Pharmaceutical Analysis

(Separation methods)

Chromatography

الأستاذ الدكتور جمعه الزهوري (دكتوراه صيدلة-ألمانيا 1991)

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Damascus university

Faculty of Pharmacy

Separation Methods

- ***An Introduction to Chromatography***
- ***The most common methods in Pharmacy :***

1. ***Thin-Layer Chromatography***
2. ***Gas Chromatography***
3. ***High-Performance Liquid Chromatography***
4. ***Electrophoresis***

Chromatography

An Introduction
to Chromatographic
Separation.

Chromatography

A physical method of separation.

Components partition between two phases.

- Stationary phase - does not move.
- Mobile phase - does move.

Solutes are separated due to differences in how they interact with the two phases.

Development of Chromatography

1903	Tswett first outlines principles
1931	Lederer & Kuhn - LC application
1936	First book on chromatography
1938	Use of TLC and ion exchange
1939	First synthetic exchange resins
1941	First LLC paper
1944	First PC paper
1950	Reverse phase LC described
1959	Gel permeation
1965	Instrumental HPLC

ميشيل تسويت نششره 1906

1952 GC

Marthin& James

التفريق اللوني

الأستشراب

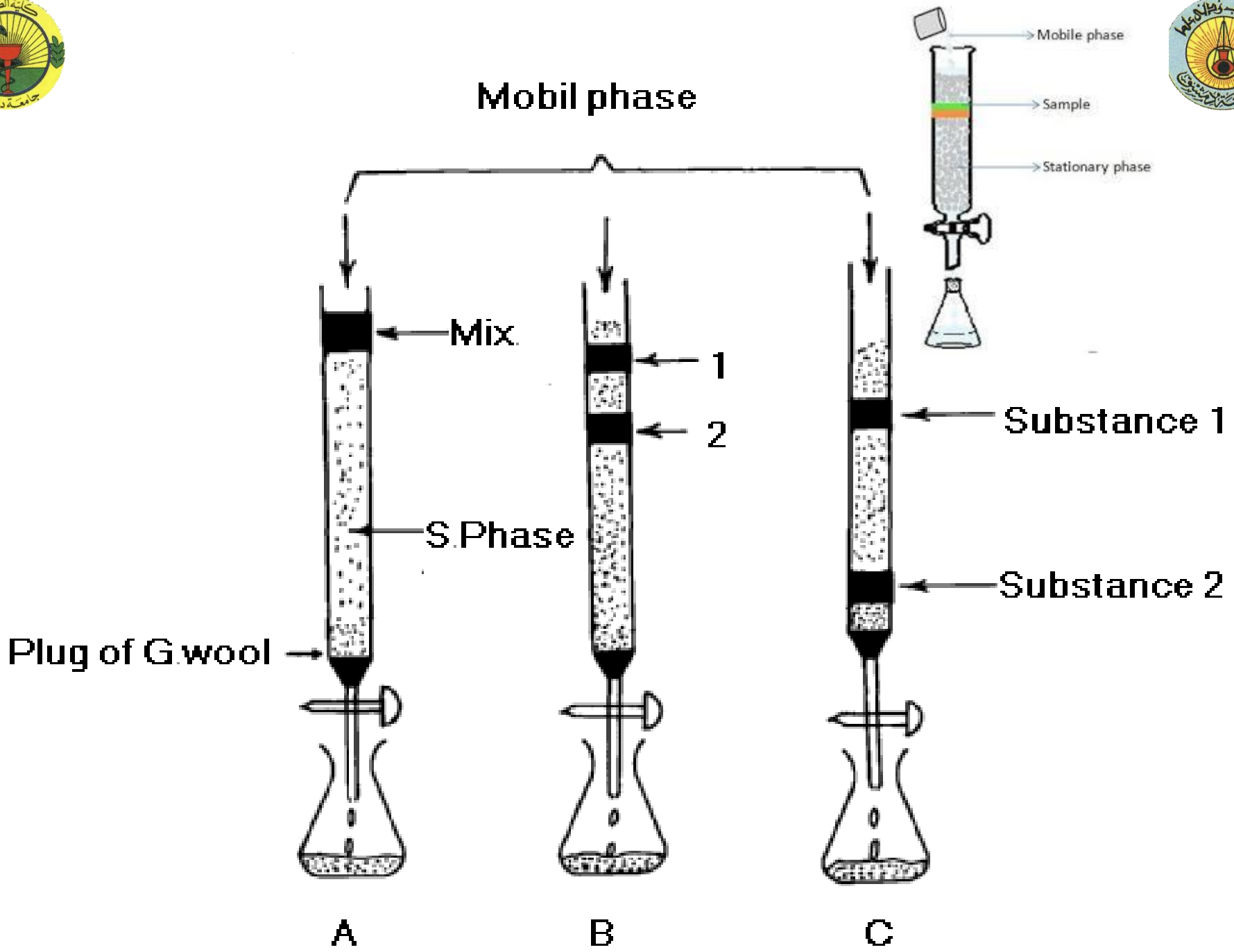
الكروماتوغرافيا

Invention of Chromatography

Mikhail Tswett invented chromatography in 1903 during his research on plant pigments.

He used the technique to separate various plant pigments such as chlorophylls

Mikhail Tswett
Russian Botanist
(1872-1919)



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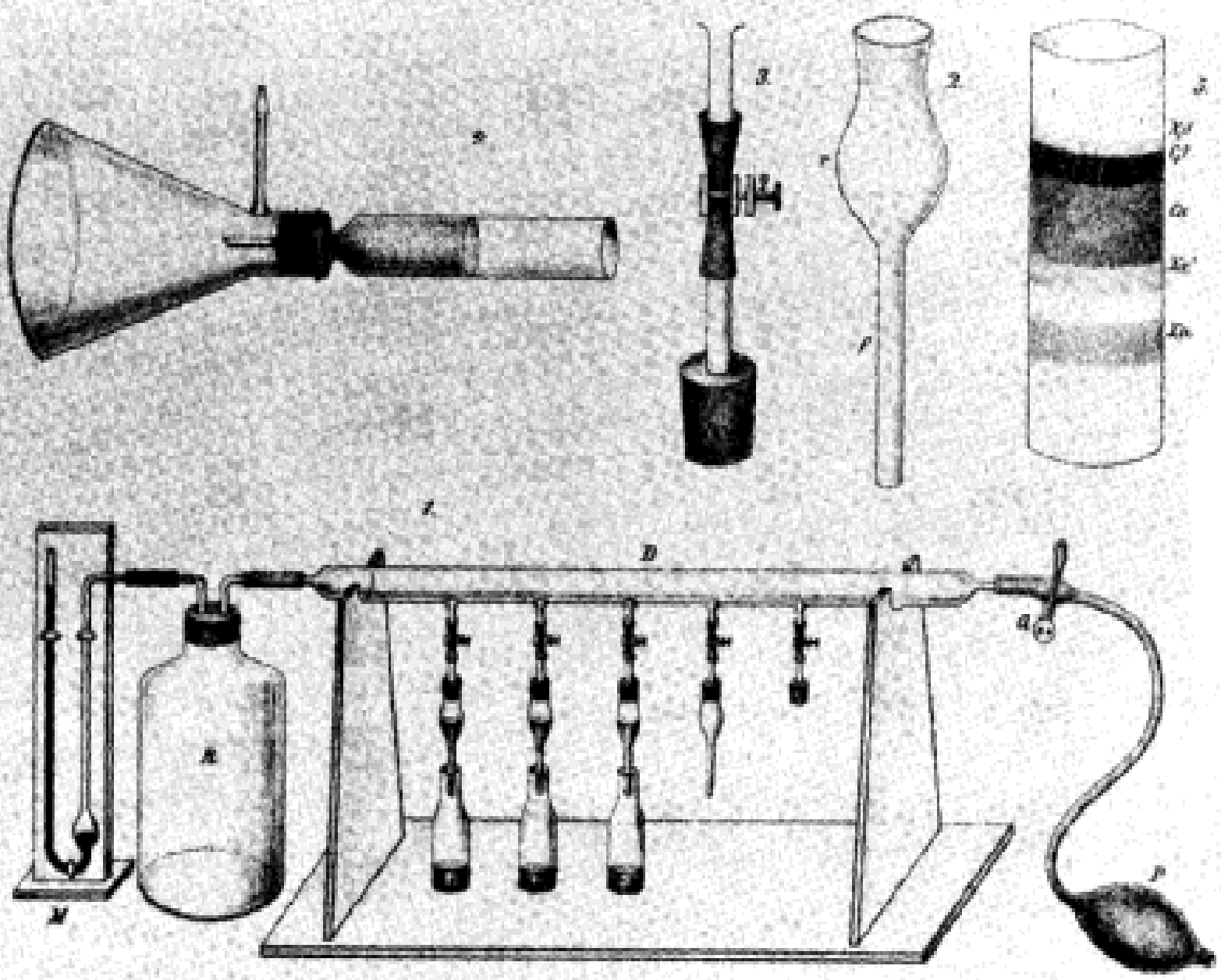

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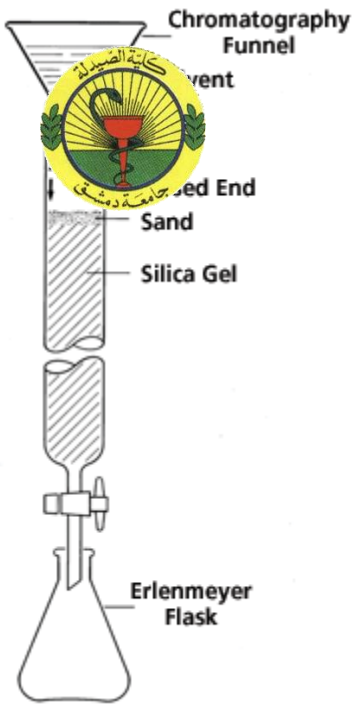
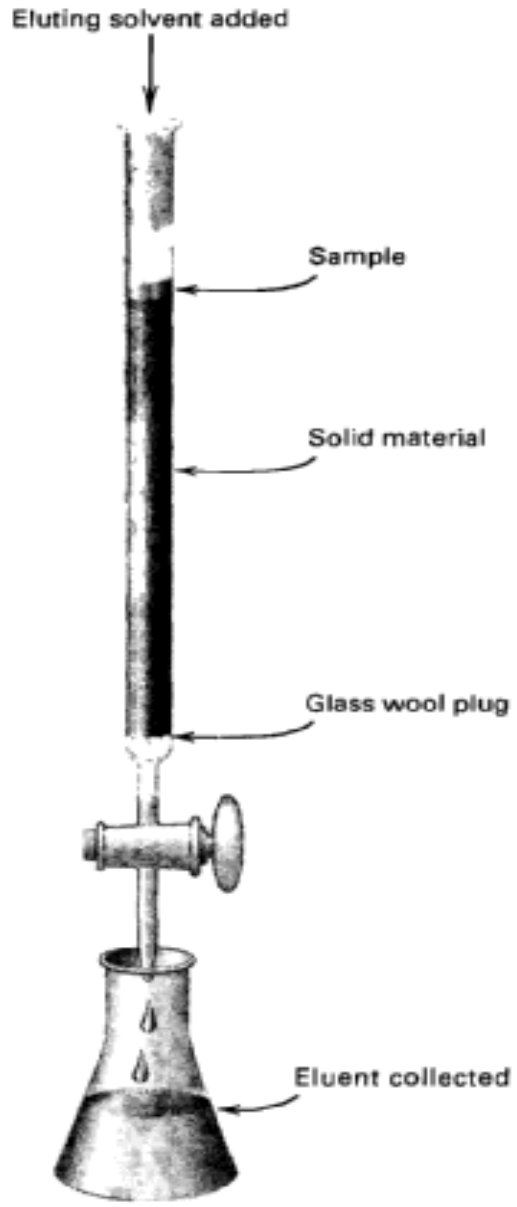


TECHNIQUES OF COLUMN CHROMATOGRAPHY



Typical chromatographic column.

Principles of Chromatography

- A solute equilibrates between a mobile and a stationary phase. The more it interacts with the stationary phase , the slower it is moved along a column.

Chromatography

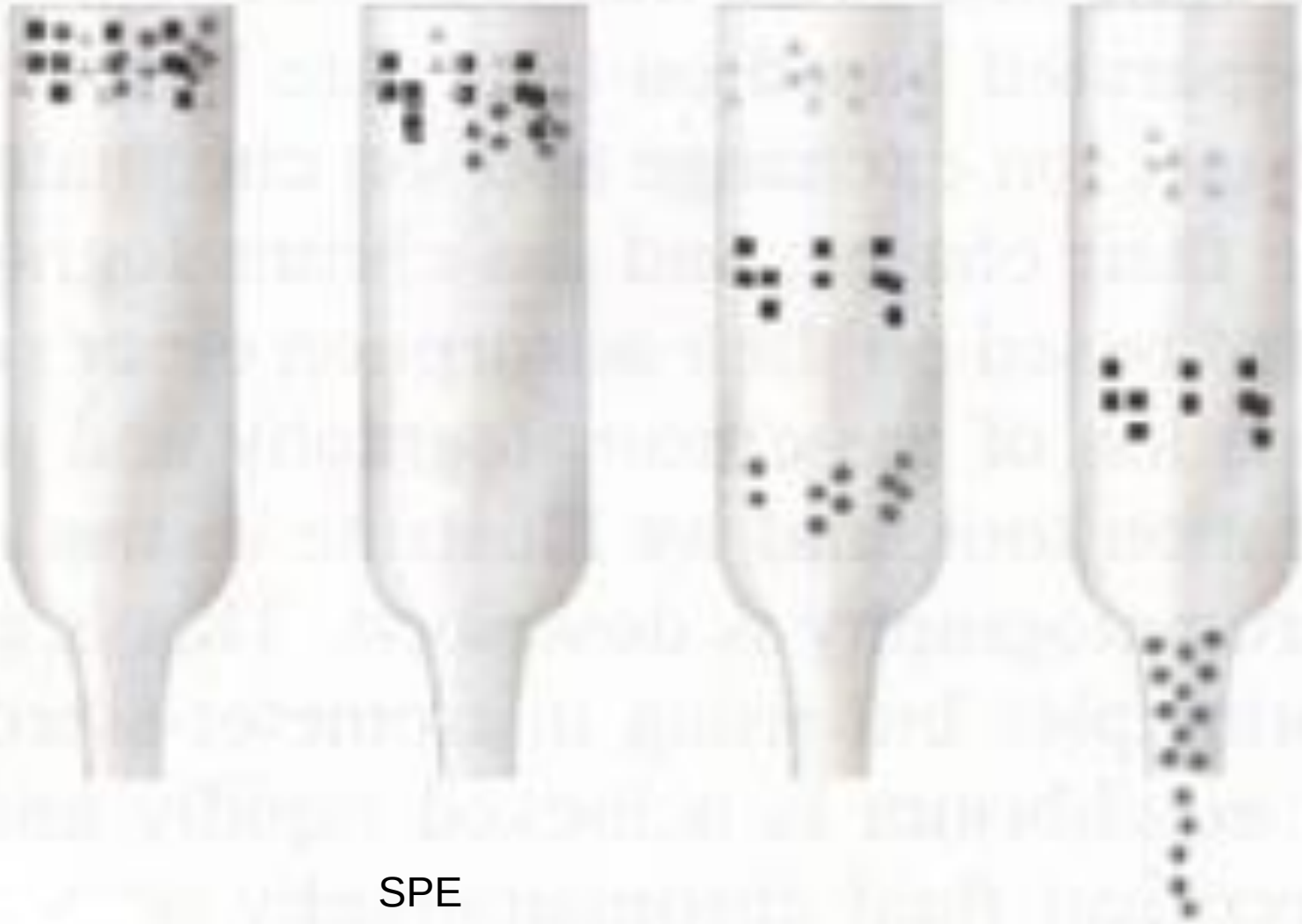
The separation process involving the interaction of one or more solutes and two phases.

Mobile phase الطور المتحرك

A gas or liquid that passes through our 'column'

Stationary phase الطور الثابت

A solid or liquid which does not move.



SPE

Principle of chromatographic separations.

Chromatography

There are several types of interaction that have been used to separate eluents.

