CLASSIFICATION OF ANALYTICAL METHODS

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Goals of Analytical Chemistry

- What is it?
- Identification
- Qualitative Analysis
- How much?
- Quantitative Analysis

- Analytical Chemistry deals with methods for determining the chemical composition of samples of matter .
- A qualitative method yields information about the identity of atomic or molecular species or the functional groups in the sample; a quantitative method, in contrast, provides numerical information as to the relative amount of one or more of these components.

Present day Instrumental Analysis

- Better and Faster
- More Data (Images)
- Miniaturization
- Better data processing methods - Chemo metrics

Classical Methods

- Separation of analytes by precipitation, extraction, or distillation.
- Qualitative analysis by reaction of analytes with reagents that yielded products that could be recognized by their colors, boiling or melting points, solubilities, optical activities, or refractive indexes.
- Quantitative analysis by gravimetric or by titrimetric techniques.

- Gravimetric Methods – the mass of the analyte or some compound produced from the analyte was determined.

- Titrimetric Methods – the volume or mass of a standard reagent required to react completely with the analyte was measured.

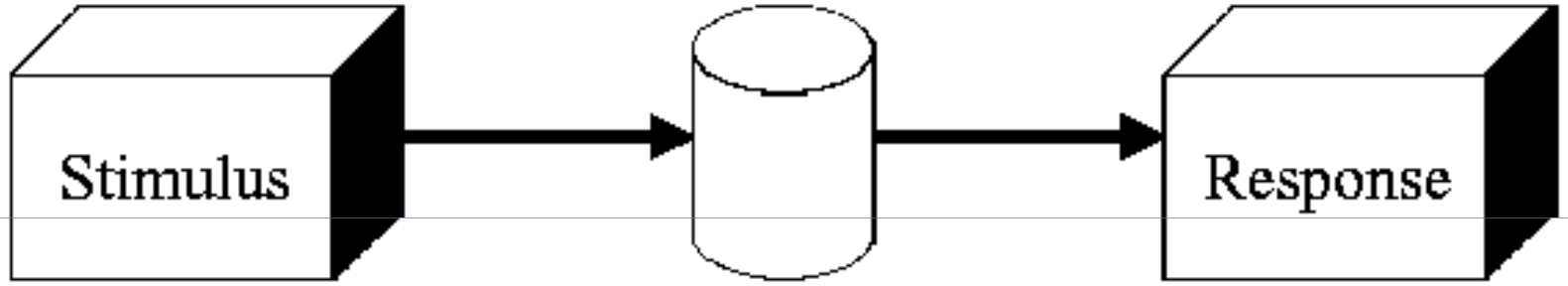
Instrumental Methods

Measurements of physical properties of analytes, such as conductivity, electrode potential, light absorption, or emission, mass to charge ratio, and fluorescence, began to be used for quantitative analysis of a variety of inorganic, organic, and biochemical analyte.

