3.3. CRYSTALLIZATION

The solid organic compounds obtained as a result of organic reactions are generally not pure. Purification of these organic chemical compounds containing impurities is usually carried out by crystallization from suitable solvent or solvent mixtures. However, direct crystallization process can not be applied to crude products with excess impurities. Because some impurities reduce the rate of crystallization, they can even prevent crystal formation completely, so that a considerable amount of matter can be lost. For this reason, pre-purification processes such as extraction or distillation should be applied before crystallization.

3.3.1. Crystallization Process

The purification of solids by crystallization are based on different solubility characteristics in the appropriate solvent or solvent mixture. The phases of the crystallization process are as follows:

1. Solubility of the impure substance in the appropriate solvent at the boiling point or near the boiling point.
2. Separation of hot solution from undissolved matter or dust by filtration.
3. Providing crystallization of the dissolved substance by cooling the hot solution.
4. Filtration of the crystals from the solvent phase and drying

After drying the resulting crystals are checked for their purity based on their melting point, if not pure, recrystallized with solvent. This process is called **recrystallization**. This process is continued until the melting point is fixed.

The separation of impurities by crystallization is as follows:

For example; the impurity in substance A is substance B (It is generally assumed that there is about 5% impurity in one substance). The solubility of A and B in a selected solvent and at a certain temperature is S_A and S_B. In this case, 3 possibilities can be mentioned;

1. The impurity may be highly soluble from the substance to be purified. $S_B > S_A$
2. The impurity may be slightly soluble from the substance to be purified. $S_A > S_B$
3. Their resolutions may be equal. $S_A = S_B$

As can be seen, in the first possibility it may be obtain pure substance A by crystallization, in which case the impurity will remain in the main solution.

FOR EXAMPLE: Solubility of 100 g of a substance A in a 100 ml of a suitable solvent at 15°C is S_A = 10 g / 100 ml and S_B= 5 g / 100 ml. If this substance is purified by crystallization technique;

In 100 g, there is 5 g of impurity (B) and 95 g of substance A. Whole substance B is dissolved at 15°C in 100 ml of solvent, 10 g of A is dissolved; according to this, 85 g of substance A can be crystallized purely.

3.3.2. Properties of Crystallization Solvent

1. The substance to be purified should be solved a lot at high temperature, little at low temperature
2. It should give the well-formed crystals of the substance to be purified.
3. It should be readily separable from the crystals of the substance to be purified and boiling point should be low.
4. It should not react with the substance to be purified
5. It should not be volatile, flammable and toxic, should be easy to find.

Some of the most commonly used solvents in crystallization are: Water, ether, acetone, chloroform, methanol, carbon tetrachloride, ethyl acetate, ethanol, benzene, petroleum ether etc. Heating in the presence of flammable solvents such as ether, acetone, methanol, ethanol, ethyl acetate, benzene, petroleum ether should not be carried out in the naked flame, water bath should be used. The most commonly used solvents and properties in the crystallization process are shown in the following table:

Solvent	**B.P.**	**Properties**
Distilled water	100	Used wherever convenient
Diethyl ether	35	Flammable
Acetone	56	Flammable
Chloroform	61	Nonflammable, steamy toxic poisonous
Methanol (99 %)	64.7	Flammable, toxic
Carbon tetrachloride	77	Nonflammable, steamy toxic poisonous
Ethyl acetate	78	Flammable
Methanol (Technical, 95 %)	77.8	Flammable
Ethanol	78	Flammable
Petroleum ether	40-60	Flammable
Acetic acid	118	Pungent

Since the ether is too volatile, it climbs up the sides of the beaker, therefore it should not be used as much as possible in crystallization processes. In addition, the material remains as residue in the bottom of the beaker because the ether volatilizes in a short time. Carbon sulfur also should not be used as much as possible because it forms low flash point mixtures in air.

The choice of solvent in crystallization is usually determined experimentally. Practically, if 0.1 g of the substance is dissolved in 1 ml of solvent without heating or 0.1 g of the substance is not dissolved by heating with 3 ml of solvent, it is not suitable.

