

Chemistry technique

التقنيات المختبرية

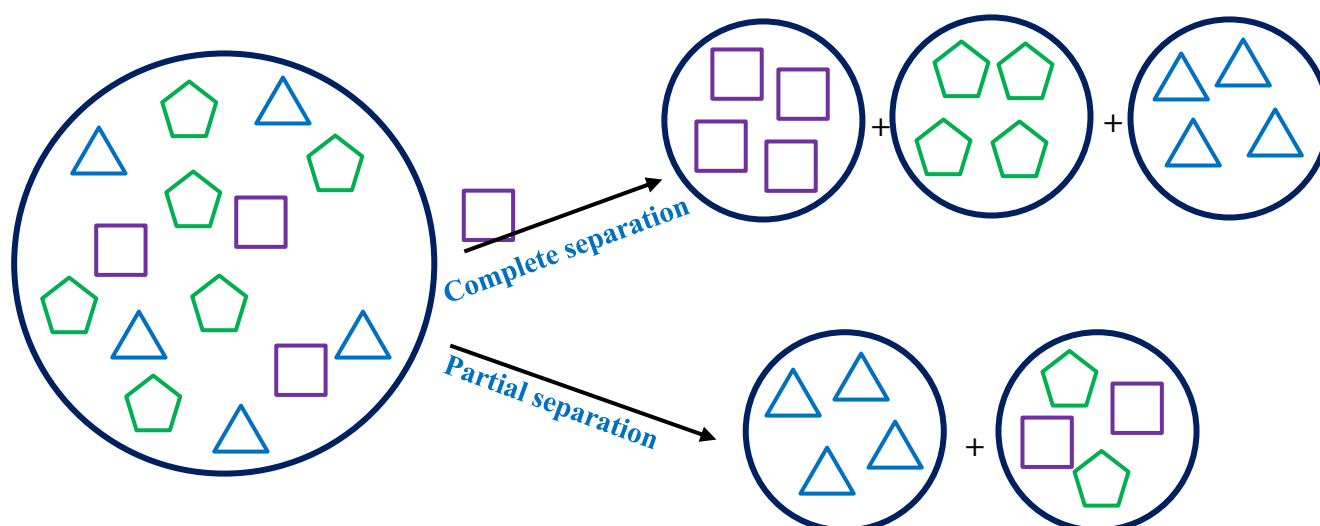
Chemistry technique include the basic of difference method:

1. Method of chemical separation and purification.
2. Base of chemical analysis.
3. Base of chemical technical.
4. Base chemical of lab. technology.

In general, the concept of chemistry technique includes technical preparatory and technical analysis.

Basis Definitions:

Separation: the isolation of the target chemicals from sample matrix, the separation can be complete or partial, and there is a difference of separation:



Separation can be achieved by differences in physical or chemical properties. The separation method that are in common use including:

1. Chemical or electrolytical precipitation.
2. Distillation.
3. Solvent extraction.
4. Ion exchange.
5. Chromatography.
6. Electrophoresis.
7. Field – Flow fraction cation.
8. Filtration.

9. Crystallization.
10. Centrifugation.
11. Fraction distillation.
12. Steam distillation.
13. Sublimation.
14. etc.

Filtration : to separate insoluble solid from a liquid particularly when the solid is suspended through the liquid slow.

Centrifugation: to separate an insoluble solid from liquid small amount of suspension fast

Crystallization: the process of forming crystals by cooling a hot concentrates solution and the recrystallization to separate solid from other solid based on their different solubilities in suitable solvent.

Extraction: to separate a compound from a mixture with suitable solvent.

Sublimation: to separate a mixture of two solids but only one sublimes.

Fraction distillation: to separate miscible liquids with widely different boiling points.

Steam distillation: to separate liquid which are immiscible with water and decompose easily below their b.p.

Chromatography: to separate mixture of substance.

Phase: the state of substance when are homogenous:

Two liquids immiscible → two phases.

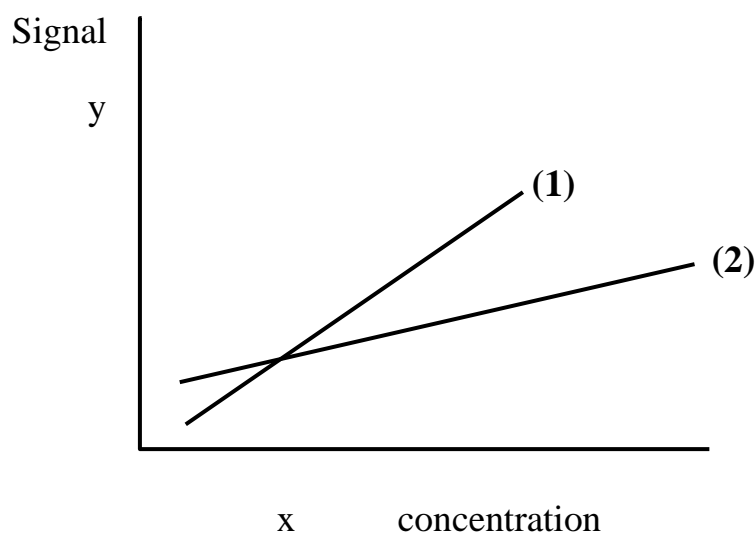
Sugar + salt = tow phases, but the solution of the sugar and salt are one phase.

Two solid substance in different crystallization forms are two phases.

الدقة (Accuracy): اختلاف القيمة الحقيقية عن القيمة العملية.

التوافقية (Precision): مدى تقارب النتائج مع بعضها البعض عند تكرارها.

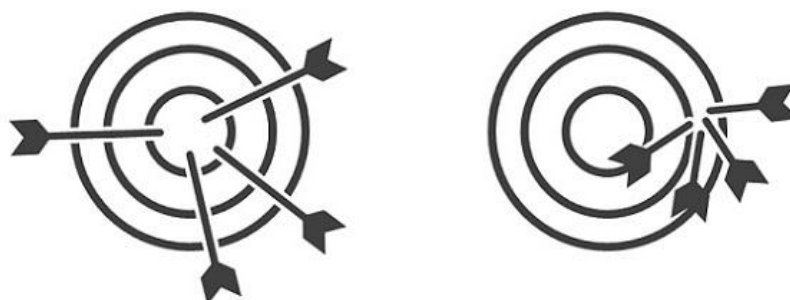
الحساسية (Sensitivity): مدى تغير الإشارة المقاسة مع تغير التراكيز.



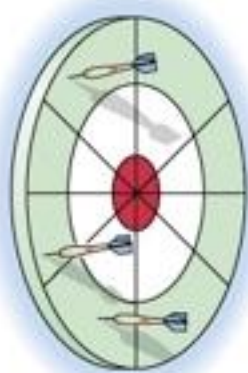
$$y = ax + b$$

a = slope

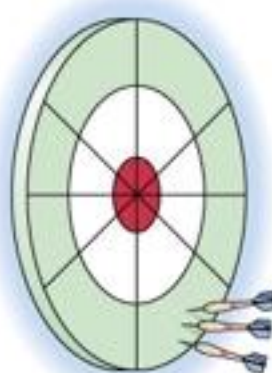
(1) more sensitivity than (2)



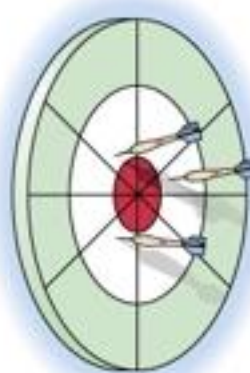
Accuracy Vs Precision



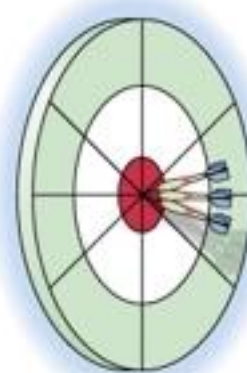
(a) *low accuracy*
— *low Precision*



(b) *low accuracy*
high Precision



(c) *high accuracy*
low Precision



(d) *high accuracy*
high Precision

Extraction:

Theory of extraction: distribution of solute between two phases is an equilibrium condition described by partition theory, this is based exactly on how the analyte moves from one phase to another. Extraction often uses two immiscible phases to separate solute from one phase into the other.

Type of extraction:

1. Liquid – liquid extraction. (L.L.E.)
2. Solid – phase extraction. (S.P.E.)
3. Solid – phase micro extraction. (S.P.m.E.)

Modern variations extraction:

1. Supercritical carbon dioxide extraction.
2. Ultra-sonic extraction.
3. Heat reflux extraction.
4. Microwave assisted extraction.

Liquid – Liquid Extraction (L.L.E.):

It's extracting a compound from a mixture with suitable solvent. Water is the solvent used to extract salts from a mixture containing salt and sand, and a nonaqueous solvent can be used to extract organic compounds.

Selection of the solvent in L.L.E.:

The selection of the solvent in L.L.E. depends upon:

1. Selectivity (high selectivity required).
2. Capacity (to reduce the amount of necessary solvent, the capacity of the solvent has to be high).
3. Miscibility (the miscibility of solvent and primary solvent, has to be low).
4. Recovery (high recovery required).
5. Density (high difference in density required).

6. Viscosity (low viscosity required).
7. Vapor pressure (low vapor pressure is required).
8. Polarity (like dissolve like).

Factor influence the L.L.E.:

1. Polarity:

أي ما يعبر عنه بالتجاذب القطبي بين المادة والمذيب، لذا فإن الأيونات اللاعضوية تتواجد بشكل أكبر في الطبقة المائية، بينما المركبات العضوية (غير القطبية) تتواجد بشكل أكبر في الطبقة العضوية وفق قاعدة (like dissolve like).

2. distribution affection (K_D) معامل التوزيع

$$K_D = \frac{C_{org. layer}}{C_{aq. layer}} \quad \begin{array}{l} \text{التركيز في الطبقة العضوية} \\ \text{التركيز في الطبقة المائية} \end{array}$$

For example: extraction of benzoic acid (B.A.) by ether from aqueous:

$$K_D = \frac{C_{BA(ether)}}{C_{BA(aq.)}}$$

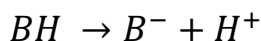
It means K_D is the essentially the ratio of the concentration of the solute in two different solvent once the system reaches equilibrium. At equilibrium, the molecules naturally distribute their selves in the solvent where they are more soluble.

