

# CHROMATOGRAPHY

## A Separation and Purification Technique

### INTRODUCTION

Chromatography is a technique that may be used to separate components of a mixture as well as to identify organic substances and examine their purity. Chromatography encompasses several techniques

مبدأين ( TLC & Paper)

مبدأين

Thin layer (inert)

مطلية بطبقة سيلكا بيضاء  
polar (SiO<sub>2</sub>.xH<sub>2</sub>O)

Two principles are basically involved in chromatography: adsorption (as in thin-layer chromatography) and partition (as in paper chromatography), and certain terms are common to both types of chromatography.

In adsorption chromatography, separation depends on the selective desorption of the components of a mixture by the eluent (mobile phase) from the surface of a solid adsorbent (stationary phase). The adsorbent may be packed in a column (column chromatography) or spread as a thin layer on a glass plate as in thin-layer chromatography.

يعتمد الفصل اللوني على الاختيارية للمكونات بين السائل المتحرك وبين المادة الصلبة الثابتة

معبأة في

In partition chromatography, separation depends on partition of the components of a mixture between the stationary and mobile phases. The mobile phase may be a liquid (liquid-liquid partition chromatography) or a gas (gas-liquid partition chromatography).

يعتمد على توزيع عناصر الخليط بين السائل المتحرك والمادة الصلبة (يجزئهم)

### ANALYSIS OF CHROMATOGRAMS

In thin layer and paper chromatography, substances are characterized by their  $R_f$ -values (retardation factor). The  $R_f$ -value is a number (less than one) which is characteristic of a compound for a given adsorbent and developing solvent. It is defined as:

$$R_f = \frac{\text{distance traveled by the compound}}{\text{distance traveled by the solvent}}$$

المسافة التي قطعها المركب / المسافة التي قطعها السائل

In gas-liquid chromatography, compounds are characterized by their retention times.

**THIN-LAYER CHROMATOGRAPHY (TLC)**

This is one application of adsorption chromatography in which an adsorbent, usually silica gel or alumina, is spread out as a thin layer on an inert surface, such as a glass plate or microscope slide. The mixture is applied at one end of the coated plate and, as the mobile phase (a liquid) moves up the solid adsorbent by capillary action, the adsorbed components of the mixture get desorbed and carried along at different rates by the moving solvent.

Adsorption of the components of the mixture, on the surface of the adsorbent, occurs to differing extents depending on their structural features and polarity. **The more strongly adsorbed a given compound is, the slower it is transported by the mobile phase, and conversely, the more weakly adsorbed the compound is, the faster it is transported up the stationary phase.** The result is that the components of the mixture are separated into different zones or spots (Figure 20).



كلما زاد التصاق المواد  
بالجزء الصلب كلما قلت  
قدرة السائل على نقلها  
لمسافة أطول والعكس  
صحيح

