

# **15EI321E**

# **ANALYTICAL INSTRUMENTATION**

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# UNIT 1

## INSTRUMENTAL ANALYSIS

# INTRODUCTION

- The chemical analysis consists of collecting a sample, possibly treating the sample either physically or chemically, performing a laboratory or nonlaboratory measurement on the sample, mathematically manipulating the data as required to obtain a meaningful result, and reporting the result.
- Chemical analysis is concerned with determining either the identity of the chemical substances or the amount of a particular substance in a sample.

# CONT...

- Chemical analysis is divided into classical and instrumental analysis.
- Classical or non-instrumental analysis is the group of analytical methods that only requires the use of chemicals, a balance, calibrated glassware, and other commonplace laboratory apparatus, such as funnels, burners or hot plates, flasks, and beakers.

# CONT...

- Instrumental analysis requires the use of an analytical instrument in addition to the apparatus that is used for classical analyses.
- Classical and instrumental methods can be used for qualitative and quantitative analysis.
- An analytical instrument is a device that is used to determine the identity or amount of one or more components in the analyzed substance (the analyte).

# CHEMICAL INSTRUMENTAL ANALYSIS

- Analytical instruments are devices that measure a physical or chemical property of the assayed substance or that measure some factor that enables determination of a property of the substance.

## CONT...

Instrumental analyses are divided into three categories.

- (1) The spectral methods - use or measure some form of radiation.
- (2) The electroanalytical methods - apply an electrical signal to the sample and/or monitor an electrical property of the sample.
- (3) The separative methods - separation of the components of a sample prior to measuring a property of the components.

# SPECTRAL METHODS

- The spectral methods of analysis use an instrument to measure the amount of radiation that is absorbed, emitted, or scattered by the sample.
- If the amount of absorbed radiation is measured, the technique is absorptiometry or absorption spectrophotometry.

## CONT...

- If the absorbed energy is electromagnetic radiation in the x-ray, ultraviolet, or visible region of the spectrum, the subsequently emitted electromagnetic radiation is a form of luminescence termed either fluorescence or phosphorescence depending upon the manner in which the deexcitation takes place.

# **ELECTROANALYTICAL METHODS**

- Instrumental methods of chemical analysis in which either an electrical signal is applied to one of the electrodes dipping into the sample solution or an electrical property of the solution is measured are the electroanalytical methods.

## CONT...

- The electroanalytical methods are divided into categories,
- Amperometry is the method in which the potential between the two electrodes is controlled and the current is measured.
- When the current between the electrodes is controlled (usually at nearly zero) and the potential is measured, the technique is potentiometry.

# CONT...

- During electroanalytical studies using coulometry.
- Electrogravimetry, either a potential or a current can be applied to the electrodes in the solution.
- When the quantity  $Q$  of electricity that is consumed during an electrochemical reaction is measured, the technique is coulometry.

## CONT...

- When the mass of a reaction product (often a metal) after an exhaustive electrolysis is measured, the method is electrogravimetry.
- Conductometry is the electroanalytical method in which the electrical conductance  $G$  of the sample solution is measured.
- Voltammetry is the technique in which a potential is applied to one of the electrodes by a potentiostat while the current flowing through the electrode is

# SEPARATIVE METHODS

- The separative methods take advantage of physical or chemical properties of the components of a mixture to separate the components.
- After the separation, the components can be individually assayed either qualitatively or quantitatively.

## CONT...

- The instrumental separative techniques are divided into categories,
- Chromatography is the method by which a mixture is separated into its components as a result of the relative ability of each component to be flushed along or through a stationary phase by a mobile phase.

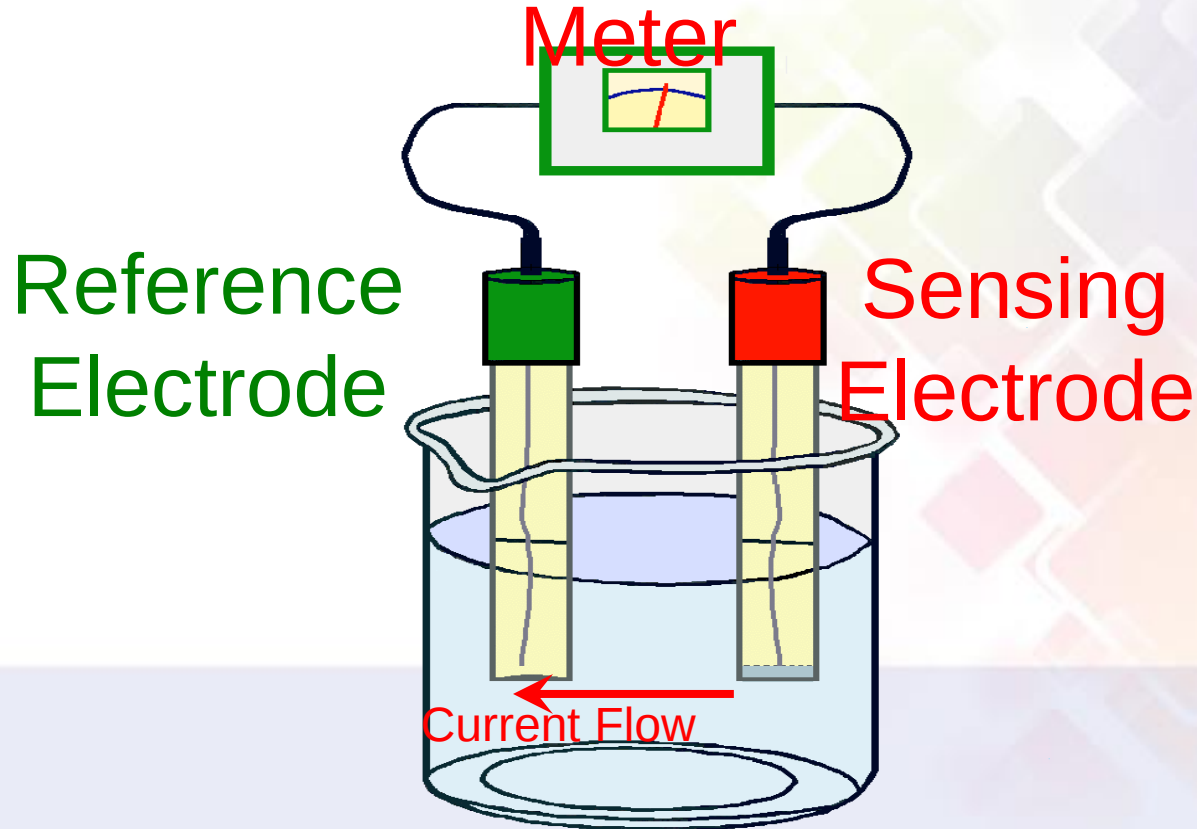
## CONT...

- Electrophoresis is the separative method that takes advantage of the relative mobility of ions toward an electrode of opposite charge (to the ion) and away from an electrode of similar charge.
- Separations using mass spectrometry are based upon the relative motion in an electrical or magnetic field of the components of a gaseous mixture of sample ions.

# ION SELECTIVE ELECTRODES(ISE)

- An **ion-selective electrode (ISE)**, also known as a specific **ion electrode (SIE)**, is a transducer (or sensor) that converts the activity of a specific **ion** dissolved in a solution into an electrical potential.

# CONT...



# WORKING OF ISE

- **Ion selective electrodes** are membrane **electrodes** that produce a potential by converting the activity of **ions** dissolved in a solution.
- The potential can be measured using a pH meter or voltmeter.

# pH

- pH is a unit of measure which describes the degree of acidity or alkalinity (basic) of a solution.
- It is measured on a scale of 0 to 14.
- The formal definition of pH is the negative logarithm of the hydrogen ion activity.
- $\text{pH} = -\log[\text{H}^+]$

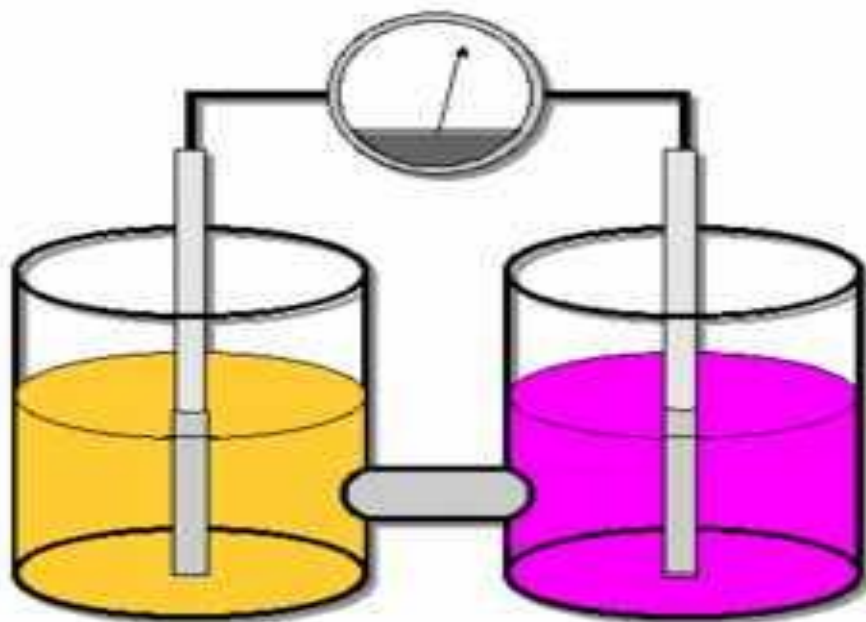
# pH MEASUREMENT

- A pH measurement system consists of three parts: a pH measuring electrode, a reference electrode, and a high input meter.
- The pH measuring electrode is a hydrogen ion sensitive glass bulb.
- The reference electrode output does not vary with the activity of the hydrogen ion.