Distribution coefficient play a **large role in the efficacy of a drug in order for a drug to be absorbed in to a brain cell**, it must pass through what is called the blood – brain barrier, into the brain cell. The drug must have enough water solubility to dissolve in the blood and be carried to the brain, however to pass through the cell wall which consists largely of water insoluble fatty lipids with solubility properties similar to an organic solvent solubility too. Cell membranes use the same fundamental solubility principles as the extraction process.

(معامل التوزيع يلعب دوراً في انتقال الدواء داخل الجسم، مثلما يحدث في خلايا الدماغ حيث يصل الى جدار الخلية وهناك توجد احماض دهنية تستخلص الدواء من الطور المائي (الدم)).

3. pH:

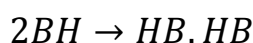
It possible to explain the pH effect on extraction by example of extraction of benzoic acid from water:



The ionic forms are difficult to be extracted.

$$K_D = \frac{[B^{-}][H^{+}]}{[BH]}$$

So that the extraction achieved by acidity the aqueous layer:

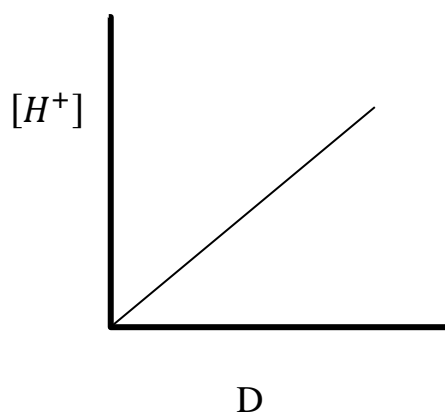


$$K_D = \frac{[HB.HB]}{[BH]^2} = \frac{[HB.HB]^{\frac{1}{2}}}{[HB]}$$

And distribution ratio (D):

$$D = \frac{K_D}{1 + K_a/[H^{+}]}$$

So that when increased $[H^{+}]$ (Acidity increased) the $K_a/[H^{+}]$ decreased and $1 + K_a/[H^{+}]$ decreased, therefore (D) increased.



Since the K_D is a ratio, unless K_D is very large not all the solute will reside in the organic layer in a single extraction, usually two or more extraction of the aqueous layer with an organic solvent are carried out in sequence in order to remove as much of the desired product from the aqueous layer as possible that called

Successive Extraction (الاستخلاص المتعاقب).

So that many smaller extraction (success extraction) are more efficient than one large extraction. It means the extraction with several small portions of organic solvent is more efficient than extraction in single **batch** with the whole volume of organic solvent.

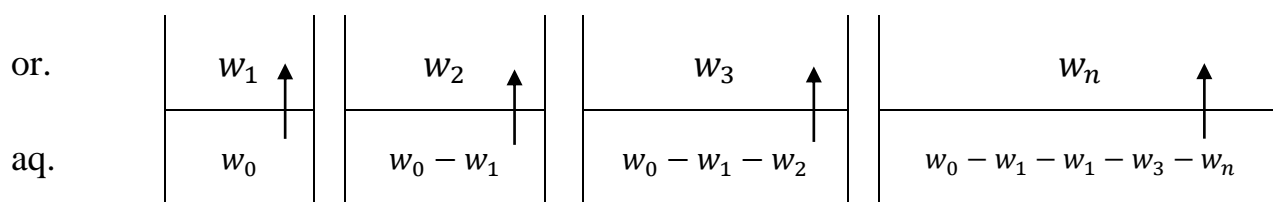
مع هذا، رغم K_D عالية و D عالية أيضاً، لكن ليس كل المادة انتقلت الى الطور العضوي وهذا في الاستخلاص المفرد.

This phenomenon can be proved mathematically by equation.

$$w_n = w_0 \left(\frac{V_A}{V_A + K_D V_B} \right)^n$$

V_A = حجم الطبقة المائية. V_B = حجم الطبقة العضوية. w_0 = الحجم الأصلي.

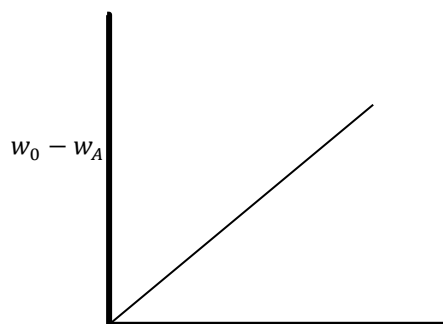
w_n = وزن المتبقي في الطبقة المائية.



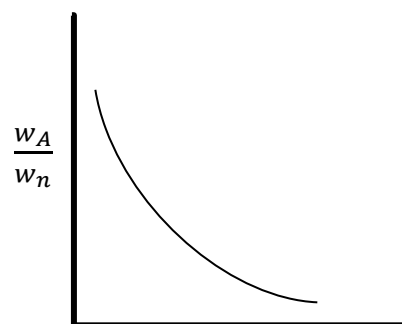
org. = organic layer.

aq. = aqueous layer.

w = weight.



n = عدد سمات



n
الاستخلاص

Emulsion:

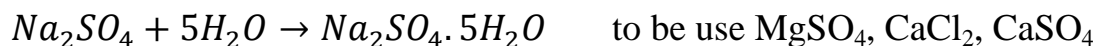
An emulsion is a suspension of tiny droplets of one solvent mixture into the other. Emulsions are common in extraction because proper mixing is essential. Additives are added to the decreasing in order to keep the two normally immiscible solvents. **Immiscible in a L.L.E. or** however an emulsion will lead to a poor separation.

Gentle shaking and swirling the separatory funnel is the most best technique to avoid emulsion. However, if an emulsion occurs there are several simple methods to destroy it as follows:

1. Time: over time the layer will eventually separate.
2. Salt water: added the salt water to the mixture.
3. If there is more difficult emulsion separate the layer as much as possible and dry the organic layer with a drying agent, the water will be removed from organic layer along with drying agent.

Dehydration methods:

Extract from L.L.E. often contain water **originating** from the sample, sample concentration can not be done directly. Thus, it's necessary to dehydrate the extracted. Dehydration is most commonly achieved by using anhydrous sodium sulfate, sample are dehydrated by adding the anhydrous sodium sulfate directly to the extract and filtering or by passing the extract sample through a column or funnel packed with anhydrous sulfate, and there are many other choices for dehydration agents:



Concentration:

The concentration of extract sample may be achieved by different methods like:

1. Rotary evaporator.
2. Evaporate solvent by water bath or by nitrogen gas or any technique doesn't effect to quality and quantity of sample extracted.

Example 1:

Assume that 4gm of butyric acid is to be extracted from 500mL of water with 500mL of ether. The K_D is equal 3.0 at 25°C if the ether is used in single batch. Calculate the butyric acid weight in ether.

Solution:

$$K_D = \frac{C_{org.}}{C_{aq.}} \implies 3 = \frac{(4-x)/0.5}{x/0.5} \quad \text{where the } (x) \text{ is the weight of butyric acid remaining in the water layer.}$$

$$x = 1\text{gm}$$

$$4 - 1 = 3\text{gm} \quad \text{the weight of butyric acid in ether.}$$

If the ether is used in two successive 250mL portions, calculate the weight of butyric acid remaining in water layer. **HomeWork**

Example 2:

One gram of benzoic acid, dissolved in 100mL of water, then extracted with 100mL of ether ($K_D = 100$, $K_a = 6.5 \times 10^{-5}$). Calculate the distribution ratio (D) if the aqueous layer is at pH = 3 and pH = 6.

$$D = \frac{K_D}{1 + K_a/[H^+]}$$

$$D = \frac{100}{1 + 6.5 \times 10^{-5} / 10^{-3}} = 39.9 \quad \text{at pH} = 3$$

$$D = \frac{100}{1 + 6.5 \times 10^{-5} / 10^{-7}} = 0.15 \quad \text{at pH} = 7$$

Solid phase extraction (SPE):

SPE solve several problems associated with LLE such as :

- 1- Incomplete phase separation.
- 2- Poor qualitative recoveries.
- 3- Large quantities of organic solvents.

SPE is generally more efficient than LLE, giving quantitative extractions that are easy to carry out rapid, and which allow automation, time are reduced solvent use reduced.

SPE is used most often to prepare liquid samples and extract semi _

volatile or non volatile analytes but it can also be used with solids that are pre-extracted into solvents.

SPE products are excellent for :

- 1- Sample extraction.
- 2- Concentration.
- 3- Clean _ up.

It used in very wide classes of applications as :

- 1- Biological sample and natural compounds.
- 2- Pharmaceutical and drugs.
- 3- Food and beverages.
- 4- Environmental samples and pollutants.

There are two very basic approaches to SPE:

- 1- The analyte of interest can be retained and the matrix interferences are washed through.
- 2- The analyte of interest is washed through and the matrix interferences are retained.

SPE cartridge are available in wide variety of chemistries, adsorbents and size, selecting the most suitable product for each application and sample can be very important.

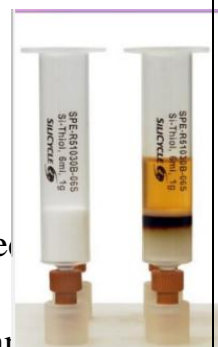
Wrong guide of mechanism the phase cartridge with the analyte in SPE interest :

- 1- reversed phase (extraction hydrophobic analyte (non polar) from aqueous matrix).
- 2- normal phase (extraction of polar analytes from non polar organic solvent).
- 3- Ion exchange (extraction of charged analytes from aqueous or non polar organic sample).
- 4- Phase with combined multiple interaction mechanism are called mixed _ mode phase.

The typical cartridge system for SPE are packed with the compounds can be non polar, moderate polar or polar.

For example:

- 1- Charcoal



- 2- XAD resin
- 3- silica gel chemically bonded with ODS.
- 4- High polymer resin such as polystyrene and polycarbonate.
- 5- Other.

How to use SPE:

SPE extraction is used to separate compounds of interest from impurities in different ways choose most appropriate way for your sample, and there is general process :

- 1- select the proper SPE tube.
- 2- Condition the SPE tube.
- 3- Add the sample (liquid or solid dissolved in liquid).
- 4- Wash the packing.
- 5- Elute the compounds of interest.