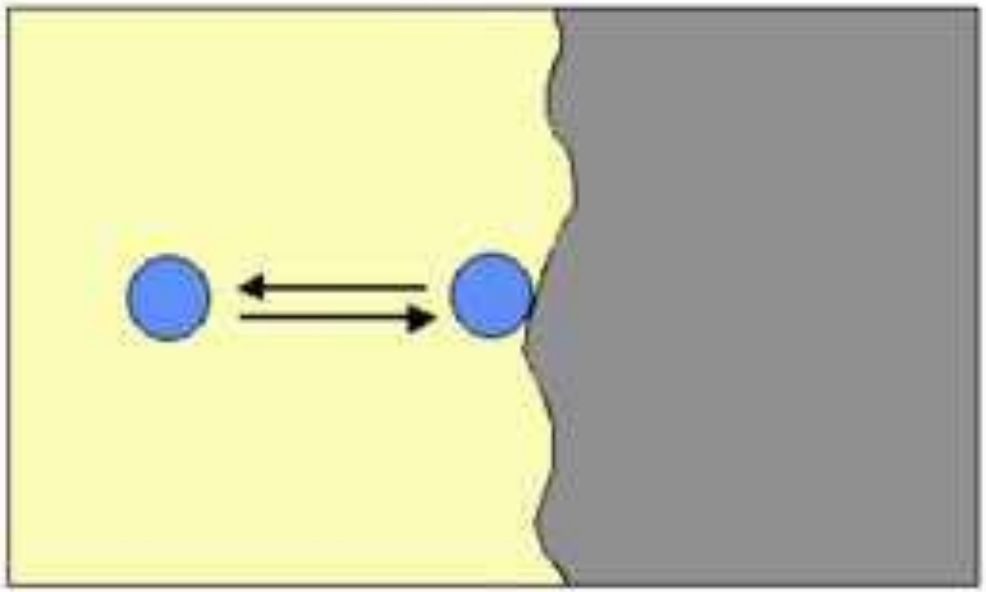
Major Categories	الفئات الرئيسية
surface adsorption	
solvent partitioning	
ion exchange	
relative solute size (Gel filtration)	الهلامي

Affinity Chromatography

Adsorption chromatography

الأدمصاص أو الأمتزاز

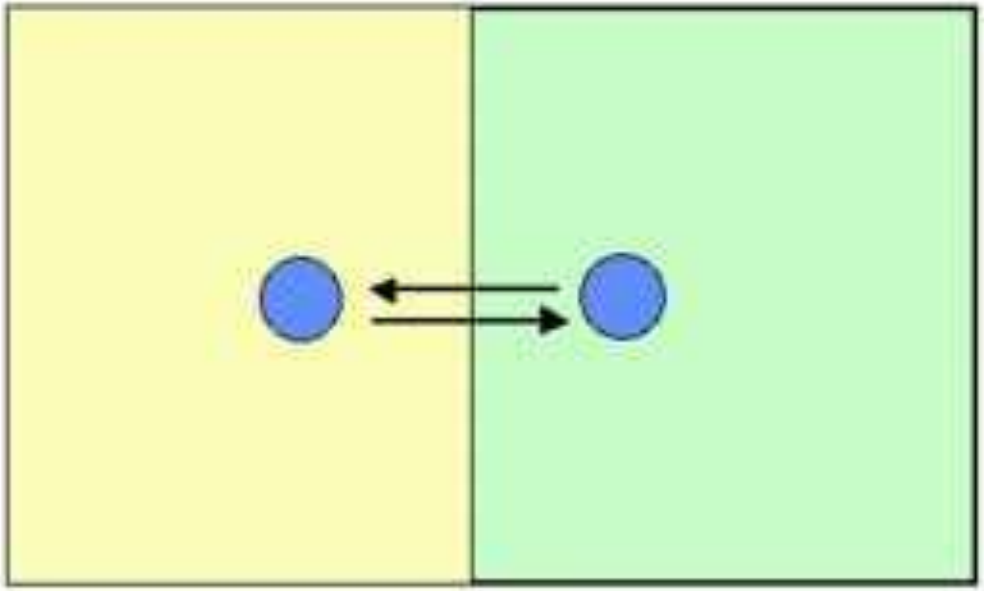
The stationary phase is a solid. Separation is due to a series of adsorption/desorption steps.



Partition chromatography

التوزع

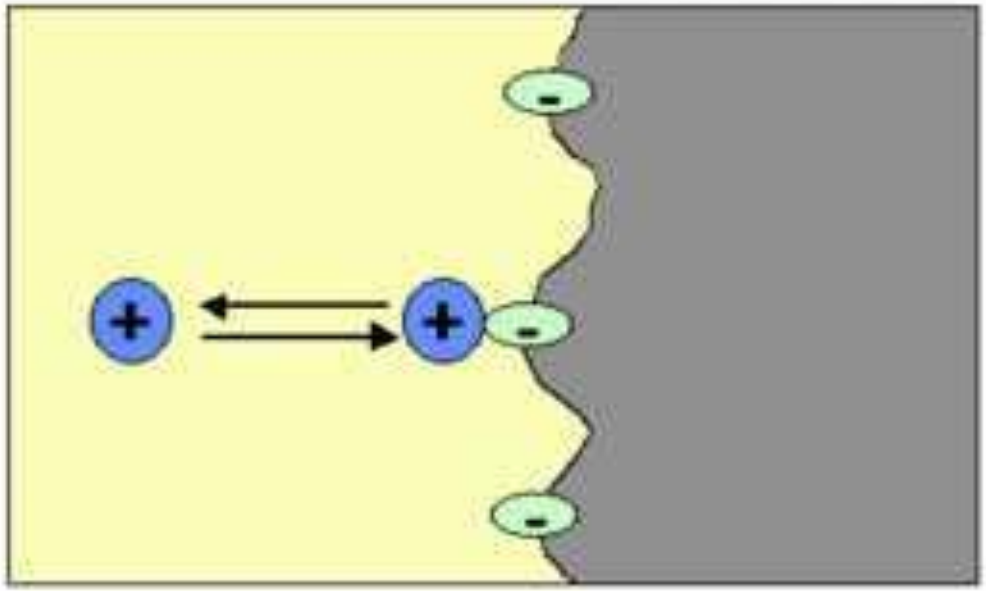
Separation is based on solute partitioning between two liquid phases.
(relative solubility)



Ion exchange chromatography

التبادل الأيوني

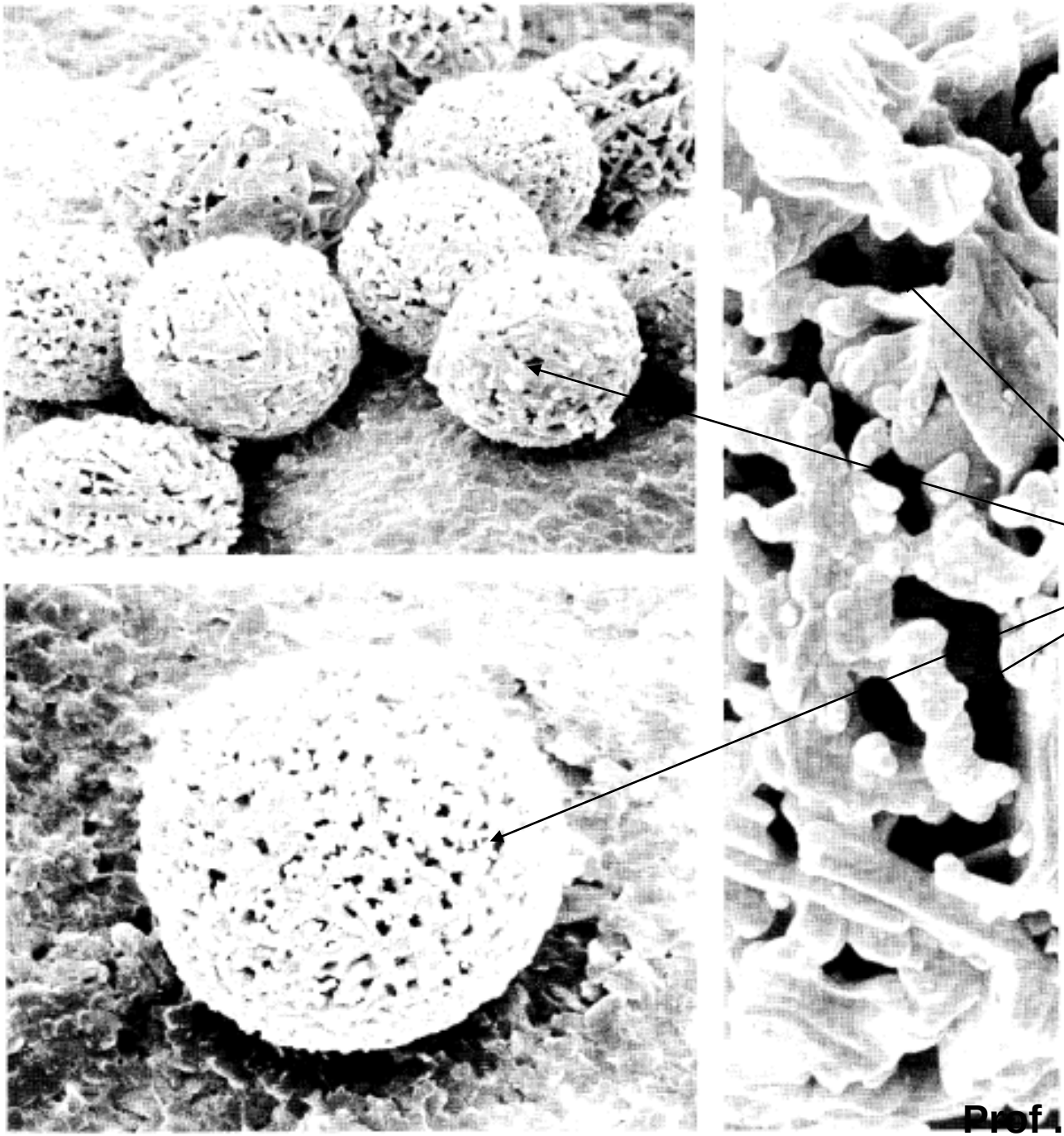
The stationary phase has an ionically charged surface, opposite that of the eluents.



Size exclusion Chromatography or Gel Filtration Chromatography

- **Is a separation technique based on differences in molecular sizes rather than chemical properties.**

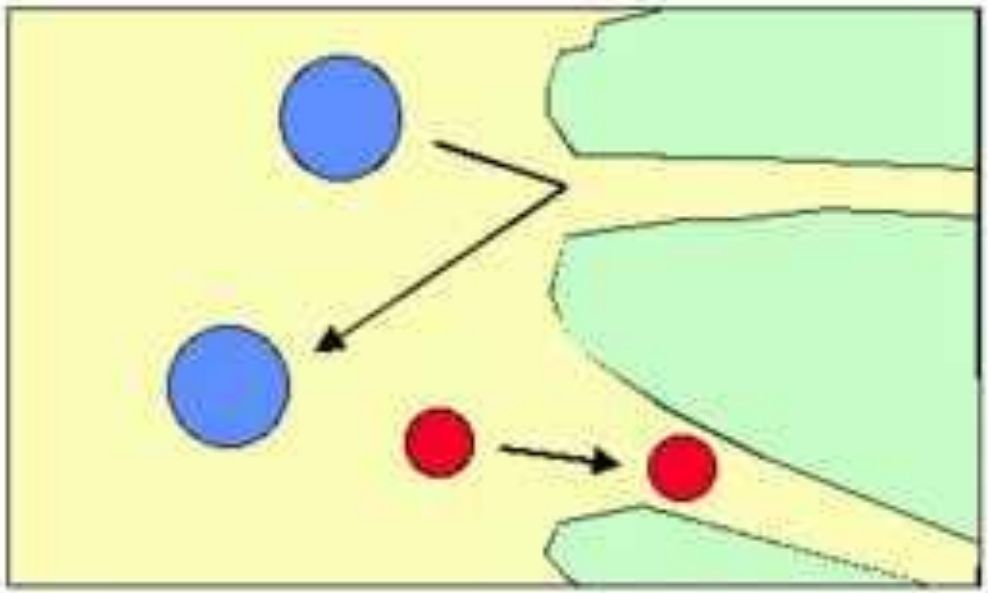
- **A column is pre-packed with porous gel beads (Stationary phase), usually across-linked polysaccharide material . The porosity of the gel is chosen so that the smaller molecules in the mixture can penetrate the beads , whereas the larger ones cannot.**



- The Sample is applied to the top of the column , and eluted out with a buffer (mobile phase).
- As the sample move down the column, the larger molecules move faster, for they cannot enter the gel beads and can only flow through the interstices between them. The small molecules can wander in to the gel beads and therefore loiter behind.
- If fractions are collected , the earlier fractions will contain the larger molecules.

Size exclusion chromatography

Separation is based on molecular size. Stationary phase is a material of controlled pore size. Also called gel permeation.



Size exclusion chromatography

Columns can be obtained that will separate specific size ranges.

Larger species will elute first - they can't pass through as many pores so their path is shorter.

Useful for determining size and size range for polymers, proteins, ...

Affinity Chromatography

It is depends upon the reversible adsorption of bimolecular through biospecific interactions of the ligand.

The most common type are performed in three main stages :

1- Equilibration

2- Sample application and wash.

3- Elution

1- Equilibration :

Equilibration of the stationary phase to the desired start conditions.

2- Sample application and wash

الهدف The goal in this step is to bind the target molecules and wash out all unbound material . Non binding molecules will pass through in the flow through during washing with binding buffer.

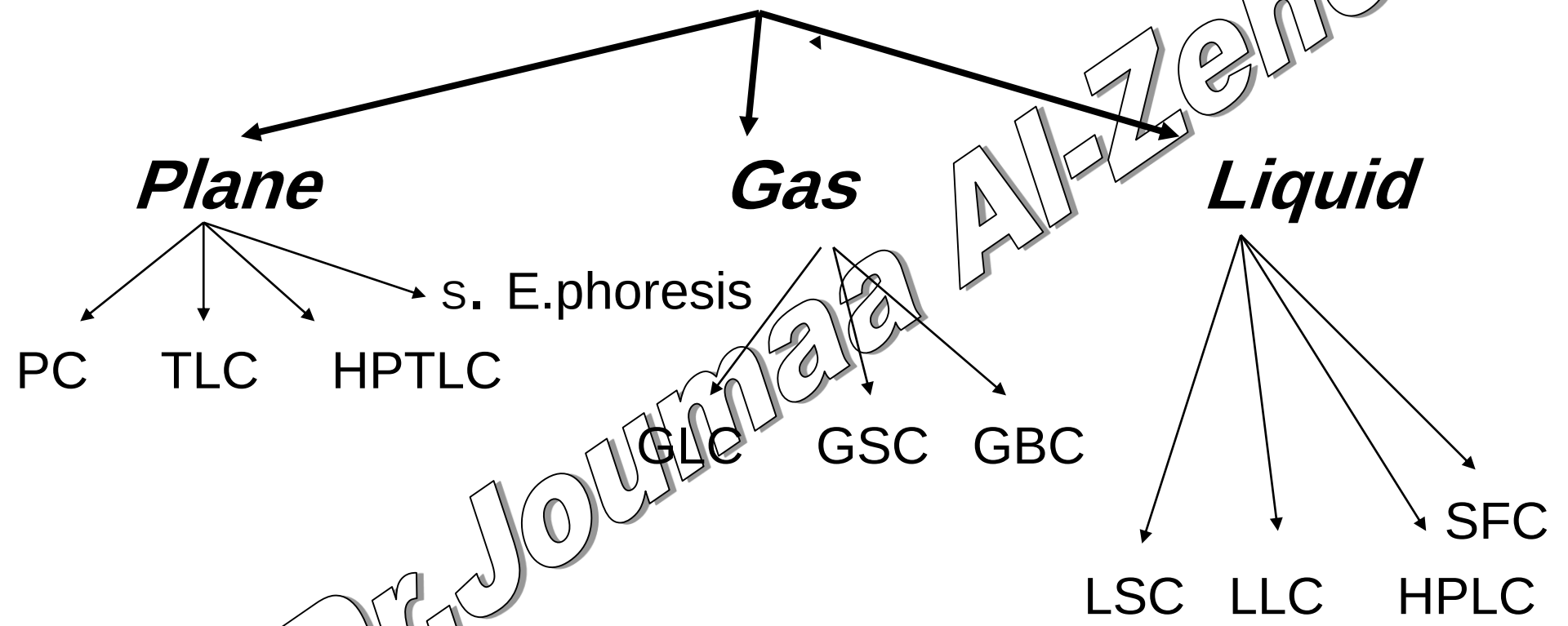
3- Elution

Bimolecular are released from the biospecific ligand in to the elution buffer by change in the buffer composition .

A Common way is to increase pH of the buffer.

Affinity Chromatography

Classification of Chromatographic Methods



Thin Layer Chromatography

Thin Layer Chromatography

- Thin Layer Chromatography is a type of Chromatography in which the stationary phase is in the form of a layer on a glass, an aluminum, support.
- Type of adsorption chromatography.
- The term “planar chromatography” is often used for TLC and Paper chromatography (PC) and slab electrophoresis because each employs a planar stationary phase.

Thin Layer Chromatography

- TLC is inexpensive
- Easy technique that requires little instrumentation
- TLC used for separation of simple mixtures and for qualitative identification or semi quantitative, visual analysis of sample
- HPTLC (up1975) is capable of producing fast,high-resulution separations and qualitative and quantitative results.
- TLC and HPTLC are available commercially

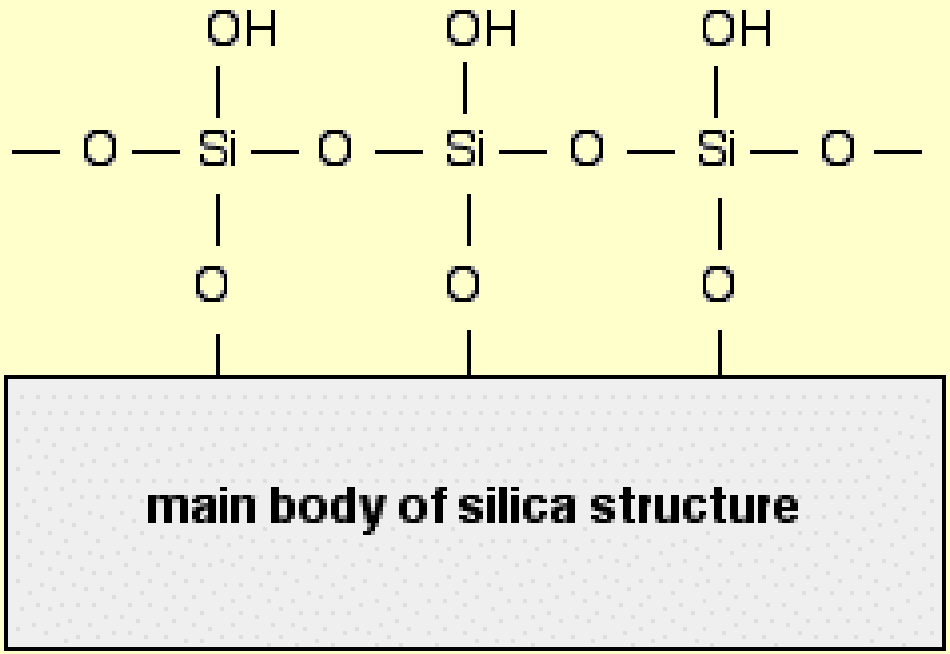
Adsorption chromatography

Silica and alumina are common stationary phases.