## Potentiometric methods

**Reference electrode**

The part of the cell that is held constant



**Indicating electrode**

The part of the cell that contains the solution we are interested in measuring

# WORKING OF pH MEASUREMENT

- A **pH meter works** like a voltmeter.
- It measures the voltage (electrical potential) produced by the solution and compares it with the voltage of a known solution, the potential difference gives the measurement of pH.

# UNIT 2

## GAS ANALYSERS

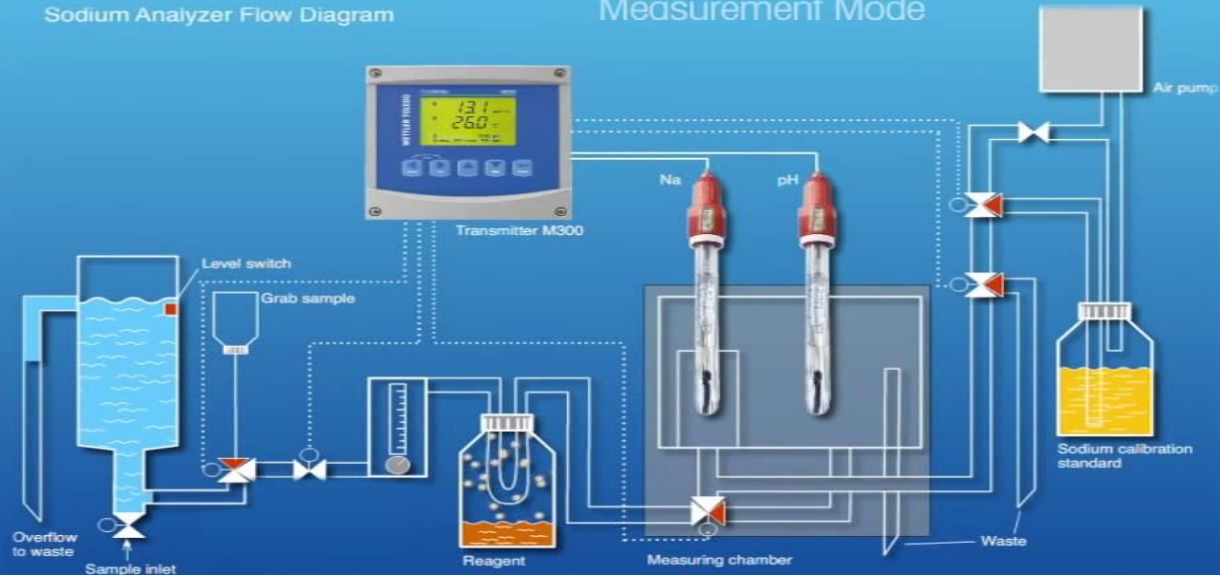
# SODIUM ANALYSER

- The sample flows through the heat exchanger, passes through constant head (used to stabilize minor changes in flow rate) and through the three solenoid valves.
- A vapor entrainment mechanism adds alkaline buffer vapor to the sample before it enters the sodium measuring flow cell and flows to drain.
- Calibration and grab sample are controlled automatically by the analyzer.

# CONT...

Sodium Analyzer Flow Diagram

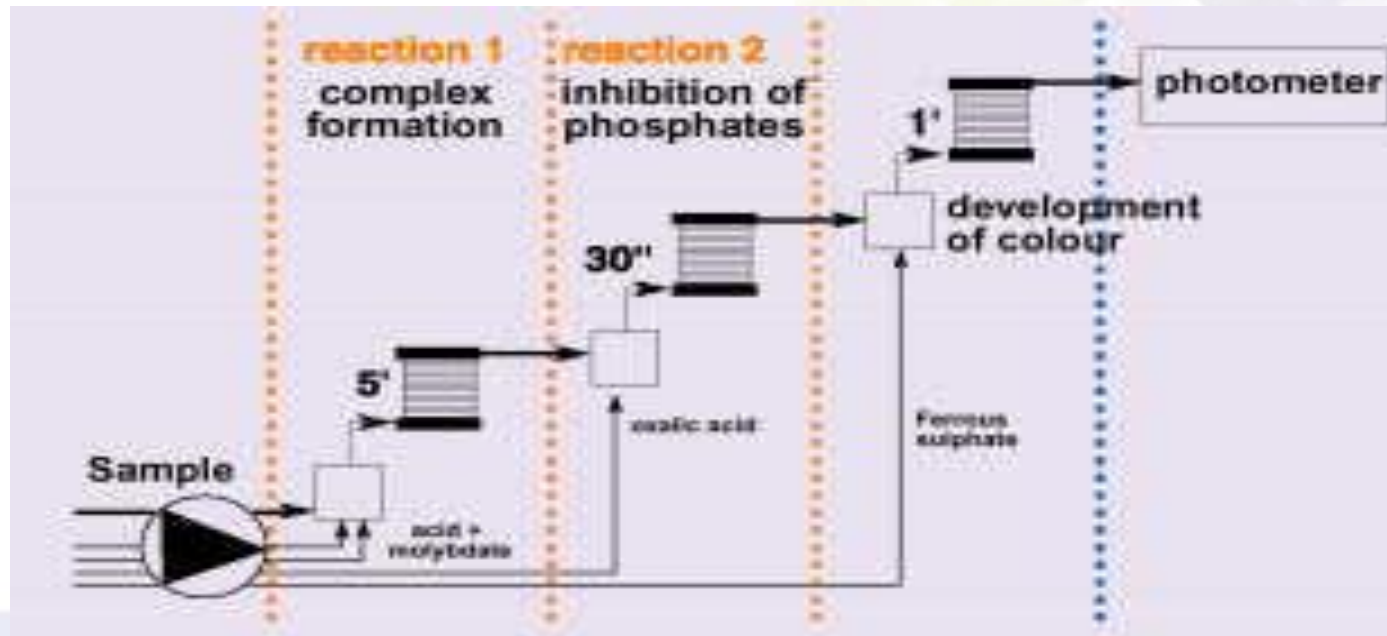
Measurement Mode



# SILICA ANALYSER

1. Silica reacts with molybdate in low pH conditions to form a yellow silicomolybdic complex.
2. Oxalic acid eliminates interference from phosphates and intensifies color of the complex.
3. Ferrous sulphate reduces the silicomolybdic complex to blue silicomolybdenum.
4. A degassing system is used to remove air bubbles which increase the signal stability.
5. The color intensity, measured by photometry at 820nm, is proportional to the silica concentration.

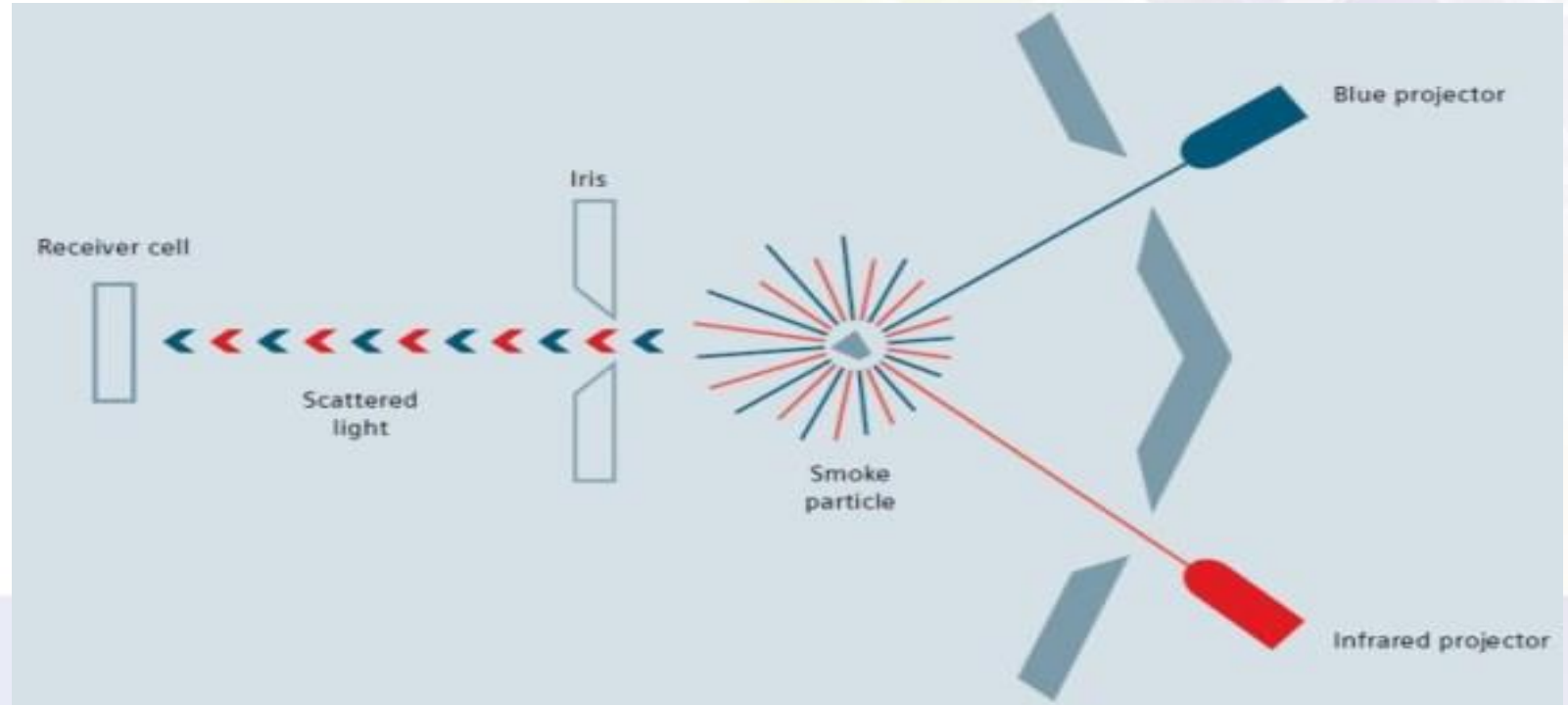
# CONT...