If the substance to be crystallized dissolves easily in one solvent and poorly in another, pairs of solvents can be used for a good crystallization operation. However, these two solvents must be

intermiscible. For this, the substance is first dissolved in the solvent in which it is dissolved a lot and then the solvent in which the substance is poorly dissolved is added little by little as hot. When slight turbidity occurs, little of the first solvent is added and it is allowed to crystallize in the cold. Alcohol-water, benzene-petroleum ether, acetone-petroleum ether are among the most commonly used solvent pairs. Theoretically, two properties are used in solvent selection;

1. A substance is very soluble in the solvent where its chemical and physical properties are similar.
2. A polar substance dissolves more in a polar solvent than in an apolar solvent. Polar compounds are very soluble in water. Especially if they have hydrogen bond, their solubility in water increases even more. For organic structures containing carboxylic acid, alcohol, amine and amide, the solubility in water is high. Besides, the salts of the organic substances are easily soluble in water. All hydrocarbons and alkyl halides are insoluble in water. Nonpolar solvents, such as ether, benzene, dissolve most of the nonionic compounds. In general, as the fragment of hydrocarbons increases in organic compounds, that is, as the number of C increases, their solubility in water decreases and their solubility in nonpolar solvents increases. As the molecular weight increases, the solubility in water decreases. Nevertheless, there are exceptions. For example, the nonsubstituted amides are less soluble in water than the substituted amides. Because an association occurs in nonsubstituted amides *via* hydrogen bridges, so that case reduces the solubility in water. Thus, the addition of halogen into the structure is another effect that reduces the solubility in water. Water, formic acid, acetic acid, methanol for polar solvents; benzene, acetone, chloroform for apolar solvents give examples.

3.3.3. Crystallization Techniques

A suitable solvent for the chemical compound is found and the solution is heated until the boiling point is reached. The boiling solution should be filtered rapidly before being allowed to cool. For this, it is usually used pleated filter paper and a funnel with a large short neck. The large and short neck prevents the formation of crystals by cooling down the solution and difficulties in filtration by clogging. No crystals remain on the filter paper with a good filtration. The filtered solution is covered with a watch glass and allowed to cool. The size of the crystals formed depends on the cooling process. Rapid cooling provides small crystal formation; slow cooling provides large crystal formation. Large crystals contain significant amounts of solvents. Small crystals may adsorb more impurities because the unit surface volume will increase. For this reason, cooling should usually done in the middle heat. The resulting crystals are filtered through flat filter paper or vacuum using a Buchner Funnel. Drying process can be carried out in the open air, under the melting point, in the fixed incubator or vacuum desiccator.

3.3.4. Difficulties in Crystallization

1. Decolorization: The crude product may contain color impurities. Because the crystals can adsorb these impurities, colored and dirty crystals are obtained. They can be removed by using substances that are capable of adsorbing impurities such as activated charcoal. A small amount of activated charcoal up to 1-2% of the weight of the crude product is added and boiled. The charcoal with high adsorption power usually adsorbs easily the large molecular impurities and removes the color of the solution. Activated charcoal should not be used in excess, otherwise it will be also absorbed the main substance and the efficiency will be reduced. The solution can be separated from the activated charcoal by filtration.
2. Crystallization difficulty and separation in oil-form: If the substance separates in oil-form (this may be due to rapid cooling or concentrating solution), it is dissolved by heating. Then the single-phase clear solution is cooled slowly or the cooling solution is stirred vigorously to prevent separation in oil-form, so that, even if the oil particles are formed, they are prevented from clustering and crystallization is provided. Sometimes crystallization does not start from saturated solutions, in which case the following steps are carried out to initiate crystallization;
    - Adding the seed crystal
    - Scratching with glass rod
    - Cooling down to very low temperatures
    - Volatilizing a little bit of solvent in the solution

3.3.5. Filtration Process in the Crystallization

The process applied to separate the liquid phase from the solid phase in a mixture is called filtration. Filtration is a very demanding process, since purification by good crystallization depends on perfect filtration. Filtering during crystallization is usually carried out twice:

- The filtration of the prepared hot solution before crystallization,
- The crystals are filtered out from the main solution, after the crystallization is completed.

1. The filtration of the prepared hot solution before crystallization:

Heat loss should be avoided as much as possible during the filtration of hot solutions. Otherwise, crystals form on the filter paper and funnel pipe in order to the solubility declines as a result of decrease the temperature, so filtration becomes more difficult. For prevent cooling should be filtered quickly, heated the funnel and filter paper to the boiling solution temperature, and prevented evaporation as much as possible. To achieve these conditions, flow pipe cut funnel and pleated filter paper are used. The funnel, which has filter paper soaked with solvent on it by covering with the watch glass, is placed on a beaker with some pure solvent in it, and the beaker is heated down. Then the pure solvent in the beaker is transferred to another beaker and immediately filtered into the same beaker. The funnel heating process can also be carried out using the solution itself to be filtered, or it can also

be heated by placing on the solution in the beaker or Erlenmeyer flask. Thus, the funnel heats up as it functions as a condenser, after that, the solution is immediately filtered by the funnel heated.

