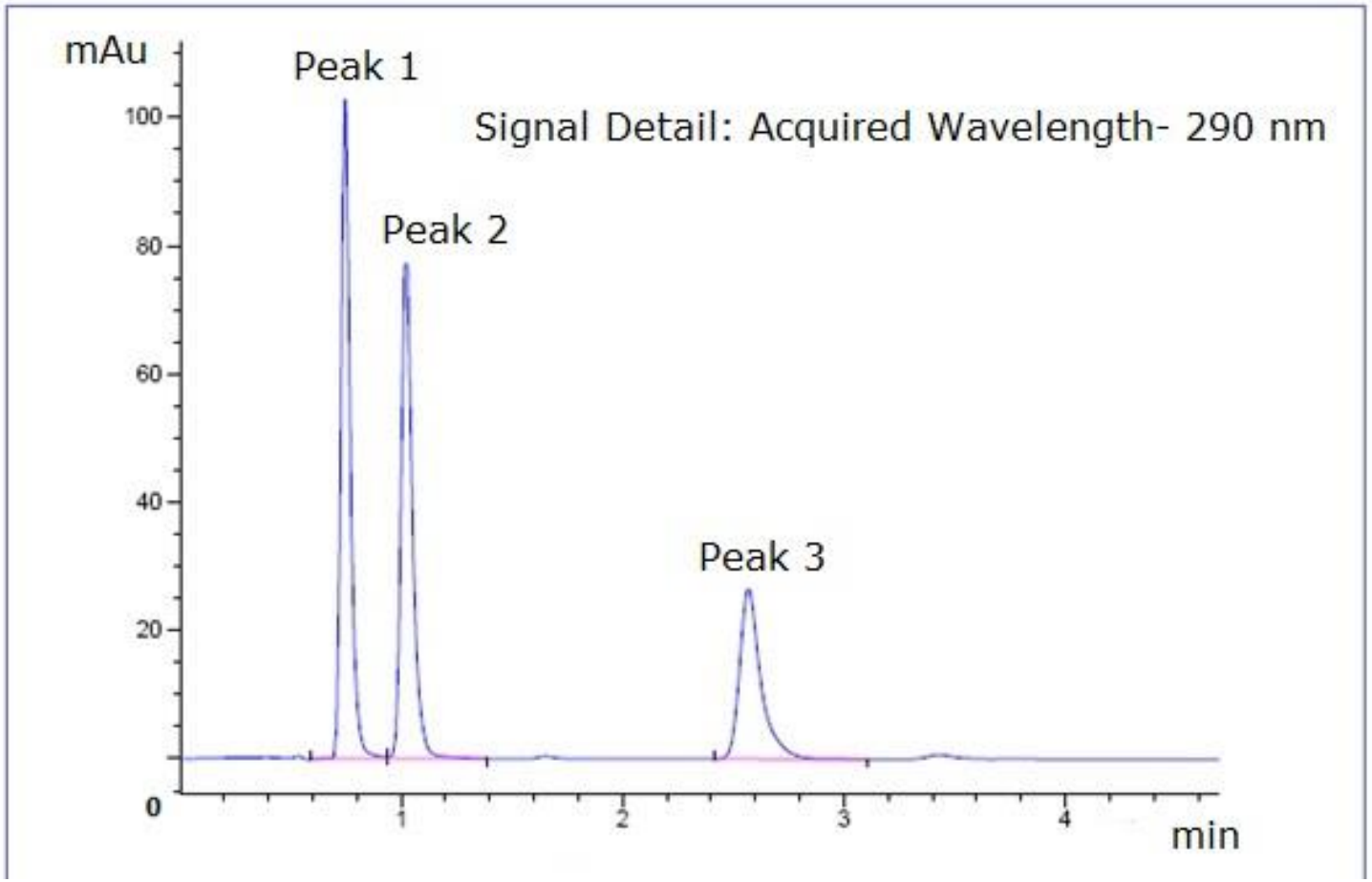


Flame-Ionization Detector Working Principle

- After chromatographic separation the rods are burned and ionized in a hydrogen flame. An electrode in the detector is disposed above the hydrogen flame and the ionized gas. Between this electrode and the gas burner there is a high voltage, which generates a positive polarity for the burner, and a negative polarity for the collector electrode. This makes negative and positive ions migrates respectively to the burner and collector electrode. An electric current, proportional to the amount of each separated substance, is detected quantitatively and amplified by a detector electrode surrounding the negative electrode. A data processing unit converts the amplified signal and calculates the percentage area of each peak in the run as a percentage of total area of all peaks, which in turn corresponds to the relative amount of each components. The peaks are identified as the different fractions based on a retention time specified for each reference peak in the reference method (Figure 6).