Selection an SPE tube size:

Amount of sample	Tube size used
< 1 mL (less than one mL)	1 mL
1 mL to 250mL and the extraction speed is not critical	3 mL
1 mL to 250 mL and faster extraction procedure is required	6 mL
10 mL to 250 mL higher sample capacity is need	12 , 20 or 60 mL
< 1 liter and extraction speed is not critical	12 , 20 or 60 mL

Application:

always some difficulties sample matrices should be pretreated before using applied to SPE device like proper pH of the sample and other pretreated some samples :

- 1-Biological matrices (serum , plasma , whole blood....)
- 2- Milk
- 3- Cell culture media.
- 4- Oils
- 5- Fish and animal tissues, plant tissues
- 6- Pharmaceutical

The disadvantages of SPE:

- 1- although solvent use is small the solvent flow rate effect the recovery rates.
- 2- samples which includes suspended solid (SS) it is necessary to separate SS composition.
- 3- in order to have high and stable recovery rates it is important to choose the most appropriate solid phase for the target compounds.

Solid phase micro extraction (SPME):

SPME is a method used to both extract and concentrate organic compounds in which a fiber needle attachment which has been chemically coated with fused silica equivalent to GC liquid phase, is dipped directly into liquid sample or exposed to the head space vapors from liquid or solid samples. It used recently with instruments like GC, HPLC, GC_MS, HPLC_MS

Soxhlet extraction :

organic compounds in solid samples are extracted from matrix by continuously washing the solid with volatile solvent in specialized piece of glass ware (soxhlet extraction apparatus) the newly developed extraction method know as supercritical fluid extraction, however soxhlet extraction frkes long time to get high efficiency and is not suitable for organic compounds which are thermally unstable.

Basic components of a soxhlet apparatus:

- 1- condenser: to cool the solvent vapor cause it to condense.
- 2- porous container : to hold the solid sample and allow for the condensed solvent to saturate and pass through sample like filter.
- 3- distilling pot : to hold the solvent poole and serve us a reservoir for the concentration materials.

the extraction process soxhlet extraction consist of there steps :

- 1- boiling
 - 2- rinsing
 - 3- evaporating
- extraction process are used to separate soluble components from a solid samples

for example :

- 1- determine the fat content in foods.
- 2- determining an impurity in soil samples.
- 3- examining the components of natural substances.

The soxhelt extraction has three main selections a percolate (boiler and

reflux) which circulates the solvent, a thimble (usually of which filter paper) which retains the solid to be laved , and a siphon mechanism.

The advantage of soxhlet extraction is isolate desired oil when it has limited solubility in a solvent and when impurities insoluble in a solvent, and the sample is constantly in contact with the solvent.

While the disadvantage is the long time process and poor extraction of polar lipids, large volume of solvent required.

Ultrasonic extraction (UE):

UE use ultrasonic vibrations to extract sample with polar solvents in an ultrasonic bath, this is often used for chemical extraction from solid samples because it is simple.

Supercritical fluid extraction(SFE)

Gas such as CO₂ and NO₂ become fluid when temperature and pressure a critical point called the supercritical phase, in SFE this fluid is used to extract the target substance in solid phases.

The SFE is a relatively new method which has recently begun to be applied extensively , instrument connection the SFE apparatus and online GC have been developed to be extraction have been automated, the fluid used for SFE included CO₂ , NO₂ , SF₆ methanol and water however the supercritical CO₂ is very nonpolar so modifiers for CO₂ include methanol , dichloromethane, acetonitrile, water.... etc.

Modifiers are mixed with CO₂ the amount of organic solvent used is much smaller than other extraction methods.

Air samples : particulates and gas sample preparation methods :

1- solvent extraction from filter papers or adsorbents, filter paper is used to trap the particulates while adsorbents are used for sample containing substance used ultrasonic or Soxhlet extraction for filter paper or adsorbent.

2- thermal desorption:

Adsorption followed by thermal desorption come introduce the whole trapped target substance into GC or GC_MS this method suitable for analyzing volatile compounds and sample containing low concentration of target analysis.

Hot acid extraction (digestion):

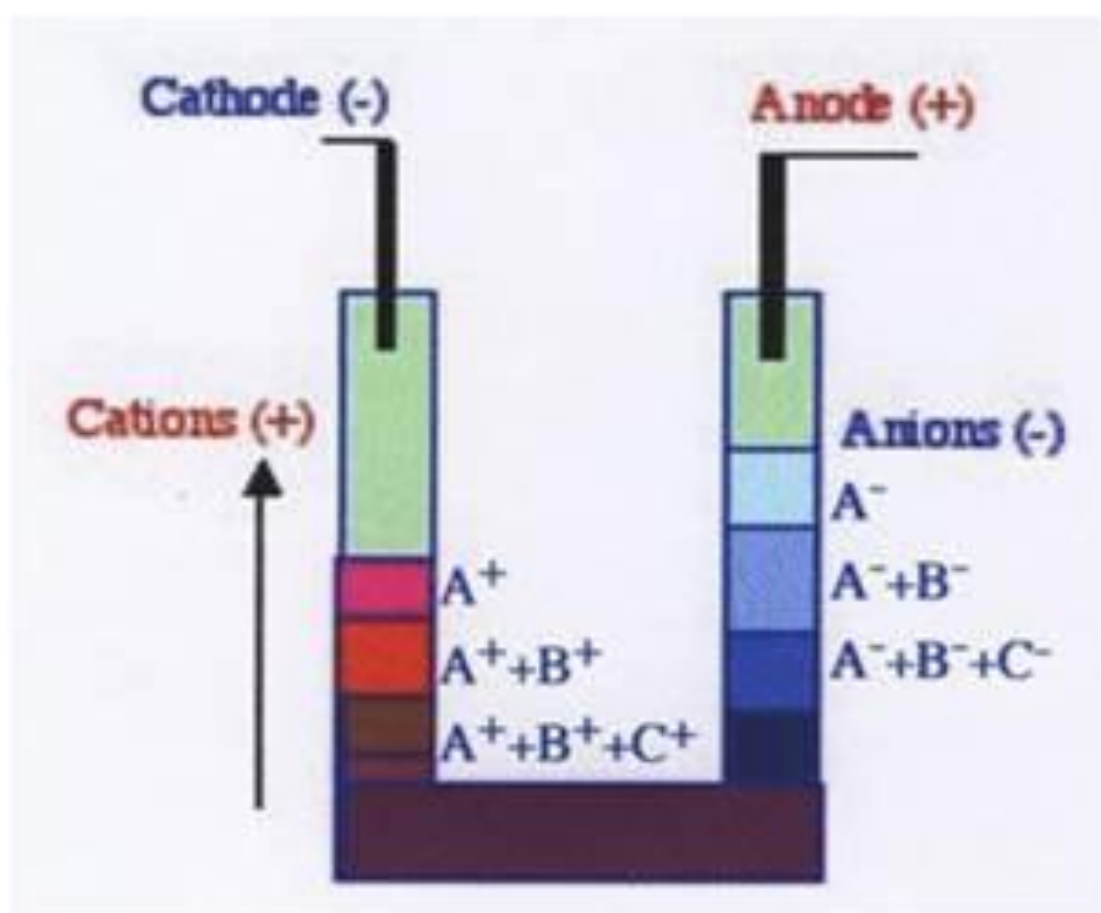
This method of extraction used for extraction heavy metals from matrix by concentrated acid with digestion by heating, the system including reflux and cooling system to avoid losses of inorganic metals through volatile.

Electrophoresis (الهجرة الكهربائية)

Electrophoresis is the separation methods depended on the migration of charged particles by using fields

1-moving boundary Electrophoresis الانتقال الكهربائي الحر

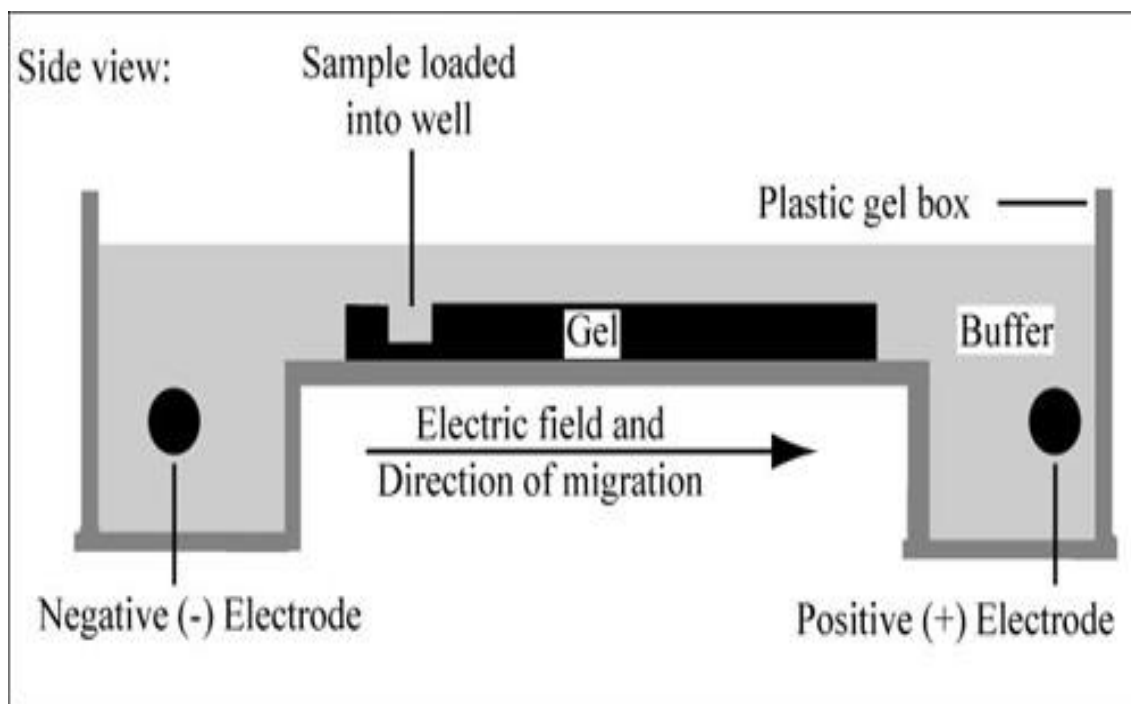
اول من جاء بهذا الاسلوب العالم tisebus ونال جائزه نوبل لهذا الاختراع وتلخصت التجربة بانه مزيج من البروتينات ووضعه في وسط انبوبة بشكل حرف U مجهز بقطبين وعند امرار تيار كهربائي ابتدأت الجزيئات المشحونة بالانتقال باتجاه الاقطاب المناسبة حيث حصل على فصل جزئي غير تام.