Both solute and solvent are attracted to the polar sites on the stationary phase.

If solutes have differing degrees of attraction to the phase, a separation is possible.


at the surface of the silica gel you have Si-O-H bonds inste of Si-O-Si bonds. The diagram shows a small part of the silica surface.

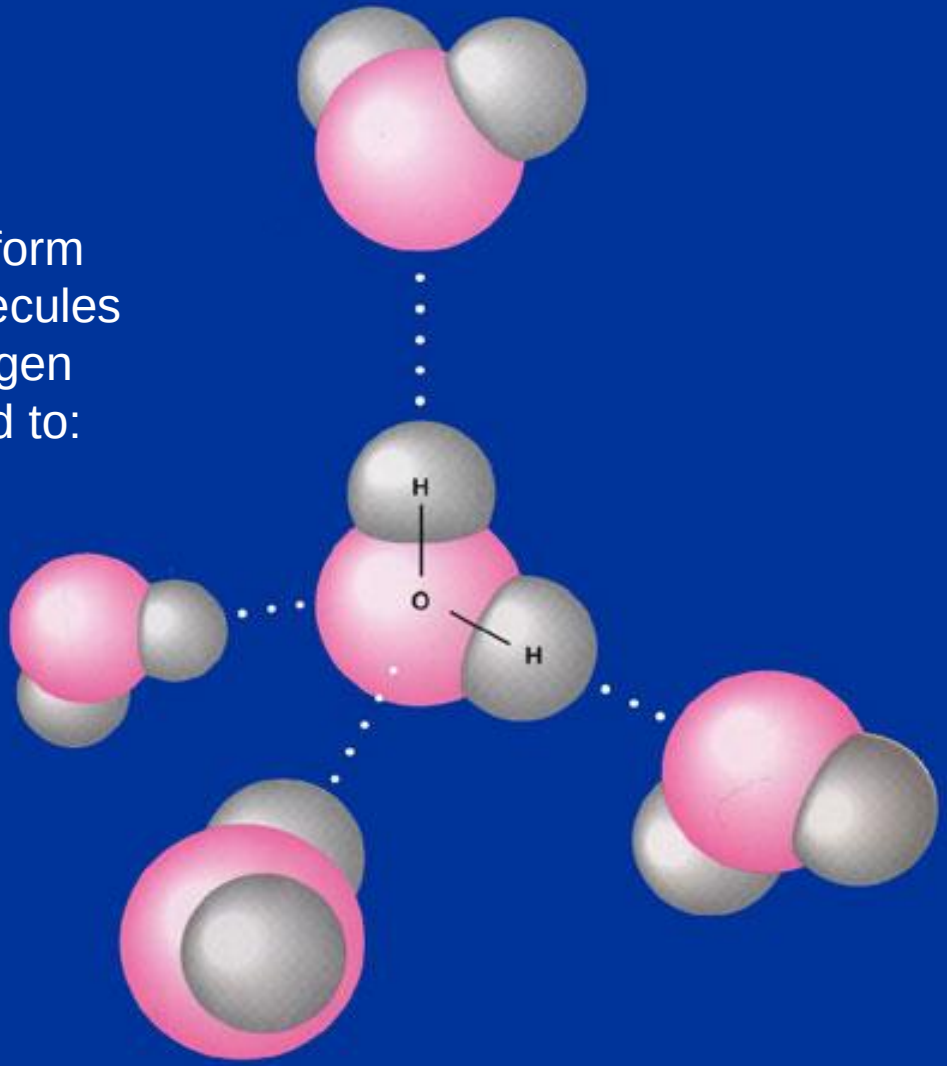


The surface of the silica gel is very polar and, because of the -OH groups, can form hydrogen bonds with suitable compounds around it as well as van der Waals dispersion forces and dipole-dipole attractions.

ثنائي القطب

HYDROGEN BONDS BETWEEN WATER MOLECULES

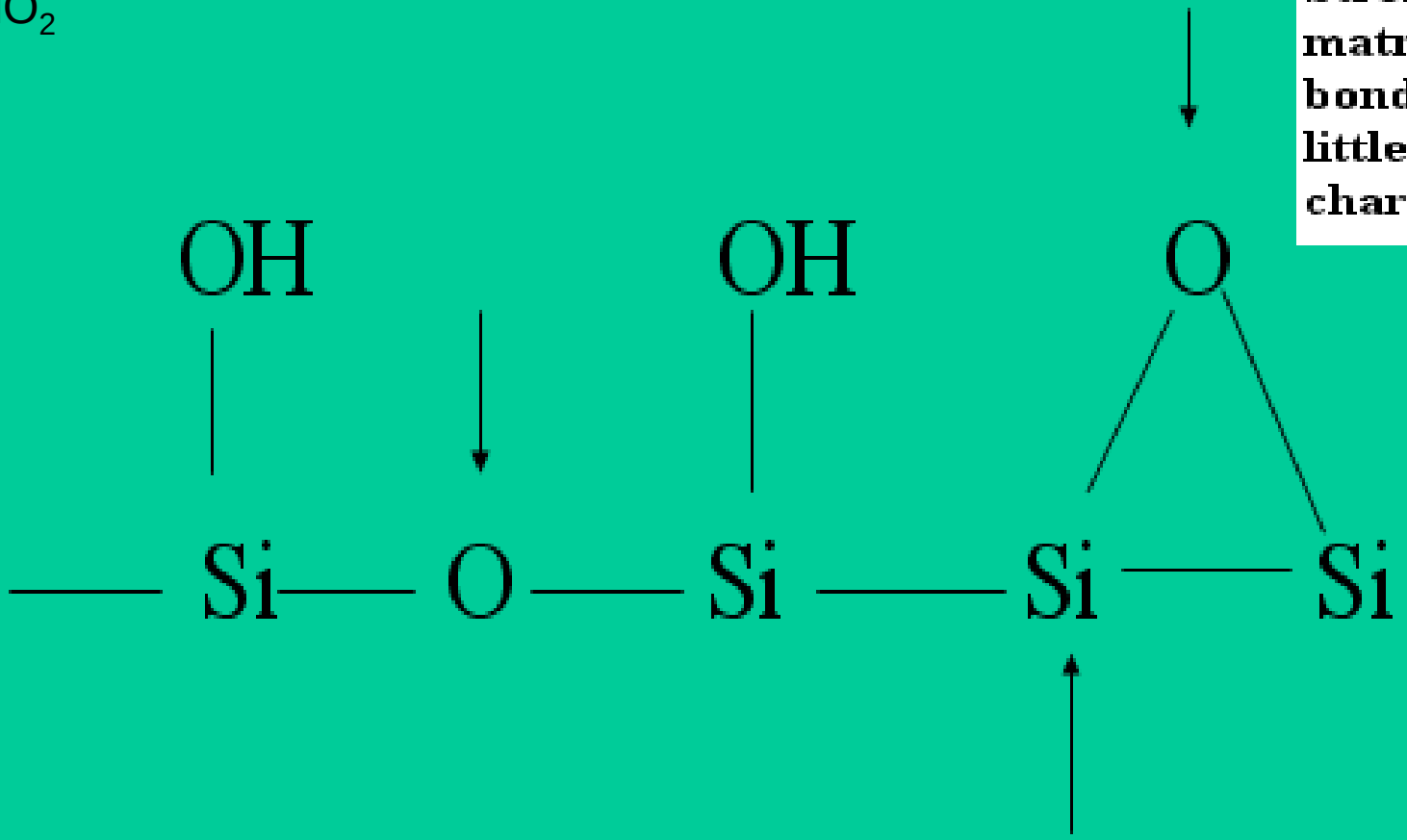
Hydrogen bonds can form between any two molecules that each have hydrogen atoms directly bonded to:
N , O , or F Atoms



Active Position

Silica Gel

SiO_2

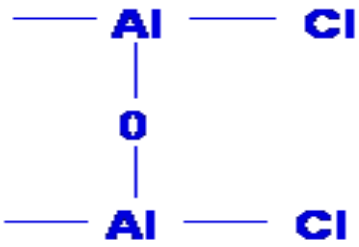


Silica Gel

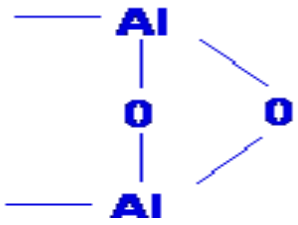
Strongly Polar matrix of Siloxyl bonds exhibiting little dispersive character.

Alumina (Strongly Polar more than Silica)

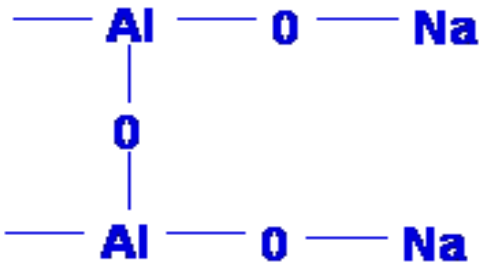
Acidic Alumina

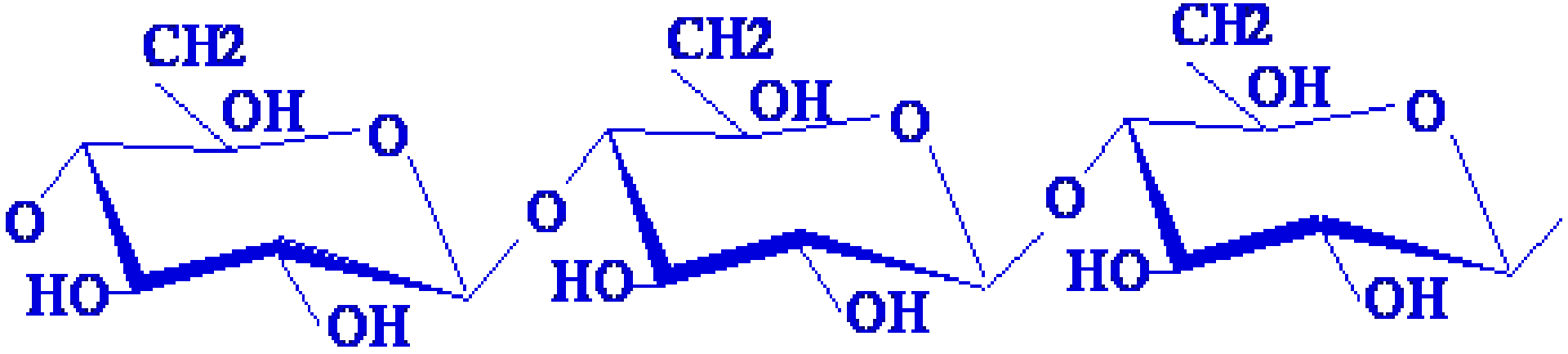


Neutral Alumina



Basic Alumina





Adsorption chromatography

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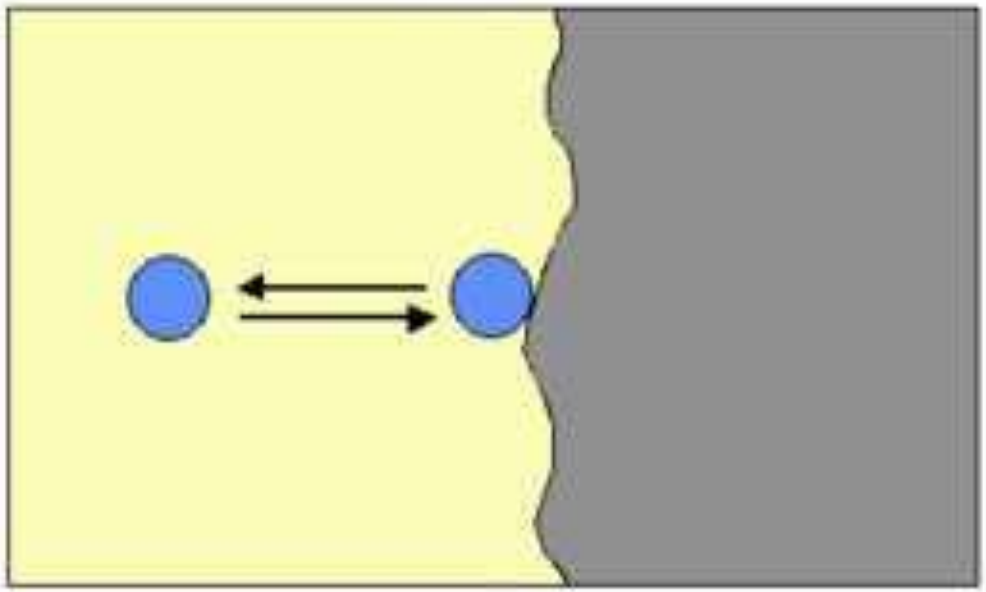

