CHROMATOGRAPHY

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One small positive thought in the morning can change your whole entire day.

Introduction

*(Greek = **chroma** "**color**" and **graphein** "**writing**")*
Michael Tswett *named this new technique as chromatography. Separation is based on* ***Color.***

- ***It 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.***

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

Milestones in Chromatography

* ***Invented by Mikhail Tswett (1903) –***
  plant pigments chlorophylls, xanthophylls and carotenoids.
  separated on chalk ($CaCO_3$) columns by ***Pet ether***
* ***1931*** *Lederer & Kuhn -* ***LC of carotenoids***
* ***1938*** *TLC and ion exchange*
* ***1950*** *reverse phase LC*
* *Studied in detail by Martin & Synge*
  1954 (Noble Prize)
* ***1959*** *Gel permeation (type of SEC) for polymers*
* ***1965*** *instrumental LC*
* ***12 Nobel prizes*** *were awarded between 1937 to 1972 alone for Chrmatography.*

Purpose & Advantages

Purpose:

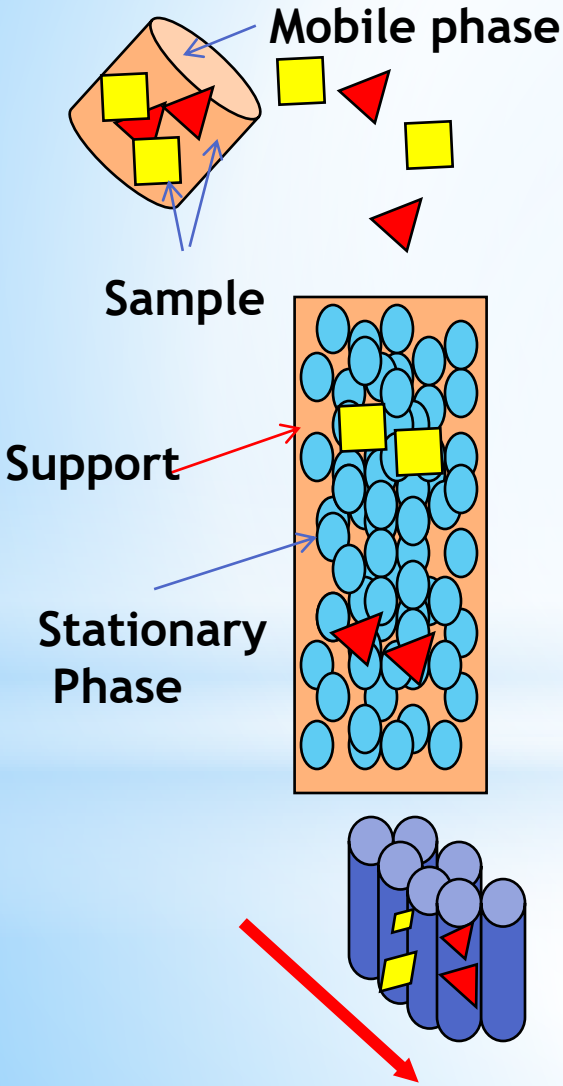
- ***Separation & Detection of components from colored, colorless, simple & mixtures of sample.***
- ***Purification & collection***

Advantages:

1. *Chemically Similar & Physically Closely resembling samples can be separated*
2. *Separation is due to diff. in physical properties at R.T. or below.*
3. *Sensitive, micro & picogram sample can be separated & detected. i.e. Versatile technique.*
4. *Simple, rapid & eligant(stylish) method, so complex mixture can be separated & detected*
5. *Simple & inexpensive equipment.*

Principle of Chromatography

Components of mixture subjected to two opposing forces one by stationary & other by mobile



- Mixture placed in a system
- Mobile phase percolates through interstices or on the stationary phase
- Mobile phase exerts driving force on solutes of mixture & tend to carry them alongwith itself due to solvation power.
- Simultaneously stationary phase tends to retain solutes by them due to adsorption power.
- But extent of these two forces are different.
- Therefore component distribute between stationary & mobile phase.

$$\text{Distribution Coefficient } k = \frac{\text{Conc. of Solute in Stationary Phase}}{\text{Conc. of Solute in Mobile Phase}}$$

Higher K , Greater opposing force , lesser movement of component & vice versa

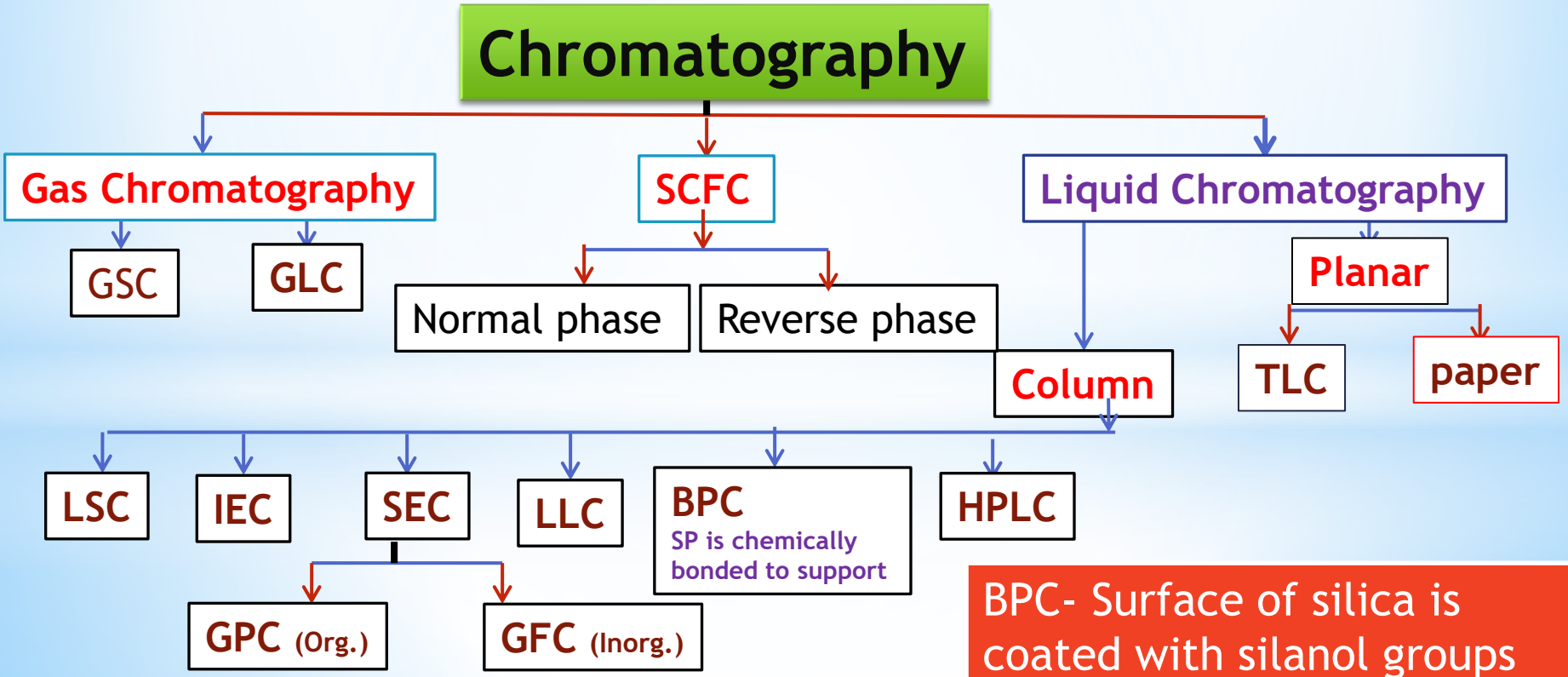
https://www.youtube.com/watch?v=0m8bWKHmRMM

Classification of Chromatography

Based on nature of forces operating:

1. Adsorption - Surface active
2. Partition - Distribution
3. Ion exchange - Ion exchange
4. Affinity - Ligand attractive
5. Permiation(Exclusion) - Molecular sieving

Classification based on Stationary & Mobile phase



BPC- Surface of silica is coated with silanol groups

"दुनिया में कोई काम "
impossible" नहीं
बस होसला और
मेहनत की जरूरत है

"Impossible" को
गौर से देखो वो
खुद कहता है
""I m possible

Paper Chromatography

- ***Liquid-Liquid type Chromatography***
- *Paper provides mechanical support to stationary liquid phase*
- ***Principle:*** *Compound partitioning itself between two liquid phases*
- ***Stationary Phase****: Paper absorbs & hold water*
- ***Mobile Phase:*** *Non- aqueous Solvent*
- *Mobile phase travels along the paper, the components of loaded mixture distributes themselves between two phases in a ratio, characteristic of their distribution coefficient.*

- **Component more soluble in stationary phase move slowly.**
- Component more soluble in mobile phase move faster with mobile phase.
- As a result of differential movement they get distributed.

$$\text{Distribution Coefficient } k' = \frac{\text{Conc. Of Solute in Stationary Phase}}{\text{Conc. Of Solute in Mobile Phase}}$$

Only those components which differ in their K value under the given set of conditions are separated

Important Aspects of paper Chromatography

Quality of separation & purity of sample depends on

1) ***Filter Paper:*** *Different Running character (Slow, medium, fast).*

- ***Stationary Phase:***

Cellulose fibers of paper absorbs water & hold it strongly.
water content range:115-195 %
Aqueous phase is held so strongly that , under normal conditions it is impossible to remove.

- ***Special Case:***

For separation of fatty acids, lipids
stationary phase - organic solvent. mobile phase - water
Whatman paper No. 1 is used : uniform flow rate, grain size, texture.
Sometimes papers are acid washed to remove tracer impurity.

2) Solvent System: ***Prepared by saturating organic solvent with water.***
But several organic solvent incorporate very small amount of water
So, polar components of mixture fail to separate in such binary system.
Therefore, HCl, CH_3COOH,HCOOH, NH_3 , Pyridine & complexing agents, etc used.
it helps to retain more water in organic solvents.
Generally three component system is used.

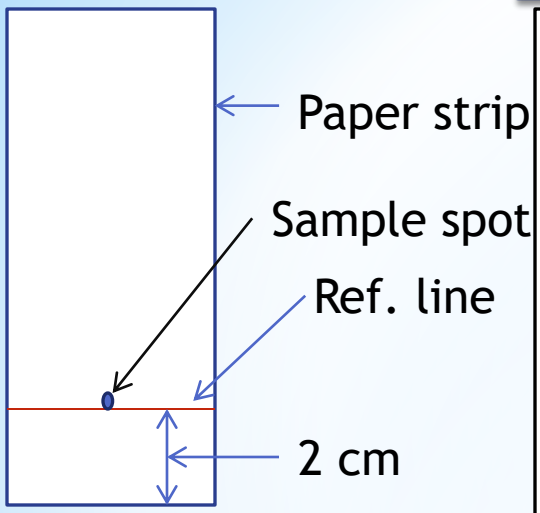
3) Spraying Reagent: *To locate colorless components by spraying reagent.*

These reagent reacts with component & develop color in region where they are present.