# DUST AND SMOKE MEASUREMENT

- Dusty air is drawn through a damping chamber which saturates the air with water.
- The strokes of the pump are standard and valve is opened, allowing the air to enter the head.
- On passing through the jet, which is in the form of an oblong slit, a reduction of pressure takes place and some of the water vapour condense on the dust particles.
- The stream of air then impinges on the cover slip and a change in direction takes place.
- The moist dust particles deposits on the cover slip
- due to pressure and temperature rise at this point, the water evaporates leaving the dust traces on the cover slip.
- The cover slip is then removed from the head and mounted on a microscope slide and the counting operation is carried out.

# CONT...



## **UNIT 3**

# **CHROMATOGRAPHY**

# INTRODUCTION TO CHROMATOGRAPHY

- Chromatography is a physical method of separation in which the components to be separated are distributed between two phases.
- one is stationary (stationary phase) while the other (the mobile phase) moves through it in a definite direction.
- The chromatographic process occurs due to differences in the distribution constant of the individual sample components.

# CLASSIFICATION

Classification of chromatography according to mobile phase:

- 1- Liquid chromatography: mobile phase is a liquid. (LLC, LSC).
- 2- Gas chromatography : mobile phase is a gas. (GSC, GLC).

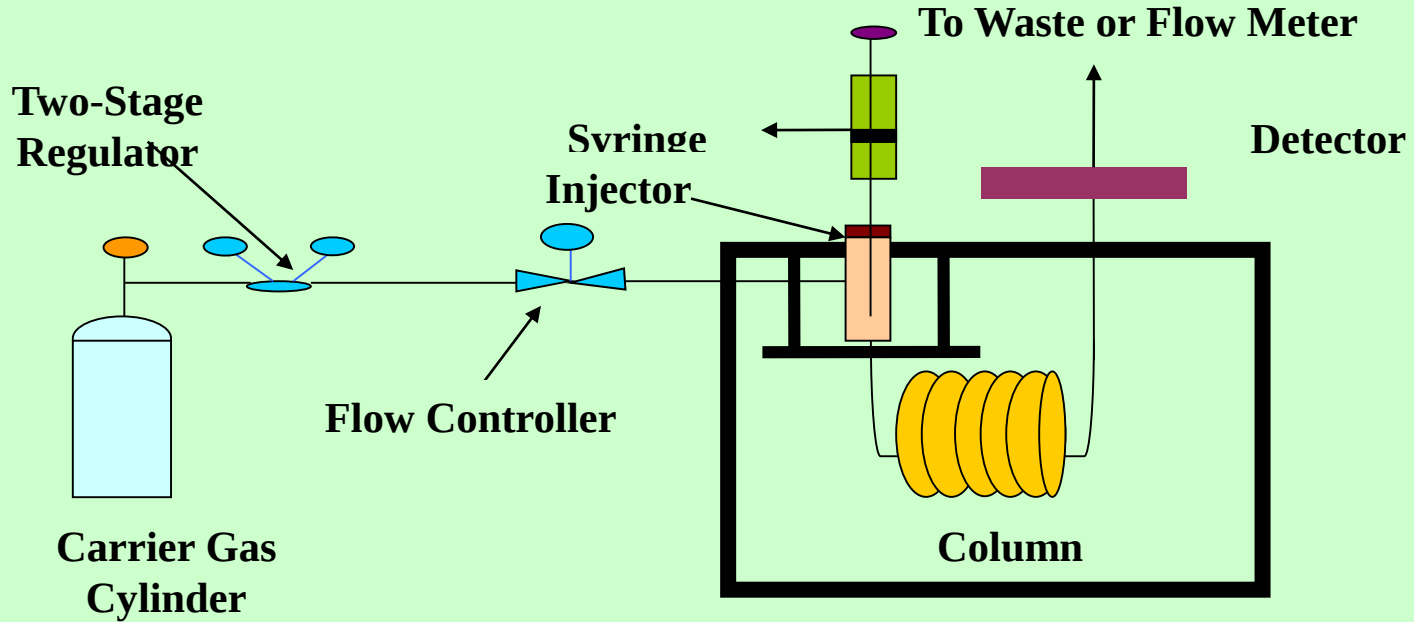
# GAS CHROMATOGRAPHY

- Gas chromatography is a technique used for separation of **volatile substances**, or **substances that can be made volatile**, from one another in a gaseous mixture at high temperatures.
- A sample containing the materials to be separated is injected into the gas chromatograph.
- A mobile phase (carrier gas) moves through a column that contains a wall coated or granular solid coated stationary phase.

## CONT...

- As the carrier gas flows through the column, the components of the sample come in contact with the stationary phase.
- The different components of the sample have different affinities for the stationary phase, which results in differential migration of solutes, thus leading to separation .
- Gas chromatography can be used for both qualitative and quantitative analysis.

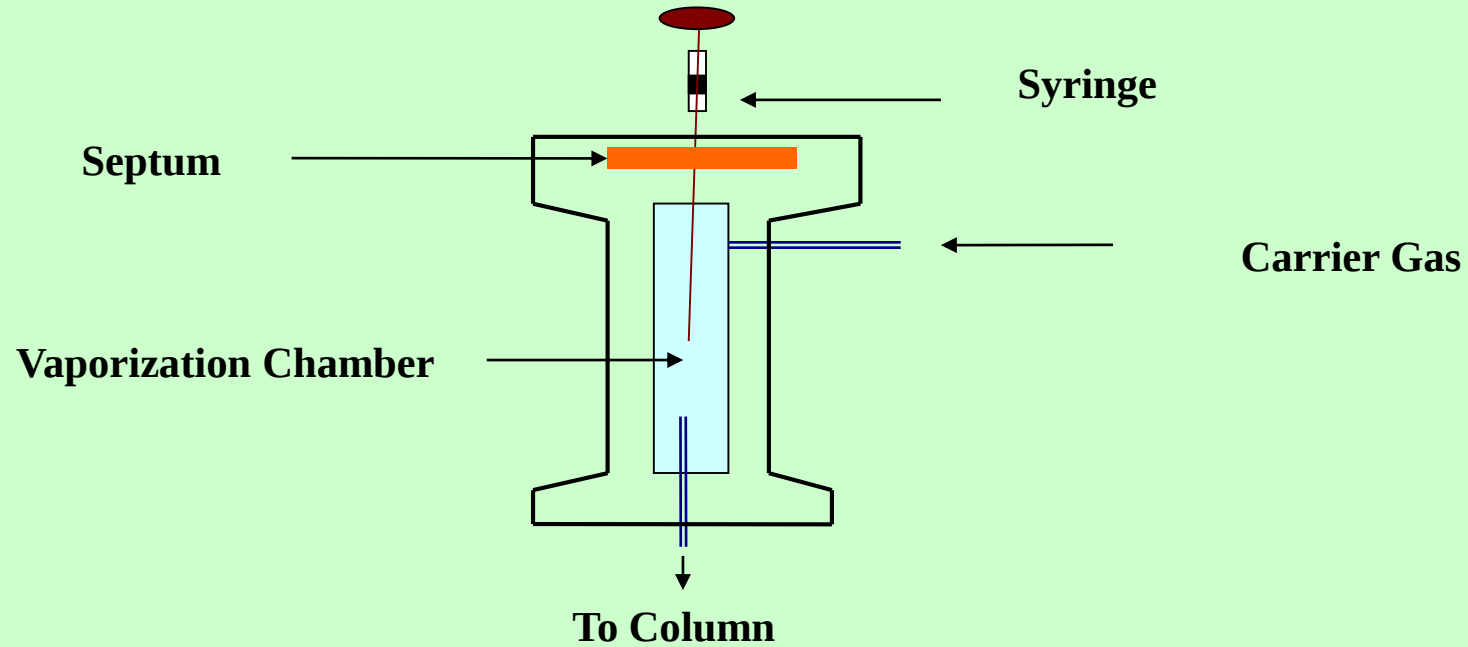
# CONT...



# CARRIER GAS

- The carrier gas pressure ranges from 10-50 psi. Higher pressures potentially increase compression possibility while very low pressures result in large band broadening due to diffusion.
- Depending on the column dimensions, flow rates from 1-150 mL/min are reported.
- Conventional analytical columns (1/8") usually use flow rates in the range from 20-50 mL/min while capillary columns use flow rates from 1-5 mL/min depending on the dimensions and nature of column.

# INJECTORS



## CONT...

- Septum type injectors are the most common. These are composed of a glass tube where vaporization of the sample takes place.
- The sample is introduced into the injector through a self-sealing silicone rubber septum. The carrier gas flows through the injector carrying vaporized solutes.
- The temperature of the injector should be adjusted so that flash vaporization of all solutes occurs.
- If the temperature of the injector is not high enough

# COLUMN CONFIGURATIONS

- The column in chromatography is undoubtedly the heart of the technique. A column can either be a packed or open tubular.
- Packed columns are relatively short (~2meters) while open tubular columns may be as long as 30-100 meters.
- Packed columns are made of stainless steel or glass while open tubular columns are usually made of fused silica.