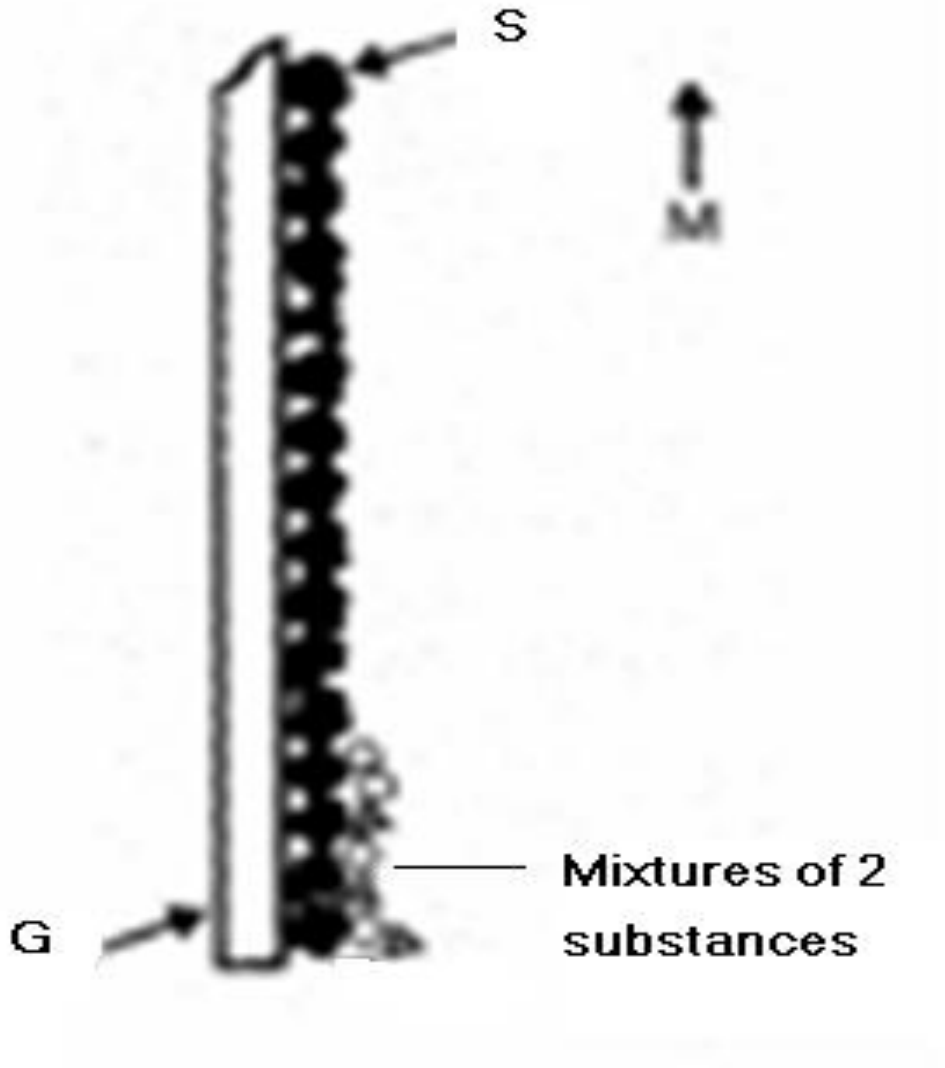

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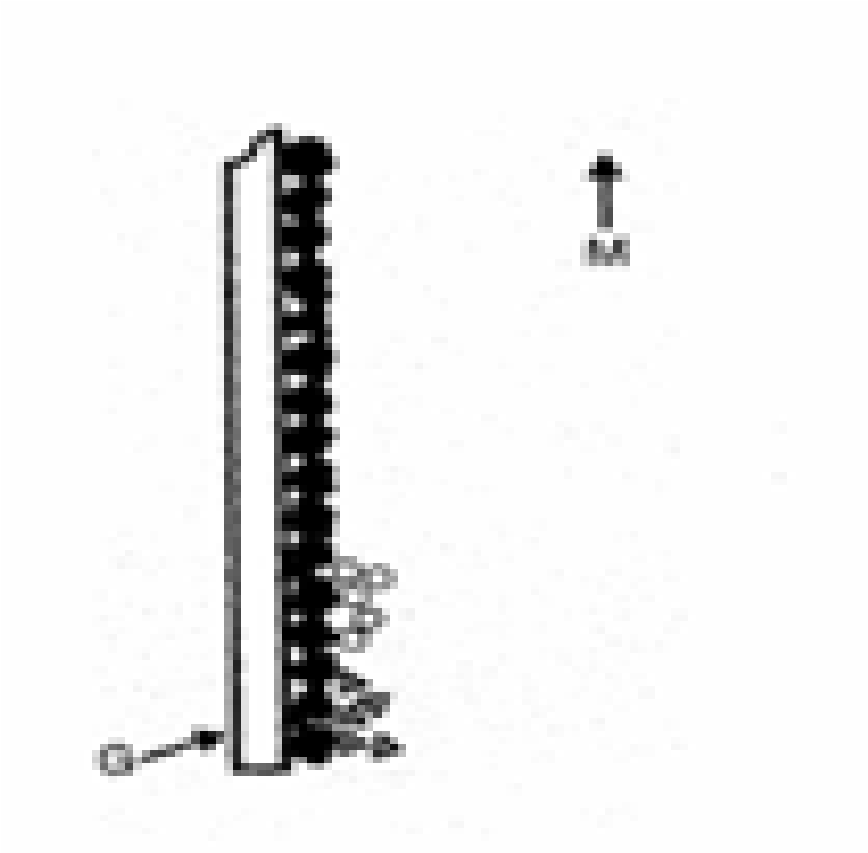
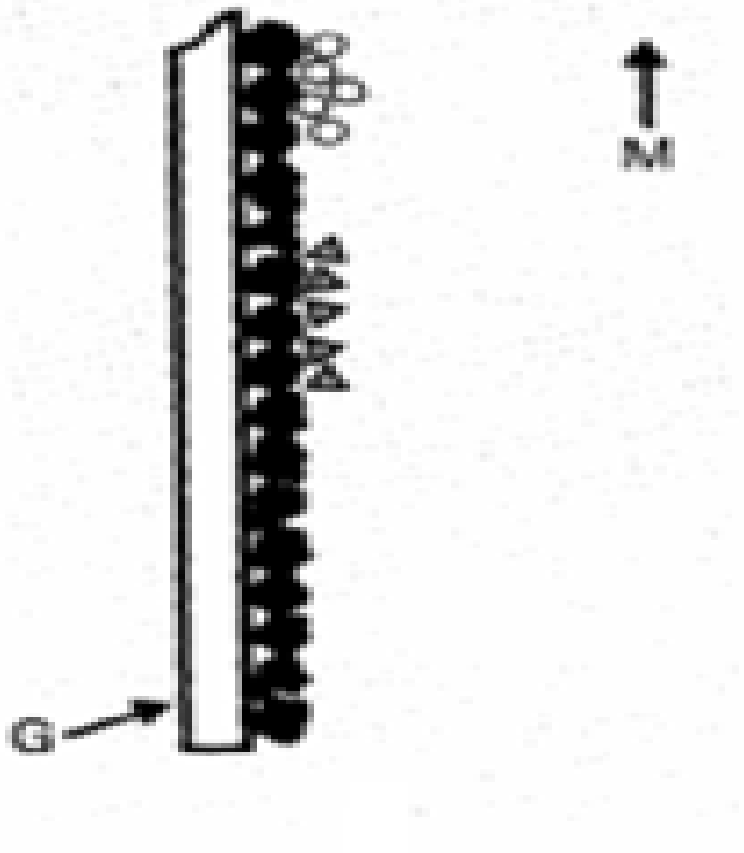
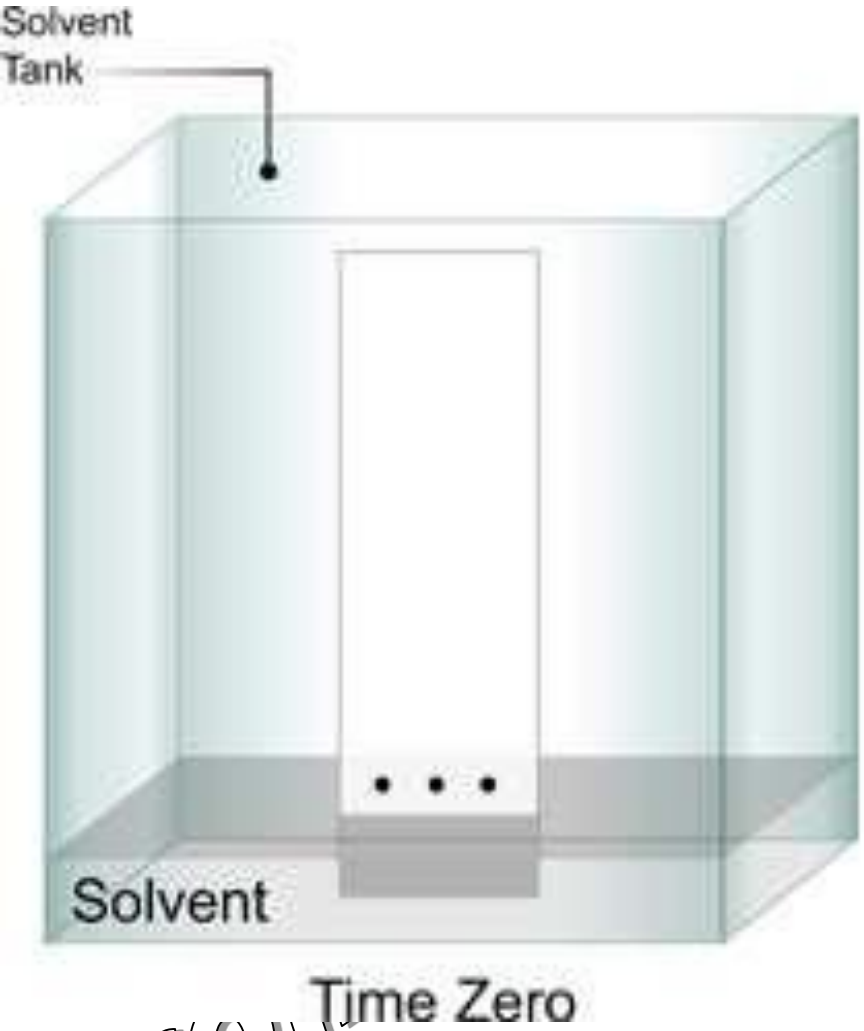
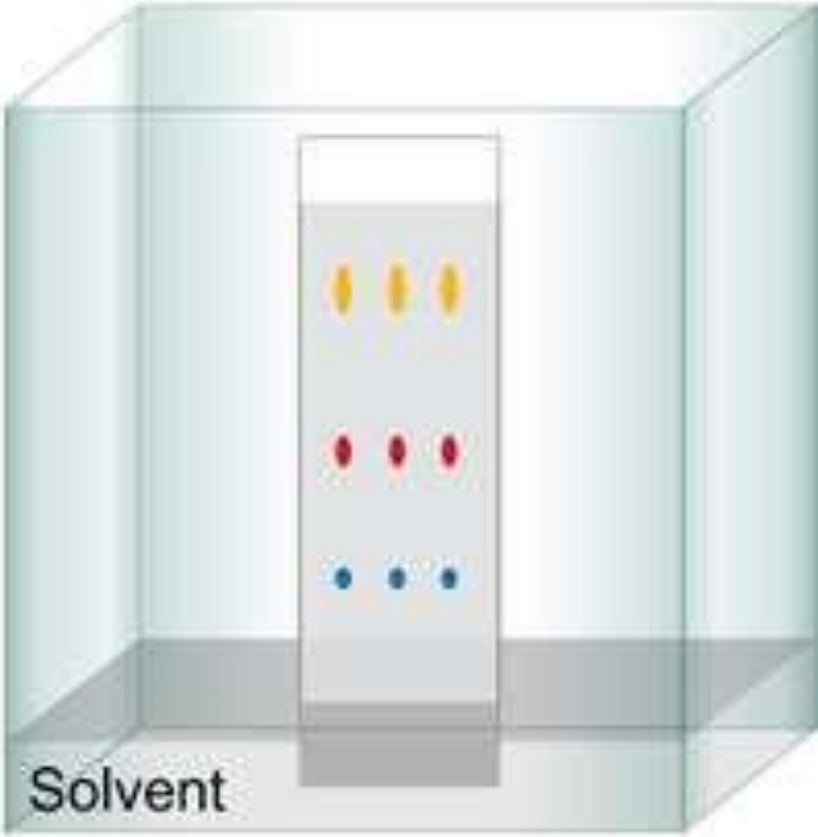


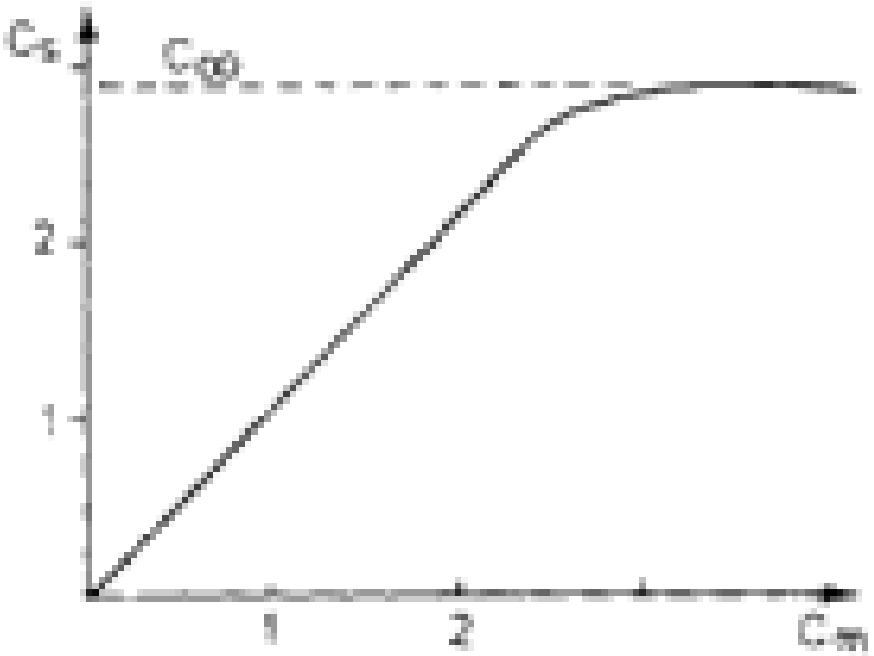




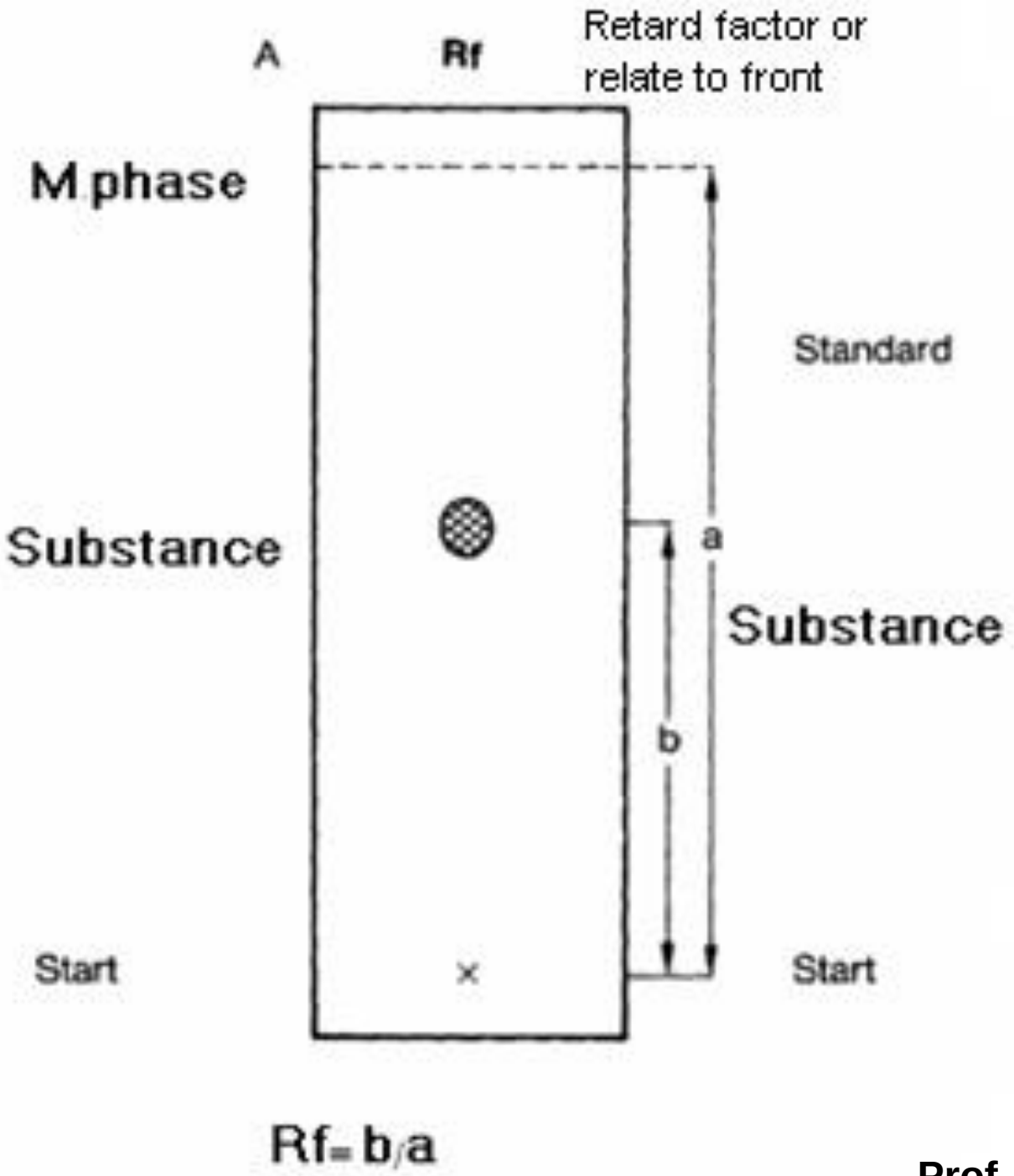
Time Zero



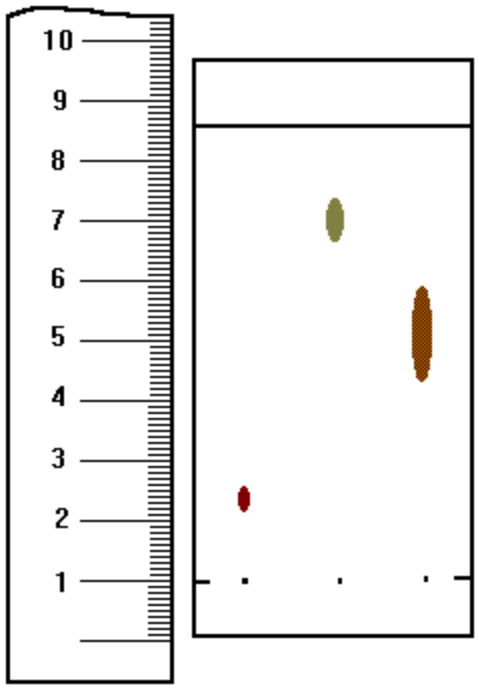
After Ten Minutes



$$K = \frac{Cs}{Cm}$$



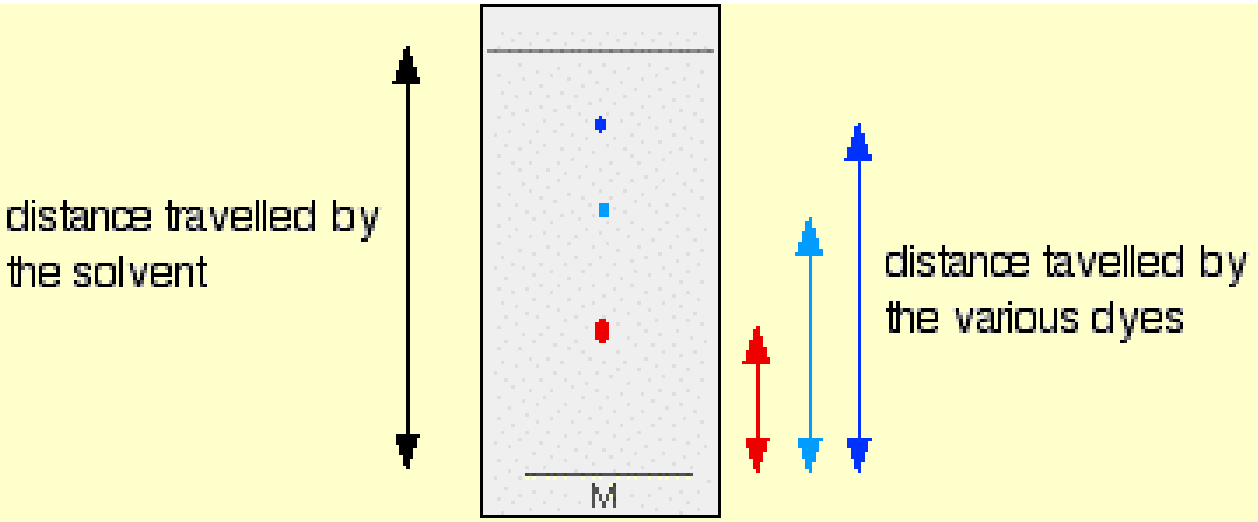
Calculate R_f Value



Spot 1: $R_f = \frac{1.4\ cm}{7.7\ cm} = 0.18$

Spot 2: $R_f = \frac{6.0\ cm}{7.7\ cm} = 0.78$

Spot 3: $R_f = \frac{4.1\ cm}{7.7\ cm} = 0.53$



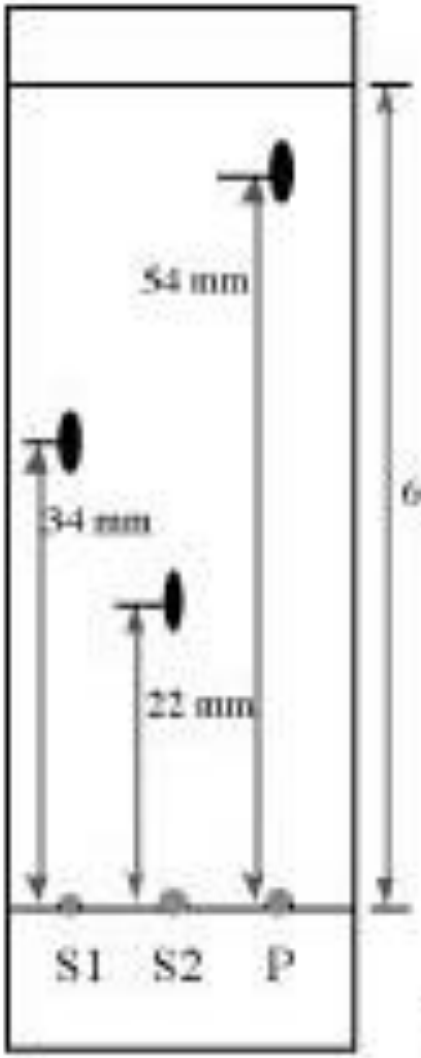
The R_f value for each dye is then worked out using the formula:

$$R_f = \frac{\text{distance travelled by component}}{\text{distance travelled by solvent}}$$