2. The crystals are filtered out from the main solution, after the crystallization is completed:

The process of filtering out from the main solution of the crystals is always done by applying vacuum, not by ordinary filtration. The vacuum is provided by a tromp fitted to the tap. The vacuum pump should not be used in this process because the solvent evaporates in order that the vapor pressure of the filtrate is high and increases the vapor pressure by dissolving in the oil of vacuum pump. Vacuum accelerates filtration as well as separates completely the crystals from the main solution. Buchner Funnel and Nuche Erlenmeyer flask are used if many substances are dissolved. After the filter paper is cut according to the funnel and placed in the funnel, first vacuum is made by operating the tromp, then the substance to be filtered is started to be poured into the funnel. Slow suction in the filtration process is more effective than rapid suction because very thin grains eluate onto the filter paper at rapid suction, which reduces the permeability of the paper. Because of the filtration, some main solution holds on to the crystals, which remain on the filter paper, in this case it is removed by washing with a small amount of pure solvent. As soon as the solvent dripping to Nuche Erlenmeyer finishes, the vacuum is cut off. The rest of the funnel is placed on the watch glass together with the filter paper by the aid of a forceps, or the funnel is turned upside down on the watch glass and the crystals are blown onto a watch glass by attaching a rubber hose to the pipe part of the funnel.

Glass filters (Gooch crucible) made by sintering glass powders at the filter part are used in the filtration of solvents to be reacted with filter paper (for concentrated acidic or basic substances). As they are expensive materials, special care should be taken in their use, no excess vacuum should be made and chromic acid solution should be used as a cleaner. Instead of the porcelain Buchner Funnel, perforated porcelain plated filters are used in the filtration of small amount of substance.

LABORATORY PRACTICE

Crystalization of Impure Acetanilide

0.5 g of dirty acetanilide is weighed. It is added as soluble as water on it in the beaker and is heated burner until boiling. While boiling, it is added a bit of activated charcoal, and then (later 1-2 minutes) it is filtered through plated filter paper (on the funnel and tripot). The filtrate is left (at the window edge) to crystallize. The resulting crystals are filtered through (a Buchner Funnel or) a flat filter paper, and then dried in the incubator or in the room heat. At end of the practice, the melting point (M.P.) is determined through a gun tube. Each pure chemical material has a fixed melting point. If this value is not reached as a result of the experiment, the purification process is repeated.

For the determination of the M.P., one terminal of a capillary tube is closed; the result material is put from other side (opened terminal) into the capillary tube. Capillary tube is connected to thermometer and they are put into the gun tube. One of the substances, which has high boiling point and can distribute the heat homogeneously like liquid Vaseline, glycerin (glycerol), liquid paraffin, is put into the gun tube and it is heated down via burner.

Acetanilide: $C_6H_5NHCOCH_3$

Acetanilide M.P.: 113-115°C

Questions

1. Write how to separate impurities in the crystallization process.
2. List the properties of the solvent to be used in the crystallization.
3. What can be done in the crystallization to remove the color of the colored product and prevent oil formation?

3.4. CHROMATOGRAPHY

Chromatography was first developed and used by Russian-Italian botanist Mikhail Tsvet in the early 1900s. Tsvet observed that the solution with petroleum ether of the plant extract had passed through the CaCO3 adsorbent in a glass column and that there was a separation with yellow, green bands on the column. The development by attracting other researchers' attention was in the 1930s because of the fact that the first publications in this area was Russian.

Chromatography is a method that allows different chemical substances forming a mixture to be separated from each other based on the dispersion balances or the different interactions between the two phases, which do not mix. Chromatography with another definition is a generic term for separating different chemical substances in a mixture based on such principles as adsorption, solubility, capillarity, ion exchange or molecular sieve between two separate phases.

The substances separated by chromatography can be identified, which is also a purification method, since they can be isolated. In other words, chromatography is a qualitative and quantitative method of identification, since it enables the identification and quantitation of separated substances.

Common to all chromatographic methods:

Stationary phase: Stable (static) phase ……...may be solid and liquid,

Mobile phase: Movable phase…….……may be liquid and gas.

Components forming the mixture (sample): They exhibit different migrations between these two phases (stationary-mobile) which do not mix and thus can be separated from each other.