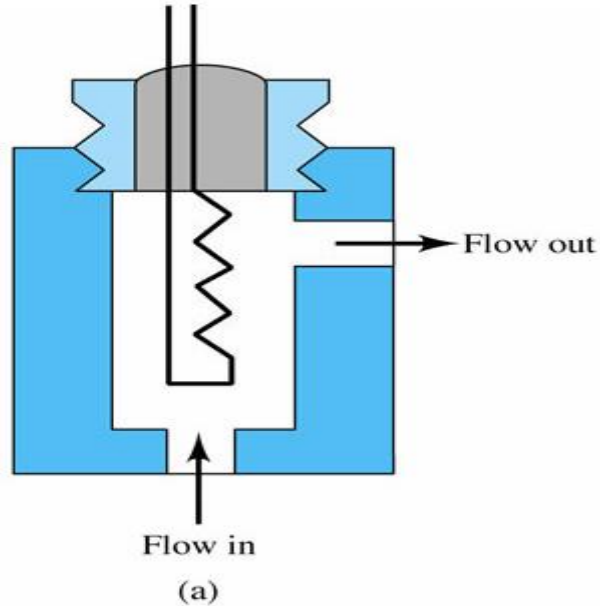
## CONT...

- The temperature of the column is adjusted so that it is close to the average boiling point of the sample mixture.
- The temperature of the column is assumed to be the same as the oven which houses the column.
- The oven temperature should be stable and easily changed in order to obtain reproducible results.

# DETECTION SYSTEMS

- Several detectors are available for use in GC.
- Each detector has its own characteristics and features as well as drawbacks.

# THERMAL CONDUCTIVITY DETECTOR (TCD)



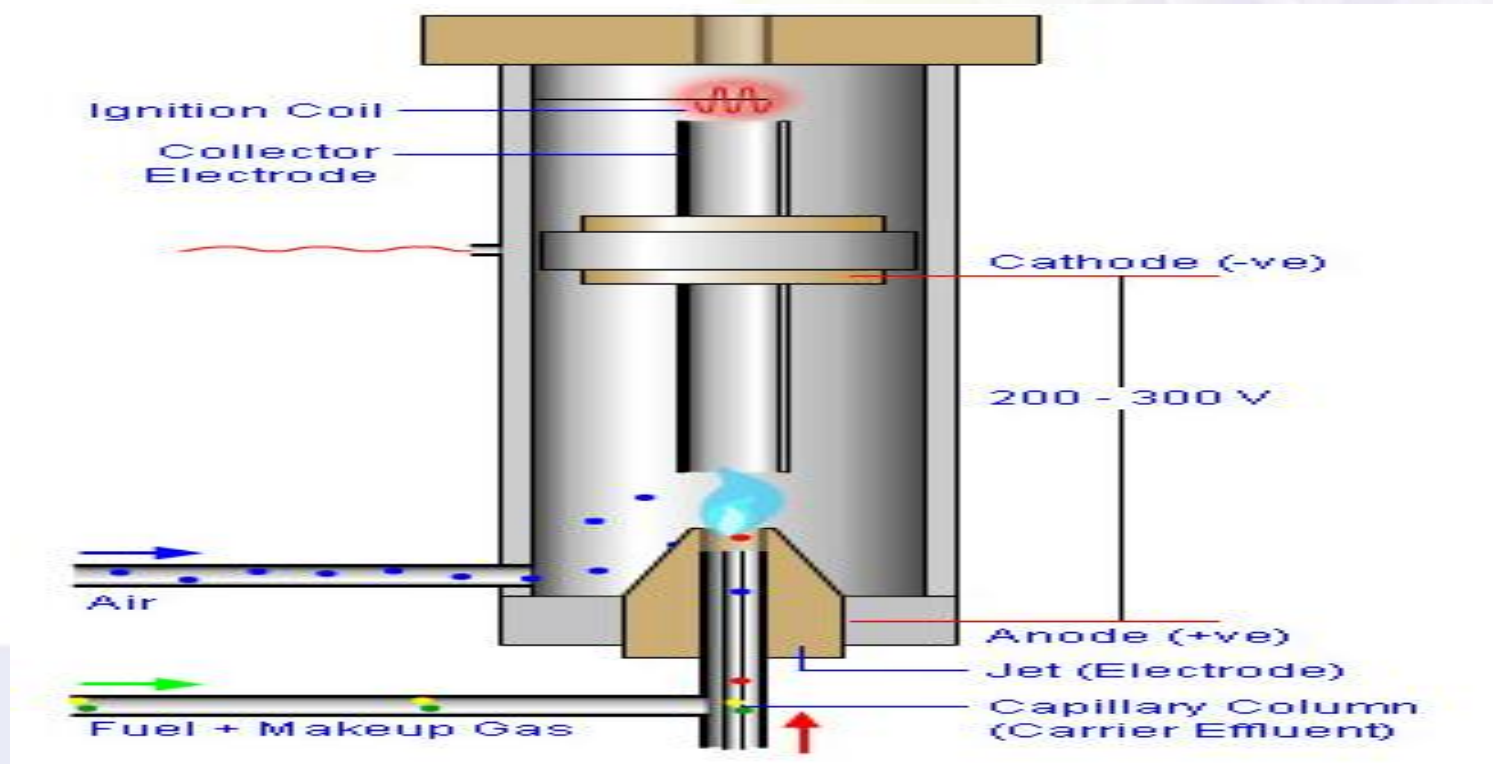
## CONT...

- This is a nondestructive detector which is used for the separation and collection of solutes to further perform some other experiments on each purely separated component.
- The heart of the detector is a heated filament which is cooled by helium carrier gas.
- Any solute passes across the filament will not cool it as much as helium does because helium has the highest thermal conductivity.

## CONT...

- This results in an increase in the temperature of the filament which is related to concentration.
- The detector is simple, nondestructive, and universal but is not very sensitive and is flow rate sensitive.

# FLAME IONIZATION DETECTOR (FID)



## CONT...

- This is one of the most sensitive and reliable destructive detectors.
- Separate two gas cylinders, one for fuel and the other for O<sub>2</sub> or air are used in the ignition of the flame of the FID.
- The fuel is usually hydrogen gas. The flow rate of air and hydrogen should be carefully adjusted in order to successfully ignite the flame.

## CONT...

- The FID detector is a mass sensitive detector where solutes are ionized in the flame and electrons emitted are attracted by a positive electrode, where a current is obtained.
- The FID detector is not responsive to air, water, carbon disulfide.
- This is an extremely important advantage where volatile solutes present in water matrix can be easily analyzed without any pretreatment.

# GAS LIQUID CHROMATOGRAPHY

- Packed columns are fabricated from glass, metal, or Teflon with 1 to 3 m length and 2 to 4 mm in internal diameter.
- The column is packed with a solid support (100-400  $\mu$ m particle diameter made from diatomaceous earth) that has been coated with a thin layer (0.1-5  $\mu$ m) of the stationary liquid phase.
- Efficiency increases with decreasing particle size.
- The retention is based on absorption of analyte (partition into the liquid stationary phase) where solutes must have differential solubility in the stationary phase

# **GAS SOLID CHROMATOGRAPHY**

- Gas-solid chromatography is based upon adsorption of gaseous substances on solid surfaces.
- Distribution coefficients are generally much larger than those for gas-liquid chromatography.
- Gas-solid chromatography is useful for the separation of species that are not retained by gas-liquid columns, such as the components of air, hydrogen sulfide, carbon disulfide, nitrogen oxides, and rare gases.
- Gas solid chromatography is performed with both

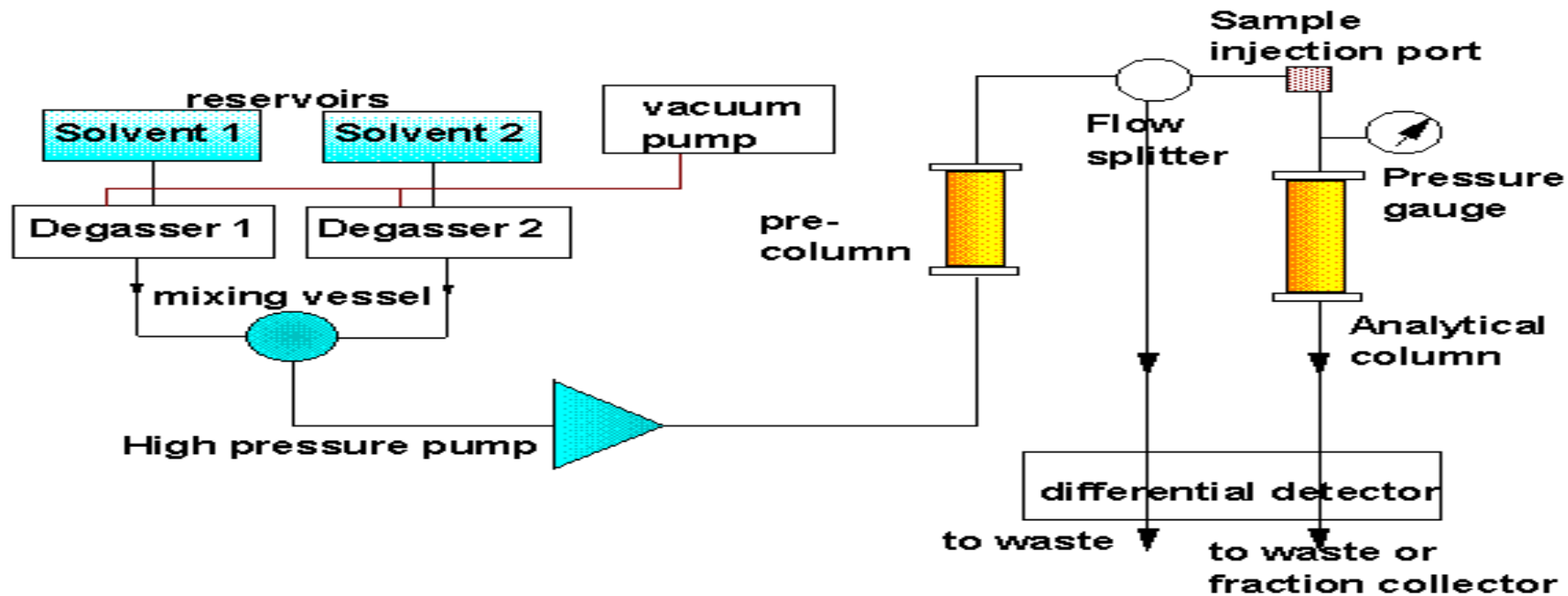
# HIGH PRESSURE LIQUID CHROMATOGRAPHY

- HPLC is a form of liquid chromatography used to separate compounds that are dissolved in solution.
- HPLC instruments consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector.
- Compounds are separated by injecting a sample mixture onto the column.

