

# Safety in the Laboratory

Prepared by Assist. Lec.  
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## Introduction

Laboratory safety rules : are a major aspect of every clinical lab.

- Each student in clinical laboratory must follow specific safety rules and procedures.

Why is lab safety important ?

Lab safety rules and symbols are needed so that students do not injure themselves or their classmates.

## Lab safety rules

- 1- wear protective clothing
- 2- laboratory personnel should not wear sandals, jewelry, and loose or baggy clothing
- 3- avoid touching objects (e.g. pencils . cell phones , door handles) while wearing gloves
- 4- long hair must be tied back or covered to minimize fire hazarded or contamination of experiments
- 5- do not eat food or drink water in the lab do not use lab glassware as food or water containers

- 6- protect your hands safety : wash hands after every lab handle glassware carefully.
- 7- electrical safety : unplug electrical equipment after use.
- 8- chemical safety : never touch, taste or smell a chemical unless instructed to do so, and keep lids on chemical containers when not in use.
- 9- dispose of waste products according to instructions.

## **Safety in the laboratory**

Many laboratory workers encounter daily exposure to biological hazards. These hazards are present in various sources throughout the laboratory such as blood and body fluids, culture specimens, body tissue and cadavers, and laboratory animals, as well as other workers.

These are biological agents (e.g., viruses, bacteria, fungi, and toxins ) that have the potential to pose a severe threat to public health and safety, to animal or plant health, or to animal or plant products

# Health hazards come from a variety of sources:

## 1 - Chemical hazards:

all chemicals are to varying extents, capable of causing damage to the body. They may be irritants and cause a short-term effect on exposure. Alternatively they may be corrosive and cause severe and often irreversible damage to the skin. Examples include strong acids and alkalis



## **2 - Biological hazards:**

Examples include human body fluids that may carry infections such as HIV. Sources of biological hazards may include bacteria, viruses, insects, plants, birds, animals, humans, cell tissue cultures, and all microorganisms. These sources can cause a variety of health effects ranging from skin irritation and allergies to infections.



# BIOLOGICAL HAZARDS

**VIRUSES**



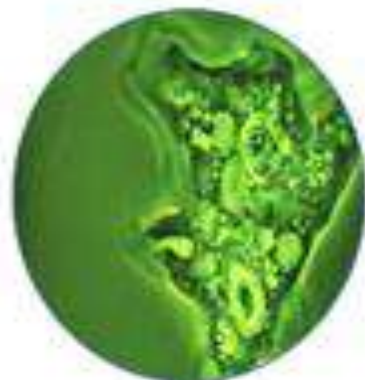
**FUNGI**



**BACTERIA**



**PARASITES**





### **3 - Electrical and Mechanical Hazards:**

all electrical apparatus should be used and maintained in accordance with the manufacturers' instructions.

Electrophoresis equipment presents a particular potential for safety problems. Centrifuges, especially high-speed varieties, also need careful use especially in the correct use and balance of the rotors.

### **4 - General laboratory hazards:**

Common examples include syringe needles, broken glassware and liquid nitrogen flasks

## **How Protect yourself, others, and the environment?**

- 1- Wash your hands after removing gloves, before leaving the laboratory, and after handling a potentially hazardous material.
- 2- While working in the laboratory, wear personal protective equipment - eye protection, gloves, laboratory coat.
- 3- Properly and dispose of all laboratory waste.

# Fire Safety

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**Fire** is a chemical reaction that requires three elements to be present for the reaction to take place and continue. The three elements are:

1- Heat, or an ignition source

2- Fuel

3- Oxygen

These three elements typically are referred to as the “fire triangle.” Fire is the result of the reaction between the fuel and oxygen in the air.. Heat, fuel and oxygen must combine in a precise way for a fire to start and continue to burn. If one element of the fire triangle is not present or removed, fire will not start or if already burning, will extinguish.

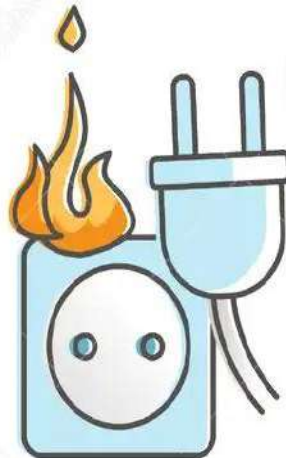
# **FIRE CLASSES**



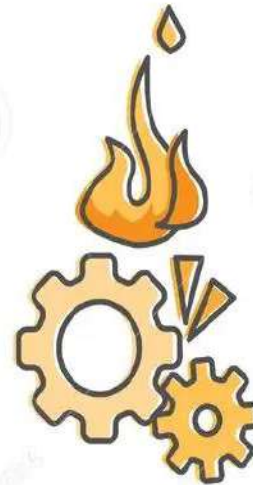
**Ordinary  
Combustibles**



**Flammable  
Liquids**



**Electrical  
Equipment**



**Combustible  
Metals**

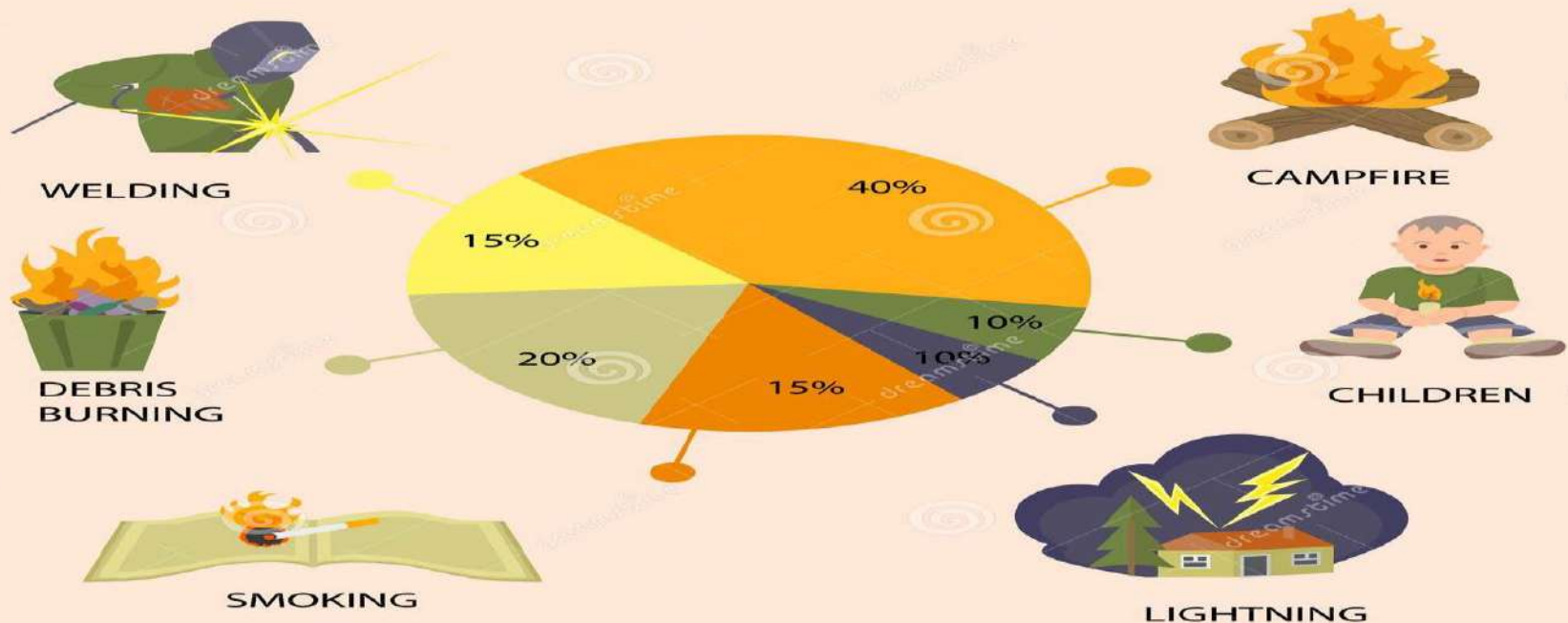


**Combustible  
Cooking**

## The major causes of fire include:

- Smoke, matches, cigarette ends etc.
- Trees on power lines.
- Faults or misuse of electrical equipment.
- Burning or faulty generating equipment.
- Careless cooking fires

### FIRE CAUSES INFOGRAPHIC
























## **What Does Fire Extinguisher Mean?**

A fire extinguisher is a handheld active fire protection device usually filled with a dry or wet chemical used to extinguish or control small fires, often in emergencies.



# KNOW YOUR FIRE EXTINGUISHER COLOUR CODE

Water	Dry powder	Foam	CO <sub>2</sub> Carbon dioxide	Wet Chemical
				
<b>For use on</b>  Wood, Paper, textiles etc.	<b>For use on</b>  Wood, Paper, textiles etc.  Flammable liquids  Gaseous fires  Live electrical equipment	<b>For use on</b>  Wood, Paper, textiles etc.  Flammable liquids	<b>For use on</b>  Flammable liquids  Live electrical equipment	<b>For use on</b>  Wood, Paper, textiles etc.  Cooking oil fires
 <b>Do not use on</b>  Flammable liquids  Live electrical equipment		 <b>Do not use on</b>  Live electrical equipment	 <b>Do not use in a confined space</b>	



# How To Use A Fire Extinguisher



Remember the **PASS** word (**P**ull-**A**im-**S**queeze-**S**weep)

**P**

**Pull**

Pull The Pin



Break seal  
and test  
extinguisher



**A**

**Aim**

Aim At  
The Base  
Of Fire



Ensure you have  
a means of  
escape



**S**

**Squeeze**

Squeeze The  
Operating  
Handle



To operate  
extinguisher  
and discharge  
the agent



**S**

**Sweep**

Sweep From  
Side To Side













Completely  
extinguish  
the fire



## Type of Fire extinguisher

- **Class A:** fire extinguisher: Contain water for use against fires involving ordinary combustibles like paper, wood, cloth, and most plastics.
- **Class B:** fire extinguisher: Use dry chemicals to put out fires caused by gasoline, oil and solvents.
- **Class C:** fire extinguisher: Contains carbon- dioxide for use against fires.
- **Class D:** fire extinguisher: Spray dry powder on combustible metals like magnesium, titanium, aluminum, sodium and potassium.
- **Class K:** fire extinguisher: Use a wet potassium acetate based, low pH agent to put out "cooking" fires in which there are animal or vegetable oils and fats.

# Different Classes of fire require different type of extinguisher

Class of Fire	Type of Fire	Type of Extinguisher	Extinguisher Identification	Symbol
<b>A</b>	Ordinary combustibles: wood, paper, rubber, fabrics, and many plastics	Water, Dry Powder, Halon		
<b>B</b>	Flammable Liquids and Gases: gasoline, oils, paint, lacquer, and tar	Carbon Dioxide, Dry Powder, Halon		
<b>C</b>	Fires involving Live Electrical Equipment	Carbon Dioxide, Dry Powder, Halon		
<b>D</b>	Combustible Metals or Combustible Metal Alloys	Special Agents		No Picture Symbol 
<b>K</b>	Fires in Cooking Appliances that involve Combustible Cooking Media: Vegetable or Animal Oils and Fats			

## Fire Safety

- 1- Respect how serious fire can be.
- 2- Learn to recognize and control all types of fire hazards at your facility.
- 3- Practice safe work habits.
- 4- Dispose of oily rags and other flammable waste in fire-proof containers.
- 5- Prevent fires – don't fight them.



# **Disposal of Hazardous Materials**

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## **Hazard and special waste**

Hazardous waste is waste which may cause hazard or harm to health or the environment because of its chemical or physical or some other property.



