KING ABDULAZIZ UNIVERSITY
FACULTY OF SCIENCE

PHYSICAL BIOCHEMISTRY (BIOC 341)

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2020 3

BIOC 341

The performance in this course will be evaluated in five areas : home works , lab. Exam and two exams

- First exam 15 %
- Second exam 15 %
- Lab. Exam 25 %
- Final exam 40 %
- Activity and homework 5 %

- **Course Description:** The goal of "Physical Biochemistry" course is to introduce undergraduate students at the Biochemistry Department to basic concepts of physical biochemistry. The instructor will address the fundamentals of thermodynamics, importance of knowing the type of intermolecular forces, membrane equilibrium and major techniques used in physical biochemistry.

Contents

INTRODUCTION

PHYSICAL BIOCHEMISTRY

- Physical biochemistry is a branch of biochemistry that deals with the theory, techniques and methodology used to study the physical properties of biological macromolecules, including proteins, RNA, DNA, and other biological polymers.
- This gives us the advantage of using the tools of the physical science to explore the complexities of biological systems.
- These physical properties provide a description of their structures at various levels, from the atomic level to large multi-subunit components.

CHAPTER I:
INSTRUMENTATION (PHYSICAL TECHNIQUES USED IN BIOCHEMISTRY)

Polarimeter

Polarimetry

[Polarimeter]

Polarimetry is an instrumental analytical method using rotation of polarized light by some substances as a measure of their concentration in a solution. The instrument used is called a **polarimeter**. When it is adapted for measuring quality of sugar the name saccharimeter is used. In both instruments it is the rotation of polarized light by a substance in a solution which is measured.

In the human body there are some compounds that have the ability to rotate the plane of polarization of light; these compounds are called " **Optically active**" compounds, such as sucrose, glucose, fructose, and amino acids.

This activity is due to the presence of **one or more** of the **chiral atom (*active atom*)** in these compounds. Each compound has a constant rotation angle called "**specific rotation angle**".

Optical Active Compounds

Any substance to be optically active must have chiral atom and able to rotate plane polarized light to the right and the other rotates it to the left.

If it rotates light to right → substance has D "dextro" (+)

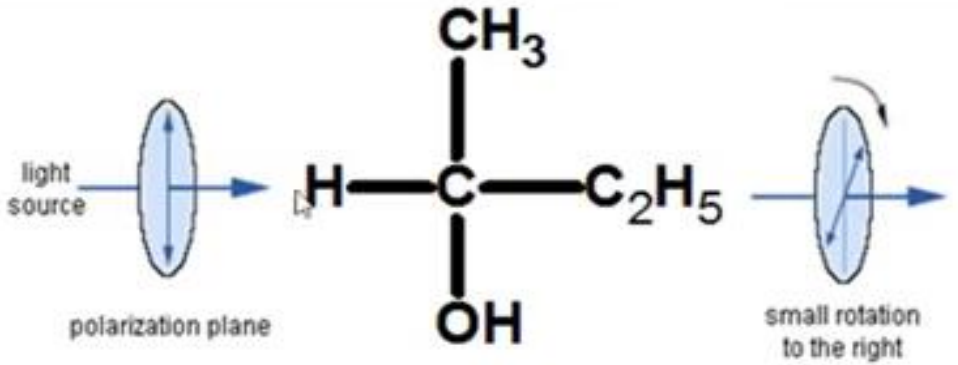
If it rotates light to left → substance has L "levo" (-)

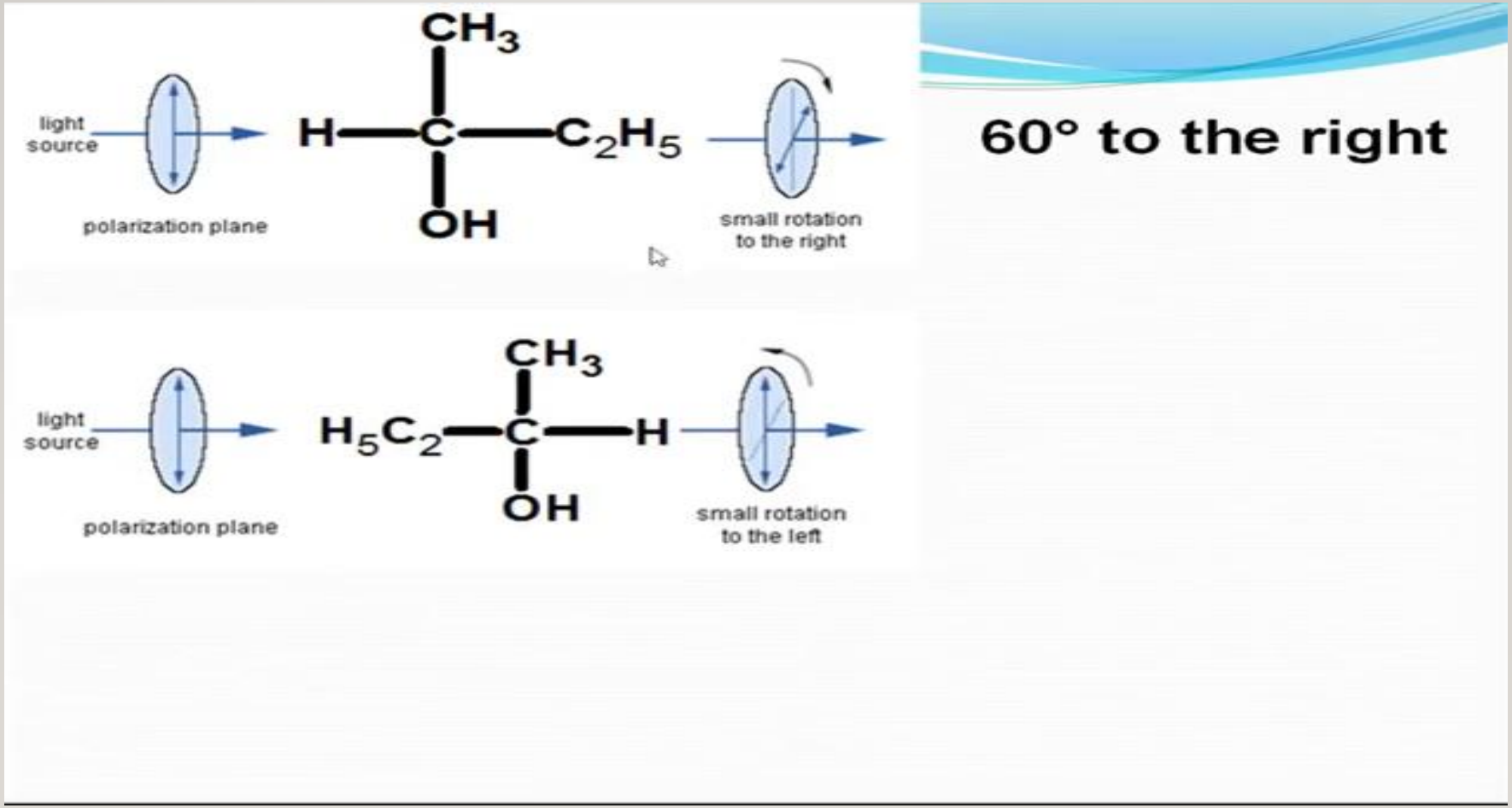
Racemic mixture: is a mixture which contains equal amounts of each enantiomer.(50% D and 50% L) and is **optically inactive** because the rotation cancel each other.

Optical Activity

- The ability of organic compound to rotate plane polarized light either to right or left

- How can we measure Optical Activity?
  - By Apparatus polarimeter Using monochromatic light




light source
polarization plane
CH3
H
C
C2H5
OH
small rotation to the right
60° to the right
light source
polarization plane
CH3
H5C2
C
H
OH
small rotation to the left

Enantiomers

Mirror images and non superimposable

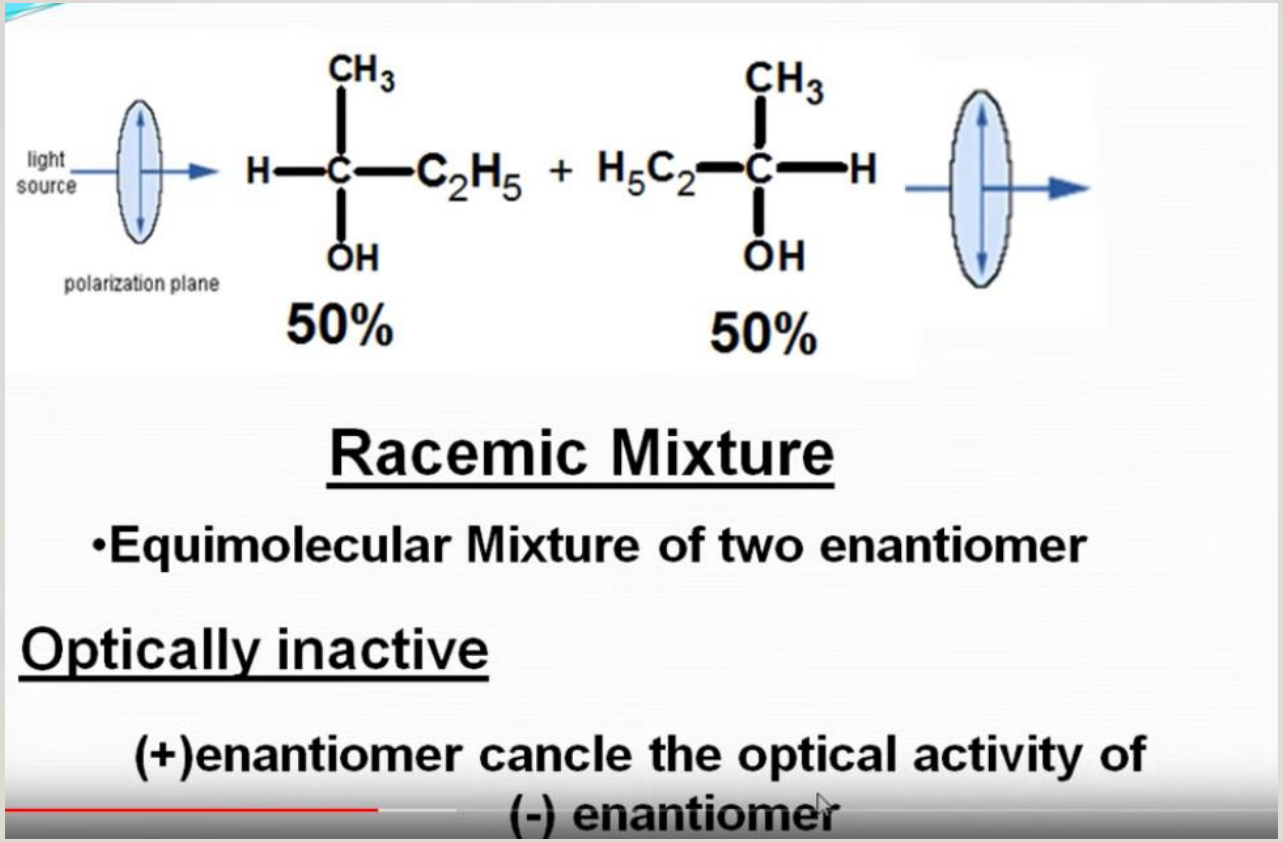
Compound contain one chiral center"Axis" or more

Have the same physical properities As:

- Melting point
- Boiling point
- Solubility

Have the same chemical properities

They rotate plane polarized light in equal angles but opposite directions


light source
polarization plane
CH3
H
C
C2H5
OH
50%
+
H5C2
CH3
C
H
OH
50%
Racemic Mixture
•Equimolecular Mixture of two enantiomer
Optically inactive
(+)enantiomer cancle the optical activity of
(-) enantiomer

To test the optical activity, we will use the **polarimeter** instrument. It is a device that measures the angle of plane polarized light that is rotated when passing through a solution.

Or it is an instrument which is used to determine optical activity of a compound.

Instrumentation and Principle

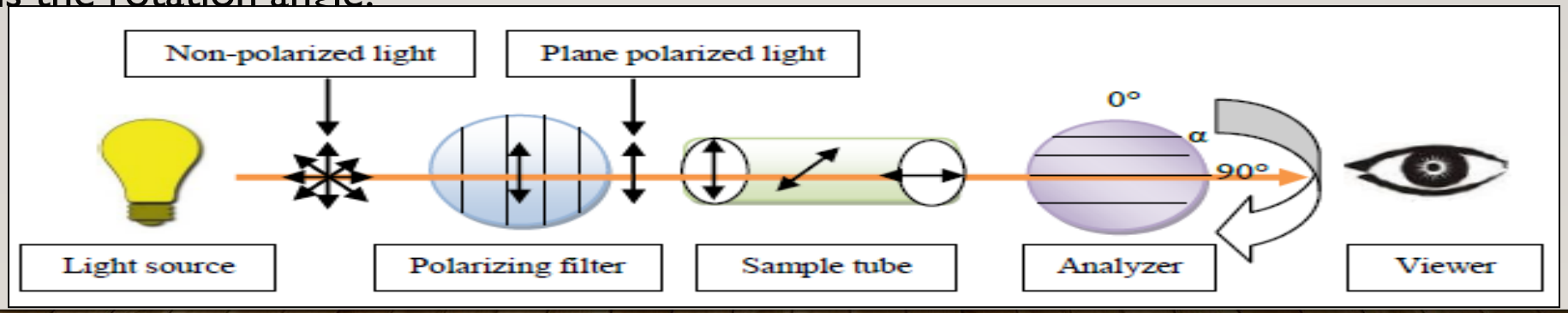
The light source: the light is unpolarized and moves in all directions.

Polarizer: (usually made of quartz), it takes light vibrating in all planes (unpolarized) , and make it vibrating in one plane only (polarized).

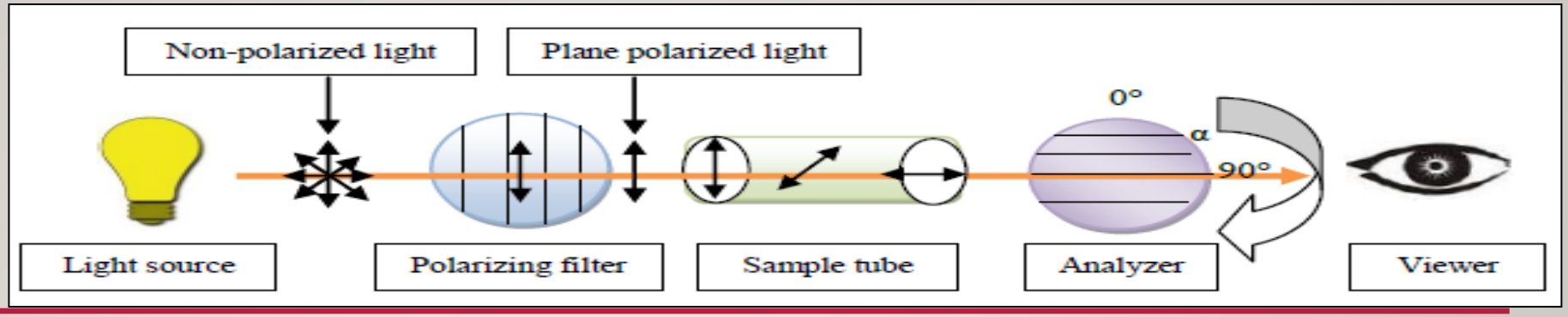
Sample tube: holds the optically active sample and has a known length (L).

Analyzes: detects the rotation of the polarized light that passes through the sample.

Detector: reads the rotation angle.



Principle



1-Unpolarized light from the light source is first polarized.

2-This polarized light passes through a sample cell.

3-If an optical active substance is in a sample tube, the plane of the polarized light waves is rotated.

4-The rotation is noticed by looking through the analyzer as a change in intensity of illumination.

5-To reach the same illumination as was without an optical active sample the analyzer must be turned around for an angle.

6- Readings are taken in degrees (rotation angle)

Calculation of specific rotation:

We use **Biot's law** to calculate specific rotation.

$$[\alpha] = \alpha / lc$$

Where,

[α]= specific rotation **[**This value is characteristic for a given compound**]**,

α (alpha)=observed rotation in degrees.

l = cell path length in decimeters.

c= concentration(g%)

The measured angle of rotation depends upon many variables:

1-The type or nature of sample (ex. sugar solution)

2-Concentration of the optical active components

3-The wavelength of the light source

4-Temperature of the sample.

5- The length of the sample tube

Specific rotation is determined at a specified temperature (usually 20 C) and a wavelength of light source (usually sodium lamp at 589.3 nm).

1-The type or nature of sample (example: sugar solution)

Some substances rotate the light to the right (or clockwise), we sign this rotation as [+], some to the left (or anticlockwise), signing α as [–]

Example:

Specific rotation [α] of some sugars solution dissolved in H2O as follows:

sucrose + 66.54

glucose + 52.74

fructose - 93.78

maltose + 137.5

lactose + 55.3

[α]= specific rotation [This value is characteristic for a given compound]

2-Concentration of the optical active components

For example: Sucrose (cane sugar) solution specific rotation = + 66.54 at a concentration of 1 g/ml.

$[\alpha] = \alpha/lc$

3-The wavelength of the light source

The influence of the wavelength of a light source on specific rotation of sucrose solution is seen from the following table:

Light source	Wavelength [nm]	Specific rotation
Mercury, green	546.23	+ 78.4178
Sodium, yellow	589.44	+ 66.5485
HeNe Laser	632.99	+ 57.2144
Near Infrared (NIR)	882.60	+ 28.5462

4-Temperature of the sample.

The influence of temperature on specific rotation for sugar solutions is seen from the following

Temperature (C)	Rotation of a sugar solution (α)
20	40.000
21	39.981
25	39.906

Notice the decrease of the rotation of sucrose solution with rising temperature. Also the effect of temperature is relatively small.

Applications

1. **Measuring specific rotation and optical rotation** are used to determine the purity of products regards to how much D and L (it is used in quality control).
2. **Pharmaceutical:** only one isomer is pharmaceutically active, so production of highly pure compounds increases the quality and cost of the product.
3. **Food industry:** To check the purity of raw material as flavor, fragrance and essential organic oil.
4. **Chemistry:** Use optical and specific rotation to identify natural polymers, and synthetic polymer.

Viscometer

Viscosity

What Is Viscosity?

Viscosity is commonly described as the thickness of the fluid. For exp., honey is more viscous than water (i.e. flow more slowly than water).

Viscosity is a physical property which describes the fluid resistance to flow. It is based on the theory that the fluid composed of different layers, when fluid moves frictional force is created between these layers which resists fluid flow. The force required to cause a layer to move will increase as the friction increases, which is called "**shear**". This shearing effect will happen as a fluid is physically moved.