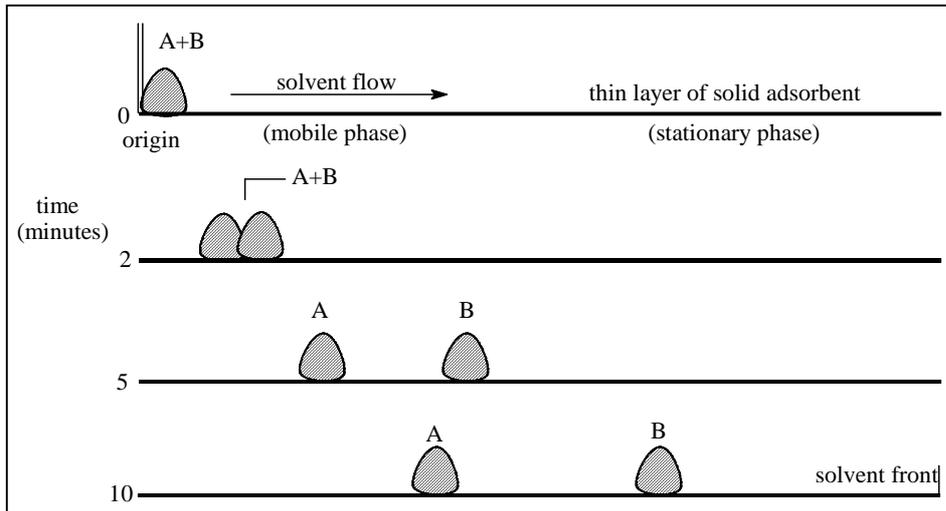


Figure 20. Separation by thin-layer chromatography

1 Separation by thin-layer chromatography depends on the kind and activity of the adsorbent (stationary phase), the polarity of the eluent (mobile phase) 2 and on the chemical nature of the components of the mixture. The most

3

common adsorbents employed in *TLC* are silica ( $\text{SiO}_2 \cdot x\text{H}_2\text{O}$ ) and alumina ( $\text{Al}_2\text{O}_3 \cdot x\text{H}_2\text{O}$ ), and the activity of these adsorbents is largely determined by their water content. For a given adsorbent and compound, the greater the polarity of the eluent, the greater is its ability to dislodge a compound from the surface of the adsorbent, and therefore the higher the  $R_f$ -value.

Eluting power of solvents:

Acetic acid > Ethyl alcohol > Acetone > Diethyl ether > Dichloromethane > Hexane.

← Increase the polarity &  $R_f$

### GENERALIZED EXPERIMENTAL PROCEDURE

**Preparation of *TLC* Plates.** Large glass plates (20x20 cm) are commonly used for quantitative separations, while microscope slides are usually used for qualitative purposes. A homogeneous slurry of the adsorbent in a volatile organic solvent (chloroform or dichloromethane) is poured over the glass plates and allowed to air-dry at room temperature. Microscope slides can be coated, two at a time, by dipping them into the slurry for sometime then holding them vertically to air-dry. The jar of adsorbent must be shaken thoroughly before each use to homogenize the slurry.

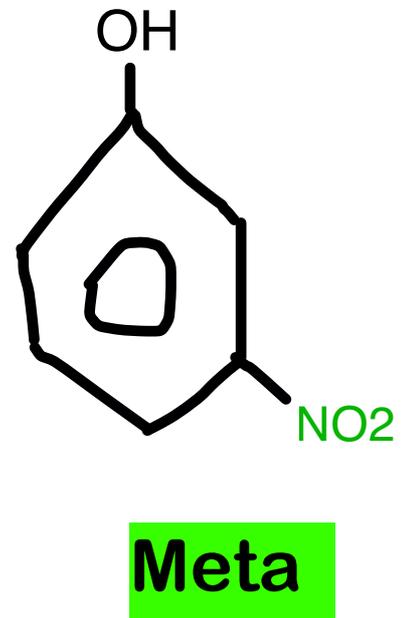
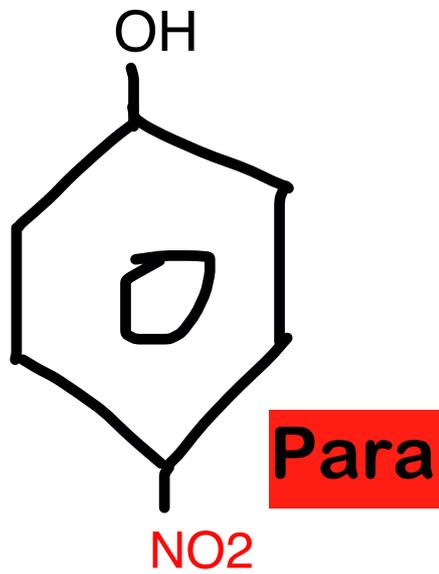
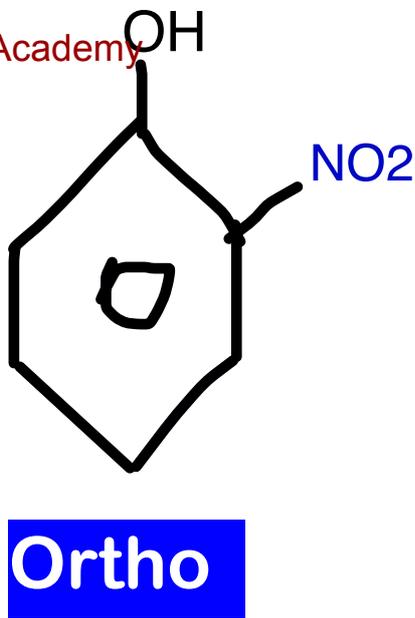
**Spotting.** The mixture to be analyzed is dissolved in a suitable solvent (1% solution). With a drawn capillary tube, a small amount of this solution is spotted on the *TLC* plate about 1 cm from the bottom (Figure 21). The spots should have a diameter not larger than 1-2 mm, since larger spots result in "tailing" and overlapping of close spots. Once the solvent evaporates from the spots, the plate is ready for developing.