## Typical Waste

- 1- Infectious Waste
- 2- Biological Waste
- 3- Chemical Waste
- 4- Radioactive Waste



# Infectious Waste

- Cultures, samples and Stocks of infectious agents
- contaminated animals materials and equipment





# **Classification of Biological Hazard**

(bacteria, Viruses , Parasites and Fungal)

- 1- agents presenting a risk or potential risk to the well – being of man or other animals
- 2- infection either directly or indirectly through disruption of the environment

## **Final Disposal**

- Chemical disinfection
- steam sterilization (Autoclaving)
- incineration (burning to ash)

# Biological Waste

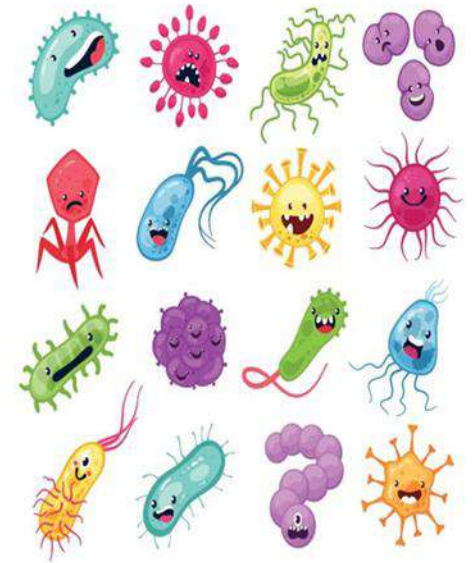
Materials and equipment's contaminated by blood and other secretions

## Packing

- Leak-proof plastic bag or container (red color)
- Storage place cold room or freezer

## Final disposal

- 1- landfilling as special waste
- 2- incineration or autoclaving



# Chemical Waste

Waste which has one or more of the following characteristics:

- Explosive
- Oxidizing
- Highly flammable/ flammable
- Irritating
- Damaging
- Very toxic/ toxic
- Corrosive
- Teratogenic
- Mutagenic
- Ecotoxic



## **Segregation and Packing**

- Segregation of different waste
- Storage of incompatible chemicals  
indifferent cabinets/ room

## **Final disposal**

chemical waste needs to be collected and stored in a tenable facility by the chemical companies and then removed from the site in safe containers to the designated disposal or recycling plant.

**Chemical Hazards** Hazardous chemicals present physical and/or health

threats to workers in clinical, industrial, and academic laboratories. Laboratory chemicals include cancer-causing agents (carcinogens), toxins (e.g., those affecting the liver, kidney, and nervous system), irritants, corrosives, sensitizers, as well as agents that act on the blood system or damage the lungs, skin, eyes, or mucous membranes.

**Material Safety Data Sheets (MSDSs)** for chemicals received by the

laboratory must be supplied by the manufacturer, distributor, or importer and must be maintained and readily accessible to laboratory workers. **MSDSs** are written or printed materials concerning a hazardous chemical. Employers must have an MSDS in the workplace for each hazardous chemical in use.

## **Radioactive West**

Radioactive waste is a result of many activities, including nuclear medicine, nuclear research, nuclear power generation and nuclear weapons reprocessing



## **Packaging**

- Packages shall be marked appropriated
- The storages area or the waste packages must be marked with a sign indicating a danger or radiation

## **Final disposal**

- waste is placed in under ground facility designed to ensure radioactivity from escaping due to natural barriers
- It is considered the best solution for final disposal of the most radioactive waste produced

# **What are methods hazardous wastes disposal ?**

## **1- Segregation**

Segregate hazardous biological waste from non-hazardous biological waste. Any waste that could cause a laceration or puncture must be disposed of as "Sharps. Sharps must be segregated from other waste. Do not mix biological waste with chemical waste or other laboratory trash.

Liquid wastes must be stored in leak proof containers

## **2-Handling and Transport**

Transport bio hazardous waste outside of the lab in a closed, leak-proof bag or container. Contain and label all treated waste before transporting it to the incinerator or dumpster



### **3- Labeling**

Clearly label each container of untreated bio hazardous waste and mark it with Symbol. Label non hazardous waste as "NONHAZARDOUS BIOLOGICAL WASTE".

### **4- Disposal Methods**

Incinerate the carcasses or send them to a commercial rendering plant for disposal. Liquid wastes should be disinfected by thermal or chemical treatment and then discharged into the sanitary sewer system

# Spectrophotometry

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# Introduction

Spectrophotometry is a measurement of how much a chemical substance absorbs or transmits.

Spectrophotometry is widely used for quantitative analysis in various areas (e.g., chemistry, physics, biology, biochemistry, material and chemical engineering, clinical applications etc.). Any application that deals with chemical substances or materials can use this technique.



## principle

The basic principle is that each compound absorbs or transmits light over a certain range of wavelength.

Spectrophotometry is an experimental technique that is used to measure the concentration of solutes in a specific solution by calculating the amount of light absorbed by those solutes.

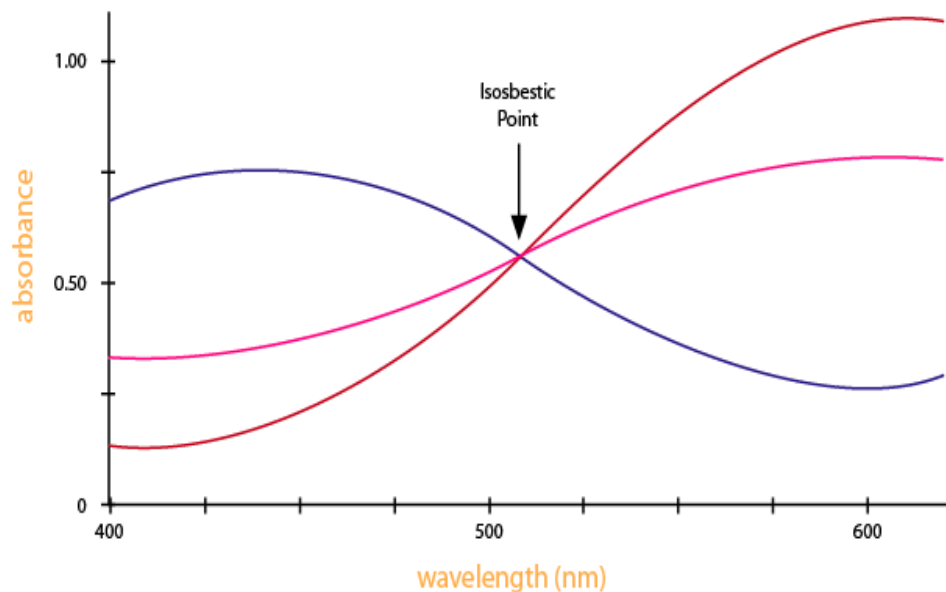
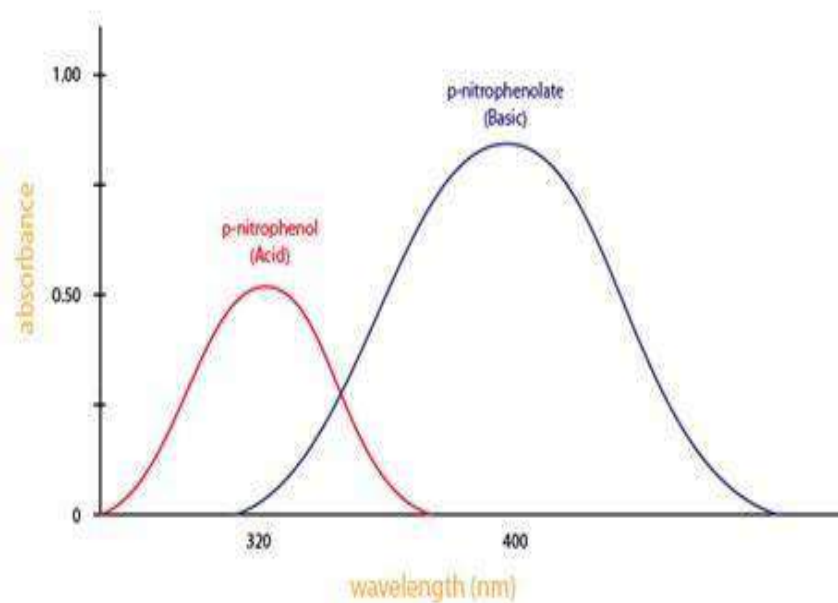
This technique is powerful because by analyzing the light that passes through the solution, you can identify particular dissolved substances in solution and how concentrated those substances.

Depending on the range of wavelength of light source, it can be

**classified into two different types:-**

- 1- UV-visible spectrophotometer: uses light over the ultraviolet range (185 - 400 nm) and visible range (400 - 700 nm) of electromagnetic radiation spectrum.
- 2- IR spectrophotometer: uses light over the infrared range (700 – 15000 nm) of electromagnetic radiation spectrum.

you need a spectrometer to produce a variety of wavelengths because different compounds absorb best at different wavelengths. For example, p-nitrophenol (acid form) has the maximum absorbance at approximately 320 nm and p-nitrophenolate (basic form) absorb best at 400 nm



## Beer-Lambert Law

Beer-Lambert Law (also known as Beer's Law) states that there is a linear relationship between the absorbance and the concentration of a sample.

Beer's Law can only be applied when there is a linear relationship.

**Beer law include :** The absorbance is directly proportional to the concentration ( $c$ ) of the solution of the sample used in the experiment.

**The general Beer-Lambert law is usually written as:**

$$A = Ebc$$

E = is the molar absorptivity (extinction coefficient )  
with units of  $\text{L mol}^{-1} \text{ cm}^{-1}$

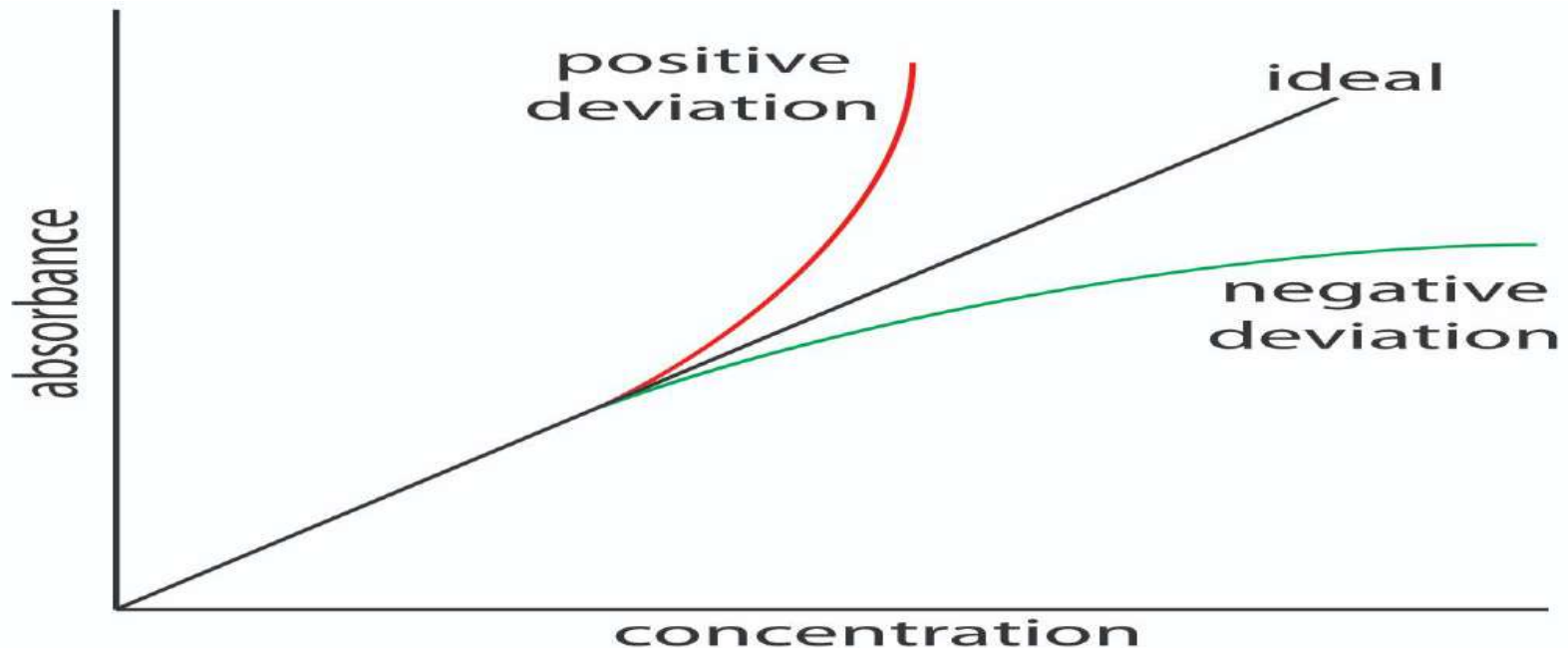
b = is the path length of the sample . We will express  
this measurement in  
centimeters.

c = is the concentration of the compound in solution,  
expressed in  $\text{mol L}^{-1}$



# Deviations from the Beer–Lambert law

Spectral measurements depend on the linearity of the relationship between absorption and concentration. When deviating from the straight line, either a negative deviation or a positive deviation both cause a Wrong in the spectral measurements. There are several reasons Causes of nonlinearity include:



# Causes deviation from Beer–Lambert law

- 1- High concentration
- 2- Aggregation
- 3- polymerization
- 4- Denaturation
- 5- Chemical reaction
- 6- Stray light

## Factors affecting UV/Vis absorption

Biochemical samples are usually buffered aqueous solutions, which has two major advantages (internal factors).