Commonly used Solvent systems & Spraying reagents:

Sr. No.	Compounds	Solvent System	Spraying Reagent
1.	Amino acids	Butanol:Pyridine:water(33:33:33) Methanol :Pyridine: water(25:12:63) Phenol: Water(80:20)	Ninhydrin
2.	Carbohydrates	Butanol: acetic acid: water 2-Propanol:pyridine:water:CH_3COOH (8:8:4:1)	Ammo. $AgNO_3$ Alk. $KMnO_4$ Aniline, diphenylamine
3.	Chromophylls & Carotenoids	Propanol: Pet ether	Self
4.	Metal ions	Ethanol: HCl (90:10)	Rubenic acid, NH_3

Methodology



Three unit operation

1) Sample loading:

- F.P. of suitable dimension is chosen and saturated with solvent vapour.
- **Ref. line at 2 cm distance from edge of paper.**
- Sample is loaded repeatedly as fine spot (5 mm) over reference line.

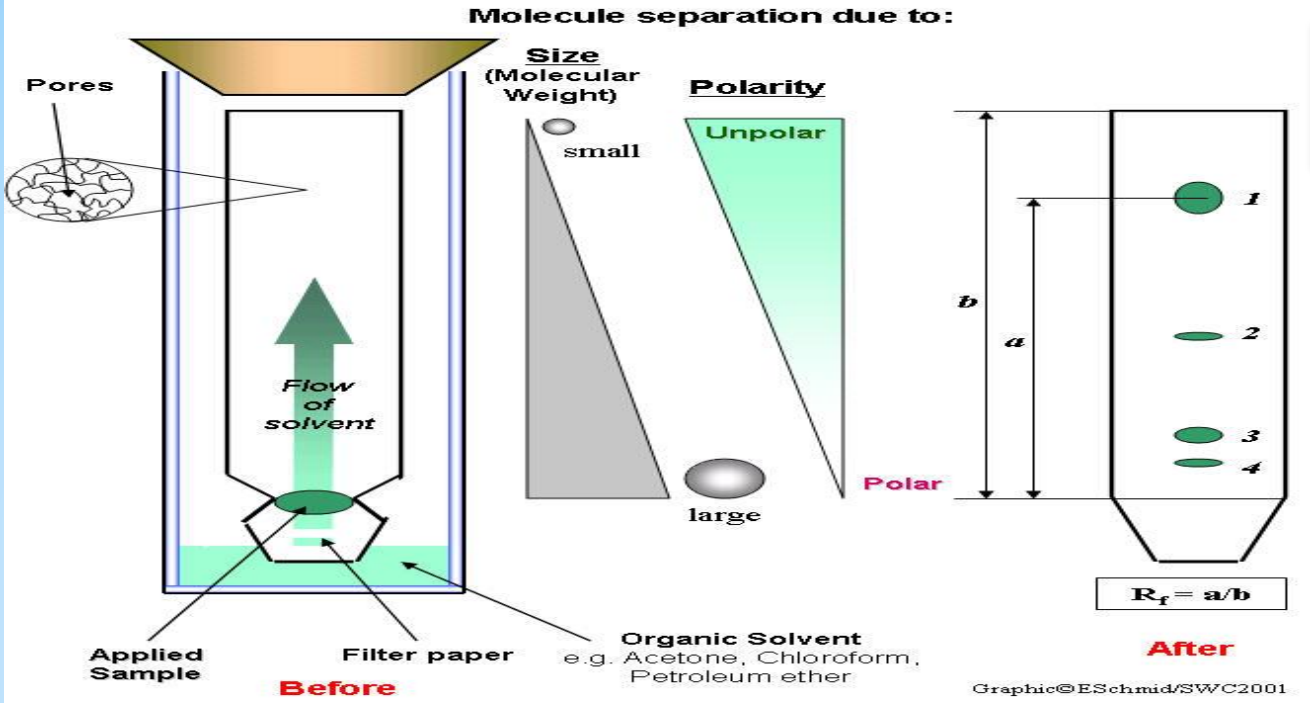
2) Developing: **i) saturation of Sample loaded strip in chromatographic jar containing solvent but without dipping (to saturate paper with solvent vapour)**
ii) Now strip is developed by running solvent through paper.

Types:

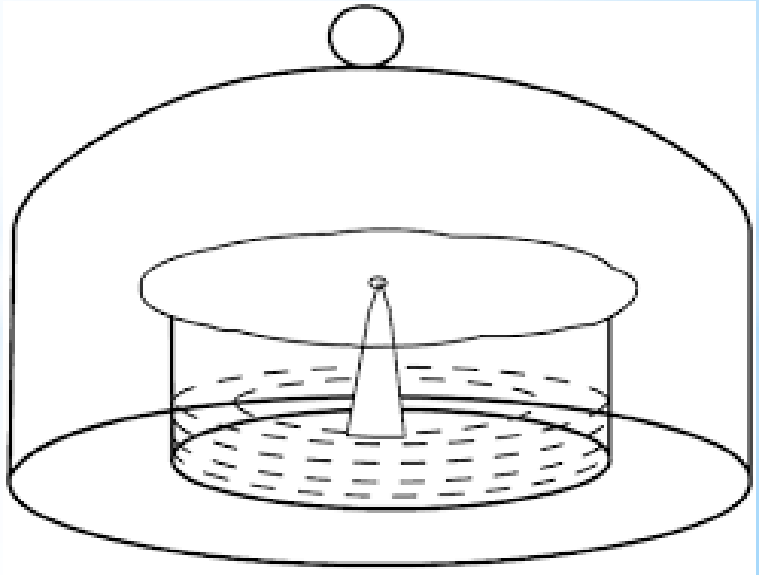
a) **Ascending:** Solvent moves upward by capillary action
b) **Descending:** Solvent moves downward. Spotted end is at top. faster flow.
c) **Radial or Circular:** plane of paper kept horizontally & solvent moves laterally from centre.

In preparative paper chromatography sample recovered by two method Elution or extraction

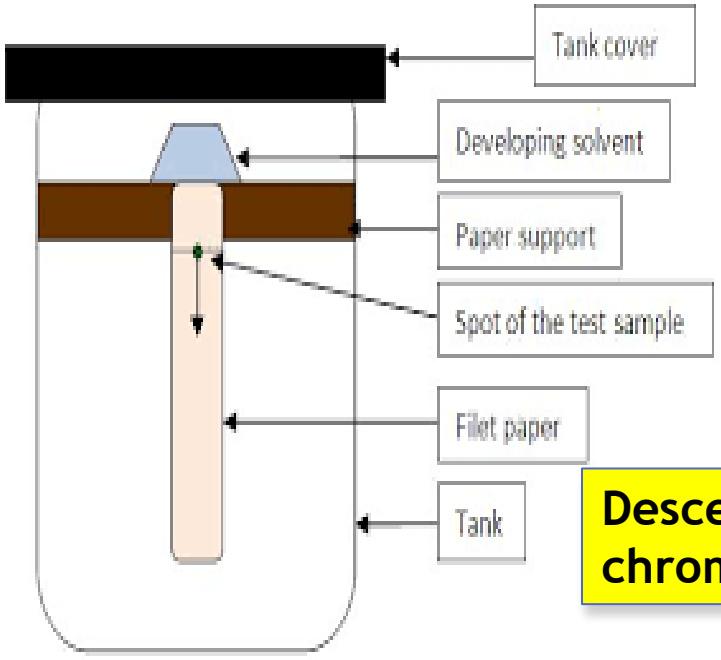
Ascending chromatography



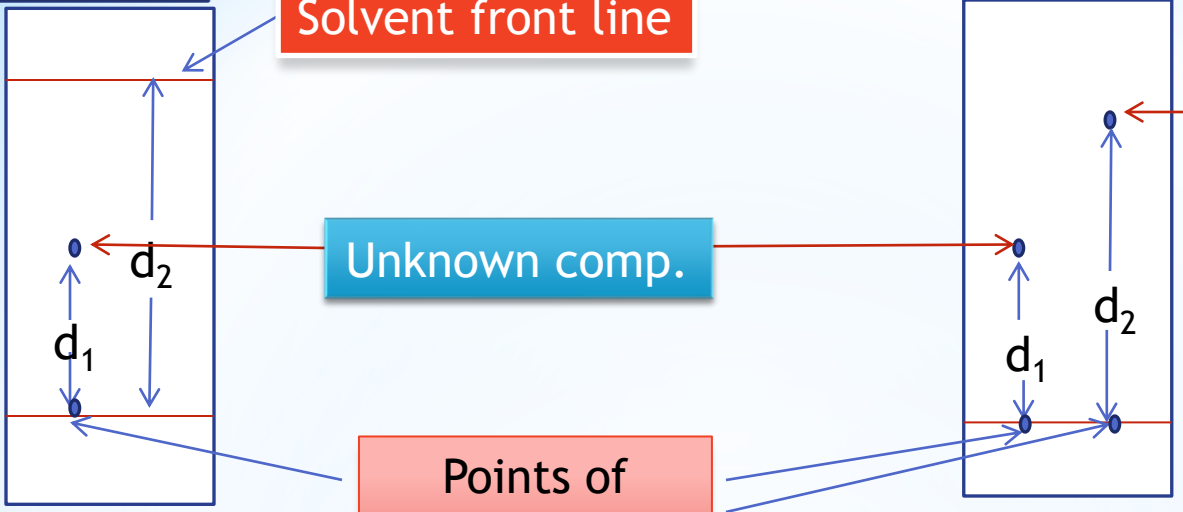
Radial flow chromatography



Descending chromatography



Rate of flow



Standard reference

When solvent needs to be run off the end of F.P. for separation, then Ref. is used.

$R_f = d_1 / d_2$

$R_x = d_1 / d_2$

Rate of flow R_f : Ratio of distance moved by solute to the distance moved by solvent front

$$R_f = \frac{\text{Distance moved by solute}}{\text{Distance moved by solvent}}$$

$$R_x = \frac{\text{Distance moved by Substance}}{\text{Distance moved by standard}}$$

R_f value of sub. Depends on:

1) *Quality and dimension of paper*
2) *Solvent system,flow rate & direction of flow.*
3) *Tempt. of environment.*
4) *Size of chromatogram developing vessel*

When these factors constant then it is possible to compare Rf values of known & unknown

Applications of Paper Chromatography

1. Separation of amino acids, peptide, alkaloids, sugar, lipid,metal ions, etc.
2. To limited extent can be used as Preparatory method.
3. To study structure & amino acid composition of proteins.
4. Analysis of blood ,urine , hemoglobin ,etc.

