Instrumental Analysis- Introduction

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Chromatography

Characteristics

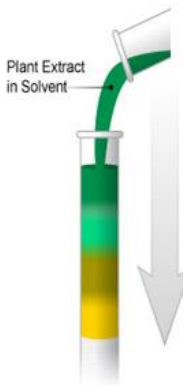
Classification

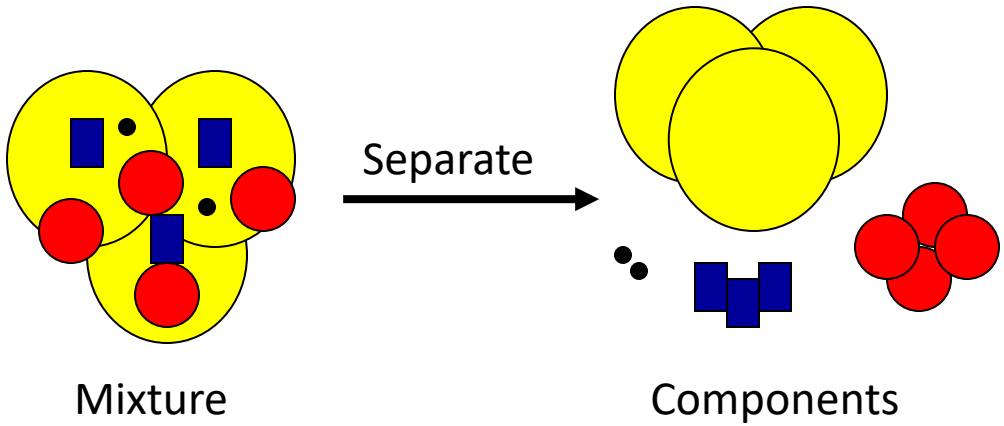
Chromatography

- First by: Mikhail Tswett (20th Century)

Chroma: color; graphlein: write

- Physical reversible separation of a mixture into its components
- Major factors in chromatography: mobile phase & stationary phase





General Classification of Chromatography

- Planar Chromatography:

  Paper & Thin-Layer Chromatography

- Column Chromatography:

✓ Liquid (Column) Chromatography(LC)

  Normal / reverse phase HPLC

  Size Exclusion C., Ion Exchange C., Affinity C.,

✓ Gas (Column) Chromatography(GC)

Base of Chromatography

- Separation in chromatography depends on:

solubility of components in the mobile phase

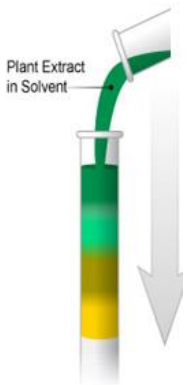
and

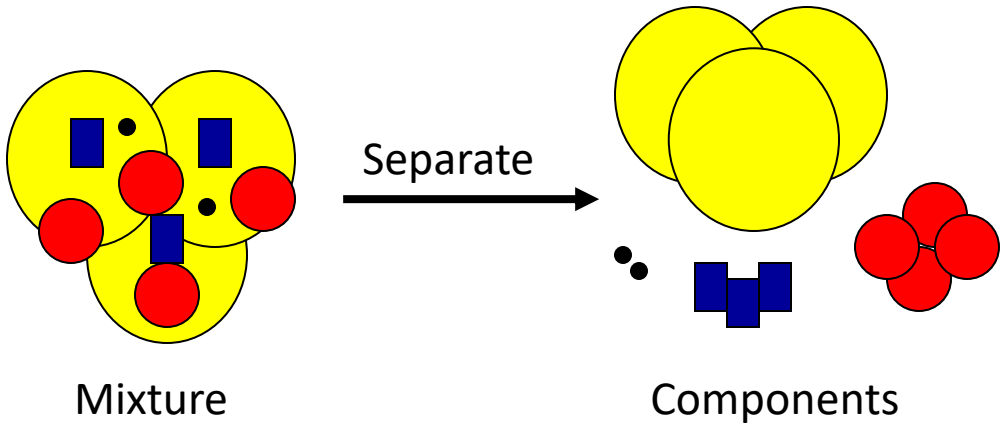
differential affinity

and

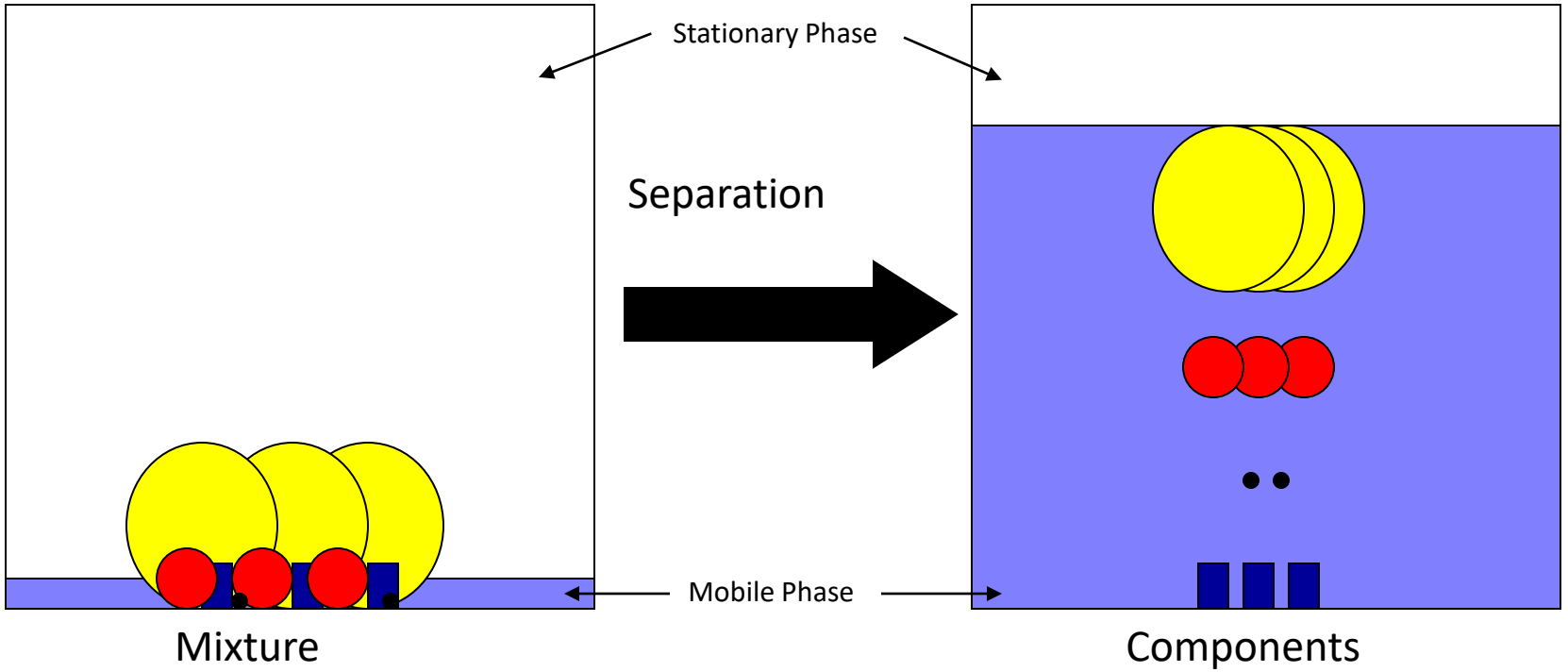
partial distribution of components to the mobile/stationary phases.

- Competitive distribution of components in mobile/stationary phases.





Chromatography by Image



Components	Affinity to Stationary Phase	Affinity to Mobile Phase
Blue	----------	Insoluble in Mobile Phase
Black	✓ ✓ ✓ ✓ ✓ ✓	✓ ✓
Red	✓ ✓	✓ ✓ ✓ ✓ ✓
Yellow	✓	✓✓✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓

Types & Application Fields of Chromatography

- Chromatography can be run as:

✓ Analytical chromatography(in small scale) for qualitative goals

✓ Analytical chromatography(in small scale) for quantitative goals

✓ Preparative chromatography(in large scale) for quantitative goals

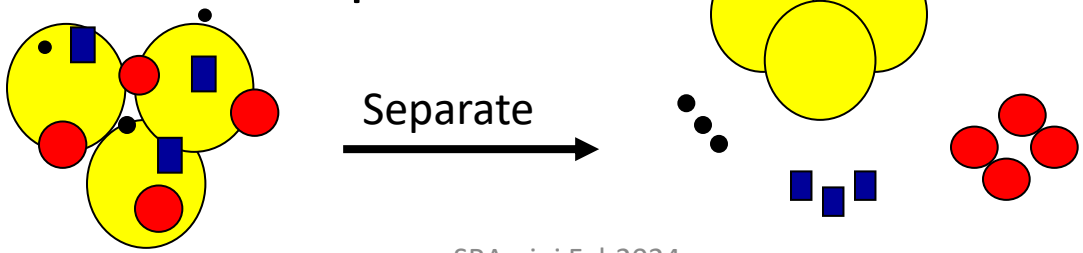
- Application fields of chromatography:

✓ isolation of components

✓ purification of a component

✓ identification/ structural elucidation of a component

✓ quantification of a component



Three Major Factors in Chromatography

- Stationary phase: coated on support:

✓ Solid: chromatography is abbreviated to (SLC; SGC)

✓ viscous Liquid: chromatography is abbreviated to (~~LLC~~; LGC)

- Mobile phase:

✓ Liquid: chromatography is abbreviated to (liq. C.: LC; SLC; ~~LLC~~)

✓ Gas: chromatography is abbreviated to (gas C.: GC; SGC; LGC)

- Sample

General Types in Chromatography Regarding Goal & Mobile Phase

- Regarding goal of analysis:

✓ analytical chromatography

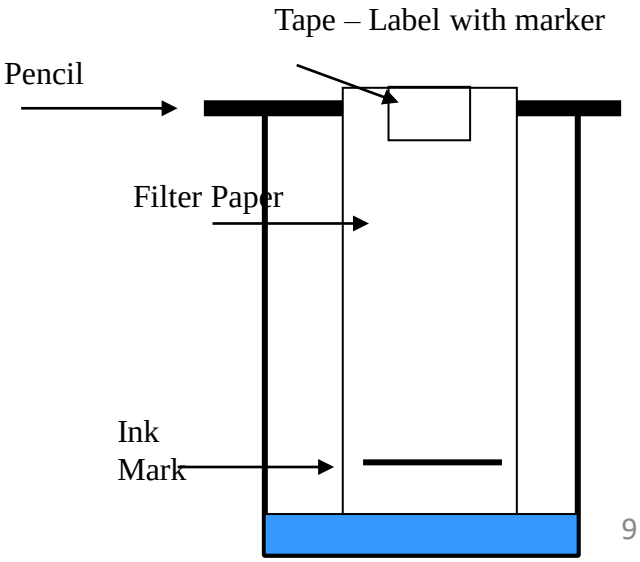
✓ preparative chromatography

- Regarding polarity of stationary & mobile phase

mostly in LC:

✓ normal Phase

✓ reverse Phase



Common Terminology for Classification in LC: Normal Phase LC & Reverse Phase LC

	Stationary phase	Mobile phase
❖Normal Phase:	Polar	Non-Polar
❖Reverse Phase:	Non-Polar	Polar