## CONT...

- The different component in the mixture pass through the column at differentiates due to differences in their partition behavior between the mobile phase and the stationary phase.
- The mobile phase must be degassed to eliminate the formation of air bubbles

# CONT...



# HPLC INJECTORS

- The function of the injector is to place the sample into the high-pressure flow in as narrow volume as possible so that the sample enters the column as a homogeneous, low-volume plug.
- To minimize spreading of the injected volume during transport to the column, the shortest possible length of tubing should be used from the injector to the column.

## CONT...

- When an injection is started, an air actuator rotates the valve: solvent goes directly to the column; and the injector needle is connected to the syringe.
- The air pressure lifts the needle and the vial is moved into position beneath the needle. Then, the needle is lowered to the vial.

# HPLC COLUMNS

- The column is one of the most important components of the HPLC chromatograph because the separation of the sample components is achieved when those components pass through the column.
- The High performance liquid chromatography apparatus is made out of stainless steel tubes with a diameter of 3 to 5mm and a length ranging from 10 to 30cm.

# CONT..

- Normally, columns are filled with silica gel because its particle shape, surface properties, and pore structure help to get a good separation.
- Silica is wetted by nearly every potential mobile phase, is inert to most compounds and has a high surface activity which can be modified easily with water and other agents.
- Silica can be used to separate a wide variety of chemical compounds, and its chromatographic behavior is generally predictable and reproducible.

# DETECTORS

- Absorbance (UV with Filters, UV with Monochromators)
- IR Absorbance
- Fluorescence
- Refractive-Index

# UNIT 4

## SPECTROPHOTOMETERS

# SPECTROPHOTOMETERS

- **Spectrophotometer** - an instrument that measures the amount of light absorbed, or the intensity of color at a given wavelength.
- The **intensity of color** can be given a numerical value by comparing the amount of light prior to passing it through the sample and after passing through the sample.
- These **quantitative measurements** of light absorbed are the **Transmittance** and the **Absorbance**.

## CONT..

- **Transmittance** is given by the equation:

$$T = I/I_0$$

where  $I$  is the intensity of the light after it has gone through the sample &  $I_0$  is the initial light intensity.

- **Absorbance** is related to the %T:

$$A = -\log T = -\log(I/I_0)$$

## CONT...

- An instrument that measures the amount of light that passes through (is transmitted through) a sample.
- Uses a type of light to detect molecules in a solution
- Light is a type of energy, and the energy is reported as wavelengths, in nanometers (nm).

# TYPES OF SPECTROPHOTOMETER

## (1) Ultraviolet (UV) Spectrophotometers.

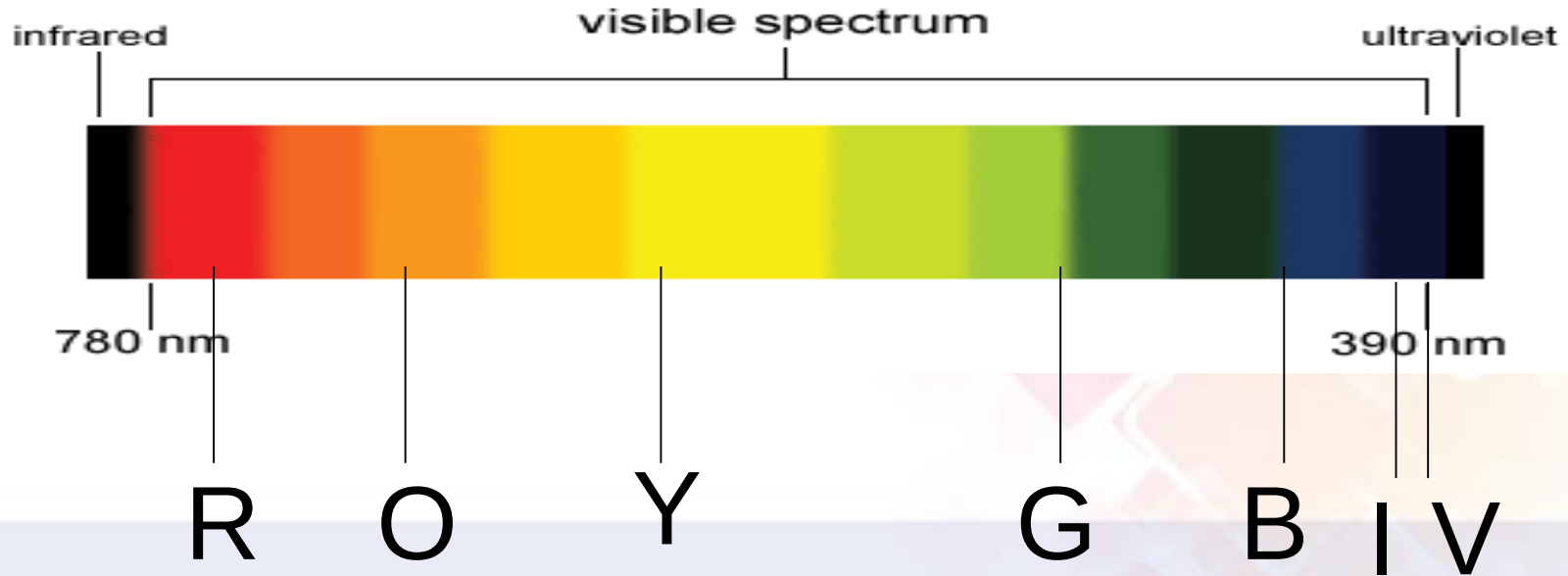
- Uses ultraviolet light of wave lengths from 200 nm to 350 nm.

## (2) Visible (VIS) Light Spectrum

- Uses visible light (white light) of wave lengths from 350 nm to 700 nm.

# VISIBLE LIGHT SPECTRUM

**The Visible Light Spectrum**



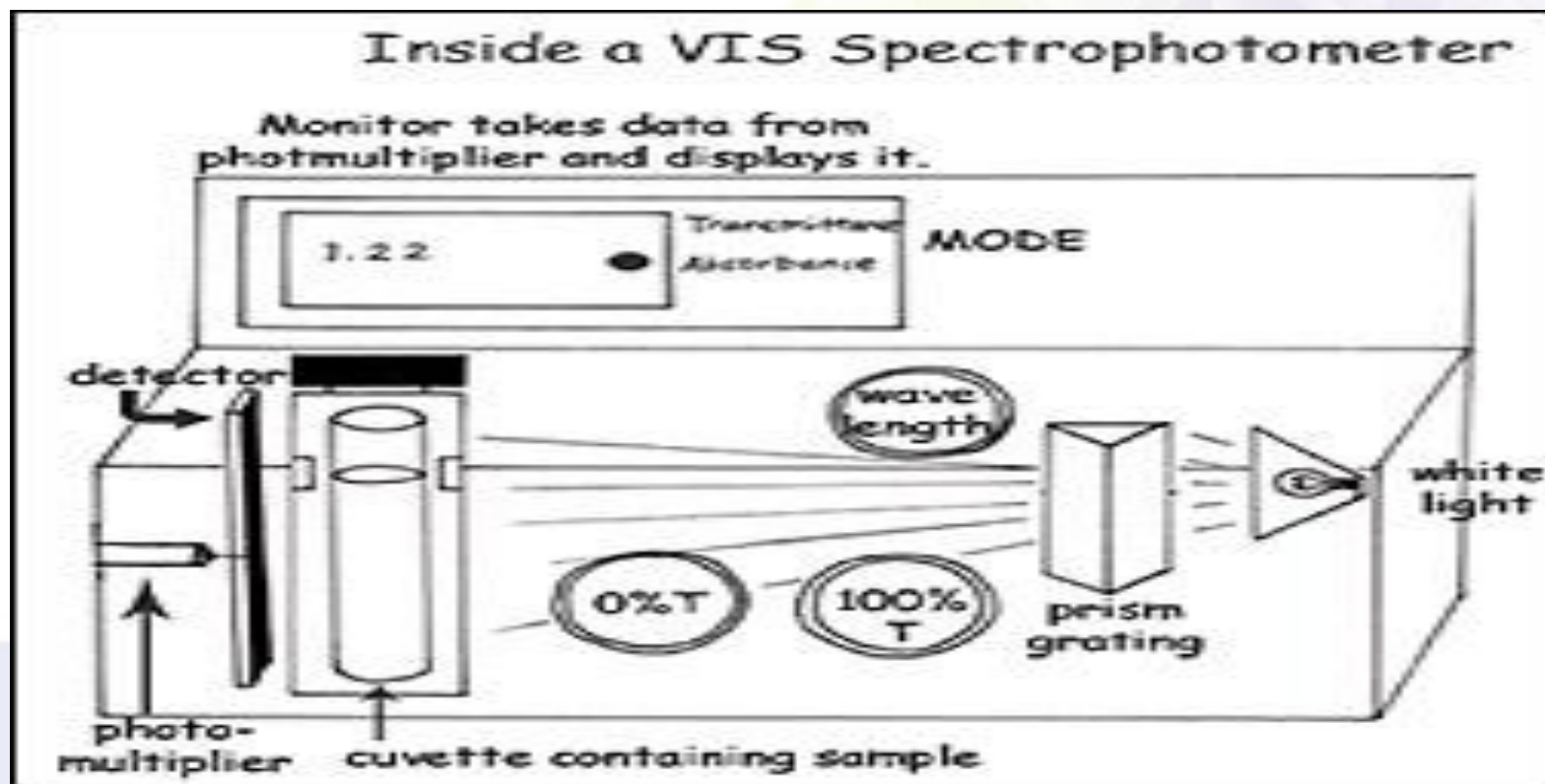
## CONT...

