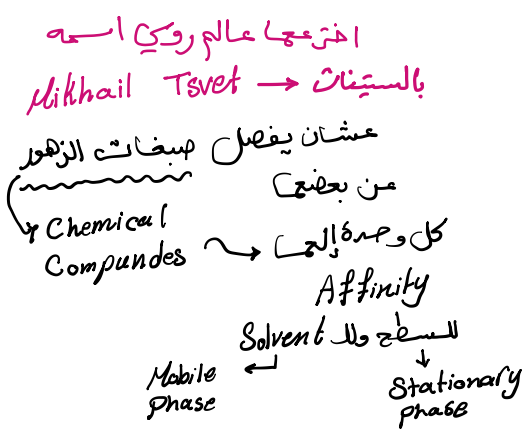
تفريغ تحليل آلي

اسم الموضوع: Chromatography Part 1

إعداد الصيدلاني/ـة: Sara Jaber

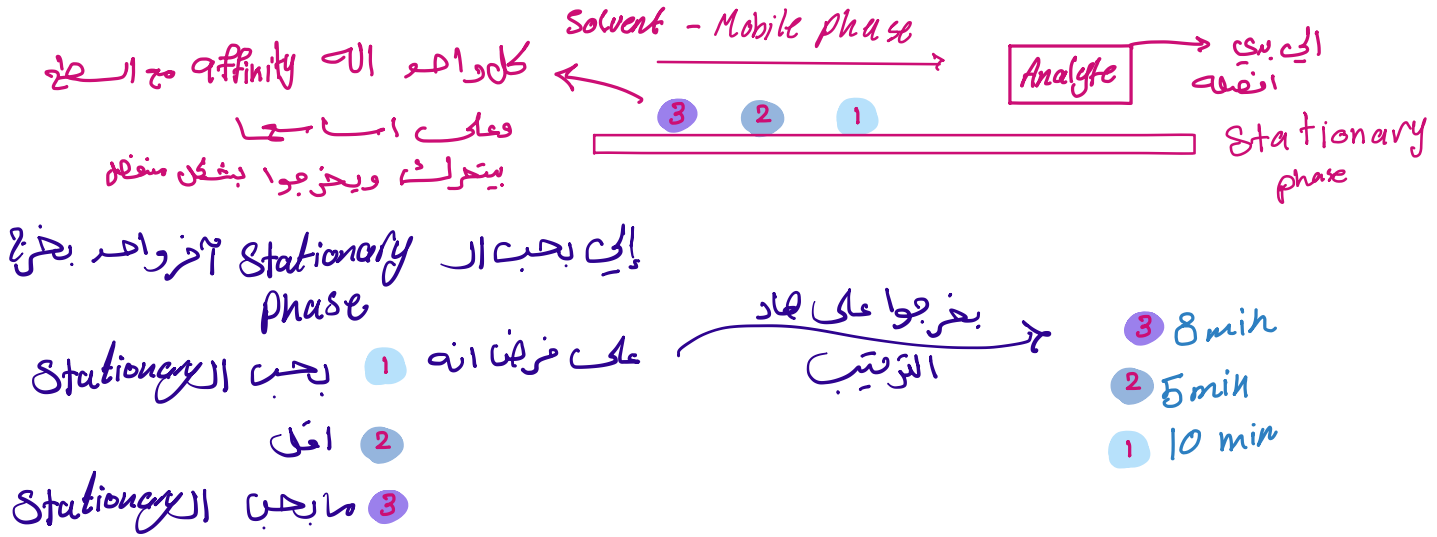
Seul Academy

لجان الدفعات



Chapter-26:

An Introduction to Chromatographic Separations



Introduction to Chromatography

Definition

Chromatography is a separation technique based on the different interactions of compounds with two phases, a *mobile phase* and a *stationary phase*, as the compounds travel through a supporting medium.

Components:

Mobile phase: a solvent that flows through the supporting medium

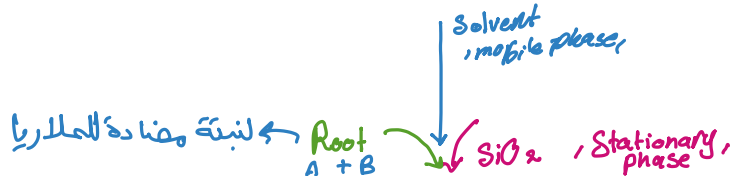
Stationary phase: a layer or coating on the supporting medium that interacts with the analytes

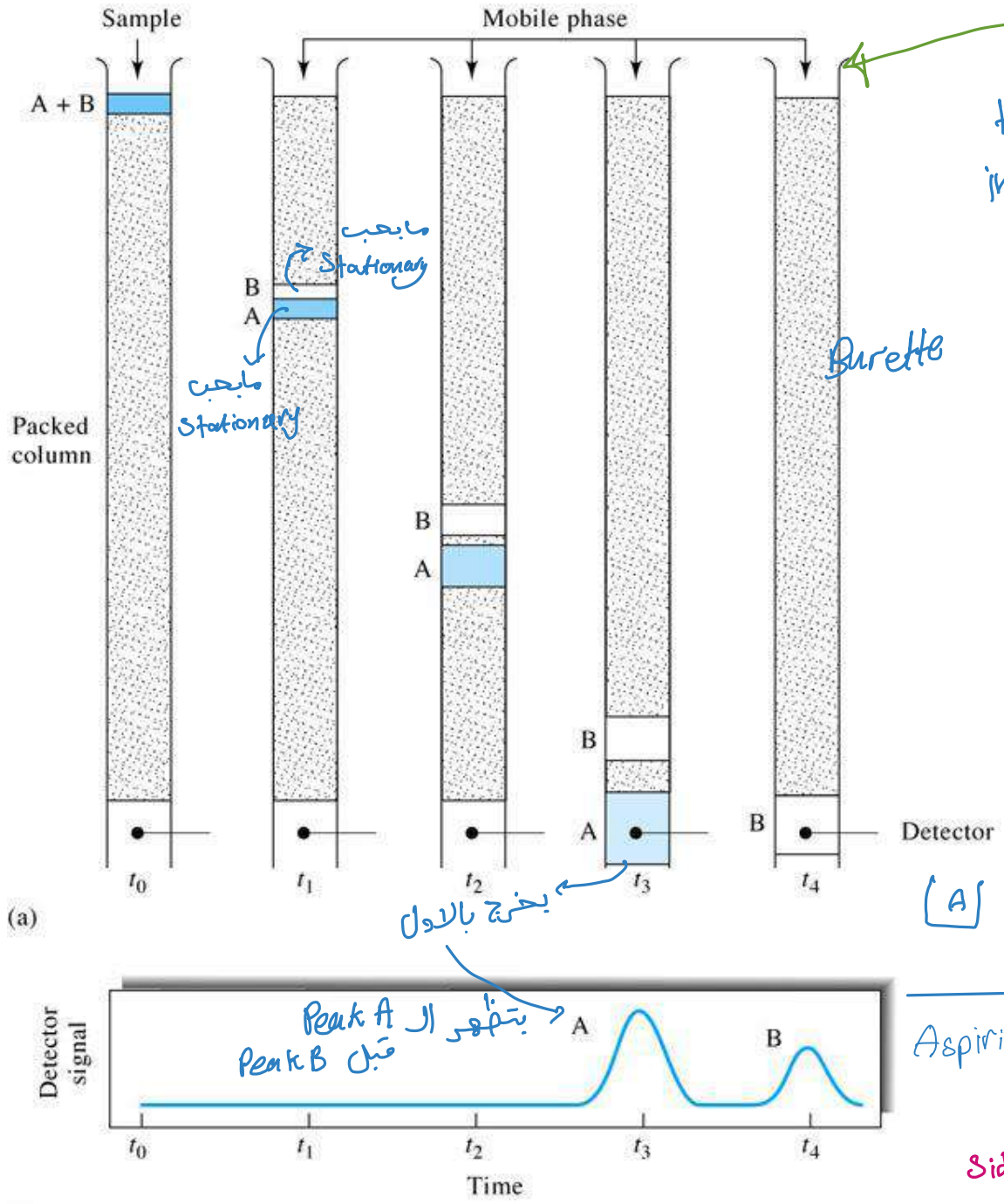
Supporting medium: a solid surface on which the stationary phase is bound or coated

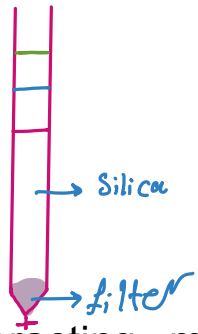
Uses for Chromatography

Real-life examples of uses for chromatography:

- Pharmaceutical Company – determine amount of each chemical found in new product
- Hospital – detect blood or alcohol levels in a patient's blood stream
- Law Enforcement – to compare a sample found at a crime scene to samples from suspects
- Environmental Agency – determine the level of pollutants in the water supply
- Manufacturing Plant – to purify a chemical needed to make a product







The analytes interacting most strongly with the stationary phase (B) will take longer to pass through the system than those (A) with weaker interactions.

These interactions are usually chemical in nature, but in some cases physical interactions can also be used.

Types of Chromatography

1) The primary division of chromatographic techniques is based on the type of mobile phase used in the system:

Type of Chromatography	Type of Mobile Phase
Gas chromatography (GC)	gas
Liquid chromatograph (LC)	liquid

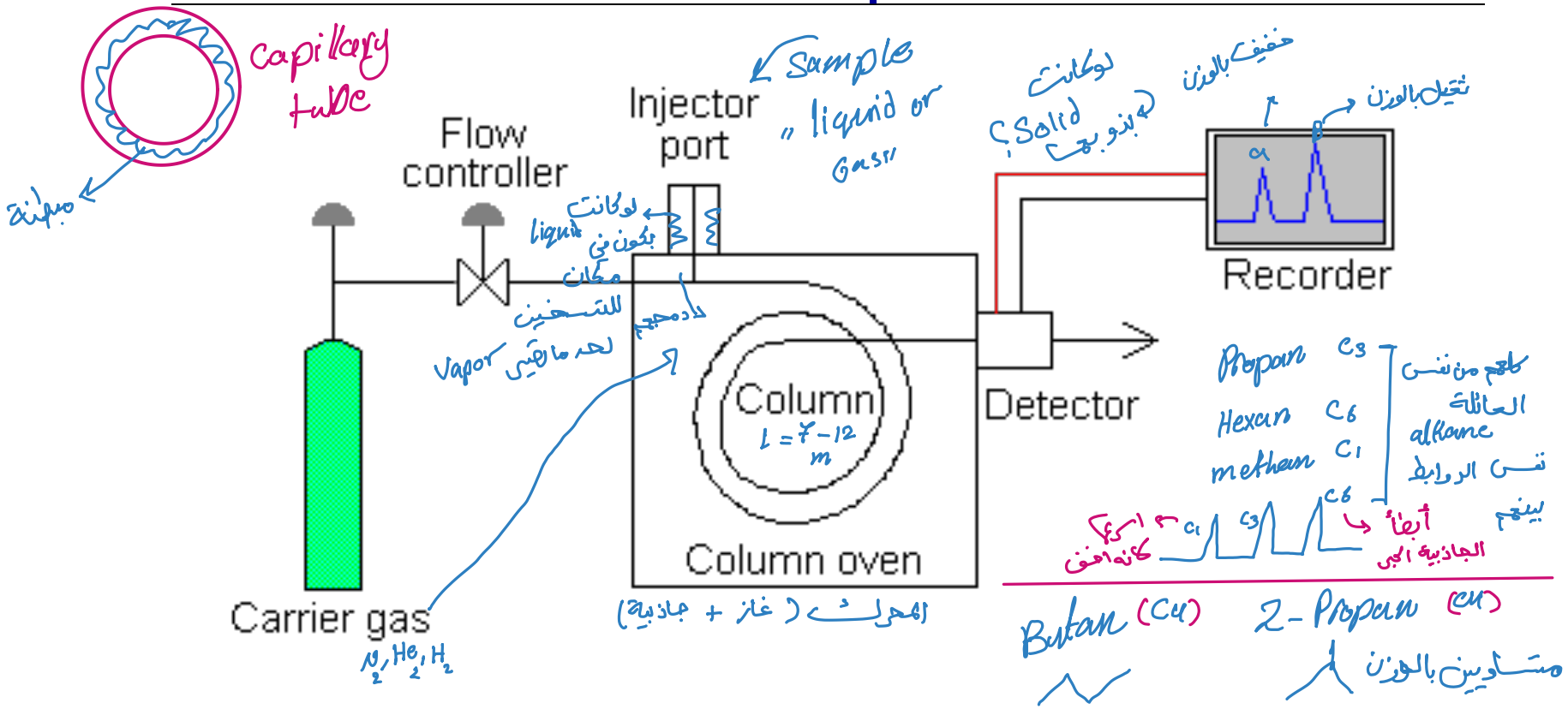
اختلاف ال mobile phase

Types of Chromatography

2) Further divisions can be made based on the type of stationary phase used in the system:

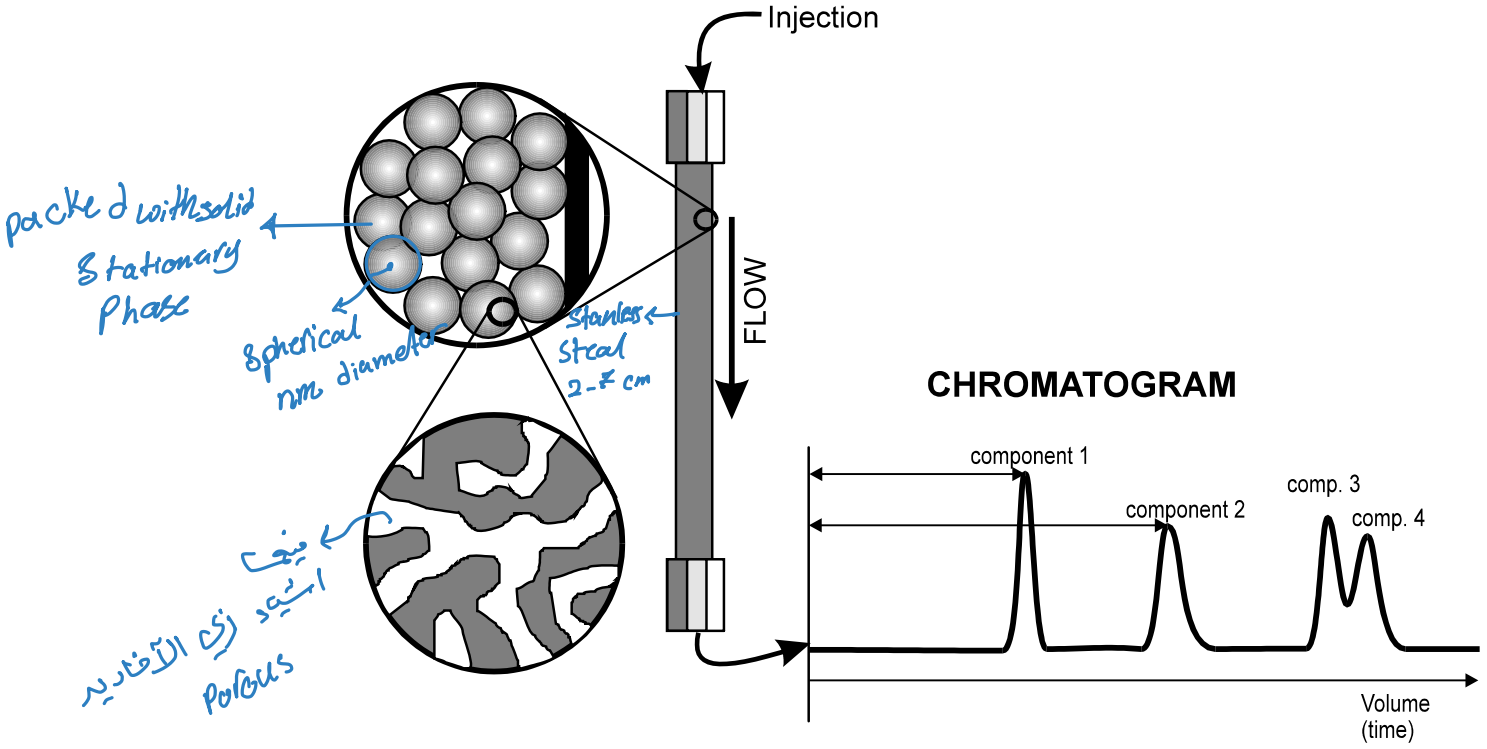
Gas Chromatography (GC)

Name of GC Method	Type of Stationary Phase
Gas-solid chromatography	solid, underivatized support
Gas-liquid chromatography	liquid-coated support
Bonded-phase gas chromatography	chemically-derivatized support



Liquid Chromatography (LC)

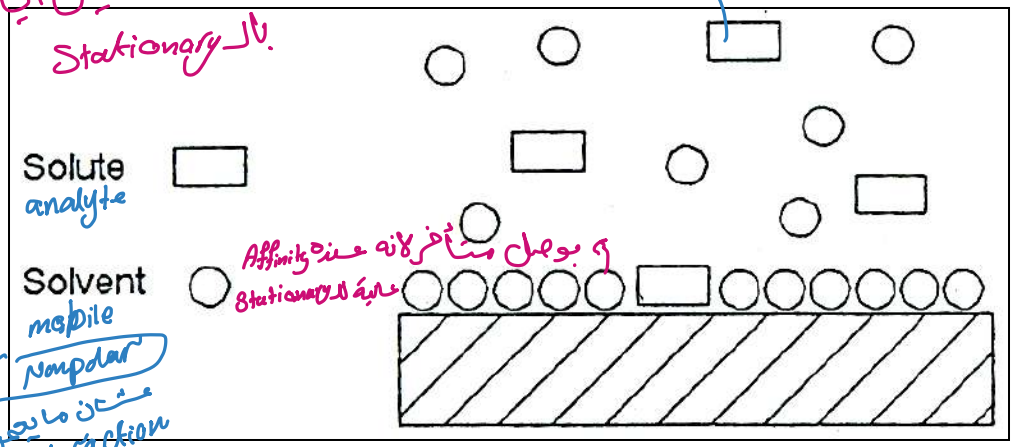
Name of LC Method	Type of Stationary Phase
Adsorption chromatography	solid, underivatized support
Partition chromatography	liquid-coated or derivatized support
Ion-exchange chromatography	support containing fixed charges
Size exclusion chromatography	porous support
Affinity chromatography	support with immobilized ligand



Adsorption Chromatography

مركبين قريبين من بعض الوزن ومختلفين بالـ Polarity

Separation based on their adsorption onto the surface of solid (stationary phase).



Normal phase-like separation

Nonpolar mobile phase for polar, non-ionic compounds

Ex; Column chromatography TLC, HPLC

Polar stationary phase Silica

Propane → non polar

Analyte → mobile phase → nonpolar

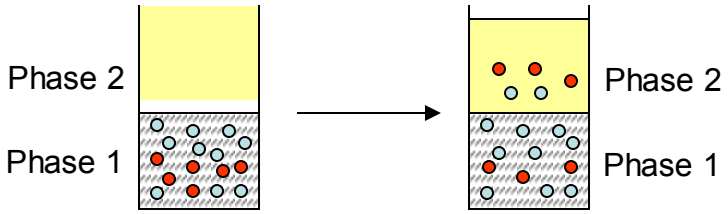
Propanol C_3H_7

Paper Chromatography

Partition Chromatography

الاثنين قريبين جدًا في ال Polarity

Solute are separated based on their partition between a liquid mobile phase and a liquid stationary phase coated on a solid support.



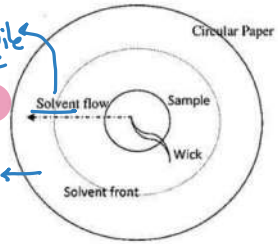
- Normal – analyte is nonpolar organic; stationary phase MORE polar than the mobile phase
- Ex : TLC, Paper Chromatography

adsobtion

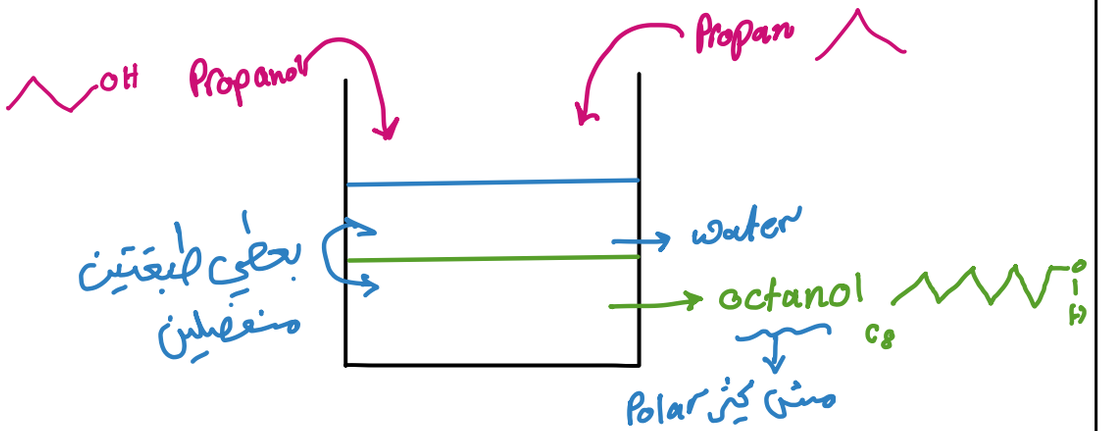
Partition

Polar mobile phase

Stationary Non Polar



- Reverse – analyte is polar organic; stationary phase LESS polar than the mobile phase
- Ex : HPLC



$$\text{Log } P = \frac{[X \text{ in octanol}]}{[X \text{ in water}]}$$

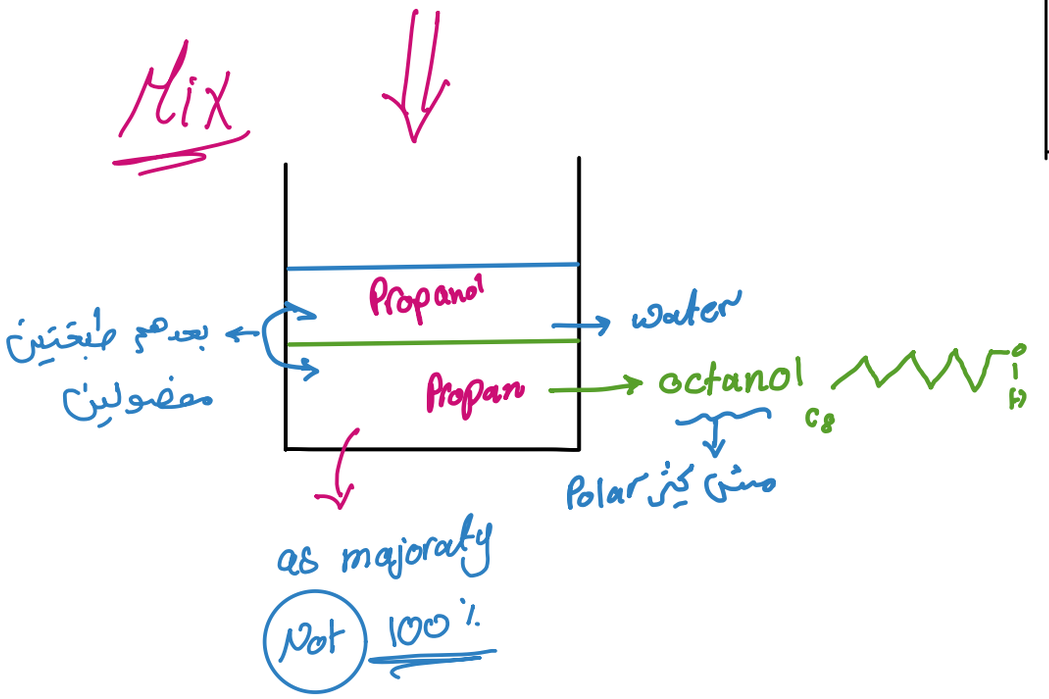
Log P ← Partition Coefficient

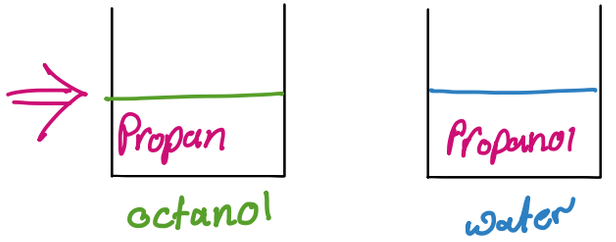
رقم نحبي ← اعرفلي Non polar

رقم صغير ← اعرفلي Polar

اقل من 2 ⇐ موجود بالماء

الادوية اللي $\log P > 4.2$ ← Toxic ← لانها بتدخل لل cell وبتواجه صعوبة بالخروج





إذا كانوا قريبين من بعض بال Polarity

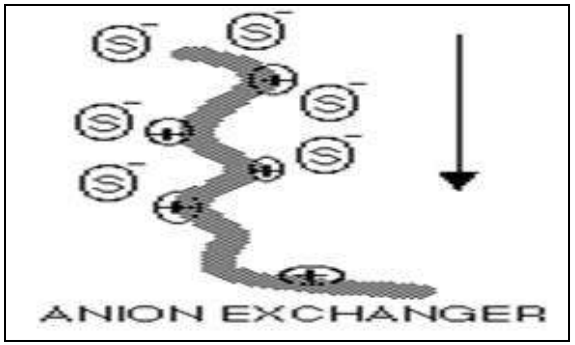
Butanol & Propanol

Butan & Propan

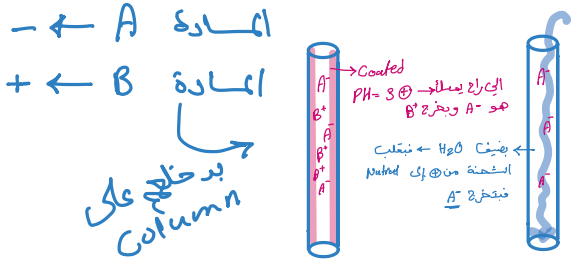
قريبين جدًا بالصفات

Ion Exchange Chromatography

- Use ionic stationary phase
  - ions separated on the basis of their tendency to displace counter ions adsorbed on stationary phase (Depends on charge, hydration, "solubility"
  - Used for analysis of aminoacids and its base pair.