For example, if the red component travelled 1.7 cm from the base line while the solvent had travelled 5.0 cm, then the R_f value for the red dye is:

$$R_f = \frac{1.7}{5.0}$$

$$= 0.34$$



calculate R_f's

S1: $R_f = \frac{34\text{ mm}}{60\text{ mm}} = 0.57$

S2: $R_f = \frac{22\text{ mm}}{60\text{ mm}} = 0.37$

P: $R_f = \frac{54\text{ mm}}{60\text{ mm}} = 0.90$

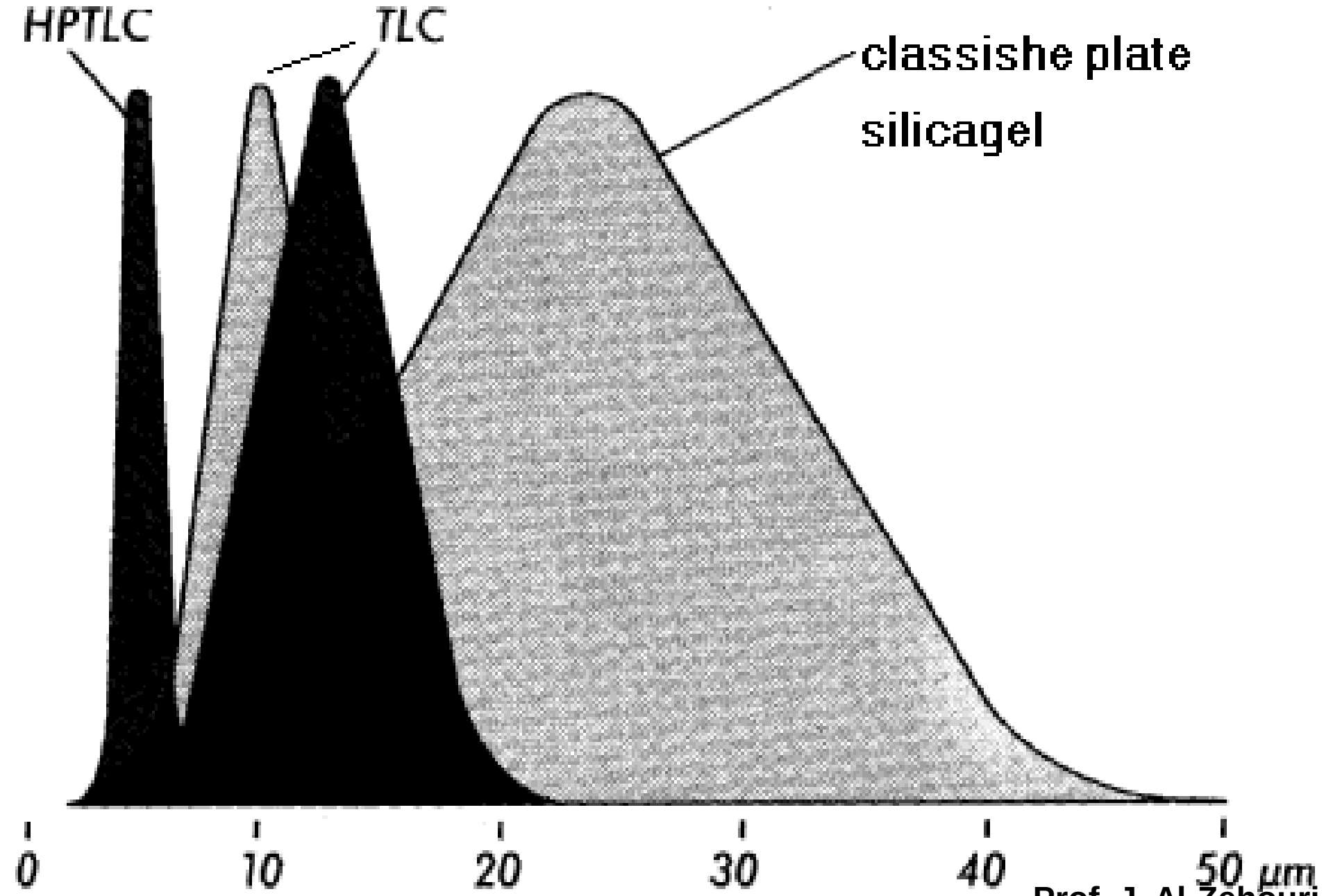
Stationary Phases

- TLC and HPTLC are commercially available in the form of precoated layers supported on glass, or aluminum foil.
- HPTLC plates are smaller (10x10 cm) have a thinner (0.1-0.2 mm) more uniform layer composed of smaller diameter particles (1-5μ) ,and are developed over shorter distances (ca.3-7cm) .

Stationary Phases

- Commercially TLC plates(20x20 cm) which have a 0.25 mm thick layer of (8-20µm) particle size and developed for 10-12 cm.
- In comparison with TLC,HPTLC provides better separation efficiency and lower detection limits.

Stationary phases



Stationary Phases

- Trial and error
  - one's own experience and Literature

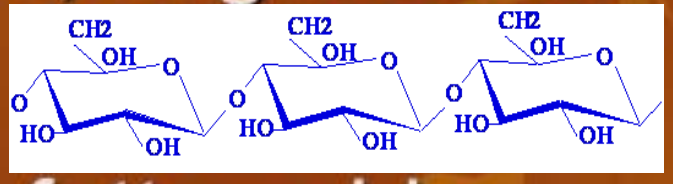
Normal phase
- Stationary phase is polar
- Mobile phase is non polar
- Non-polar compounds eluted first because of lower affinity with stationary phase
- Polar compounds retained because of higher affinity with the stationary phase

Reversed phase
- Stationary phase is non polar
- Mobile phase is polar
- Polar compounds eluted first because of lower affinity with stationary phase
- Non-Polar compounds retained because of higher affinity with the stationary phase

Selection of chromatographic layer

- Precoated plates - different support materials - different Sorber lable
- 80% of analysis - silica gel

- Basic substances, alkaloids and steroids - Aluminum oxide
- Amino acids, dipeptides, sugars and alkaloids - cellulose



. Non-polar substances, fatty acids, carotenoids, cholesterol - RP2, RP8 and RP18

Mobile Phases

- The mobile phase in TLC ,exerts a decisive influence on the separation.
- Because in TLC the mobile phase is removed (evaporated) before the zones are detected , a wide variety of solvents can be used to prepare mobile phase .such as :

يلعب دور حاسم Exerts decisive influence

n-Heptan	C_7H_{16}
n-Hexan	C_6H_{14}
Cyclohexan	C_6H_{12}
Isooctan	C_8H_{18}
1,1,2-Trichlor-trifluorethan	$Cl_2FCCClF_2$
Tetrachlor-kohlenstoff	CCl_4
Toluol	$C_6H_5CH_3$
Chloroform	$CHCl_3$
Dichlor-ethan	$ClCH_2CH_2Cl$
Dichlor-methan	CH_2Cl_2
1-Butanol	$CH_3(CH_2)_3OH$
Acetonitril	CH_3CN
2-Propanol	$CH_3CH(OH)CH_3$
Ethylacetat	$CH_3COOC_2H_5$
Aceton	CH_3COCH_3
Ethanol	C_2H_5OH
1,4-Dioxan	$C_4H_8O_2$
Tetra-hydrofuran	C_4H_8O
Methanol	CH_3OH
Wa :er	H_2O

Eluting power of organic solvents.

increasing polarity

(more powerful eluters)

alkanes (hexanes, petroleum ether)

toluene

halogenated hydrocarbons (methylene chloride)

diethyl ether

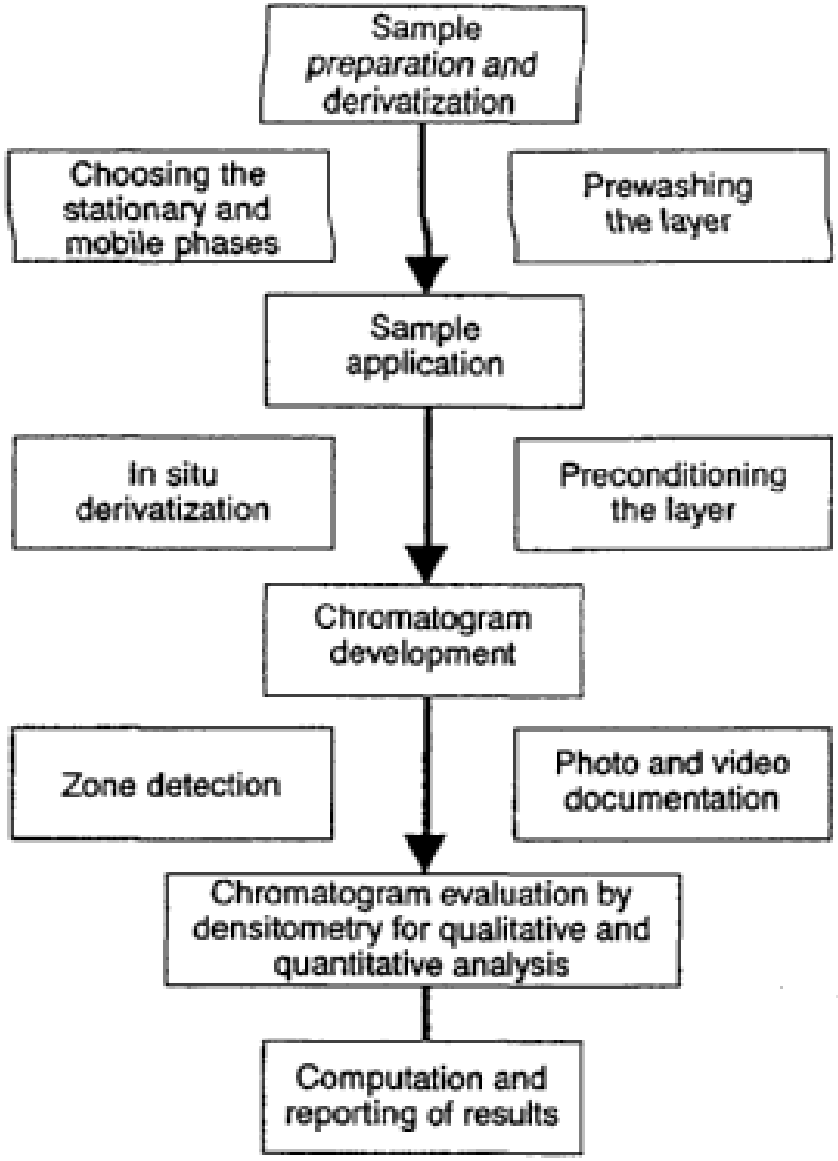
ethyl acetate

acetone

alcohols

acetic acid

TLC Procedure

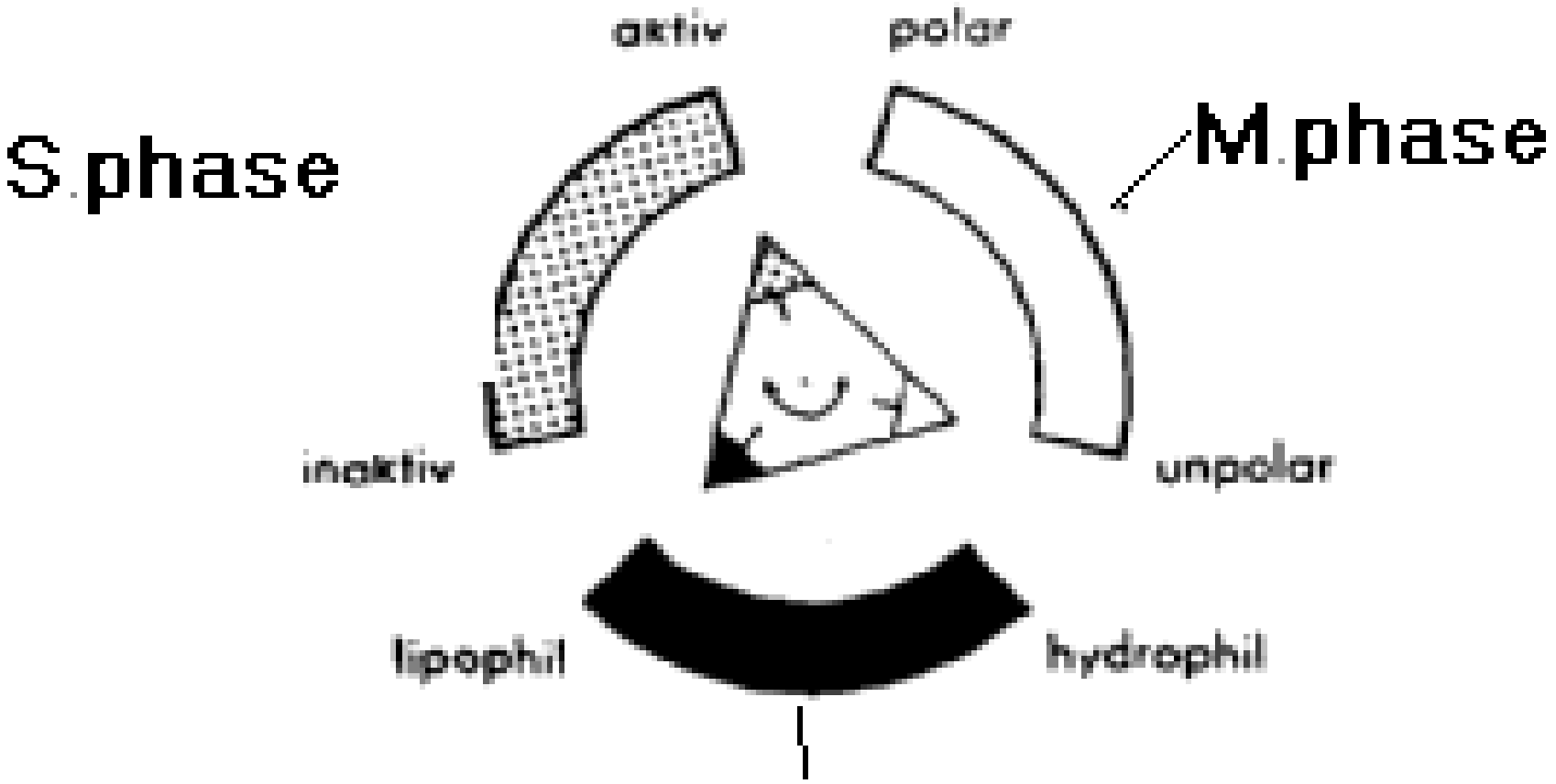


Schematic diagram of the steps in a TLC analysis.

CHOICE OF THE BEST PHASES TLC SYSTEM

The choice of the best phases system to employ in TLC , as with all Chromatography techniques ,is the most challenging and the most difficult.