- Shines a beam of light on a sample.
- The molecules in the sample interact with the light waves in of 3 ways:
  - Absorb** the energy
  - Reflect** the energy
  - Transmit** the energy between and through the atoms and molecules of the sample.

# CONT...

- The spectrophotometer measures the **amount** of light transmitted through the sample (Transmittance).
- Using an equation (Beers law), it converts the transmittance data to an absorbance value.
- The concentration of an unknown sample can be determined by comparing the absorbance data to standards of known concentration.

# CONT...



# WORKING OF VIS SPECTROPHOTOMETER

- White light hits the prism or grating, it is split into the colors of the rainbow (Visible Spectrum).
- Wavelength knob rotates the prism/grating, directing different color of light toward the sample.
- The wavelength of light produced by the tungsten lamp range from about 350 nm (Violet light) to 700 nm (red light).
- The molecules in the sample either **absorb** or **Transmit** the light energy of one wavelength or another.

## CONT...

- The detector measures the **amount** of light being **transmitted** by the sample and reports that value directly (% transmittance) or converts it to the **amount** of light absorbed in absorbance units (au) using Beers Law.
- After collecting data for your concentration an absorption spectrum graph is created.

# BEER LAMBERTS LAW

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

$$A = abc$$

A is the absorbance

“a” is molar absorptivity in  $L/[(\text{mole})(\text{cm})]$

“b” is the path length in cm

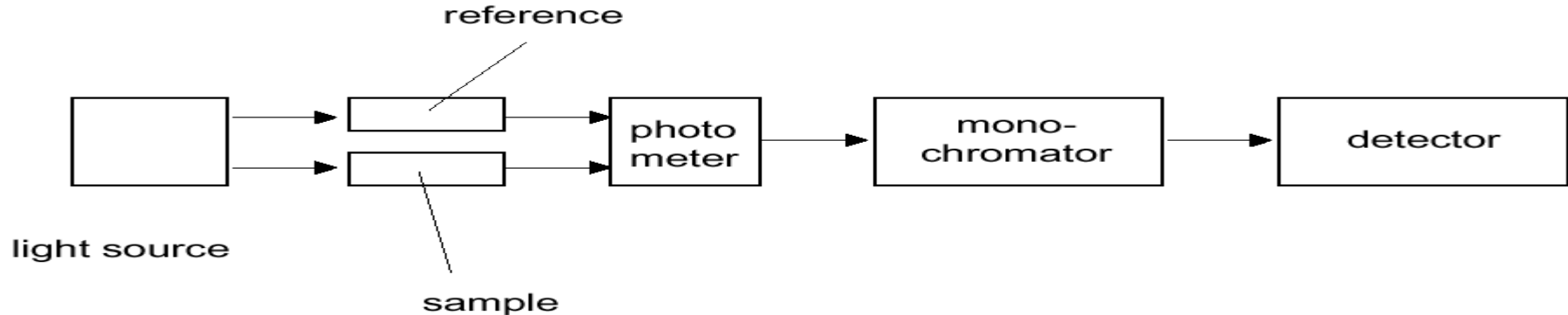
“c” is the concentration of the analyte  
(sample) in mol/L

# IR SPECTROPHOTOMETER

- IR deals with the interaction of infrared radiation with matter.
- The IR spectrum of a compound can provide important information about its chemical nature and molecular structure.
- Most commonly, the spectrum is obtained by measuring the *absorption* of IR radiation, although infrared emission and reflection are also used.
- Widely applied in the analysis of organic materials, also useful for polyatomic inorganic molecules and for organometallic compounds.

# DISPERSIVE IR SPECTROPHOTOMETERS

- Modern dispersive IR spectrophotometers are invariably double-beam instruments, but many allow single-beam operation via a front-panel switch.



# FOURIER TRANSFORM INFRARED SPECTROMETER (FTIR)

- The Fourier transform method provides an alternatives to the use of monochromators based on dispersion.
- In conversional dispersive spectroscopy, frequencies are separated and only a small portion is detected at any particular instant, while the remainder is discarded. The immediate result is a *frequency-domain spectrum*.

## CONT...

- Fourier transform infrared spectroscopy generates *time-domain spectra* as the immediately available data, in which the intensity is obtained as a function of time.
- Direct observation of a time-domain spectrum is not immediately useful because it is not possible to deduce, by inspection, frequency-domain spectra from the corresponding time-domain waveform (*Fourier transform* is thus introduced).

# GAS SAMPLES

- A gas sample cell consists of a cylinder of glass or sometimes a metal.
- The cell is closed at both ends with an appropriate window materials (NaCl/KBr) and equipped with valves or stopcocks for introduction of the sample.
- Long pathlength ( $>10$  cm) cells – used to study dilute (few molecules) or weakly absorbing samples.
- Multipass cells – more compact and efficient instead of long-pathlength cells.
- Mirrors are used so that the beam makes several passes through the sample before exiting the cell. (Effective pathlength  $> 10$  m).

## CONT...

- To resolve the rotational structure of the sample, the cells must be capable of being evacuated to measure the spectrum at reduced pressure.
- For quantitative determinations with light molecules, the cell is sometimes pressurized in order to broaden the rotational structure and all simpler measurement

# LIQUID SAMPLES

- Pure or soluted in transparent solvent – not water (attacks windows).
- The sample is most often in the form of liquid films (“sandwiched” between two NaCl plates).
- Adjustable pathlength (0.015 to 1 mm) – by Teflon spacer

# SOLID SAMPLES

- Spectra of solids are obtained as alkali halide discs (KBr), mulls (e.g. Nujol, a highly refined mixture of saturated hydrocarbons) and films (solvent or melt casting)

# FAR-INFRARED SPECTROMETRY

- Almost all FIR studies are now carried out with FTIR spectrometers.
- The far-IR region can provide unique information.
  - i) The fundamental vibrations of many organometallic and inorganic molecules fall in this region due to the heavy atoms and weak bonds in these molecules.
  - ii) Lattice vibrations of crystalline materials occur in this region,
  - iii) Electron valence/conduction band transition in semiconductors often correspond to far-IR wavelengths.

# NEAR-INFRARED SPECTROMETRY

- NIR shows some similarities to UV-visible spectrophotometry and some to mid-IR spectrometry. Indeed the spectrophotometers used in this region are often combined UV-visible-NIR ones.
- The majority of the absorption bands observed are due to overtones (or combination) of fundamental bands that occur in the region 3 to 6  $\mu\text{m}$ , usually hydrogen-stretching vibrations.
- NIR is most widely used for quantitative organic functional-group analysis.
- The NIR region has also been used for qualitative analyses and studies of hydrogen bonding, solute-solvent interactions, organometallic compounds, and inorganic compounds.

## **UNIT 5**

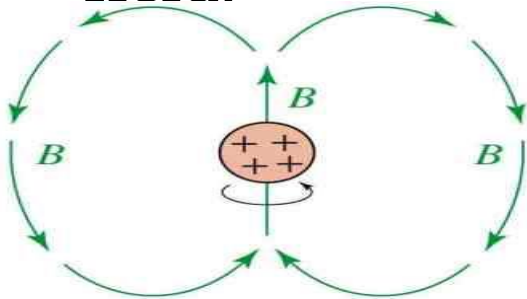
# **NUCLEAR MAGNETIC RESONANCE**

# NMR SPECTROMETERS

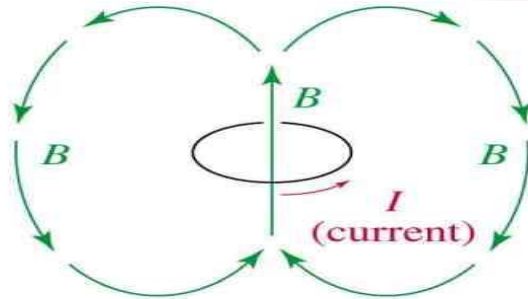
- NMR is the most powerful tool available for organic structure determination.
- It is used to study a wide variety of nuclei.