Instrumentation is divided into two Category

- Detection
- Quantitation

Analytical Instruments



Energy Source

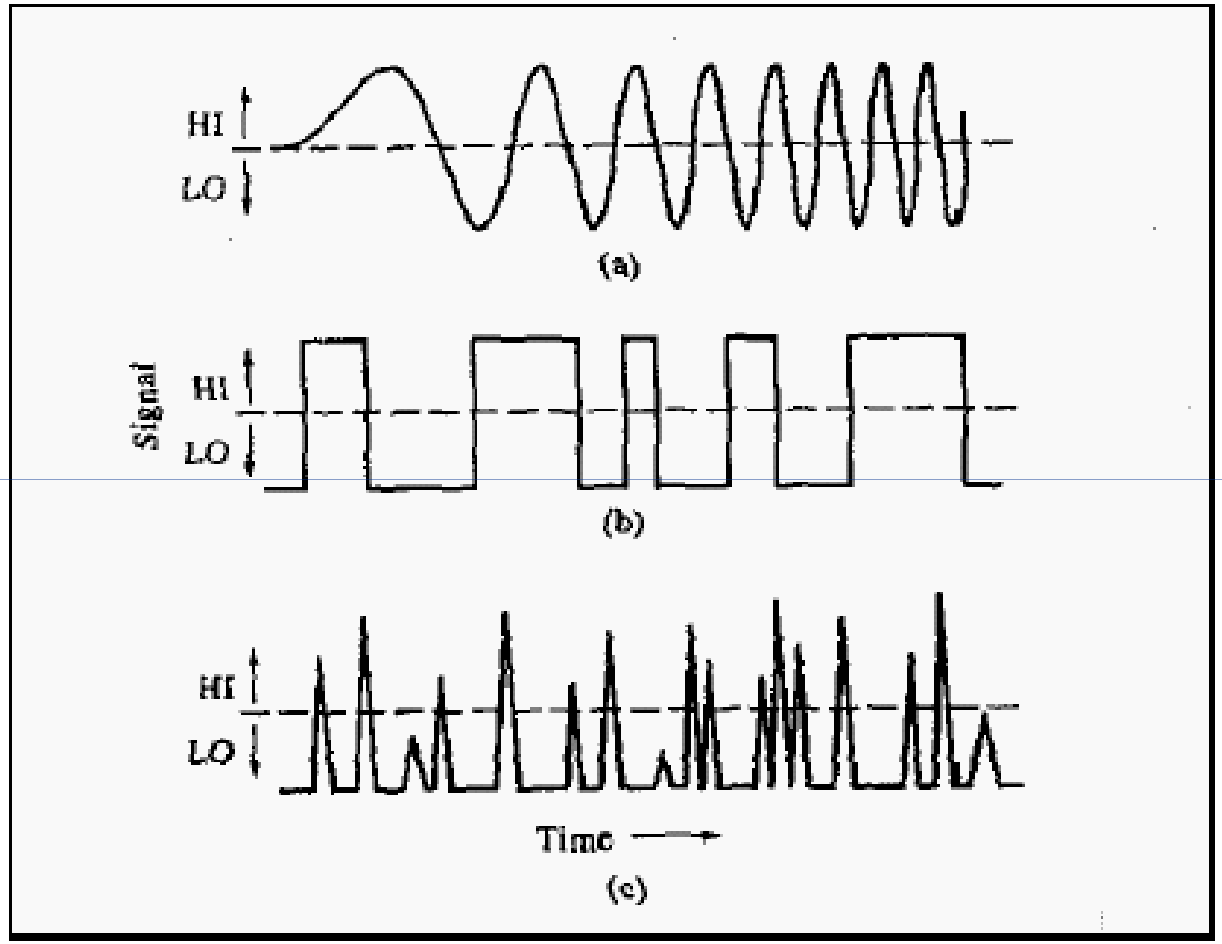
System studied

Analytical Information

Analytical Signals

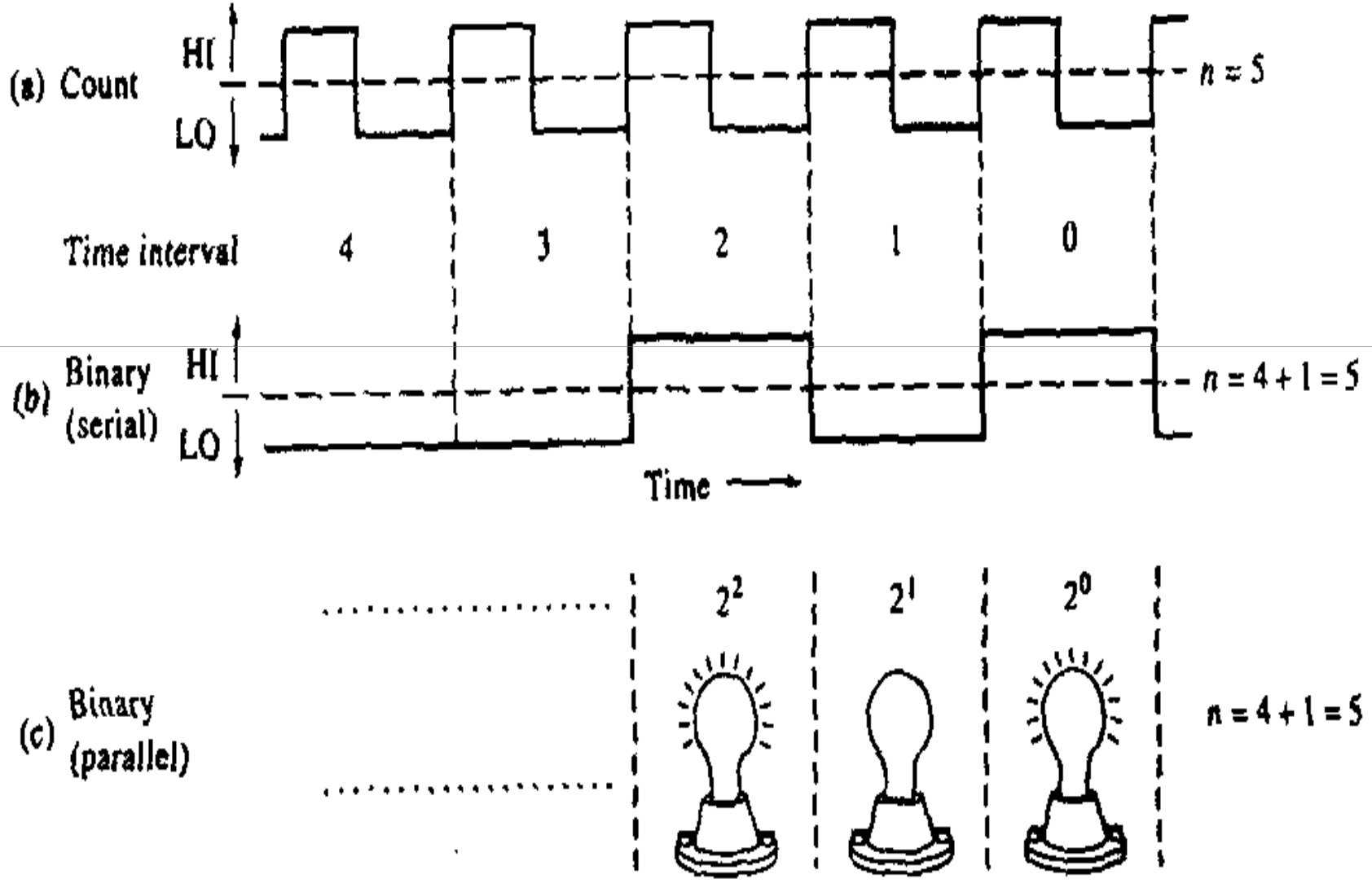
- Data Domain – information encoded
- Non-electrical Domains (scale, number, chemical)
- Electrical Domains – (volts, current, charge)
- Analog Domains – continuous quantities (volts, current)
- Time Domains– (pulses, slopes)
- Digital Domains – (Off/On or Hi/Lo)