How to Measure Viscosity?

- Viscosity can be measured with a **viscometer**, an equipment that measures the force necessary to move through a liquid.
- The idea behind measuring viscosity is to determine how resistant that material is to flowing.

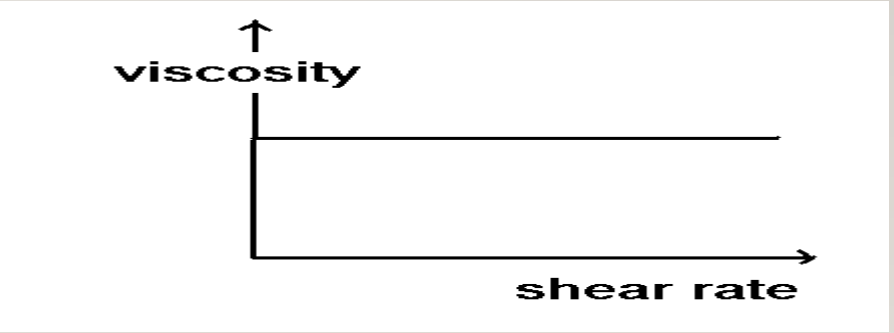
[More force therefore is required to move highly viscous fluids].

High frictional force>>> higher resistance >>> higher viscosity

- **Types of fluids**

1- Newtonian fluids (**Ideally viscous):** If a fluid's internal flow resistance is independent of the external force (shear rate), it is ideally viscous. Such fluids are named Newtonian liquids. Typical Newtonian liquids are water or motor oil.

2- Non-Newtonian fluids **(not ideally viscous):** If a substance is not ideally viscous, its viscosity changes with the shear rate. Non-Newtonian fluids include paints, emulsions, tomato ketchup, etc.



- Water, for example, is a **Newtonian fluid**. Regardless of whether you shake the cup of water, the viscosity/thickness or rate of flow doesn't change. **Ketchup**, on the other hand, is a different beast. **Ketchup** is a non-**Newtonian fluid** because, unlike water, its viscosity is dependent on shear rate **Blood**, on the other hand with a knife in it, is a non-**Newtonian fluid**. Its viscosity changes depending on how much stress is placed on it. ... It's a so-called "shear-thinning" **liquid**–the more **blood** is agitated the less viscous it becomes. But **blood** is just one type of **fluid** that flows unlike what you'd expect

• Factors Affecting Viscosity (η)

A substance's flow behavior depends on three factors:

1- The substance's inner molecular structure. The tighter the molecules are linked, the more the substance will resist deformation.

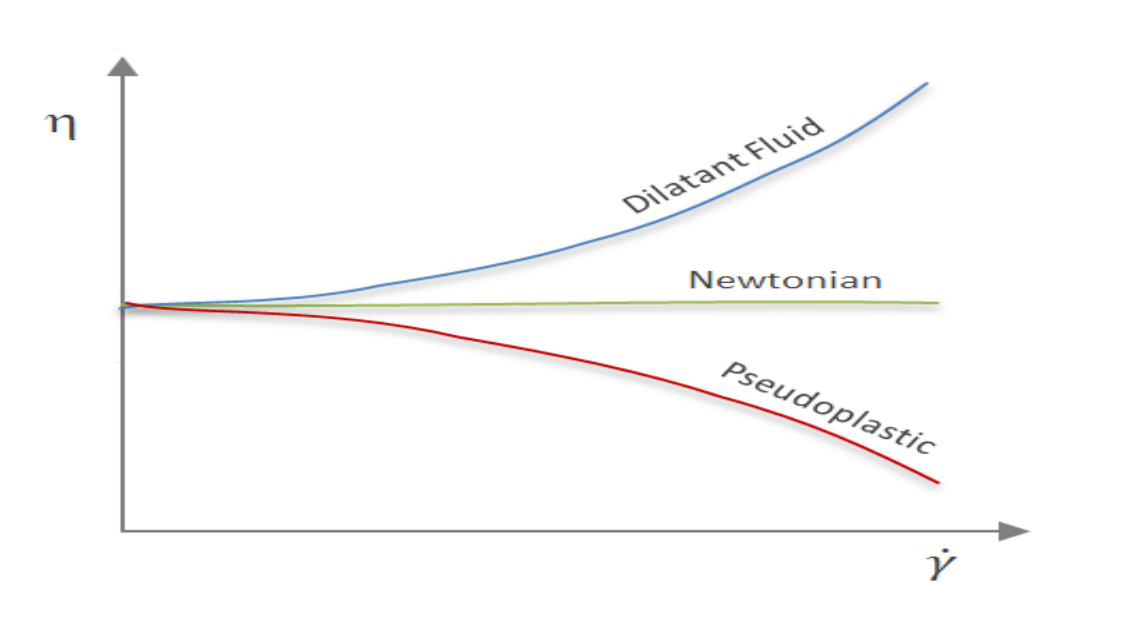
2- The external forces acting upon the substance that deform it or make it flow. Both the *intensity* of the external force as well as the *duration* has an influence. The external force can have the form of pushing or tearing a substance. In viscometry, the external forces define as **shear rate** or **shear stress**

Shear rate is the **rate** at which a fluid is sheared or "worked" during flow. In more technical terms, it is the **rate** at which fluid layers or laminae move past each other. ... The **shear stress**, τ, is the force per area, dynes/cm^2. The viscosity, η, is the relationship **between** the **shear stress** and the **shear rate**.

3- The surroundings conditions.

a-Temperature (°C): when temperature increases the viscosity decreases,

b-Pressure: when pressure increases the viscosity increases.



Viscosity Units

The most commonly used unit for dynamic viscosity is the *centipoise* (cP), which is equivalent to 0.01*Poise* (P). This unit is used in honor of French physicist, Jean Léonard Marie Poiseuille (1797-1869).

The SI unit for dynamic viscosity **η** is the *Pascal-second* (**Pa-s**).

The *millipascal-second* (mPa-s) is often used instead.

Note that **1 mPa-s = 1 cP**

What is the importance of viscosity in Biochemistry?

1-Viscosity affect on the diffusion rate of molecules throughout the cell

2-Viscosity play an important role in cell homeostasis [ex. body temperature and body fluids]

3-Viscosity is very important for cytoplasm movements.

4-The viscosity of biological membranes is very strictly controlled, depending on temperature and degree of unsaturation in the phospholipid chains.

5-Measuring blood viscosity is very important to improve patient outcomes.

Factors Affecting Blood Viscosity

1- Erythrocyte deformability. This refers to the ability of RBCs to bend and fold themselves in order to make their way through the capillaries. RBC deformability is inversely correlated with blood viscosity, meaning that the more deformable the RBCs are, the less viscous the blood.

2- Plasma viscosity refers to the viscosity of the non-cellular matrix of the blood. An important determinant of plasma viscosity is hydration status. Dehydration increases blood viscosity

What causes blood viscosity?

Increased **blood viscosity** can be caused by an increase in red cell mass or increased red cell deformity, increased plasma levels of fibrinogen and coagulation factors, and dehydration

What does high viscosity in blood mean?

"**Viscosity** is an indication of the 'thickness' of the **blood**, or its resistance to flowing normally. ... She says that **blood viscosity** can increase because of many factors, such as certain medications, too many red **blood** cells, **high** lipid levels, and other conditions, including diabetes and cancer

3- RBC sedimentation/aggregation is the tendency of RBCs to be attracted to each other and stick together. There are numerous factors that can increase sedimentation and aggregation. Blood viscosity correlates directly with both RBC aggregation and plasma viscosity.

4-Hyperlipidemia increase the viscosity of the plasma

5-Inflammation increases cytokines that affect the polarity of RBCs, making them stickier and more attracted to each other.

Blood Viscosity and Diabetes

1-It is has been demonstrated by many investigators that diabetics have elevated blood viscosity.

2-It is also known that red cell deformability of the blood affected by uncontrolled blood glucose. Diabetics have a higher proportion of red cells that are relatively non-deformable.

Blood Viscosity and Cognitive Decline

Multiple forms of cognitive decline, including dementia and Alzheimer's disease, are affected by *increased blood viscosity*. Blood viscosity is an important determinant of the circulatory flow of blood and was shown to be significantly linked with cognitive function.

SPECTROPHOTOMETRY

WHAT IS SPECTROSCOPY ?

- It is the branch of science that deals with the study of interaction of electromagnetic radiation with matter.
- Using electromagnetic radiation as a probe to obtain information about atoms and molecules that are too small to see.
- Spectroscopy can tell us so many things about the structure and different types of motions within an atom or a molecule

1-Types of Radiation

- There are various kind of **radiation** which can be classified in electromagnetic radiation and particle radiation

Radiation is classified into:

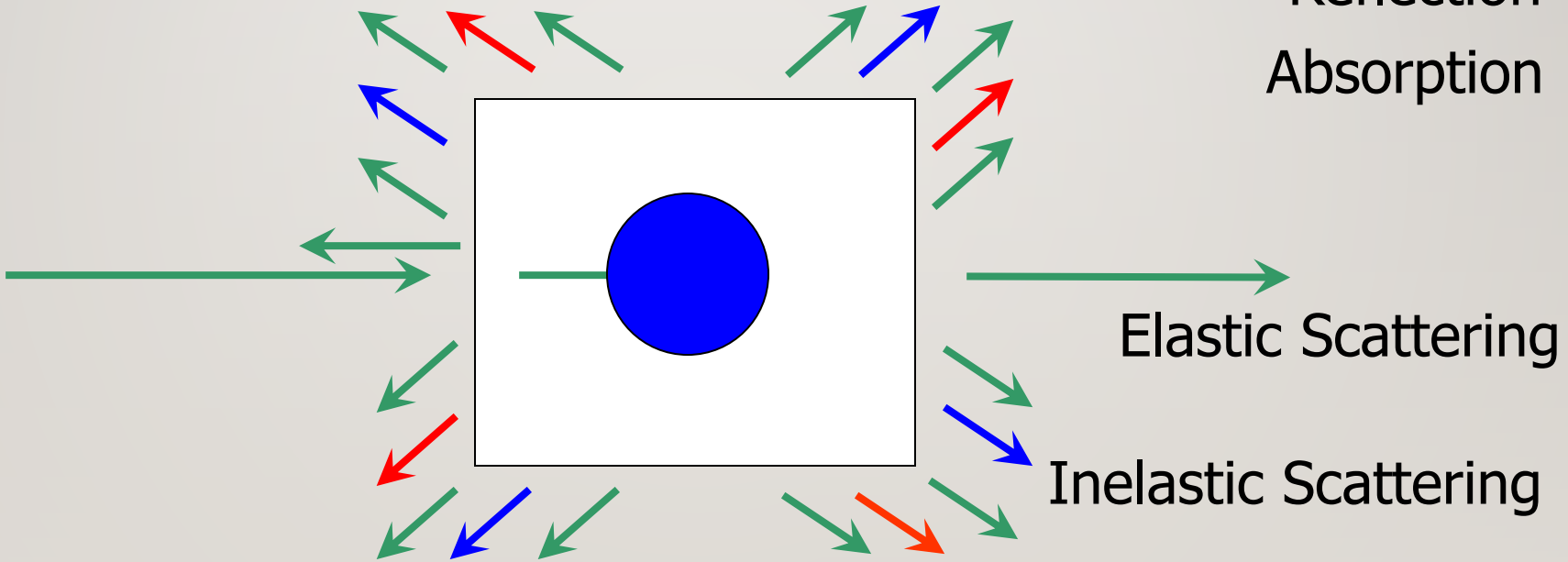
1. Ionizing radiation
2. Non-ionizing radiation

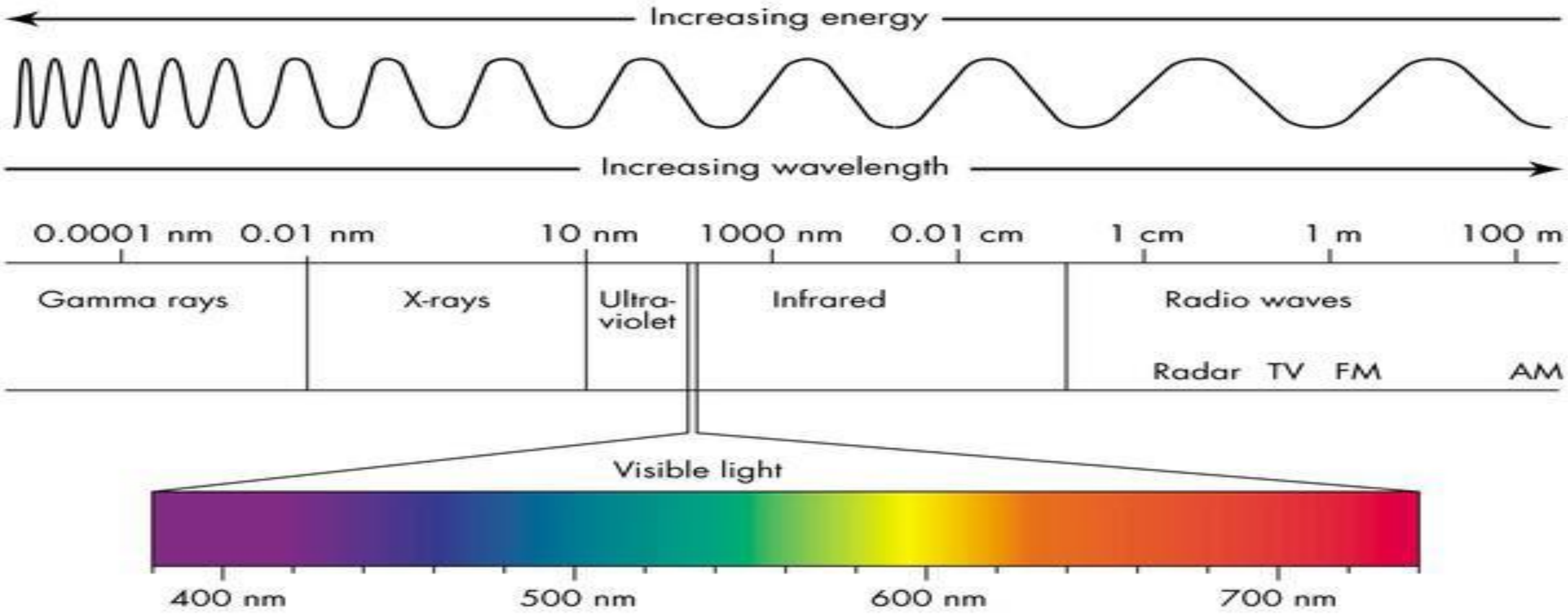
- **Ionizing Radiation**
  - Higher energy electromagnetic waves (gamma) or heavy particles (beta and alpha).
  - High enough energy to pull electron from orbit.

- Non-ionizing Radiation
  - Lower energy electromagnetic waves.
  - Not enough energy to pull electron from orbit, but can excite the electron.
  - Definition:
  - “ They are electromagnetic waves incapable of producing ions while passing through matter, due to their lower energy.”

Introduction

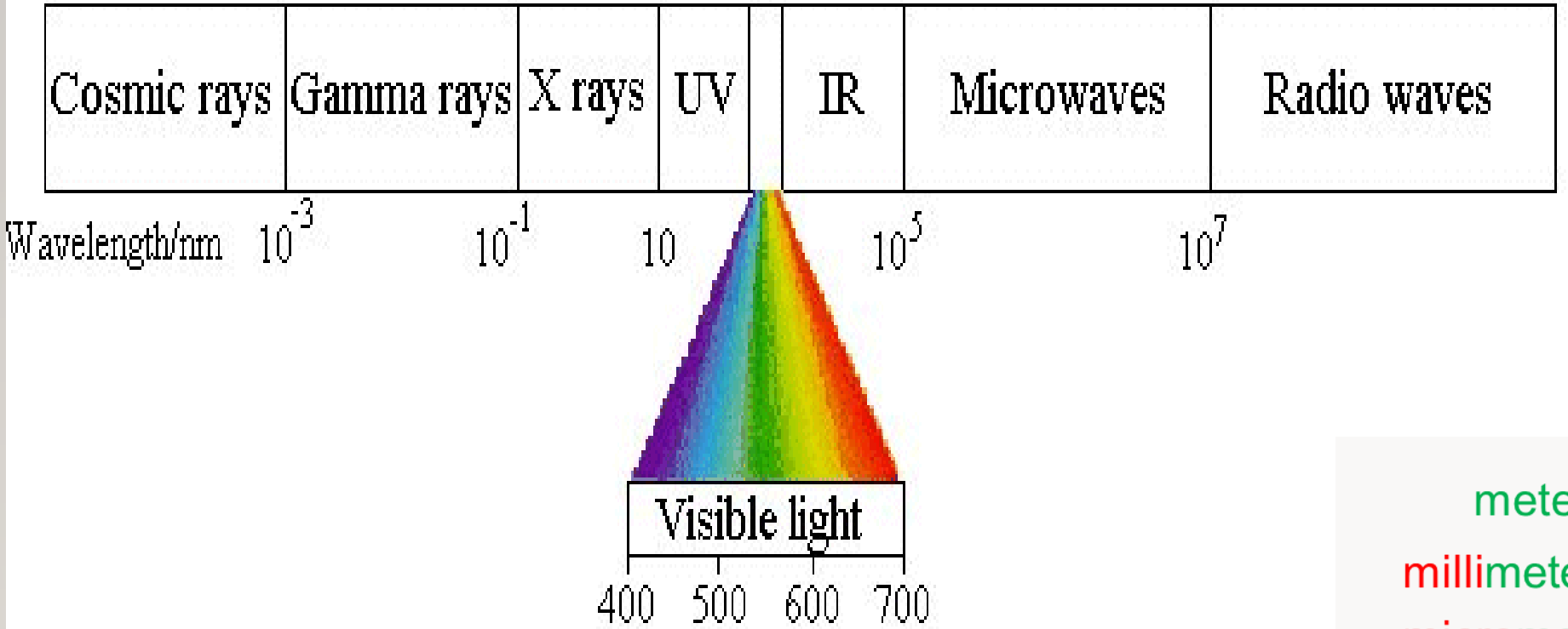
WHAT HAPPENS WHEN LIGHT FALLS ON A MATERIAL?





The higher the frequency, the more energetic the radiation. Thus, ultraviolet radiation, X rays, and ϒ rays are high-energy radiation.