# NUCLEAR SPIN

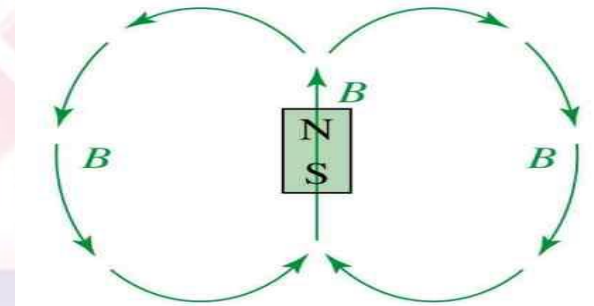
- A nucleus with an odd atomic number or an odd mass number has a nuclear spin.
- The spinning charged nucleus generates a magnetic field.



spinning proton



loop of current

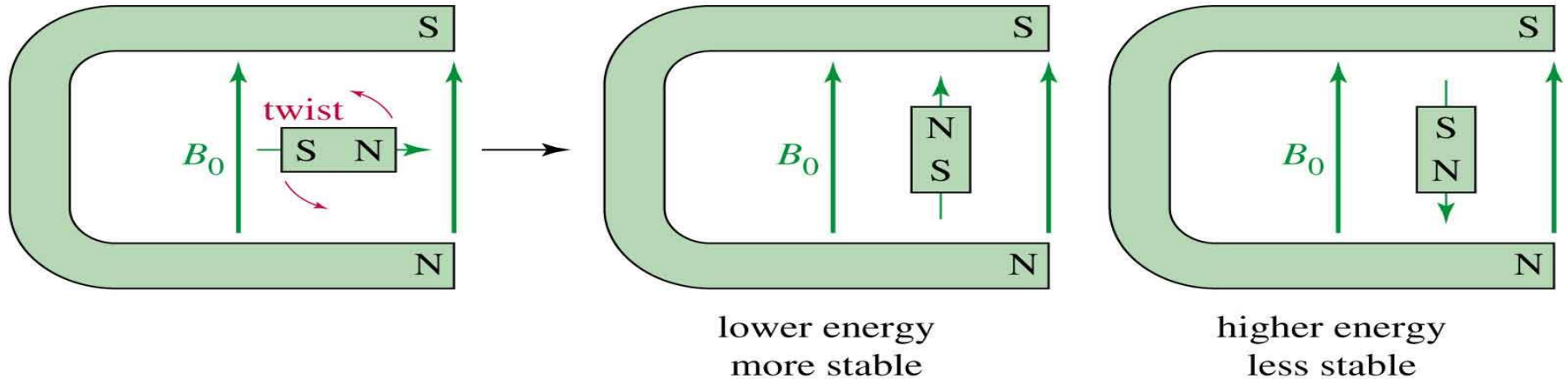


bar magnet

=>

# EXTERNAL MAGNETIC FIELD

- When placed in an external field, spinning protons act like bar magnets.



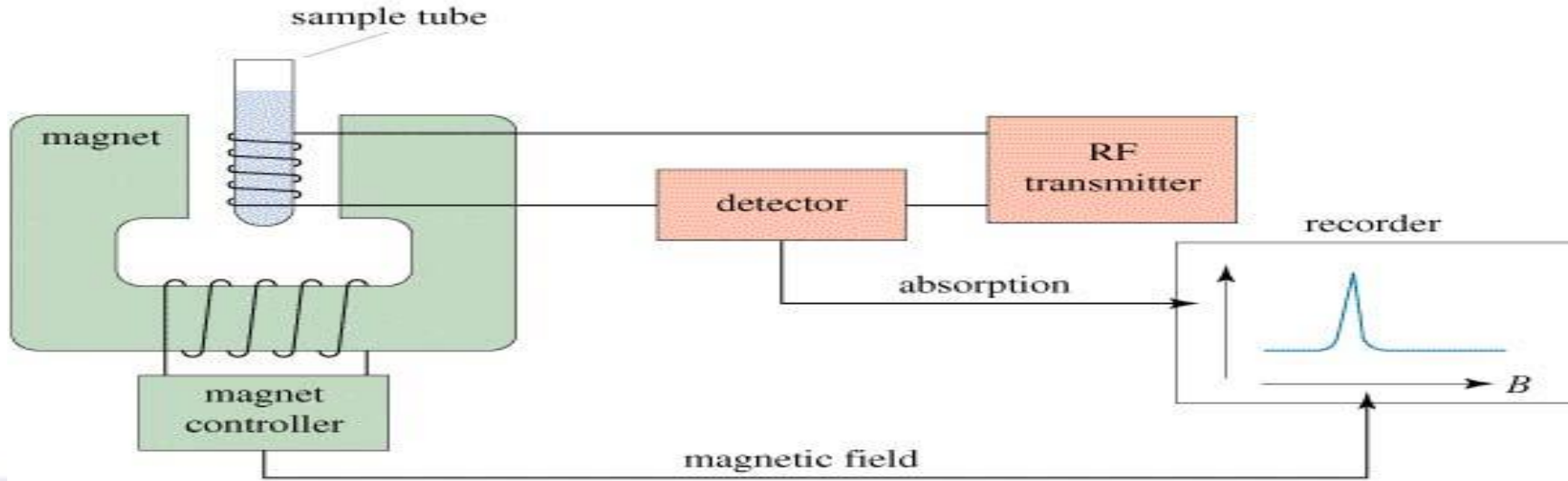
# TWO ENERGY STATES

- The magnetic fields of the spinning nuclei will align either *with* the external field, or *against* the field.
- A photon with the right amount of energy can be absorbed and cause the spinning proton to flip.

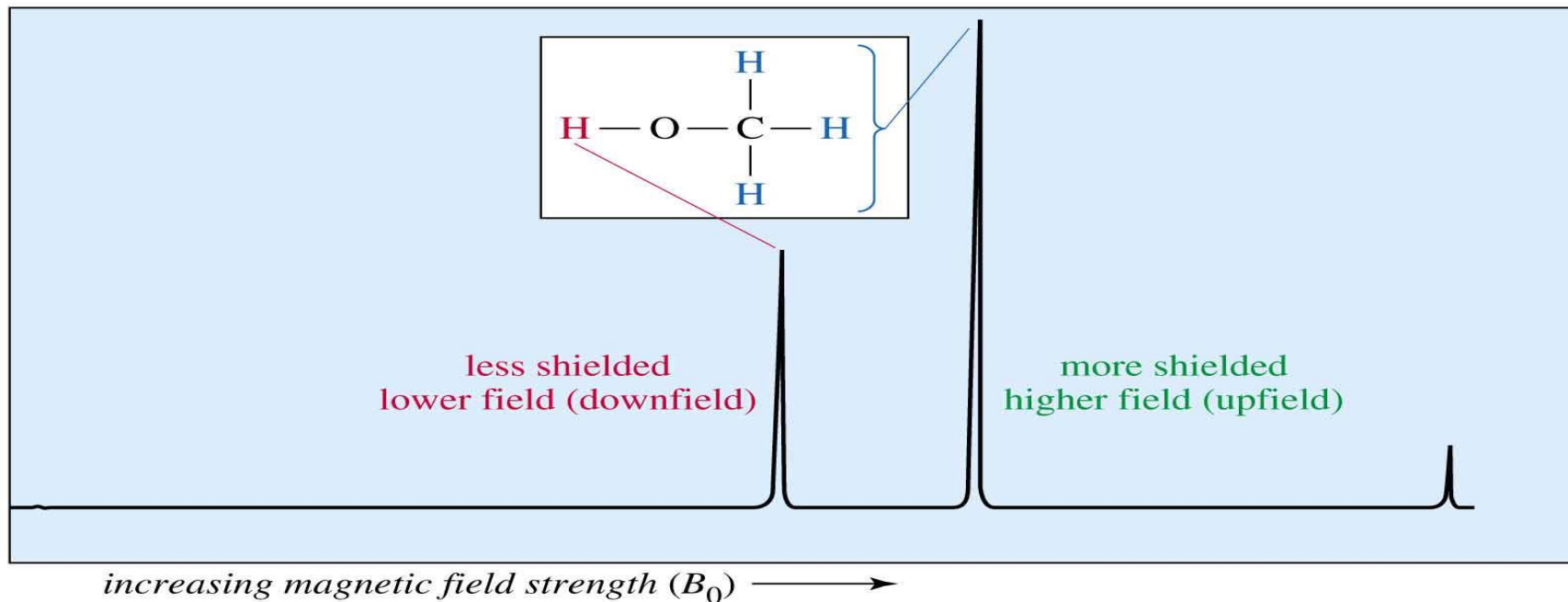
# NMR SIGNALS

- The number of signals shows how many different kinds of protons are present.
- The location of the signals shows how shielded or deshielded the proton is.
- The intensity of the signal shows the number of protons of that type.
- Signal splitting shows the number of protons on adjacent atoms.