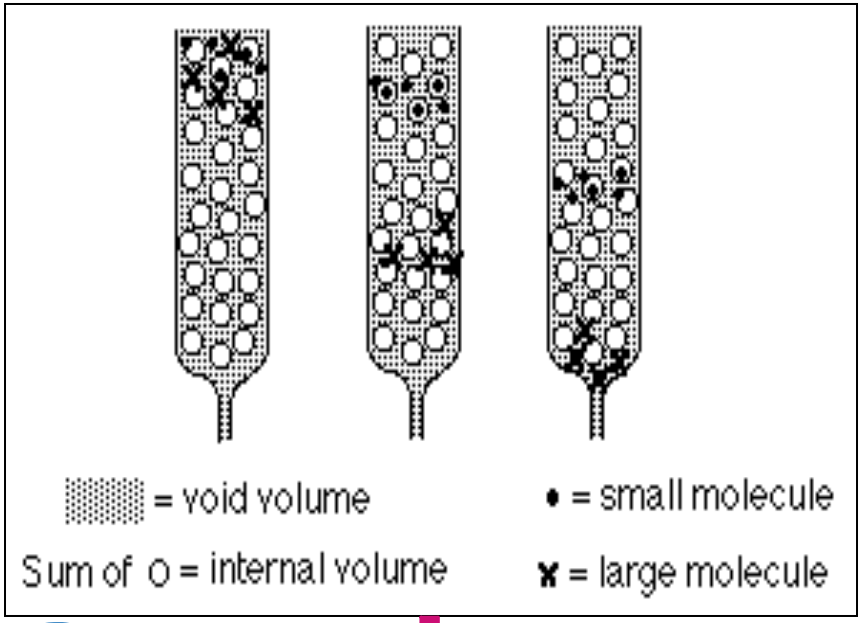
- Anionic stationary phases: used for cation separation
- Cationic stationary phases : for anion separation for ionic compounds
- - Ex : HPLC

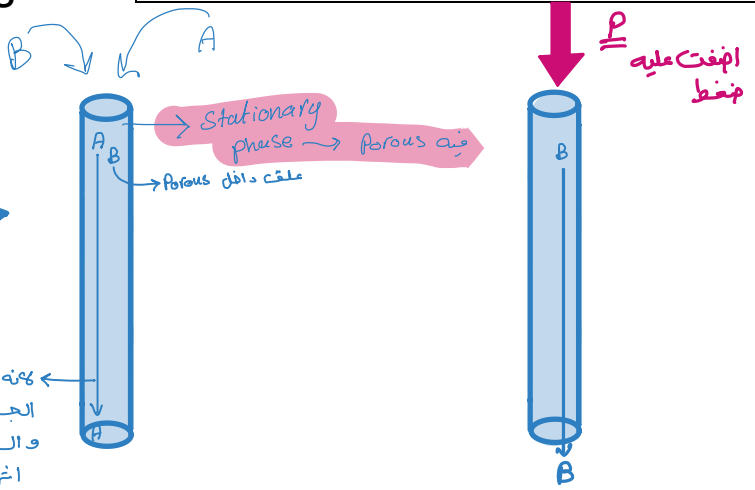


Size Exclusion Chromatography

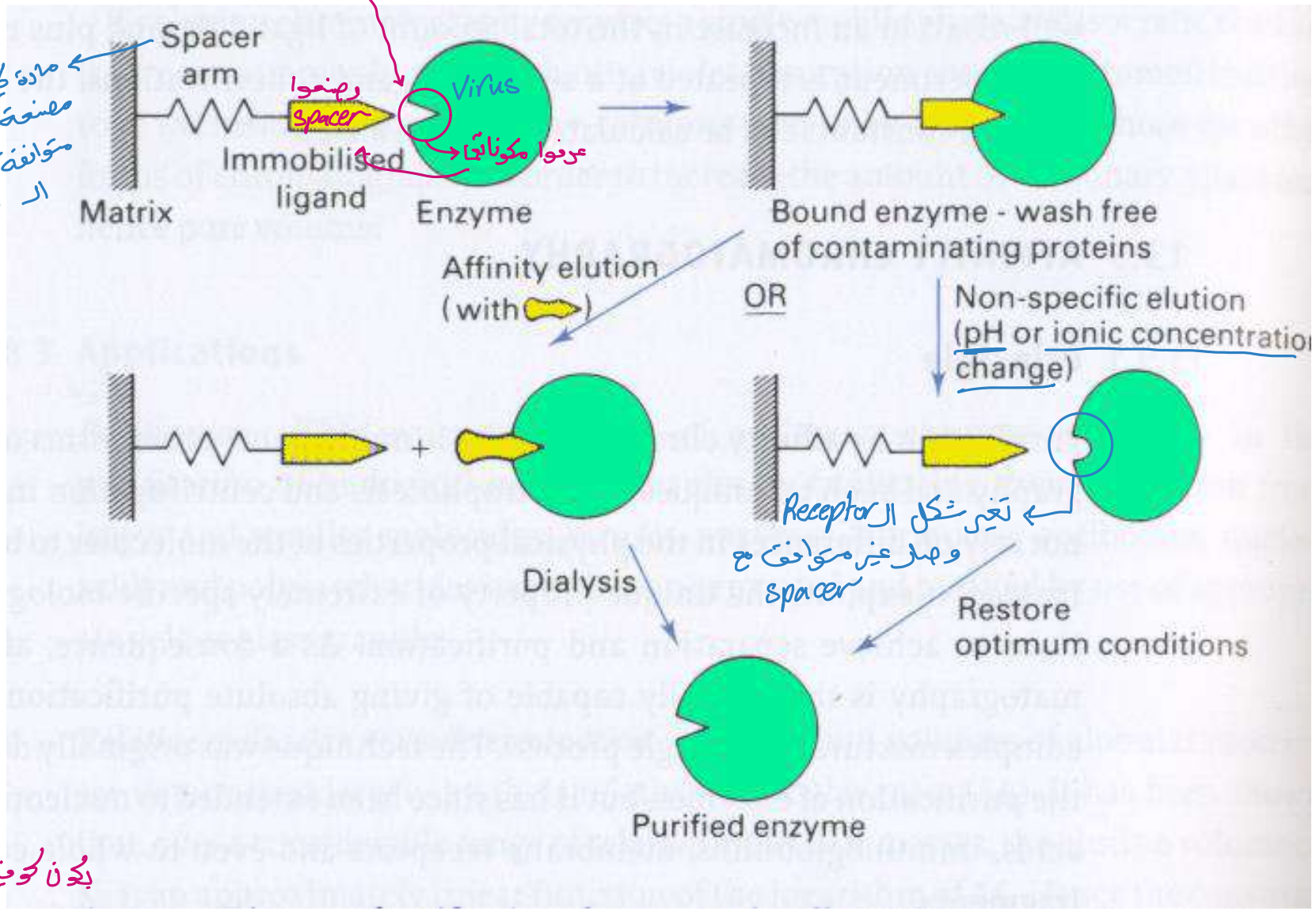
- Separation is a result of "trapping" of molecules in the pores of the packing material

- Very large molecules can't get into the pores – unretained
- Very small molecules get hung up in to pores for a long time - most retained – longest retention time
- stationary phase is a porous matrix
- Ex: GC, HPLC



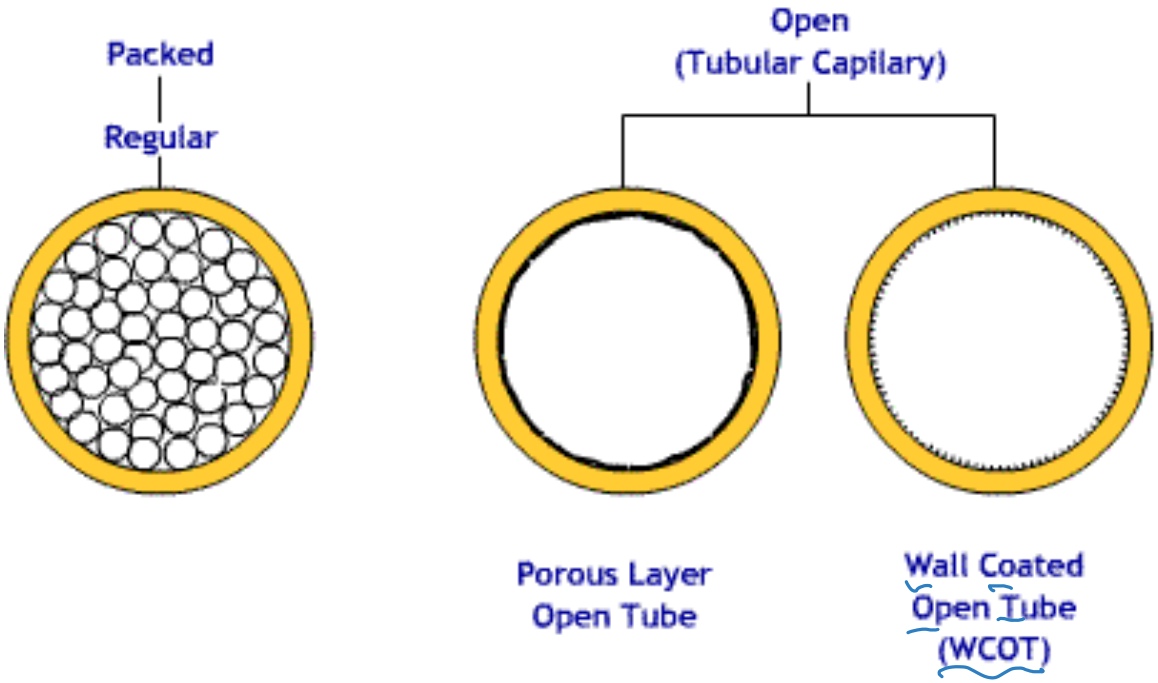


Affinity chromatography



3) Chromatographic techniques may also be classified based on the type of support material used in the system:

Packed bed (column) chromatography
Open tubular (capillary) chromatography
Open bed (planar) chromatography



Some chromatography terms

Analyte

- Substance that is to be separated during chromatography

Immobilized phase

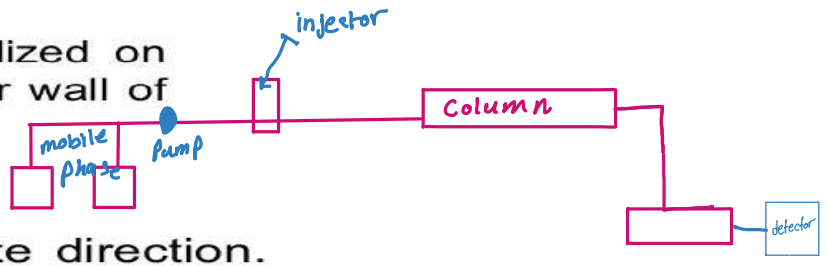
- Stationary phase which is immobilized on the support particles or on the inner wall of the column tubing

Mobile phase

- Phase which moves in a definite direction. (liquid/gas/fluid).
- Consists of the sample being separated/ analyzed and the solvent that moves the sample through the column.

Effluent

- Mobile phase leaving the column.



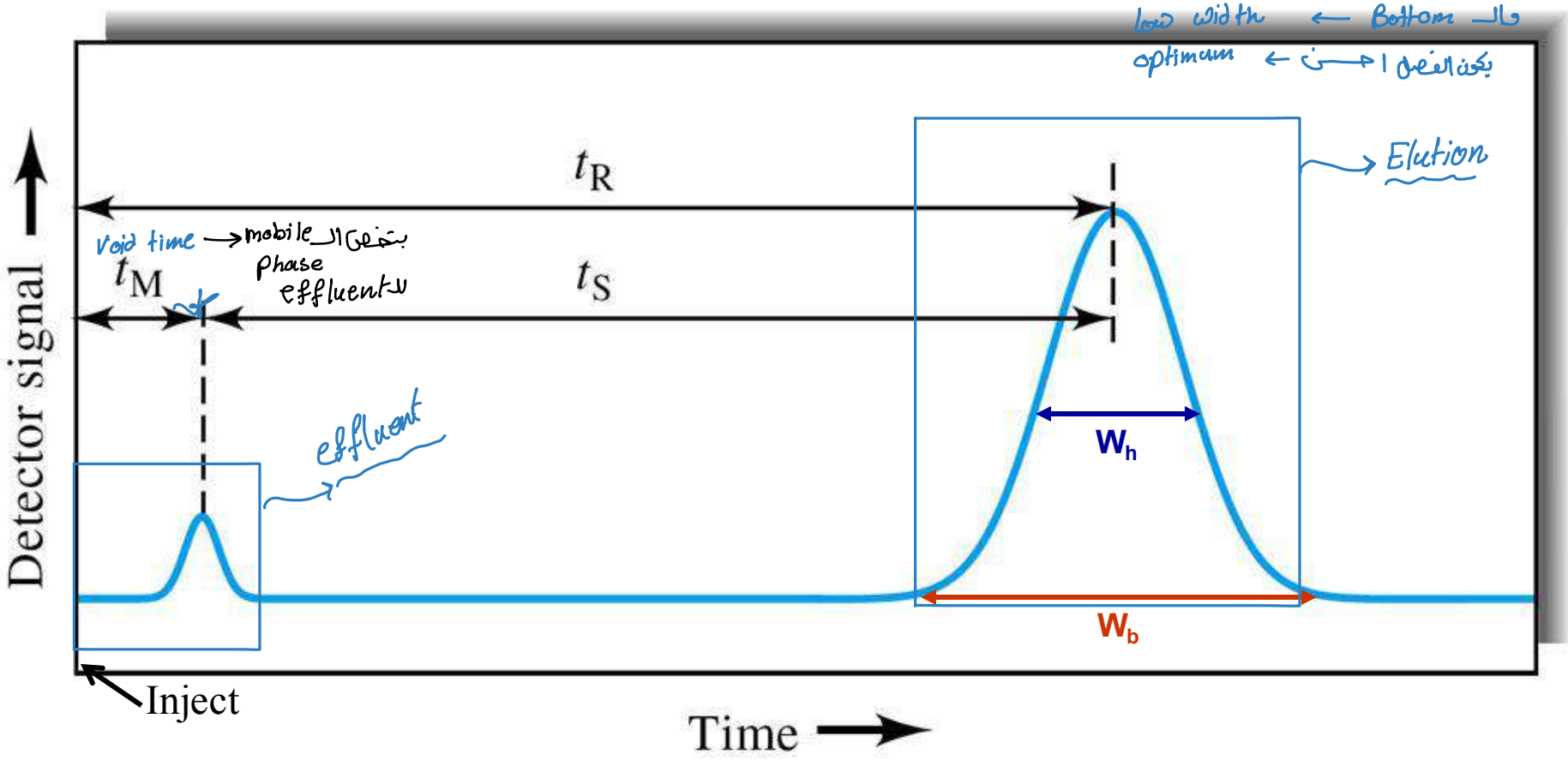
← اول اشي بخرج هو الـmobile phase واسمه الـ Effluent