Characteristics of Stationary Phase in Chromatography

- Completely homogenous
- Porous or finely divided solid or liquid
- Coated on an inert supporting material
- Theoretical description for stationary phase in any type of Chromatography:

✓ Theoretical Plate (TP)

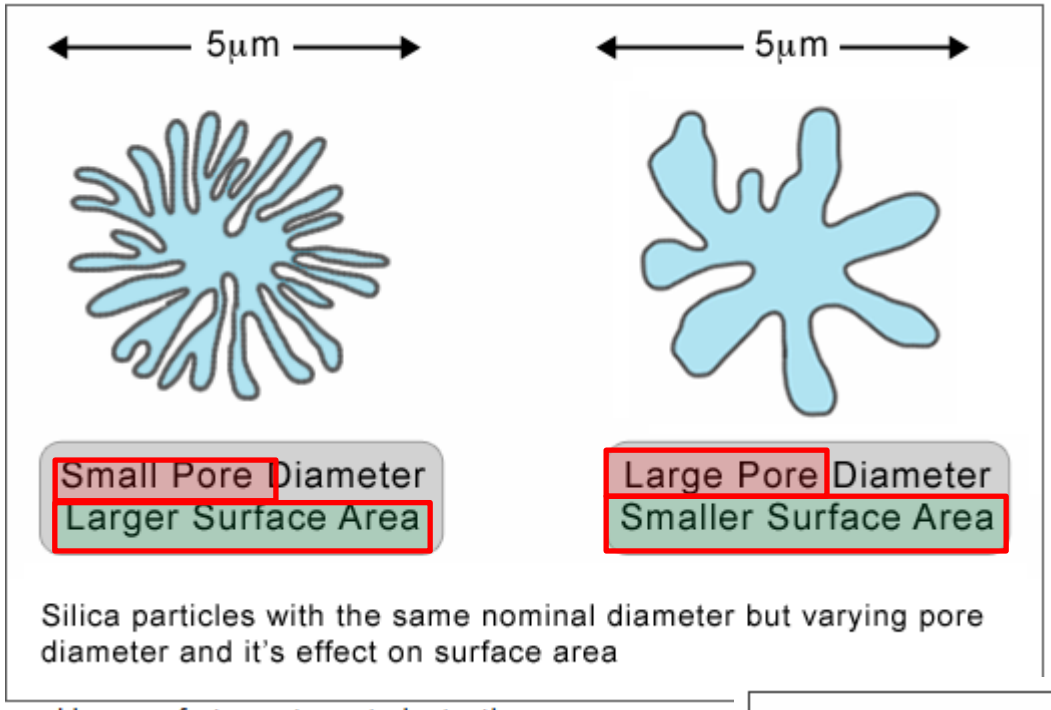
✓ High Efficient TP (HETP)

Characteristics of Stationary Phase as Adsorbent

- homogenous condition
- Absorptivity
- Surface activity: depends on:
  - Surface area
  - Particle size
  - Pore size

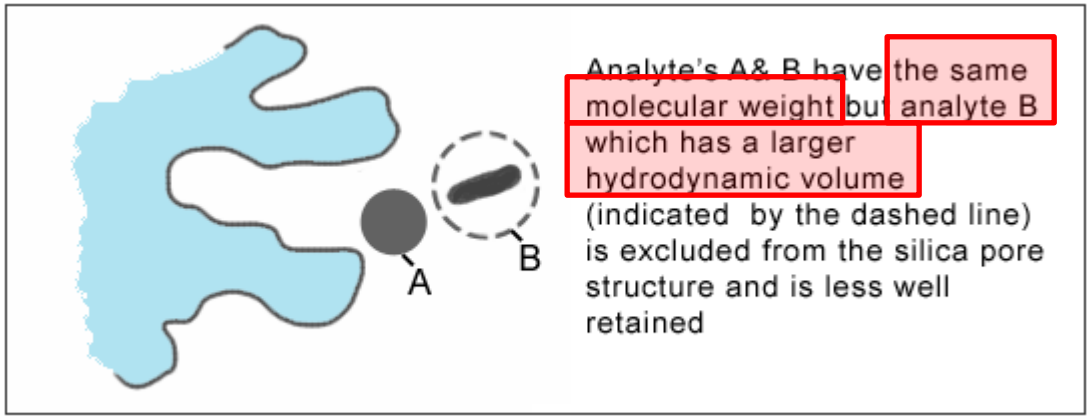
- Examples:
  - ✓ Sucrose
  - ✓ starch,
  - ✓ Talc
  - ✓ calcium carbonate
  - ✓ calcium phosphate
  - ✓ magnesia
  - ✓ magnesium silicate
  - ✓ silica gel: SiO_2
  - ✓ alumina: Al_2O_3

Surface Activity Characteristics of Stationary Phase Includes: Surface Area/ Particle Size/ Pore Size



Silica particles with the same nominal diameter but varying pore diameter and it's effect on surface area

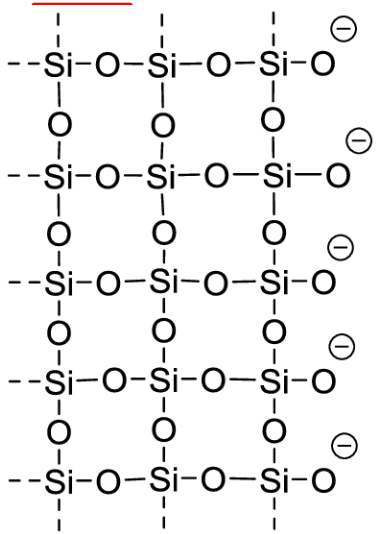
Grade of Compression of Solid Phase



Silica Gel as Introduced in Company Catalogue

http://www.sigmaaldrich.com/catalog/product/aldrich/717177?lang=en&region=IR

- Manufacture Co.: Sigma-Aldrich®
- Grade: technical grade
- particle size: 63-200 µm: 70-230 mesh
- pore size: 0.7 - 0.85 cm^3/g
- pore volume: Approx. 60 Å
- surface area: ≥480 m^2/g (approximately)
- Bp: 2230 °C
- Mp: >1600 °C



H7631 SIGMA

Silica gel

10 µm mean particle size, average diameter 100 Å

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Properties

mean particle size	10 µm
pore size	100 Å average diameter
surface area	300 m^2/g

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H7506 SIGMA

Silica gel

5 µm mean particle size, average diameter 100 Å

MSDS | SIMILAR PRODUCTS

Purchase | Safety & Documentation | Peer-Reviewed Papers

Properties

mean particle size	5 µm
pore size	100 Å average diameter
surface area	300 m^2/g

Characteristics of Mobile Phase in Liquid Chromatography

- Completely homogenous
- Simple solvent or miscible solvents in mixture
- Elutropic value
- Dielectric constant: polarity
- Solubility parameter: miscibility
- Volatility

Solvent Polarity Chart Based on Solvent Functional Groups

Solvent Polarity Chart

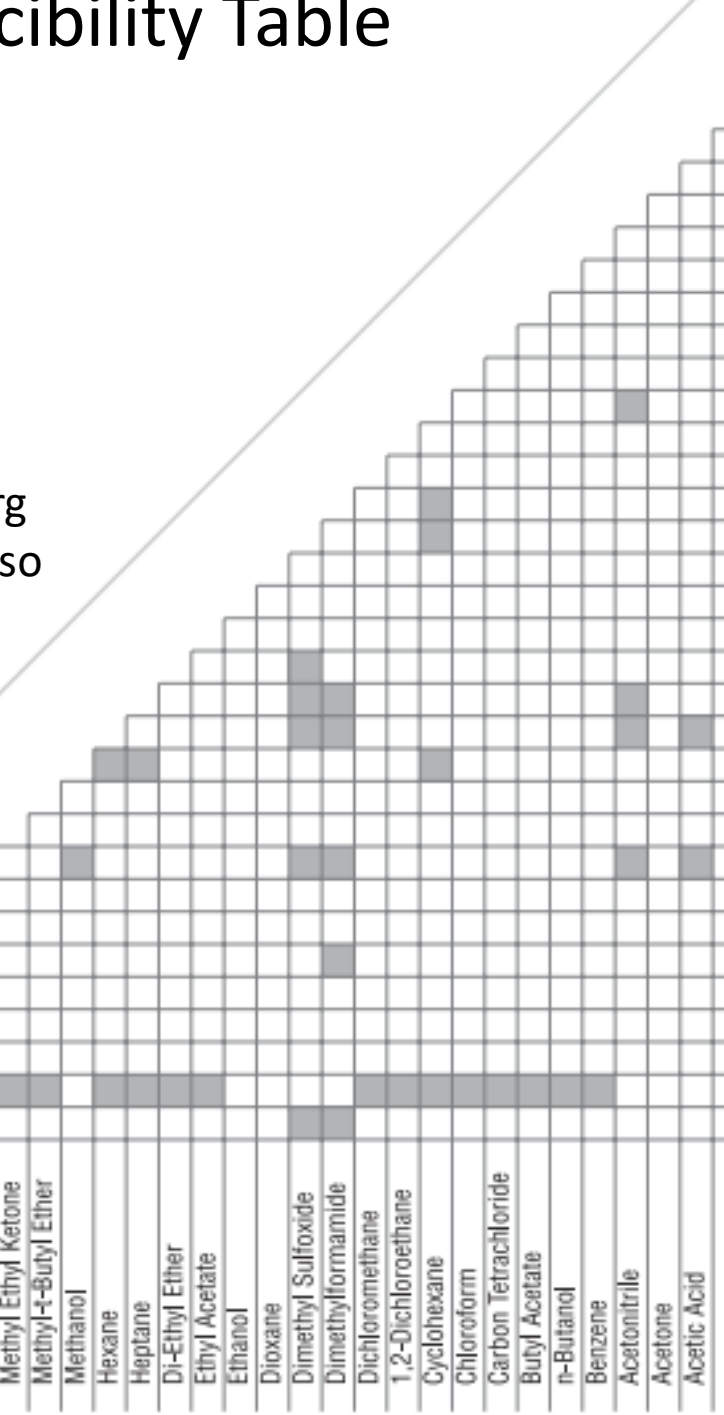
Relative Polarity	Compound Formula	Group	Representative Solvent Compounds
Nonpolar	R - H	Alkanes	Petroleum ethers, ligroin, hexanes
	Ar - H	Aromatics	Toluene, benzene
	R - O - R	Ethers	Diethyl ether
	R - X	Alkyl halides	Tetrachloromethane, chloroform
	R - COOR	Esters	Ethyl acetate
	R - CO - R	Aldehydes and ketones	Acetone, methyl ethyl ketone
	$R - NH_2$	Amines	Pyridine, triethylamine
	R - OH	Alcohols	Methanol, ethanol, isopropanol, butanol
	$R - COHN_2$	Amides	Dimethylformamide
	R - COOH	Carboxylic acids	Ethanoic acid
Polar	H - OH	Water	Water