3.4.1. CLASSIFICATION OF CHROMATOGRAPHIC ANALYSES

3.4.1.1. Classification of Principles (Based on Mechanism of Separation)

a- Adsorption chromatography
b- Partition chromatography
c- Ion exchange chromatography
d- Ion pair chromatography
e- Molecular sieve chromatography
f- Affinity chromatography
g- Electrochromatography

a- Adsorption Chromatography:

Adsorption is the superficial interaction between a solid substance and a liquid soluble compound. Here, the stationary phase is a high adsorption capacity (Al_2O_3, Silicagel) and the mobile phase is gas or mostly liquid.

Bonds that play role in adsorption;

- Van-der-Waals bonds
- Dipole-dipole interaction power
- Hydrogen bond
- Ionic bonds
- Chelate bonds
- Rarely irreversible covalent bonds.

The basic principles of this type of chromatography are:

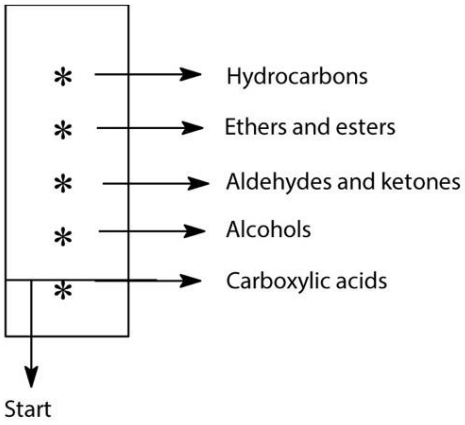
a- Saturated hydrocarbons are hardly adsorbed, so they migrate very quickly. The adsorption of unsaturated hydrocarbons increases with the number of double bonds and their conjugation numbers. As a result, a non-polar solvation is required with an active adsorbent for separation. As a result, a non-polar solvent is required with an active adsorbent for separation.

b- Generally, adsorption affinity increases with formation of functional group on a hydrocarbon. Among the functional groups, the following ranking can be made.

-CH3 ⟶ **-O-alkyl** ⟶ **C=O** ⟶ **-OH** ⟶ **-COOH**

⟶ polarity, adsorption increase

For example, if benzene is used as the solvent, in the chromatography plate the ethers and esters eluate to the upper part of the plate, the ketones and aldehydes are relatively in the middle, the alcohols are below them and the acids remain at the beginning. Therefore, the separation takes place according to the polarity of the compounds.



c- If there are many substituents in one molecule, it can be said that adsorption affinities are roughly interrelated. Especially steric effect is important for functional groups in aromatic rings

b- Partition Chromatography:

In partition chromatography, both phases are usually liquid. For this reason, liquid-liquid chromatography is also called. The stationary phase is usually more polar than the mobile phase

(mostly water) and is adsorbed as a thin layer of film on a solid support (kieselguhr, cellulose, etc.). The mobile phase is less polar than the stationary phase and another liquid that does not mix with it. In liquid-liquid chromatography it is sometimes desirable that the stationary phase is less polar. This chromatographic scheme is called “reverse phase liquid-liquid chromatography”.

This method is often used to separate homologous serials. In partition chromatography, the mobile phase can sometimes be gaseous. In this case, the stationary phase is a liquid impregnated with an inert solid which is not an adsorbent. With this method, the chromatographic separation of the readily volatile or gaseous substance is provided.

In partition chromatography, the partition coefficient of Nerst is valid. According to Nerst; "The ratio each other of the concentration in two seperate liquid phases of a third substance dissolved in the two liquids mixture, which do not mix with one another, is fixed."

$K = C_s / C_m$

K : Partition coefficient

C_s : Concentration at the stationary phase

C_m : Concentration at the mobile phase

If the K value is large, the concentration at the stationary phase is higher than at the mobile phase. It means that the molecule stays at the stationary phase longer.

c- Ion Exchange Chromatography:

Some solid substances may be ion exchanged between solute (mixture to be separated into components) and solid when they are exposed to ionizable substance solutions. These solid things are called “ion exchangers”. The ion exchangers may be inorganic or organic. Examples of inorganics include clay and zeolite. Organics are frequently used in the analysis process and are called "ion-exchange resins". Ion exchange resins are polymer compounds containing ionizable groups and are usually present in the form of small spheres or granules. Cation-exchange resins and anion-exchange resins are divided into two. In the cation exchange resin, the resin part of the polymer has anionic character and the polymeric cation is replaced by another cation in the solution. In the anion-exchange resin, the resin part has cationic character. The ion-exchange resins are generally styrene and divinyl benzene copolymers. In ion-exchange chromatography, the stationary phase forms the resin. The mobile phase is only liquid. In this method, the analyzed solution is distributed according to the ionic equilibrium rules between the mobile and the stationary phase.

d- Molecular Sieve Chromatography:

This method is used to separate macromolecules. The stationary phase and the mobile phase are the same structure and composition. In this method, a porous structure which supports the stationary phase is needed. For this purpose, hydrophilic or hydrophobic gels are used.