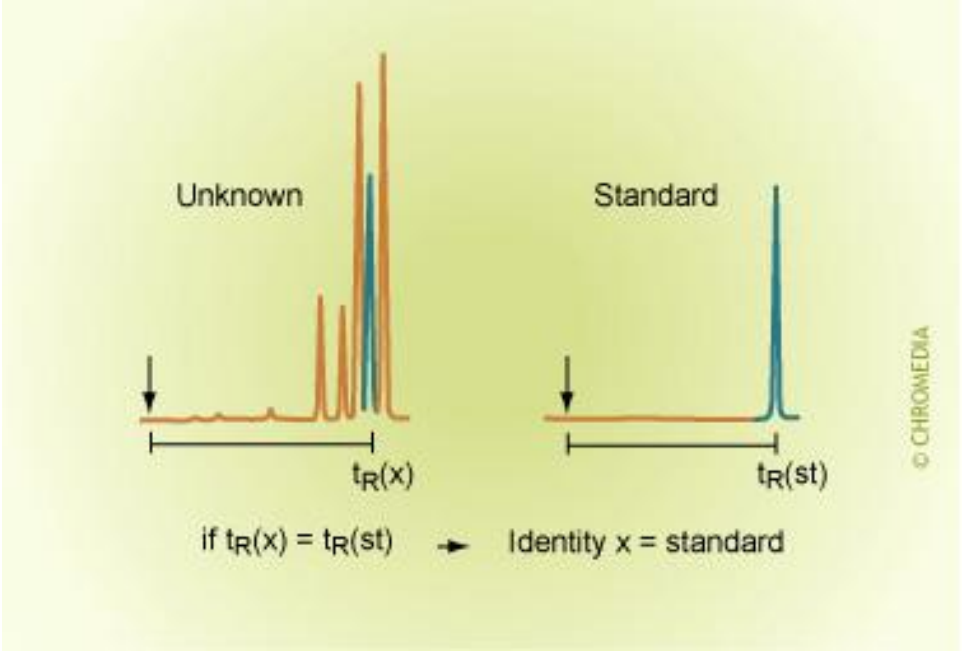


Example for a chromatogram



3. Monitorizing and Quantification (Continued)

- Identification of the peaks
- The compounds (peaks) of chromatograms are identified by retention time. Since the retention time is a specific property of a component, it may be used to identify the component. The retention time of the unknown component (peak) is compared to the retention time of a so-called standard.



3. Monitorizing and Quantification (Continued)

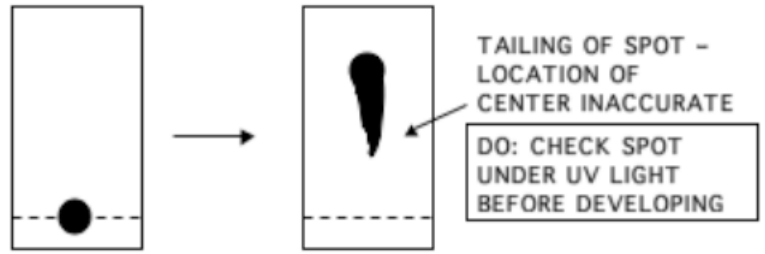
$$\text{Peak 1 \%}\left(\frac{g}{g}\right) = \frac{area\ of\ peak\ 1}{total\ peak\ area\ of\ the\ peaks\ (1+2+3)} \times 100$$

$$\text{Peak 2 \%}\left(\frac{g}{g}\right) = \frac{area\ of\ peak\ 2}{total\ peak\ area\ of\ the\ peaks\ (1+2+3)} \times 100$$

$$\text{Peak 3 \%}\left(\frac{g}{g}\right) = \frac{area\ of\ peak\ 1}{total\ peak\ area\ of\ the\ peaks\ (1+2+3)} \times 100$$

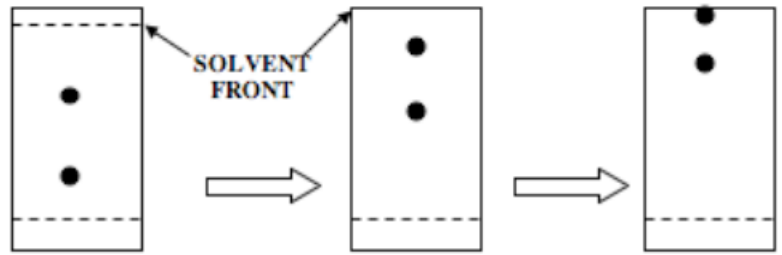
THINGS TO WATCH OUT FOR.