ADSORPTION



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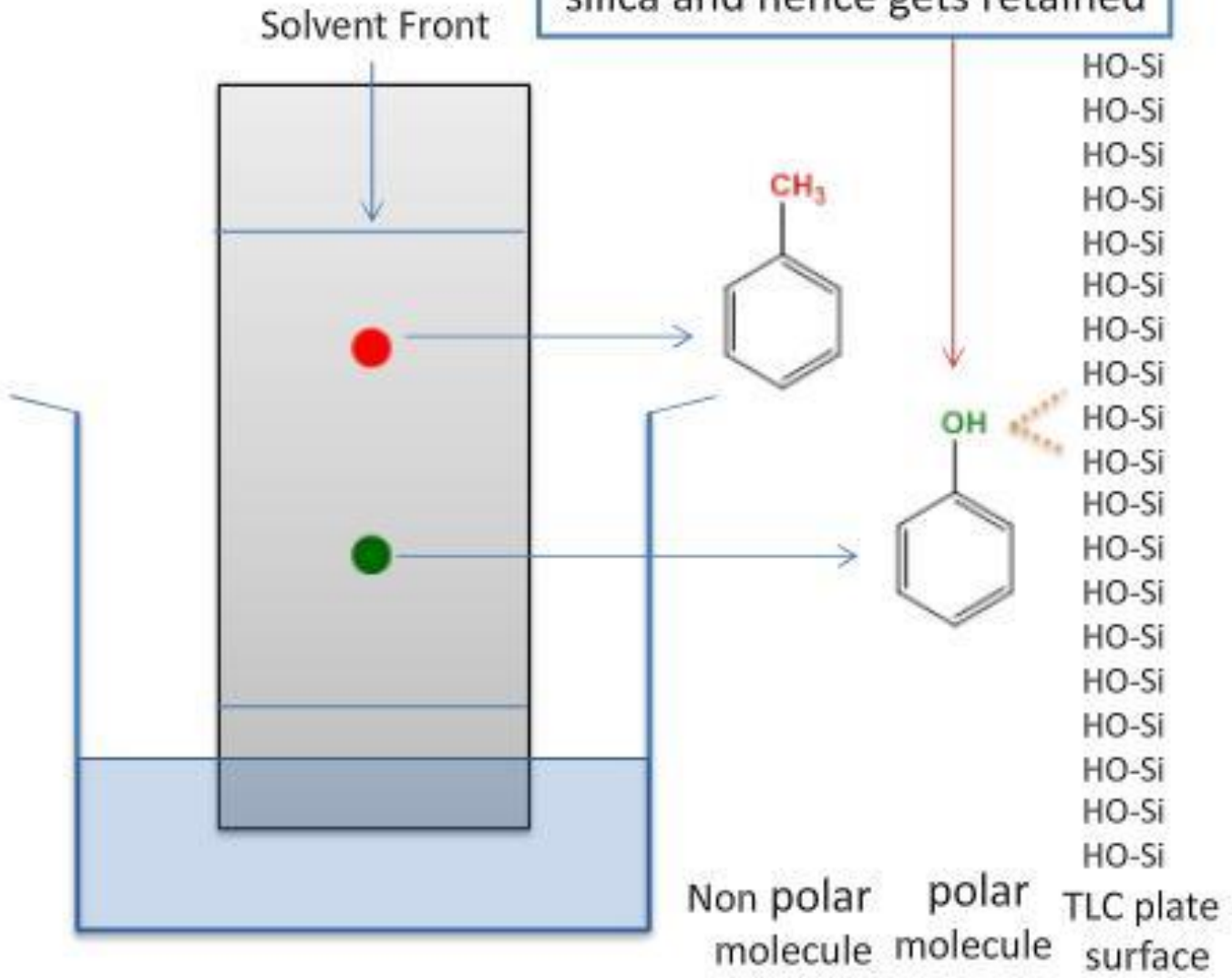
Selection of chromatographic layer

- Precoated plates - different support materials - different Sorber lable
- 80% of analysis - silica gel

- Basic substances, alkaloids and steroids - Aluminum oxide
- Amino acids, dipeptides, sugars and alkaloids - cellulose
- Non-polar substances, fatty acids, carotenoids, cholesterol - RP2, RP8 and RP18

As the solvent rises the mixture gets separated/ resolved

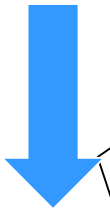
Polar molecule is capable of H-bond interactions with silica and hence gets retained



Solvents

- Choose a solvent depending on the polarity of the compound

Least Polar •



More polar •

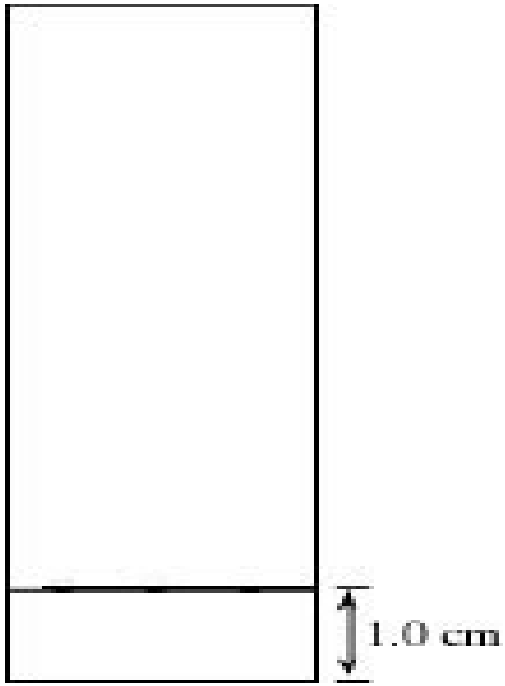
The air Saturated with solvent Vapor contained in th Chamber not only prevents solvent evaporation from the plate surface, but also allows the Surface coating that controls the retention of the Solutes

Sample Application

- Application of small ,exactly positioned initial zones of sample and standard solutions having accurate and precise volumes, without damaging the layer surface.
- The volumes applied and the method of application depend on the type of analysis to be performed (qualitative or quantitative),
- For TLC 0.5-5 µl volumes are usually applied manually with micropipette to produce initial zones with diameters in the 2-4 mm range.

ring the Plate

Do not touch the TLC plate on the side with the white surface .In order to obtain an imaginary start line, make two notches on each side of the TLC plate. You can also draw a thin line with pencil .**Do not use pen** .***Why ?***The start line should be 0.5-1 cm from the bottom of the plate.



illary spotters

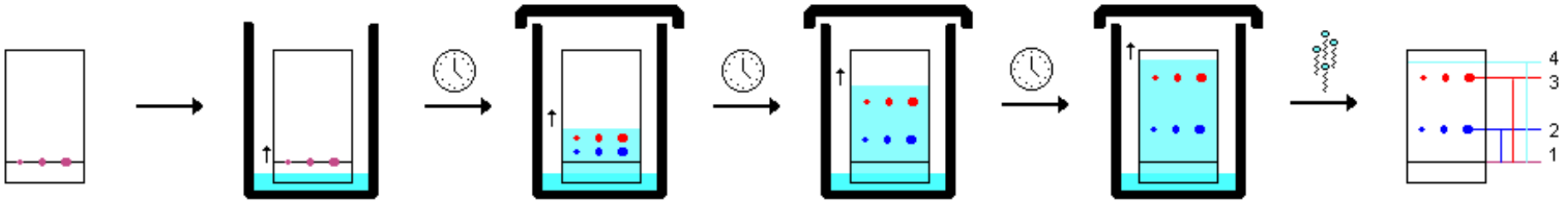
Place a melting point capillary and in the dark blue part of the Bunsen burner flame. Hold it there until it softens and starts to sag. Quickly remove the capillary from the flame and pull on both ends to about 2-3 times its original length. If you pull the capillary inside the flame, you will have a "piece of art", but not a good spotter. Allow the capillary to cool down, and then break it in the middle. Make sure that you break off the closed end on one of them .*Do not use gloves when you pull capillaries. You will have much better control without them!*

Spotting the plate

thin end of the spotter is placed in the dilute solution; the solution will rise up in the capillary (capillary forces). Touch the plate briefly at the start line. Allow the solvent to evaporate and spot at the same place again. This way you will get a concentrated and small spot. Try to avoid spotting too much material, because this will deteriorate the quality of the separation considerably (‘tailing’). The spots should be far enough away from the edges and from each other as well. If possible, you should spot the compound or mixture together with the starting materials and possible intermediates on the plate. They will serve as internal reference since every TLC plate is slightly different

Chromatogram Development

- TLC development times are typically in the range of 3-60 min, depending on the layer, mobile phase, and development method chosen.



x

TLC- Development methods

1. UV Lampe
2. Chemical reagent
3. Densitometry chromatogram

Visualization

UV light:

non-destructive, long wavelength (background green, spots dark), short wavelength (plate dark, compounds glow), **Do not look into the UV !!!lamp**

Omeprazole

Action and use
Treatment of peptic ulcer

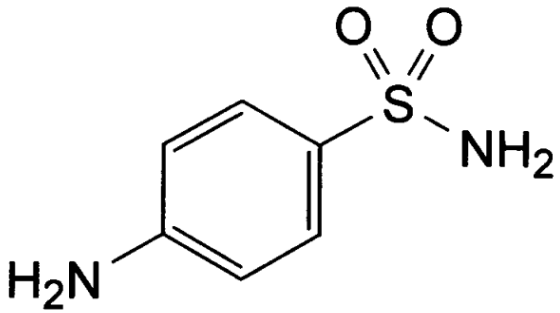
Impurity C

using a *TLC*

silica gel F 254plate R. ….

Examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a) with a higher *Rf* value than that of the spot due to omeprazole is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.1 per cent). …

Sulfanilamide



Test solution

Reference solution

Action and use

Antibacteria

Related substances

Examine by **thin-layer chromatography** *(2.2.27)*, using a *TLC silica gel F 254plate R …….*

Apply to the plate 5 µl of each solution. Develop over a path correspondingto two-thirds of the plate height using a mixture of 3 volumes of *dilute ammonia R1*, 5 volumes of *water R*, 40 volumes of *nitromethane R* and 50 volumes of *dioxan R*. Dry the plate at 100 °C to 105 °C and

Chemicals reagent

1. Sulfuric acid with Oxidant or not/heat: destructive, leaves charred blots behind
2. Ceric stain: destructive, leaves a dark blue blot behind for polar compounds
3. Iodine: semi-destructive, iodine absorbs onto the spots, not permanent
4. Ninhydrin for amino acid

Ibuprofen

Action and use

Anti-inflammatory; analgesic.

مثبط COX-inhibition
للسيكلو اكسيجيناز

IDENTIFICATION

Test solution Dissolve 50 mg of the substance to be examined in *methylene chloride R* and dilute to 10 ml with the same solvent ….

Mobile phase ***anhydrous acetic acid R*, *ethyl acetate R*, *hexane R* (5:24:71 *V/V/V*).**

Application **5 µl.**

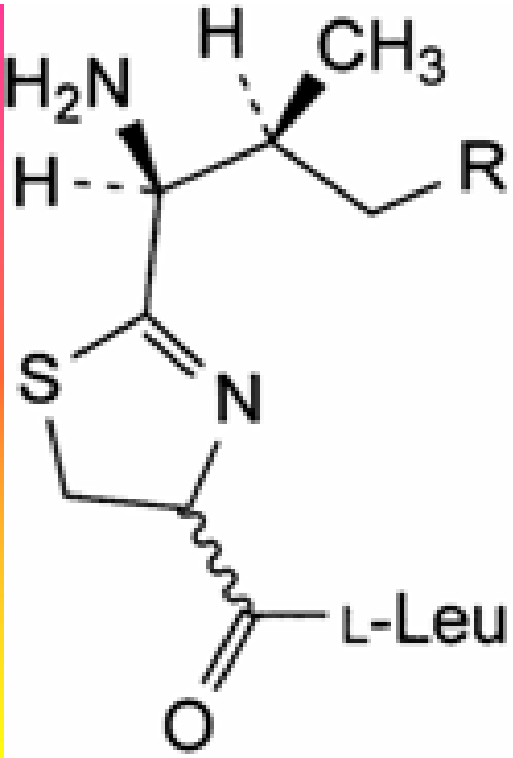
Development **Over a path of 10 cm.**

Drying **At 120 °C for 30 min.**

Detection **Lightly spray with a 10 g/l solution of *potassium permanganate R* in *dilute sulphuric acid R* and heat at 120 °C for 20 min….**

Bacitracin

Antibacterial



L-Leu → D-Glu → Y → L-Lys → D-Orn → X → D-Phe

L-Asn ← D-Asp ← L-His

Bacitracin

Action and use

Antibacterial.

IDENTIFICATION

A.

Test solution **Dissolve 10 mg of the substance to be examined in a 3.4 g/l solution of *hydrochloric acid R* and dilute to 1.0 ml with the same solution.**

Reference solution **Dissolve 10 mg of *bacitracin zinc CRS* in a 3.4 g/l solution of *hydrochloric acid R* and dilute to 1.0 ml with the same solution.**

Plate ***TLC silica gel plate R.***

Mobile phase ***glacial acetic acid R*, *water R*, *butanol R* (1:2:4 *V/V/V*).**

Application **10 µl.**

Development **Over half of the plate.**

Drying **At 100-105 °C.**

Detection **Spray with *ninhydrin solution R1* and heat at 110 °C for 5 min.**

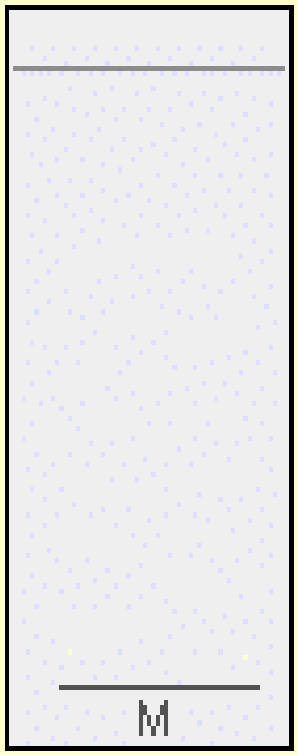
Results **The spots in the chromatogram obtained with the test solution are similar in position, size and colour to the spots in the chromatogram**

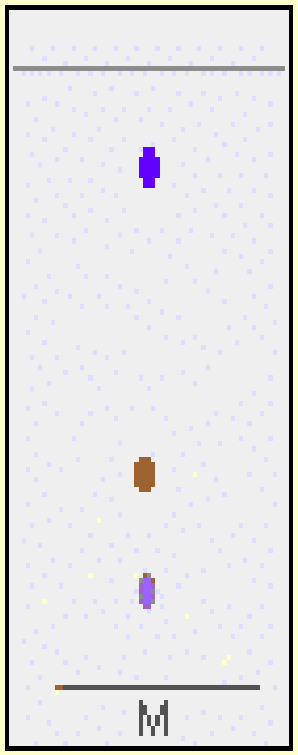
IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using a ……

After removal of the plate, allow it to dry in air, expose it to iodine vapour until spots appear and examine in daylight …..

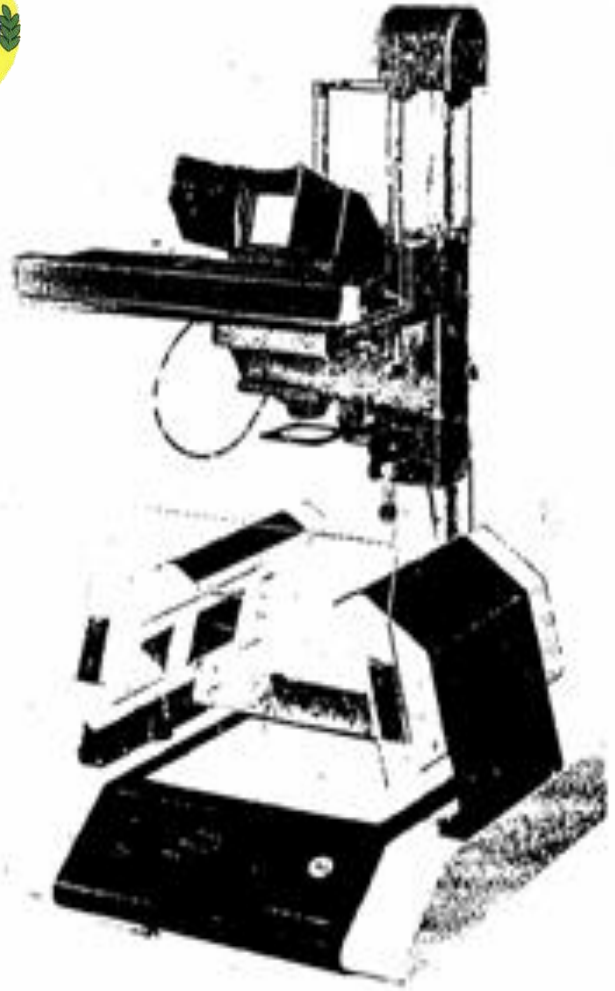
e chromatogram is allowed to dry and is then sprayed w
solution of ***ninhydrin***. Ninhydrin reacts with amino acids to give coloured compounds, mainly brown or purple.





before spraying with ninhydrin

after spraying with ninhydrin



Densitometry = reflectance spectrophotometry

ماسح الكثافة الضوئية = مقياس الطيف الضوئي الأنعكاسي

anning Densitometry

In situ scanning of TLC plates employing optical instrumentation has been extensively developed over the past decade and is now considered essential for both the *accurate identification* of the spot position and the *precise quantitative estimation* of its content. In most instruments, the plate surface can be examined employing either reflected light, transmitted light or fluorescent light. In addition the incident light may be adsorbed, diffusely scattered, or transmitted through the plate. The normal procedure is to measure the light scattered, reflected or generated by fluorescence from the spot and compare it electronically with light from a part of the plate where no sample has passed (*e.g.* the channel between the spots). Single beam and double beam instruments are available and both forms are diagrammatically depicted in figure

- ***Reflected light***
- ***scattered light***
- ***Fluorescent Light***

TLC-Evaluation

Qualitative
-Rf

semi quantitative
-colors intensity
- Zones diameter

quantitative

Extraction

can be performed after manually scraping off the separate zones of samples and standards and elution of the substance from the layer material with a strong, volatile solvent, the eluates are concentrated and analyzed with suitable analytical methods.

direct
- Densitometry

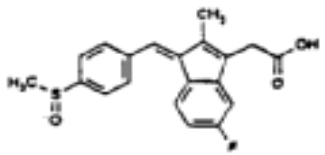
Application of TLC

TLC has been applied virtually in all areas of analysis, including :

- Chemistry
- Biochemistry
- Biology
- Industrial
- agriculture
- Environmental
- Food
- Pharmaceutical
- Clinical
- Natural product
- Toxicology
- Forensics
- Plant science
- Bacteriology
- Parasitological
- And entomology

Sulindac

Analgesic



$C_{20}H_{17}FO_3S$ 356.42

1*H*-Indene-3-acetic acid, 5-fluoro-2-methyl-1-[[4-(methylsulfinyl)phenyl]methylene]-, (*Z*)-.

cis-5-Fluoro-2-methyl-1-[(*p*-methylsulfinyl)benzylidene]indene-3-acetic acid [*38194-50-2*].