Advantages:
Simple , inexpensive & sensitive

Disadvantages:
Large time required
Not suitable for preparative method.

The two most powerful warriors are patience and time.

Leo Tolstoy

Spirit Science

Column Chromatography

Stationary phase is in the form of column
Liquid –solid type Chromatography

Principle:

- *Partition of solute between solid & liq. phase takesplace.*
- *The loosely held species desorbs faster & move fast to forward along with unabsorbed Species.*
- *But these species readsorbs at new location.*
- *This process of adsorption & desorption repeats continuously.*
- *As a result components which differ in their adsorption abilities migrate at different speed forming bands on the column & get separated.*

The success of adsorption chromatography depends on Adsorbent & Solvent.

- ***Adsorbents:***

-Choice depends on nature of mix. to be separated.

-Adsorption power depends on chemical nature of surface , availability of area & its pretreatment.

-By varying water content absorptive activity can be controlled.

Eg- Alumina, Charcoal, Silica gel, Sucrose, Starch, powdered cellulose, $CaCO_{3,etc.}$

- ***Solvent:***

-More & too slow eluting power solvents are not suitable.

-Eluting power should practically parallel to dielectric constant.

-Choice is on the basis of solid phase, solubility of solute

Eg- Pet-ether, ether, acetone, benzene, ester, alcohol, pyridine, AA, CCl_4***, mix. of solvent,etc.***

Methodology of Column Chromatography

1. Column Packing:

Length: Breadth (5:1 or 8:1), sintered glass disc at bottom

i) Wet Packing:

-Viscous solution of adsorbent poured steadily.

-Adsorbent particles settle down & solvent flows out

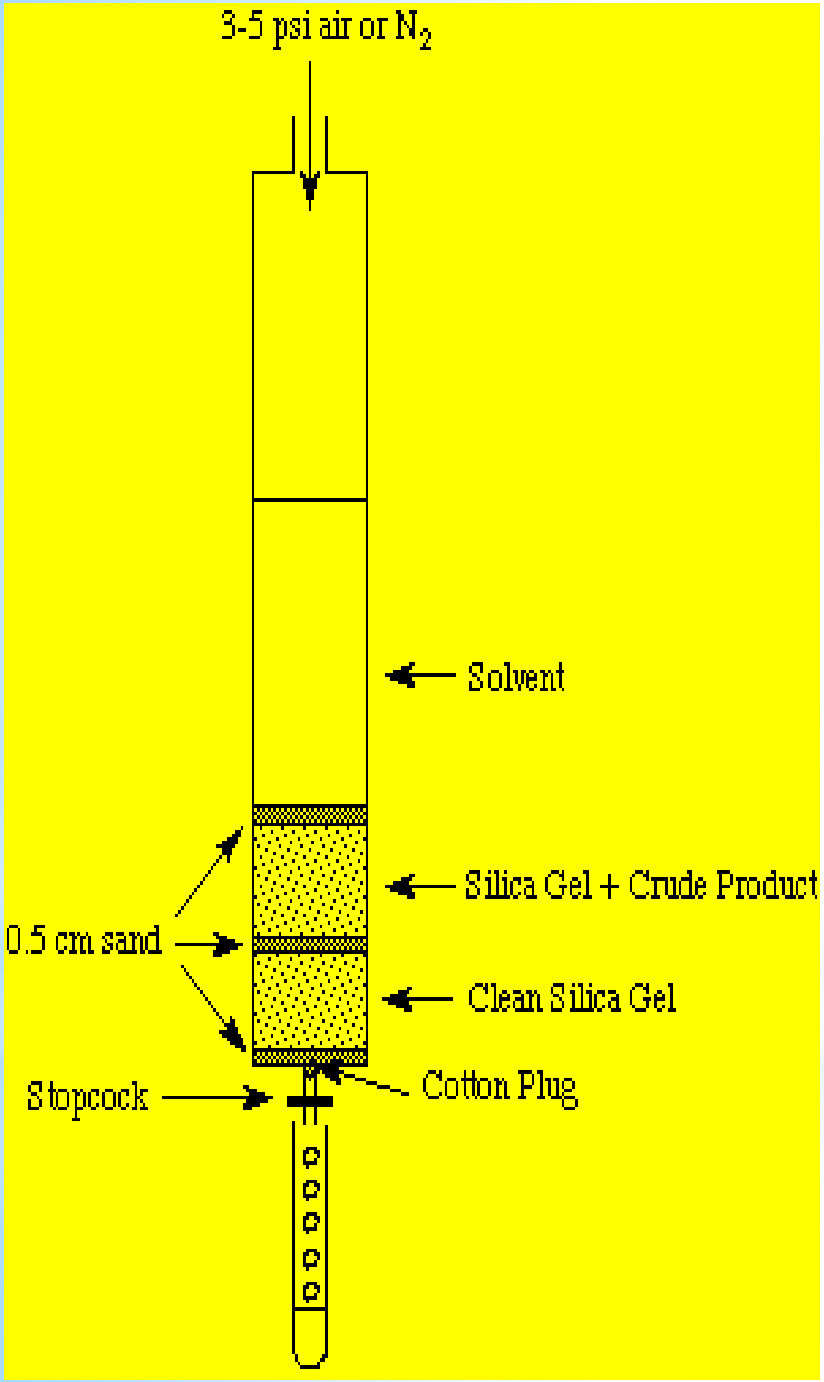
-Addition of slurry continued till desired height of column.

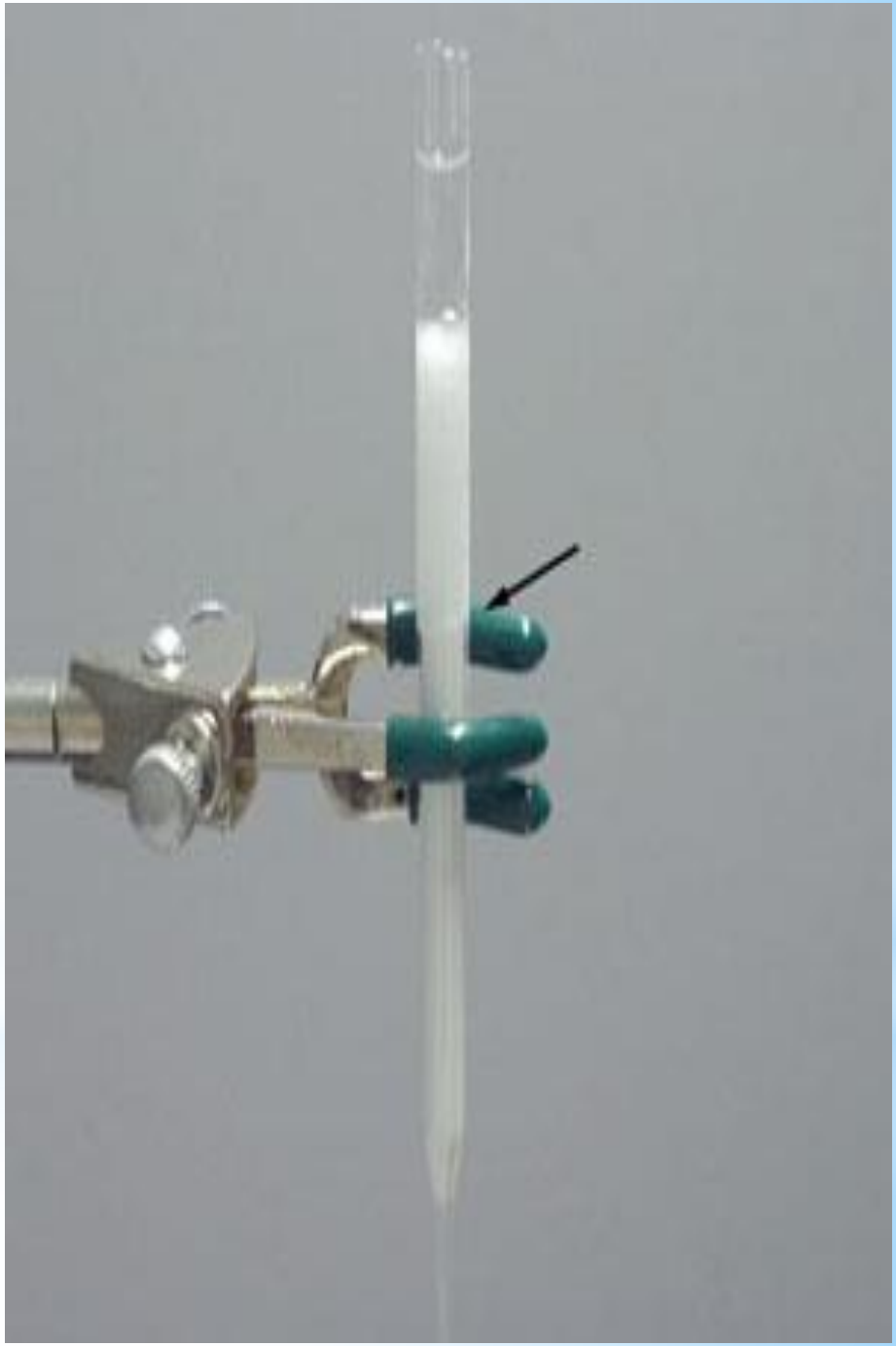
ii) Dry Packing:

-Dry powder is poured into the column with constant tapping.

-Tampering with glass rod can also done, this helps to avoid air trapping.