* OVERLOADING THE SPOT (SPOT TOO LARGE)

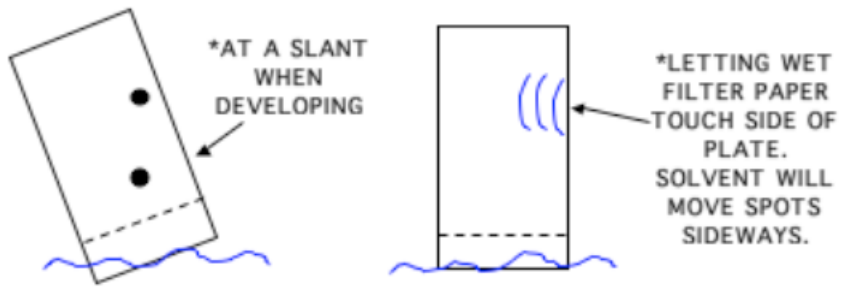


* SPOT TOO SMALL – DIFFICULT TO SEE

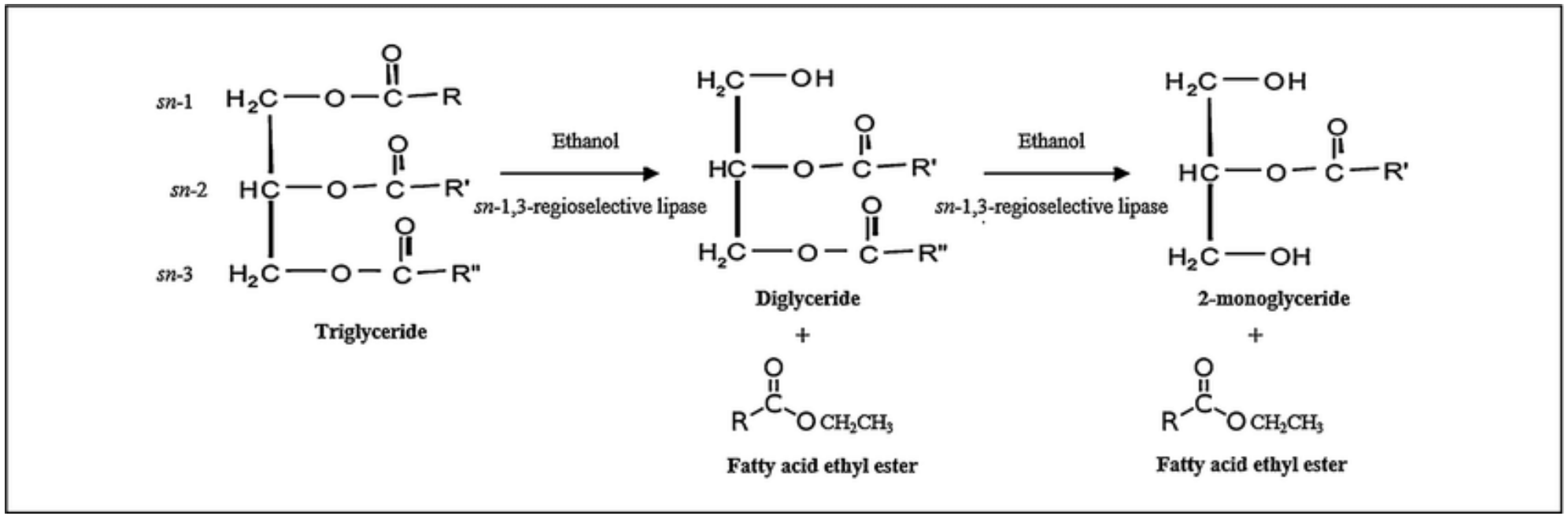
* ALLOWING SOLVENT FRONT TO REACH TOP OF PLATE



IF THE SOLVENT FRONT CANNOT BE SEEN, THE R_f CANNOT BE CALCULATED. STOP BEFORE FRONT REACHES TOP OF PLATE.



REPORT


sn-1
sn-2
sn-3
Triglyceride
Ethanol
sn-1,3-regioselective lipase
Diglyceride
+
Fatty acid ethyl ester
Ethanol
sn-1,3-regioselective lipase
2-monoglyceride
+
Fatty acid ethyl ester

REPORT

- 1. Purpose (10 p.)
- 2. Theory (10 p.)
- 3. Material/Metod (10 p.)
- 4. Procedure (5 p.)
- 5. Calculation (20 p.)
- 6. Discussion (45 p.)

CALCULATION PART

- Calculate the percentages of each peak (each component).

Discussion Part

- Identify the ethanolysis reaction products (2-monoacylglycerol, diacylglycerol, and fatty acid ethyl ester) and unreacted triacylglycerol on the chromatogram considering their polarities. Explain the reason.
- Explain why activation is done before the analysis.