ELECTROMAGNETIC SPECTRUM



UV: extends from about 100-400 nm

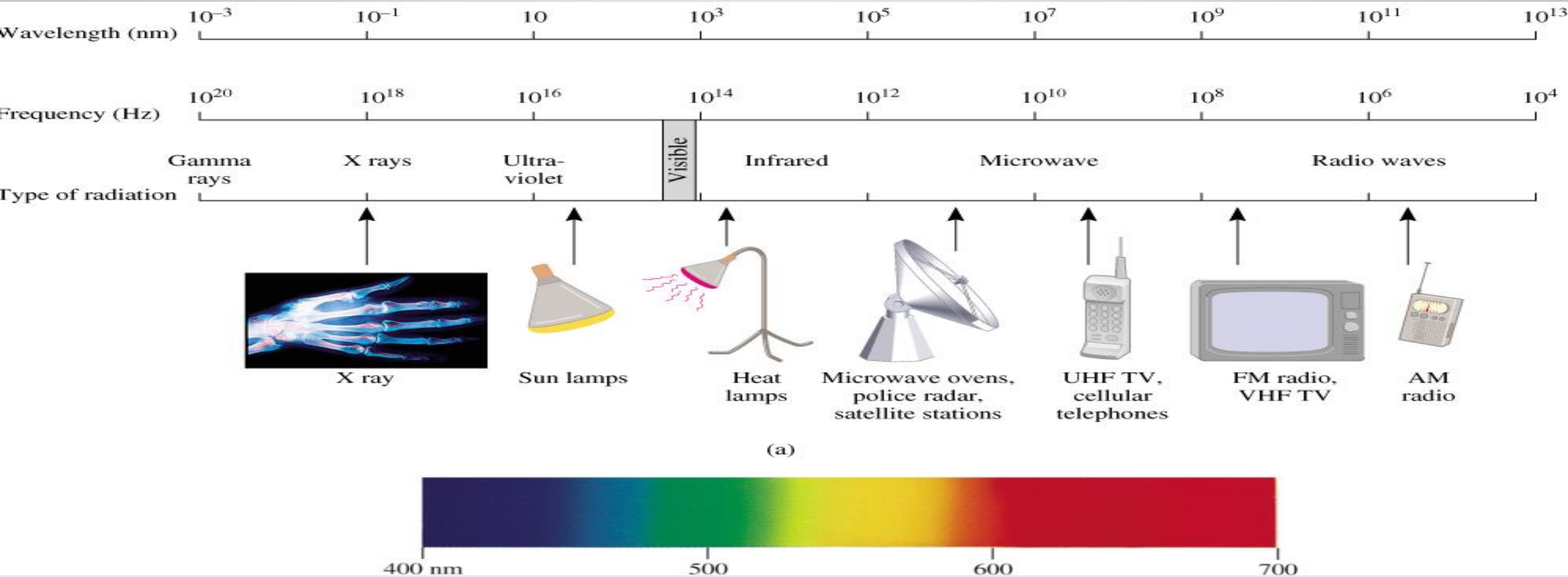
Visible: extends from about 400-800 nm

IR: 0.8 → 25 μm (800-25000 nm)

meter	m
millimeter	mm
micrometer	μm
nanometer	nm
picometer	pm

1 Angstrom unit = 1 Å = 10^{-10} meter = 10^{-8} cm

2-TYPES OF ELECTROMAGNETIC RADIATION



- The figure shows various types of electromagnetic radiation, which differ from one another in wavelength and frequency.
- The radiation with longer wavelength has lower frequency.

3-SOURCE OF EM

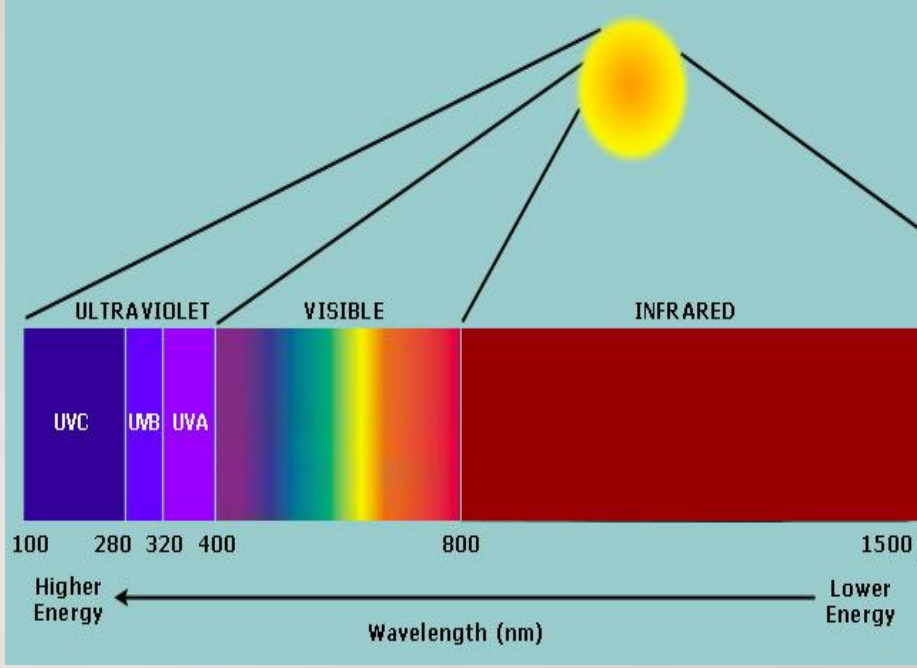
The Sun's Radiation Spectrum: Sun rays are electromagnetic waves

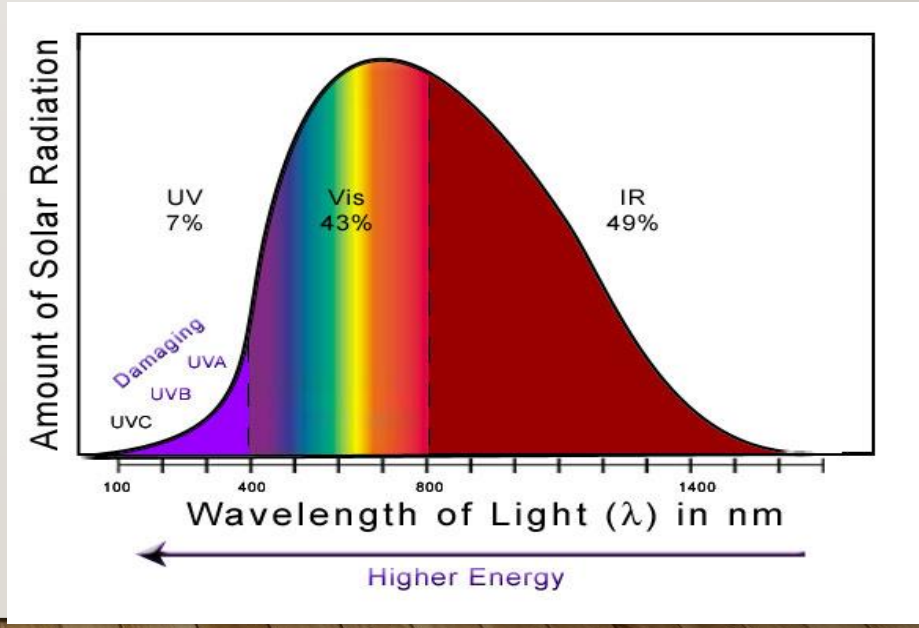
Each kind has a wavelength, frequency and energy

The sun emits several kinds of electromagnetic radiation which is the main cause of sunburn.

Infrared (IR), Visible (Vis), and Ultra Violet (UV) Sun rays are electromagnetic waves(Each kind has a wavelength, frequency and energy)

EM energy from the sun travels in (8) minutes across the intervening 150 km of space to the earth





4-SPECTROCHEMICAL METHODS

- These methods are based on measurement of:

(i) Radiation absorbed (e.g. ulltraviolet, visible, infrared, and atomic absorption spectroscopy.

(ii) Radiation emitted (e.g. flame photometry and atomic fluorescence Spectroscopy)

(iii) Radiation diffracted (e.g. x-ray diffraction)

(iv) Radiation scattered (e.g. Raman spectroscopy)

5-Chemical and physical properties used in Instrument methods.

Chemical and Physical Properties Used in Instrumental Methods

Characteristic Properties	Instrumental Methods
Emission of radiation	Emission spectroscopy (X-ray, UV, visible, electron, Auger); fluorescence, phosphorescence, and luminescence (X-ray, UV, and visible)
Absorption of radiation	Spectrophotometry and photometry (X-ray, UV, visible, IR); photoacoustic spectroscopy; nuclear magnetic resonance and electron spin resonance spectroscopy
Scattering of radiation	Turbidimetry; nephelometry; Raman spectroscopy
Refraction of radiation	Refractometry; interferometry
Diffraction of radiation	X-ray and electron diffraction methods
Rotation of radiation	Polarimetry; optical rotary dispersion; circular dichroism
Electrical potential	Potentiometry; chronopotentiometry
Electrical charge	Coulometry
Electrical current	Amperometry; polarography
Electrical resistance	Conductometry
Mass	Gravimetry (quartz crystal microbalance)
Mass-to-charge ratio	Mass spectrometry
Rate of reaction	Kinetic methods
Thermal characteristics	Thermal gravimetry and titrimetry; differential scanning calorimetry; differential thermal analyses; thermal conductometric methods
Radioactivity	Activation and isotope dilution methods

Q:What kind of information do we obtain from spectral data????

-Molecular formula

-Types of functional groups

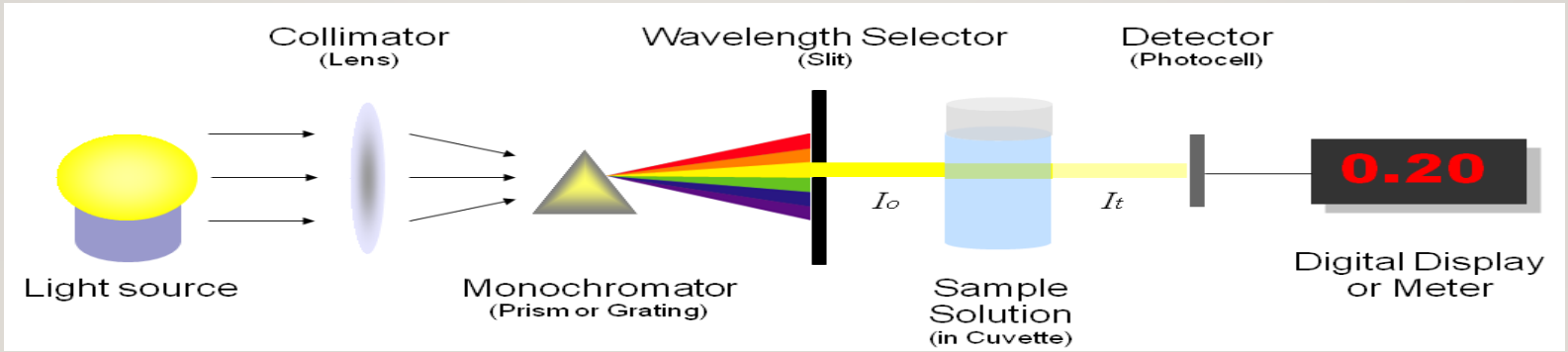
-Connectivity

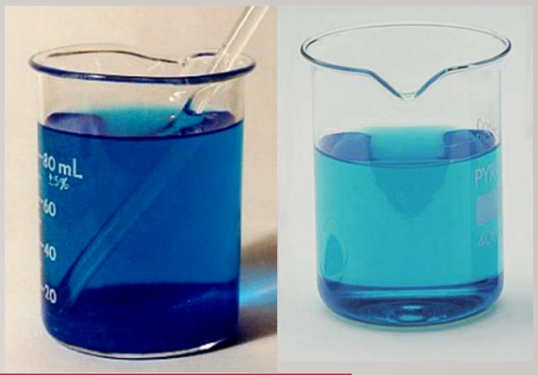
-Concentreation

-Position of substituent's, functional gps. on carbon skeleton

-Stereochemistry

Spectrophotometry is a method to measure how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through sample solution. The basic principle is that each compound absorbs or transmits light over a certain range of wavelength





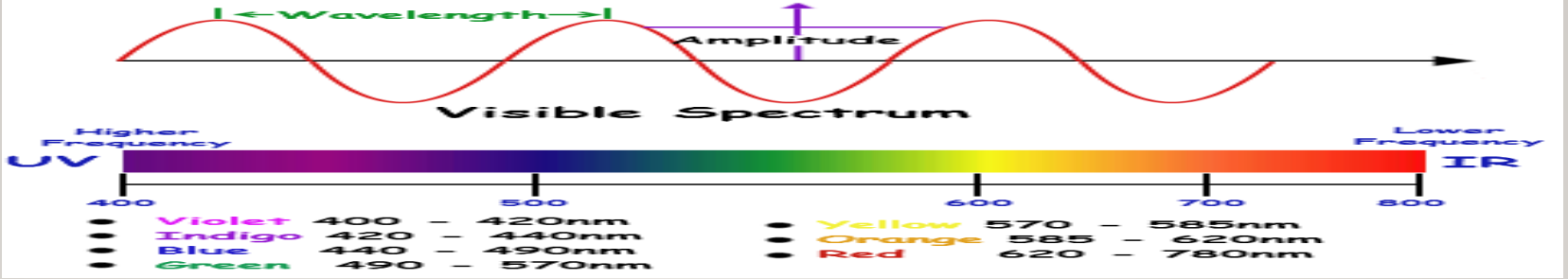
Suppose you look at two solutions of the same substance, one a deeper color than the other. Your common sense tells you that the darker colored one is the more concentrated.

In other words, as the color of the solution deepens, you conclude that its concentration also increases. This is an underlying the first principle of spectrophotometry: **the intensity of color is a measure of the concentration of a substance in solution.**

A second principle of spectrophotometry is that **every substance absorbs or transmits certain wavelengths of radiant energy but not other wavelengths.**

For example, chlorophyll always absorbs red and violet light, while it transmits yellow, green, and blue wavelengths. The transmitted and reflected wavelengths appear **green**—the color your eye "sees." Thus, the absorption or transmission of specific wavelengths is characteristic for a substance, and a spectral analysis serves as a "fingerprint" of the compound.

In recent years spectrophotometric methods have become the most frequently used and important methods of quantitative analysis. They are applicable to many industrial and clinical problems involving the quantitative determination of compounds that are colored or that react to form a colored product.



The color we see in a sample of solution is due to the selective absorption of certain wavelengths of visible light and transmittance of the remaining wavelengths. If a sample absorbs all wavelengths in the visible region of the spectrum, it will appear **black**; if it absorbs none of them, it will appear **white**. For example, the wavelength we sense as green is 495 nanometers.

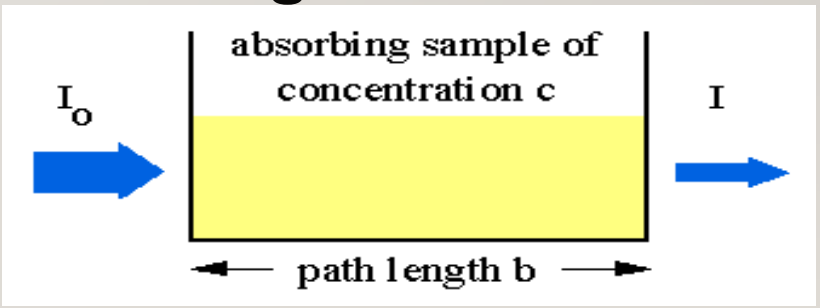
You should remember, of course, that the visible range is only a very small part of the electromagnetic spectrum.

TRANSMITTANCE, ABSORBANCE, AND THE BEER-LAMBERT LAW

Transmittance is defined as the ratio of the amount of light transmitted to the amount of light that initially fell on the surface.

Transmittance =

intensity of transmitted light/ intensity of incident light



Experimental measurements are usually made in terms of **transmittance** (T), which is defined as:

$$T = I / I_o$$

where **I** is the **light intensity** after it passes through the sample and I_o is the **initial light intensity**.

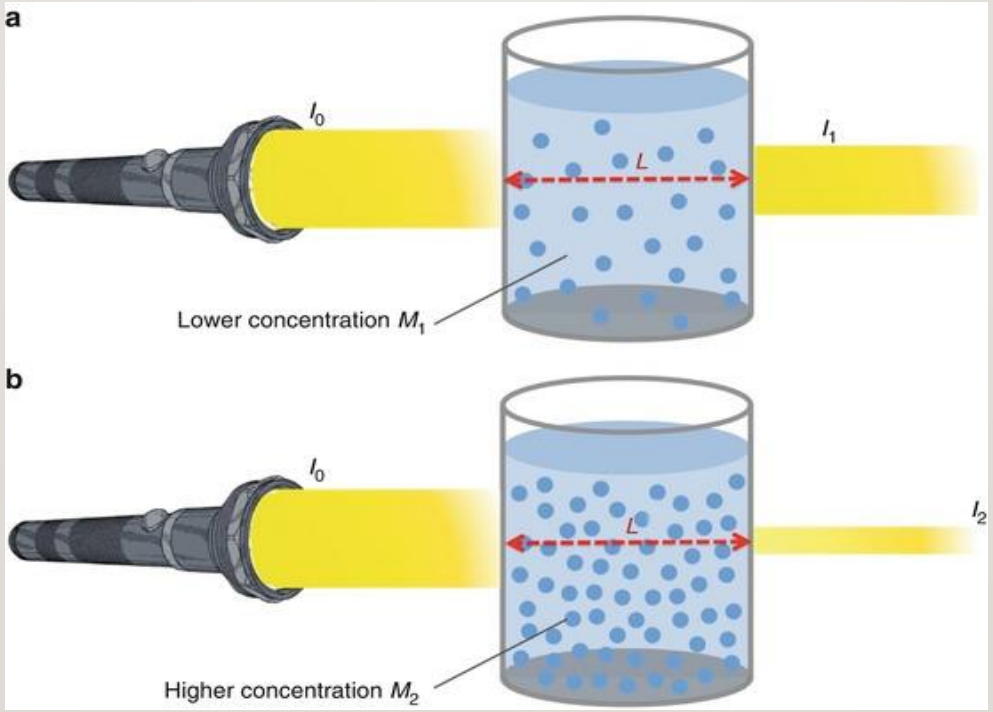
- The relation between A and T is:

$$A = -\log T = -\log (I / I_o)$$

Absorption of light by a sample

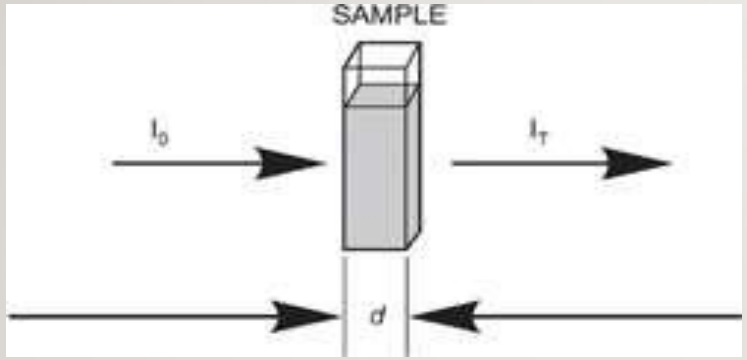
Absorbance is defined as the negative logarithm of the transmittance

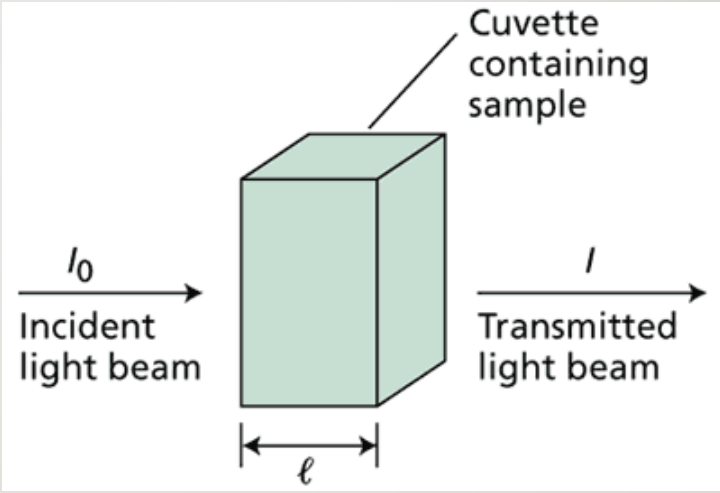
According to the **Beer-Lambert law**, the amount of light absorbed is directly proportional to the concentration of the chemical species. ……..(1)



Also, according to the **Beer-Lambert law**

absorbance increases as pathlength increases. …….(2)





The two observations described above (those dealing with the relationship between [absorbance and concentration] and [absorbance and path length] constitute the **BEER-LAMBERT LAW.**

The Beer-Lambert Law

The **Beer-Lambert law (or Beer's law)** is the linear relationship between absorbance and concentration of an absorbing species.