**Development of the Chromatogram.** The eluent, also called developing solvent, is chosen on the basis of the nature and polarity of the compounds being studied. It is best to choose the solvent that will give a satisfactory

نشاطها يعتمد على محتواها من الماء

كلما زادت قطبية المادة السائلة يزداد ارتباط المركبات بها وبذلك تزداد المسافة التي تقطعها المركبات فتزداد قيمة  $R_f$

حفظ



الترتيب حسب القطبية **Para > meta > ortho**

كلما ازدادت قطبية المركبات يزداد ارتباطها بالمادة الصلبة وبذلك تقل المسافة التي يقطعها المركب وبالتالي نقصان قيمة **R<sub>f</sub>**

هذا بالنسبة ل :

**Polarity of the compound**

أما بالنسبة لقطبية المادة الصلبة  
**polarity of the stationary phase**  
تزداد القطبية بزيادة عدد جزيئات الماء بداخلها

Number of H<sub>2</sub>O



Polarity



R<sub>f</sub>



separation within the range of 0.2-0.8  $R_f$  values. The plate is placed in a developing chamber (e.g. a covered beaker) containing the solvent and lined with filter paper soaked in the solvent to help saturate the atmosphere with solvent vapors. When the solvent front reaches the finish line, the plate is removed from the beaker and placed on the bench top to air-dry.

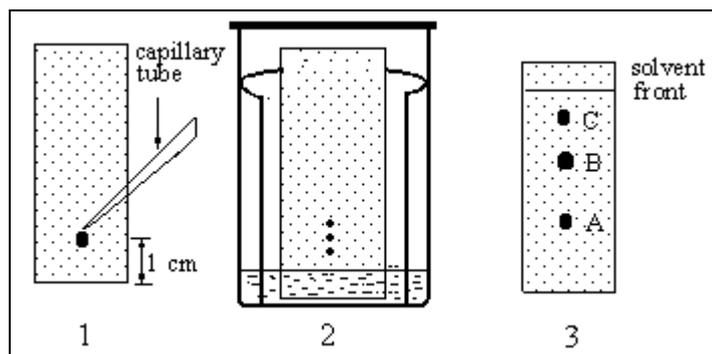


Figure 21. Steps in the TLC technique

إظهار النقاط التي تحتوي على المركبات

**Visualization of Spots.** Compounds on the plate are located according to their characteristics:

- If the spots are colored, they can be observed in ordinary light.
- If the compounds are colorless, they can be seen under UV-light where they appear as dark spots on a white background.
- Colorless spots may also be located with an indicator. Most organic compounds form complexes with iodine giving dark brown spots when the plate is exposed to iodine vapor. Sulfuric acid may also be used to make colorless spots visible. Most organic compounds turn black when sprayed with sulfuric acid.

إذا كانت النقاط ملونة فيمكن رؤيتها بالضوء العادي

1

2

3

### OBJECTIVES

- Determining the  $R_f$ -value for *o*- and *p*-nitroaniline by *TLC*.
- Separating a mixture of two dyes by paper chromatography.
- Determining the constituents of an analgesic drug by *TLC*.