Hydrophilic gels: They are used with aqueous solvent and swell in aqueous media. This type of molecular sieve is called “gel filtration chromatography”. It is often used in biochemical applications to especially remove the salt of the protein solution.

Hydrophobic gels: They are used with organic solvent. This chromatographic application is called “Gel permeation chromatography”.

e- Affinity Chromatography:

Polyamide is used as the stationary phase in affinity chromatography.

Hydrogen bonds are formed between the phenol or nitro compounds present in the mixture to be analyzed and the polyamide stationary phase, thus adsorbing. Here the mobile phase is often liquid, and rarely gas.

3.4.1.2. Classification by Application Technique (Based on Shape of Chromatographic Bed)

Chromatographic methods can be seen as a rich area of analysis with different application techniques.

A - Planar Chromatography	A_1 - Thin layer chromatography
	A_2 - Paper chromatography
	A_3 - Preparative thick layer chromatography
	A_4 - Electrochromatography (electrophoretic chromatography**)**
B - Column Chromatography	B_1 - Column chromatography
	B_2 - Gas chromatography
	B_3 - High pressure liquid chromatography (HPLC)
	B_4 - Capillary electrochromatography

A_1 - Thin Layer Chromatography (TLC)

The TLC is a physicochemical separation method. It has an important place with fast results, good resoluion and economic application advantages. The most commonly used adsorbents for the stationary phase are silicagel, aluminum oxide, kieselguhr, cellulose and its derivatives and polyamides. The adsorbent material used as stationary phase in İTK is coated on glass, plastic or aluminum plates as a thin layer and in homogeneous thickness. For coating the plates, the adsorbent is mixed well with a 1: 1.5 ratio distilled water until a homogeneous mixture is obtained, taking care not to form air bubbles in a wide-necked ball. About 0.5%, more distilled water is added and mixed again. This mixing time should not exceed 90 seconds. The suspension prepared in this way is applied onto the plate with the help of the spreader. The prepared plates are used after being activated by holding the body 30 ' at 110 ° C. Activation is important. This is because the adsorbent has a significant effect on the separation.

The mobile phase used in the TLC may consist of one or several solvents. Solvent elution in adsorption chromatography is grouped under the so-called eluotropic series according to their effect, i.e. their dragging power, and is increased by the polarity of the elution effect of a solvent. Polarity is proportional to the "dielectric constant" of a substance. In this case, the substance, which has bigger dielectric constant, has greater polarity, and accordingly the effect of elution is higher.

Solvent	**ϵ (dielectric constant) at 20 ºC**
n-Hexane	1.890
Heptane	1.924
Cyclohexane	2.023
CCl_4	2.238
Benzene	2.284
$CHCl_3$	4.806
Ether	4.34
Ethyl acetate	6.02 (25 ºC)
Pyridin	12.30 (25 ºC)
Acetone	20.70 (25 ºC)
Ethanol	24.30 (25 ºC)
Methanol	33.62
Water	80.35

Benzene or chloroform can be initially selected for an unknown substance. If the substance remains at the start, a second solvent is added to the used solvent. On the contrary, if the substance is rapidly migrating and drifting near the front, a weaker should be selected.

There are a number of factors that affect the development. These:

a- Polarity of the substance: The more polar substance, the more held by the adsorbent.

b- The polarity of the solvate forming the mobile phase: The more polar the mobile phase, the weaker the bond between the substances and the stationary phase. So the substance drifts so much over the plaque.

c- Activity of Adsorbent: The more active adsorbent, the greater interaction with the substance.

TLC cuvettes or tanks: It is important to ensure good atmospheric saturation when working with high precision. This can be achieved by using filter paper. Small tanks are preferred due to the minimum volume and the advantages associated with it.

Some of the terms used in chromatographic analyzes are:

Solute: Mixture of substances (sample) to be separated into their components.