# NMR SPECTROMETER



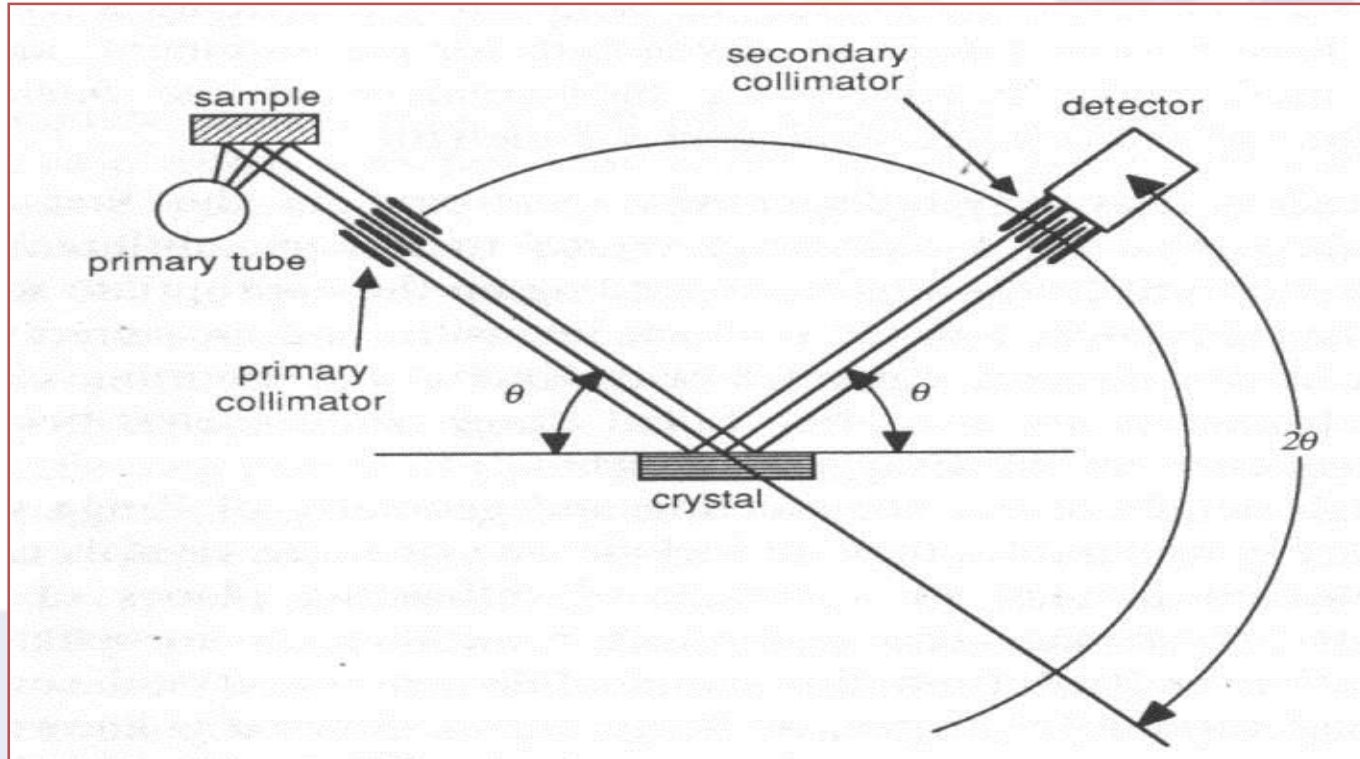
# NMR GRAPH



# X-RAY SPECTROMETER

- An XRF spectrometer is very similar to an electron microprobe: just replace the electron gun with an x-ray tube located very close to the specimen; both the characteristic and the continuum x-rays cause (secondary) fluorescence of the specimen, and the resulting x-rays are focused using collimators in either WDS (crystal + counter) or EDS (solid state detector) mode .

# CONT...



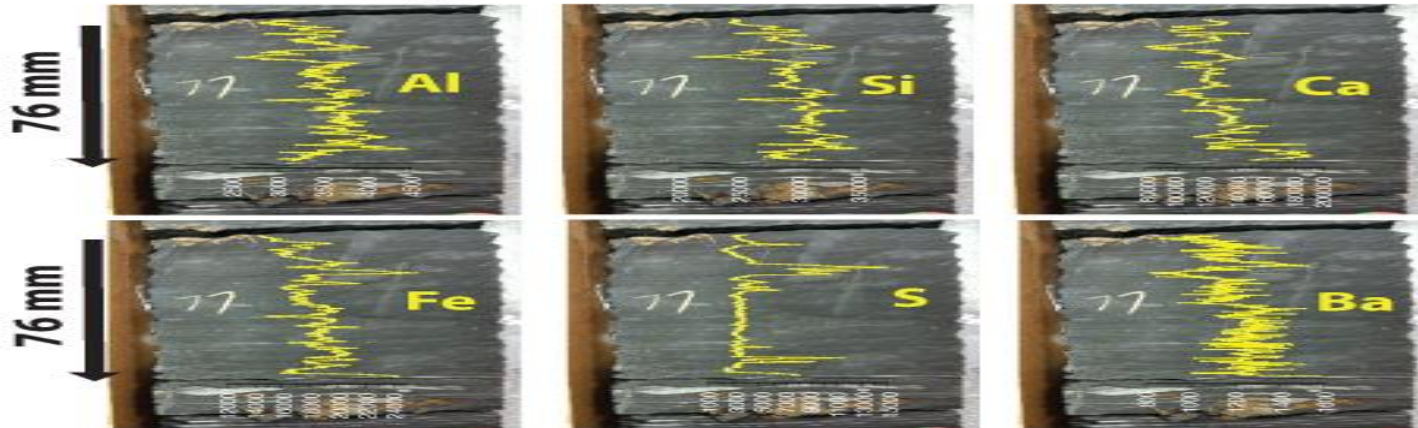
# SAMPLE PREPARATION

- Samples and standards (fine powders) are mixed with a flux (e.g., a glass disk with  $\sim 90\%$   $\text{LiBO}_4$  for major elements, a pressed pellet with  $\sim 75\%$  cellulose for traces).
- The purpose is to minimize “particle size / micro-absorption effects” by producing a more uniform absorption path for samples made of discrete phases that may not have been ground down into submicron sizes.

# UW XRF CORE SCANNER

- Application of the XRF Core Scanner: deep sea sediments and search for chemical signature of cyclical behavior in sediment deposition

**UW-Madison XRF Scanner Lab**  
**Angus Core Scan: 200 microns**



S.R. Meyers, UW-Madison Department of Geoscience

# MASS SPECTROPHOTOMETERS

- A technique for measuring and analyzing molecules, that involves introducing enough energy into a (neutral) target molecule to cause its ionization and disintegration.
- The resulting primary ions and their fragments are then analyzed, based on their mass/ charge ratios, to produce a "molecular fingerprint."

# COMPONENTS OF MASS SPECTROMETERS



*Ionisation*

Electron Ionisation (EI)

*Ion Separation*

Quadrupole

*Ion Detection*

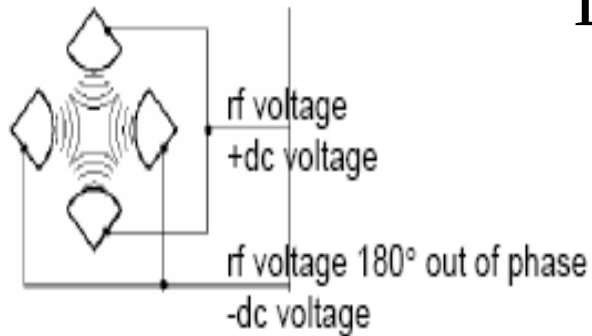
Electron Multiplier

# IONISATION METHODS

- Removal/addition of electron(s)
  - $M + e^- \rightarrow (M^+ \cdot)^* + 2e^-$
  - electron ionization
- Removal/addition of proton(s)
  - $M + (\text{Matrix})-H \rightarrow MH^+ + (\text{Matrix})^-$
- chemical ionization (CI)
- atmospheric pressure CI (APCI)
- fast atom bombardment (FAB)
- electrospray ionization (ESI)
- matrix assisted laser desorption/ionization (MALDI)

# MASS ANALYSER

- Four parallel rods or poles through which the ions being separated are passed.
- Poles have a fixed DC and alternating RF voltages applied to them.



Depending on the produced electric field, only ions of a particular  $m/z$  will be focused on the detector, all the other ions will be deflected into the rods.

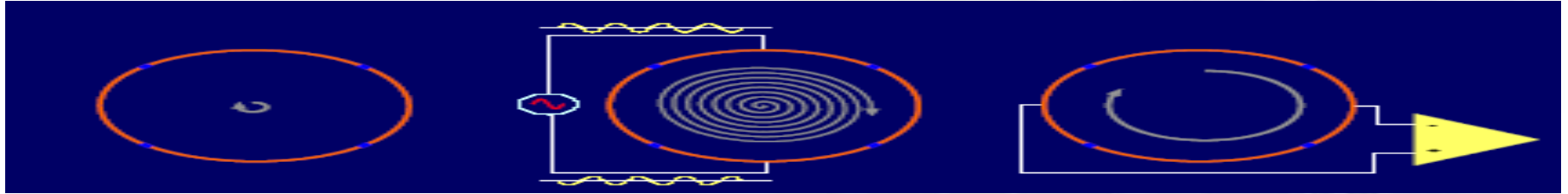
# NEW DIMENSIONS OF HIGH PERFORMANCE MASS SPECTROMETRY

- A high-frequency mass spectrometer in which the cyclotron motion of ions, having different  $m/z$  ratios, in a constant magnetic field, is excited essentially simultaneously and coherently by a pulse of a radio-frequency electric field applied perpendicularly to the magnetic field.
- The excited cyclotron motion of the ions is subsequently detected on receiver plates as a time domain signal that contains all the cyclotron frequencies excited.

## CONT...

- Maximum energy is gained by ions that satisfy the cyclotron resonance condition and as a result these are separated from ions of different mass/charge.
- Fourier transformation of the time domain signal results in the frequency domain FT-ICR signal which, on the basis of the inverse proportionality between frequency and  $m/z$  ratio, can be converted to a mass spectrum.
- The ions are to be detected, with a selected  $m/z$  ratio, absorb maximum energy through the effect of a high-frequency field and a constant magnetic field perpendicular to it.

# CONT...



- Ions are trapped and oscillate with low, incoherent, thermal amplitude
- Excitation sweeps resonant ions into a large, coherent cyclotron orbit
- Preamplifier and digitizer pick up the induced potentials on the cell.