-Once the column is packed the liq. level is never allowed to go below packing.-


3-5 psi air or N_2
Solvent
Silica Gel + Crude Product
0.5 cm sand
Clean Silica Gel
Stopcock
Cotton Plug



2. Application of Sample:

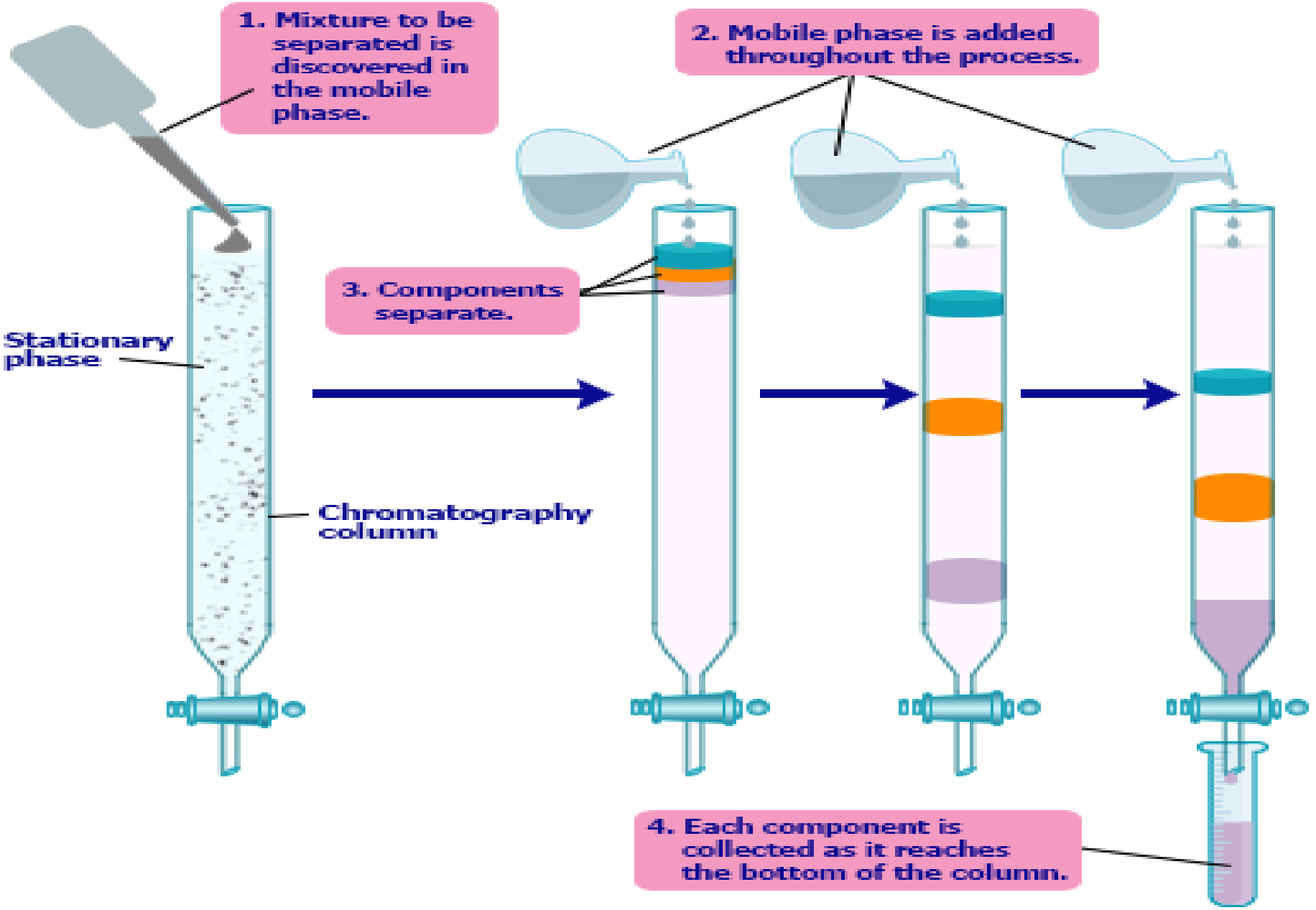
Concentrated soln of sample is prepared in more soluble solvent

1. ***Apply sample from wall of column by pipette,never poured directly over the column.***
2. ***Alternatively,*** *mix. is dissolved in volatile solvent. Then* ***one or more F.P. discs are immersed*** *in this soln & allowed to dry.*

Now, this discs are slowly placed at the top of column.

3.Development:

- *Solvent is introduced from side of column & eluent is allowed to run off column.*
- *Flow rate is adjust, so that it permitts max. separation & it must be constant.*
- *Flow rate depends on- particle size, dimension of column, viscosity of fluid.*
  i.e. flow rate is (1 cm/ min)
- *Flow is continued untill separation of all component.*


1. Mixture to be separated is discovered in the mobile phase.
2. Mobile phase is added throughout the process.
3. Components separate.
Stationary phase
Chromatography column
4. Each component is collected as it reaches the bottom of the column.

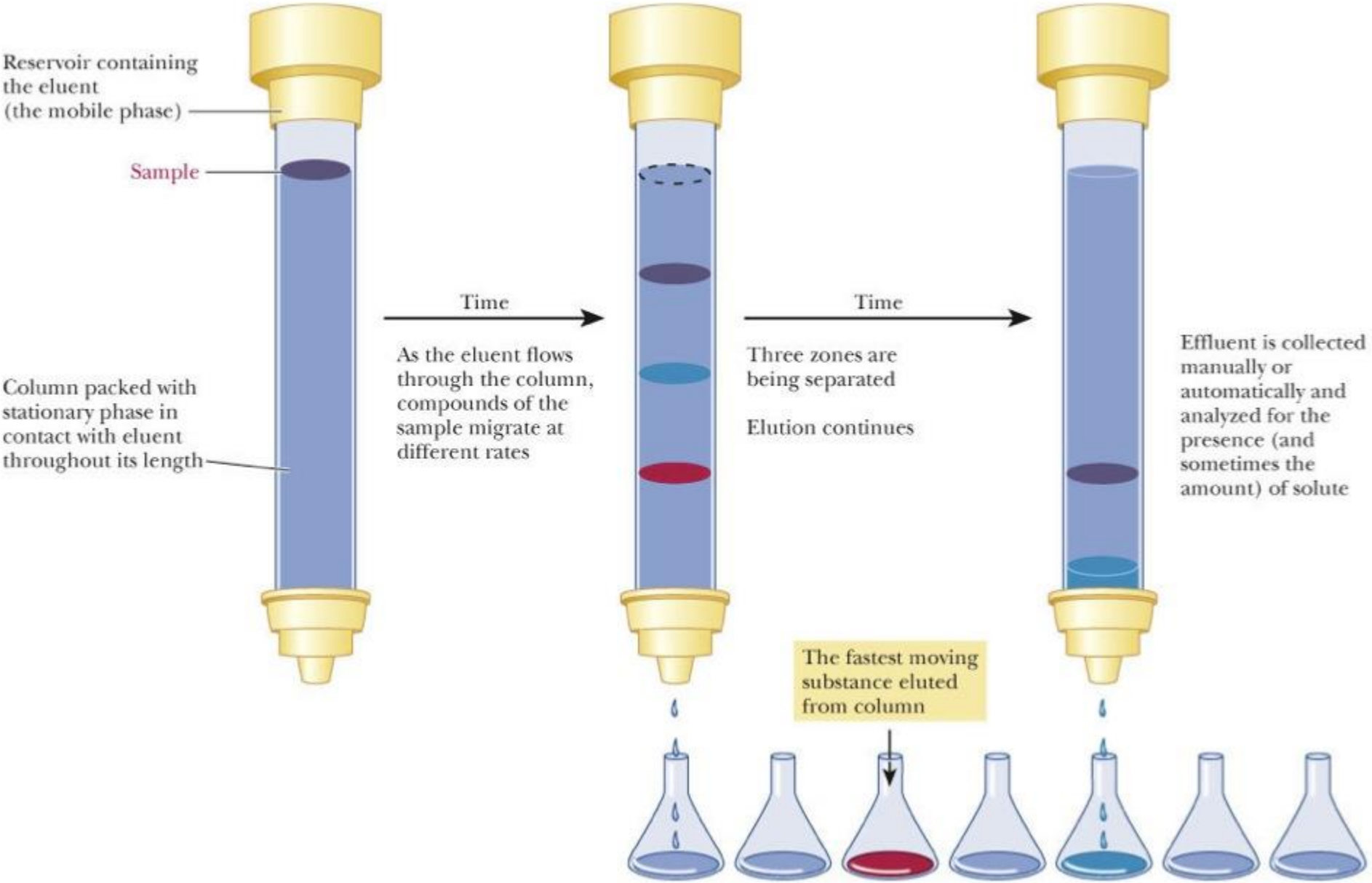


Fig. 5-2, p. 126

4. Detection Methods:

- *Coloured components can be directly detected by **visual inspection**.*
- *For colorless components no. of **optical detectors** are used.*
- *use of **color developing reagent** to indicate the position of band.*

5. Recovery of separated components:

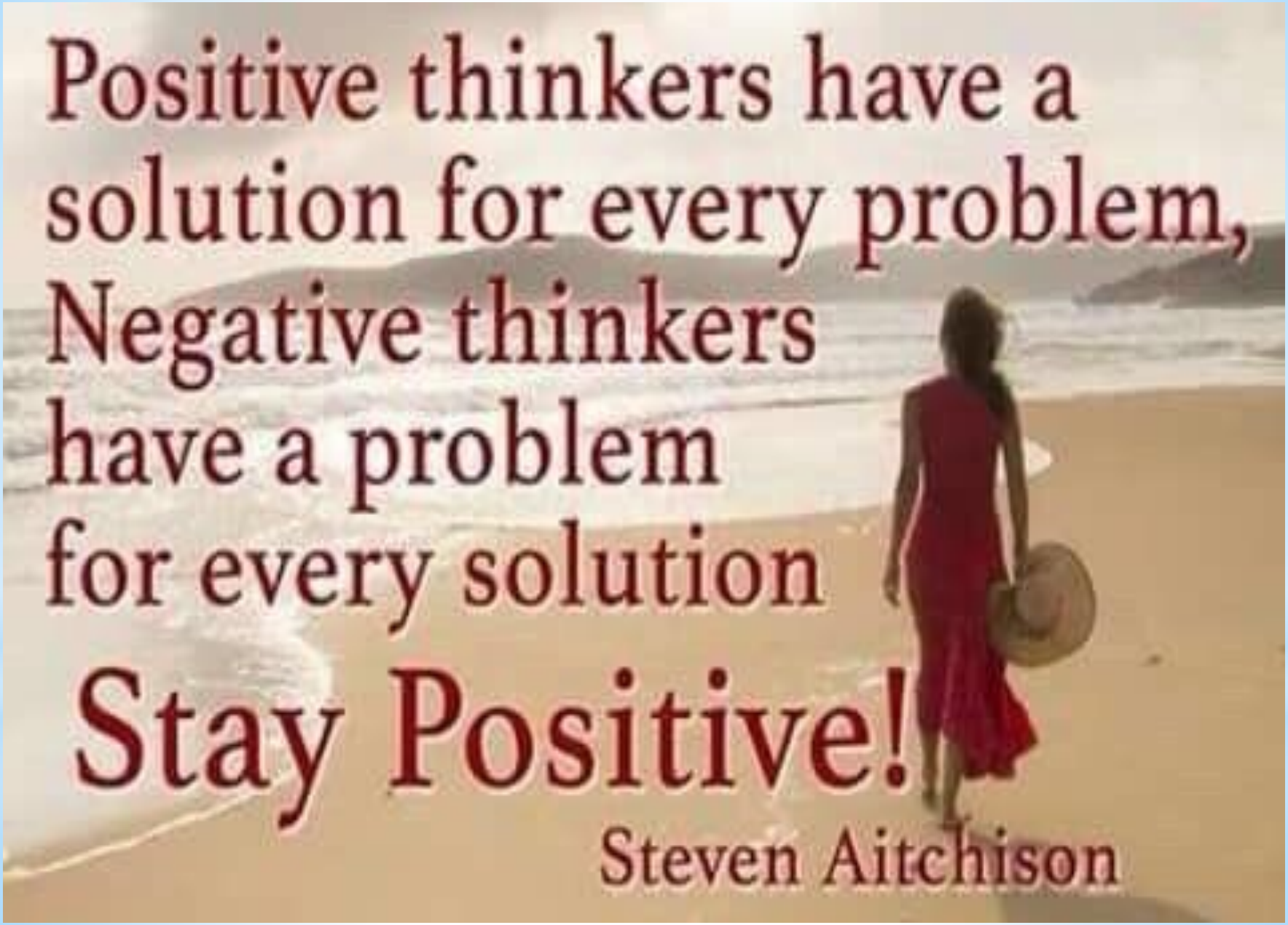
a) Elution technique:

Continuous elution of solvent & collection of fraction of eluent. Each fraction is examined separately & like fractions are collected together.

b) *Extrusion method :* *Column packing is carefully extruded & streaked with color developing reagent to indicate position of band*

Applications :

1. ***Separation of geometrical isomers***
2. ***Separation of amino acids from protein hydrolase.***
3. ***Separation of distereomers,racemates & tautomeric mixture***
4. ***Separation of 17 keto steroids.***eg-estrone, androsterone


Positive thinkers have a solution for every problem, Negative thinkers have a problem for every solution
Stay Positive!
Steven Aitchison

Thin layer Chromatography

- ***Modified form of Adsorption Chromatography.***
- *TLC is regarded as* ***Partition Chromatography***
- *Performed on open layer of adsorbent supported on glass plate.*
- *Simple form of Column Chromatography.*

Principle:

- *Partition of solute between solid & liq. phase takesplace.*
- *The loosely held species desorbs faster & move fast to forward, along with unabsorbed Species.*
- *But these species readsorbs at new location.*
- *This process of adsorption & desorption repeats continuously.*
- *As a result components which differ in their adsorption abilities migrate at different speed forming bands on the plate & get separated.*
- *Here Partition effect is predominant & adsorption effect diminish. (when polar solvent used as running solvent)*

1.Adsorbents :

- *Success of TLC depends on* ***Adsorbents***
- ***Choice*** *of adsorbents depends on* ***nature of comp****. to be separated &* ***solvent system***

eg- Alumina, Silica gel & florisil(activated mg-silicate)

- *Alumina is highly active, strongly adsorbing polar comp. Available in neutral, acidic & basic in washed form*
- *Adsorption Order:*

Cl^- ,Br^-,I^-< C=C< $-OCH_3$ < -COOR < C=O < -CHO < -SH< -OH < -COOH

2.Solvent System:

- *Variety of solvents available*
- ***Adsorption*** *effect is max. in* ***nonpolar*** *solvent*
- ***partition*** *effect is max. in* ***polar*** *solvent.*

eg- hexane, hexene, toulene, CCl4, Chloroform, water, AA, methanol,pet ether, Mix. of solvents can be also used

Methodology

1.Preparation of TLC plate :

-A Slurry of adsorbents & binder $CaSO_4$ in water spread uniformly on clean glass plate

-Can be done manually or with applicator.

-Dry & incubated at 383 K

-This process activates adsorbing particles.

-Thickness = 0.25 mm

2. Sample Application :

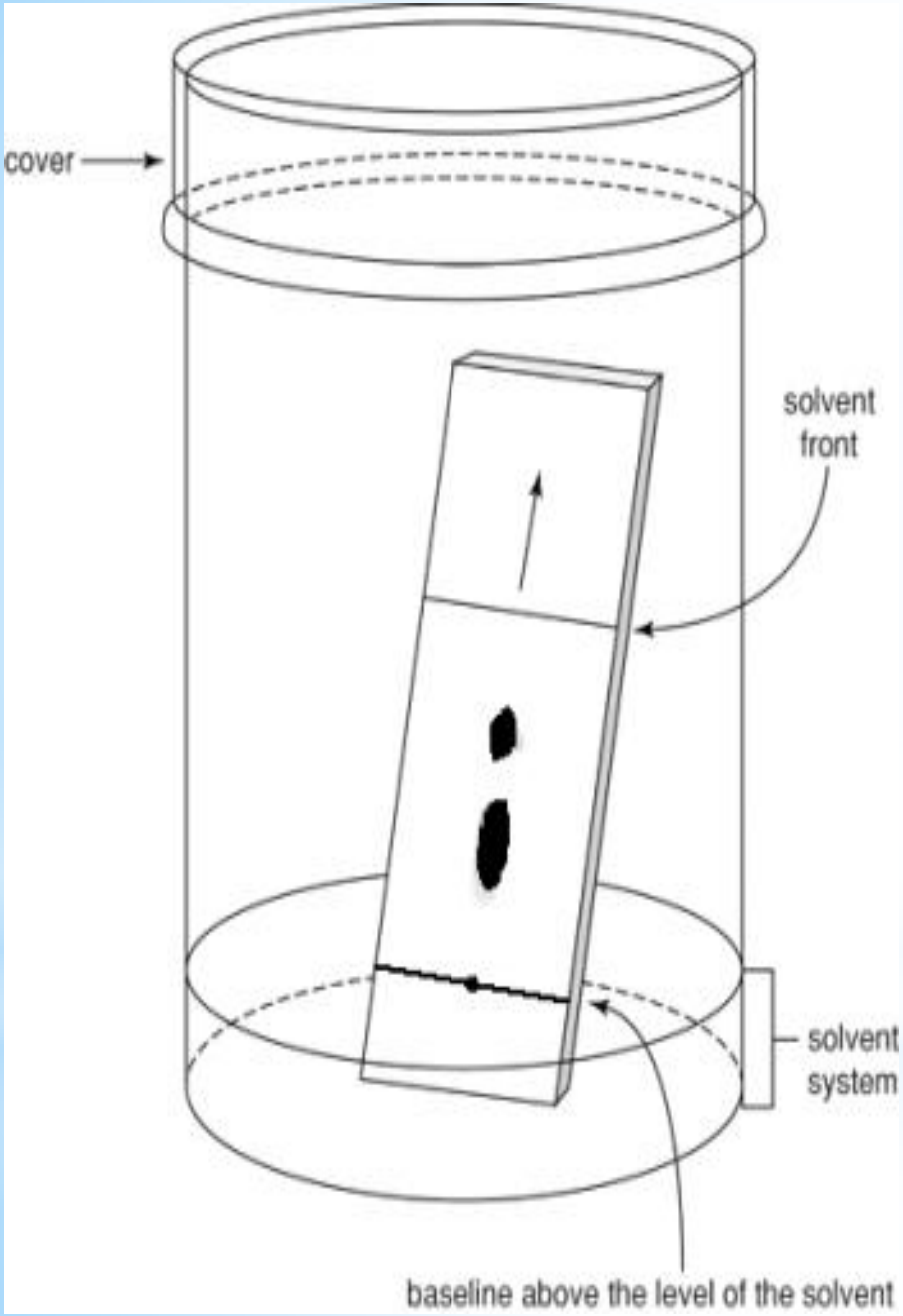
- ***Draw fine ref. line at 2 cm from bottom edge of TLC.***
- ***Point of sample application is marked***
- ***Mix. to be separated is applied after drying each.***
- ***Ref. soln also spotted***
- ***Finally TLC dried gently***

3.Development :

-TLC plate is placed in development chamber at angle 45°
-Tank is filled with developing solvent about 1 cm height.
-Three sides of jar are lined with **solvent impregnated paper**.(to saturate TLC plate by solvent vapor)
-Top is covered with lid.
-When solvent move up to certain height plate is taken out.
-Solvent front is carefully marked.
-Plate is dried at atmosphere.

4. Location of substance:

- **Colorless compounds are detected under UV light or by spraying reagent with ninhydrin**
- **Most suitable method is iodine saturated chamber, Conc. HCl, sulfuric acid, Chromic acid can also used.**
- **Strong heating method can also used.**



TLC Methodology

Application:

1. *For quantitative & qualitative analysis of comp. like amino acid, carbohydrate, lipids, alkaloids, vitamin ,terpins, etc.*
2. *Can be used as preparative method*

Advantages:

- ***Versatility in choice of adsorbents***
- ***Thin layer of variable thickness can be prepared***
- ***Very rapid & Separation can be completed within 1 hr.***
- ***Possible to detect low concentration comp.***
- ***Simple , rapid, in expensive method, routinely used in research.***

Disadvantages:

Rf value is not reproducible , affected by thickness


" आपण घेतलेला
कोणताच निर्णय
हा कधीच
चुकीचा नसतो..
फक्त तो बरोबर
आहे हे सिध्द
करण्याची जिद्द
आपल्यात हवी
असते.....