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

$$A \text{ (O.D)} = \varepsilon cL$$

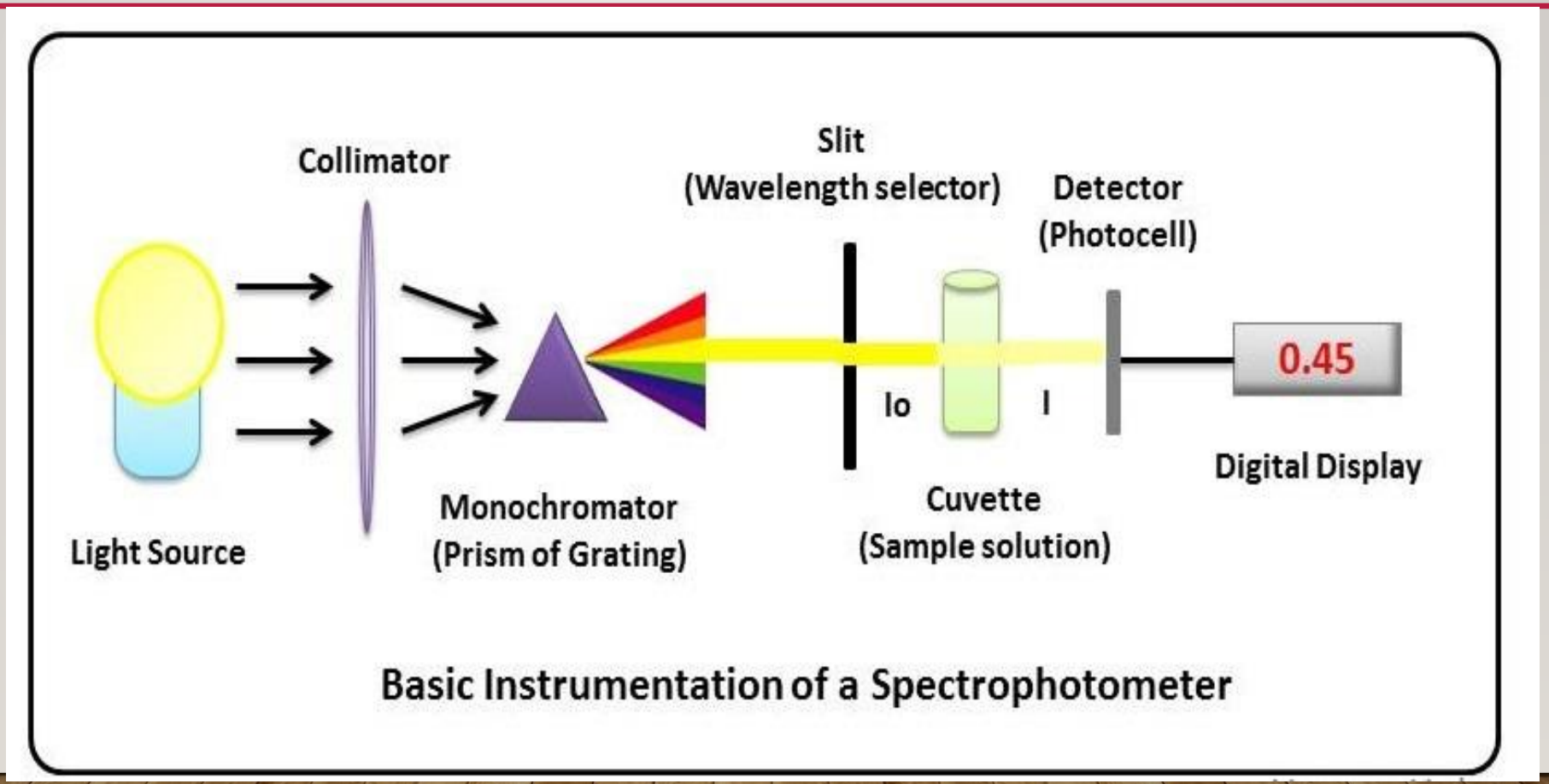
- where **A** is the measured absorbance (Optical Density)
- **ε** is the wavelength-dependent molar absorptivity coefficient with units of M^{-1} cm^{-1}
- **L** is the path length (cm)
- **c** is the sample concentration (g%)

Spectrophotometer

A spectrophotometer is an instrument that measures the amount of light absorbed by a sample.

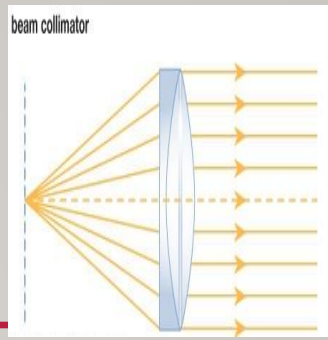
A spectrophotometer optically determines the **absorbance** or **transmission** of characteristic wavelengths of light by a substance in solution.

Principle

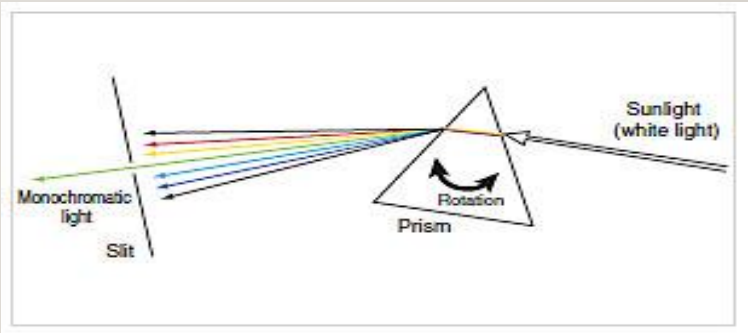

Collimator
Slit
(Wavelength selector)
Detector
(Photocell)
0.45
Io
I
Light Source
Monochromator
(Prism of Grating)
Cuvette
(Sample solution)
Digital Display
Basic Instrumentation of a Spectrophotometer

Instrumentation

- It consists of a light source, a collimator, a monochromator, a wavelength selector, a cuvette for sample solution, a photoelectric detector, and a digital display or a meter.
- **Light source**: Tungsten Halogen Lamp, it is the most common light source used in spectrophotometer. This lamp consists of a tungsten filament enclosed in a glass envelope, with a wavelength range of about 400 to 800 nm, are used for the visible region.



- **Collimator**: is a device that narrows a beam of waves.
- **Monochromator:** is an optical device that transmits a narrow band of wavelenghts of light. The device is based on the separating capability of prism
- **Wavelength selector:** limits the radiation absorbed by a sample to a certain wavelength or a narrow band of wavelengths.



Spectrophotometer applications

A spectrophotometer is used in many areas of science including microbiology, biochemistry, physics, chemistry, and medical health.

1. Qualitative Analysis:

The visible and UV spectrophotometer may be used to identify classes of compounds.

2.Quantitative determination of DNA, RNA, and proteins.

3.Enzyme Assay: This is the basic application of spectrophotometry

4. Molecular Weight determination:

Molecular weights of sugars and many aldehyde and ketone compounds have been determined by this method.

5-Detection of impurities:

UV absorption spectroscopy is one of the best methods for determination of impurities in organic molecules

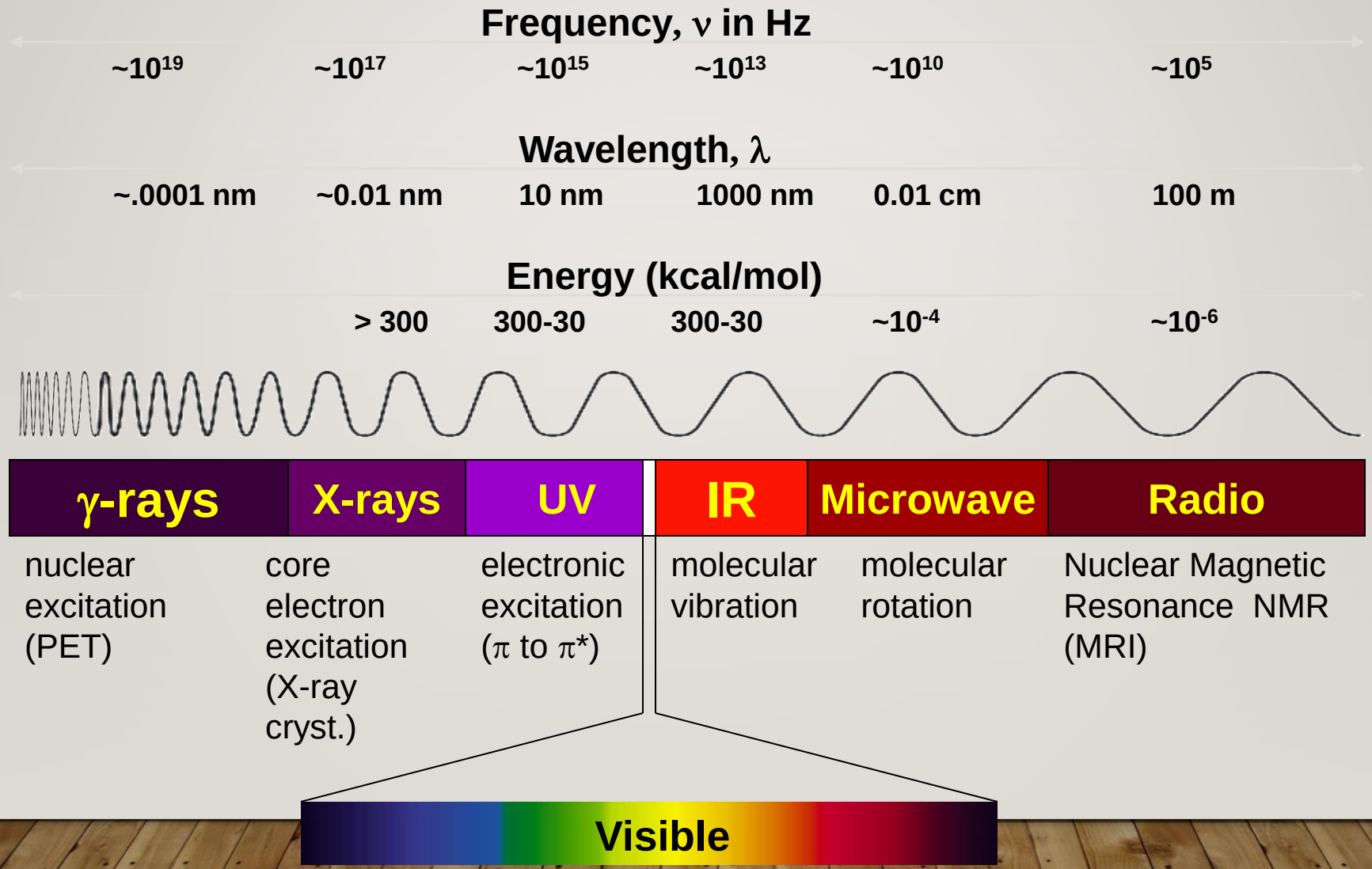
6-Detection of Functional Groups: Absence of a band at particular wavelength regarded as an evidence for absence of particular group.

INFRARED SPECTROSCOPY

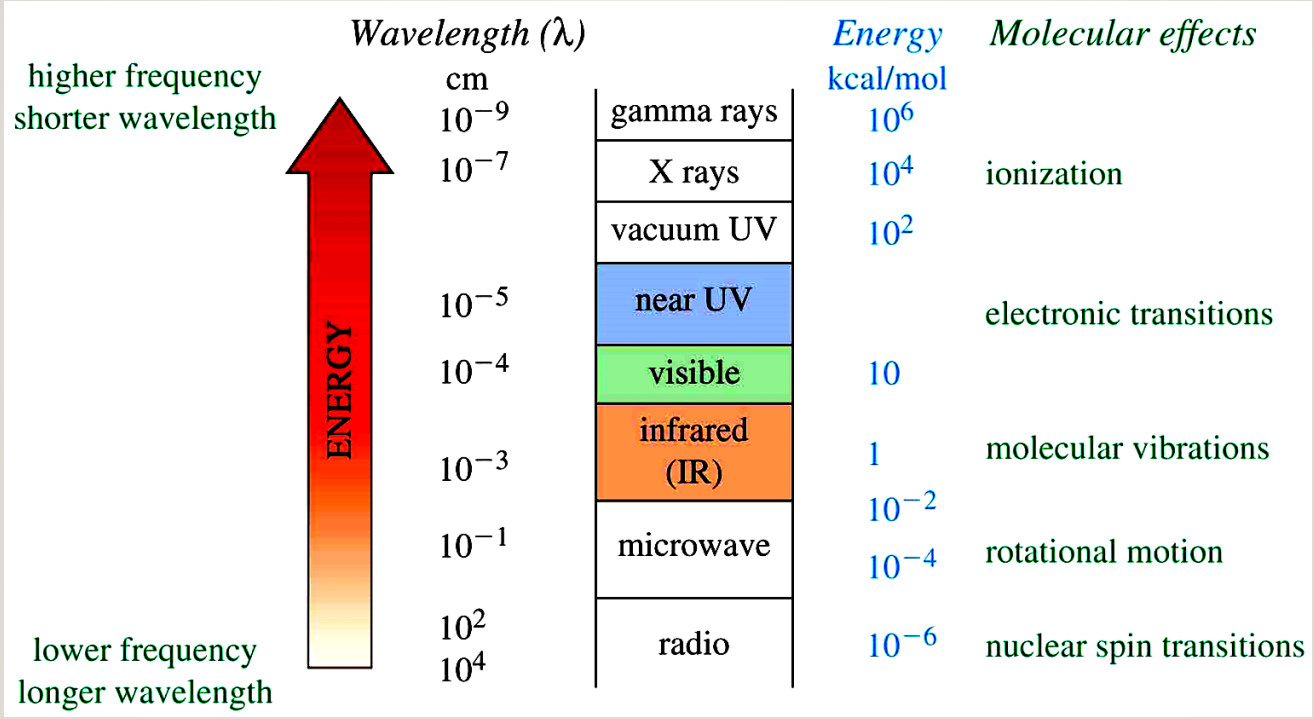
IR Spectroscopy

I. Introduction

8. The entire electromagnetic spectrum is used by chemists:



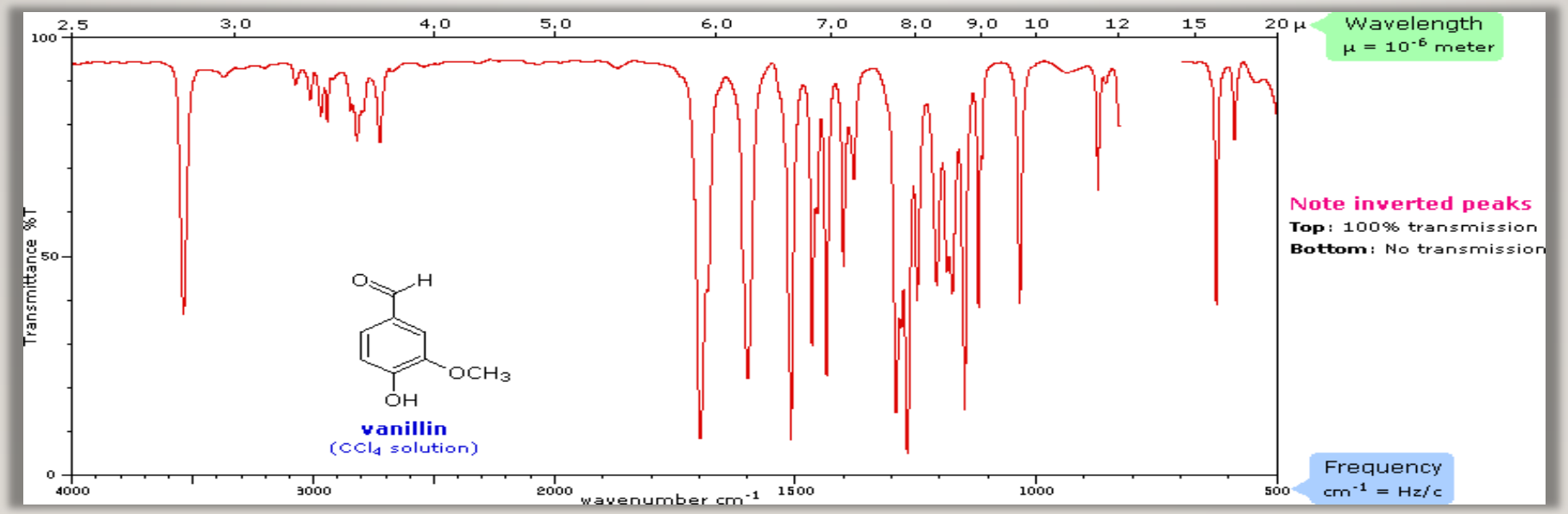
EFFECT OF ELECTROMAGNETIC RADIATION ON MOLECULES



Graphics source: Wade, Jr., L.G. *Organic Chemistry*, 5th ed. Pearson Education Inc., 2003

WHAT IS INFRARED SPECTROSCOPY?