Start: The point where the soluton is applied on the stationary phase.

Front: The distance the mobile phase reached on the stationary phase.

Development: The solution of the mixture of the substances to be separated in a suitable solvent is applied on the plate in small droplets and this plate is placed in a tightly sealed tank containing a suitable solvent system (mobile phase). On the mobile phase adsorbent, while the capillaries move up, the substances in the mixture are separated from each other. This is called developing the plaque.

In a normal development, the distance between start and front is 10 cm. The mobile phase is also utilized in a gradual, bi-directional and circular development which can provide better discrimination than simple development with a 10 cm rise.

TLC Application

After the mobile phase has been filled in the tank at a height of 5-8 mm, a clean filter paper is placed so as to surround the tank inner wall all around. This ensures that the tank is saturated with solvent vapor.

The solution of the sample in the appropriate solvent is spotting on the plate that it is 1 cm from the side edge of the adsorbed-coated plate and 1.5 cm from the lower edge. The distance between the spots should not be less than 10 mm and the diameter of the spots should be 3-5 mm. In this way, spotting on a line 1.5 cm from the bottom edge of the plate with glass tubes.

The prepared plate is placed in the saturated tank and the plate is removed from the tank after the end of the mobile phase rise (separation of the substance mixture from each other) (development) is over. The front is marked with a sharp-pointed pen.

What is important here is that the sample solution and the reference mixture are always spotted side by side on the plate. Otherwise, if the reference solution is not spotted, comments can not be made or if they are not side by side, faulty interpretations can be made.

When any synthetic environment is examined chromatographically, the following information can be accessed.

a- The reaction is not progressing (only if there is staining of the starting materials in the chromatogram)

b- The reaction proceeds over time (if the chromatogram contains both starting materials and product stains)

c- The starting substances have been depleted or have been completely converted into products,

d- Transition to the final product on an intermediate product, etc.

In order to identify the substances that are discriminated in the manner described above, in other words, to reveal the spots;

a- Physical methods

b- Chemical methods

c- Biological and enzymatic methods are applied.

It is simple to identify spots on the chromatogram if the excluded substance absorbs itself in the UV region or if it shows fluorescence when irradiated with UV rays at 254 nm or 366 nm. Otherwise, a chromatographic marker is sputtered and substance spots become apparent by utilizing the process of forming colored derivatives of a substance by a chemical reaction. In some cases, spots can also be detected with biological methods.

For the identification of the substance; the distance between the midpoint of the visible spots and the start is precisely measured with a millimetric ruler (A). This distance is proportional to the development distance (B), the distance between start and front.

R_f (Resolusion factor) is a constant in certain conditions that determine the position of a substance in an TLC plate.

$$R_f = \frac{\text{The distance between the midpoint of the spot and start (A)}}{\text{The distance between start and front (Development distance) (B)}}$$ Always $R_f < 1$

Under certain conditions, the R_f value of a compound is a physical constant and helps identify the compound by determining other properties of the compound. It is not only true to speak of an absolute R_f value. Because it may change depending on the circumstances.

Factors Affecting R_f Value:

Adsorbent quality: Adsorbent particle size is important. However, this has no effect on the R_f value when affecting the development time.

Layer thickness: The standard thickness of the coating technique with aqueous method is 0.25 mm. However, for a reproducible R_f according to Stahl, the thickness should not be less than 0.15, although it falls below this value as a result of drying. It can rise up to 5mm thick in preparative works. The layer thickness does not change R_f but it changes the speed of development.

Activation of the layer: 30-60 minutes - 105-110 °C to dry, desiccator is required to keep. (Cellulose is 10 minutes at 105 ˚C)

Quantity of substance: 10-20 μg for many substances. Excessive substance may cause increased or decreased R_f value.

Solvent quality: For solubility analysis, pure solvent should be used. Due to evaporation, the rate of the solvate system will change, so it should be renewed frequently.

Temperature: Adsorption chromatography is less affected by temperature than dispersion chromatography. Generally, an increase in R_f values with temperature is observed. (Not important between 18-38 ˚C)

Tank atmosphere: If the atmosphere of the tank or bath is saturated with the solvent system, the development time is shortened. The R_f value in the unsaturated atmosphere rises.

Application technique: Chromatographic plate placed in the tank can change R_f even if the plate gradient is small. However, different R_f's are achieved with the descent, descent, or horizontal technique.

Adsorbent pre-adsorption of solvated vapors: Important for dispersion chromatographic technique. R_f values.