Gas Chromatography

Martin and James (1952)

Technique in which ***volatile*** *components of mix. are* ***separated*** *by* ***partitioning*** *between* ***Stationary solid or liquid*** *phase &* ***mobile gaseous*** *phase.*

Stationary phase : Solid (C, Ca-silicates,polymers) → GSC

Stationary phase : liquid → GLC

Construction:

1. ***Carrier Gas Tank:*** *Chemically inert gas ,having high coefficient of thermal conductivity. Must be dry & pure.*
   Pressure regulating valve maintain pressure.
   eg- He, N_2, H_2, CO_2,etc

2. Sample Injector:

-Imp to introduce sample in shortest time & in smallest volume

- Injected directly into column through inlet

- For high boiling solvent , to an heated injection chamber

- Sample is vaporized & vapors are swept by carrier gas into column

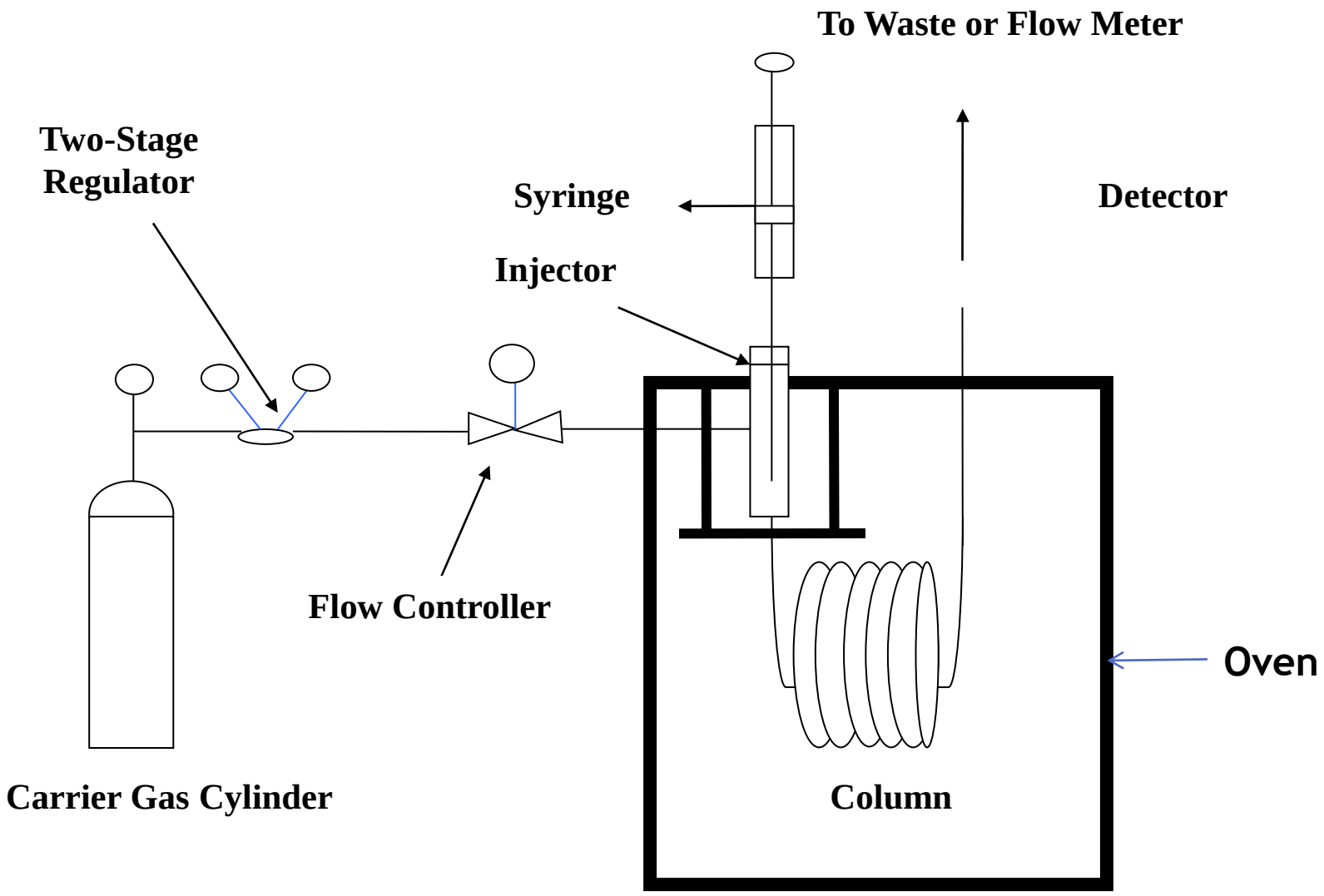


Fig: Gas Chromatography

Gas Chromatograph

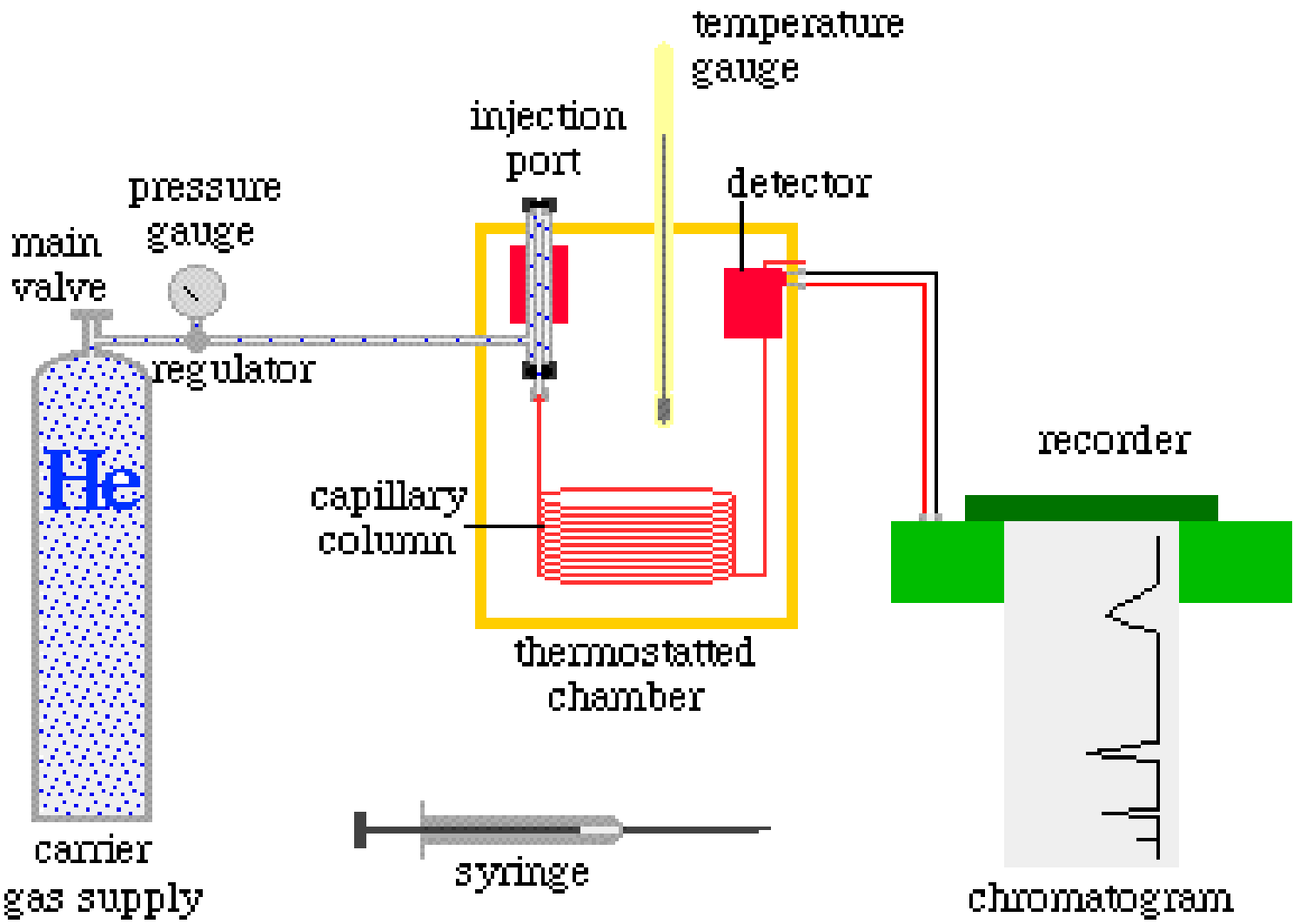


Fig: Gas Chromatography

3. Column : Glass or metallic tube (Cu, Al, SS)
'U' or 'W' bent, placed into oven.

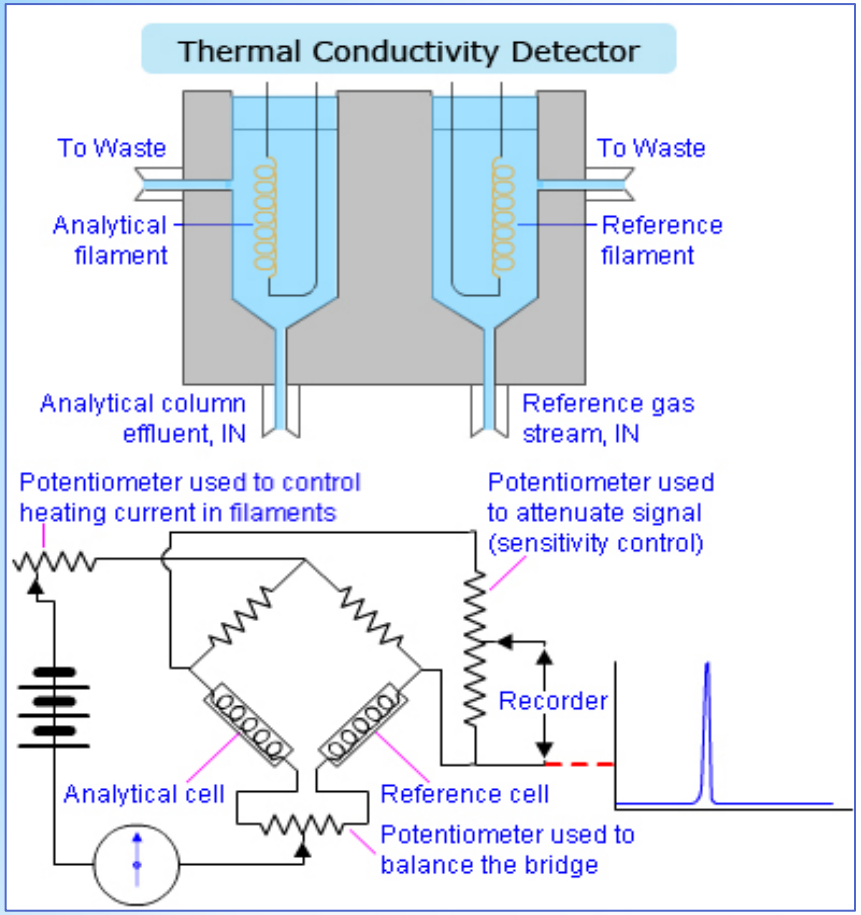
i) Packed Column :
Tubing 0.5 cm dia, 1-20 cm long, uniform size, inert, thermostable, solid support(diatomaceous earth, teflon powder, glass beds) coated with liq. film.