2-Zone Electrophoresis الانتقال الكهربائي المنطقي (مناطق)

حيث يتم الانتقال خلال طبقة رقيقة من السائل مطلية على ورقة او مادة مسامية في مجال كهربائي، الجهاز النموذج يتمثل بأن يبيل شريط من الورق المستخدم كحامل في محلول الكتروليتي ثم ينشف بورق نشاف وتغمر كل من نهايتيه في مستودع من

المحلول ويوضع النموذج وسط الشريط ويسلط جهد DC بين القطبين يتراوح بين ١٠٠ الى عدة الاف فولت ويكون المذيب عادة محلول مائي منظم ذات اس هيدروجيني يعمل على شحن بعض المكونات فتتجذب باتجاه احد القطبين فاذا نزحت مكونات النموذج تجاه الاقطاب المختلفة او حتى باتجاه القطب الواحد بدرجات متفاوتة وبذلك تتم عملية الفصل.



Simple zone electrophoresis

Theory Basics of Electrophoresis **الاساس النظري للانتقال الكهربائي**

ان مفهوم انتقال الايونات (θ) يمثل بالمعادلتين:

$$\theta = \frac{q \pm}{6\pi r \eta} \text{ ————— } 1$$

$$S = \frac{\theta E t}{L} \text{ ————— } 2$$

حيث (S) تمثل المسافة التي يقطعها المذاب بشحنه q^{+} ونصف قطر (r) و (E) تمثل الفولتية المسلطة عبر الحامل الذي طوله (L)

η : لزوجة الطور السائل و t الوقت اللازم للتظهير

من المعادلتين (١) و (٢) توضح ان انتقال الايونات (θ) تتناسب طرديا مع شحنة الايونات (q^{+}) لذا اسهل عمليه فصل هي فصل الايون الموجب (Cation) عن الايون السالب (anion) بالإضافة ان الايون الثنائي التكافؤ ينتقل ضعف المسافة التي يقطعها الايون احادي التكافؤ او بالتساوي وبشكل عام فان مسافه الفصل Δs بين الايونين A و B اللذان يحملان نفس الشحنة يكون :

$$\Delta s = S_A - S_B = (\theta_A - \theta_B) \frac{Et}{L} \text{ ————— } 3$$

ولأجل التوصل الى فصل جيد يجب ان تتوفر الشروط التالية:

١- Δs كبيره.

٢- اختلافات عالية في انتقال الايونات (θ)

٣- طول الفترة الزمنية.

٤- قيمه E/L عالية.

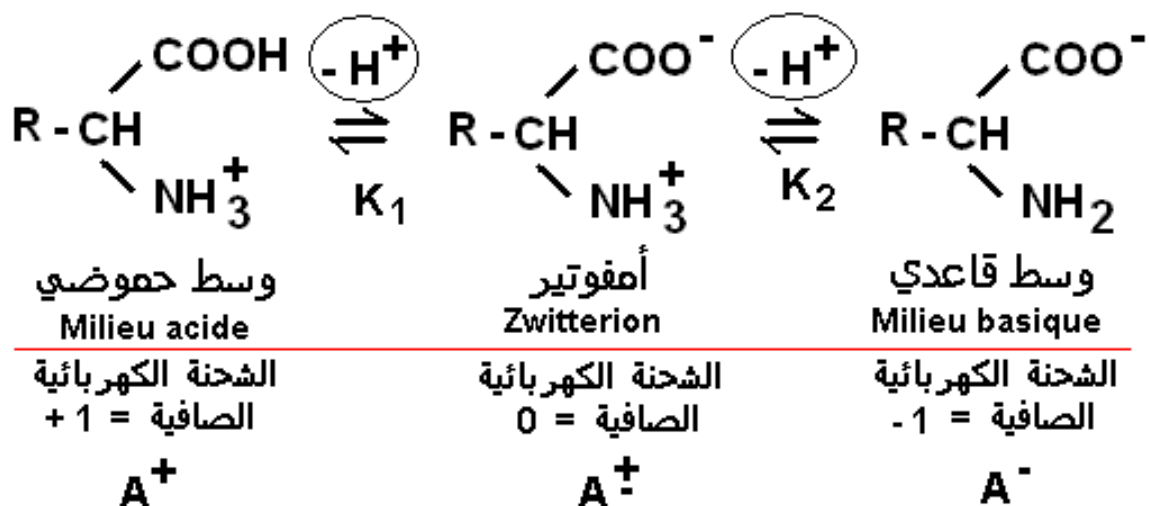
العوامل المؤثرة على عمليه الفصل

The factors effecting the separation process

من خلال المعادلة (١) فان كل من نصف القطر (r) اللزوجة (η) تأثير على انتقال الايونات وبالنتيجة تأثر على الفصل ' حيث يفضل المذيب ذو اللزوجة الواطئة لان يزيد قيمه (θ) اما انصاف الاقطار (r) كل من الايونين يجب ان يكونا بقدر الامكان مختلفين اضافه الى عوامل اخرى تؤثر على عمليه الفصل ومنها درجه التأين ' تكوين المعقد ' تركيز الالكتروليت.

١-درجة التأين (degree of ionization)

ان المذيب المستعمل في ال Electrophoreses غالبا هو محلول منظم (Buffer) وبصوره عامه كلما ارتفع مقدار ال pH كلما زاد التأين وبالتالي يزداد نزوح او انتقال الايونات. ولأجل فصل حامضين ضعيفين بهذه التقنية يجب اختيار pH مناسب بحيث يحصل فرق كبير في التأين.



يلاحظ من المثال اعلاه ان تحلل الحامض الاميني اعلاه له علاقه بالرقم الهيدروجيني pH فاذا قلت قيمه ال pH انزاح التحلل الى اليسار اي تولد ايون موجب وعندما يصل الرقم الهيدروجيني pH الى مقدار معين لا يحدث تحلل تسمى هذه النقطة iso-electric point اما اذا ارتفع مقدار ال pH فان التحلل يتجه الى اليمين اي يتكون ايون سالب وهنا يتبين الدور المهم الذي يلعبه المحلول المنظم (Buffer) يفضل استعمال محلول منظم عضوي وذلك لسرعه تبخره بالتسخين البسيط.

٢-تكوين المعقد Complex formation

عندما تتكون المعقدات بين المذاب والمذيب (المحلول المنظم) يصبح بالإمكان تغيير اثره الايون الموجب الى السالب ومثال ذلك الكربوهيدرات لا تتأين بسهولة ولكنها تكون معقدات ايونية مع ايون البورات في محلول قاعدي لذلك يمكن فصلها بطريقة الانتقال الكهربائي ويمكن تكوين المعقدات السالبة مع عدد كبير من الحوامض الاوكسجينية غير العضوية.

٣- تركيز الالكتروليت Electrolyte concentration

ان وجود الالكتروليت اساسي جدا في طريقه الانتقال الكهربائي فبالإضافة الى عمله كمنظم وعامل معقد فهو ضروري جدا لنقل التيار الكهربائي وان تركيز الالكتروليت العالي يمكن ان يكون غير مفيد احيانا لان انتقال الايونات يتناسب عكسيا مع الجذر التربيعي للشدة الأيونية حيث كلما زادت الشدة الأيونية يقل انتقال الايونات.

الاساليب التقنية في الانتقال الكهربائي

Apparatus and techniques in Electrophoresis

ان طريقة الانتقال الكهربائي تتشابه الى حد مع طريق ال TLC ويمكن توضيح مكوناتها:

١- العلبة والحامل Chamber and support

حيث تستعمل اوساط عديده كحوامل ومنها

١- الورق

٢- اسيتات السيليلوز

٣- المنشأ الجيلي

٤- الاكريليك

٥- انواع عديده اخرى

٢- العوامل التجريبية الاخرى

other experimental parameter

يشكل الالكتروليت اهميه كبيره في الطريقة وفي الغالب يتكون من محلول منظم (Buffer) بتركيز 0.1 مولاري ويفضل ان يكون بحجم كبير لكي يقلل انتشار نواتج التفاعل للاقطاب ويمكن منع الانتشار ايضا يوضع حاجز بين جزئ الالكتروليت الحاوي على الشريط. وكذلك يجب توفر مستودعان متماثلان في الحجم واحد لكل قطب وان يتكون الاقطاب من البلاتين او الكربون.

٣- الفولتية او الجهد الكهربائي Voltage

يستخدم بصورة عامه 400 فولت وينتج تيار مقداره 100 مايكرو أمبير على شريط طوله 40 سم باستعمال 40 واط تنتج كميته من الحرارة لذلك فان التبريد في بعض الحالات ضروري لمنع ازدياد درجة الحرارة التي تؤدي الى تبخر المذيب وبالتالي اختلاف تركيز المحلول المنظم ويسمح بمرور تيار اكبر وينتج حراره اكبر . بينت الدراسات ان الفولتية العالية الى 4000 فولت اكثر فائدة في تقليل فترة التحليل وتحسن عمليات الفصل . وان الفولتية العالية تقلل الوقت اللازم للتحليل وهو المطلوب ويمكن تلافي الحرارة العالية باستخدام اجهزه تبريد.

العينات Sampling

حيث توضع العينات على الشريط الورقي او الصفيحة بواسطه ماصه ميكروية (micropipette) او ابره ميكروية (micro syringe) ويمكن اتباع طريقتين الاولى يبلل الشريط بالمذيب ويوضع في العلبة المشبعة بنفس المذيب قبل وضع العينة وبذلك تكون بقعه النموذج اوسع عند وصلها على شريط مبلل. اما الطريقة الثانية فتوضع العينة بشكل بقع على شريط جاف فتبقى البقع صغيره ثم يغمر الشريط مباشرة في المذيب ليلل دفعه واحده بدل ان يبلل بصورة تدريجيه بفعل الخاصية الشعرية من النهايتين.

التكنيك Technique

يستخدم التظهير الاحادي البعد one-dimension او يستخدم التكنيك ذي البعدين two-dimension

محاسن طريقه الانتقال الكهربائي

Advantage of Electrophoresis method

- ١- تطبيقات واسعه على نماذج قابله للتأين ومن ضمنها الجزيئات الكبيرة.
- ٢- يمكن دمجها مع الطرف الكروماتوغرافية الصفائحية TLC للحصول على كفاءه اعلى.
- ٣- اسرع من طرق اخرى مكافئه مثل الترشيح الجيلي.
- ٤- بساطه الأجهزة والتكنيك.

مساوئ طريقه الانتقال الكهربائي

disadvantage of Electrophoresis method

- ١- الفولتية العالية قد تشكل خطرا.
- ٢- ليست بكفاءة عالية جدا.
- ٣- يصعب احيانا التقدير الكمي بتراكيز معينه.