- Is the spectroscopy that deals with the infrared region of the electromagnetic spectrum, that is light with a longer wavelength and lower frequency than visible light.



It is a method of measurement of the extent, at a various wave numbers, of absorption of infrared radiation when it passes through a layer of substances.

The **infrared range** is usually divided into three **regions**:

1-Near **infrared** (nearest the visible spectrum), with wavelengths 0.78 to about 2.5 micrometres (a micrometre, or micron, is 10^{-6} metre); not hot at all

2-Middle **infrared**, with wavelengths 2.5 to about 50 micrometres;

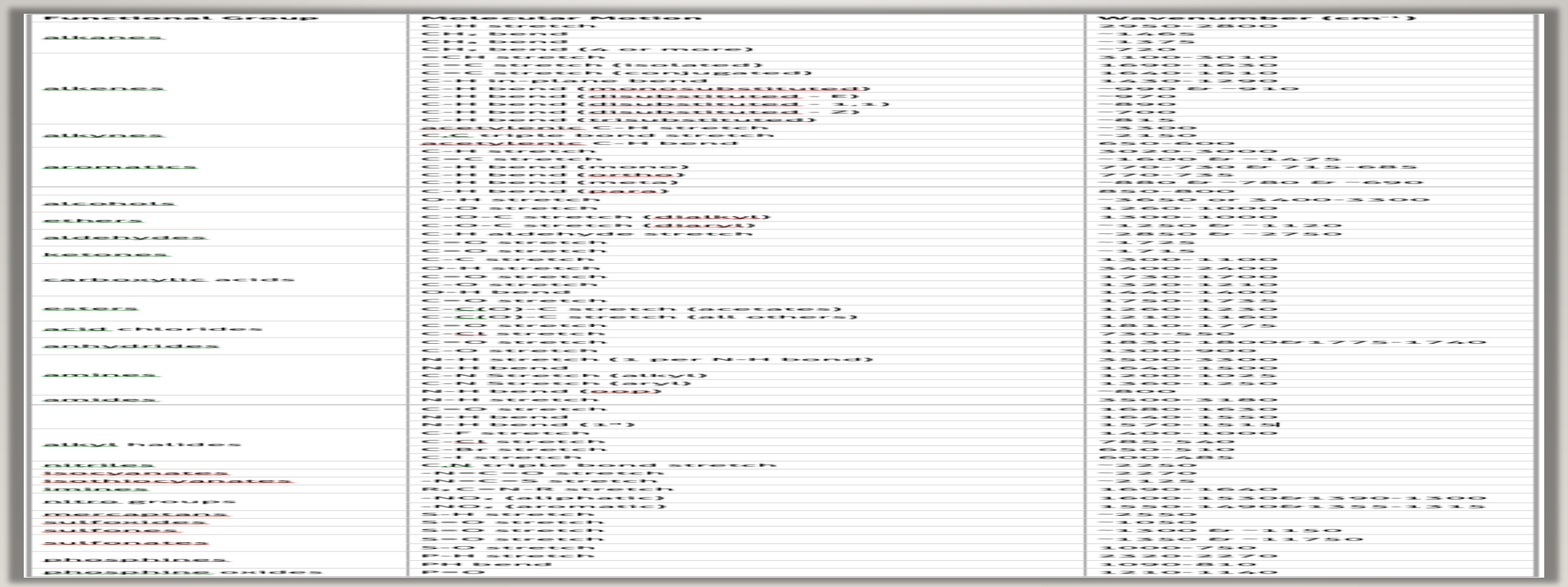
3-Far **infrared**, with wavelengths 50 to 1,000 micrometres.

INFRARED SPECTROMETER

- Infrared Spectrometer determines the wavelength and absorbance of a sample in the infrared region of the electromagnetic spectrum.

FUNCTIONAL GROUPS

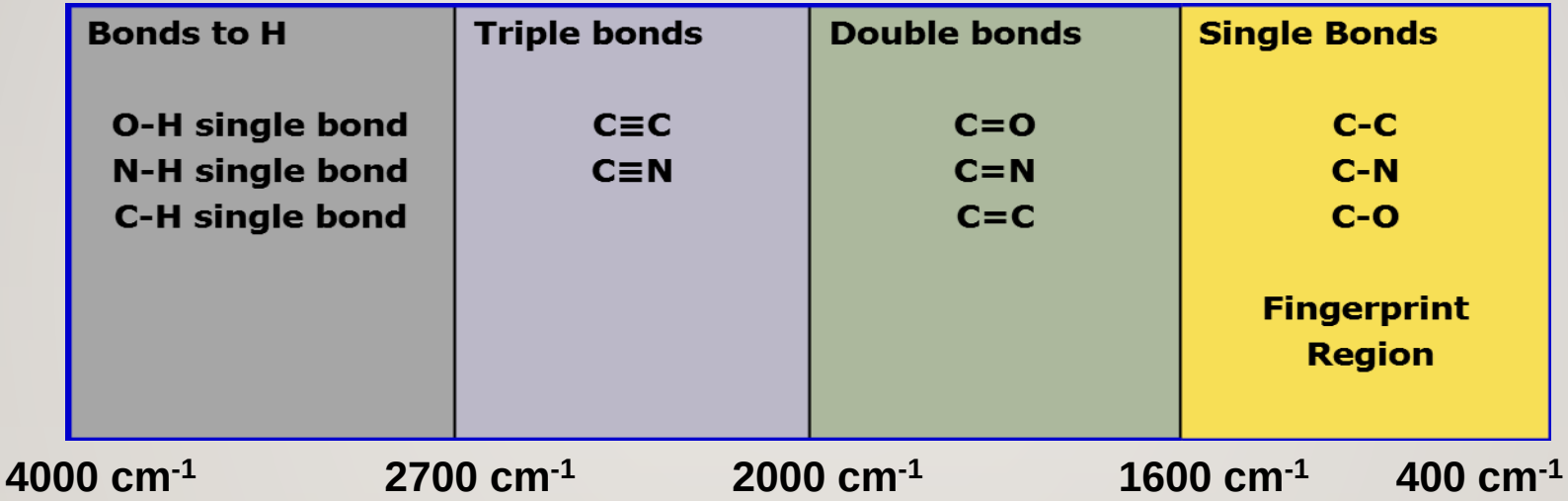
Different groups absorb at different wavelengths--characteristic frequencies. Carbonyl groups absorb at certain frequencies, primary amines at others, phenyl groups at still others, and so on.



Functional Group	Molecular Motion	Wavenumber (cm^{-1})
alkanes	C–H stretch	2950–2800
	CH_2 bend	~1465
	CH_3 bend	~1375
	CH_2 bend (4 or more)	~720
alkenes	=C–H stretch	3100–3010
	C=C stretch (isolated)	1690–1630
	C=C stretch (conjugated)	1640–1610
	C–H in-plane bend	1430–1290
	C–H bend (monosubstituted)	~990 & ~910
	C–H bend (disubstituted – E)	~970
	C–H bend (disubstituted – 1,1)	~890
	C–H bend (disubstituted – Z)	~700
	C–H bend (trisubstituted)	~815
alkynes	acetylenic C–H stretch	~3300
	C≡C triple bond stretch	~2150
	acetylenic C–H bend	650–600
aromatics	C–H stretch	3030–3000
	C=C stretch	~1600 & ~1475
	C–H bend (mono)	770–730 & 715–685
	C–H bend (ortho)	770–735
	C–H bend (meta)	~850 & ~780 & ~690
	C–H bend (para)	850–800
alcohols	O–H stretch	~3650 or 3400–3300
	C–O stretch	1260–1000
ethers	C–O–C stretch (dialkyl)	1300–1000
	C–O–C stretch (diaryl)	~1250 & ~1120
aldehydes	C–H aldehyde stretch	~2850 & ~2750
	C=O stretch	~1725
ketones	C=O stretch	~1715
	C–C stretch	1300–1100
carboxylic acids	O–H stretch	3400–2400
	C=O stretch	1725–1700
	C–O stretch	1320–1210
	O–H bend	1440–1400
esters	C–O stretch	1750–1735
	C=O)–C stretch (acetates)	1260–1230
	C=O)–C stretch (all others)	1210–1160
acid chlorides	C=O stretch	1810–1775
	C–Cl stretch	730–550
anhydrides	C=O stretch	1830–1800 & 1775–1740
	C–O stretch	1300–900
amines	N–H stretch (1 per N–H bond)	3500–3300
	N–H bend	1640–1500
	C–N stretch (alkyl)	1200–1020
	C–N stretch (aryl)	1360–1250
	N–H bend (oop)	~850
amides	N–H stretch	3500–3180
	C=O stretch	1680–1630
	N–H bend	1640–1550
	N–H bend (1°)	1570–1515
alkyl halides	C–F stretch	1400–1000
	C–Cl stretch	785–540
	C–Br stretch	650–510
	C–I stretch	600–485
nitriles	C≡N triple bond stretch	~2250
isocyanates	–N=C=O stretch	~2270
isothiocyanates	–N=C=S stretch	~2125
imines	R–N=C–N–R stretch	1690–1640
nitro groups	–NO_2 (aliphatic)	1600–1530 & 1390–1300
	–NO_2 (aromatic)	1550–1490 & 1355–1315
mercaptans	S–H stretch	~2550
sulfonides	S=O stretch	~1050
sulfones	S=O stretch	~1300 & ~1150
sulfonates	S=O stretch	~1350 & ~1175
	S–O stretch	1000–750
phosphines	P–H stretch	2320–2270
	P–H bend	1090–810
phosphine oxides	P=O	1210–1140

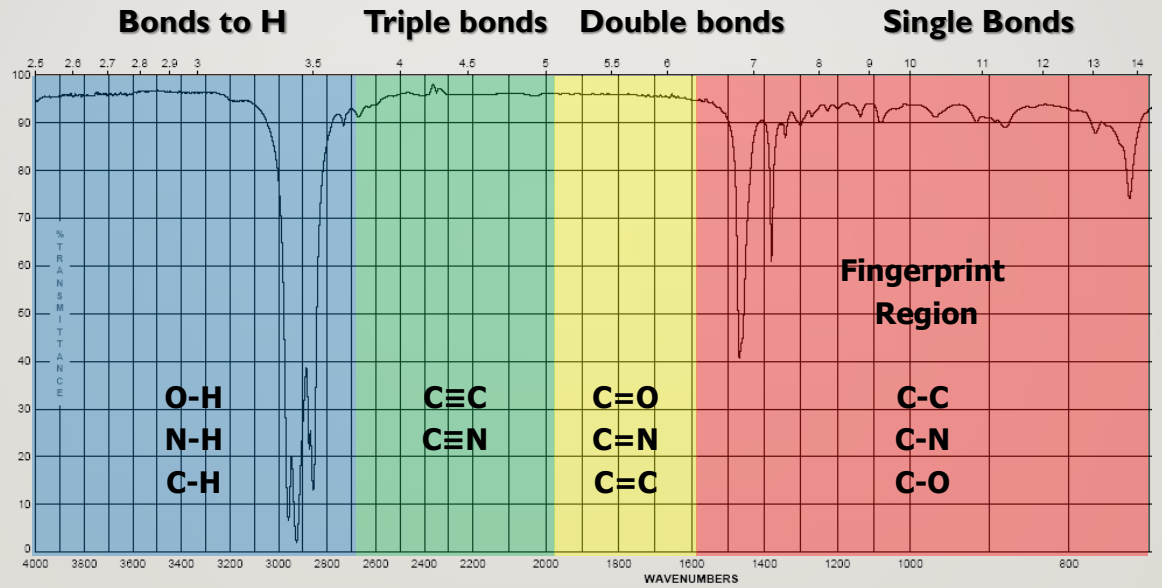
IR FREQUENCY RANGE

- *The infrared spectrum is divided into three portions, near, mid, and far infrared, covering wave numbers (related to frequency) from 10-14,000 cm^{-1}. The range 500 - 4000 cm^{-1} is used for basic laboratory work.*



Infrared Spectroscopy

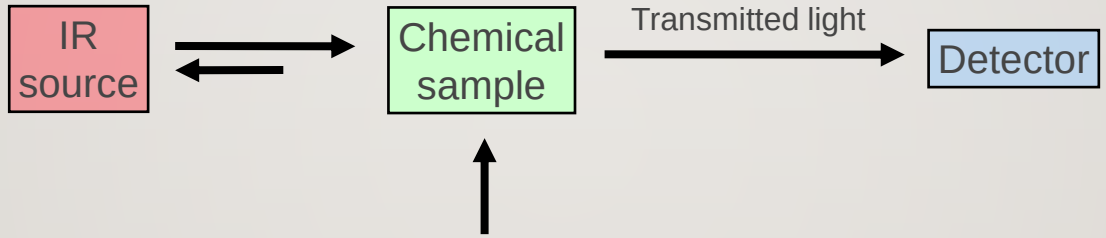
The four primary regions of the IR spectrum



4000 cm^{-1} 2700 cm^{-1} 2000 cm^{-1} 1600 cm^{-1} 600 cm^{-1}

TRANSMISSION VS. ABSORPTION

When a chemical sample is exposed to the action of **IR LIGHT**, it can **absorb** some frequencies and **transmit** the rest. Some of the light can also be reflected back to the source.

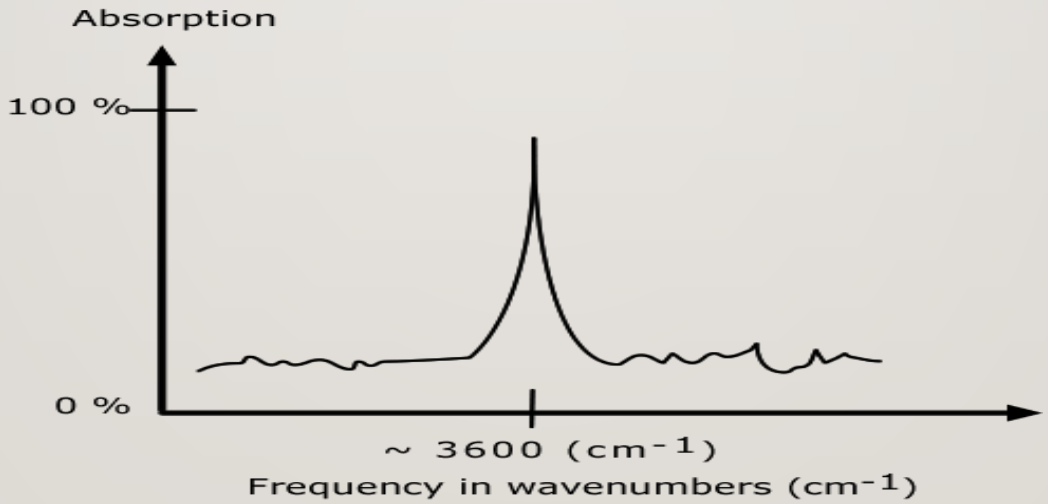


From all the frequencies it receives, the chemical sample can **absorb** (retain) **specific frequencies** and allow the rest to pass through it (transmitted light).

The detector detects the transmitted frequencies, and by doing so also reveals the values of the absorbed frequencies.

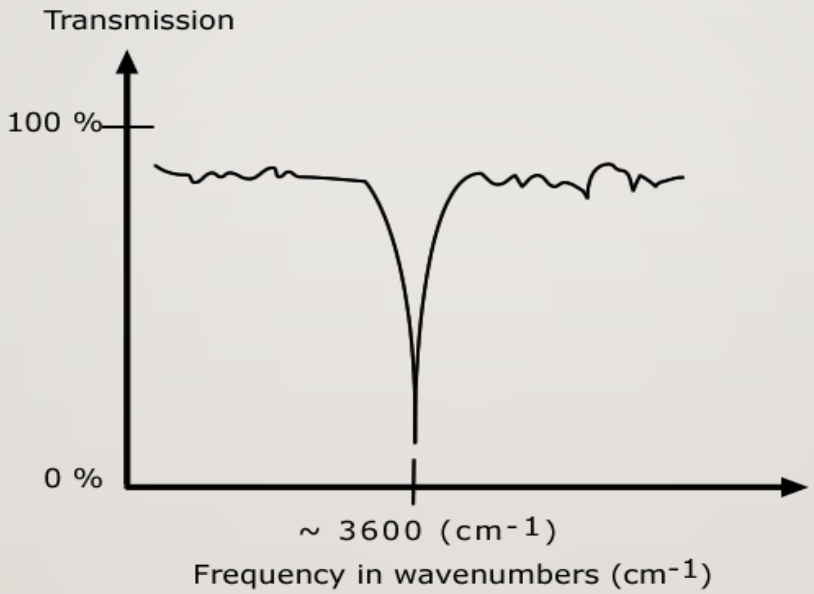
AN IR SPECTRUM IN ABSORPTION MODE

The IR spectrum is basically a plot of transmitted (or absorbed) frequencies vs. intensity of the transmission (or absorption). Frequencies appear in the *x*-axis in units of inverse centimeters (wavenumbers), and intensities are plotted on the *y*-axis in percentage units.



The graph above shows a spectrum in **absorption** mode.

AN IR SPECTRUM IN TRANSMISSION MODE

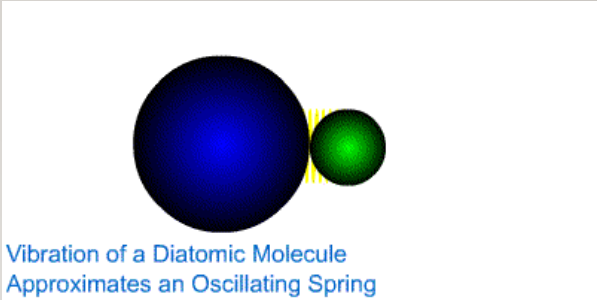


The graph above shows a spectrum in **transmission** mode.
This is the most commonly used representation and the one found in most chemistry and spectroscopy books. Therefore we will use this representation.