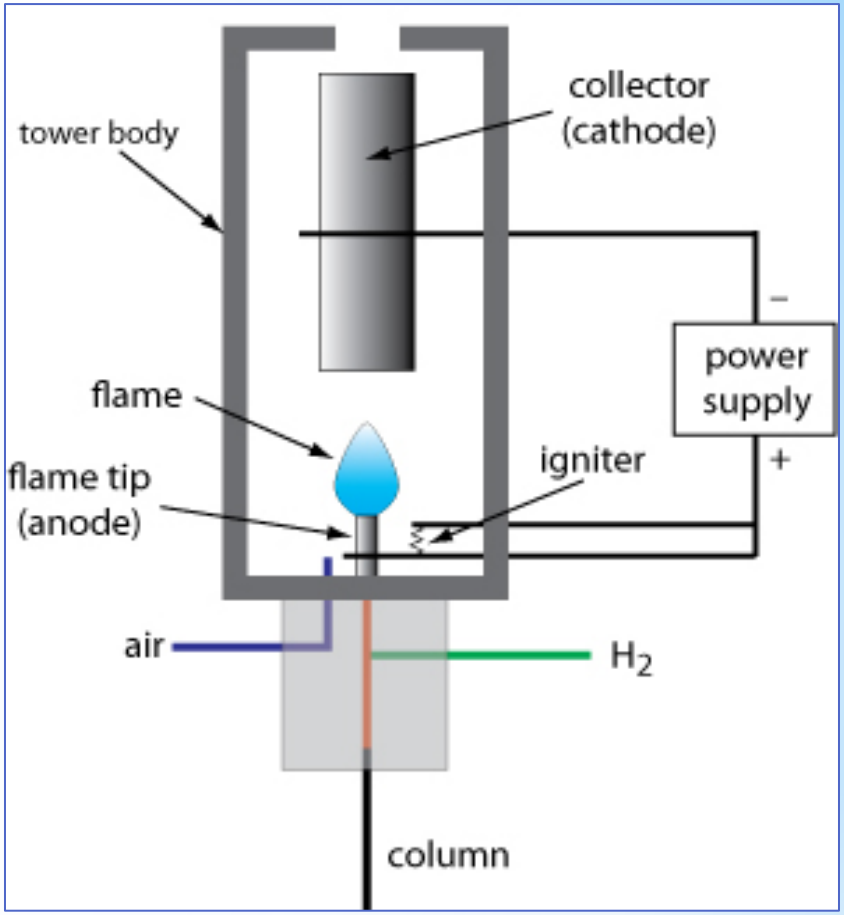
ii) Open tubular : Tubing 0.2-1.2 mm dia.
30-100 m long.

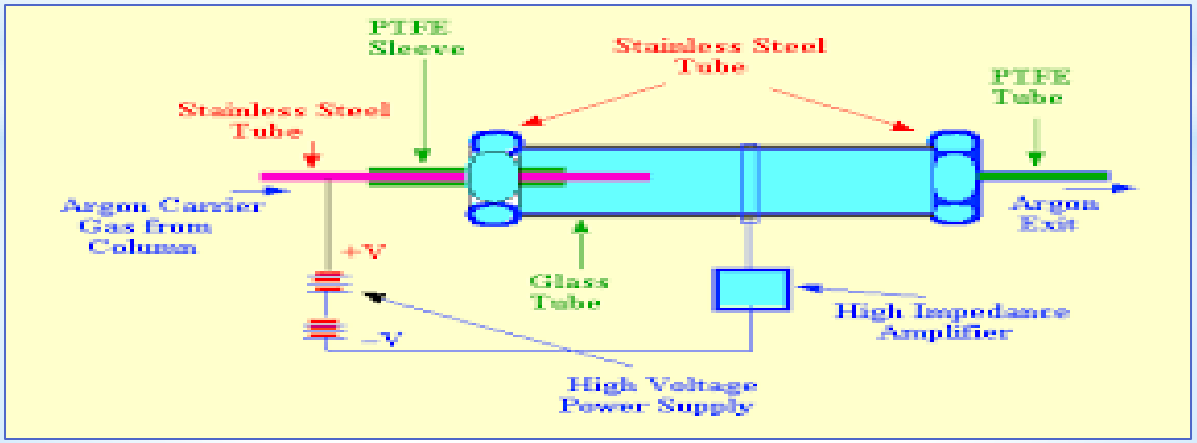
For high resolution : Tubing: 2 km long
Columns held at predetermined high tempt.

4.Detector : To record separation.
i) Thermal Conductivity detector
ii) Argon ionization detector
iii) Flame ionization detector

When carrier gas enters to detector, electrical signals are generated in proportion to conc. of sub.
These signals are recorded.


Thermal Conductivity Detector
To Waste
To Waste
Analytical filament
Reference filament
Analytical column effluent, IN
Reference gas stream, IN
Potentiometer used to control heating current in filaments
Potentiometer used to attenuate signal (sensitivity control)
Recorder
Analytical cell
Reference cell
Potentiometer used to balance the bridge


tower body
collector (cathode)
power supply
flame
igniter
flame tip (anode)
air
H_2
column


PTFE Sleeve
Stainless Steel Tube
PTFE Tube
Stainless Steel Tube
Argon Carrier Gas from Column
Argon Exit
+V
Glass Tube
High Impedance Amplifier
-V
High Voltage Power Supply

Applications

1. *Biofuel , biogas analysis.*
2. *Analysis of auto exhaust gas.*
3. *Identification of natural products.*
4. *Process control.*
5. *Biomedical Applications: Analysis of body fluid.*
6. ***Organic Analysis**: C & H can be determined by burning sample in dry stream of Oxygen.*
7. *Separation of benzene & cyclohexane.*
8. *GLC used to study reaction mechanism.*
9. *GSC is used to separate gaseous mix. of H_2, O_2,CO_2,CH_4,C_2H_2,etc.*

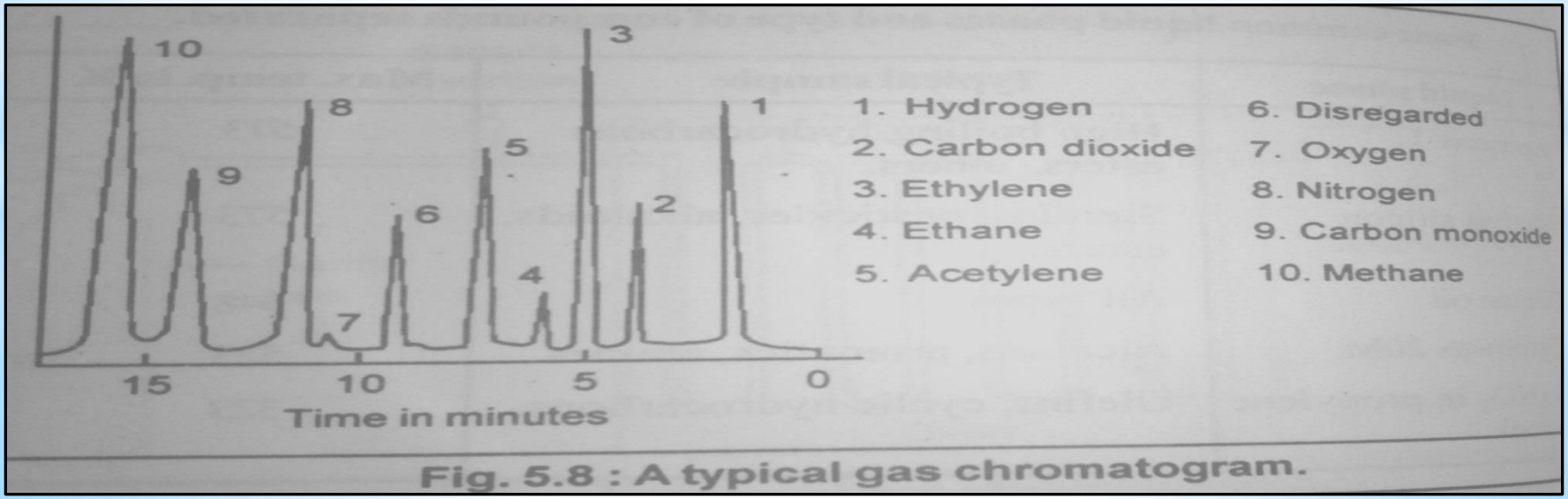


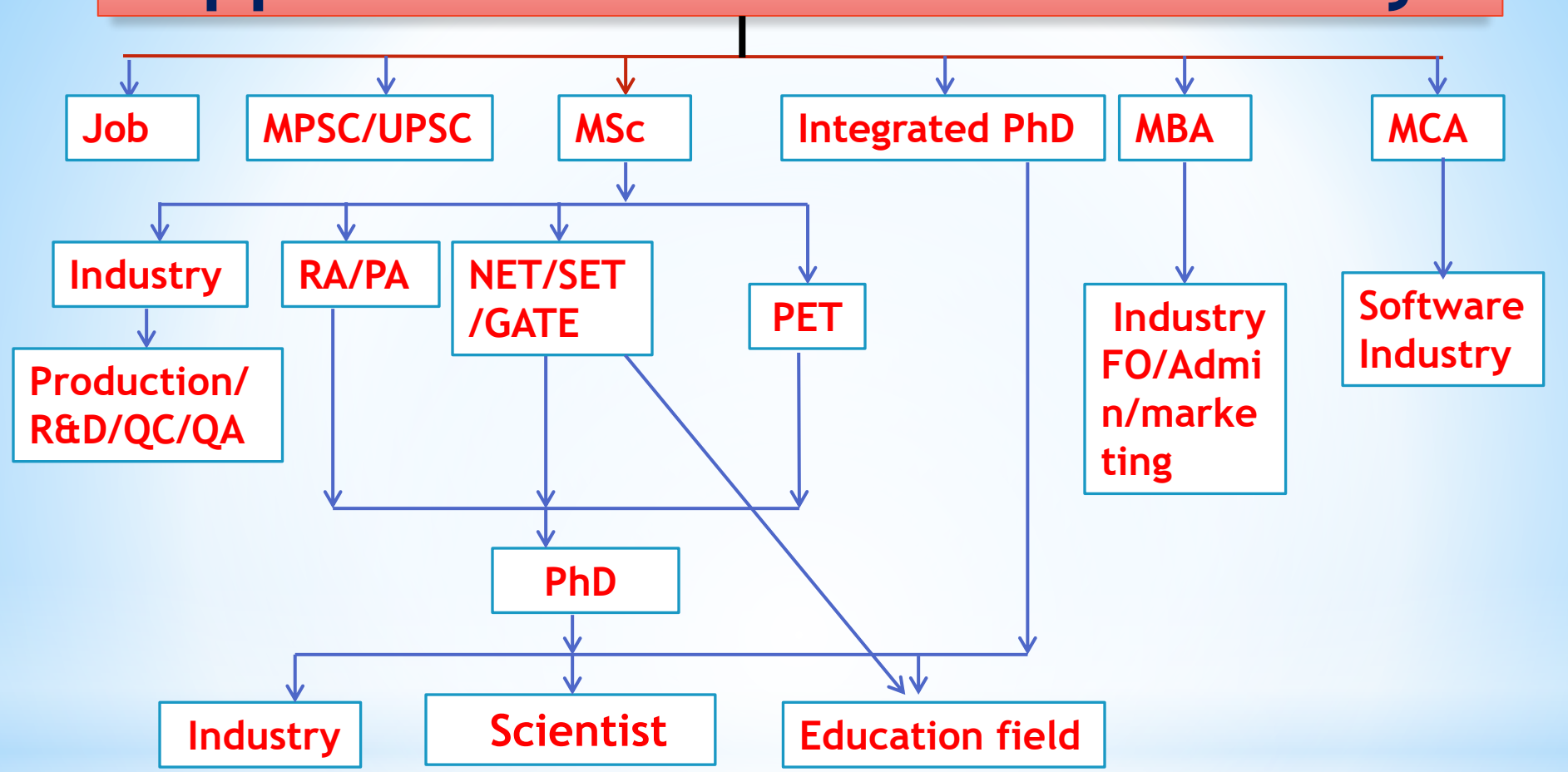
Fig. 5.8 : A typical gas chromatogram.