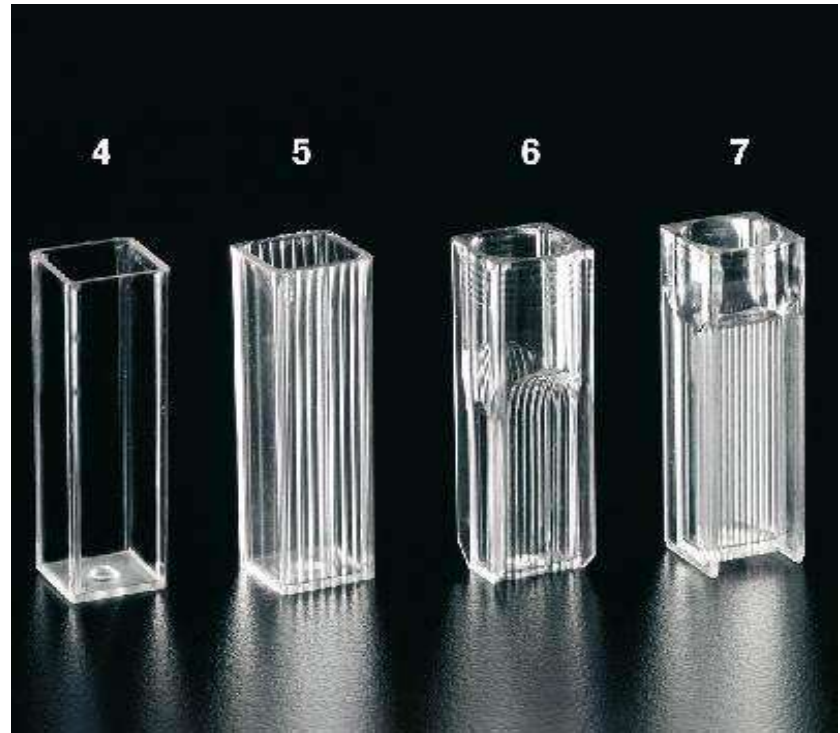
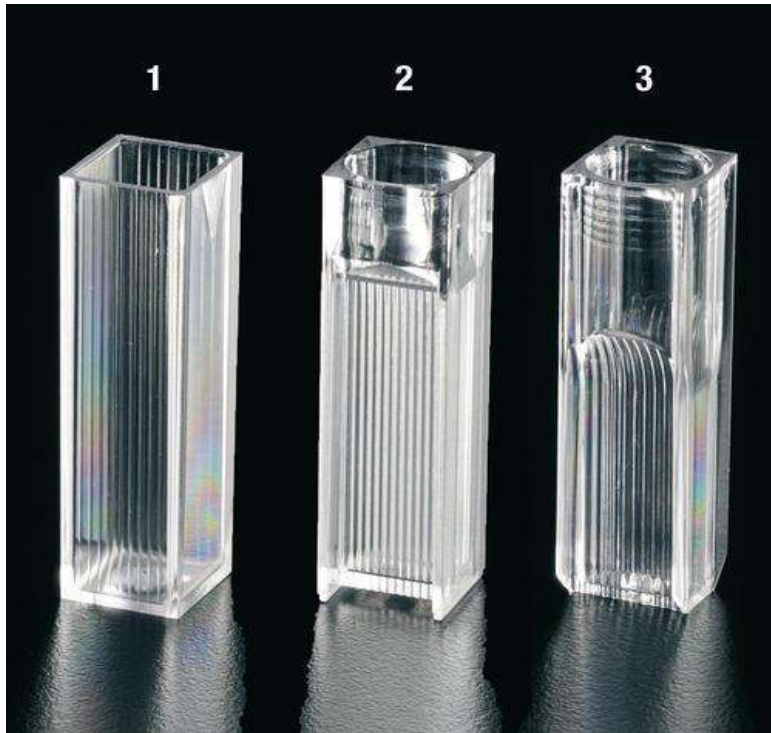
**Firstly**, proteins and peptides are stable in water as a solvent, which is also the 'native' solvent.

**Secondly**, in the wavelength interval of UV/Vis (200–700 nm) the water spectrum does not show any absorption bands and thus acts as a silent component of the sample.

**The environment (external factors)** also affects the observed spectrum, which mainly can be described by parameters: PH , Polarity solvent, Temperature, Concentration, Conformation.

## Cuvettes for samples

Cuvettes are generally made of glass, plastic or quartz. Quartz cuvettes are used when absorbance is measured at a wavelength under 320 nm, as glass and plastic absorb most of the light in this range (above 320 nm).



## **Frequently arising problems in photometry:**

- 1- If the sample is turbid, this will result in an error as the light will be scattered and part of it will never reach the detector and thus will appear as absorbed.
- 2- If the molecule is capable of association/dissociation and the absorbance's of the two forms differ, the Lambert- Beer law will no longer be valid as the degree of dissociation will depend on the concentration.
- 3- It is crucial for the cuvette to be clean and free of scratches. The sides of the cuvette in the light path should never be touched by hand

# **ATOMIC ABSORPTION SPECTROMETER**

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## Introduction

**Atomic Absorption Spectrometer** : is a very common technique for detecting metals and metalloids in environment samples.

It is very reliable and simple to use.

It can analyze over 62 elements.

It also measures the concentration of metals in the sample.



Elements detectable by atomic absorption are highlighted in pink in this periodic table

H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac															

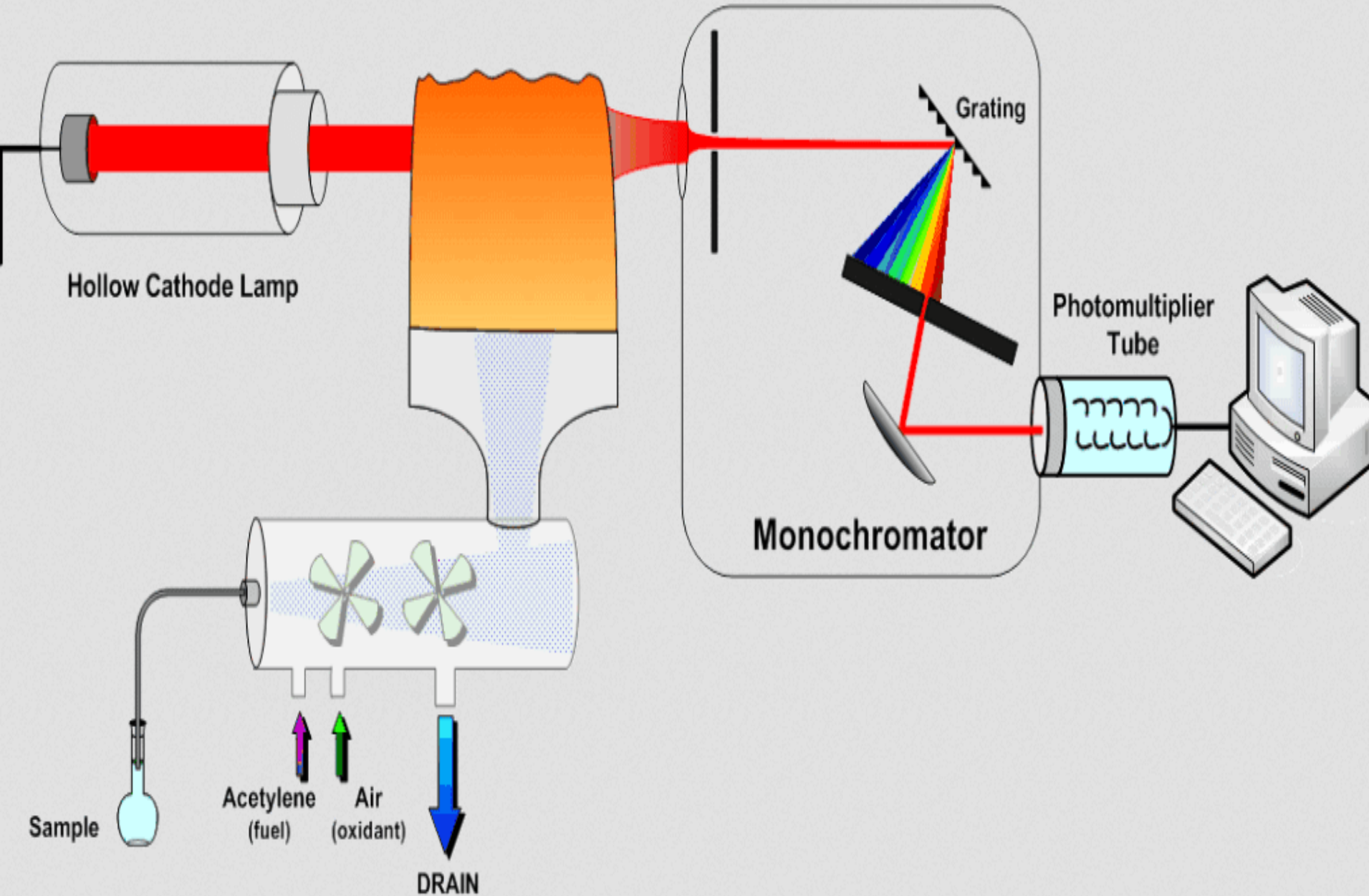
## Principle

The atoms absorb ultraviolet or visible light and make transitions to higher electronic energy levels by the flame. The analytic concentration is determined from the amount of absorption.

**The wavelength gives** information about the element and the color of the flame gives information about the amount of the element present in the sample

- Concentration measurements are usually determined from a working curve after calibrating the instrument standards of known concentration

# Atomic Absorption Spectroscopy



## **Flame Atomizer:**

**The most commonly employed flames in flame photometry can be grouped into two types:**

- a. Flames in which the fuel and oxidant as air or oxygen are well mixed before combustion, these are called pre- mix.
- b. Flames in which the fuel gas and the oxidant are first mixed in the flame itself. They are called unpre-mix.  
In most of the gases Air-acetylene flame or Nitrous oxide acetylene flame is used.  
Liquid or dissolved samples are typically used with flame atomizer

## **The following processes occur in the flame photometer.**

- 1) Nebulization: A solution containing the relevant substance to be analyzed is aspirated into the burner into the flame as a fine spray. This process is called nebulization.
- 2) Vaporization: The heat of the flame vaporizes the sample constituents. no chemical change takes place at this stage.
- 3) Atomization: At this stage the metal ions that were in the solvent are reduced to metal atoms. molecules and ions of the sample species are decomposed and reduced to give atoms.