Development size: R_f increases slightly as the distance increases.

Second substances: Especially in the adsorption technique, it changes R_f.

Advantages of the TLC:

1- The basic tools used are quite simple and economical,

2. The distinctions are quite rapid (better than the column and paper)

3- Corrosive reagents may be used to identify the spots,

4- It gives definite and repetitive results for many applications,

5- Provides the possibility of using a wide variety of adsorbents,

6- With High Performance Thin Layer Chromatography (HPTLC) system

a- Densitometric chromatogram scanning,

b- Quantitative calculation and printing of results is possible.

A_2 - Paper Chromatography

Although it is a very important method of analysis in the beginning (especially for polar-hydrophilic compounds), it has largely left its place TLC today.

It is generally a separation method based on the principle that the substances adsorbed on the filter paper, move differently on paper with the help of a suitable solvent. The filter paper naturally contains some water. For this reason, this method is a chromatograph where the liquid-liquid diffusing principle is valid. The separation of the substances applied to paper depends on the difference in dispersion between the solvent that moves on the paper and the water that the paper contains. Here, the stationary phase is water molecules, the paper serves only as support. The mobile phase consists of a solvent or solvent mixture.

The paper chromatography and TLC applications are similar. Three separate solutions are prepared for the identification of the unknown substance in both TLC and paper chromatography.

- the solution containing the substance to be analyzed,
- the solution containing the standard (reference)
- Solution containing both sample and reference substance in equal concentration.

$$R_f = \frac{R_f\ (\ddot{o})}{R_f\ (c)} = 1 \longrightarrow$$ The sample is the same as the reference.

The Rm value is also calculated using the TLC and Paper chromatographs.

Indicated by the formula: $$Rm = \log\left(\frac{1}{R_f} - 1\right)$$

Different development methods are applied in paper chromatography.

* Descending method: Solvent system is given from top to bottom.
* Ascending method: Solvent system is given from bottom to top.
* One-way and two-way chromatography
* Circular chromatography

In circular paper chromatography, the center of the special circle chromatographic paper is first marked. Approximately 1 cm away from the center, a solution of the mixture of substances to be separated in circular form is applied. A ring with a diameter of about 2 mm is then opened in the center. At the same time, a petri dish containing the appropriate solvent system is saturated with solvent vapor. Subsequently, a sheet of paper or cotton wool rolled so as to be able to bridge between the solvent and the chromatography paper is placed in the ring opened to the center of the chromatography paper and closed in the mouth of the chromatography paper petri dish. The solvent proceed in the stationary phase by drawing circles originating from a center. Meanwhile, the substances in the mixture migrate differently, forming R_f's different spot circles.

The ascending method is applied according to the tube technique. Here again, after being cut into special chromatography paper strips, the substance solution is applied approximately 1 cm above the lower edge. Once the appropriate solvation system has been placed in a given glass tube, the paper ribbon is placed in the tube in such a way that it contacts the mobile phase, and the mouth is tightly closed. In this way, the substances in the mixture are separated.

The value of R_f is again used to evaluate the results in paper chromatography. The R_f value varies depending on various factors. These:

1- The type of paper used

2- Method used

3- Used solvent

4- Concentration of substance and application area

5- Direction of development

6- Temperature

R_f values are more reliable in paper chromatography if certain conditions are provided.

A_3 - Preparative Thick Layer Chromatography

It is another chromatographic method based on the principle of TLC. The difference from the TLC is that the adsorbent layer thickness and the amount of applied substances are much. For this reason, it is possible to isolate and separate substances at the milligram or gram level. In this method, substance solution in strip form is applied to an adsorbent plate conveyed thick, and after the end of development, the strips formed are removed by scraping with a spatula, extracted with a suitable solvent, filtered. After evaporation of the liquid portion, the residue is crystallized by dissolving in a clean and suitable solvent.

A strip of compound solution is applied to a thick adsorbent plaque, and after the end of development, the strips formed are removed by scraping with a spatula, extracted with a suitable solvent, filtered. After evaporation of the liquid portion, the residue is crystallized by dissolving in a clean and suitable solvent.

B1 - Column Chromatography

The compounds to be separated from the mixture have different adsorption constants against an small particles filled in a glass tube as small particules. The adsorbent in the glass tube is called the stationary phase, while the solution containing mixture of the compounds poured into the column for separation is called the mobile phase, which is the fresh solvent that allows adsorption and desorption to proceed in the column. Solut brings adsorbed and desorbed compounds by the influence of the mobile phase as different bands. If the compounds contained in the solute are colored, they appear in colored bands; if they have the fluorescence properties, they can be rendered visible with UV light.