» Sulindac contains not less than 99.0 percent and not more than 101.0 percent of $C_{20}H_{17}FO_3S$, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

USP Reference standards ⟨11⟩—*USP Sulindac RS*.

Identification—

A: *Infrared Absorption* ⟨197M⟩.

B: *Ultraviolet Absorption* ⟨197U⟩—

Solution: 1 in 65,000.

Medium: hydrochloric acid in methanol (1 in 120). Absorptivities at 284 nm, calculated on the dried basis, do not differ by more than 3.0%.

Loss on drying ⟨731⟩—Dry it in vacuum at 100° for 2 hours: it loses not more than 0.5% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Heavy metals, *Method II* ⟨231⟩: 0.001%.

Chromatographic purity—

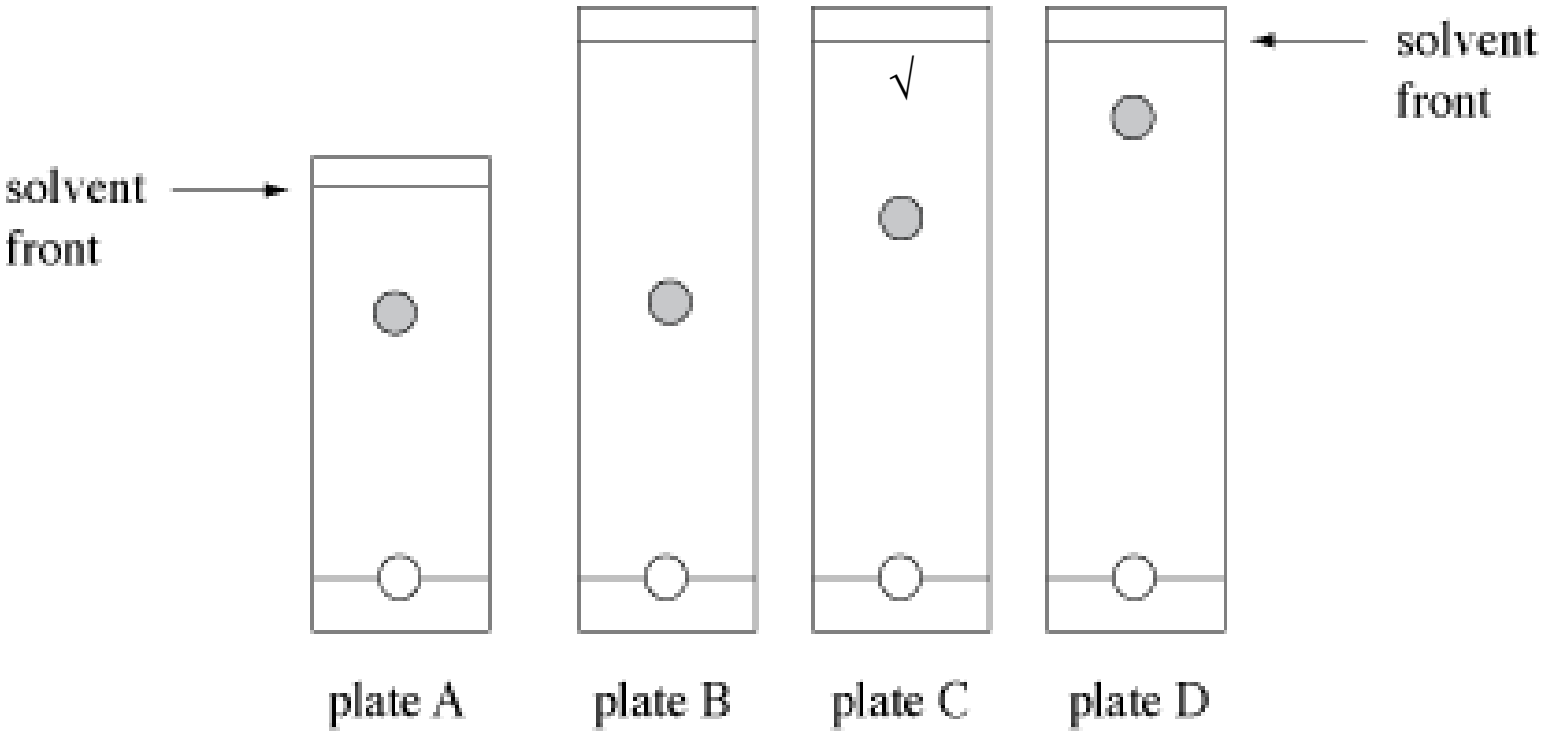
Standard preparation—Prepare a solution of USP Sulindac RS in methanol having a concentration of 25 mg per mL (*Solution A*). Prepare a second solution by diluting 1.0 volume of *Solution A* with methanol to obtain 250 volumes of solution (*Solution B*).

Test preparation—Prepare a solution of the sample in methanol having a concentration of 25 mg per mL.

System suitability—From the chromatograms obtained as directed under *Procedure*, estimate the intensity of the origin spot, if any, in the chromatogram of *Solution A*. The system is satisfactory if any spot observed at the origin is less intense than that obtained from the principal spot in the chromatogram of 4 µL of *Solution B*.

Procedure—Apply 4-µL portions of *Solution A* and the *Test preparation* and 2-, 4-, 6-, 8-, and 10-µL portions of *Solution B* to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of ethyl acetate and glacial acetic acid (97:3) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, allow the solvent to evaporate, and examine the plate under short-wavelength ultraviolet light: the chromatograms show principal

Plate A, below, represents the TLC chromatogram of a compound run in hexanes. The same compound was then spotted on a large TLC plate and again run in hexanes. Which TLC plate, B, C, or D, correctly represents how far the compound would run on the longer plate?



compound will move with the same

Rf

pla

- Se

A a

the

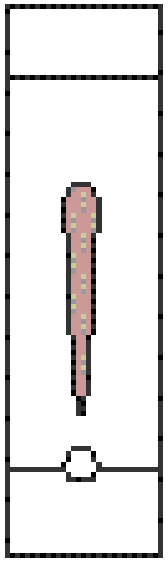
- R

- R

- So the answer is plat C

تبقى قيمة R f ثابتة ضمن نظام تفريق ثابت بصرف النظر عن طول الصفيحة لذا فإن المادة بالصفيحة A تكافىء C

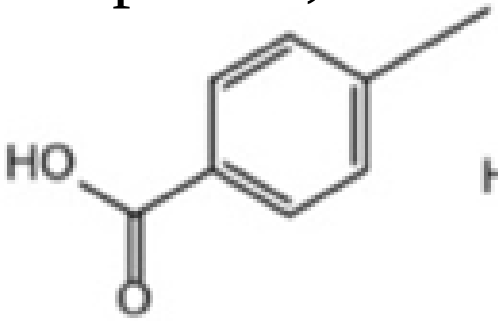
After a rather lengthy organic chemistry synthesis procedure, a student ran the product of the reaction on a TLC plate and obtained the result below. What might he/she have done wrong, if anything?



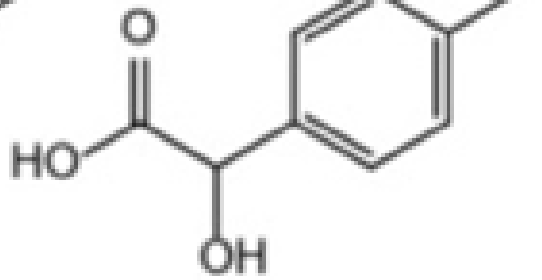
A couple things can cause a TLC plate to streak as illustrated in the diagram of the plate shown. The plate might be **overloaded,** in other words, the solution used to spot the plate is too **concentrated** (to fix this, dilute the solution and try the TLC again). Or, there are simply **too many components** in the mixture to be separated by TLC.

sider a TLC experiment of compound A,B and C using Acetone: n-Hexan 20:80 showed 3 spots under UV light.

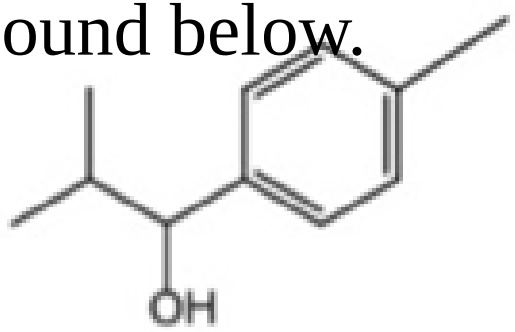
Match spots A,B and C with the 3 compound below.



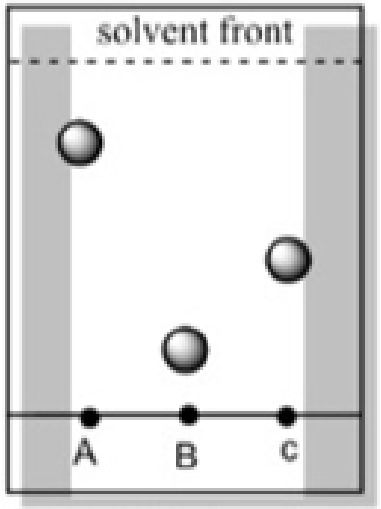
C



B



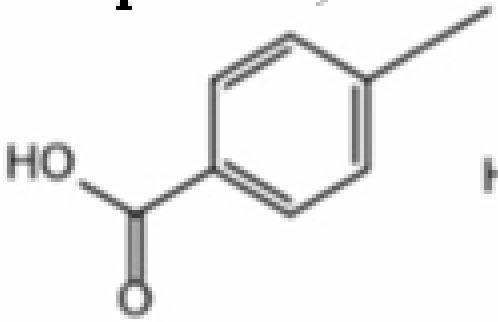
A



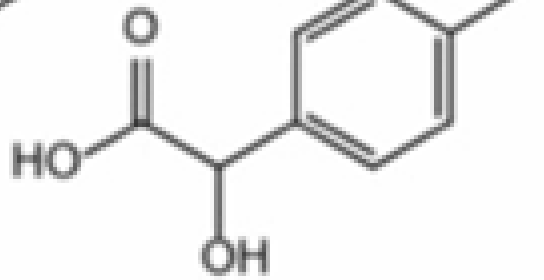
TLC Plate

sider a TLC experiment of compound A,B and C usin
Acetone: n-Hexan 20:80 showed 3 spots under UV light .

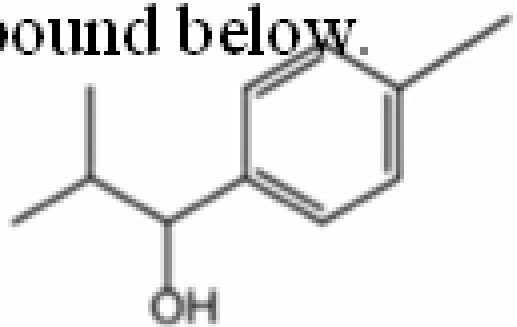
Match spots A,B and C with the 3 compound below.



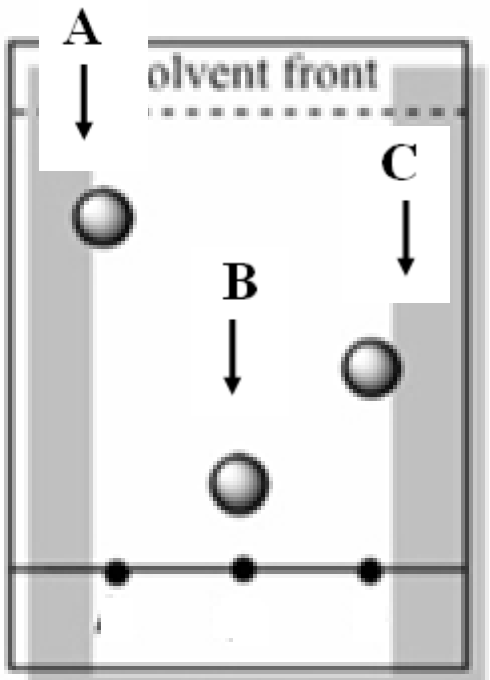
C



B

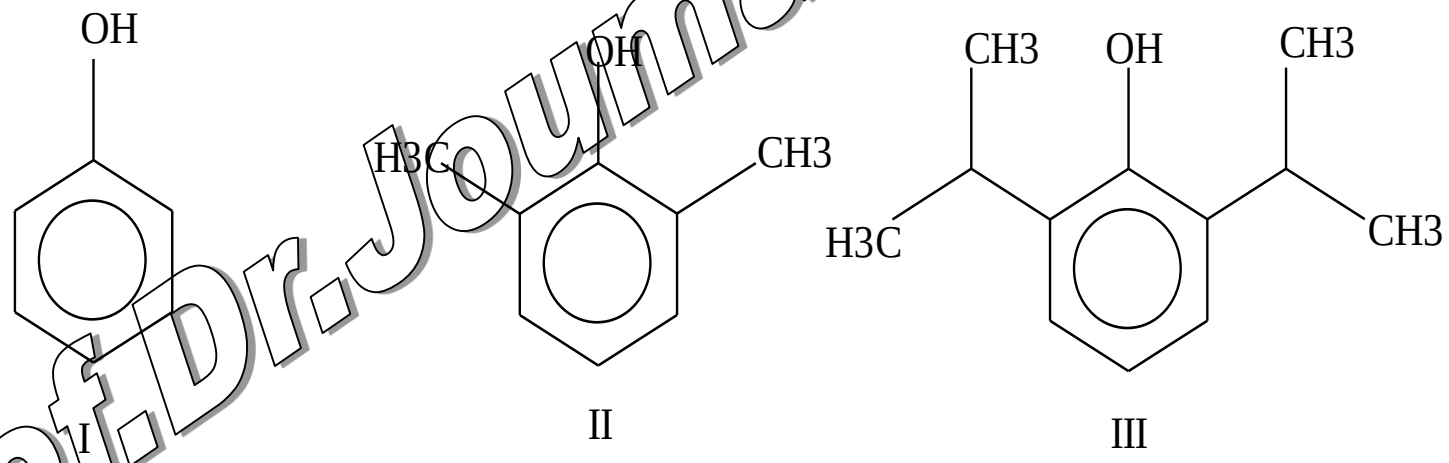


A



Example : A mixture consists of the 3 following substances was separated with TLC using NP (as stationary phase) and n-Hexan as mobile phase.

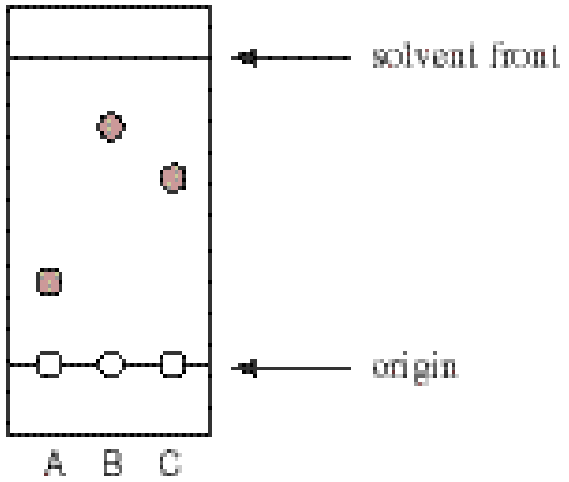
Arrange the substances according to its Rf.



Consider the following silica gel TLC plate of compounds A, B, and C developed in hexanes:

Hexan= C_6H_{14}

Aceton CH_3-CO-CH_3



A- Which compound, A, B, or C, is the most polar?

b- What would you expect to happen to the Rf if you used aceton instead of hexanes as the eluting solvent ?

c- How would the Rf values change if eluted with hexanes using alumina TLC plate ?

ompound A is the most polar because it does not travel a as the other two compounds. Remember, polar compounds stick to the adsorbent more readily, and thus do not travel as far and have a lower value for Rf.

b) Acetone is a more polar solvent than is hexanes. If it were used to elute the same three compounds, each of the compounds would travel faster because the more polar eluting solvent is more proficient at eluting the compounds from the polar adsorbent. Since each compound travels faster, each compound would have a larger Rf value if acetone were used to elute than when hexanes is used to elute the TLC plate

c) Alumina is more polar than is silica. Therefore, each of the compounds would travel slower on an alumina TLC plate than on values for each of the compounds would a silica TLC plate, the Rf .be smaller

ين :

أثناء فصل إحدى المواد الدوائية باستخدام ال TLC قطع الطور المتحرك مسافة 10 سم في حين استغرق التحليل كاملاً 15 دقيقة بينما كانت المسافة التي قطعتها المادة الدوائية = 8 سم ، المطلوب :

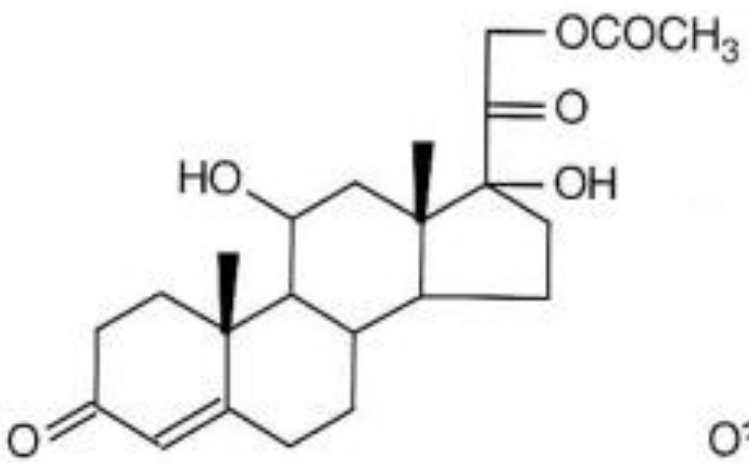
1- احسب قيمة Rf المئوية

2- الزمن الذي مكثته المادة بالطور المتحرك .