4) Excitation: The atoms at this stage are able to absorb energy from the heat of the flame.

The amount of energy absorbed depends on the electrostatic forces between the negatively charged electrons and the positively charged nucleus. This in turn depends upon the number of protons in the nucleus. As electrons absorb energy they move to higher energy levels and are in the excited state.

5) Emission of radiation: Electrons in the excited state are very unstable and move back down to the ground state or a lower energy state quite quickly.

As they do so, they emit the energy in the form of radiation of wavelength, which is measured by a detector.

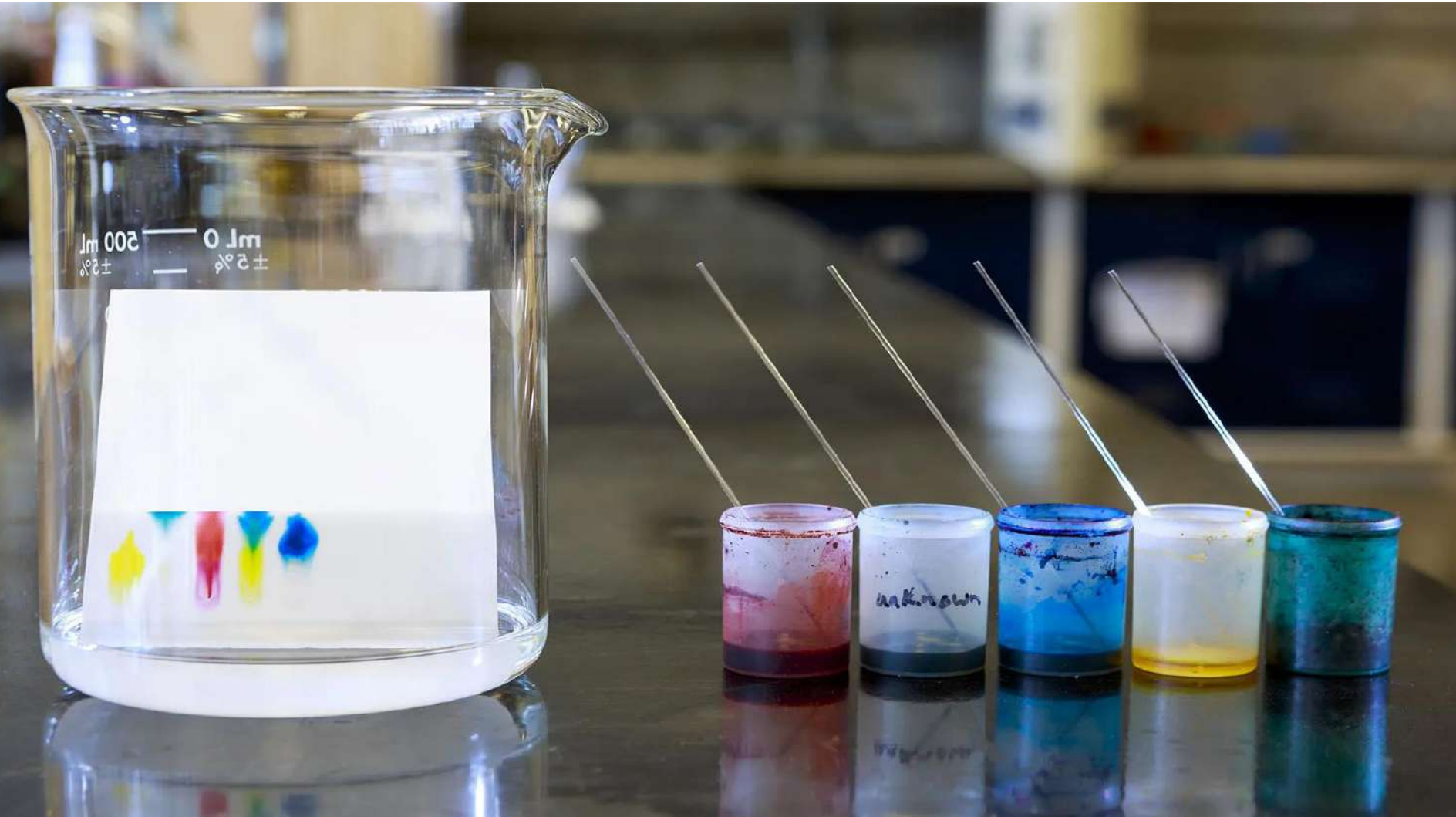
## **Applications:**

- 1- Determination of even small amounts of metals (Lead, Mercury, Calcium, Magnesium, etc.)
  - Environment at studies (drinking water, ocean water, soil)
  - Food Industry
  - Pharmaceutical Industry
- 2- It can be applied to find both for quantitative and qualitative characteristic to certain metal.



# Chromatography separate technique

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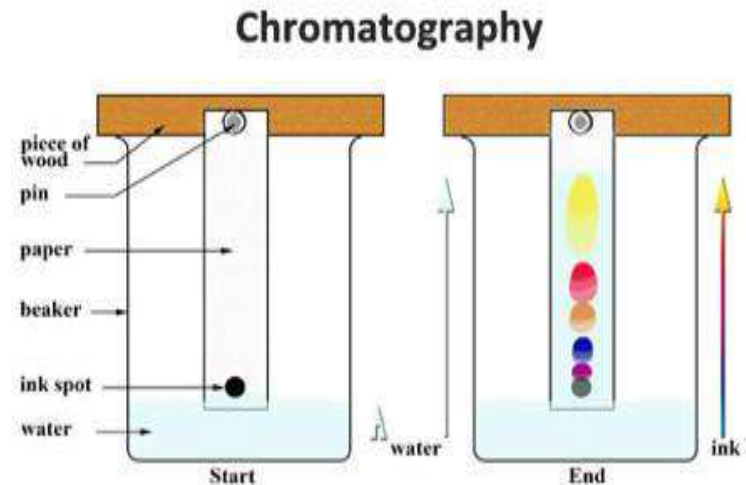


# Introduction

Chromatography is a physical process where the components (solutes) of a sample mixture are separated as a result of their differential distribution between stationary and mobile phases.

The various compounds of the mixture travel at different speeds, causing them to separate.

Greek chroma meaning "color" and graphein meaning "writing"



## Principle

Chromatography is usually based on principle of partition of solute between two phase. It usually consists of a mobile phase and stationary phase.

- **The Mobile phase:** usually refers to the mixture of the substances to be separated dissolved in a liquid or gas.
- **The Stationary phase :** is a porous solid matrix through which the sample contained in the mobile phase percolates.

# Classifications

Chromatographic separate methods can be classified in two different way:

## **1) Classification of Chromatography According to Mobile Phase and Stationary Phase:**

- 1- Liquid chromatography: mobile phase is a liquid.
- 2- Gas chromatography: mobile phase is a gas.
- 3- Thin layer chromatography (TLC): the stationary phase is a thin layer supported on glass, plastic or aluminum plates.
- 4- Paper chromatography (PC): the stationary phase is a thin film of liquid supported on an inert support
- 5- Column chromatography (CC): stationary phase is packed in a glass column

## **2) Classification According to the Mechanism of Separation :**

- 1-Adsorption chromatography
- 2- Partition chromatography
- 3-Jon exchange chromatography
- 4- Gel filtration chromatography
- 5- Affinity chromatography

## **Chromatography Applications**

- 1- Used to diagnose and identify Chemical compounds
- 2- Used for separation isolation and purify chemical compounds usually.
- 3- The technique is also useful for the determination of molecular weight of proteins
- 4- helpful for the qualitative and quantitative analysis of complex mixture.

## **Pharmaceutical Sector**

- 1-To identify and analyze samples elements or chemicals.
- 2-In drug development.

## **Chemical Industry**

- 1-In testing water samples and also checks air quality.
- 2-In various life sciences applications.

## **Food Industry**

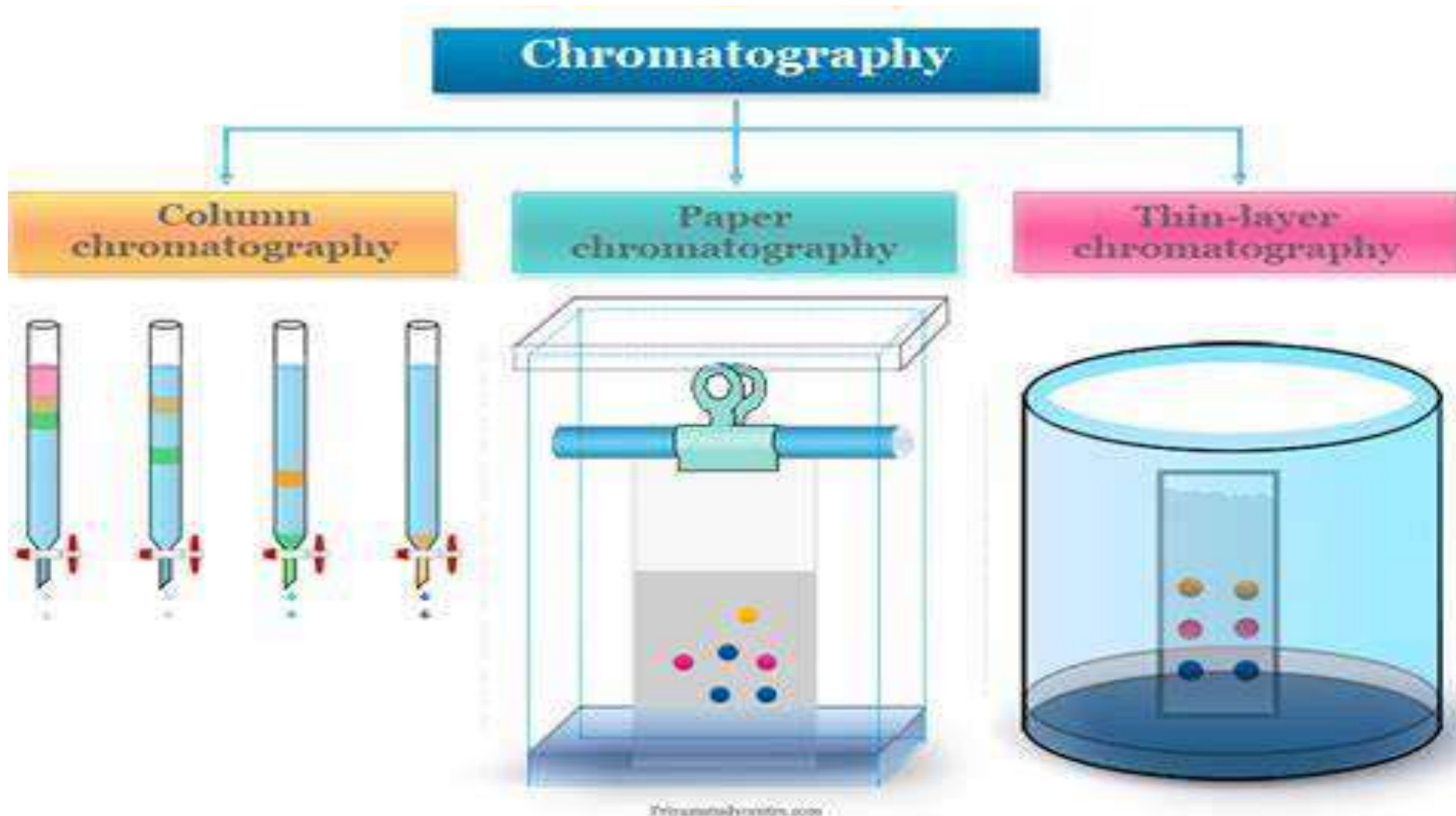
- 1-In food damage and additive detection
- 2-Determining the nutritional quality of food.