Efficiency of Electrophoresis depends on :

- 1- Electro phoresis mobility.
 - 2- Electrosmotic flow of the bulk solution (EOF)
 - 3- Joule heating.
- Electrophoresis mobility is the fundamental parameter which determines the efficiency of separation based on charge size ratio.
 - change in pH effectively alters the charge on the ions and their electrophoretic mobility.
 - EOF is in apposite direction to analyte electrophoretic flow (generally).
 - EOF refers to migration of the bluk liquid toward the cathode.

-method for decreasing joule heating

- 1- decrease applied voltage.
 - 2- dissipate heat use thin gell smaller diameter capillary.
 - 3-cold system.
- Electrophoresis separates molecule on the basic of their charge to size.

Electrophoresis techniques may by of two types :

- 1- Slab electrophoresis TLC.
 - 2- Capillary electrophoresis-column.
- The strength of electric field € depend upon
 - the charge of analyte ion.
 - the fractional retarding forces.

- plate count in electrophoresis given by formula

$$N = u_e v / 2D$$

V: voltage

D: diffusion coefficient of the so

U_e : electrophoretic mobility

N: number of plates

Slab electrophoresis

- 1- Porous layer 2——10 cm.
 - 2- Slow.
 - 3- Simple.
 - 4- Poor quantities.
 - 5- Large quantities.
 - 6- Large cross-sectional area.
 - 7- $V_{\max} = 500$ volt.
- $N = 100—1000$ low resolution.

-capillary Electrophoresis:

- narrow –bore capillaries.
- Each capillary is about 25-100 μm in internal diameter.
- small cross –sectional ; long length leading to high resistance low currents.
- $V_{\max} = 20—100\text{Kv}$
- $N = 100000 —10000000$ high resolution.

Buffer used in electrophoresis to provide ions that carry a current and maintain the pH at relatively constant value

Chromatography :-

In Greek it means chroma (color) and _graphy (writing) ,
Tswett named this new technique Chromatography based on
Fact that Separated the components of asolution by color .

There are several common chromatographic methods these in
clude

- paper chromagraphy
- thin layer chromatograpy (TLC)
- liquid chromatography (LC)
- ion chromatography
- High pressure liquid chromatogphy (HPLC) or High
performance liquid chromatography (HPLC)
- Gas chromatography (GC)
- Affinity chromatography
- Gel filtration chromatography
- others

In all chromatography methods the sample is transported in
a mobile phase , the mobile phase can be agas or liquid or a
surpercritical fluid , then the mobile phase forced thrugh the
stationary phase held in acolumn or on asolid surface
(support), the stationary phase needs to be does not react with
the mobile phase or sample.

The sample then has the opportunity to interact with the
stationary phase as it moves pas it that interact can then be
separated in to their components it means the sample
distribution between two phases and that depended upon

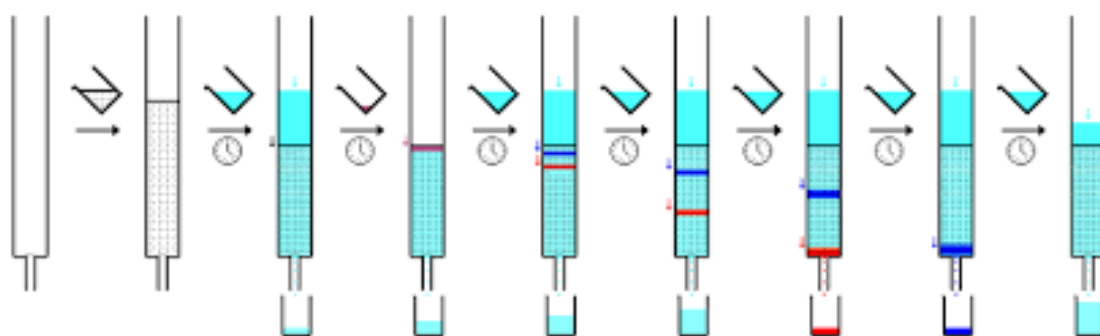
partition or Adsorption the sample between two phases , while the distribution coefficient (K) by

$$k = \frac{C_s}{C_m}$$

C_s ; concentration the solute in the stationary phase (sp)

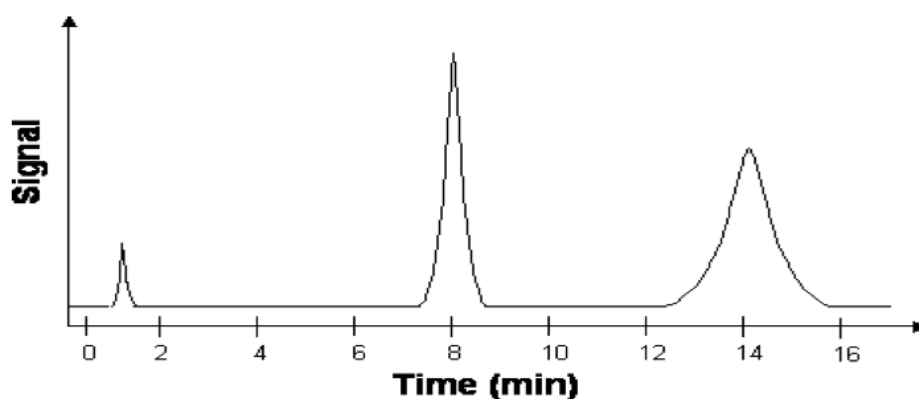
C_m concentration the solute in the mobile phase (mp)

In the mixture each component has a different (k) and then separated



After separated then eluted and the detector used to determine the component by a series of Gaussian peaks are obtained

Theoretical plate : It is based on a study in which they imagined that chromatographic column were a



analogous to distillation column, and made up numerous discrete but connected narrow layers of plates. Theoretical plates (N) measure how efficiently a column can separate a mixture into its components. This efficiency is based on the retention time of the compounds and the width of the peaks

$$N = 16 \left(\frac{t_R}{W} \right)^2$$

t_R ; is the retention time

W : is the width of peak

When t_R IS HELD CONSTANT, The column that produces peaks with narrower widths (W), will be more efficient have greater N value

Like wise a column that produces wider peaks will be less efficient. have a smaller N value.

And also the efficiency is inversely related to the thickness of the theoretical plate (H) which is termed the plate height or the height equivalent of theoretical plate (HETP), which is rearranged to

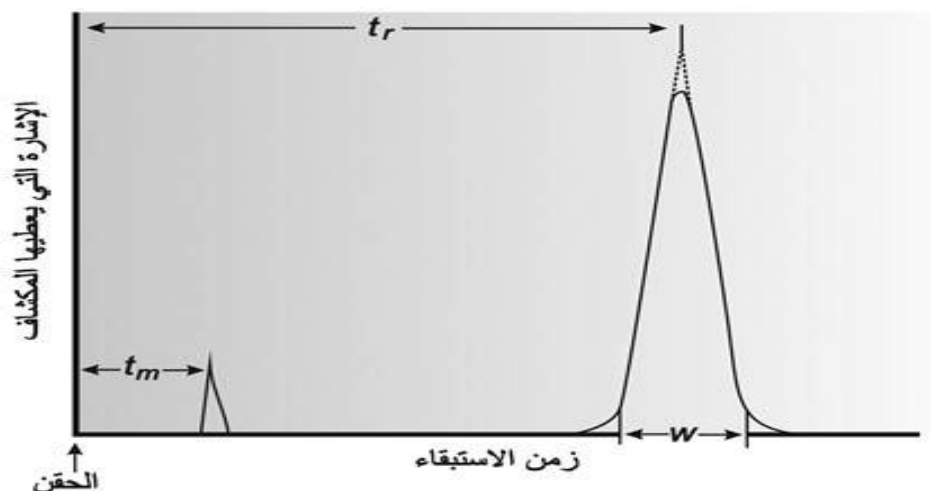
$$H = \frac{L}{N} \quad \text{or}$$

$$N = \frac{L}{H} \quad \text{Where L is the column length}$$

And from the rate theory the

$H = A + B/U + C_u \rightarrow$ van Deemter equation where A,B,C constant and H the flow rate of mobile phase

t_R : it is the difference in time from the point of injection to appearance of peak maxima , and measured in minutes or seconds.



V_R : Retention volume it is the volume of mobile phase which requires to elute 50% of the component from the column

$$V_R = t_R * \text{Flow rate}$$

If HPLC is less , the column is efficient increased

If HPLC is more, the column is efficient decreased

Factors affecting retention time

- 1- length of column
- 2- packing material of stationary phase
- 3- type of mobile phase
- 4- Flow rate of mobile phase
- 5- Temperature of column

While the resolution between peaks given by

$$R = \frac{2 (t_{R(2)} - t_{R(1)})}{(W_1 + W_2)}$$

R : Resolution

$t_{R(2)}$, $t_{R(1)}$: Retention time of compound (1) and (2)

W_1, W_2 : peak width of compound (1) and (2)

TLC Thin layer chromatography :-

TLC : Is one of the simplest , Fastest , easiest and least expensive of several chromatographic techniques used in qualitative and quantitative analysis to separate organic compounds and to test the purity of compounds

Principle of TLC :- it is based on the principle of adsorption or partition chromatography or combination of both . depending on adsorbent its treatment and nature of solvents employed

The compounds with more affinity toward stationary phase travel slower while the compounds with less affinity toward stationary phase travel faster

The basic parameter used to describe migration in TLC is the R_f value where

$$R_f = \frac{\text{distance moved by solute}}{\text{distance moved by mobile phase}}$$

R_f values vary from 0 to 1

R_f means retardation factor

Factors affecting R_f value :-

It depends on following factors

- 1-Natural adsorbent
- 2- mobile phase
- 3- Activity
- 4- thickness of layer
- 5- temperature
- 6- Equilibrium
- 7- dipping zone
- 8- chromatographic technique

There is different kinds of stationary phase in TLC like

- 1- Silica gel
- 2- Silica gel modified
- 3- Alumina
- 4- Cellulose powder
- 5- Sephadex gel
- 6- Others

Selection of adsorbents (stationary phase) in TLC :-

- 1- SOLUBILITY OF COMPOUND eg hydrophilic or lipophilic
 - 2- Nature of substance to be separated (acidic ,basic ,
 - 3- Adsorbent particle size
 - 4- Reactivity of compound with solvent or adsorbent
 - 5-Adsorbent should not adhere to plate
- The standard sizes of TLC plate are 20*5cm , 20*10cm , 20*20 cm and the standard film thickness is 250µm and surface be flat without irregularities

- After spreading plates are allowed to dry in air and further dried and activated by heating at about 100 c for 30 minutes.