CLASSIFICATION OF IR BANDS

IR bands can be classified as **strong** (s), **medium** (m), or **weak** (w), depending on their relative intensities in the infrared spectrum. A strong band covers most of the *y*-axis. A medium band falls to about half of the *y*-axis, and a weak band falls to about one third or less of the *y*-axis.

THE IR SPECTROSCOPIC PROCESS



Vibration of a Diatomic Molecule
Approximates an Oscillating Spring

1. The quantum mechanical energy levels observed in IR spectroscopy are those of molecular vibration

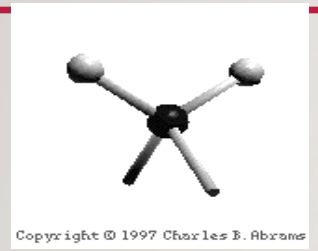
2. We perceive this vibration as heat

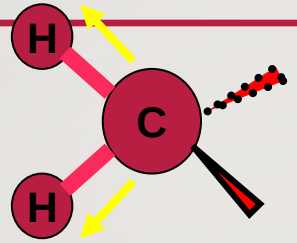
3. When we say a covalent bond between two atoms is of a certain length, we are citing an average because the bond behaves as if it were a vibrating spring connecting the two atoms

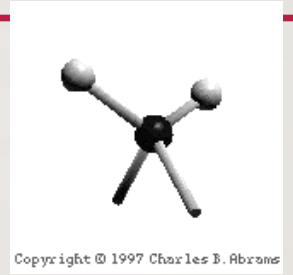
4. For a simple diatomic molecule, this model is easy to visualize:

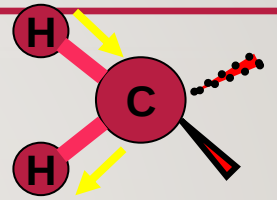
5. There are two types of bond vibration:

- ***Stretch – Vibration or oscillation along the line of the bond***







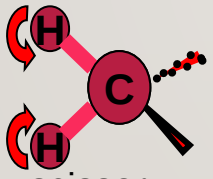


- ***Bend – Vibration or oscillation not along the line of the bond***

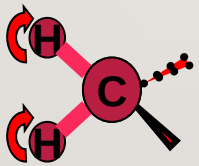
symmetric

asymmetric

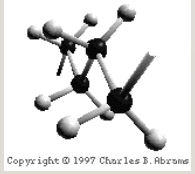
in plane

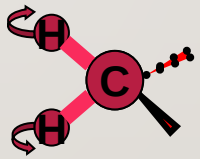


scissor

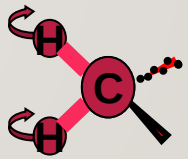


rock





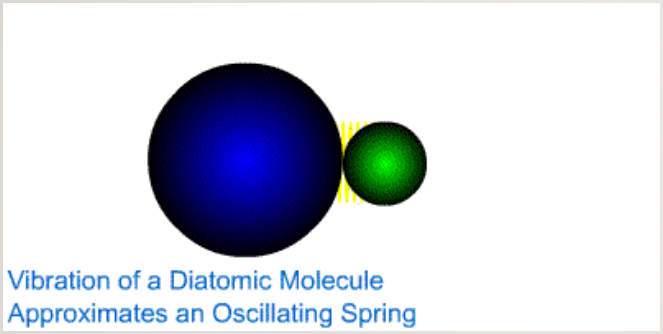
twist

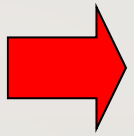


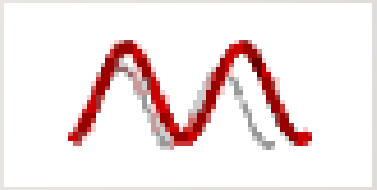
wag

out of plane

6. As a covalent bond oscillates = vibrating – due to the oscillation of the dipole of the molecule – a varying electromagnetic field is produced.





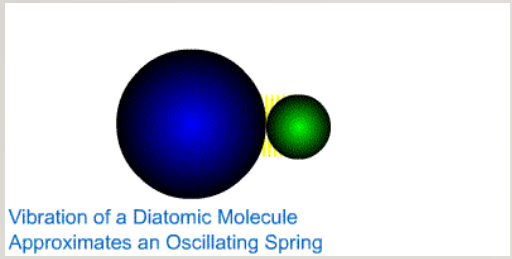


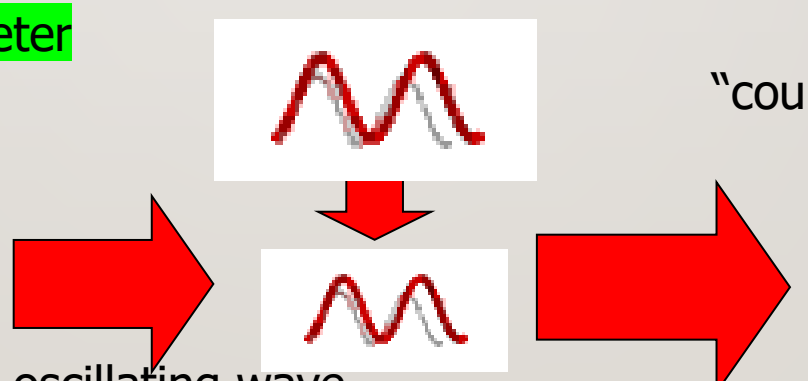
7. The greater the dipole moment change through the vibration, the more intense the EM field that is generated.

8. When a wave of infrared light encounters this oscillating EM field generated by the oscillating dipole of the same frequency, the two waves couple, and IR light is absorbed

9. The coupled wave now vibrates with twice the amplitude

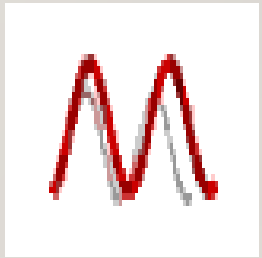
IR beam from spectrometer





EM oscillating wave
from bond vibration

"coupled" wave



USES AND APPLICATIONS

- **Infrared spectroscopy** is widely used in industry as well as in research. It is a simple and reliable technique for measurement, quality control and dynamic measurement. It is also employed in forensic analysis in civil and criminal analysis
- **Infrared** (IR) light is used by electrical heaters, cookers for cooking food, short-range communications like remote controls, optical fibres, security systems and thermal imaging cameras which detect people in the dark

- IR has been successfully **used in diagnosis** of breast cancer, diabetes neuropathy and peripheral vascular disorders. It has also been **used** to detect problems associated with gynecology, kidney transplantation, dermatology, heart, neonatal physiology, fever screening and brain imaging.

- Chemical Analysis: Testing Pill Quality. According to "Medical News Today," scientists at the University of Maryland have been successful in using the method of near-infrared spectroscopy (NIR) to make a prediction regarding quick dissolution of pills inside the body. The success of the experiment can help drug manufacturers in checking the quality of pills to benefit consumers in the health industry.

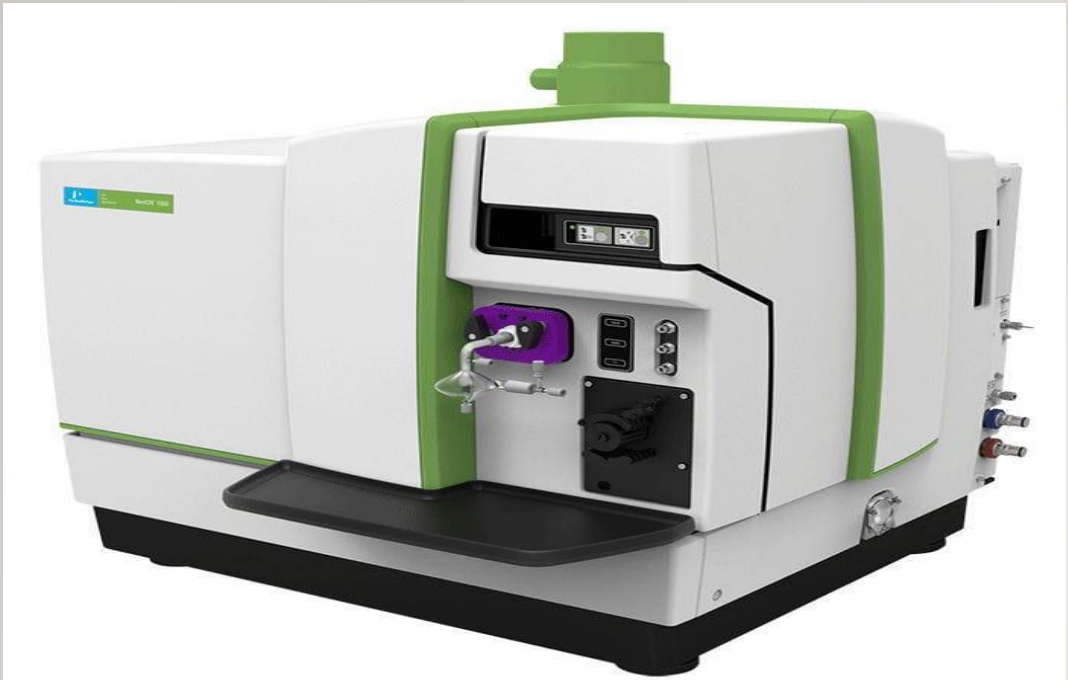
WHAT IS THE DIFFERENCE BETWEEN IR & RAMMAN

- IR(A&T) SELECTING RULE :it must be changed in a dipole moment during the vibration of the molecule

- IR &Raman complementary to each other

  Thus N_2 and O_2 do **not** absorb in the IR range.

- The selection rule for Raman scattering is that the polarizability of the molecule must change with vibration in order to have a transition to different energy level.
- [The polarizability α : is a measure of how easy it is to induce a dipole in a molecule by applying an electric field]
- **Polarizability**:The ability of a bond or molecule to be polarized by changing its electron cloud.
- Thus, N_2 and O_2 will show vibrational Raman spectra because their vibrations cause a change in polarizability.



ICP-OES

ICP-MS

ICP

Inductively Coupled Plasma

ICP-OES is an analytical technique used for the detection of chemical elements

INDUCTIVELY COUPLED PLASMA (ICP)

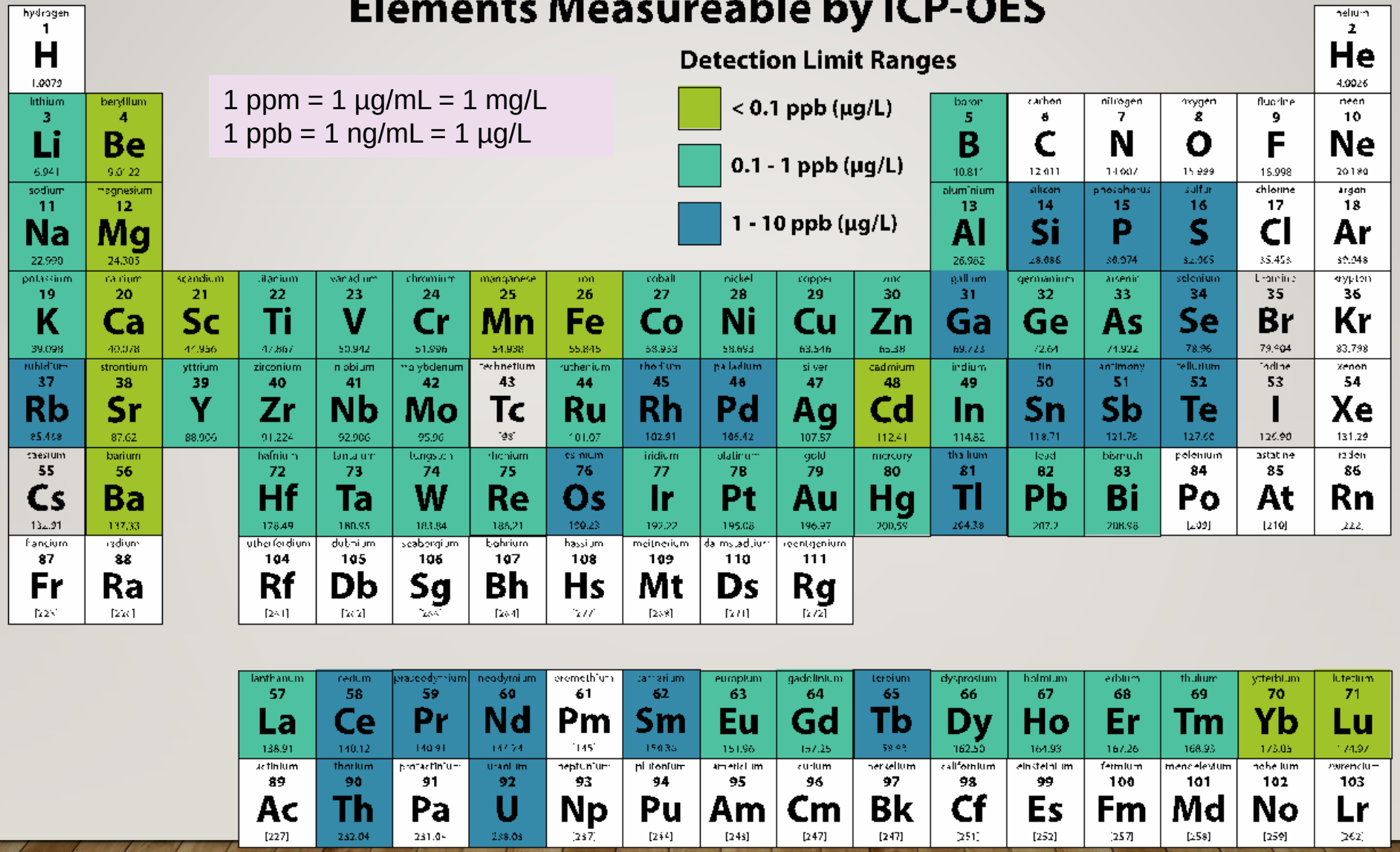
- It has become a widely applied in both routine research as in more specific analysis purposes.
- It uses ICP to ionize the sample.
- It atomizes the sample and creates a small polyatomic ions , Which are then detected.
- It is used for it to detected metals and several non-metal in liquid sample at very low concentration

Elements Measureable by ICP-OES

1 ppm = 1 µg/mL = 1 mg/L
1 ppb = 1 ng/mL = 1 µg/L

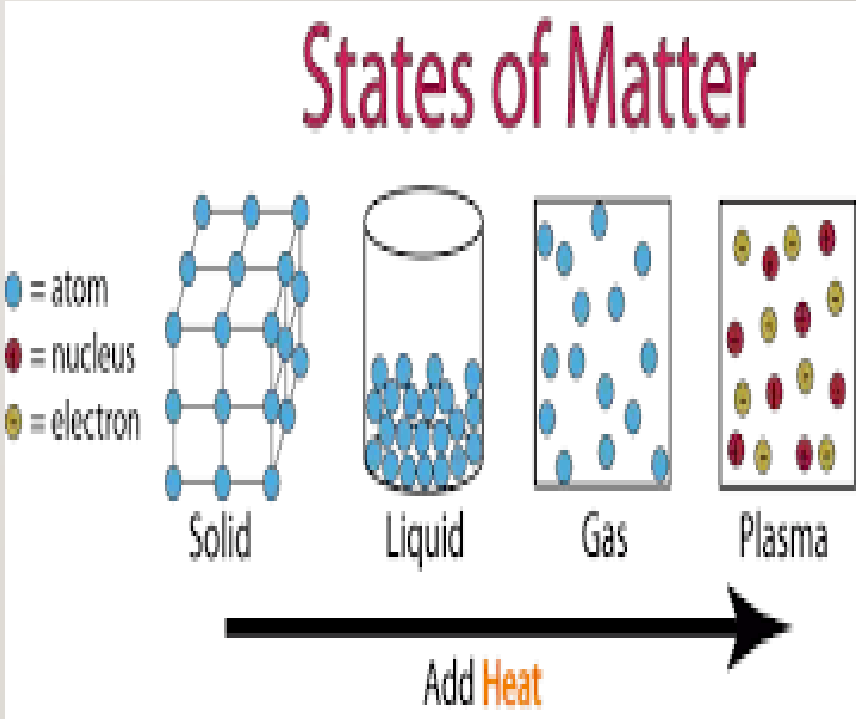
Detection Limit Ranges

- < 0.1 ppb (µg/L)
- 0.1 - 1 ppb (µg/L)
- 1 - 10 ppb (µg/L)



DEFINITION OF PLASMA

- A plasma is an electrical conducting gaseous mixture containing a significant concentration of cations and electrons. (The concentrations of the two are such that the net charge approaches zero).
- In the argon plasma used for atomic spectroscopy, argon ions and electrons are the principal conducting species.

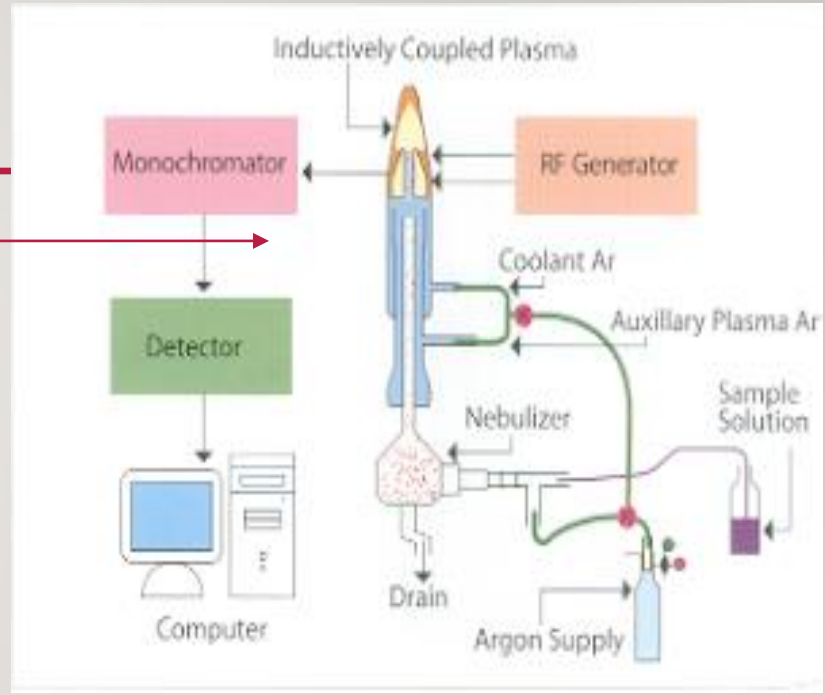


(ICP)

- Inductively Coupled Plasma (ICP) is an analytical technique used for the detection of trace metals in environmental samples. The primary goal of ICP is to get elements to emit characteristic wavelength specific light which can then be measured. The technology for the ICP method was first employed in the early 1960's with the intention of +. Since then, ICP has been refined and used in conjunction with other procedures for quantitative analysis.
- An ICP is a very high temperature (7000-8000K) excitation source that efficiently desolvates , vaporizes, excites, and ionises atoms. Molecular interferences are greatly reduced with this excitation source but are not eliminated completely.