← بعدهيك تخرج العينة بصورة بطيئة بتنفصل من الـSolid و بتنتهي عندي

Elution: is the process of removing analyte from stationary phase by mobile phase

1) Typical response obtained by chromatography (i.e., a chromatogram):

chromatogram - concentration versus elution time



Where:

t_R = retention time t_S = solute retention time

t_M = void time

W_b = baseline width of the peak in time units

W_h = half-height width of the peak in time units

High performance liquid chromatography

- HPLC is an extension of conventional liquid chromatography.
- Powerful tool in analytical techniques
- Columns are tightly packed, and the eluent is forced through the column under high pressure(up to 5,000 psi) by a pump.
- Allows to use a very smaller particle size for the column packing material which gives a much greater surface area for interactions between the stationary phase and the molecules flowing through it.
- Allows a much better separation of the components of the mixture.

mobile
* ممكن نوقف واحد
* لو الحش من واحد
لكن النسبة ثابتة
ونفس النوع
طول مدة الـ Operation

	A	B
1min	50%	50%
3min	50%	50%
10min	50%	50%

ثابت نوعًا
وكمًا

HPLC can classify base on elution method

اكتر اشي mobile
وبيتغير تركيزهم خلال الزمن

	A	B
1min	50%	50%
3min	60%	40%
10min	50%	50%

سؤال عليهم
[A B C D]
* لو ضلت التراكيز ثابتة مع الزمن
* لو اختلفت التراكيز

Isocratic

Gradient

Liquid Chromatography: Adsorption chromatography

Isocratic

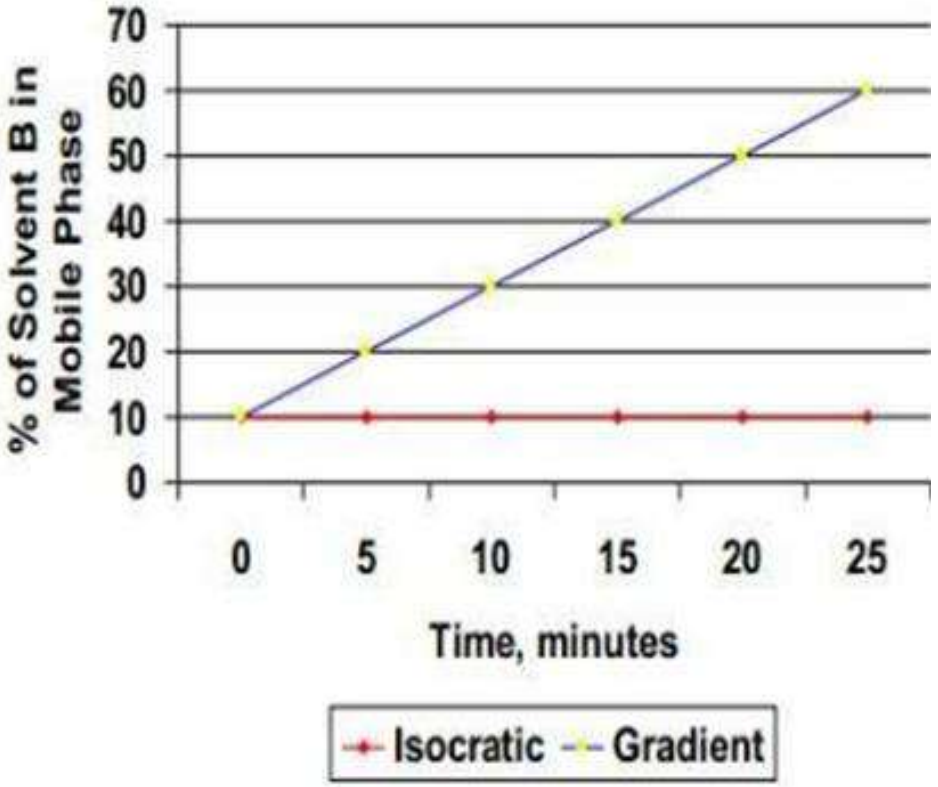
mobile phase solvent composition remains constant with time

- Best for simple separations
- Often used in quality control applications that support and are in close proximity to a manufacturing process

Gradient

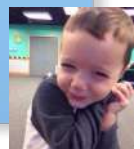
mobile phase solvent ("B") composition increases with time

- Best for the analysis of complex samples
- Often used in method development for unknown mixtures
- Linear gradients are most popular (for example, the "gradient" shown at right)



Normal → Stationary → Polar
Mobile → Non polar

Reverse (Normal العكس)



HPLC can be classified based on separation mode to

- **1- Normal Phase;** when the stationary phase is more polar than the mobile phase
- **2- Reverse Phase;** when the mobile phase is more polar than the stationary phase
- **Reverse phase chromatography;** The silica gel is polar and to be used for the reverse phase separation, its polar surface has to be changed. This can be done by attaching different functional groups such as hydrocarbons mostly C-8 and C18 (none polar).
- As a result we create a none polar phase. This type is used more than the Normal Phase, and the reason why it is more popular is that its weak mobile phase is the high polar water, therefore, the samples are applied in this weak mobile phase i.e applied in aqueous status such as biological compounds.
- This makes it especially attractive in clinical chemistry for drug confirmation, amino acid analysis and hormone separations.

Normal

SiO_2

Polar Stationary phase

mobile phase

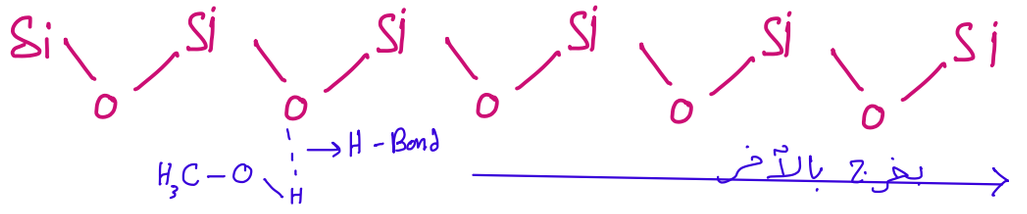
Non polar

(Acetonitrile $CH_3C{\equiv}N$)

Analyte

CH_4 + CH_3OH → Polar

Methan → Non Polar Methanol



CH_4 يخرج بالاول

- ترتيب المواد حسب سرعة الخروج

① Non polar

② weak polar

③ Polar

Reverse

$SiO + C_{18}$

Non Polar Stationary phase

mobile phase

polar

(methanol , water)

Analyte

CH_4 + CH_3OH → Polar

Methan → Non Polar Methanol

CH_4 يخرج بالآخر

H_3C-O-H يخرج بالأول

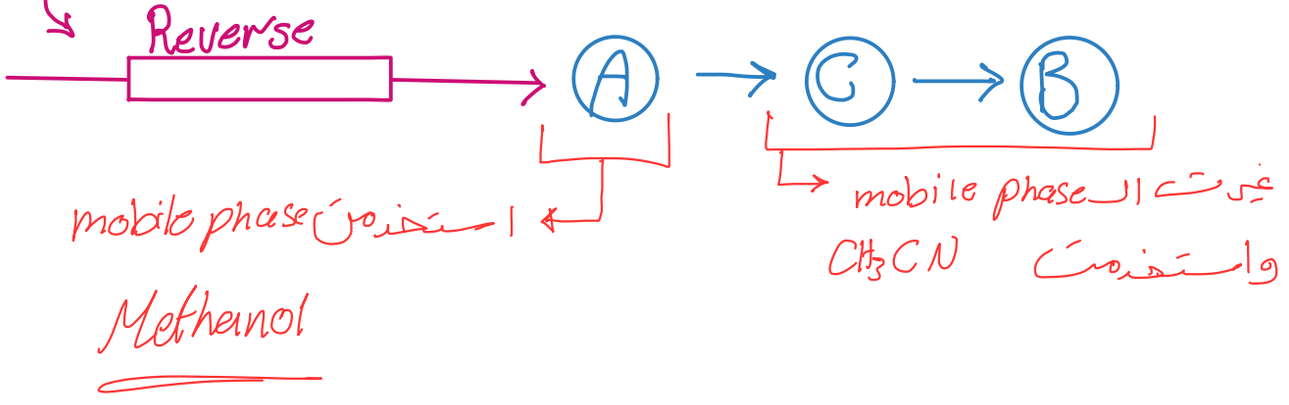
الترتيب

① Polar

② Second

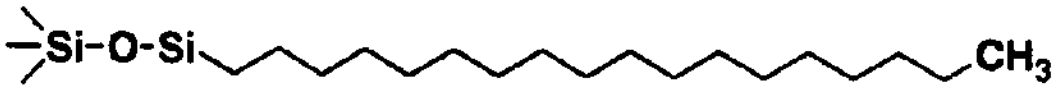
③ low polar

A	B	C
Polar	Non Polar	low Polar

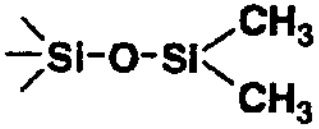


mobile / time	Methanol	CH_3CN
1min	100 %	0 %
3 min	50 %	50 %
5min	0 %	100 %

Common Reverse Phase (RP) Packings

Octadecyl - 

Octyl - 

Dimethyl - 

NORMAL PHASE:

Cyanopropyl - 

Aminopropyl - 

Qualitative & Quantitative

HPLC Technique

High Performance Liquid Chromatography

- Utilizes liquid mobile phase to separate the mixture
- Analytes are first dissolved in a solvent then through the column under high pressure of up to 400 atm

By the pump ⇒ في عملية الضغط يتم اضافة ضغط

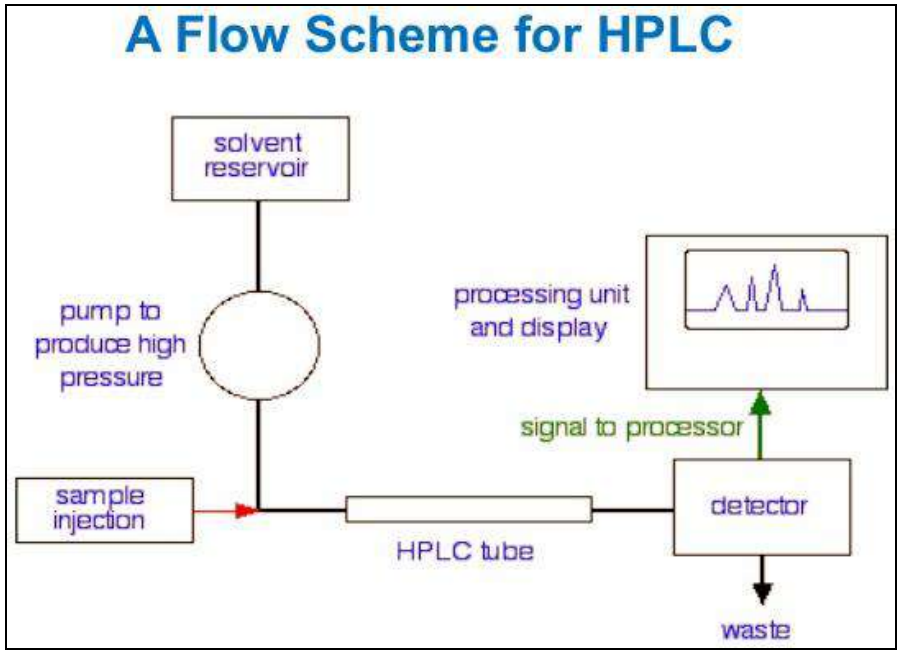
- Mixture is resolved into its components in the column
- The total separation time is often 5 or 10 minutes rather than hours or even days required for some separations by gravity flow with the larger systems.