## **Molecular Biology Studies**

- 1-Various techniques in chromatography such as applied in the study of metabolism.
- 2-This kind of chromatography technique is used in the purification of enzymes, insulin purification, and plasma fractionation.

# Type chromatography 1

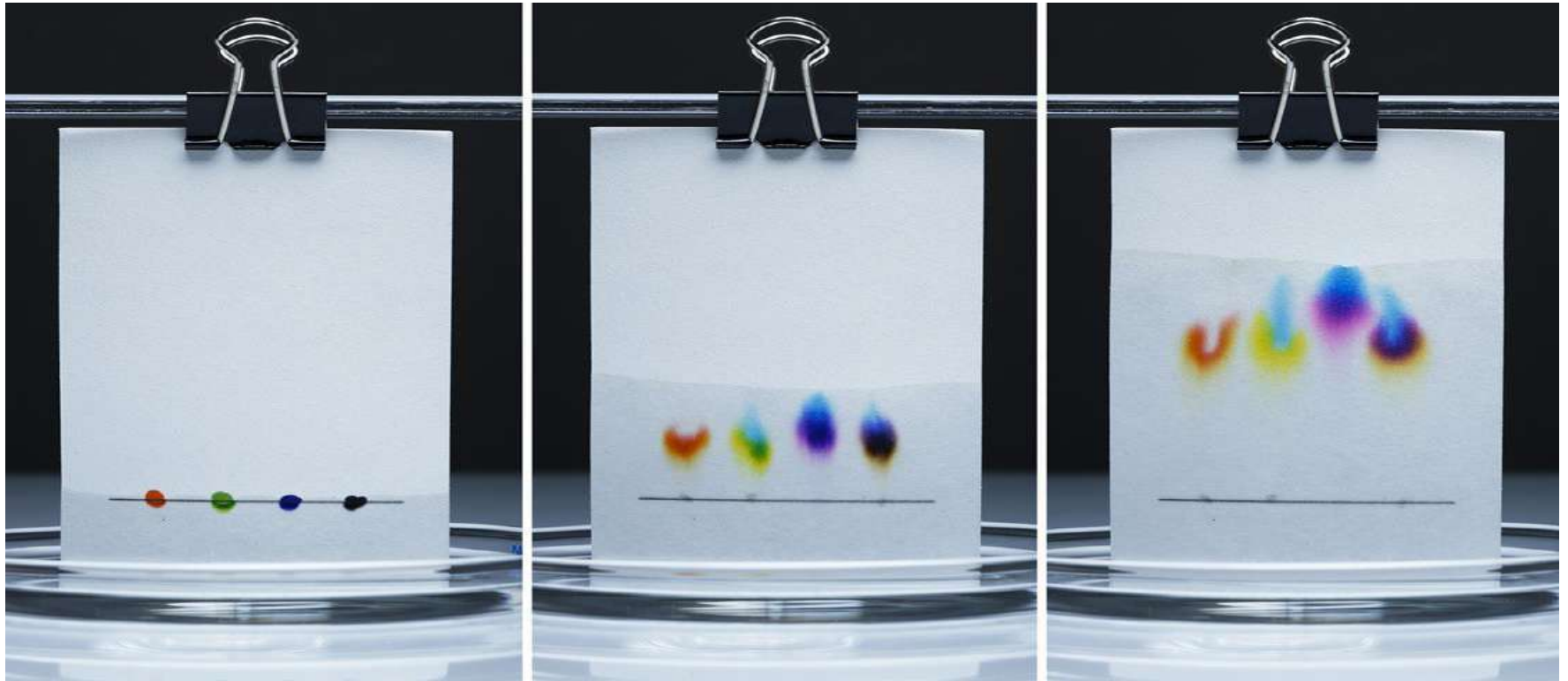
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# Paper Chromatography (PC)

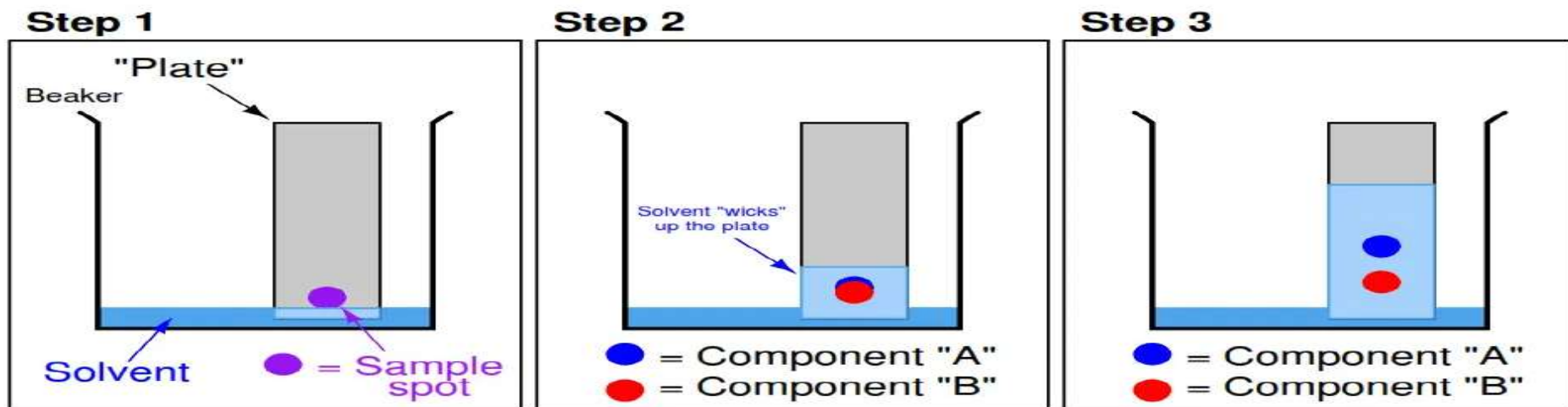
Paper chromatography: is one of the simplest forms of chromatography and the oldest. Paper chromatography is an analytical method that is used to separate colored chemicals substances, is generally used to separate water-loving compounds such as amino acids, peptides and sugars.



# Thin layer chromatography (TLC)

is a method for identifying substances and testing the purity of compounds is a chromatography technique used to separate organic compounds mixtures, Thin Layer Chromatography is performed on a sheet of glass, plastic or aluminum foil which is coated with the thin layer of absorbent material usually silica gel, aluminum oxide, or cellulose. **TLC** is a useful technique because it is relatively quick and requires small quantities of material

## *Thin-layer chromatography*

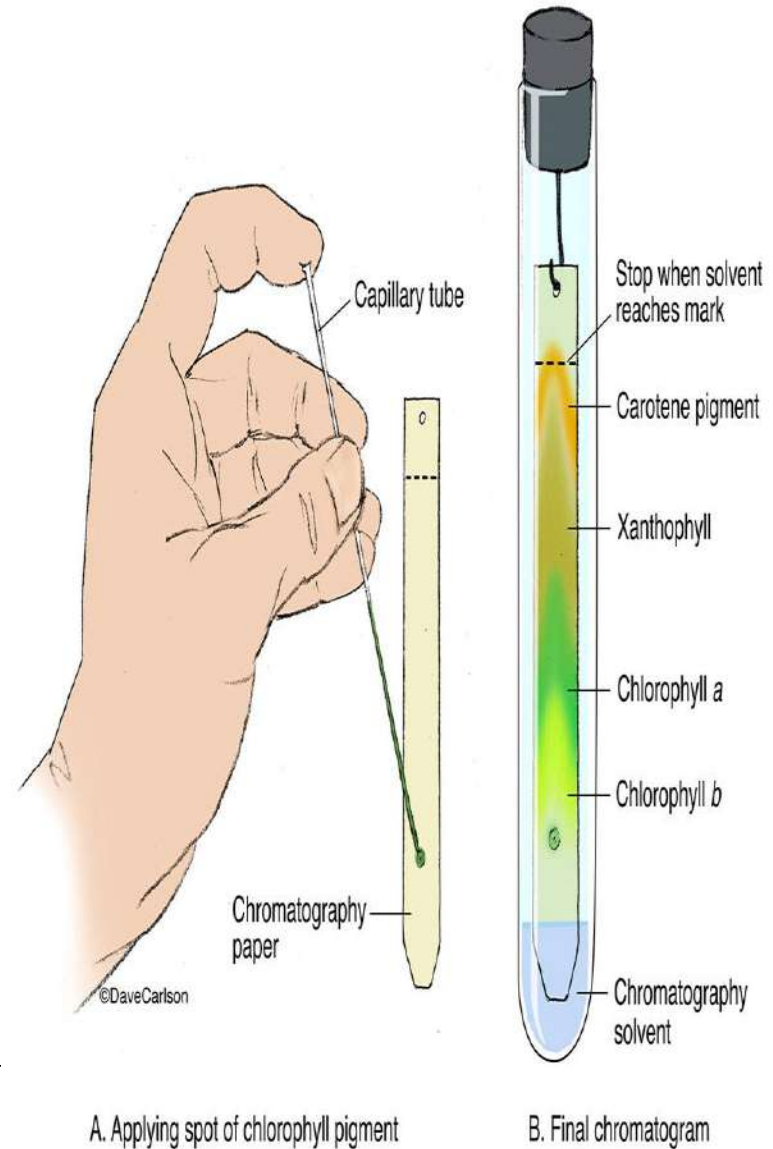


## **Advantages of TLC compared to over Paper Chromatography:**

- 1- In case of paper chromatography it takes 14-16 hrs. for the separation of the components but in TLC it takes 3-4hrs.
- 2- It is easier to separate and visualizer the components by this methods. 2- It is easier to separate and visualizer the components by this methods.
- 3- It has capacity to analyze multiple samples in a single run.
- 4- It is relatively a low cost.
- 5- Increased separation efficiency

# Procedure

- 1- A small of sample is applied to a strip of chromatography paper about from the two centimeters away base of the plate.
- 2- This sample is absorbed into the paper and may form interactions with it
- 3- The paper is then dipped into a solvent such as ethanol or water taking care that the spot is above the surface of solvent and placed in a sealed

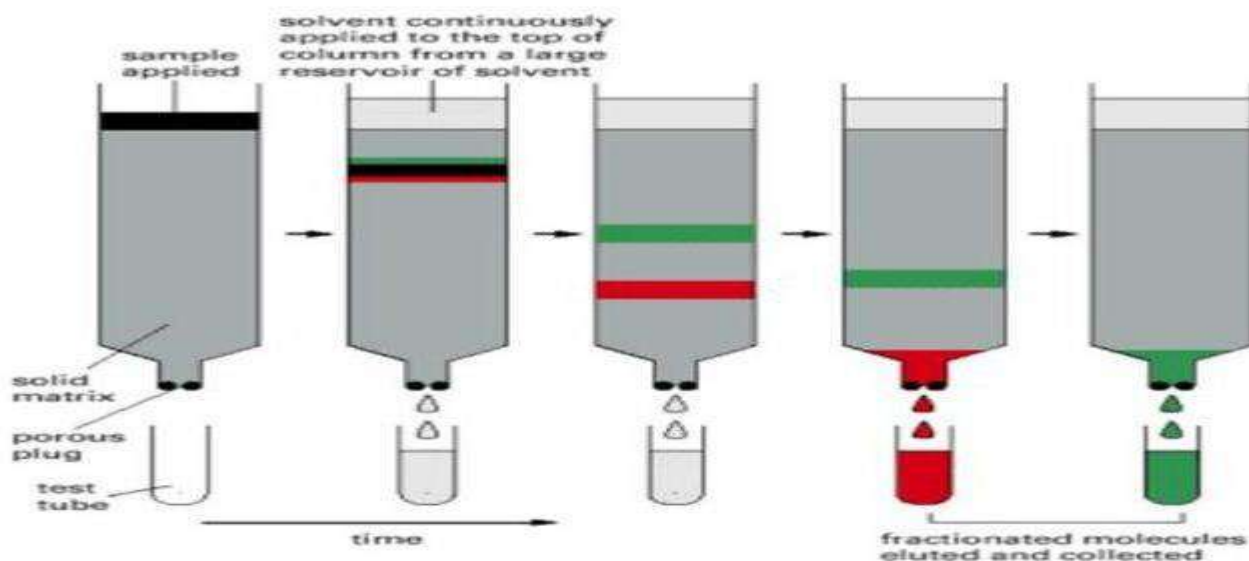


- 4- The solvent moves up the paper by capillary action and dissolves the sample mixture which will then travel up the paper with the solvent solute sample.
- 5- Different compounds in the sample mixture travel at different rates.
- 6- it takes several minutes to several hours.