Increasing Polarity

https://www.erowid.org/archive/rhodium/pdf/solvent.miscibility.pdf

cibility Table

rg
so



Solvent	Polarity Index	Refractive Index @20°C	UV(nm) Cutoff @1AU	Boiling Point(°C)	Viscosity (cPoise)	Solubility in water (%w/w)
Acetic Acid	6.2	1.372	230	118	1.26	100
Acetone	5.1	1.359	330	56	0.32	100
Acetonitrile	5.8	1.344	190	82	0.37	100
Benzene	2.7	1.501	280	80	0.65	0.18
n-Butanol	4.0	1.394	254	125	0.73	0.43
Butyl Acetate	3.9	1.399	215	118	2.98	7.81
Carbon Tetrachloride	1.6	1.466	263	77	0.97	0.08
Chloroform	4.1	1.446	245	61	0.57	0.815
Cyclohexane	0.2	1.426	200	81	1.00	0.01
1,2-Dichloroethane[1]	3.5	1.444	225	84	0.79	0.81
Dichloromethane[2]	3.1	1.424	235	41	0.44	1.6
Dimethylformamide	6.4	1.431	268	155	0.92	100
Dimethyl Sulfoxide[3]	7.2	1.478	268	189	2.00	100
Dioxane	4.8	1.422	215	101	1.54	100
Ethanol	5.2	1.360	210	78	1.20	100
Ethyl Acetate	4.4	1.372	260	77	0.45	8.7
Di-Ethyl Ether	2.8	1.353	220	35	0.32	6.89
Heptane	0.0	1.387	200	98	0.39	0.0003
Hexane	0.0	1.375	200	69	0.33	0.001
Methanol	5.1	1.329	205	65	0.60	100
Methyl-t-Butyl Ether[4]	2.5	1.369	210	55	0.27	4.8
Methyl Ethyl Ketone[5]	4.7	1.379	329	80	0.45	24
Pentane	0.0	1.358	200	36	0.23	0.004
n-Propanol	4.0	1.384	210	97	2.27	100
Iso-Propanol[6]	3.9	1.377	210	82	2.30	100
Di-Iso-Propyl Ether	2.2	1.368	220	68	0.37	
Tetrahydrofuran	4.0	1.407	215	65	0.55	100
Toluene	2.4	1.496	285	111	0.59	0.051
Tichloroethylene	1.0	1.477	273	87	0.57	0.11
Water	9.0	1.333	200	100	1.00	100
Xylene	2.5	1.500	290	139	0.61	0.018

Immiscible

Miscible

Immiscible means that in some proportions two phases will be produced

Synonym Table
[1] Ethylene Chloride
[2] Methylene Chloride
[3] Methyl Sulfoxide
[4] tert-Butyl Methyl Ether
[5] 2-Butanone
[6] 2-Propanol

Comparison of Characteristics of Some Common Solvents in LC

	Dielectric constant (ε)	Polarity (D)
Ethanol		
Ethyl acetate		
chloroform		
THF		
Acetonitrile		

Planar Chromatography
TLC, HPTLC & Plate Chromatography

- Liquid chromatography: LC
- Base of separation: differential affinity by polarity

✓ adsorption

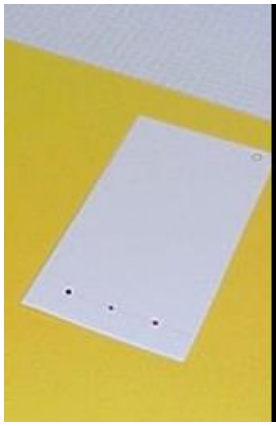
✓ partition distribution or partial distribution

- Follow solvent flow up through capillary action
- Different migration rate

Compare Three Types of Planar Chromatography

Type	Stationary Phase	Mobile Phase	Sample	Qual./Quan.	Analytical/ Preparative	Reporting
TLC						
HPTLC						
Plate C.						

Thin Layer Chromatography



- ➢ Stationary phase:
- ✓ silica (polar phase): SiO_2, $H(-O-Si-O-Si-O)_nH$: strongly polar
- • Solid support: plastic polymer, aluminum layer
- ➢ Mobile phase: tank solvent system: miscible solvent mixtures
- ✓ polarity gradient; solvent with similar or non-similar functional groups
- ✓ acid or base added to prevent tailing
- ➢ Sample loading: using capillary tube

- • Reporting items
- • Application

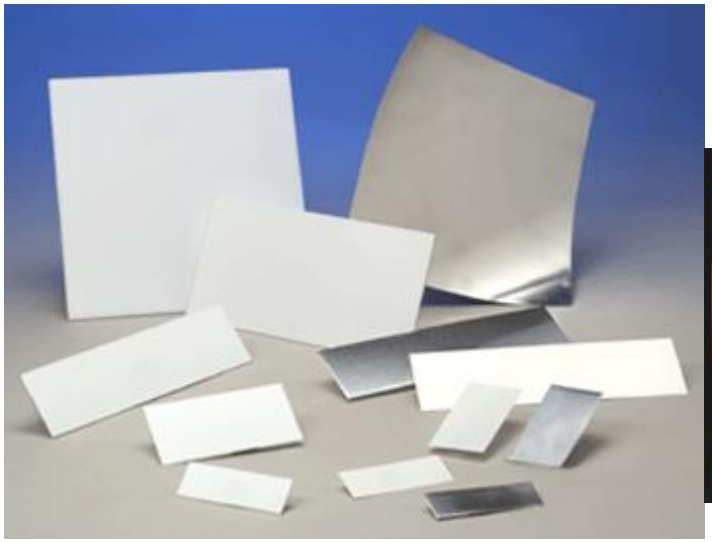
Stationary Phase in TLC: TLC Sheets

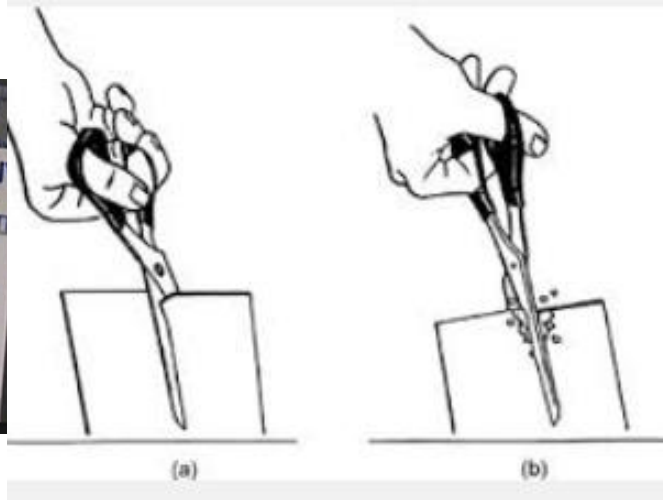
- TLC silica gel 60G F254, 25 sheets, 20×20
- Using binder for uniform hard surface layer
- Layer thickness: 100-250 micron

60: particle size

G: Gypsum binder that mixed w

254F: Fluorescent indicator with absorption in 254 nm





Items to Run TLC

1. Cutting TLC silica sheet in suitable size:
2. Preparation of sample solution:

dissolving sample in suitable solvent

3. Loading a sample spot from sample solution on TLC sheet:

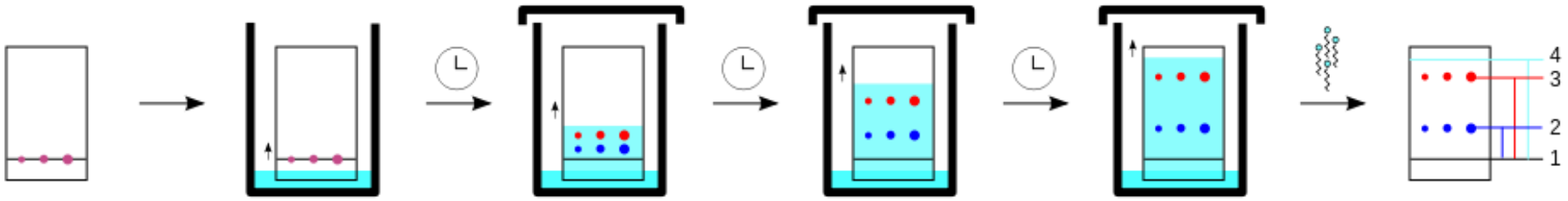
✓ sample spot in a right line

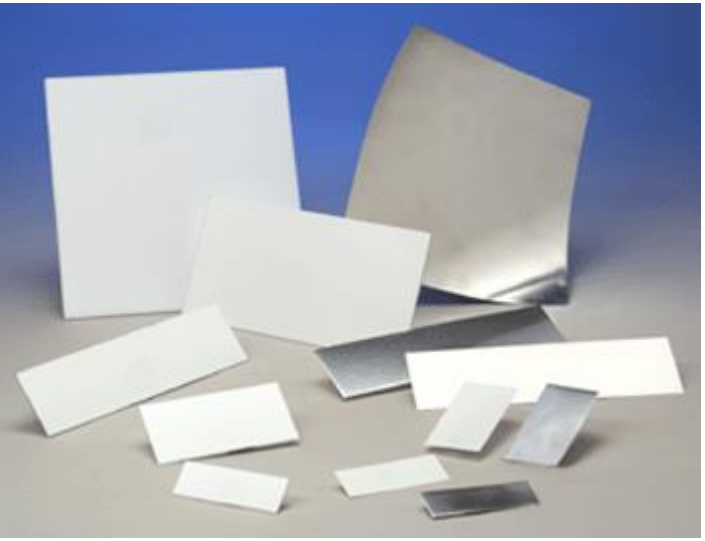
4. Selection of optimum mobile phase

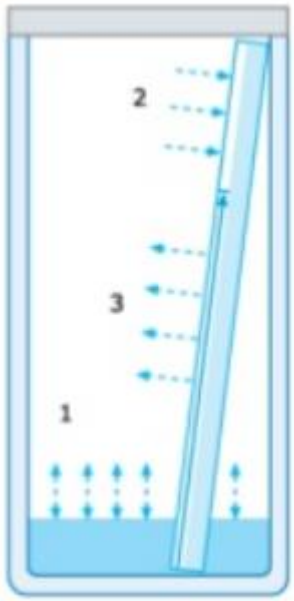
✓ preparation of solvent tank

5. TLC run
6. Stop solvent run at suitable solvent level: dry TLC sheet
7. Visualize the spots
8. Report R_f

TLC Separation Process

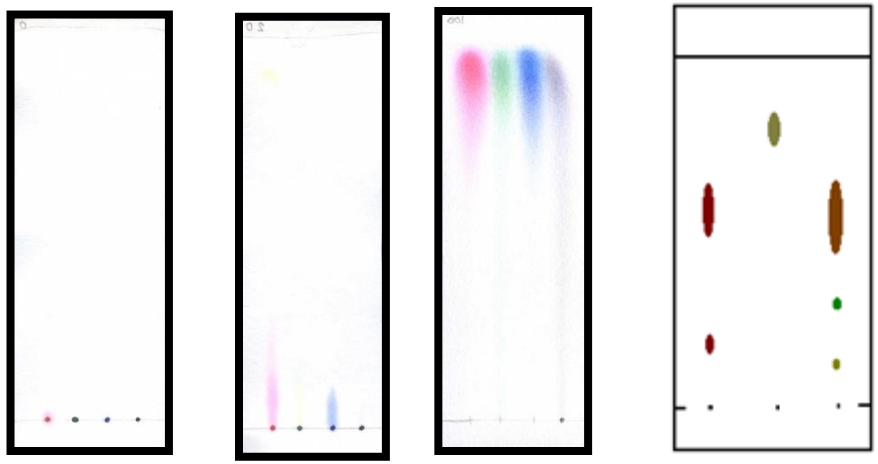






Thin Layer Chromatography-contd.