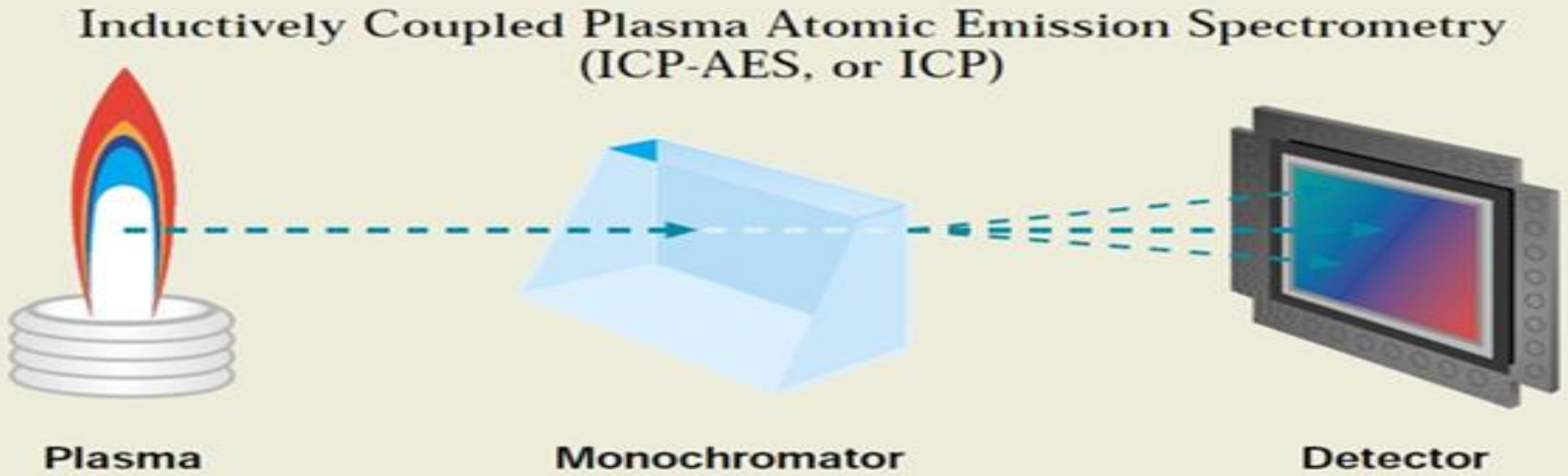
INSTRUMENT COMPONENTS OF ICP

- An ICP typically includes the following components:
  - Sample introduction system (nebulizer)
  - ICP torch
  - High frequency generator
  - Transfer optics and spectrometer
  - Computer interface



- An ICP requires that the elements which are to be analyzed be in solution. An aqueous solution is preferred over an organic solution, as organic solutions require special manipulation prior to injection into the ICP.

INSTRUMENTS FOR ICP



ICP-AES is a multi-element analysis technique that uses an inductively coupled plasma source to dissociate the sample into its constituent atoms or ions, exciting them to a level where they emit light of a characteristic wavelength. A detector measures the intensity of the emitted light, and calculates the concentration of that particular element in the sample

ICP TYPES

ICP-MS
(INDUCTIVELY COUPLED PLASMA-MASS-SPECTROMETRY)

- ICP-MS is a technique to determine low-concentrations (range: ppb = parts per billion = µg/l) and ultra-low-concentrations of elements (range: ptt = parts per trillion = ng/l). Atomic elements are lead through a plasma source where they become ionized. Then, these ions are sorted on account of their mass.
- **Mass spectrometry** is an analytical tool useful for measuring the **mass**-to-charge ratio (m/z) of one or more molecules present in a sample. These measurements can often be **used** to calculate the exact molecular weight of the sample components as well.
- Mass spectrometry is a powerful analytical technique used to quantify known materials, to identify unknown compounds within a sample, and to explain the structure and chemical **properties** of different molecules

MSJChem
Tutorials for IB Chemistry

ICP-MS

Inductively coupled plasma mass spectrometry is a type of mass spectrometry which is capable of detecting metals at very low concentrations.

ICP-MS combines a high temperature ICP (inductively coupled plasma) source with a mass spectrometer.

The ICP source converts the atoms of the elements in the sample to ions.

These ions are then separated and detected by the mass spectrometer.

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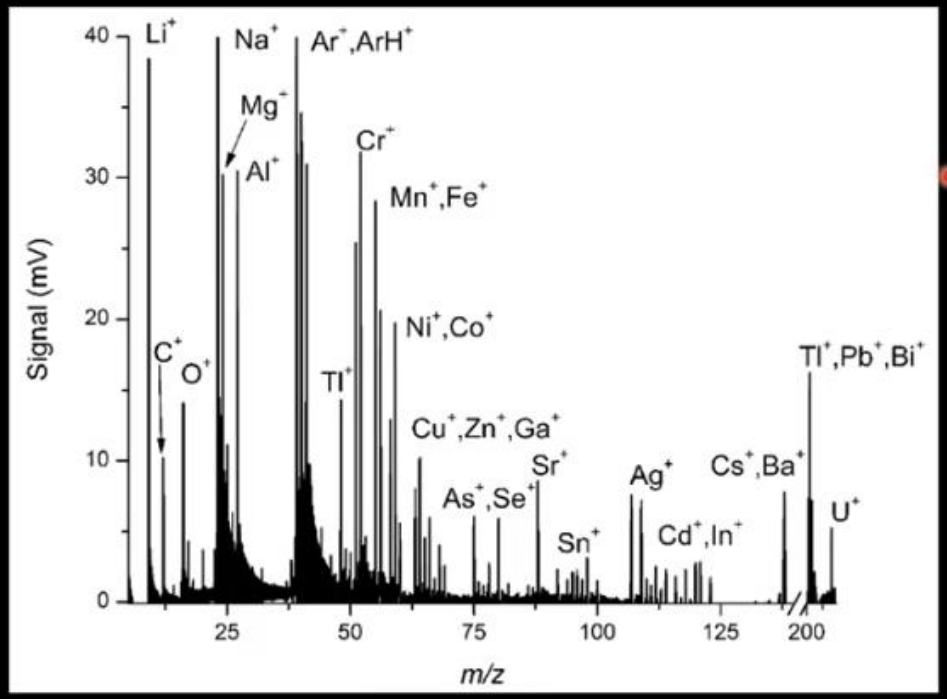
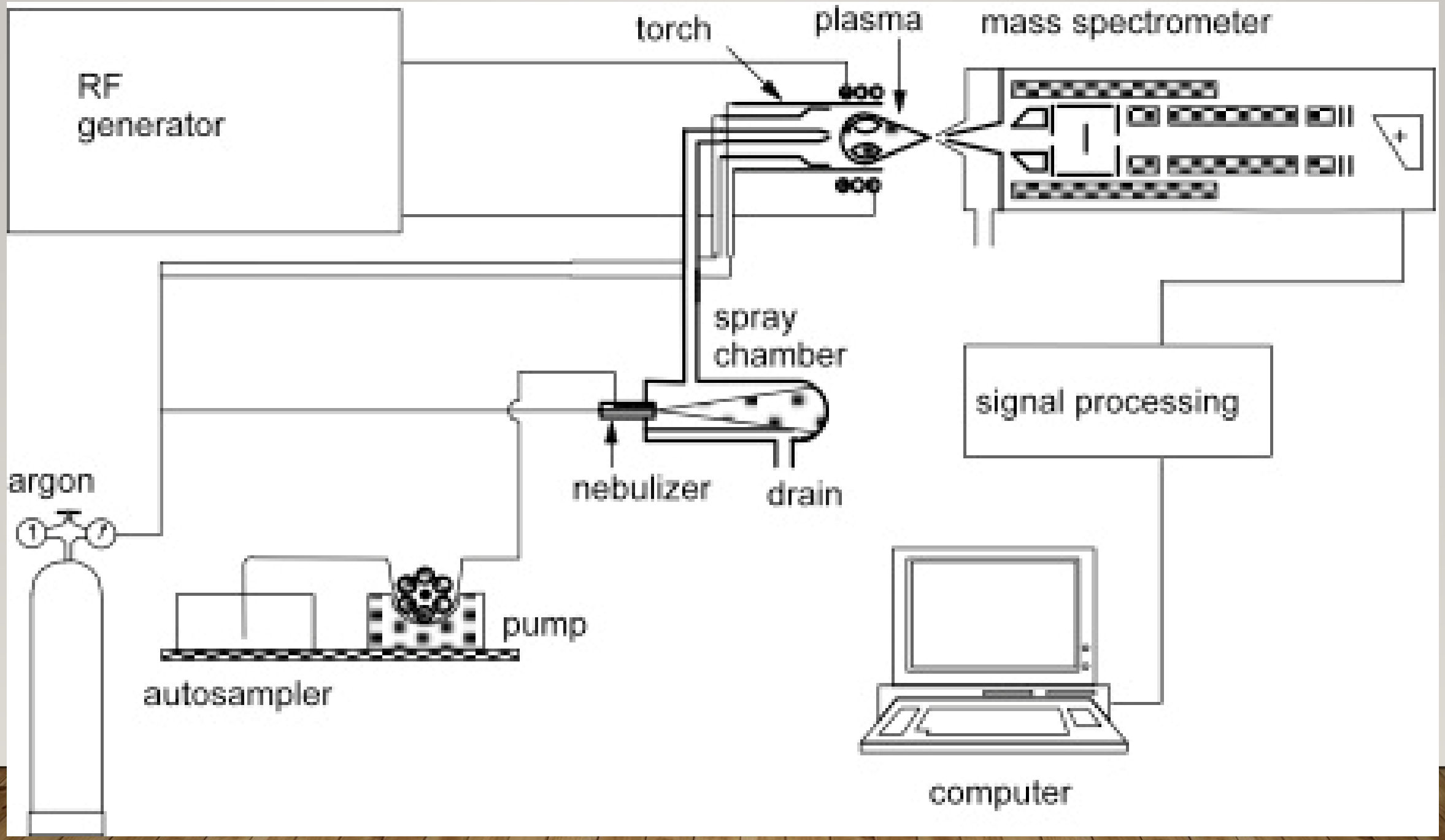
ICP-MS

The ions enter into an electric field and are separated according to their mass/charge (m/z) ratio.



- The signal intensities are directly proportional to the concentrations of the elements in the sample.


RF
generator
torch
plasma
mass spectrometer
spray
chamber
nebulizer
drain
signal processing
argon
pump
autosampler
computer

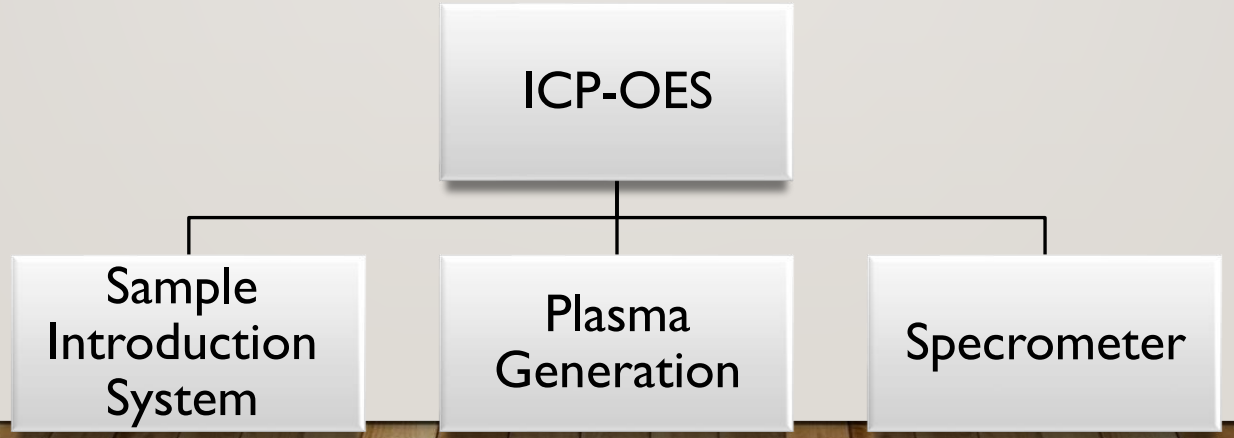
PRINCIPLE ICP-OES

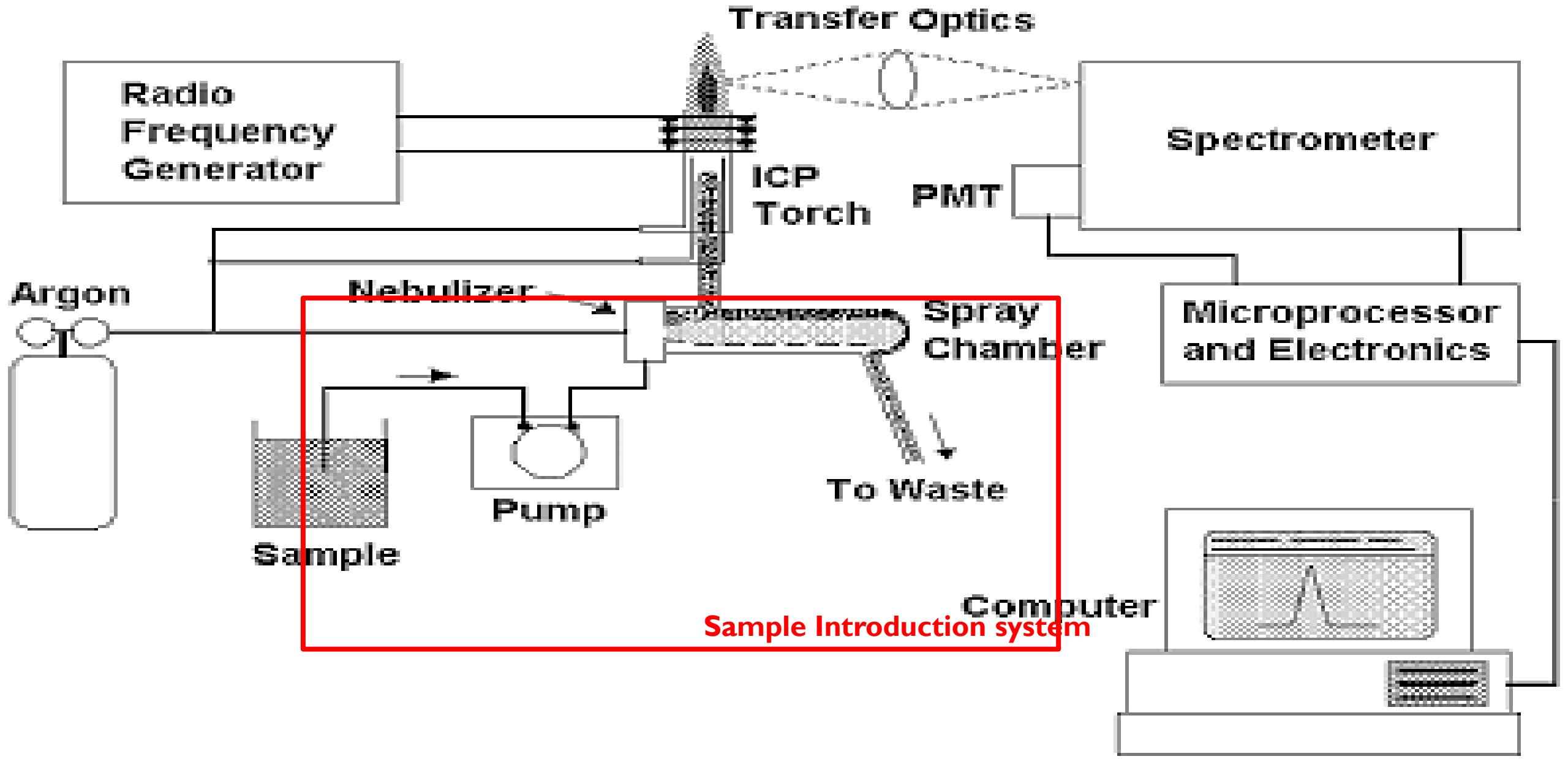
inductively coupled plasma atomic emission spectroscopy

- Like for the ICP-OES, the sample solution is introduced into the device by means of a peristaltic pump. There it becomes nebulized in a spray chamber. The resulting aerosol is injected into an argon-plasma that has a temperature of 6000-8000 K. Inside the paerosollasma torch, solution is removed from the sample and also atomization and ionization occur. Only a small amount part of the ions produced in the plasma

ICP-OES

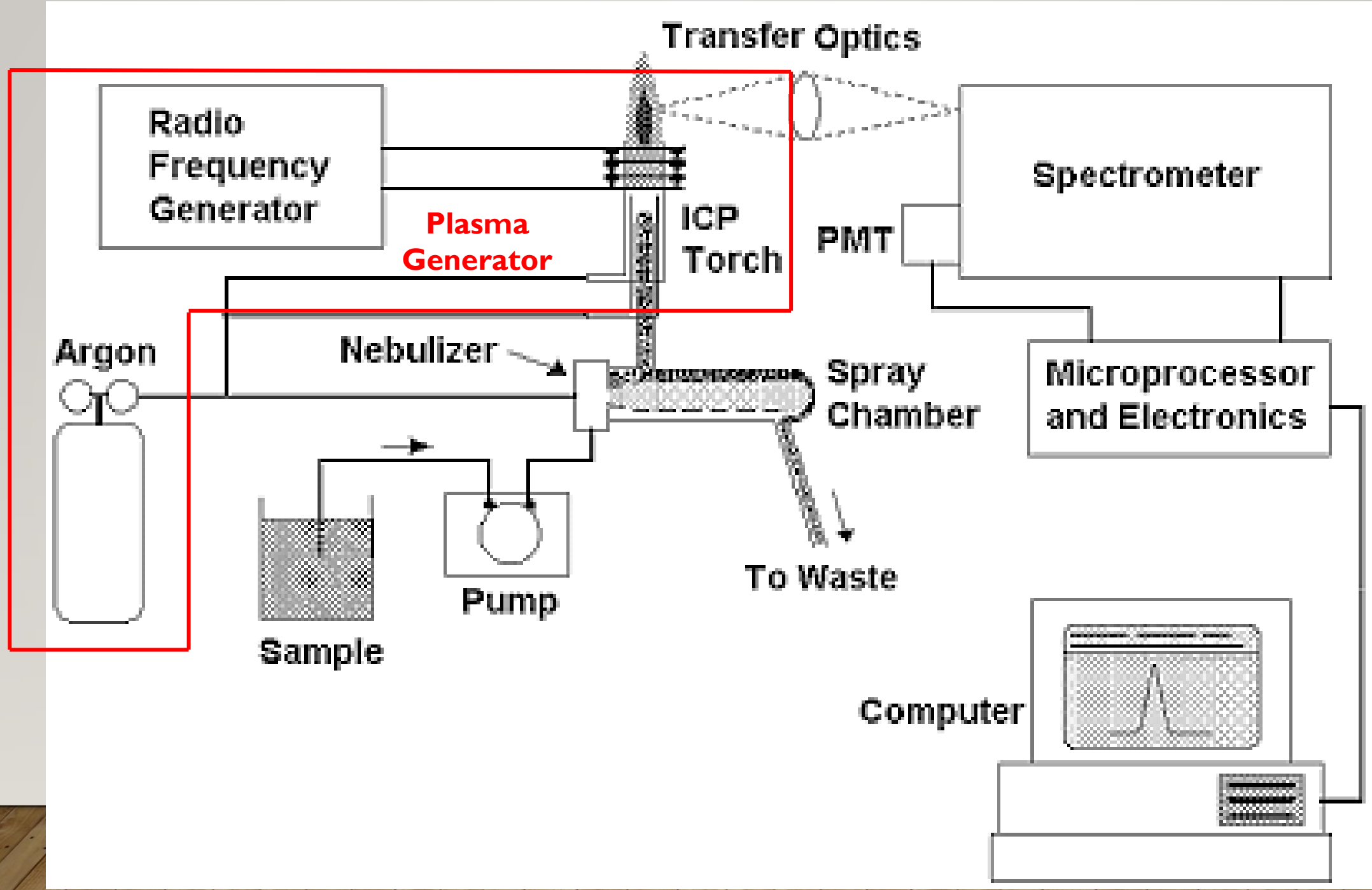
ICP-OES (Inductively coupled plasma - optical emission spectrometry) is a technique in which the composition of elements in (mostly water-dissolved) samples can be determined using plasma and a spectrometer.
The technique has been commercially available since 1974 and thanks to its reliability, multi-element options and high throughput, it has become a widely applied in both routine research as in more specific analysis purposes.