**Analysis:** Spots corresponding to different compounds may be located by their color, UV. Light, Ninhydrine or by treatment with iodine vapors.

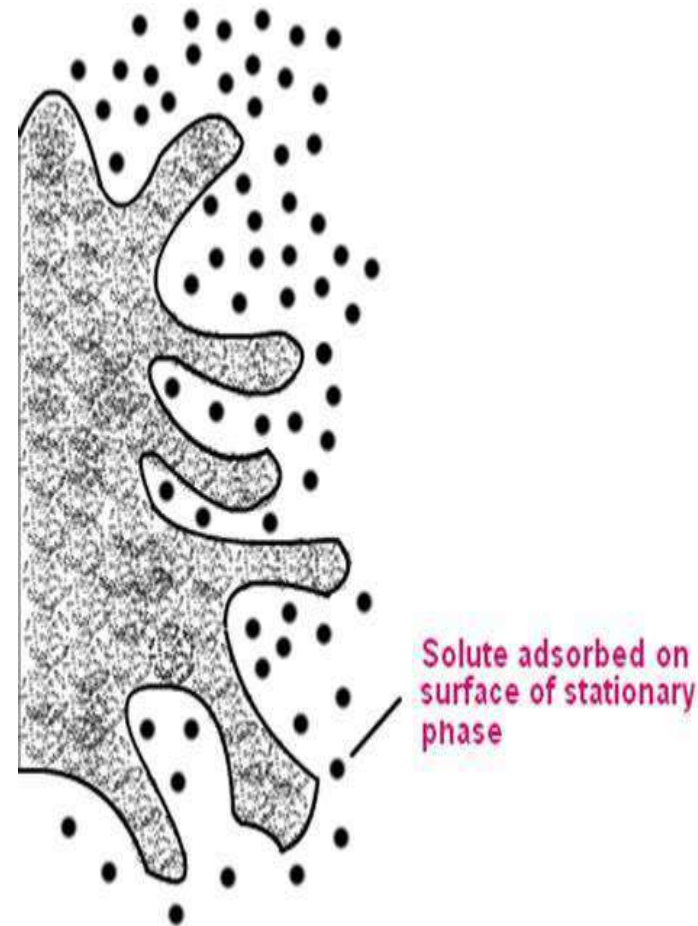
# Column Chromatography(CC)

Column Chromatography in chemistry is a chromatography method used to isolate a single chemical compound from a mixture. compounds move through the column at different rates, allowing them to be separated into fractions. The technique is widely applicable. It is often used for preparative applications on scale from micrograms up to kilograms



## Adsorption Chromatography:

Adsorption Chromatography is probably one of the oldest types of chromatography. It utilizes a mobile liquid or gaseous phase that is adsorbed into the surface of a stationary solid phase. The equilibration between the mobile and stationary phase account for the separation of different solute.

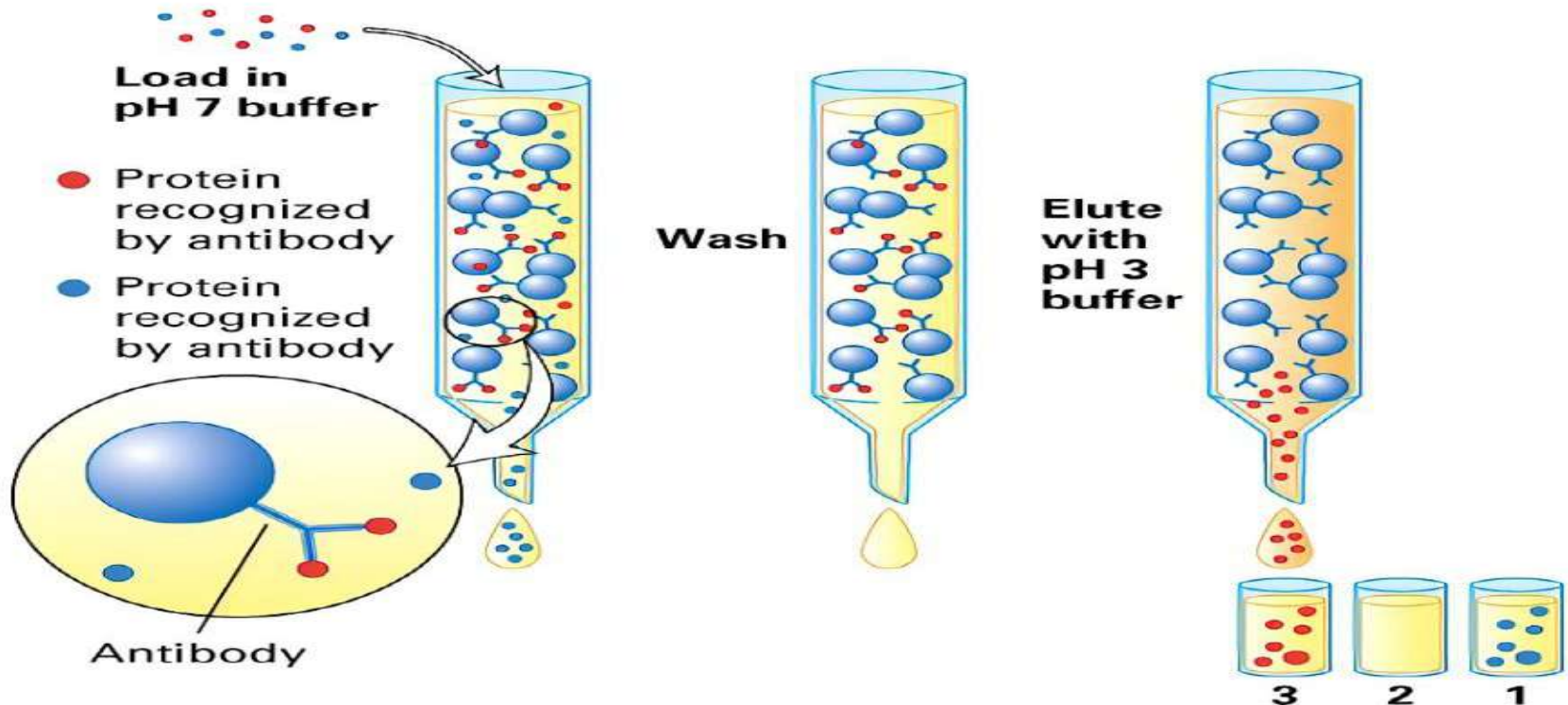




# Affinity Chromatography

Affinity Chromatography: is a method of separating biochemical mixture based on a highly specific interaction such as that between antigen and antibody, enzyme and substrate, or receptor and ligand.

(c) Antibody-affinity chromatography





# Type chromatography 2

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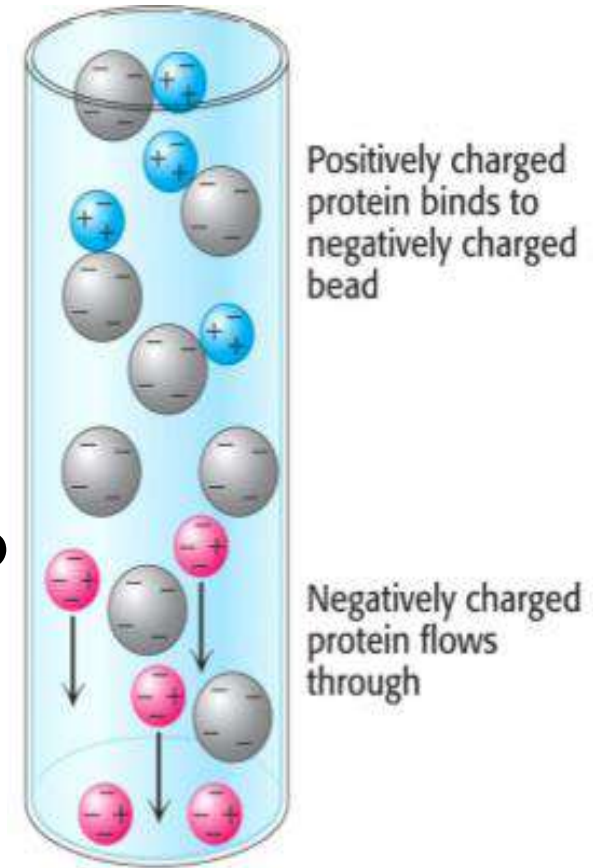


# **Ion Exchange Chromatography:**

**Ion Exchange Chromatography:** is a process that allows the separation of ions and polar molecules based on their electrical charges.

It can be used for separate almost any kind of charged molecules including large proteins small nucleotide and amino acids.

It is often used in protein purification, water analysis and quality control.



# Principle

**The stationary phase** is an ion exchange resin that carries charge functional groups that interact with oppositely charged groups of the compound to retain.

**The principle of separation** is thus by reversible exchange of ions between the target ions present in the sample solution to the ions present on ion exchangers resin.

**In this process two types of exchangers can be used:**

- 1- Cationic Exchangers: possess negatively charged group, and these will attract positively charged cations. These exchangers are also called "Acidic ion exchange" materials
- 2- Anionic Exchangers: have positively charged groups that will attract negatively charged anions. These are also called "Basic ion exchange" materials.