- Visualizing spots:



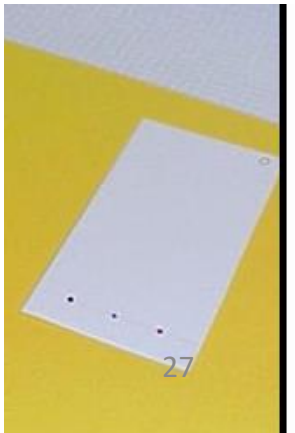
✓self colored

✓UV detection

✓Iodine detection

✓visualizing solutions: staining reagents

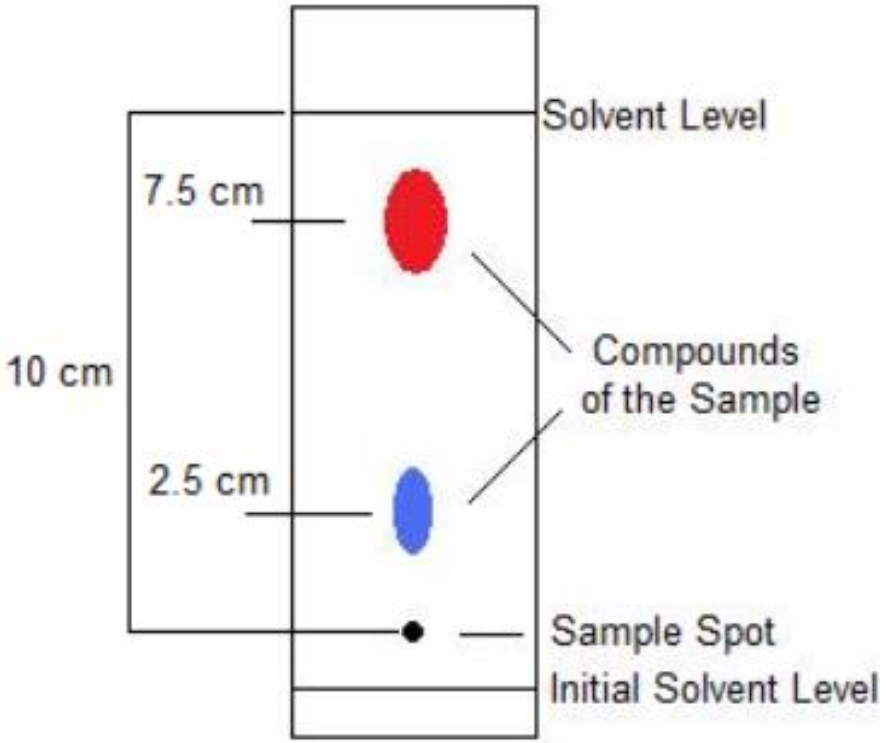
- R_f: Retardation Factor: $0<R_f<1$:what is ideal range for R_f?



Staining Reagents in TLC

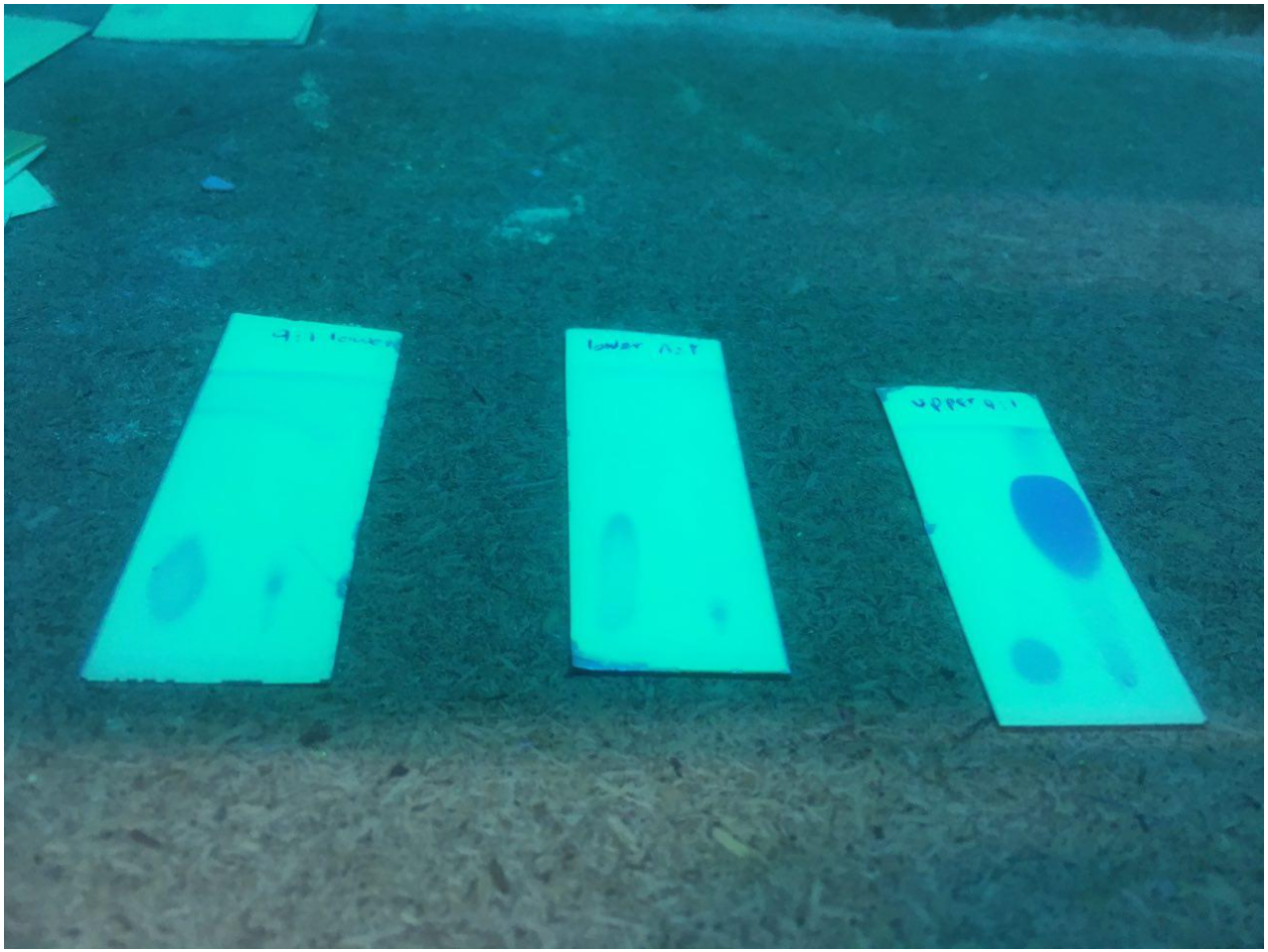
- Ferric Chloride
- Morin Hydrate
- Ninhydrin
- Di-nitro-phenyl-hydrazine (DNP)
- Vanillin
- Potassium permanganate: $KMnO_4$

TLC Characterization & Report: R_f



$$Rf = \frac{Migration\ Distance\ of\ Substance}{Migration\ Distance\ of\ Solvent\ Front}$$

TLC Run Results Using UV as Visualizer



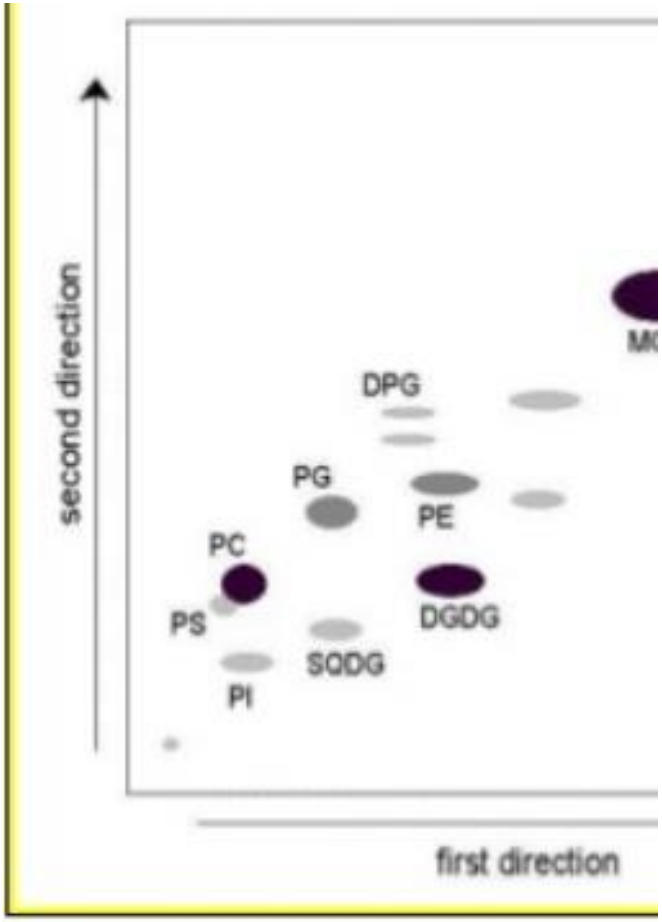
Application of TLC

- Analytical separation
- To detect synthesis reaction progress
- To detect purity of a sample
- To detect number of components in a sample
- To identify organic compounds comparing to control
- For samples which are not good candidate for other types of chromatography: why?
- One dimension or two dimension

Advantage of TLC Run

- Simple
- Fast
- easy
- The least expensive

Running 2-D TLC



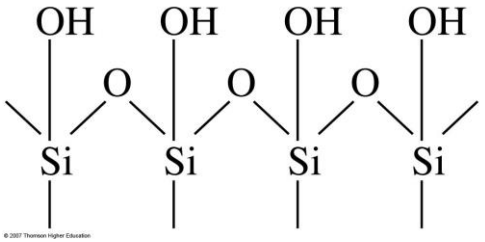
HPTLC:
High Performance TLC

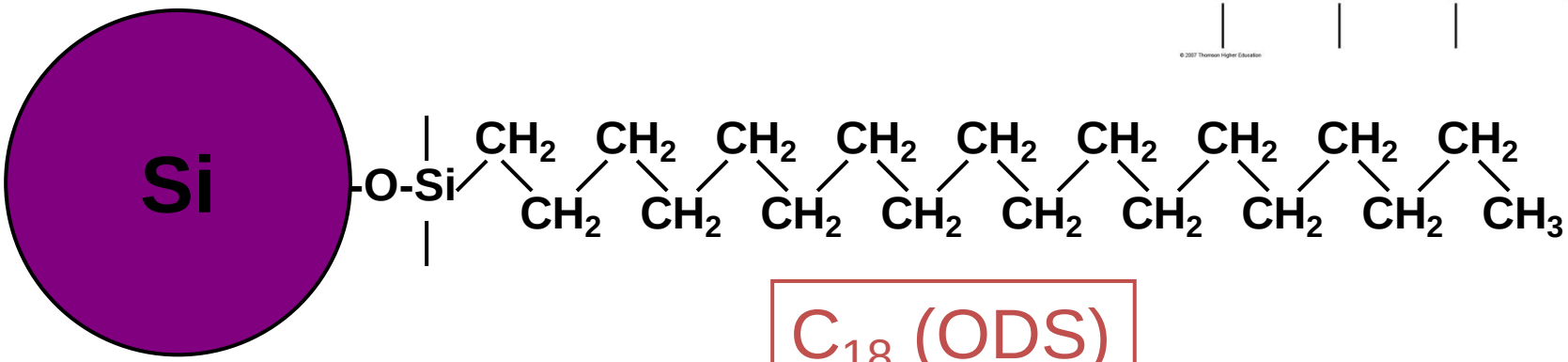
HPTLC characteristics:

- ➢ Stationary phase:
  - ✓ silica (polar phase): SiO_2, $(-Si-O-Si-OH)_n$: strongly polar
  - ✓ or
  - ✓ alkylated silica: non-polar (C8;C18)
- Solid support: plastic polymer, aluminum layer

- ➢ Mobile phase: solvent system
- ➢ Sample loading: using sampling instrument & needle
- Reporting items
- Application

Alkylated Silica





C_{18} (ODS)

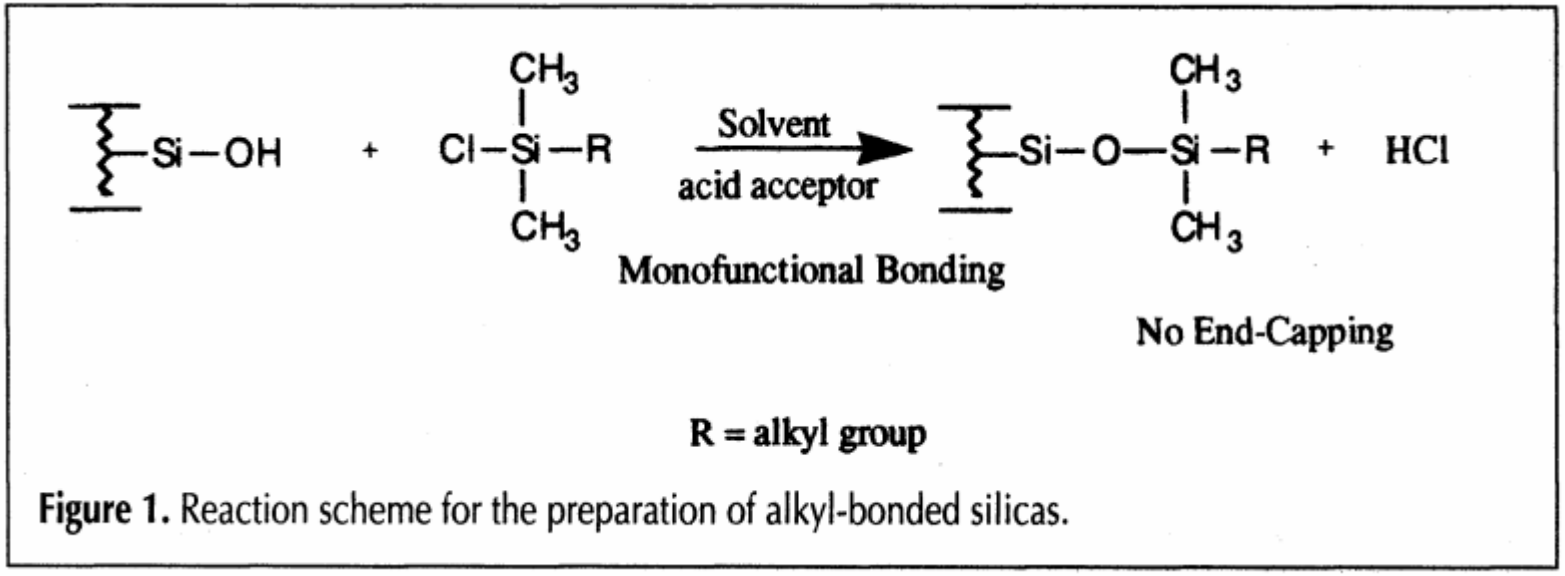
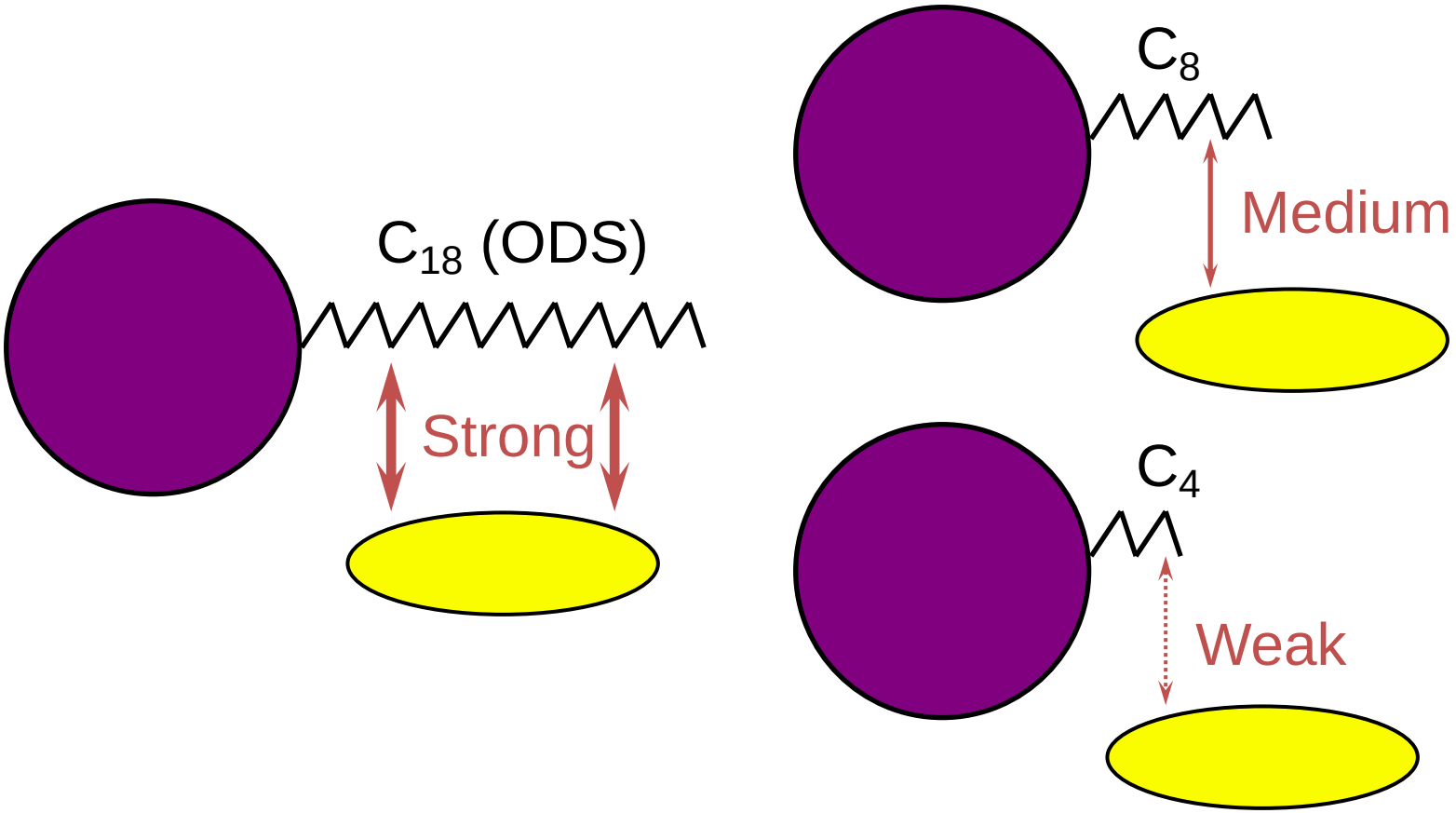


Figure 1. Reaction scheme for the preparation of alkyl-bonded silicas.

Journal of Chromatographic Science, Vol. 34, March 1996

Strength of Interaction of Components of Sample to Stationary Phase



HPTLC Sheets

- Glass ~~or aluminum~~ backing surface
- Optimized silica particle size: 5-6 micron
- Layer thickness: 100 or 200 micron

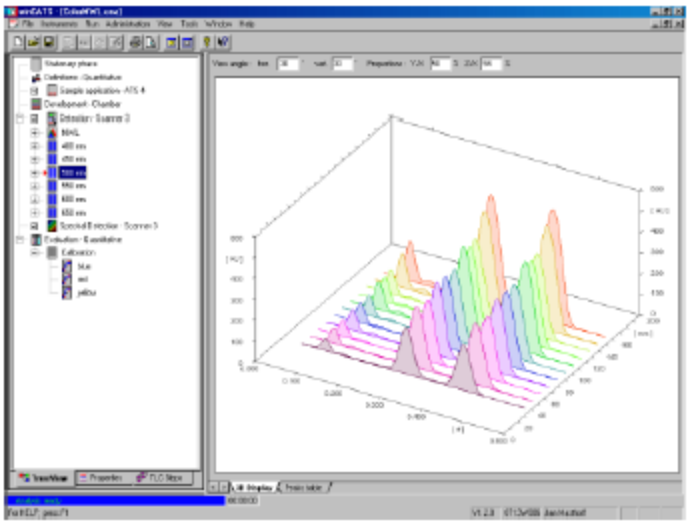
Sampling Tools

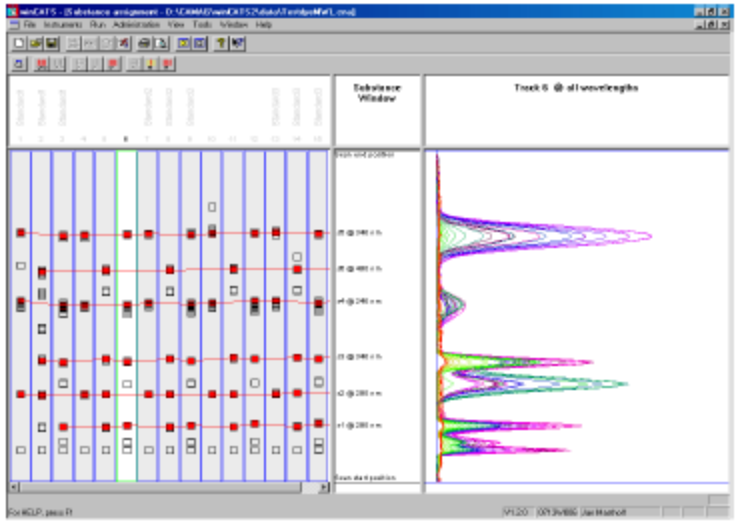
- For manual sample loading

- Chambers to support silica sheets

HPTLC Tools

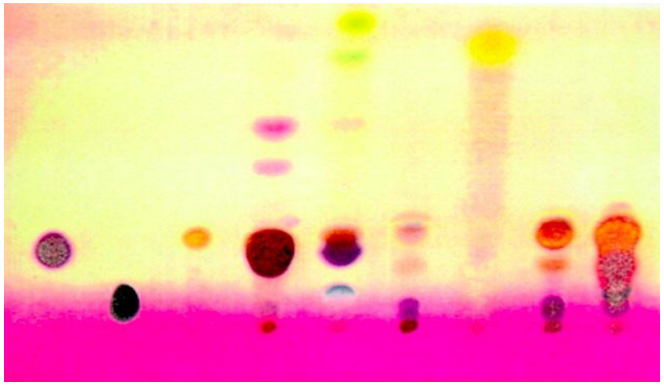
HPTLC Output File



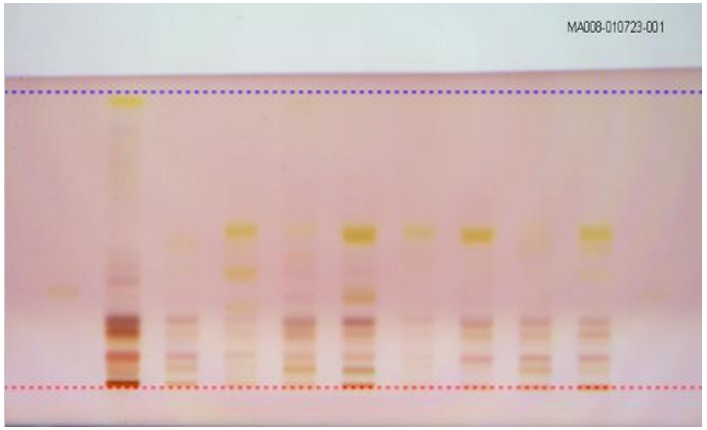


HPTLC Run Result

TLC



HPTLC



	TLC	HPTLC
Plate particle size:	10 - 25 µm	5 - 7 µm
Separation distance:	100 - 150 mm	60 mm
Development time:	30 - 200 min	3 - 20 min
Application:	manual	automated/semi-automated
Development:	manual	automated
Derivatization:	spraying	dipping
Analysis data:	no documentation	fully documented
Quantitative analysis:	no	yes
Environment:	no control	no problems
Resolution:	often poor	very good
Procedure:	flexible	fully standardized
Reproducibility:	impossible	highly attainable
cGMP Compliant:	usually not	YES!!