Advantages

1. Extraordinarily Sensitive method.
2. Many samples can be detected in small quantity like10^{-15} Kg.
3. Separation achieved in less than minute.
4. Volatile compound can be separated.
5. Gives satisfactory separation even of complex mixture.
6. Simple technique, can be used as routine laboratory practice.
7. Can be used as preparative method.(by using non destructive detectors & condensing the vapours emerging from collection end)

स्वत:ला घडविण्यात
आपला वेळ खर्च करा
म्हणजे तुम्हाला इतरांना दोष द्यायला
वेळच मिळणार नाही
तुम्ही उंच शिखरावर जरूर चढा पण
जगाने तुमच्याकडे पहावं म्हणून नव्हे
तर...त्या शिखरावरून तुम्हाला जग
पाहता यावं म्हणून..

Opportunities After BSc Chemistry

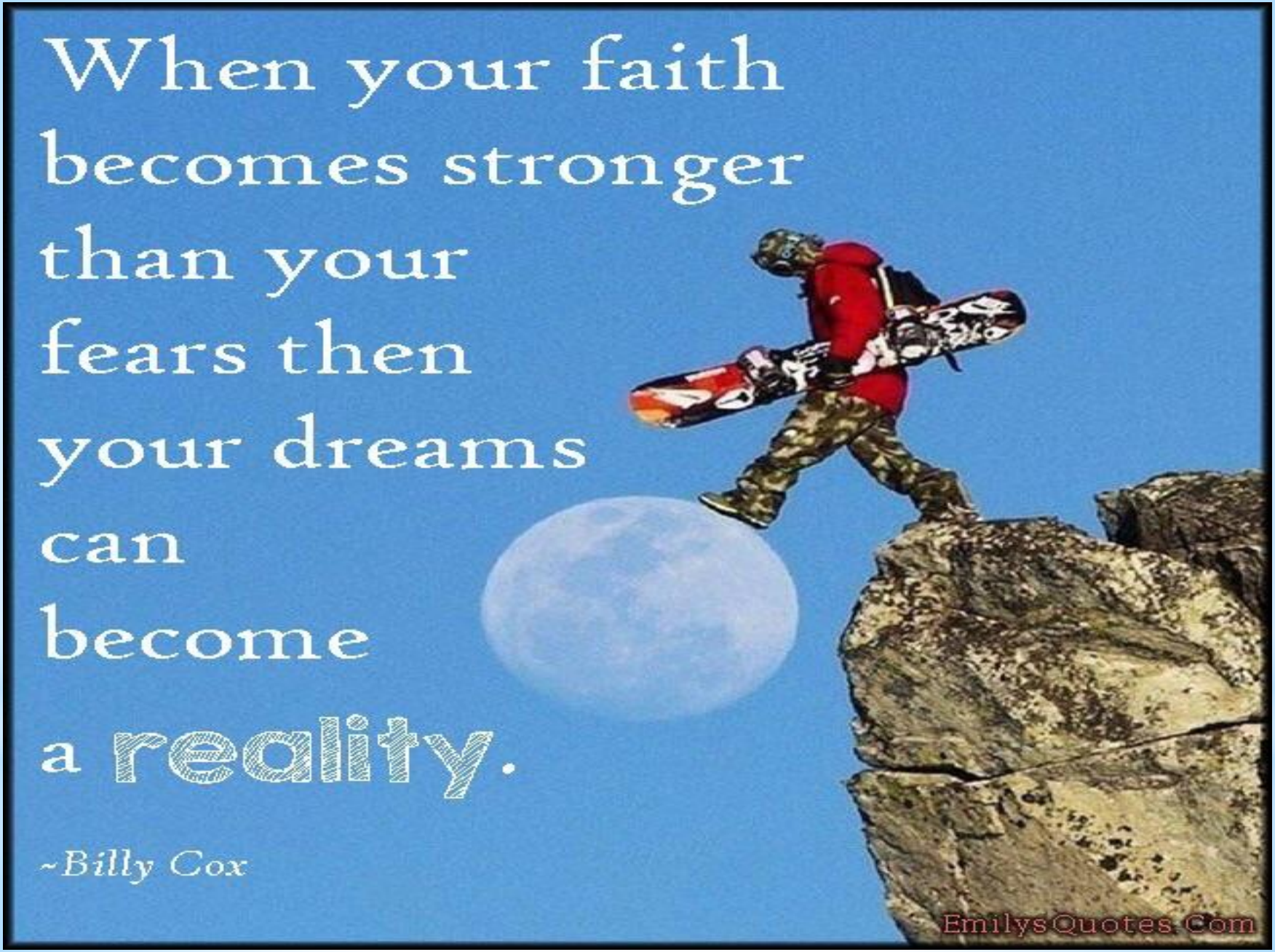


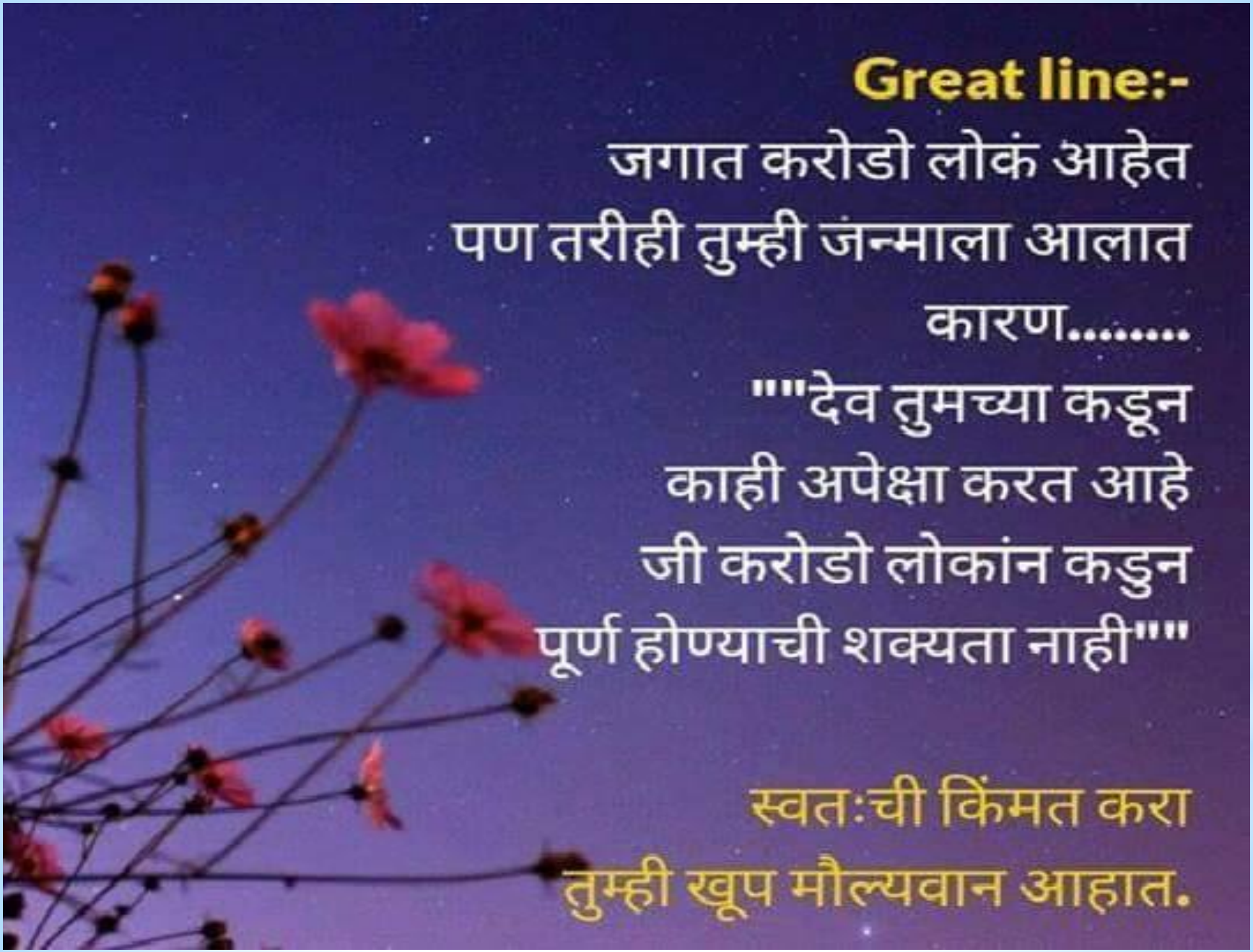
Imp branches of Chemistry for MSc

Organic | Physical | Inorg. | Ind. | Analy. | Polymer | Drug | Medicinal

AGPM | Biochemistry | Env. chem. | Astrochem. | Busi. chem | Nanoscience


दिवा कधी बोलत नाही,
त्याचा प्रकाश त्याची ओळख करून देतो...
अगदी तसंच तुम्ही तुमच्याविषयी
ही बोलण्याची गरज नाही, चांगले काम करीत रह
तेच तुमची ओळख देतील.
Be Positive


When your faith
becomes stronger
than your
fears then
your dreams
can
become
a reality.
~Billy Cox
EmilysQuotes.Com


Great line:-
जगात करोडो लोकं आहेत
पण तरीही तुम्ही जंन्माला आलात
कारण.....
""देव तुमच्या कडून
काही अपेक्षा करत आहे
जी करोडो लोकांन कडुन
पूर्ण होण्याची शक्यता नाही""
स्वत:ची किंमत करा
तुम्ही खूप मौल्यवान आहात.

Thank You

Questions???