instrument not tool

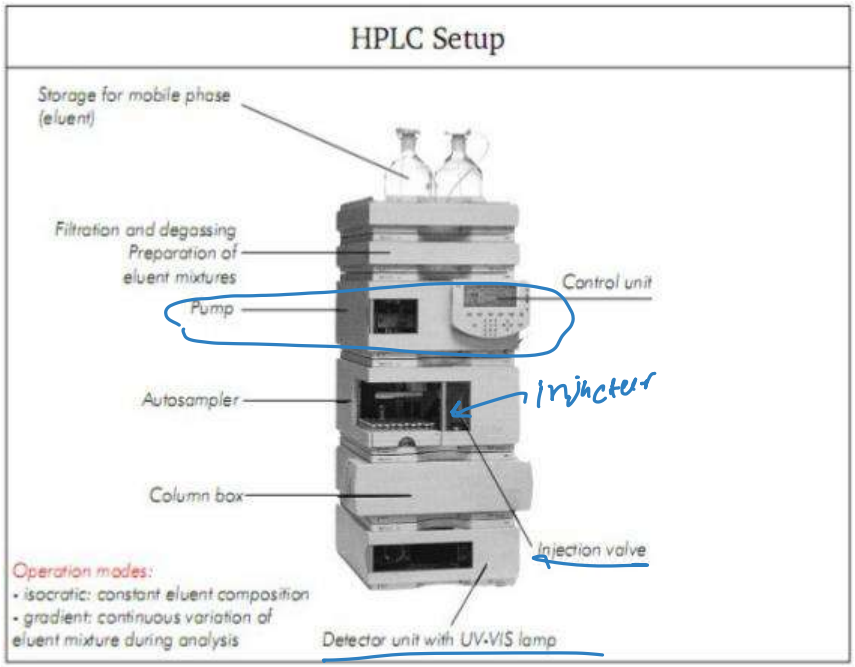
Components of HPLC

- Pump
- Injector
- Column
- Detector
- Recorder or data system

A Flow Scheme for HPLC

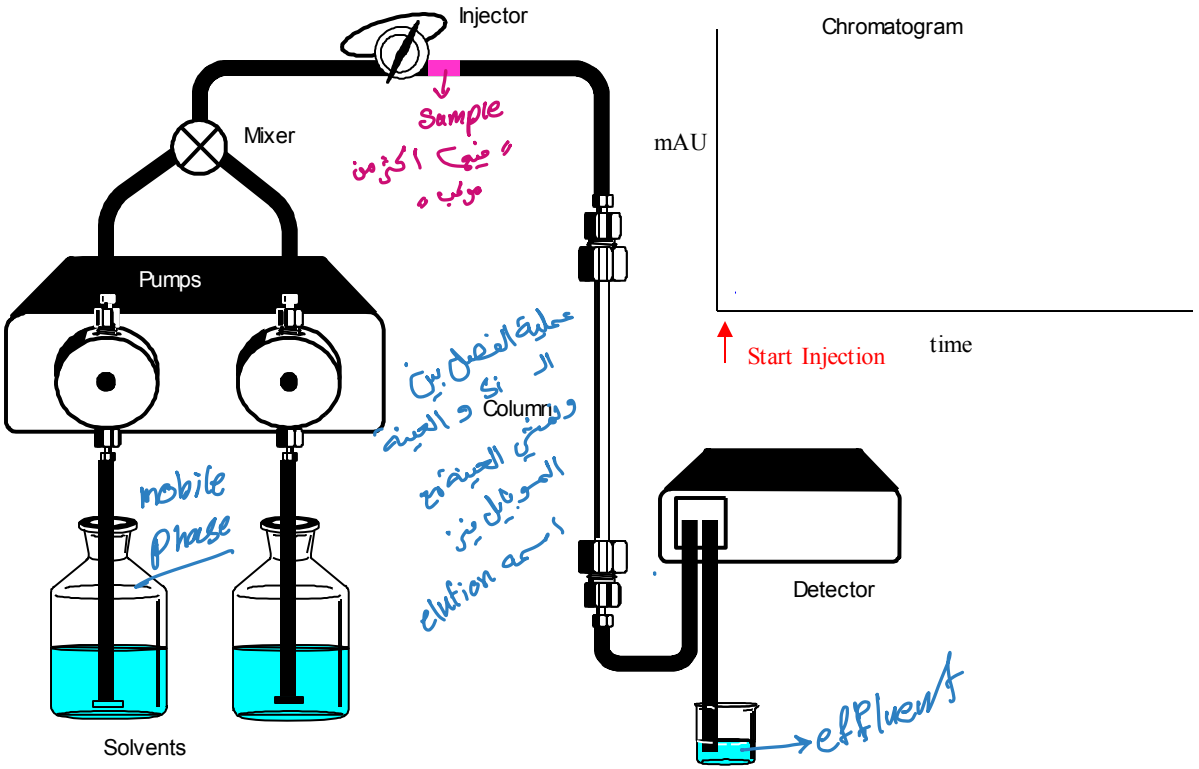


HPLC Setup



1

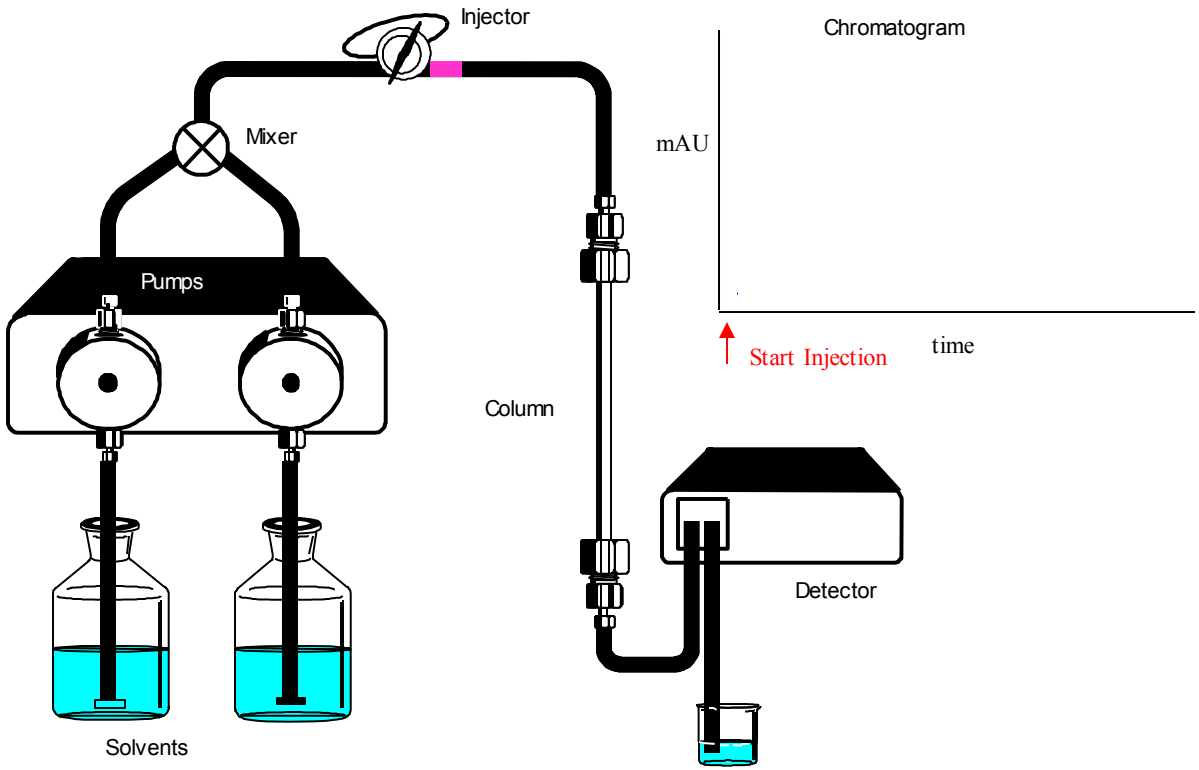
Separations



High Performance Liquid Chromatograph

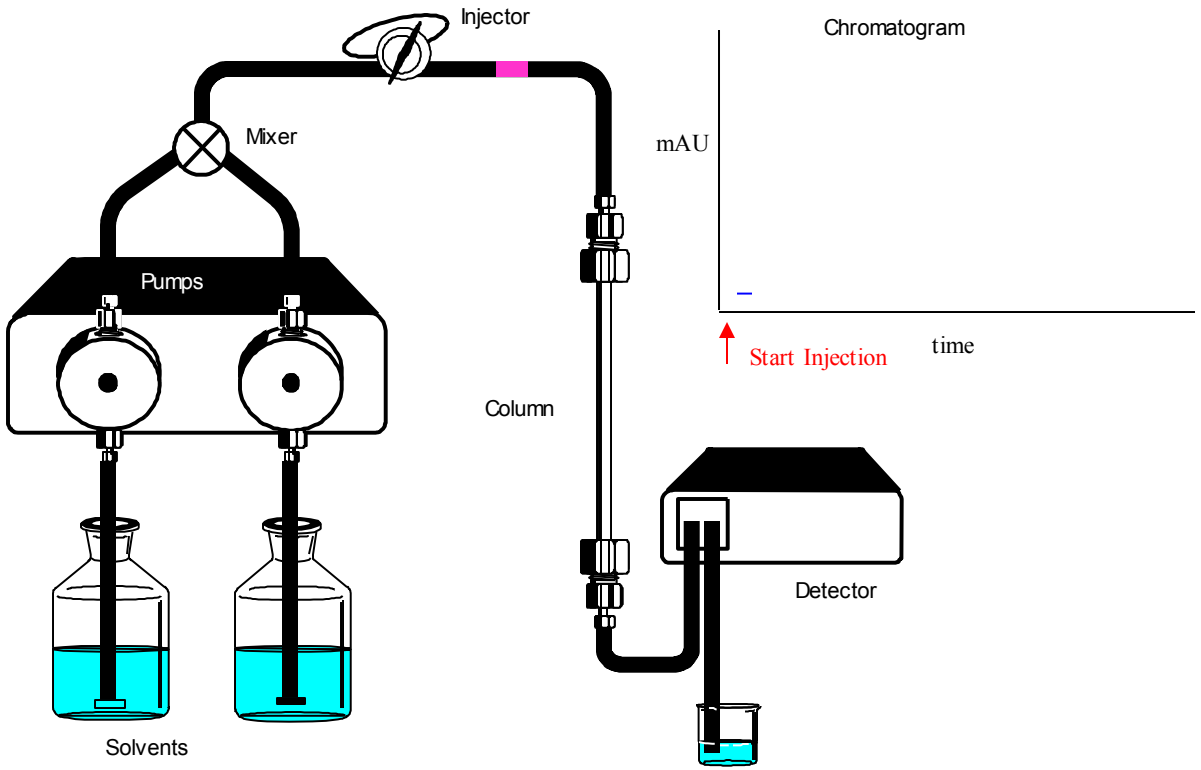
2

Separations



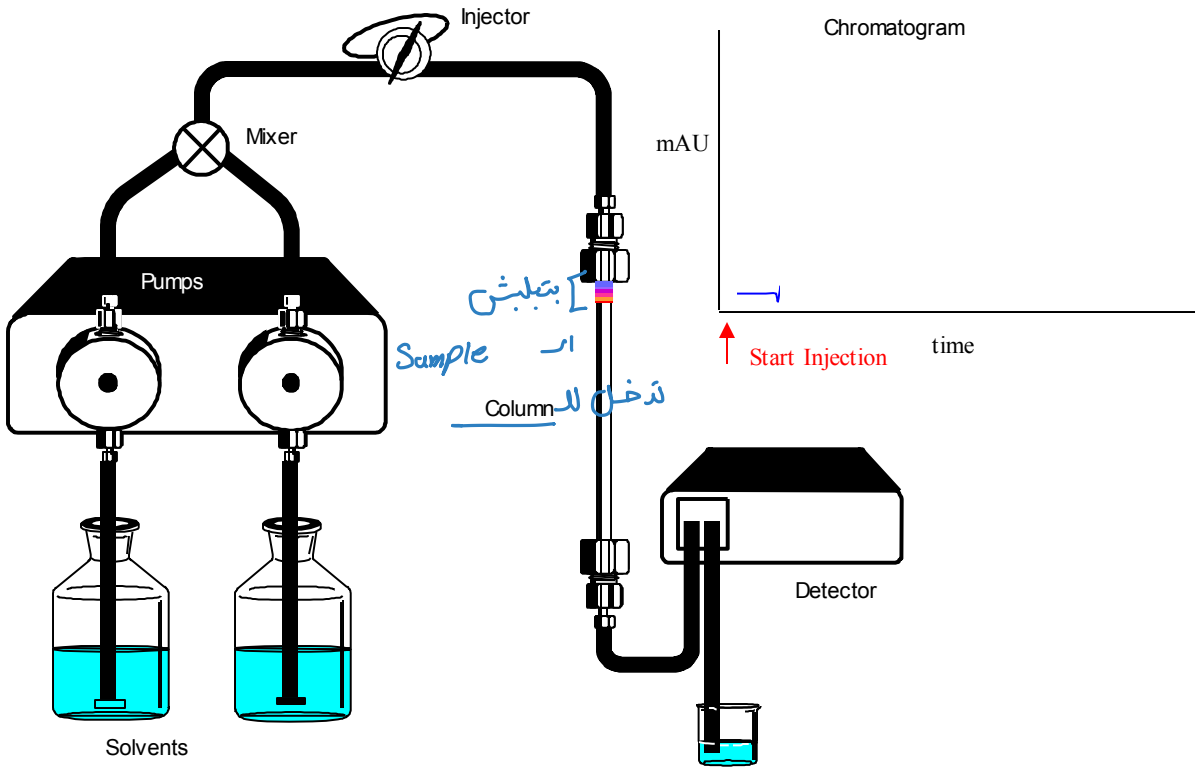
3

Separations

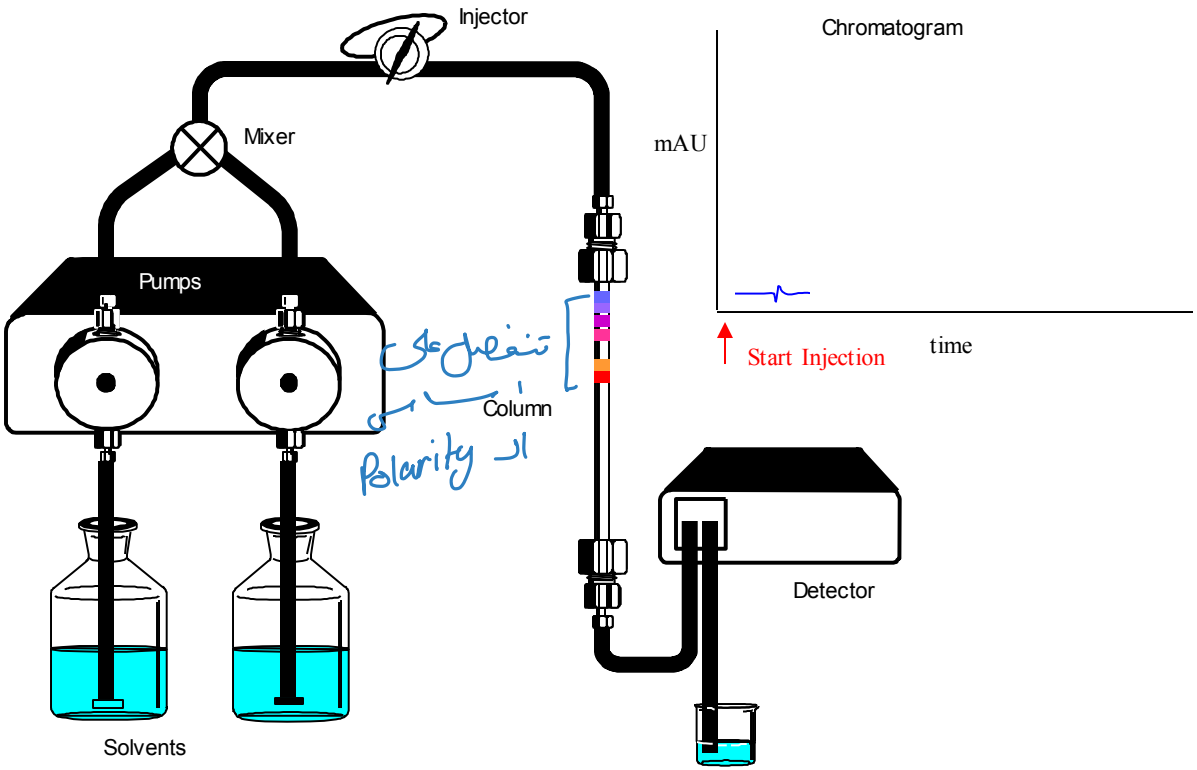


4

Separations

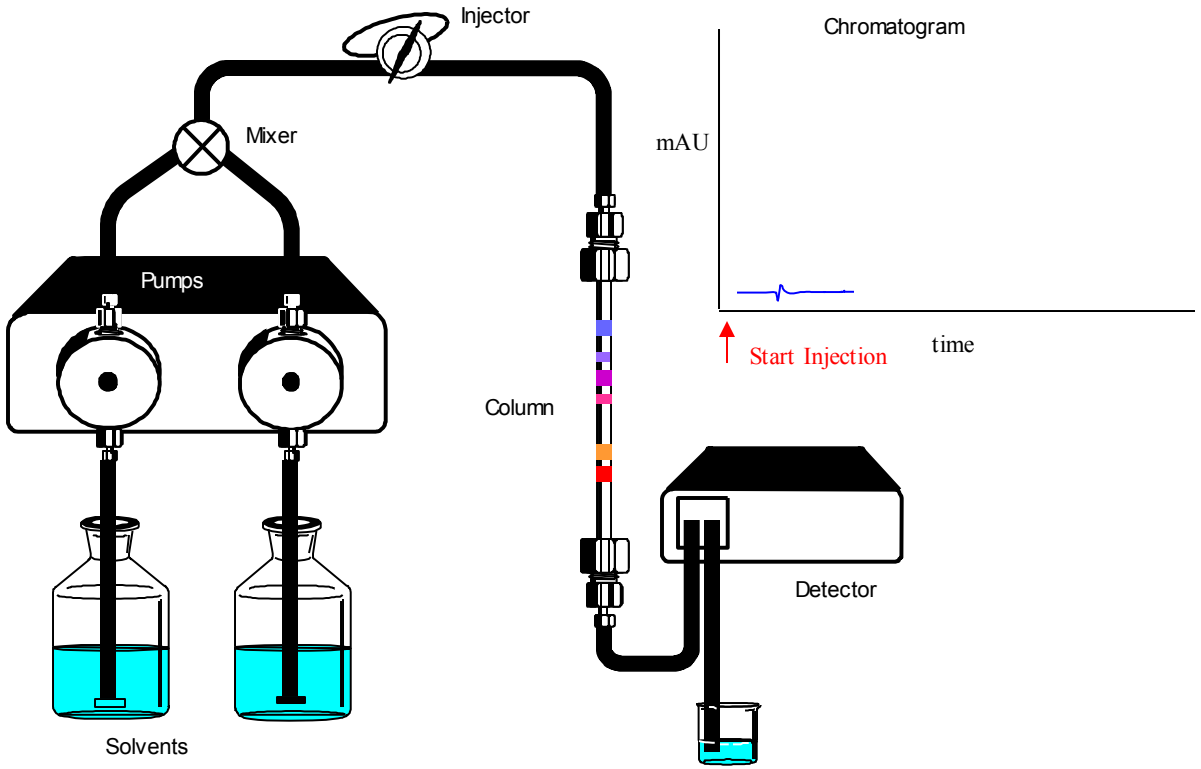


Separations

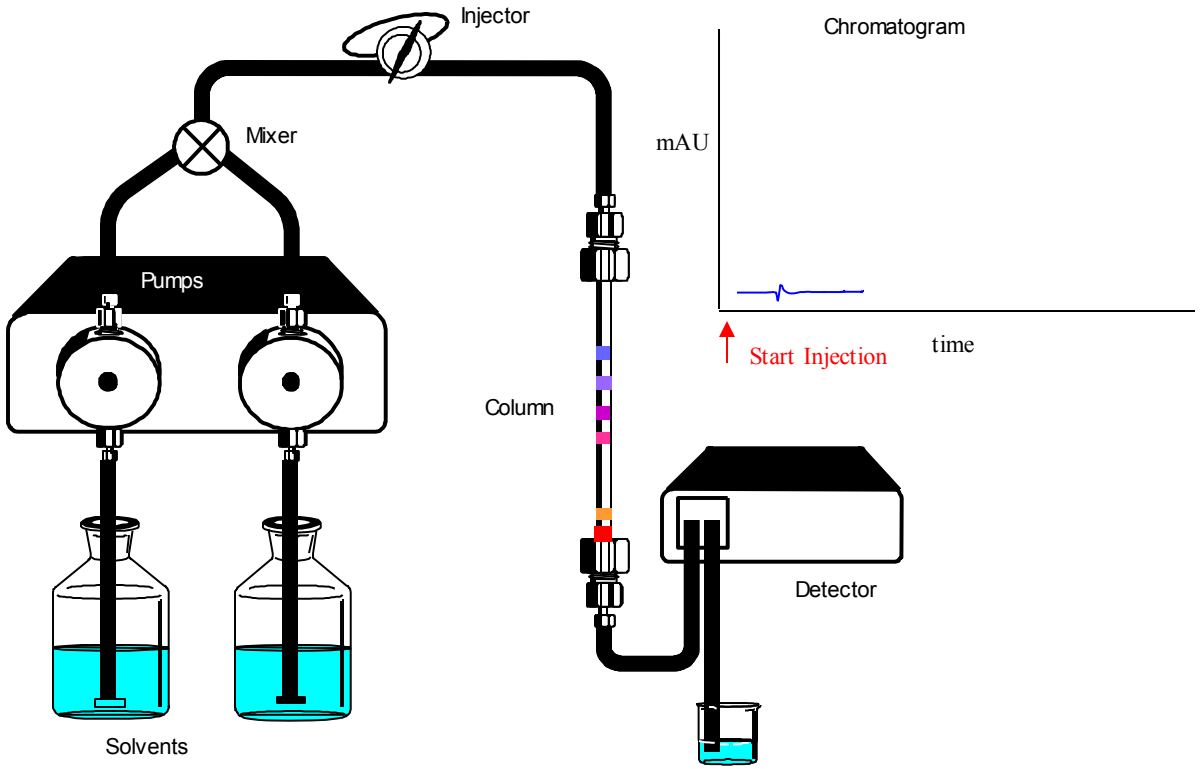


6

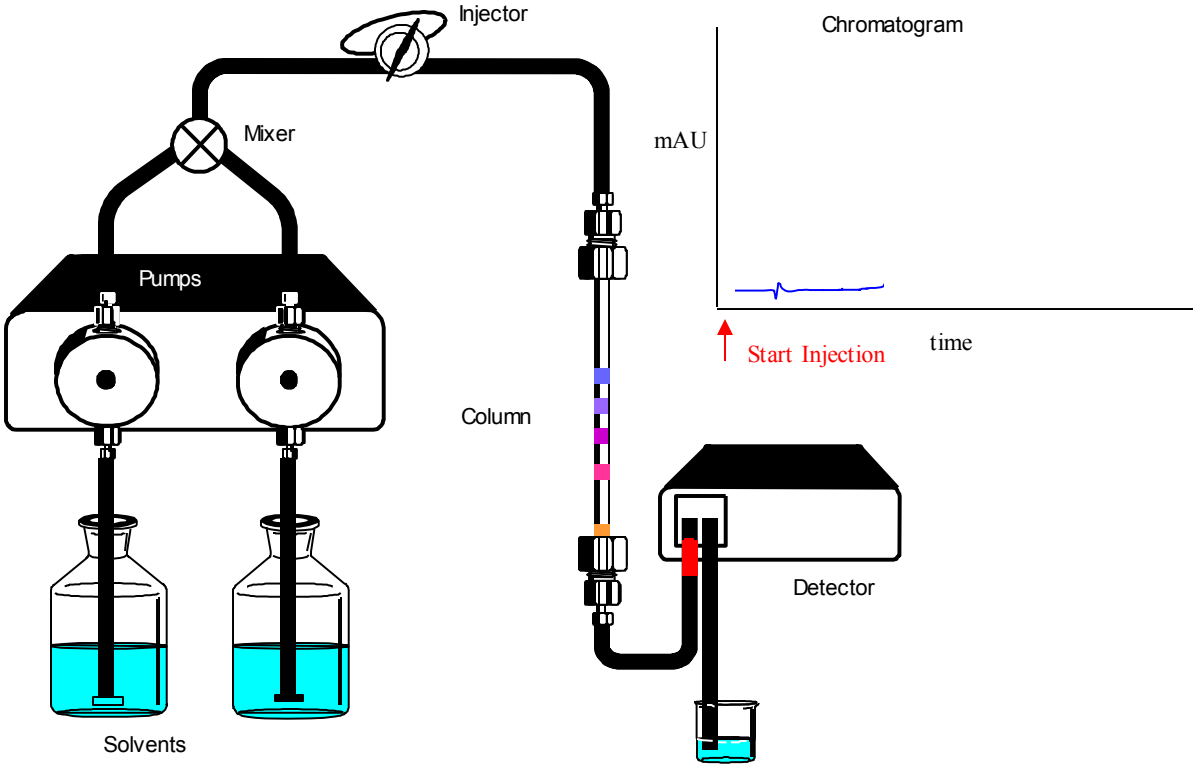
Separations



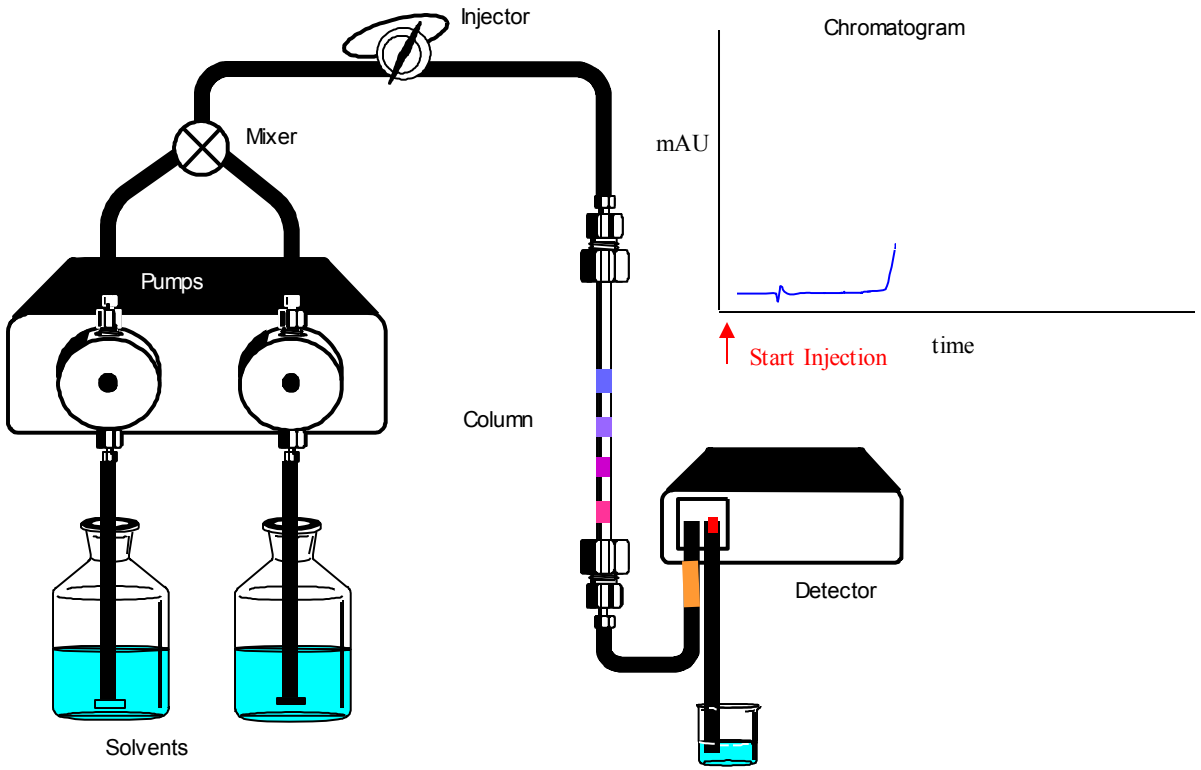
Separations



Separations

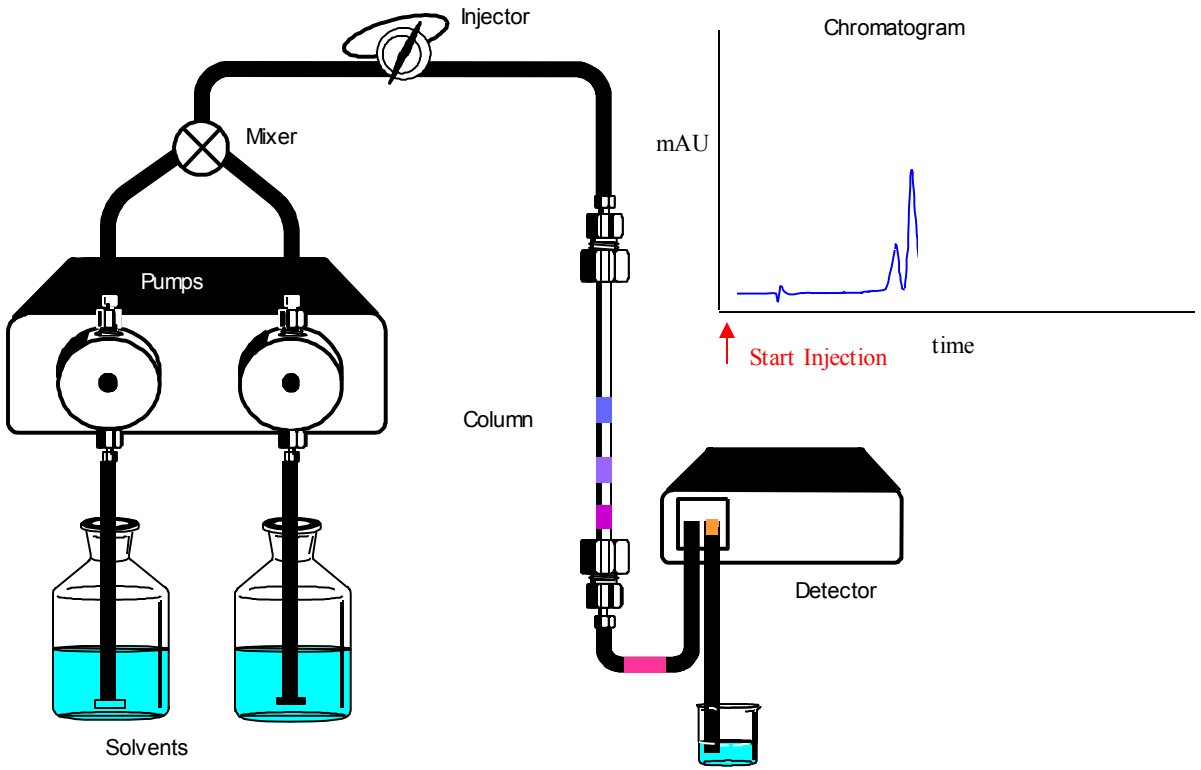