HPTLC

Comparative Characteristics:

- ✓ Fast, automated and sophisticated
- ✓ increased sensitivity; and resolution
- ✓ Lower amounts of mobile phase;
- ✓ Generate & evaluate digital images:
- ✓ Densitometry: up to 31 wavelengths
- ✓ Reproducible
- ✓ Quantitative: more accurate
- ✓ Efficient data acquisition & processing: imaging & documentation
- ✓ Parallel analysis: more than one sample / standard
- ✓ Can be run in two dimensions
- ✓ Direct biologic test on HPTLC sheets
- ✓ In identification of: hydrocarbons, alcohols, phenols, carbohydrates, organic acids, lipids, steroids, saponins, alkaloids, amino acids, peptides, proteins, enzymes, nucleic acids, vitamins, antibiotics, pesticides

	HPTLC	TLC
Efficiency	High due to smaller particle size generated of silica gel With more sensitivity	Less
Separations	3 – 5 cm	10 – 15 cm
Detectors	Use of UV/visible, fluorescence. Scanner is an advanced type of densitometer	Scanning Not possible
Sample spotting	Auto sampler (need less amount of sample)	Manual spotting
Analysis time	Shorter migration distance and the analysis time is greatly reduced	slower
Solid support	Wide choice of st. phases like silica gel for normal ph. and C8,C18 for reverse ph. modes	Silica gel, alumina
Development chamber	Less amount of mobile phase	More amount
Reporting items	Quantitative and qualitative result	R_f

Plate Chromatography

- Stationary phase:

✓ silica (polar phase): SiO_2, $H(-O-Si-O-Si-O)_nH$: strongly polar

✓ solid support: glass: 20*20 or 25*25cm

✓ depth of layer: 1-2 mm

✓ almost thick coating for preparative work (greater solute capacity)

- Mobile phase: tank solvent system miscible solvent mixtures

Polarity gradient; solvent with similar/non-similar functional groups;

- Sample loading via scraping/aspirating into Pasteur pipette;

Using spreader to coat the plates by slurry

- Reporting items
- Application

Items to Run Plate C.

1. Preparation of silica plates:
homogenous coating silica suspension on plate
through pouring/spraying; then drying and finally activation
2. Preparation of sample solution:
dissolving sample in suitable solvent
3. Loading sample solution on silica plates:
as continuous sample spot in a right line
4. Selection of optimum mobile phase
preparation of solvent tank
5. Run plate
6. Stop solvent run at suitable solvent level: dry plates
7. Visualize the target component using UV lamp
8. Scratch the target fraction of silica from plate
9. Add suitable solvent to scratched fraction: mix for a while to dissolve component in solvent
10. Filtrate the obtained suspension
11. Evaporate the filtered solution

Plate Chromatography- Contd.

- Visualizing spots:

self colored; UV detection;

~~iodine detection~~; ~~visualizing solutions~~

Spectro-densitometry: an automated method

- R_f: Retardation factor: $0 < R_f < 1$:

what is ideal range for R_f ?

Plate Run Results Under UV Lamp

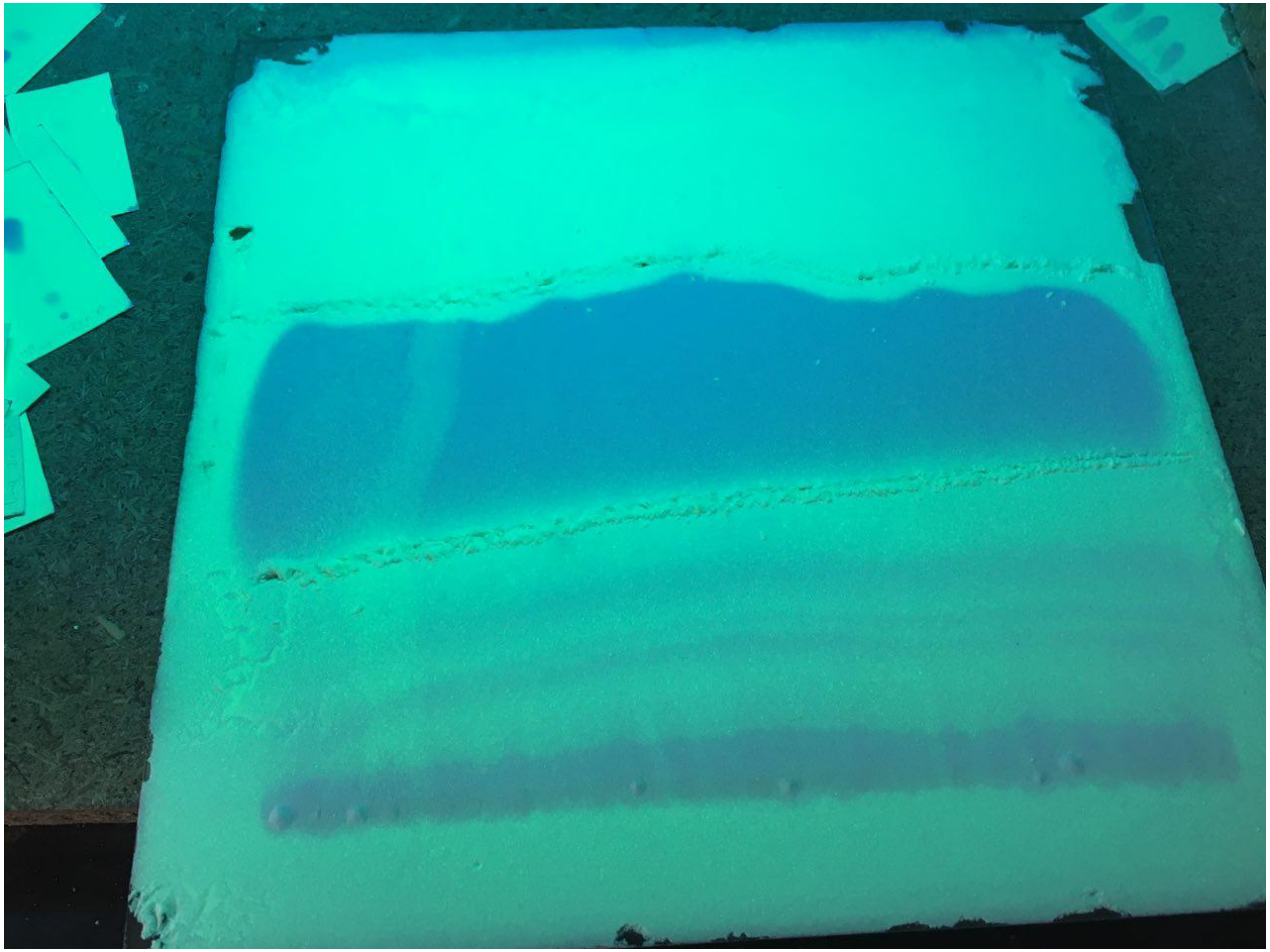
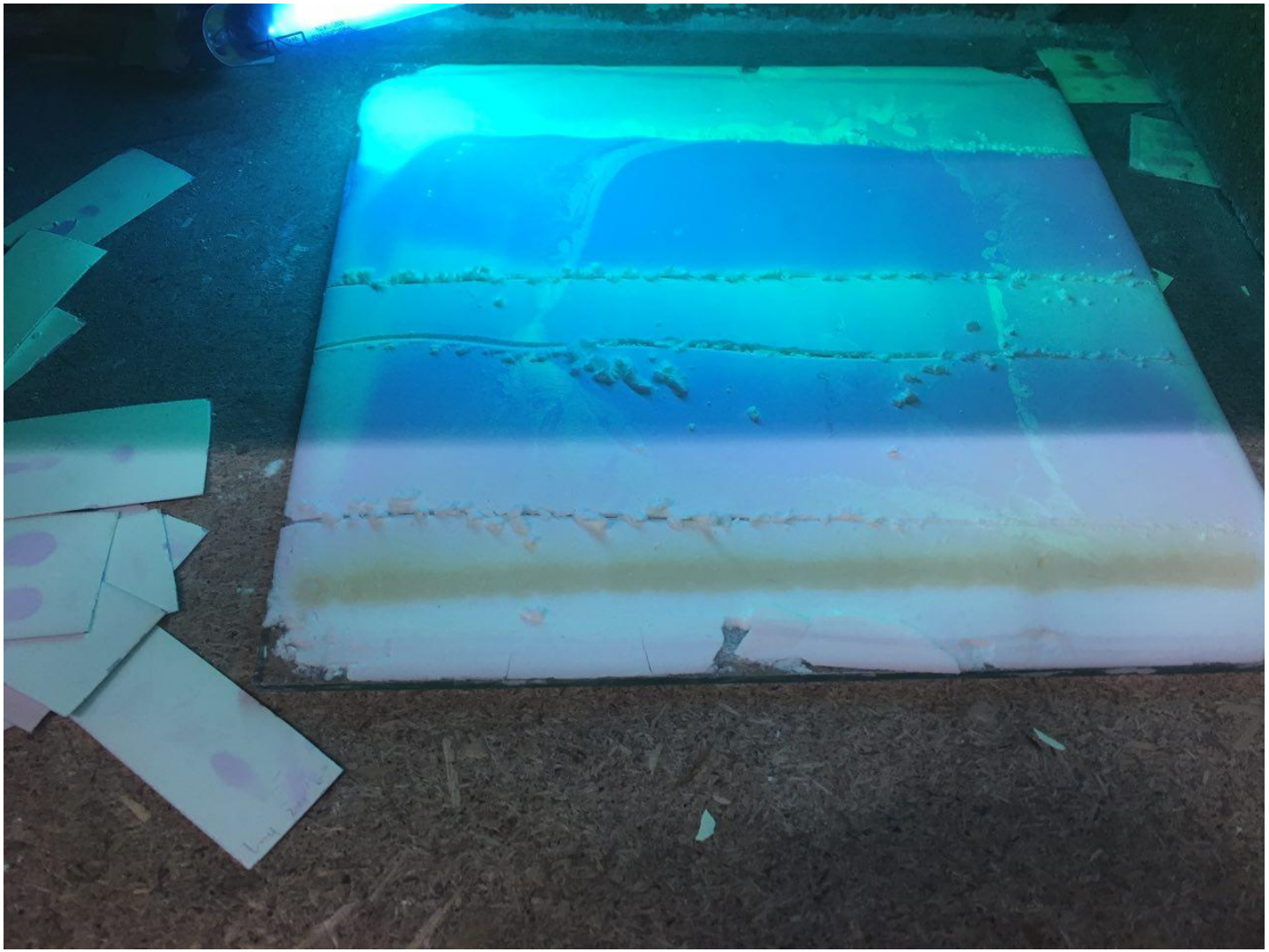