The choice of the mobile phase in TLC depends upon the following factors Nature of the substance to be separated

- 1- Nature of the stationary phase used
 - 2- Mode of chromatography (Normal phase or reverse phase)
 - 3- Separation to be achieved . Analytical or preparative .
- The organic solvent mixture of low polarity is used , highly polarity solvents are avoided to minimize adsorption of many components of the solvent mixture . use of water as solvents is avoided as it may loosen the adhesion of layer on plate.
 - The area application should be kept as small as possible for sharper and greater resolution

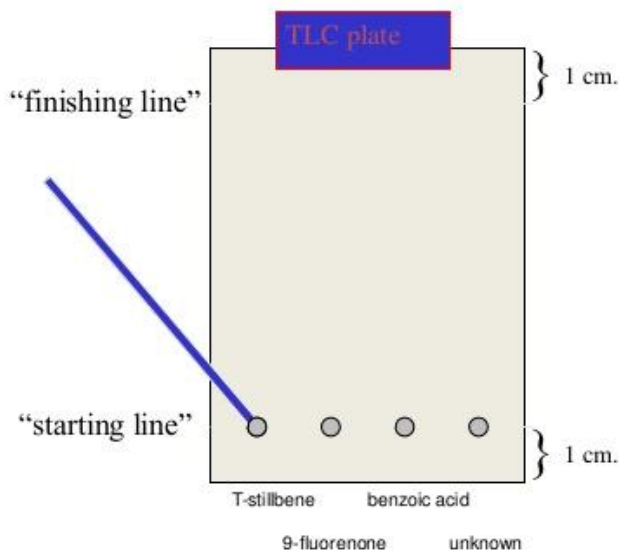
Process:

- 1- Draw guide line lightly with pencil (starting and Finishing line)
- 2- Use TLC capillary to transfer and spot dissolved sample on starting line

Sample Application (spotting)

Process

- A. Draw "guide lines"
lightly with pencil
- B. Dissolve solid
sample in CH_2Cl_2
- C. Use TLC capillary
to transfer and spot
dissolved sample



How to Run TLC :-

- 1- Prepare the TLC plate
- 2- Prepare the Developing container
- 3- Spot the sample on TLC plate (application)
- 4- Develop the plate
- 5- Visualize the spots

Visualize the spots :-

- 1- If there are any colored spots , circle them lightly with a pencil
- 2- Most samples are not colored and need to be visualized with a uv lamp
- 3- Hold a uv lamp over the plate and circle any spots you see
- 4- Use chemical reagent (general and specific) to visualize the spots.

5- Use TLC scanners (instrument) for quantitative and qualitative analysis

Development of thin-layer chromatograms :-

1- One – dimensional development

- Single development
- Vertical
- Horizontal in one direction
- Horizontal in opposite direction
- Circular
- Anti Circular

2- Two – dimensional development

- Two dimensional , one solvent system
- Two dimensional , two solvent system
- SRS (separation in 1st dimension and 2nd dimension)

* The advantage of the Two – dimensional development is the possibility of analytical (RPC) separation (Reversed phase chromatography)

* More complete separation of sample components can be achieved by two dimensional development in this process the plate is developed normally and following complete drying and then development (second) of the plate is continued , and the second development performed by using different mobile phase with different selectivity than used first development .

Application of TLC :-

- Checking the purity of samples
- Purification process
- Examination of reaction
- Identifying organic compounds (like Amino acid , Vitamins , others)
- For preparative technique

Preparative thin layer chromatography (PTLC) or PPC :-

Preparative thin layer (planner) chromatography (PPC) is a liquid chromatography technique performed with aim of isolating compounds , in amounts of 10-1000 mg , layer TLC plates called preparative plates can be used for separation of milligram quantities of materials because they contain thick layers (1-3) mm of stationary phase .

The principle of process :- of PTLC is applied more quantity of samples and after development and marking the spots using metal spatula and scrap desired spot with silica and transfer to clean glass tube and add 8-10 ml of $CHCl_3 : CH_3OH$ (2 : 1) to each glass tube then centrifuge at 3000 RPM , and then use the organic solvent ($CHCl_3 : CH_3OH$) that contain the sample separated and dry the solvent to reach the amount of sample separated , Repeat those steps and continue to accumulate the sample separated

HPTLC :-

High performance thin layer chromatography is sophisticated and automated form of TLC efficient separation in short time , it is a form of TLC that provides superior separating power using optimized loading material , novel procedures for mobile phase feeding , layer conditioning and improved sample applications.

The basic difference between conventional TLC and HPTLC is only in particle and pore size of sorbents.

The principle of separation is similar that of TLC and the HPTLC is very useful in qualitative and quantitative analysis .

Advantage of HPTLC :-

- 1- Simultaneous processing of sample and standard better analytical accuracy and precision
- 2- Lower analysis time and less cost per analysis
- 3- Sample preparation is simple

- 4- Mobile phase (solvent) does not need prior treatment (filtration , degassing)
- 5- Shorter developing time
- 6- High separation efficiencies and resolution of zones due to higher number of theoretical plates .
- 7- Automatic application
- 8- Automatic detecting

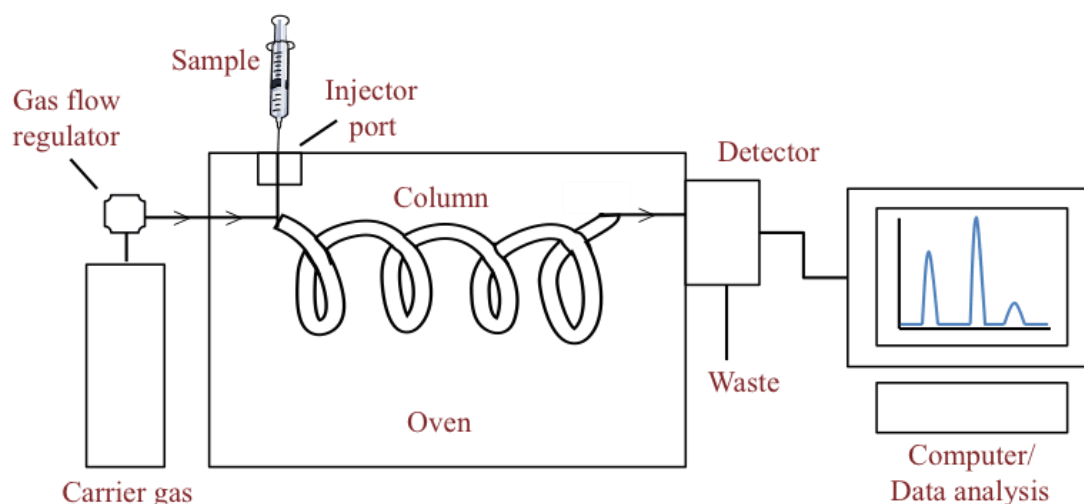
Gas chromatography(GC):

GC is common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition, typical uses of GC include testing the purity of particular substance or separating the different components of mixture. In some situation GC may help in identifying a compound. In GC the mobile phase is a carrier gas, usually an inert gas such as helium, or an unreactive gas such as nitrogen. The basic GC consists from:

1. Injector :
 - i. Splitless
 - ii. Split: divided
 - a. Split ratio = $\frac{\text{split outlet flow rate}}{\text{column outlet flow rate}}$
Split ratio typically varies between 1:20 and 1:500
2. column:
 - i. packed column
 - ii. capillary column
3. detector :the most commonly detector in GC

1. Flame ionization detector (FID): general detector
2. Alkaline flame ionization detector (AFID): Specific detector used for nitrogen and phosphorus compounds some time called (NPD)
3. Flame photometric detector (FPD): specific detector used for sulfur and phosphorus

4. Thermal conductivity detector (TCD) : general detector was non destructive detector
5. Electron capture detector (ECD): specific detector, specific for halogen and hetroatom compounds
6. Atomic emission detector (AED): specific for inorganic metals, used microwave plasma this types is two instrument combination it is (two in one) technique used for advanced diagnostic
7. Mass spectrometry detector (MSD): it is (two in one) technique used for advanced diagnostic
8. Fourier transform infrared detector(FT-IR D): It is (two in one) technique used for advanced diagnostic.

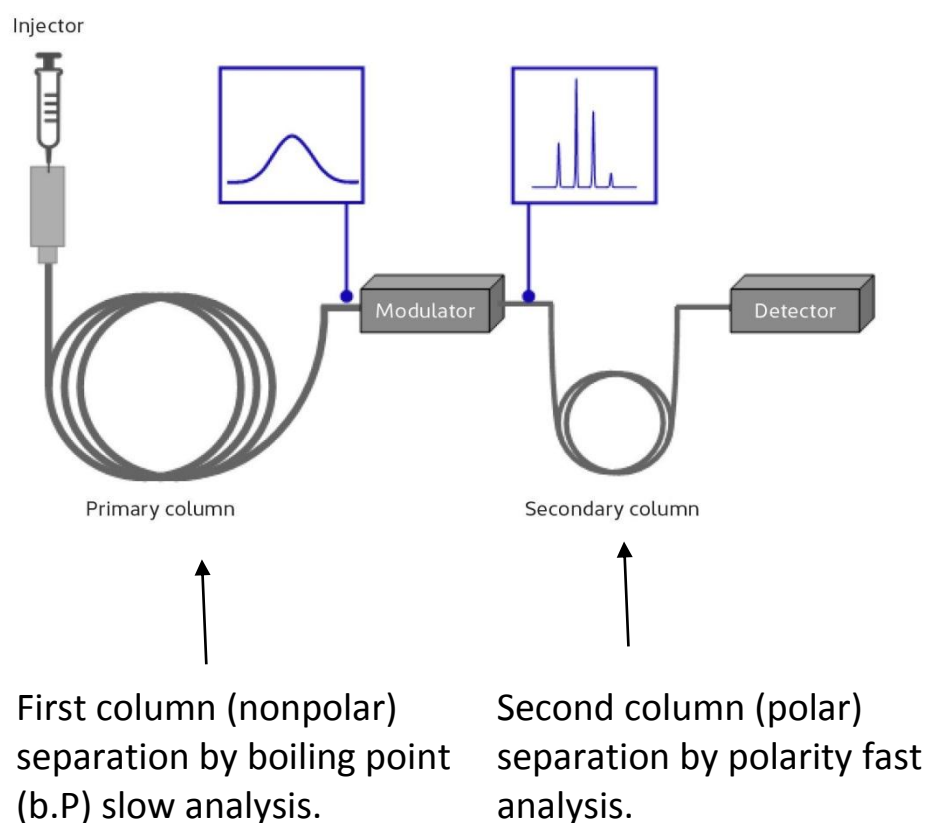


G.C. diagram

GC is superior in separating of multi-compounds but is not good at identifying peak components.