# Ion Exchange Chromatography

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graph TD; A[Ion Exchange Chromatography] --> B[Cation Exchange Chromatography]; A --> C[Anion Exchange Chromatography]; B --> D[Solute cations are attached to the negatively charged sites covalently bond to the stationary phase]; C --> E[Solute anions are attached to the positively charged sites covalently bond to the stationary phase];
```

## Cation Exchange Chromatography

Solute cations are attached to the negatively charged sites covalently bond to the stationary phase

## Anion Exchange Chromatography

Solute anions are attached to the positively charged sites covalently bond to the stationary phase

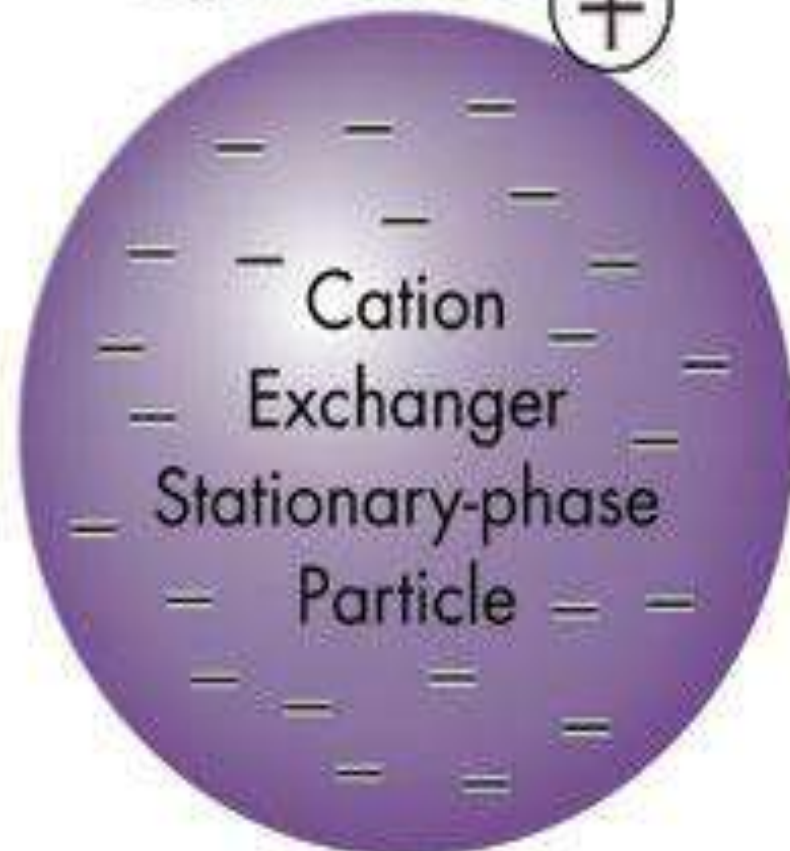
Negatively Charged  
Analyte [Anion]

Attracted to  
Positive Surface



Positively Charged  
Analyte [Cation]

Attracted to  
Negative Surface



# Applications of Ion-Exchange

1- Water softening:

Removal of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ .

2-Water demineralization:

Removal of cations & anions dissolved in water.

3-Separation of electrolytes from non-electrolytes.

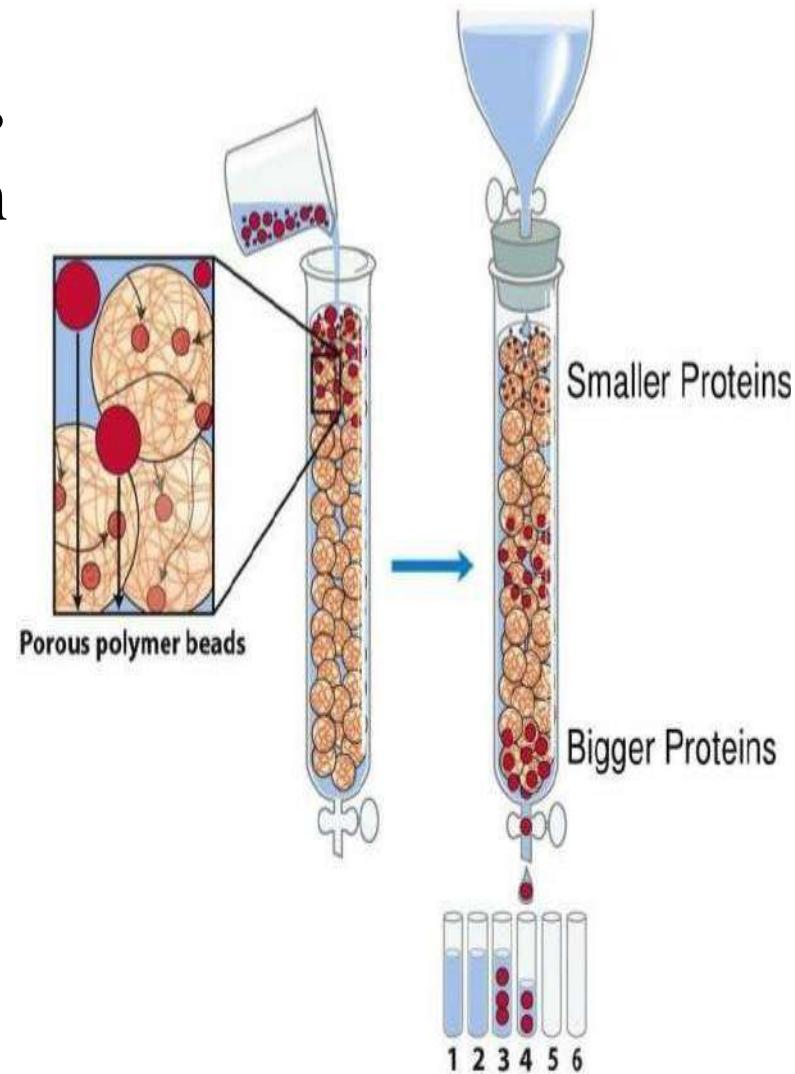
4- Separation of carbohydrates, Proteins & their derivatives.



## Gel filtration chromatography:

**Gel filtration chromatography:** is a chromatography method in which molecules in a solution are separated by their size, shape and molecular weight.

It is usually applied to large molecules or macromolecular complexes such proteins and industrial polymers.

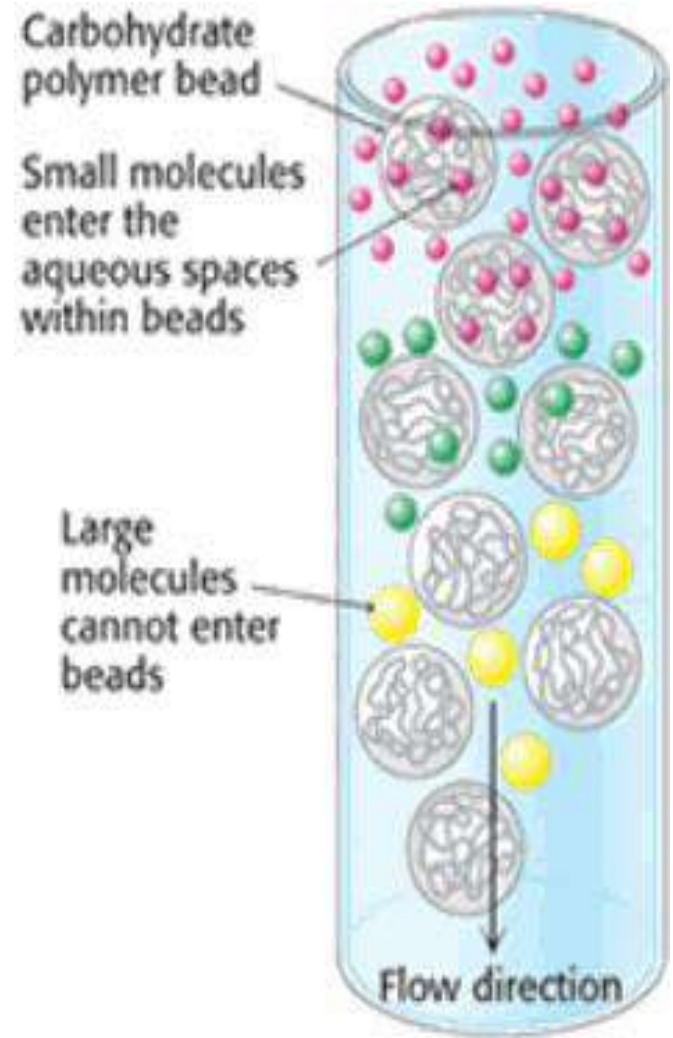


# Principle

**Large analytes** that are completely excluded from the pores will pass through the interstitial spaces between the particles and will appear first in the eluate.

**Smaller analytes** will be distributed between the mobile phase inside and outside the particles and will therefore pass through the column at a slower rate, hence appearing last in the eluate.

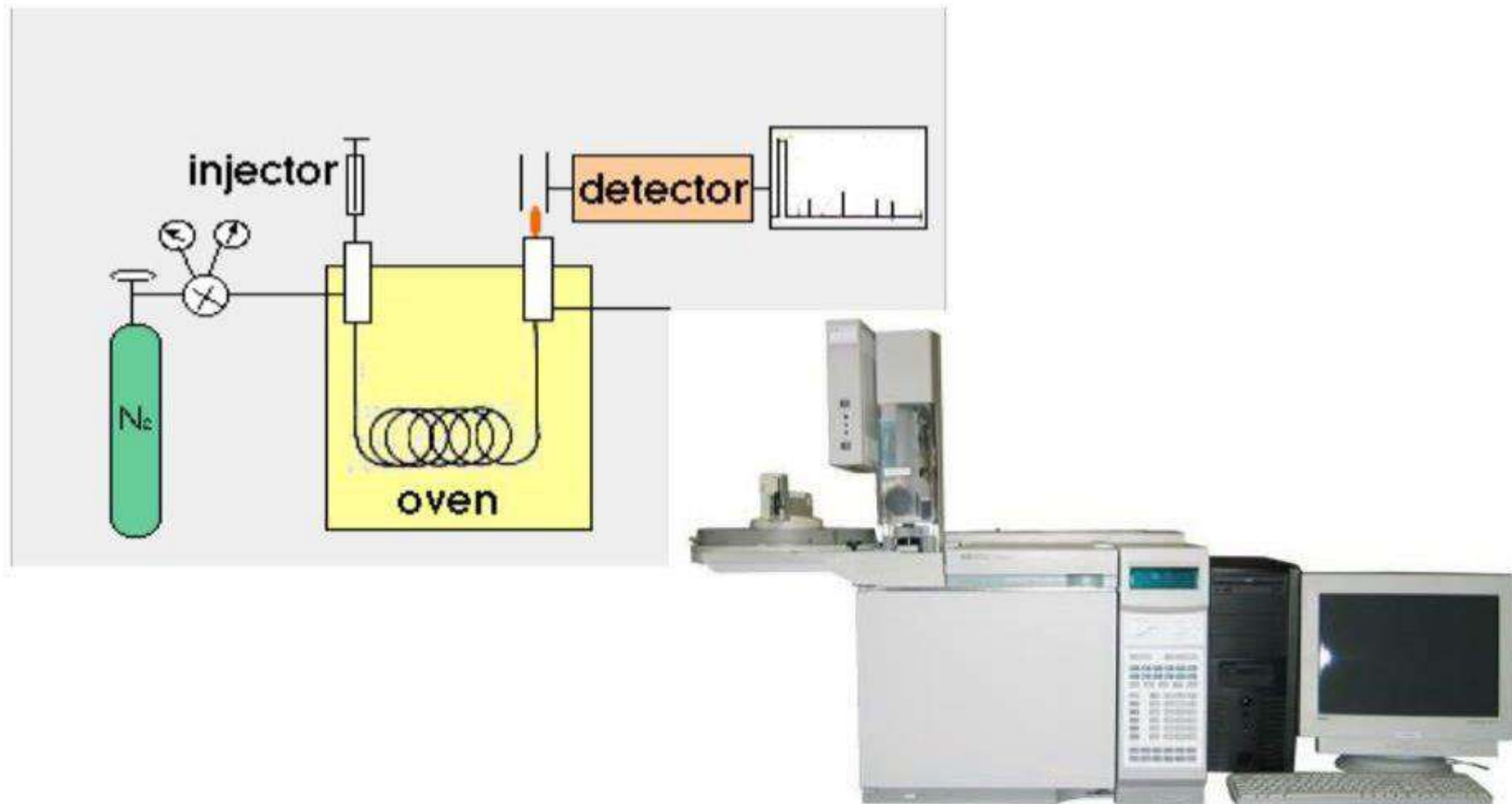
This is how the molecules are separated.





# Gas chromatography

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# Gas Chromatography (GC)

Gas chromatography (GC): is common type of chromatography technique for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture.

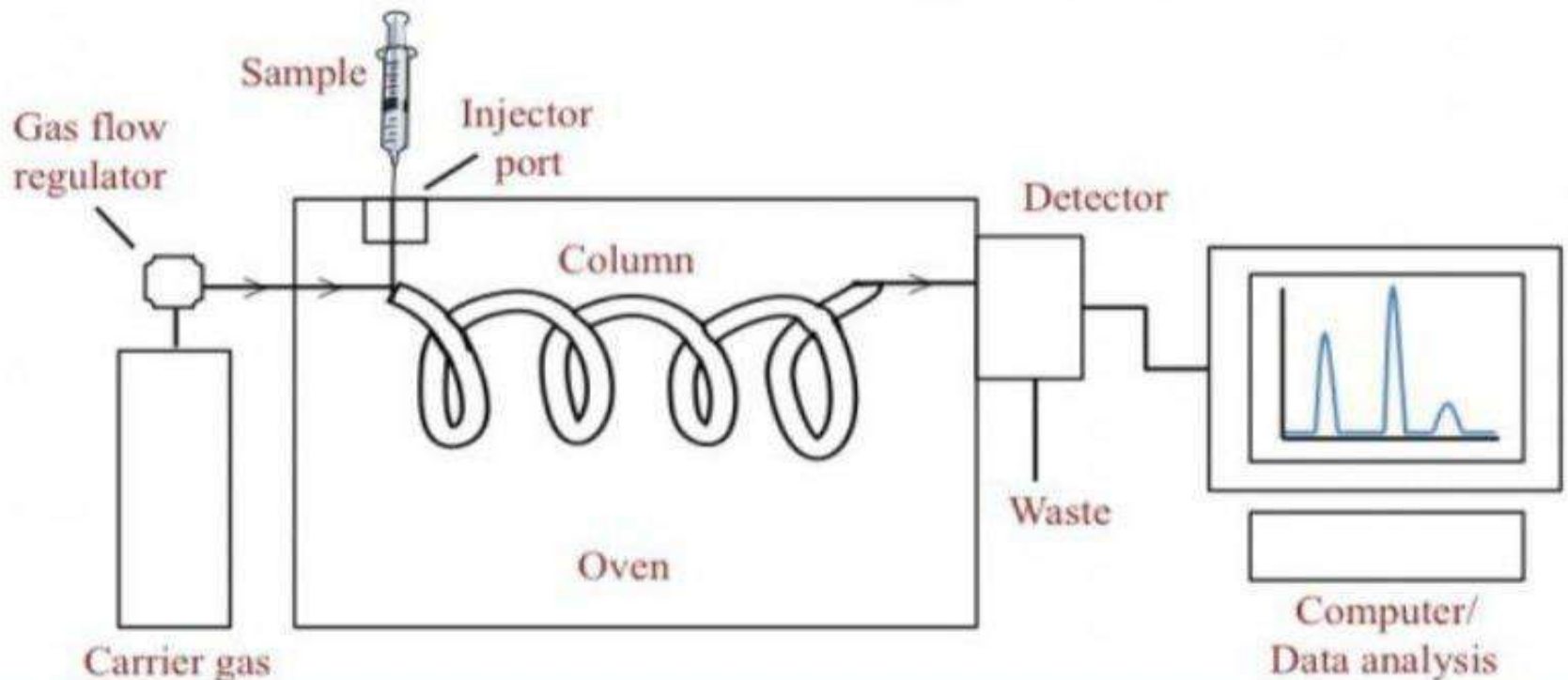


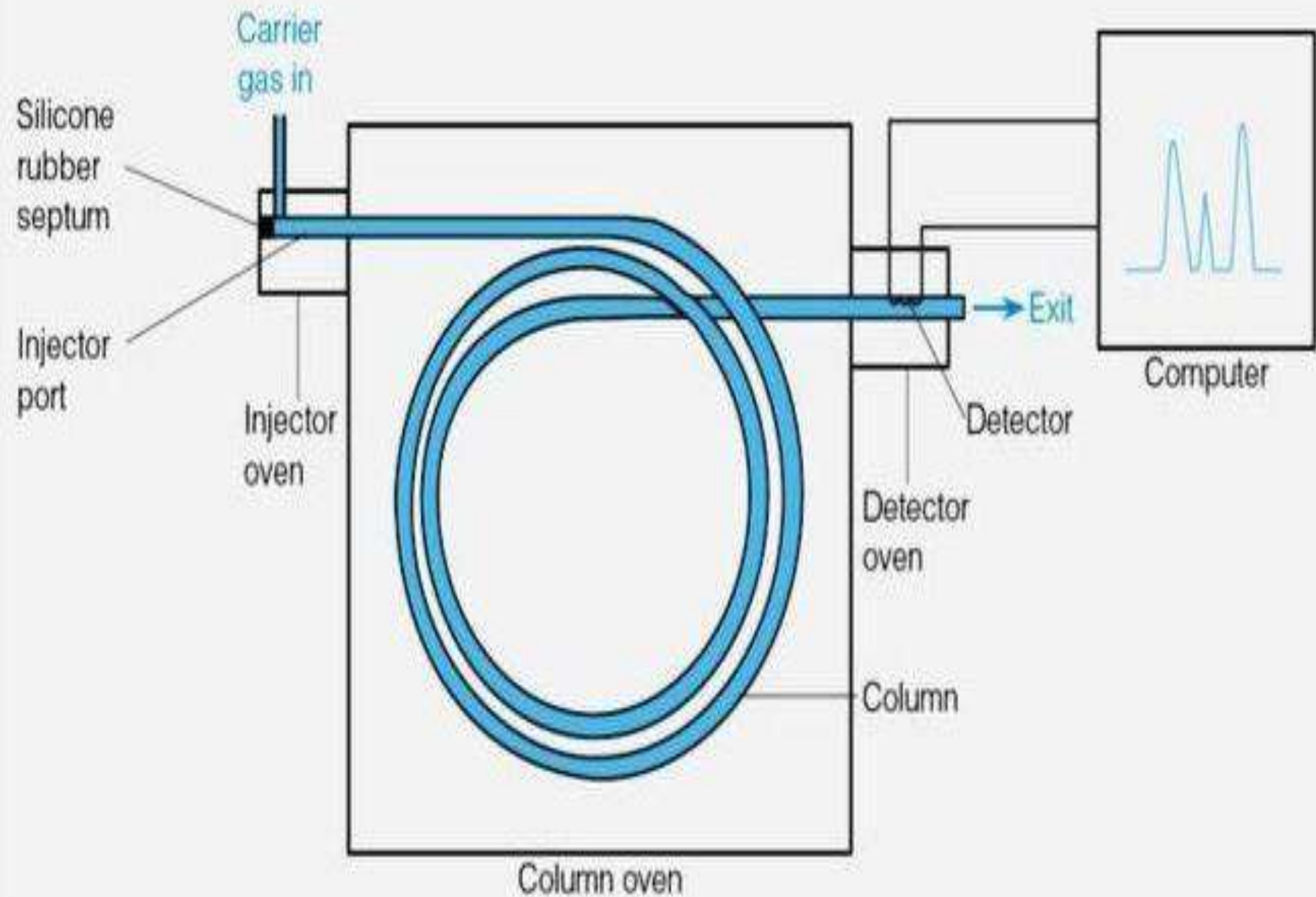
## WORKING PRINCIPLE OF GC

- It works on the principle that the organic compounds are separated due to differences in their partitioning behavior between mobile phase and stationary phase.
- The mobile phase is consists of inert gas.
- The stationary phase consists of packed column

# **GAS CHROMATOGRAPH MAIN COMPONENTS:**

- **Carrier Gas:** This is the mobile phase and should be inert gas(He, Ar, N,etc).
- **Injection Port:** The injection port consist of rubber septum through which a syringe needle is inserted to inject the sample.





# PROCEDURE OF GC

- Sample is injected in column.
- Oven heats the system to vaporize the sample and speed its passage through the column.
- The different components of the sample will be separated by the column because each of the components "sticks" to the liquid coating that on the column packing differently.
- When a substance leaves the column, it is sensed by a detector.
- The detector generates the voltage that is proportional to the amount of the substance.

# Factors Affecting GC

**1- Temperature**

**2- Carrier gas flow:** If the carrier gas flow is high, the molecules do not have a chance to interact with the stationary phase.

**3- Column length:** The longer the column is the better the separation usually is.

**4- Amount of material injected:** The injection of too much sample causes poor separation.

# Advantages of Gas Chromatography

- 1- Very good separation.
- 2- Time (analysis is short).
- 3- Small sample is needed.
- 4- Good detection system.
- 5- Quantitatively analyzed