Plate Run Results Under UV Lamp



Application of Plate Chromatography

- Preparative separation
- To separate synthesis reaction products
- To separate impurities of a sample
- To separate components in a sample

Column Chromatography

➢ Stationary phase:

silica (polar phase): SiO_2, $H(-O-Si-O-Si-O)_nH$: strongly polar

10-50 mm in diameter, 5-100 cm in length;

- Stopcock or a type of flow restrictor
- Packing: Dry powder or slurry suspension

Positive pressure / vacuum or tamping rod

➢ Mobile phase:

Eluent: gradient: from non-polar to polar range

✓ Sample loading:

direct

Or

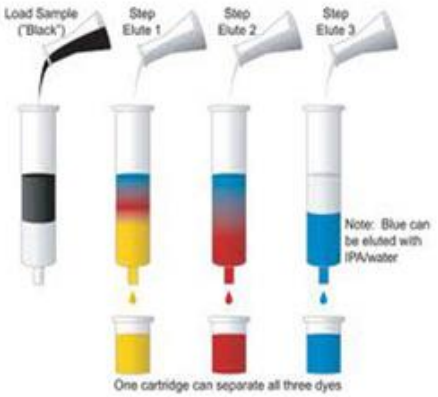
mixed with small portion of stationary phase ;

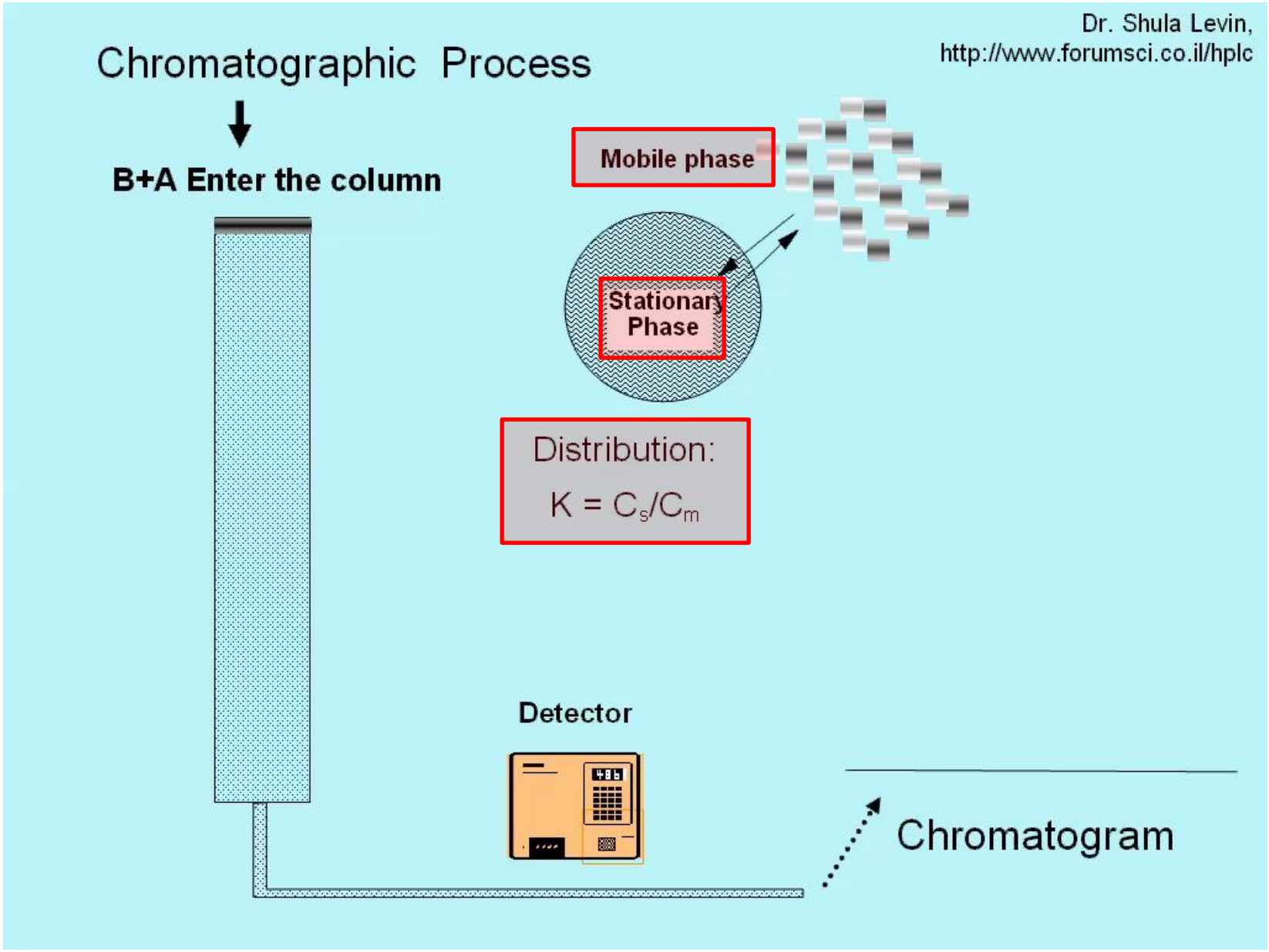
or

dissolved in the minimum volume of starting mobile phase

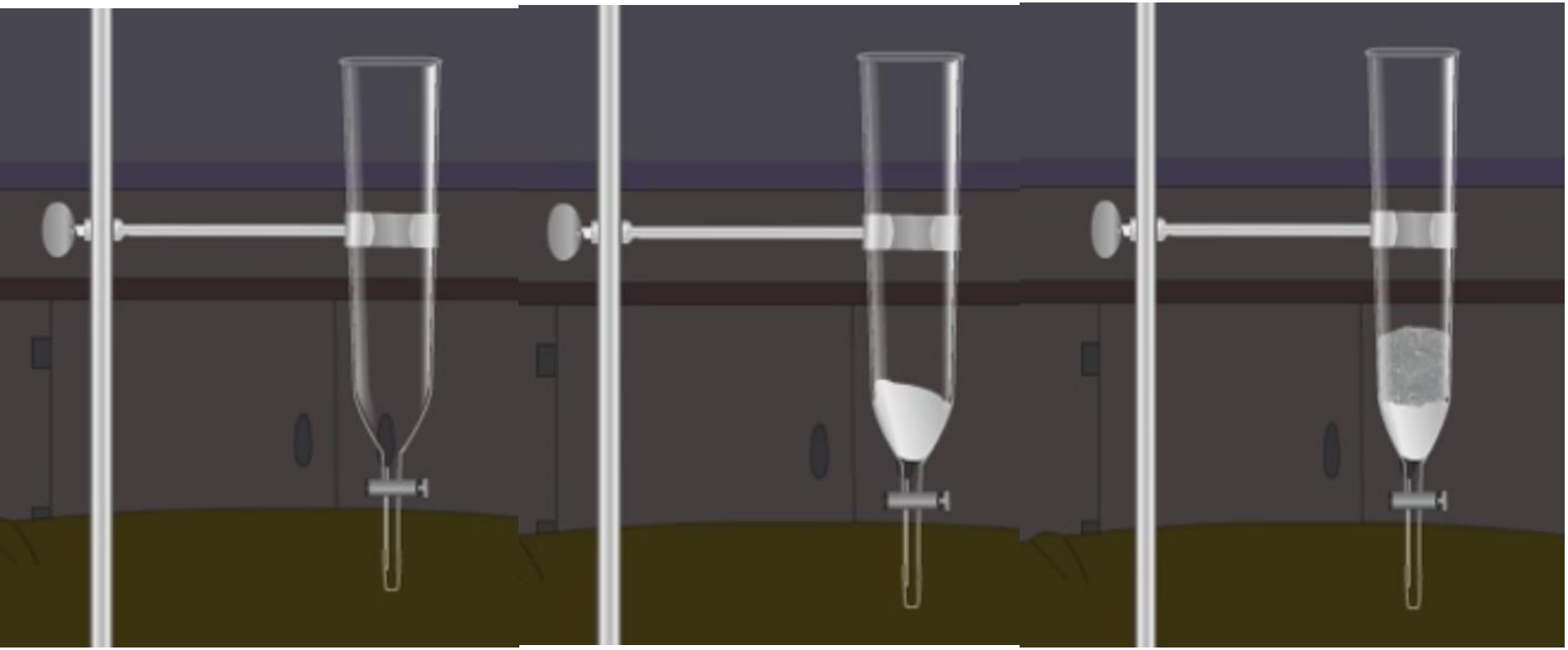