Separations

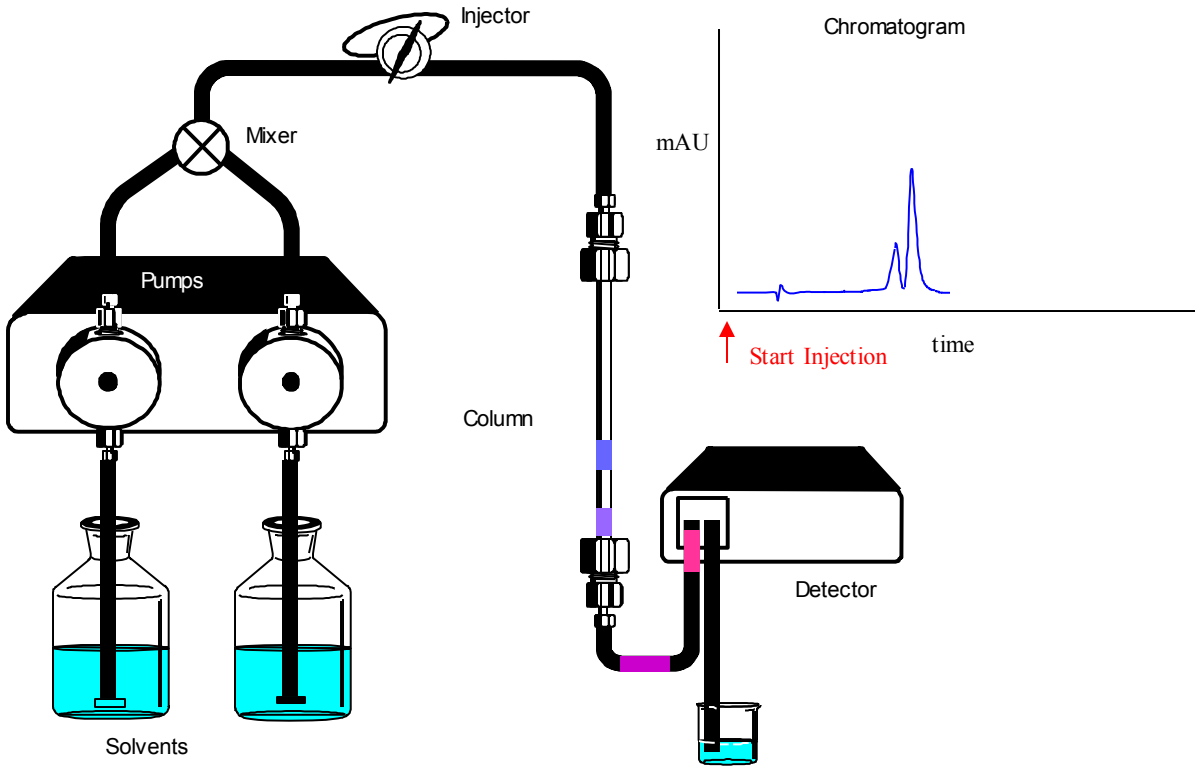


Separations

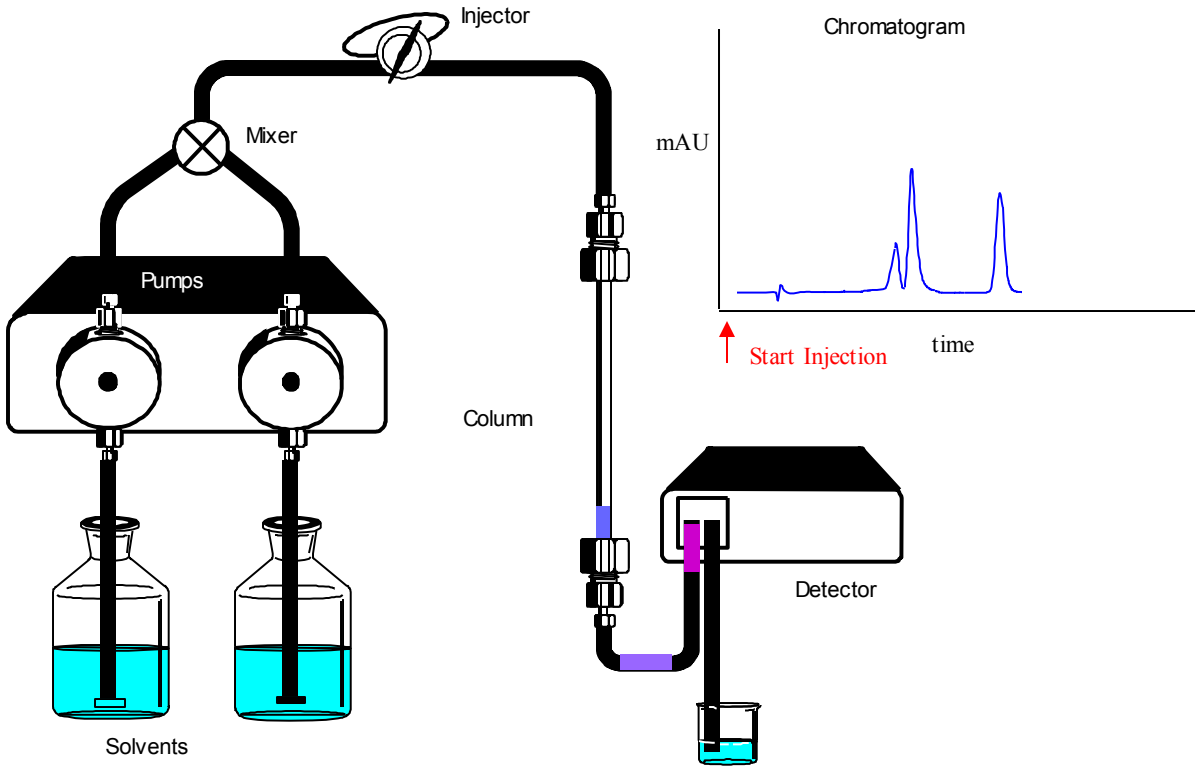
10



Separations

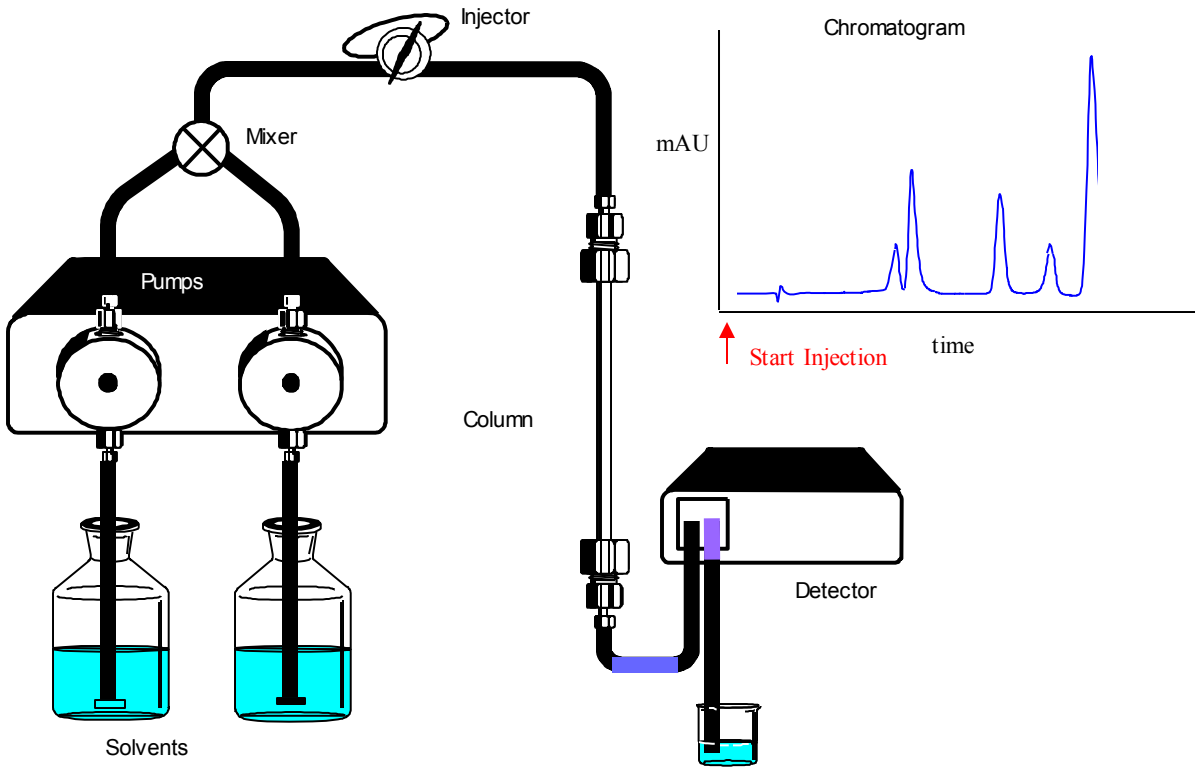


Separations

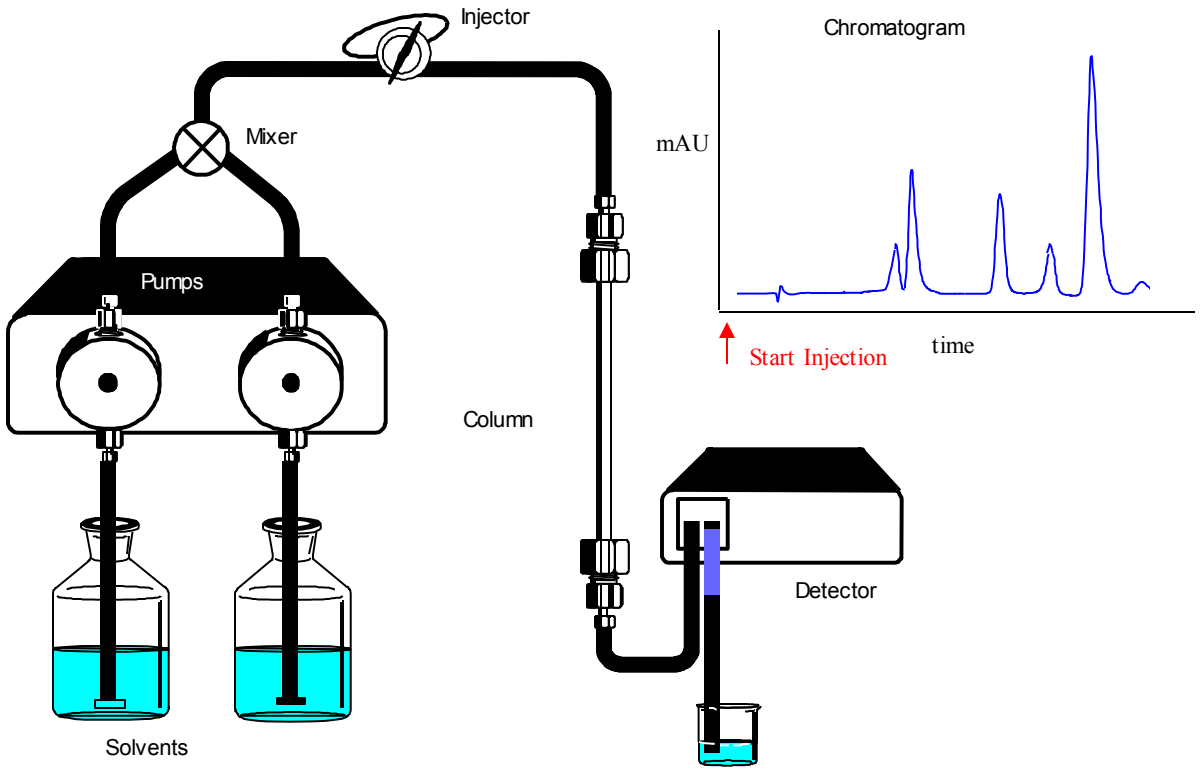


13

Separations

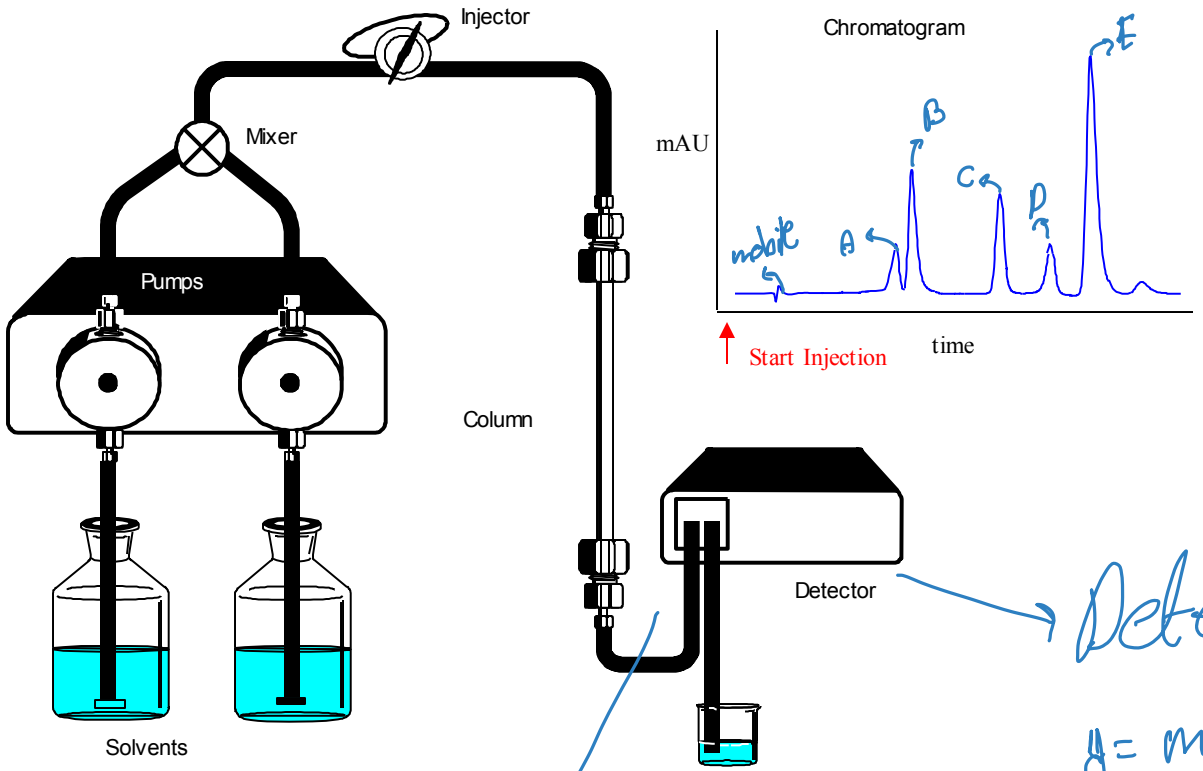


Separations



15

Separations



Separation

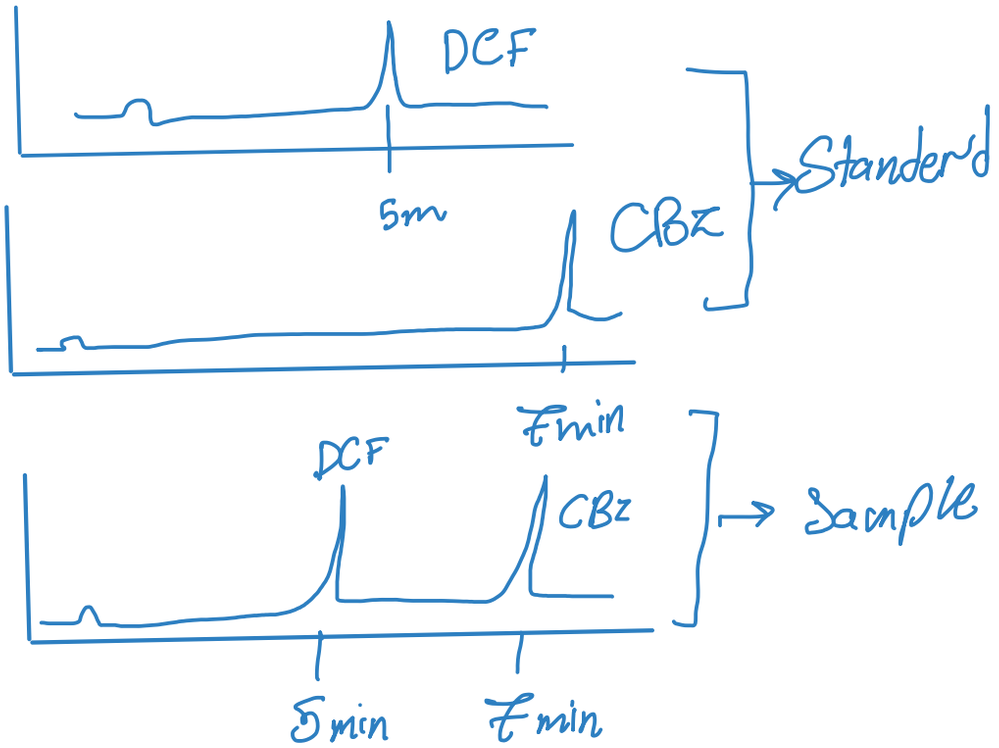
Determinactics

$y = mx \pm b$

Qualitative

⇒ Sample (DCF + CBz)

Solution بعض فيه كل مركب بتركيز معلوم وبحقنه للجهاز



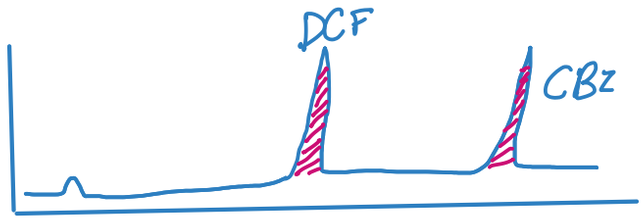
بستخدم نفس الـ mobile وبنفس الجهاز

بتطلع المادة بالعينة بنفس الـ Retentiontime الـ Standerd

* لازم ينفصلوا بأوقات مختلفة

وإذا انفصلوا بنفس الوقت ما بتزبط الـ method

Quantitative



نفس الـ Calibration

بحقنهم بتراكيز معينة (x) وباخذ استجابة الجهاز (y)