In order to have a good discrimination for the adsorbent in column chromatography,

* Having a high but selective adsorption power,
* The surface area should be large (small particle fraction, particle diameter too small).

In column chromatography, cellulose, silicagel, active magnesium silicate, active aluminum oxide are used as the stationary phase.

Column is prepared in two forms, dry and wet.

Preparing column by wet method:

Approximately 25 g of the adsorbent is thoroughly mixed with 75 ml of organic solvent to form a slurry, cotton and round cut filter paper placed into the bottom of cleaned column and the mixture poured slowly and carefully. A long glass bag is inserted into the colon to prevent air bubbles. When the column is filled with the adsorbent in the slurry, wait for the adsorbent to precipitate. The adsorbent particles adhering to the inner wall of the glass tube are washed with the same organic solvent and sent to the column. After the column is homogeneously filled, a round cut filter paper is placed on the adsorbent. The solution in the mobile phase of the sample is carefully added from the side of the column with the help of a baguette, taking care that the level of organic solvent is at least 4-5 cm higher than the filter paper. The tap speed is adjusted. With the addition of fresh solvent continuously from above, the solution is allowed to proceed in the column. By observing with TLC, different fractions of substances are obtained. If necessary, the elution can be continued with solvent mixtures according to the structures of the substances to be separated. The process is completed at the point where the final fraction does not contain any spots on the TLC.

3.4.1.3. CLASSIFICATION OF PHASE TYPES (Based on Phases)

1- Liquid Chromatography	a- **L**iquid / **S**olid **C**hromatography (LSC)
	b- **L**iquid / **L**iquid **C**hromatography (LLC)
2- Gas Chromatography	a- **G**as / **S**olid **C**hromatography (GSC)
	b- **G**as / **L**iquid **C**hromatography (GLC)

Table 3.1. Sumary of Chromatographic Methods

Stationary Phase	**Mobile Phase**	**Method of Application**	**Based on Physical Priciple**
Solid	Liquid	TLC, Column Chrom.	Adsorption (if solid phase is ion exchange resin, ion exchange)
Liquid	Liquid	TLC, Column Chrom., Paper Chrom., HPLC	Partition
Solid	Gas	Gas / Solid Chrom..	Adsorption
Liquid	Gas	Gas / Liquid Chrom.	Partition

Enantiomer Separation in Chiral Compounds:

Equal proportion of the (+) and (-) enantiomers is called racemate. The whole physicochemical properties of the enantiomers are the same except the angle of rotation of polarize light. Since the solubilities of the enantiomers are the same, the resolution of the racemic compounds (enantiomeric separation) is not as simple as the separation of the other mixtures. In general, the conversion of enantiomers to diastereomeric salts facilitates their separation by differences in their physicochemical properties. Some methods applied for this purpose in chromatographic studies are listed below. Also in these methods, the main purpose is to form diastereomeric salts.

a- Separation by using Chiral Derivatizing Agents (CDA):

In the achiral stationary phase, the reaction of the mixture (racemate) with CDA provides separation of diastereoisomeric derivatives.

b- Separation by using Chiral Mobile Phase Additives (CMA):

In the achiral stationary phase, the reaction of the mixture (racemate) with CMA provides separation of diastereoisomeric derivatives. Here, addition of Chiral-Counter-Ions (CCI) to the mobile phase is required.

c- Separation by using Chiral Stationary Phase (CSP):

In the chiral stationary phase, the separation is provided with the diastereomeric complexes are formed by the mixture enantiomers

LABORATORY PRACTICE

1- TLC Application:

During the laboratory studies, caffeine, theophylline or caffeine + theophylline samples are identified the sample by comparing with the references. As the solvent system, acetone : chloroform : *n*-butanol : ammonia (30 : 30 : 40 : 10) is used.

2- Column Chromatography Application:

During the laboratory studies, the sample mixture included gentian violet, eosin B.A., dimethyl yellow and naphthol green is separated into its components using the solvent system included acetone : chloroform : *n*-butanol : ammonia (30 : 30 : 40 : 10).

Questions

1. What are the bonds that play a role in adsorption chromatography and explain the basic principles of such chromatography?
2. Classify chromatographic methods according to the application technique.
3. Define what solute, start, front, R_f and development are.
4. List the factors that affect R_f value.