✓ Reporting items

✓ Application

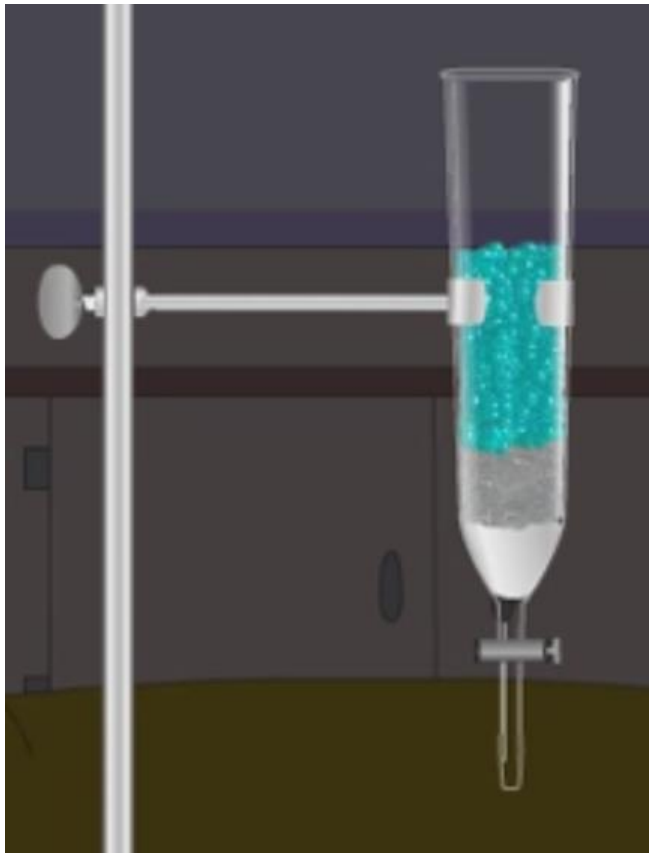


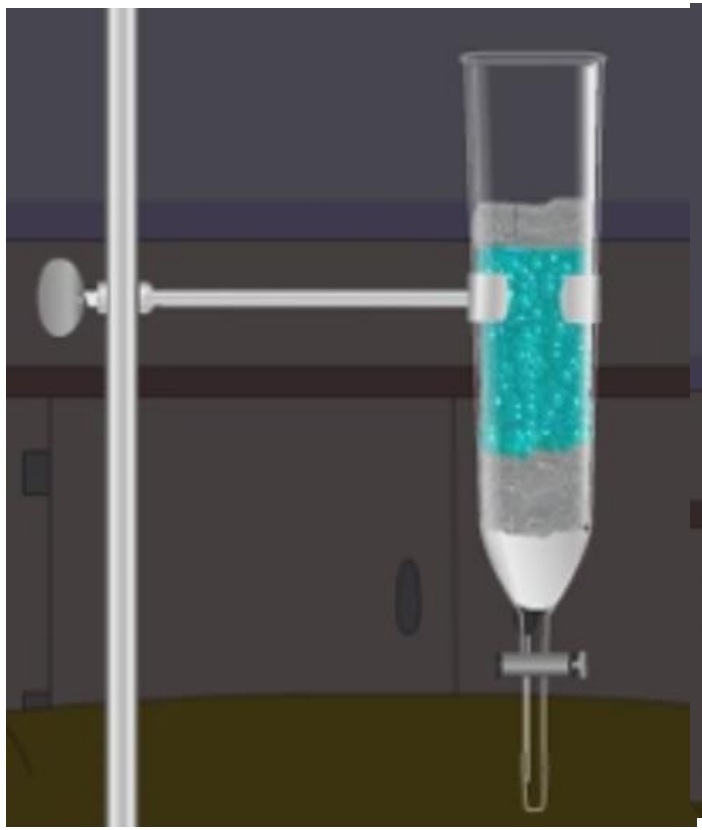

Chromatographic Process
Dr. Shula Levin,
http://www.forumsci.co.il/hplc
B+A Enter the column
Mobile phase
Stationary Phase
Distribution:
K = Cs/Cm
Detector
Chromatogram

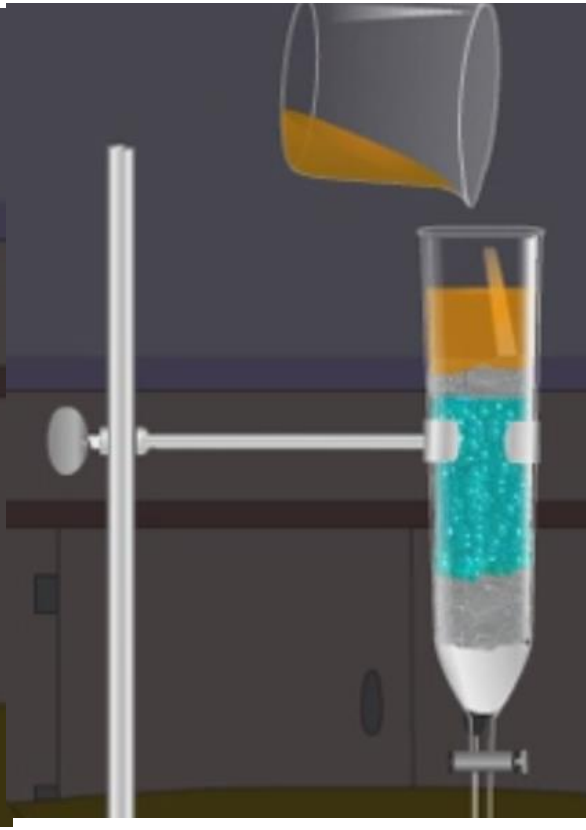
Steps to Run Column C.



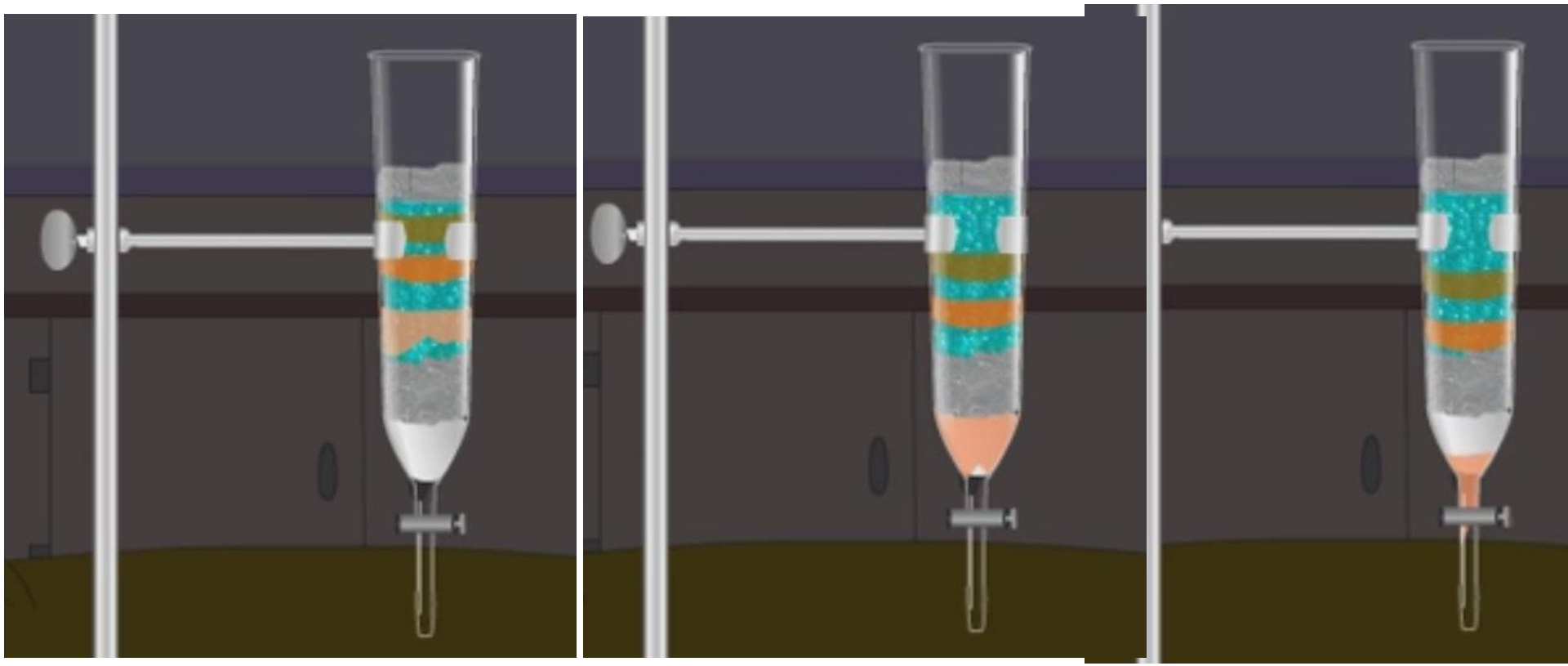
Steps to Run Column C.- Contd.







Steps to Run Column C.- Contd.



Column Chromatography

- Fraction number
- Preparative separation

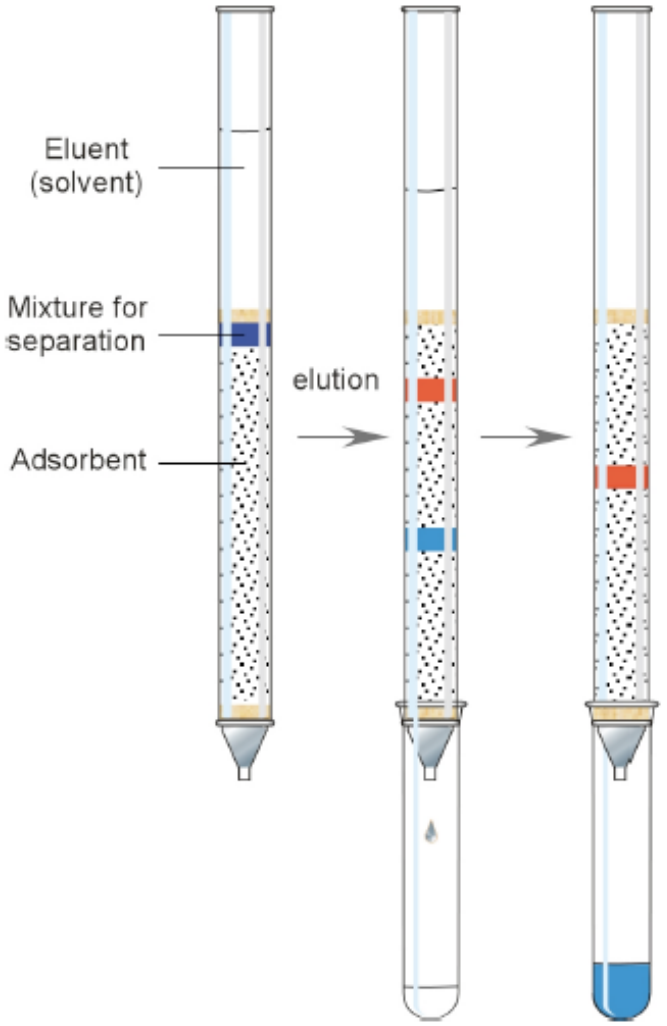
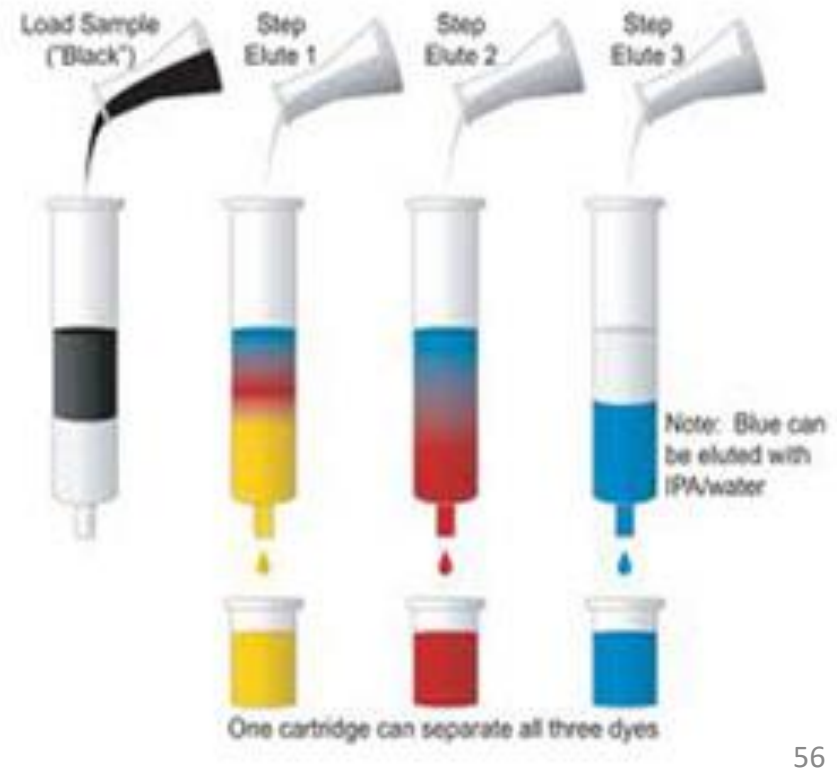


Figure 4: Separation of mixture of compounds by column chromatography.

Flash Column Chromatography

- What is flash chromatography?



Instrumental
Flash Column Chromatography