$$y = mx \pm b$$

x	y	
1ppm للمعادلة	y → DCF	y → CBz
2ppm		
⋮		
50ppm		

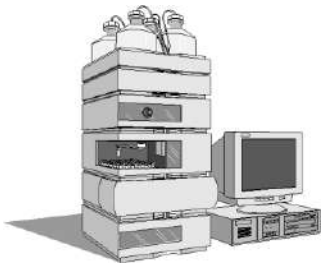
Not destructive

غالبًا بستخدم Non Polar

Stationary phase

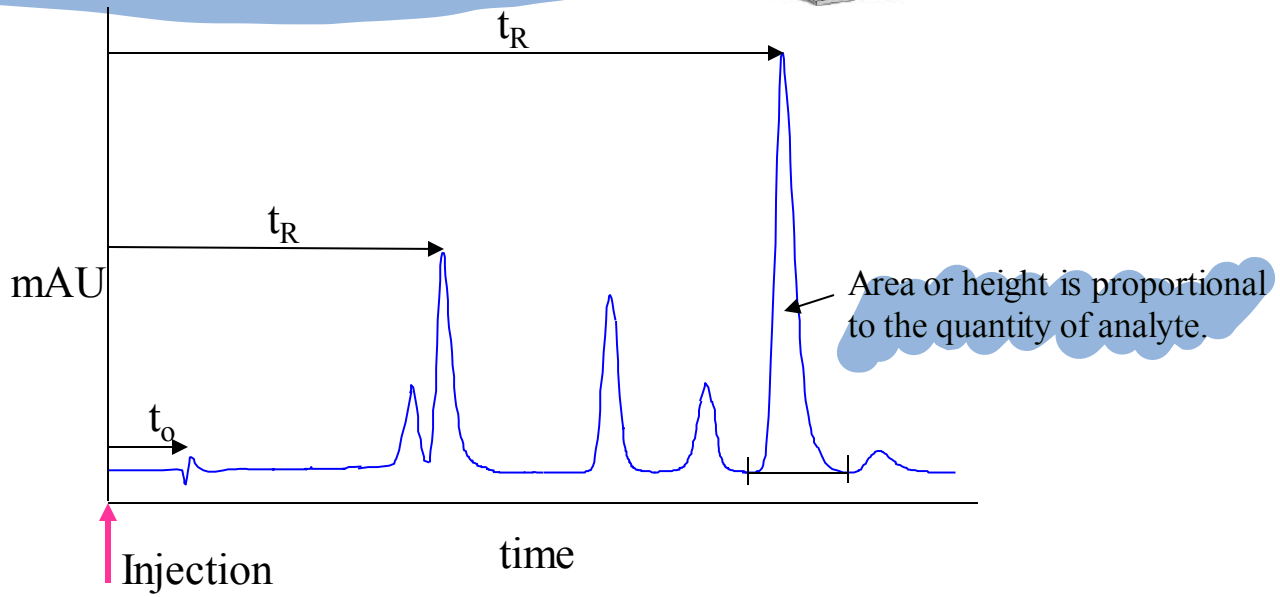
The Chromatogram

بحلل فيه الـ liquids والـ Solids اللي بتذوب
ما بقدر احلل فيه غازات
↳ بحللهم بالـ GC



t_o - elution time of unretained peak

t_R - retention time - determines sample identity



Applications

Quality control unit

- Monitoring materials that may jeopardize occupational safety or health
- Monitoring pesticide levels in the environment.
- To survey food and drug products,
- To identify confiscated narcotics
- To determine the amount of such chemical compounds found in new drugs in pharmaceutics

Summary

- The modern day technique is greatly enhanced in terms of selectivity, resolution, through miniaturization and the use of very elaborate stationary phases.
- Therefore HPLC is widely used for separation of molecules in biological, pharmaceutical, food, environmental and industrial process

advantages of HPLC

- Small diameter, reusable stainless steel columns
- Column packing with very small particles
- Control flow of mobile phase
- Precise sample introduction
- Good pumping system
- Special continuous flow detectors- can handle small flow rates and detect very small amounts
- Rapid analysis
- High resolution

disadvantages of HPLC

- Cost
- Complexity
- Low sensitivity for some compounds
- Irreversibly adsorbed compounds not detected
- Co-elution difficult to detect