GC × GC chromatography :

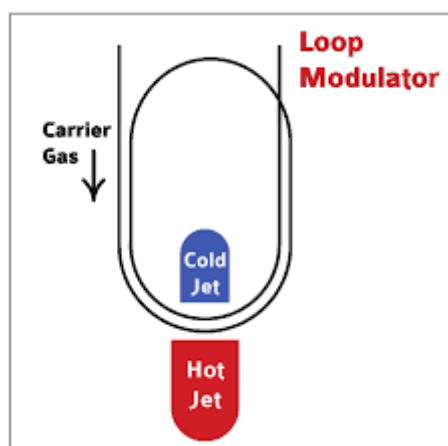
GC×GC is the last destinational techniques, mainly due to the association of two different mechanisms of separation , and is more complete toward the separation process involving a sample analysis , and employs a pair of GC column (generally, nonpolar , polar column) connected in series through modulator, the chromatogram obtained in two dimensional chromatogram with boiling point and polarity or respective axes (different separation characteristics), the instrument using specialized software for the result , dimensional chromatogram with B.P and polarity .



Digram separation GC × GC

What is modulator :

The cuts effluent from the first column and transmits it on to second column and repetitive injector for the second column.



Modulator

vade based

thermal heater

cryogenic

Detector for GC×GC is the speed of acquisition and they are very fast selectivity and sensitivity like FID,ECD,MS,.....

Vade

Characteristics of GC × GC :

1. Separation primary flowed by steady repetition of secondary separation .
2. The selectivity of the first separation phase and second stationary phase are different .
3. The time scale of the second separation is small and the resolution achieved by the first separation .
4. The total area of second separation is representative of the component concentration

Benefits of GC × GC :

- 1.** 10× increase in GC resolution .
- 2.** 5× increase in sensitivity .
- 3.** No cost in analysis time .
- 4.** Compound class information .
- 5.** Automatic operation .
- 6.** Routine quantitation .

Preparative GC (PGC) :

In preparative chromatography GC can be used to prepare pure compounds from a mixture , GC technique has largely been associated with analytical instead of preparative purpose . actually PGC is an ideal alternative technique for the preparation of pure substances especially volatile compound .

There are a number of problems associated with PGC as:

1. It is difficult to recycle the mobile phase and thus large volume of gas are necessary.
2. The sample must be fully vaporized on to the column
3. The materials of interest are eluted largely in very dilute from the column and there for must be extracted or condensed from the gas stream which is also difficult to achieve efficiently
4. The efficient packing of large GC column is difficult

In the PGC technique we can isolate pure components by condensing the organic compounds at the outlet from the detector, so for this technique we will use PGC to collect the component of the mixture. PGC is limited to relatively small sample sizes even though we are using larger diameter column than we are used in the analytical experiments. and more materials could be isolated by repeating the injections and collections multiple times

The detector that is used with PGC must have specifications that are almost opposite to those of an analytical detector, if in-line it must be non-destructive the katharometer is one of the most popular detector for (PGC) in this state, while another technique in PGC the detector not in-line (splitting by valve) normally use analytical detector, it means always destructive detector

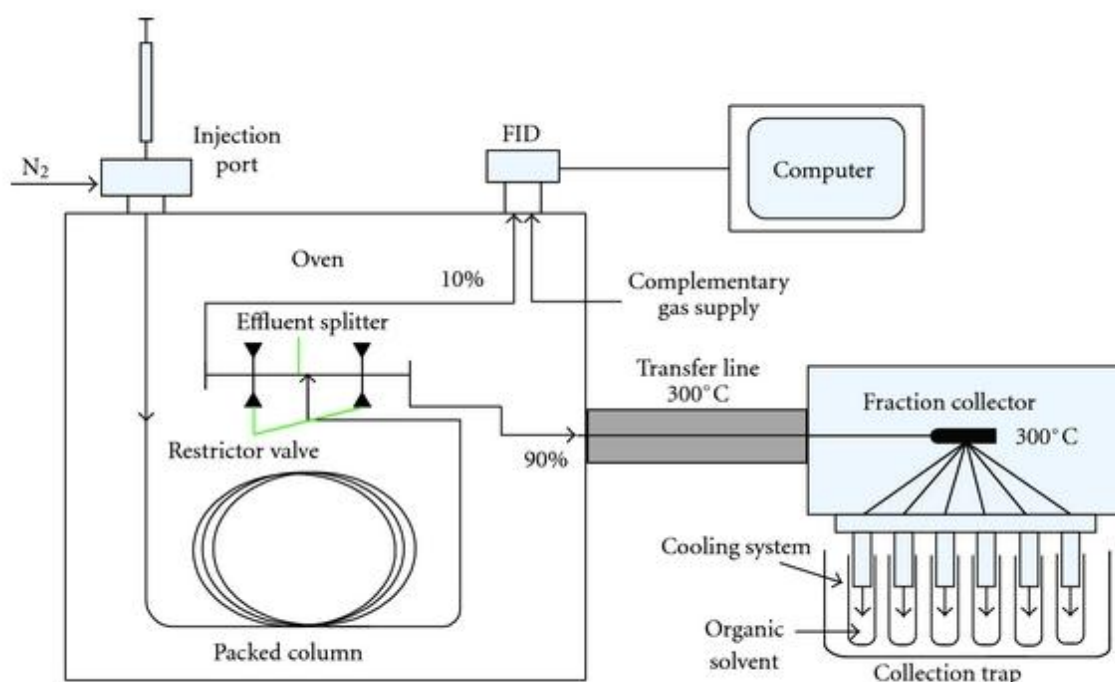
The column in PGC :

Analytical column : 1-15 m (length) \times 3-6 mm d.m

Preparative column : 3-6 m (length) \times 6-9 mm d.m

1. for a large scale preparation, large sample injection and vaporization, high loading capacity column, gas splitter at the end of column and special collecting device are important for PGC system. for general preparative the collection vessel may be cooled by different ways in specific states use liquid nitrogen (LN), and may be the eluent extracted into a specific liquid or to suitable adsorbent.

The modern preparation technique used fraction collector (FC) unites to collect compounds automatically, the (FC) is equipped with sample traps and the waste trap, traps are available in different volume size (1 ml to 100 ml) and the (FC) traps equipped with cooling system, and the unit (FC) control and connected with the (GC) software to operate with system, the switching times can be selected to within 0.01 minutes



- In diagram (1) the detector is not in-line of the collection unit (preparation) while in diagram (2) the collection unit is in-line with the detector
- The collection units are always cooled by ice or solid carbon dioxide or liquid nitrogen or other cooling technique.

Advantage of (FC) for preparation GC :

1. Automated collection of compounds or compound groups.

2. Many collection traps using cryo-trapping or adsorbent .
3. Purification of compounds from many injection of sample .
4. Separate waste trap can be cooled for residue collection .
5. Best possible recovery by software controlled

There is different and important applications of PGC like:

2. Purification of volatile compound from natural essential oils.
3. Purification isotopes.
4. Purification isomers and enantiomers.
5. General application in environmental study, research, work ,

HPLC

Other names for HPLC :

- 1- High speed liquid chromatography.
- 2- High performance liquid chromatography.
- 3- High resolution liquid chromatography (HRLC).

HPLC is an chromatographic techniques then can be separate a mixture compounds.

It is used in biochemistry and analytical chemistry to identify quantity and purity the individual components of mixture, in HPLC the mixture dissolved in a solvent (mobile phase) and then forced to flow through chromatographic column under high pressure in the column the mixture is resolved into the compound.

The separation in HPLC occurs because the components in the mixture interacts differently with stationary phase molecules that interacts strongly move slowly through the column while the molecules that interacts less strongly will move rapidly through the column.

Instrumentation:



HPLC instrument includes:

- 1-Reservoir for solvent (mobile phase)
- 2- High pressure pump.
- 3- Sample inlet device (injector).

- 4- Column.
- 5- Detector.
- 6- Computer or recorder.

Why high pressure used in HPLC?

the solvent or mobile phase used must be passed through the column at high pressure at about 1000 to 5000 Psi this because as the particle size of stationary phase around (5-10)nm so the resistance to the flow of solvent is high

Typical pumps can reach pressure in the range of 6000 - 9000 Psi

.

Treatment of mobile phase:

- 1- Filtration before entering the column.
- 2- Degassing using degasser.
- 3- Heating with stirring.
- 4- Applying vacuum.
- 5- Passing nitrogen or helium gas.
- 6- Ultrasound.

Pump:

Pump is used for forcing the mobile phase through the column, there are two types of pumps:

- 1- Constant pressure pump, its free from pulsation, resulting baseline .
- 2- Constant flow pump, its able to give constant flowrate of mobile phase .

Elution techniques :

1- Isocratic elution : The mobile phase composition remains constant through the separation procedure.

Its best for simple separation and used for quality control.

2- Gradient elution : The mobile phase composition is changed during the separation process, its best for analysis of complex and unknown mixtures, and its most popular than isocratic elution.

Advantage of gradient elution :

1- Shortening the time analysis.

2- Reduces tailing and gives sharp peak.

3- Increases the sensitivity of an analysis.

4- Decreases the retention of later eluting compounds so that they elute faster.

pH of mobile phase :

The pH of solvent may be adjusted using phosphate or perchlorate or trifluoroacetate acid or sulphate buffer.

The selectivity of HPLC is effected by:

1- Type of mobile phase.

2- The composition of mobile phase.

3- The pH of mobile phase.

Based on mode of separation in HPLC.

The classification of HPLC according to the mechanism of separation:

1- Normal phase chromatography

The stationary phase is polar and mobile phase is nonpolar.

2- Reversed phase chromatography.

The stationary phase is non polar while the mobile phase is polar.