# Application of Gas Chromatography

- 1- In assuring the quality of products in the chemical industry; or measuring toxic substances in soil, air or water.
- 2- GC is very accurate if used properly and can measure pico-moles of a substance in a 1 ml liquid sample, or parts-per-billion concentrations in gaseous sample.
- 3- The hydrocarbons are separated using a capillary column and detected by GC technique

# Specimens Collection

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# **STEPS IN THE INVESTIGATION OF A PATIENT**

1. Patient History
2. Physical Examination
3. Laboratory Tests
4. Imaging Techniques
5. Diagnosis
6. Therapy
7. Evaluation

# Biological Specimens:

- 1) Blood
- 2) Urine
- 3) Cerebrospinal fluid (CSF)
- 4) Sweat
- 5) Semen Fluid
- 6) Amniotic Fluid
- 7) Gastric juice
- 8) Kidney stone
- 9) Gall stone
- 10) Stools
- 11) Tissue Specimens and ele.

# Specimen Collection

Proper collection of specimens is important to maximize the outcome of laboratory tests for the diagnosis of infectious diseases.

A variety of laboratory tests can be performed to make a presumptive or definitive diagnosis so that therapy can begin.

A Blood sample is taken by physician in a laboratory or hospital and in many cases the blood sample is sent on to analysis, It takes about 5 minutes to take a blood sample.

Blood samples are taken in the arm, hand, finger or ear, depending on the analysis to be made

**Choice of specimen type depends on:**

- 1) The analysis to be measured.
- 2) Easy of collection

# Blood Specimen Collection

## Definition:

Venipuncture involves puncture a with a needle and collecting blood in a syringe or evacuated tube.

It is performed using the antecubital fossa. Vein of the wrist, the dorsum of the hand or foot or other accessible location.



# **Blood Can be Collected from 3 different Sources:**

- 1) Capillary blood
- 2) Venous blood
- 3) Arterial blood



# Capillary blood-Method of Collection

- 1) Select the least used finger
- 2) Cleanse the site with alcohol swab
- 3) Puncture transfer blood to a strip or small container

**Capillary sampling:** (i.e. finger or heel-pricks or rarely, an ear lobe puncture) for analysis of capillary blood specimens for all ages; examples include testing of iron levels before blood donation blood glucose monitoring and rapid tests for HIV, malaria and syphilis

Blood collection is used to obtain blood from donors for various therapeutic purposes.



# Blood Collection for Arterial Blood:

## Equipment Required:

- 2) Syringe
- 3) Alcohol swab
- 4) Bandage



After a pulse is found, a blood sample is taken from the artery

**Arterial Blood Gases (ABG):** test measures the acidity (pH) and the levels of oxygen, and carbon dioxide CO<sub>2</sub> in the blood from an artery. This test is used to find out how well your lungs are able to move oxygen into the blood and remove carbon dioxide from the blood.

# Blood Collection for Venous Blood:

Equipment Required:

- 1) Tourniquet
- 2) Vacutainer and syringe
- 3) Alcohol swab
- 4) Bandage

**Venous blood:** is the specimen of choice for most routine laboratory tests. The blood is obtained by direct puncture to a vein, most often located in the antecubital area of the arm or the back (top) of the hand.

Most laboratory reference ranges for blood analyses are based on venous blood.

## Precaution

- Venipuncture area must be cleaned/ sterilized properly
- Tourniquet should not be applied for a long time and not more than 1 minuet

# Blood Collection

The Two Sources of Blood for Examination in the Laboratory are:

- 1) Venous Blood Collection: this is obtained by venipuncture. It is preferred for most hematological examinations and drains a large volume of blood. Its color is dark red.
- 2) Capillary Blood: this is usually obtained by skin puncturing of the pulp of the finger, earlobe or the heel (in infants). It recovers a small volume of blood.

**Note**: Arterial Blood may be needed for blood gas analysis this procedure is not usually performed in the hematology laboratory