Transfer Optics
Radio Frequency Generator
Spectrometer
ICP Torch
PMT
Argon
Nebulizer
Spray Chamber
Microprocessor and Electronics
To Waste
Pump
Sample
Computer
Sample Introduction system

SAMPLE INTRODUCTION SYSTEM

1. The solution to analyze is conducted by a peristaltic pump though a nebulizer into a spray chamber.
2. the spray chamber converts the solution into a fine mist (aerosol)
3. The produced aerosol is lead into an argon plasma.

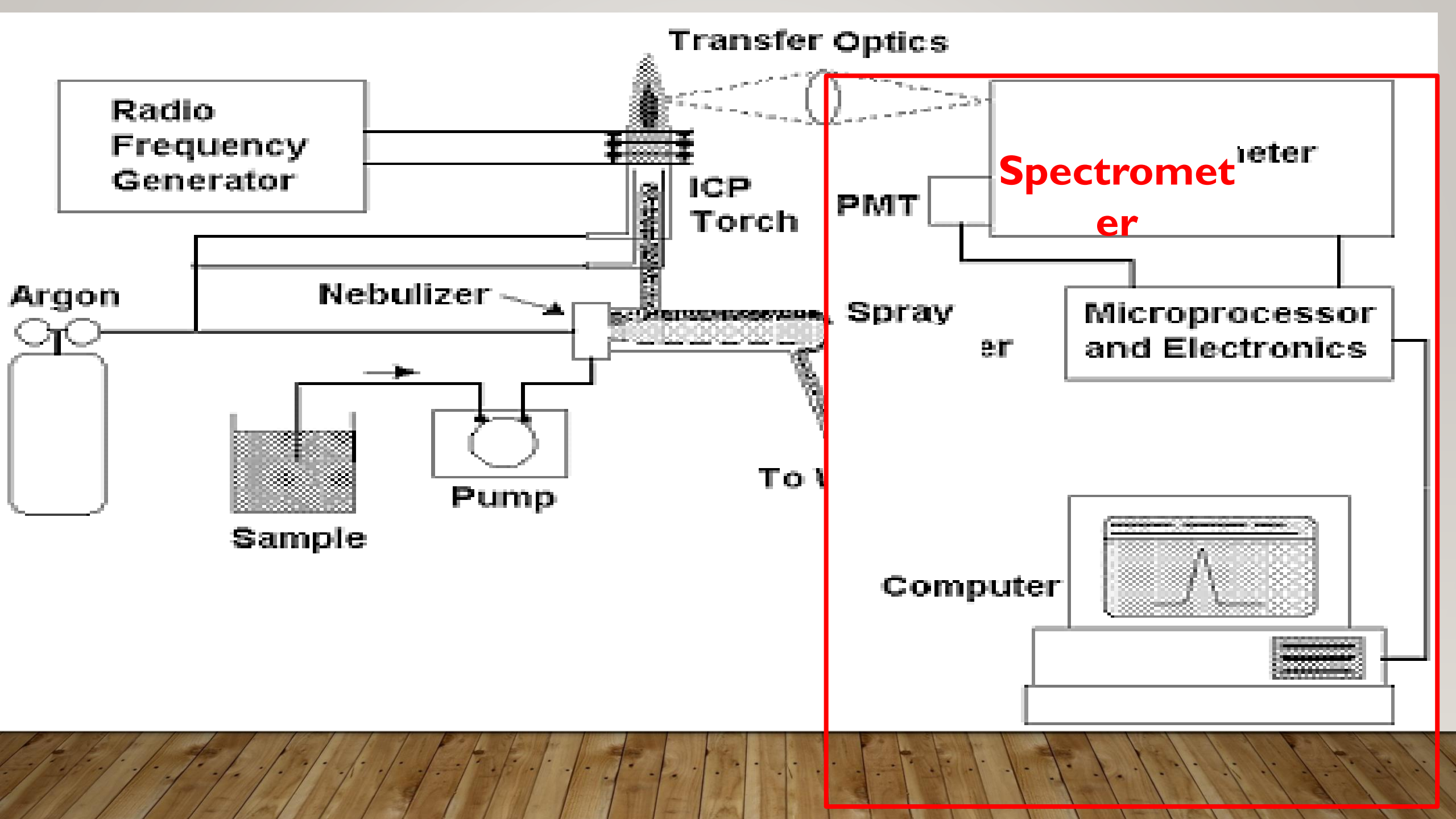

Transfer Optics
Radio Frequency Generator
Plasma Generator
ICP Torch
Spectrometer
PMT
Argon
Nebulizer
Spray Chamber
Microprocessor and Electronics
Pump
To Waste
Sample
Computer

PLASMA GENERATION

1. Plasma is the forth state of matter, next to the solid, liquid and gaseous state. In the ICP-OES the plasma is generated at the end of a quarts torch by a cooled induction coil through which a high frequency alternate current flows.
2. As a consequence, an alternate magnetic field is induced which accelerated electrons into a circular trajectory.
3. Due to collision between the argon atom and the electrons ionization occurs, giving rise to a stable plasma. The plasma is extremely hot, 6000-7000 K. In the induction zone it can even reach 10000 K.

PLASMA GENERATION

4. In the torch the sample goes through:
   a) desolvation: The removal of solvent from a material in solution to get the solids.
   b) atomization: The conversion of a vaporized sample into atomic component.
   c) ionizations: process by which an atom or a molecule acquires a negative or positive charge by gaining or losing electrons.
5. Due to the thermic energy taken up by the electrons, they reach a higher "**excited**" state. When the electrons drop back to ground level energy is liberated as light **(photons)**.


Transfer Optics
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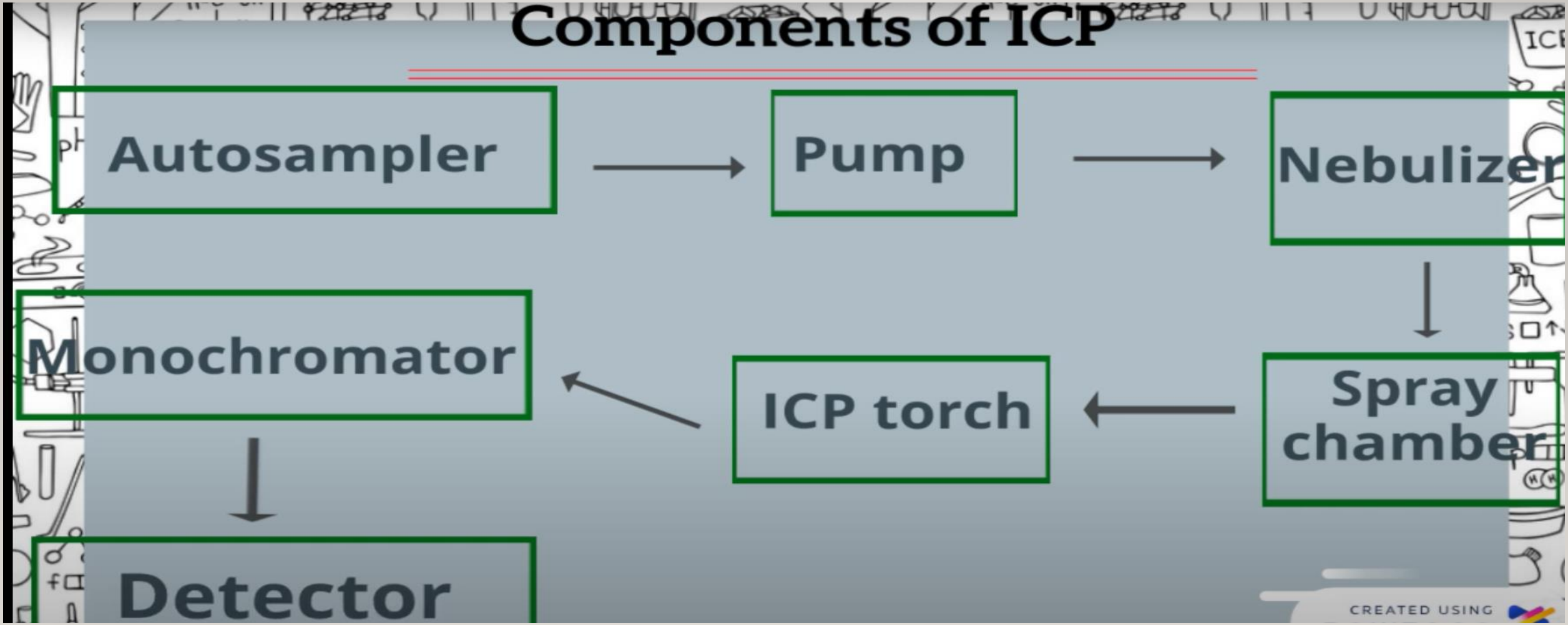
SPECTROMETER

- Each element has an own characteristic emission spectrum that is measured with a spectrometer. The light intensity on the wavelength is measured and with the calibration calculated into a concentration.


Components of ICP
Autosampler
Pump
Nebulizer
Spray chamber
ICP torch
Monochromator
Detector
CREATED USING

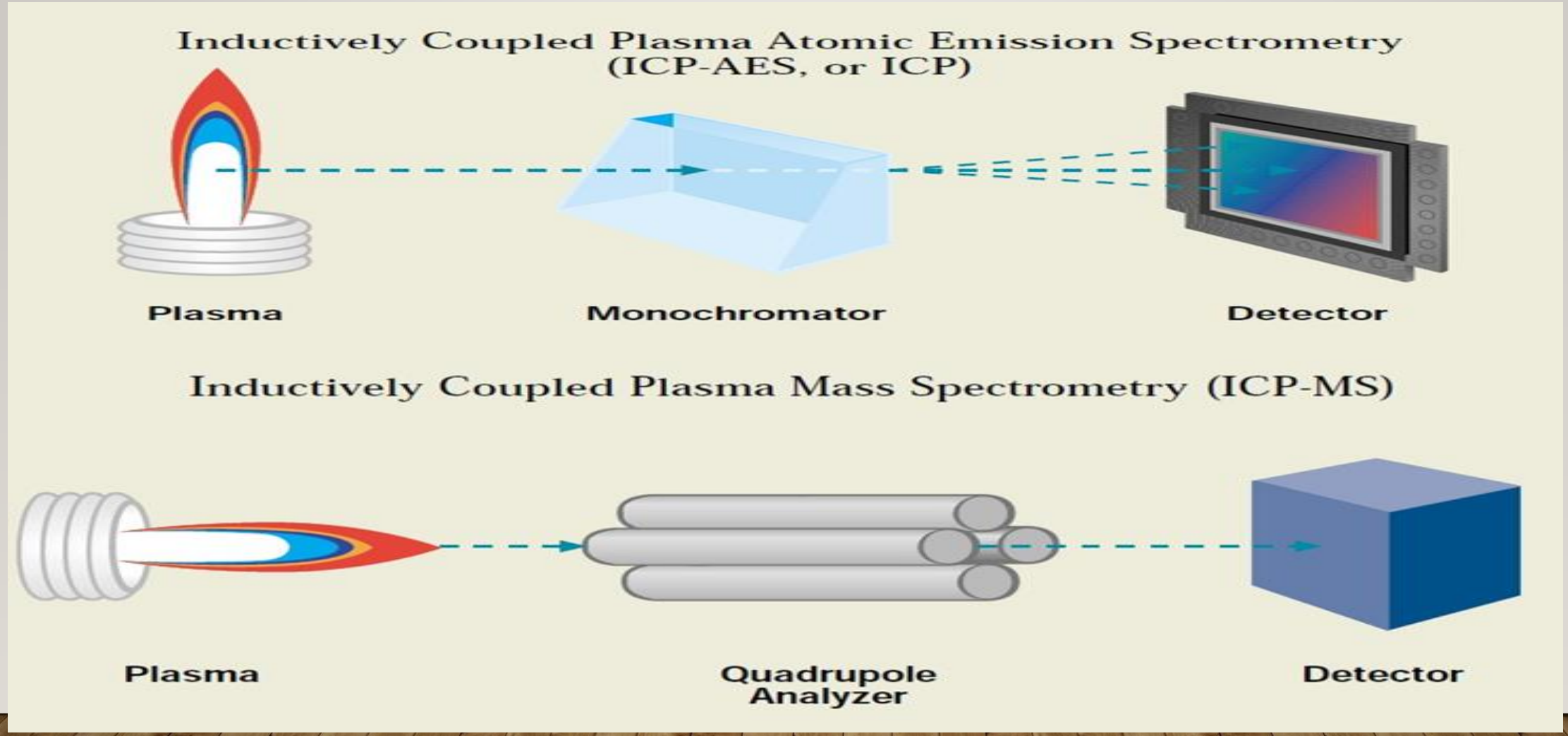
Conclusions

Steps

1. Plasma will dissociate a sample into atoms , ions.
2. Exciting them to a higher energy level.
3. They emit light at a characteristic wavelength .
4. The emitted light, will be analysing .

The instrument will know the concentration of metals inside the sample, using standard solutions.

ICP vis ICP-MS



ICP-MS VIS ICP-OES

Performance Comparison

Component	ICPMS	ICP-OES
Autosampler / Plasma	Same	Same
Detection	By Weight of Atom Hitting Surface	By Light Wavelength Emitted by Sample
Robust vs Sensitive	Maximum Sensitivity	More Robust
Instrument Cost	$$$$	$$
Analysis Price	$$	$

APPLICATIONS

- 1-medical And Forensic Field, Specifically Toxicology Ex(suspicion Of Heavy Metal Poisoning, Metabolic Concerns, And Even Hepatological Issues.)
- 2-water Testing For Municipalities Or Private Individuals All The Way To Soil, Water And Other Material Analysis For Industrial Purposes.
- 3- Industrial And Biological Monitoring Has Presented Another Major Need For Metal Analysis Via Icp-ms .Ex(individuals Working In Factories Where Exposure To Metals Is Likely And Unavoidable, Such As A Battery Factory, Are Required By Their Employer To Have Their Blood Or Urine Analyzed For Metal Toxicity On A Regular Basis.)
- 4- is also used widely in the geochemistry field for radiometric dating, which it is used to analyze relative abundance of different isotopes, in particular uranium and lead.
- 5- In the pharmaceutical industry, ICP-MS is used for detecting inorganic impurities in pharmaceuticals and their ingredients.

APPLICATIONS:

6-Simultaneous Measurement of the Trace Elements Al, As, B, Be, Cd, Co, Cu, Fe, Li, Mn, Mo, Ni, Rb, Se, Sr, and Zn in Human Serum and Their Reference Ranges by ICP-MS:

The goal of this article was to establish reference ranges of the concentration of trace elements in human serum and to compare these results with those reported by other authors. We describe the sample preparation and measurement conditions that allow the rapid, precise, and accurate determination of Al, As, B, Be, Cd, Co, Cu, Fe, Li, Mn, Mo, Ni, Rb, Se, Sr, and Zn in human serum samples by inductively (ICP-MS).

APPLICATIONS:

7- A New Method For Determining Gem Tourmaline Species By La-icp-ms:

- Gem tourmaline species cannot be determined visually in the gem and jewelry trade based on their color and appearance.
- With adequate standards and calibration, LA-ICP-MS can quantitatively measure six common major elements in tourmaline (Na, Ca, Mg, Fe, Al, and Si), allowing for species classification.
- Comparing LA-ICP-MS data to highly precise and accurate EPMA data for major elements on our samples demonstrated that some tourmaline species can be determined solely by LA-ICP-MS.

APPLICATIONS:

8-The application of ICP-MS and ICP-OES in determination of micronutrients in wood ashes used as soil conditioners:

- Extraction of micronutrients and elements from wood ashes by ICP-MS and ICP-OES

9-Analysis of Trace Elements in Teeth by ICP-MS: Implications for Caries

- Detection of trace elements in teeth to check the environmental and nutritional status reveal large amounts of data.

APPLICATIONS:

10-Determination of Lead in empty petrol by ICP-OES with use of oxygen and a cooled spray chamber.

11-Multi-element determination in brazilian honey samples by ICP- MS and estimation of graphic geographic origin with data mining techniques.

12-Determination of trace and minor elements in milk and yogurts by ICP-MS.

13-Application of ICP-MS in determination of essential and toxic materials in the blood with calibration in synthetic matrix

APPLICATIONS:

14-Application of hydrodynamic chromatography-ICP-MS to investigate the fate of silver nanoparticles in activated sludge