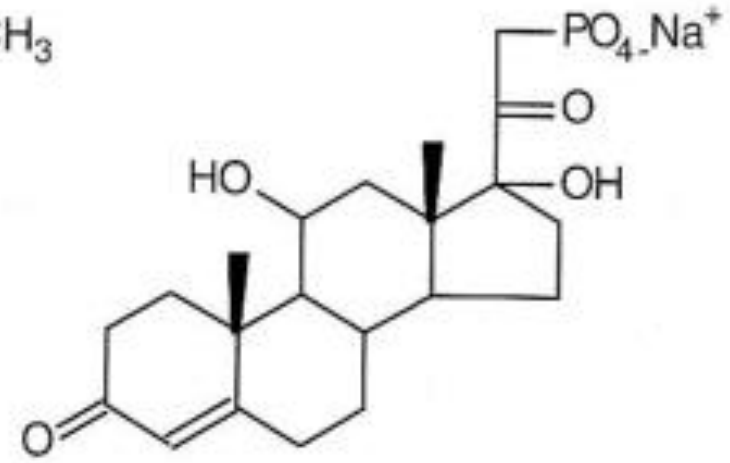
3- الزمن الذي مكثته المادة بالطور الثابت

(الأجابة : 80 ، 12 دقيقة ، 3 دقيقة)

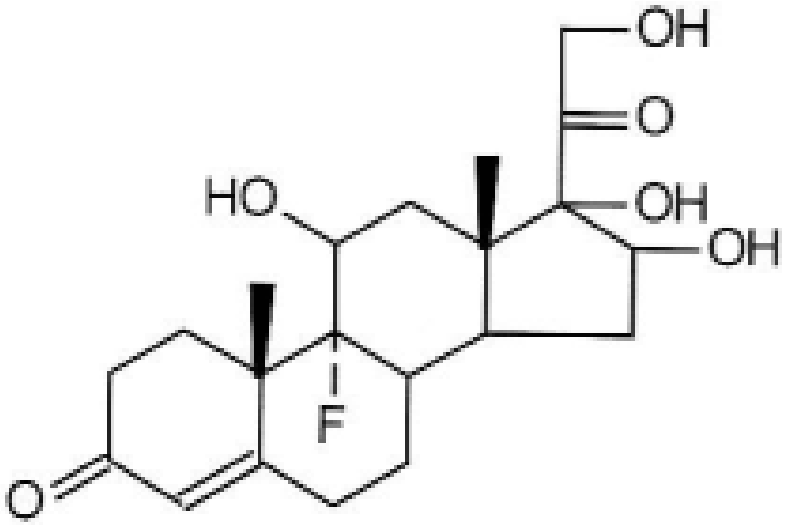
The steroids below are spotted onto a silica gel TLC plate. The plate is developed in methylene chloride/ether/methanol/water (77:15: 8:1.2) and under UV light has the appearance shown in Figure . From your knowledge of the polarity of organic molecules, match the steroids to the spots on the TLC plate



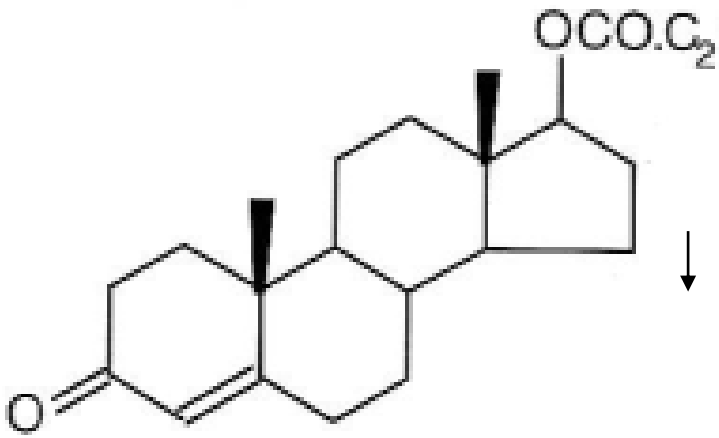
Hydrocortisone acetate



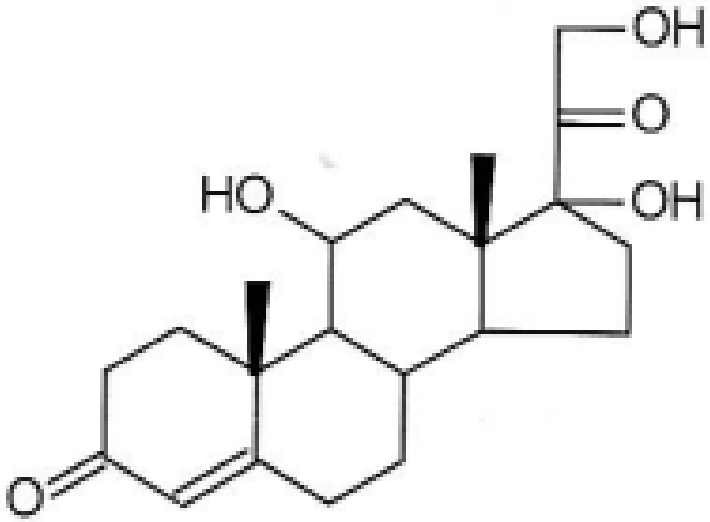
Hydrocortisone sodium phosphate



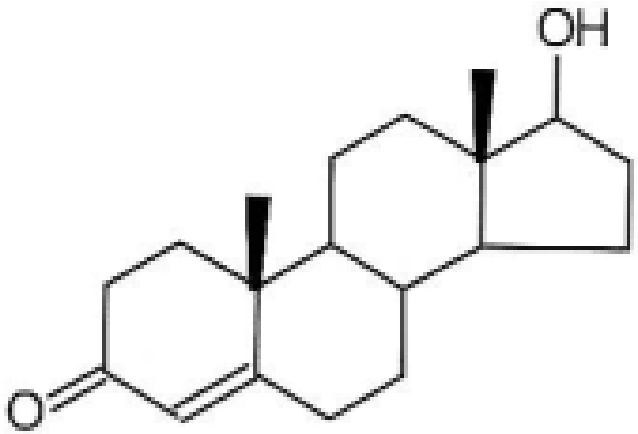
Triamcinolone



Testosterone propionate

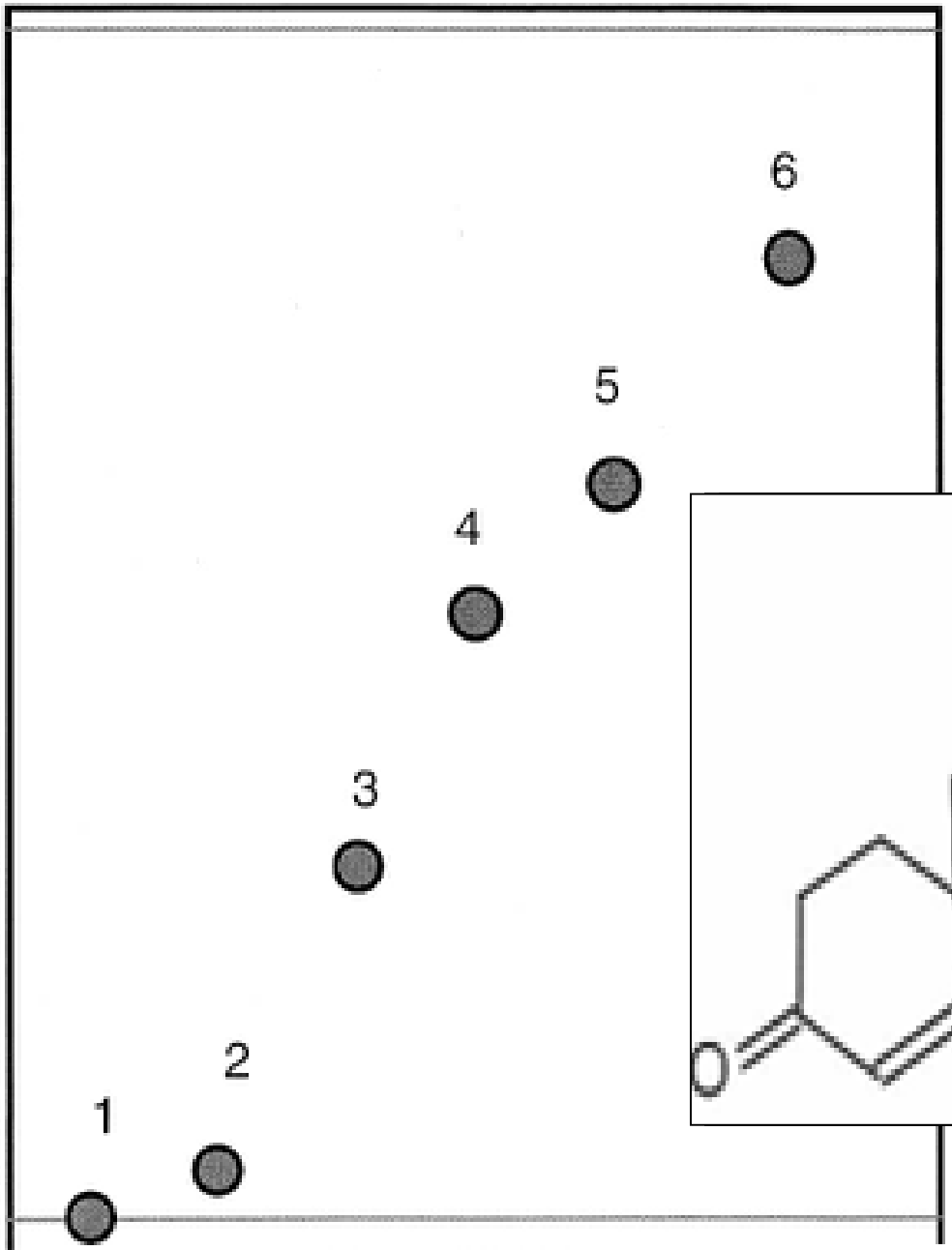


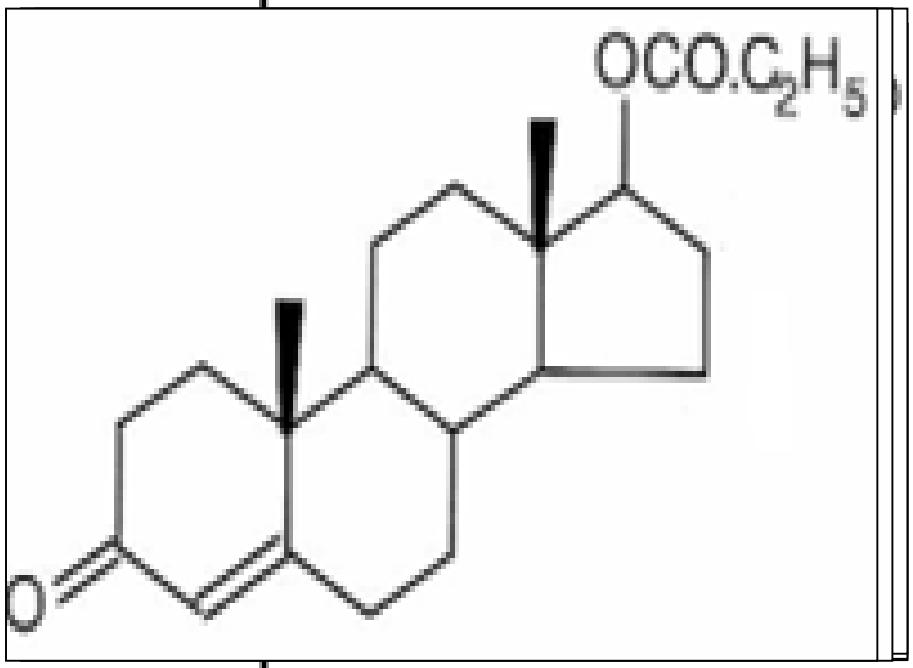
Hydrocortisone



Testosterone

Prof. J. Al-Zehouri





Migration steroids on a silica gel TLC plate.

Answer: 1. hydrocortisone sodium phosphate; 2. triamcinolone; 3. hydrocortisone; 4. hydrocortisone acetate; 5. testosterone; 6. testosterone propionate

Stationary phases which are commonly used in TLC

Stationary phase	Description	Applications
Silica gel G	Silica gel with average particle size 15 µm containing *ca* 13% calcium sulphate binding agent	Use in a wide range of pharmacopoeial tests. In practice commercial plates may be used which contain a different type of binder
Silica gel GF_{254}	Silica gel G with fluorescent agent added	The same types of applications as silica G where visualisation is to be carried out under UV light
Cellulose	Cellulose powder of less than 30 µm particle size	Identification of tetracyclines
Keiselguhr G	Diatomaceous earth containing calcium sulphate binder	Used as a solid support for stationary phases such as liquid paraffin used in analysis of fixed oils

British Pharmacology 2007

Appendix III Chromatographic Separation Techniques

Appendices
Appendix III A. Thin-layer Chromatography

Appendices
Appendix III B. Gas Chromatography

Appendices
Appendix III C. Size-exclusion Chromatography

Appendices
Appendix III D. Liquid Chromatography

Appendices
Appendix III E. Paper Chromatography

Appendices
Appendix III G. Capillary Electrophoresis

Appendices
Appendix III H. Supercritical Fluid Chromatography

Stationary phase in BP

Appendix I A. General Reagents
Silica Gel for Chromatography

Appendix I A. General Reagents
Silica Gel for Chromatography, Alkyl-bonded for use with Highly Aqueous Mobil

Appendix I A. General Reagents
Silica Gel for Chromatography, Aminohexadecylsilyl

Appendix I A. General Reagents
Silica Gel for Chromatography, Aminopropylmethylsilyl

Appendix I A. General Reagents
Silica Gel for Chromatography, Aminopropylsilyl

Appendix I A. General Reagents
Silica Gel for Chromatography, Amylose-derivative of

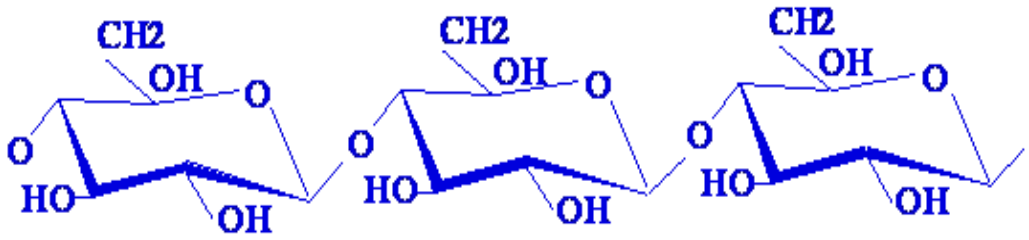
Appendix I A. General Reagents
Silica Gel for Chromatography, Base-deactivated, End-capped Octadecylsilyl

Appendix I A. General Reagents
Silica Gel for Chromatography, Base-deactivated Octadecylsilyl

Appendix I A. General Reagents
Silica Gel for Chromatography, Base-deactivated, End-capped Octylsilyl

Appendix I A. General Reagents
Silica Gel for Chromatography, Base-deactivated Octylsilyl

Appendix I A. General Reagents
Silica Gel for Chromatography, Butylsilyl



Cellulose for Chromatography

- **Cellulose for Chromatography** (*9004-34-6*) A fine, white, homogeneous powder of an average particle size of less than 30 µm.

- ***Preparation of a thin-layer*** Suspend 15 g in 100 ml of *water* and homogenise in an electric mixer for 60 seconds. Coat carefully cleaned plates with a layer 0.1-mm thick using a spreading device and allow to dry in air.

TLC Octadecylsilyl Silica Gel Plate

TLC Octadecylsilyl Silica Gel Plate
Support of glass, metal or plastic coated with a layer of octadecylsilyl silica gel. The plate may contain an organic binder.

Adsorbent : المادة المازة مثل سيليكاجل والألومنيا

Adsorption : عملية الأمتزاز مابين المواد وسطح المادة المازة وتحدث نتيجة تشكل روابط قوية مثل الروابط الهيدروجينية أو روابط أضعف مثل فاندرفاس .

TLC

KEYPOINT

Principles

- فصل المواد الدوائية عبر الطور الثابت (غالباً سيليكاجل) تحت تأثير الطور المتحرك (عادة مزيج من المحلات العضوية) التي تتحرك عبر الطور الثابت من خلال الخاصة الشعرية حيث تحدد المسافة التي تقطعها المادة من خلال مدى علاقتها الفيزيائية مع كل من الطور الثابت والطور المتحرك .

Pharmaceutical applications

- ***تحديد مدى نقاوة المواد الأولية والمنتجات الصيدلانية Impurities .***
- ***غالباً ما يستخدم كطريقة أساسية بتحديد هوية المواد الأولية الصيدلانية .***
- ***يستخدم بحالات التحقق من الصلاحية للنظافة Cleaning Validation والذي يعتبر جزء من الصناعة الصيدلانية .***

Strengths

- ***متينة ورخيصة Robust & Cheap***
- ***إمكانية الكشف بالكواشف الكيميائية لأعطاء ألوان مختلفة .***

ROBUSTNESS

Definition— The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in procedural parameters listed in the procedure documentation and provides an indication of its suitability during normal usage. Robustness may be determined during development of the analytical procedure.

- ***المرونة Flexible بسبب استخدام أنواع عديدة من الأطوار الثابتة والمتحركة .***

Limitations

- طريقة غير مناسبة لفصل المواد الصيدلانية الطيارة Volatile
- ضعف الحساسية Sensitivity is often limited
- بحال ال TLC فان عدد الصفائح النظرية يعتبر قليل بالمقارنة مع ال GC و HPLC

Prof. J. Al-Zehouri

Q&A