3- Size exclusion chromatography

The column is packed with material having controlled pore size and the sample is screened or filtered according to its molecular size there is no interaction between solute and stationary phase the large molecules rapidly washed through the column, and the smaller molecules penetrate inside the pores and elute later it is suitable for biochemistry compounds separation (proteins, enzymes....etc)

4- Ion exchange chromatography

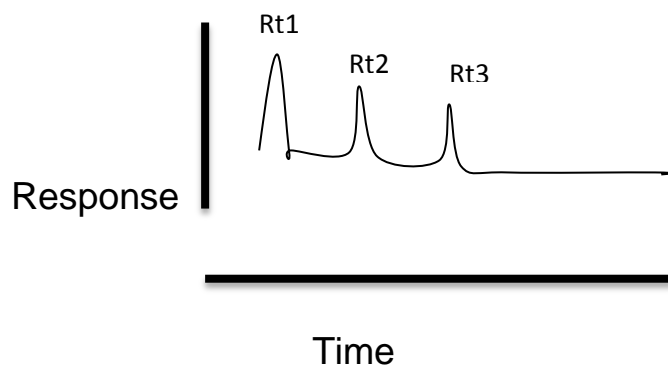
The stationary phase has an ionically charged surface of opposite charge to the sample ions.

Chromatographic process in HPLC:

1- Injection the solute onto the column (zero time).

2- The separation occurs as the analyte and mobile phase are pumped through the column.

3- Detection of compound by detector is displayed in chart or computer screen (chromatogram).



The qualitative analysis described by retention time (R_t) while the quantitative analysis described by area under the peak or peak high.

Detectors:

- 1- UV detector.
- 2- Fluorescence detector.
- 3- Refractive index detector.
- 4- Mass detector (two in one) technique (HPLC - Mass).

HPLC advantages Vs GC:

- 1- Not limited by sample volatility or thermal stability.
- 2- Two interaction phases.
- 3- Room temperature analysis.
- 4- Ease of sample recovery (preparative technique).

Notes:

Some time in HPLC technique (advanced HPLC) used guard column to remove particular matter and contamination its protect the analytical column and contains similar packing of analytical column and also connected before the analytical column.

UHPLC:

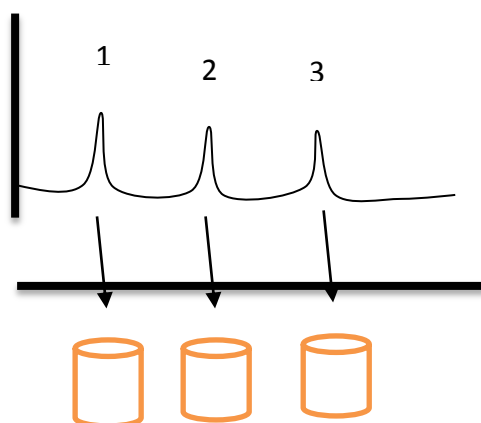
Ultra high performance liquid chromatography its different in HPLC by the size of particles of stationary phase it is < 3 nm while the size of particles of stationary phase in HPLC is (3-5) nm.

Benefits of UHPLC :

- 1- Faster analysis (typically 10x faster)
- 2- Reduced solvent consumption (typically 10x reduction)
- 3- High efficiency and higher resolution and sensitivity.

Preparative HPLC (PHPLC).

The term preparative HPLC is usually associated with large column and high flow rates, the objective of an analytical HPLC run is the qualitative and quantitative determination of compound while for preparative HPLC run it is the isolation and purification of compounds.



Scheam of preparative

The role of column in preparative HPLC typically 4-6 mm i.d for column used for small scale preparative HPLC.

7-8 mm i.d column used for semi preparative HPLC and to 20 mm i.d column for large scale preparative HPLC.

30-50 mm i.d column for higher levels scale preparative.

Working areas of preparative HPLC :

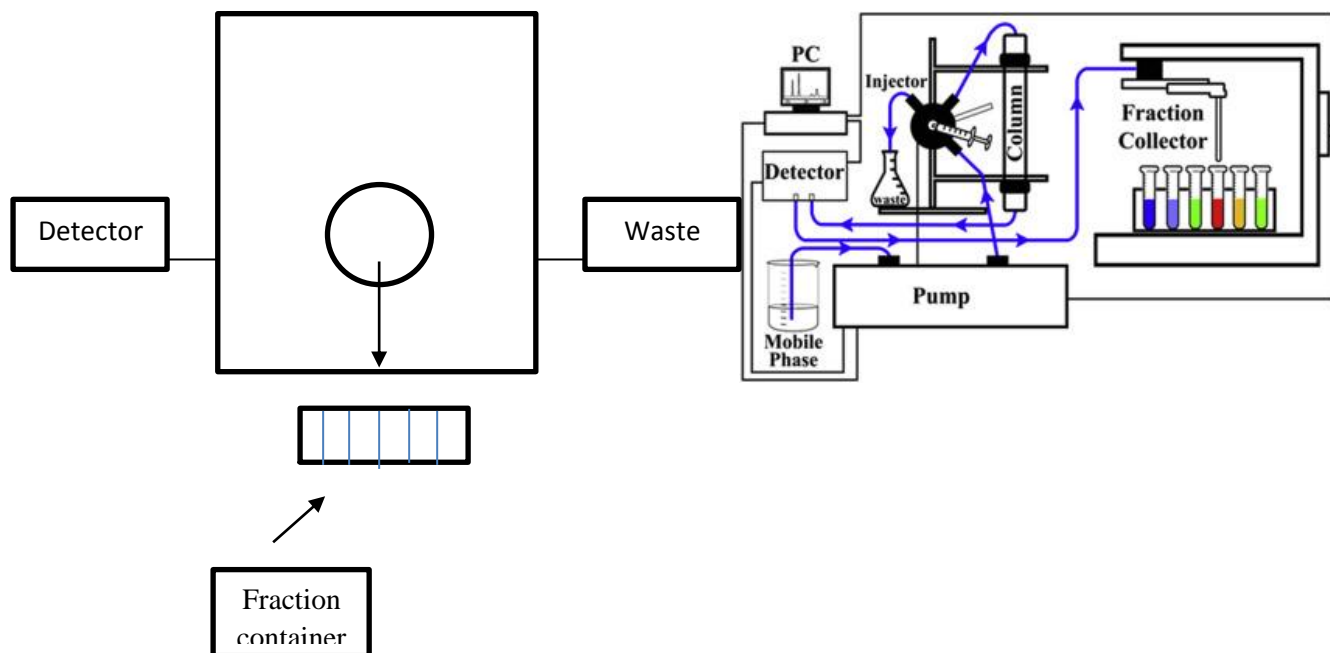
Compound amount	Working area
µg	Isolation of enzymes
mg	<ul style="list-style-type: none">- Biological and biochemical testing- Structure elucidation and characterization of:<ul style="list-style-type: none">1- Side products from production.2- Metabolites from biological matrix.3- Natural products.
gm	<ul style="list-style-type: none">- Reference compounds (analytical study).- Compounds for toxicological screening.
kg	Industrial scale , active compound , drugs.

The modern preparative instruments coupled to high efficiency and high throughput column have made the purification job of impure substance much easier

Fraction collector

In analytical HPLC the sample travels directly into the waste after it has left the detector, while in preparative HPLC the sample it goes to fraction collector, the fraction collector diverts the flow either to waste or the desired part of the injected sample, to fraction _ container vial in the fraction collector needle.

This is achieved using a diverter valve that can be switched for example by time programming or based on a detector signal.

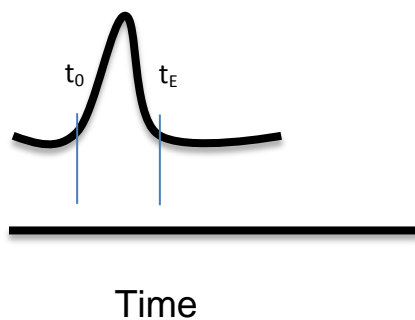


Schematics of a fraction collector

Fraction collector are commercially available in different size and designs while some can be used from very low to high flow rates, the micro fraction collector is designed for flow rates below 100 $\mu\text{l}/\text{min}$ and the preparative scale fraction collector is designed for flow rates up to 100 $\mu\text{l}/\text{min}$ in large preparative scale.

MAU

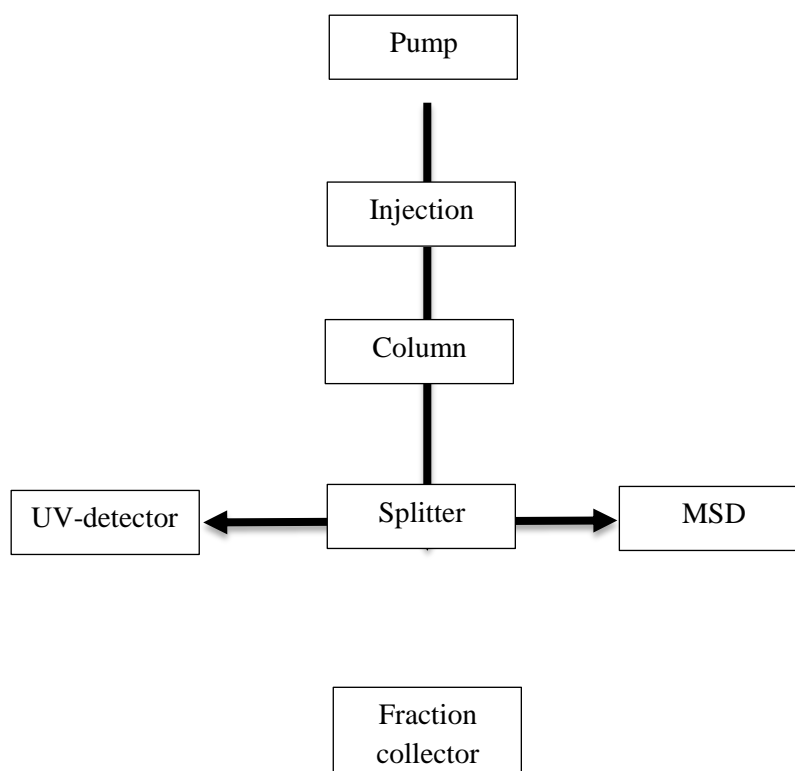
Response



And the computer software controls purification process by fraction collector unit.

Mass selective detector (MSD):

While the MSD is a destructive detector so the flow coming from the column must be split into the main flow going to the fraction collector and into the split flow going to the MSD for this is achieved using splitter.



Notes:

Column over loading occurs by :

- 1- Injection of high concentration samples.
- 2- Injection of high volume samples.

Application of PHPLC :

- 1- Clinical study.
- 2- Biochemistry study.
- 3- Food study.
- 4- Environmental study.
- 5- Manufacturing process study.
- 6- Other science fields.

Today most compounds in drug discovery are synthesized by medicinal or chemistry groups.

The chances to find an active compound are increased by synthesizing large number of compounds while medicinal chemistry takes a closer look at the drug target, PHPLC with MSD widely used in these studies (drugs discovery) .