Time => Digital Domains

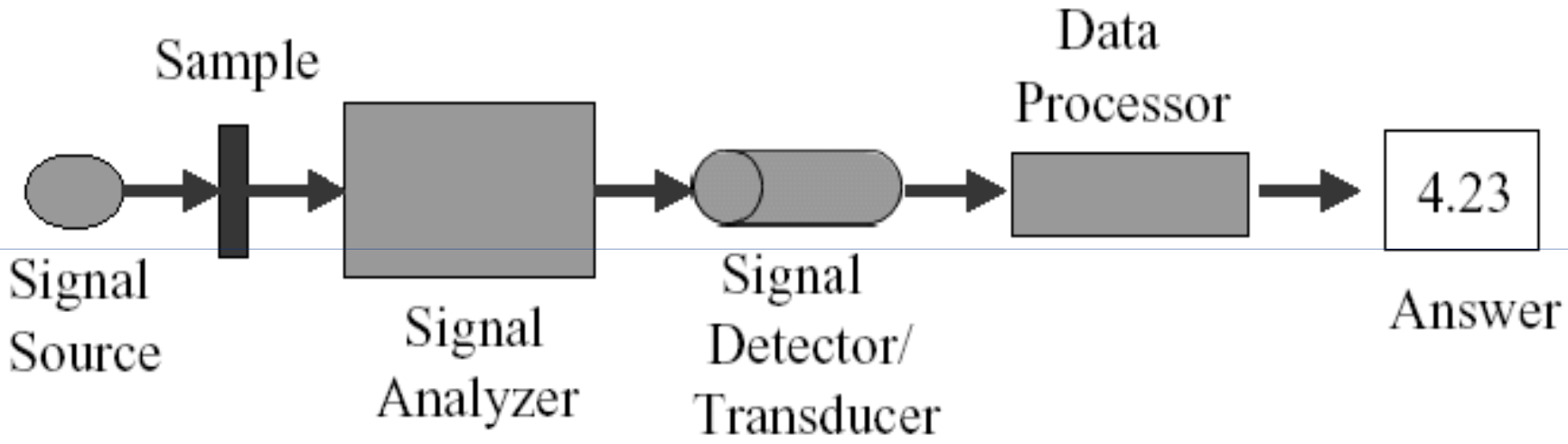


Continuous → Hi/Lo

Digital Domain



Instrument Components



Website Link

- *Useful Websites Dealing With Instrumental Analysis*
- **American Chemical Society:**
- http://www.acs.org
- **Chemical Abstracts Service:**
- http://www.cas.org
- **Chemical Center Home Page: (maintained by ACS)**
- http://www.chemcenter/org
- **Science Magazine:**
- http://www.sciencemag.org
- **Journal of Chemistry and Spectroscopy:**
- http://www.kerouac.pharm.uky.edu/asrg/wave/wavehp.html

Thank you

FLAME EMISSION SPECTROSCOPY

prienciple

Flame Spectrophotometry

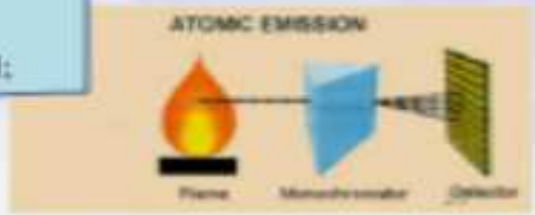
- Also known as Flame emission /Flame photometry /Atomic emission spectroscopy.
- Study of Radiant Energy
- A flame by its heat can raise atoms from lower energy to an excited state of higher energy.
- Emission through Radiation.
- Determination of radiant energy.

Instrumentation

Instrumentation

Flame Emission Spectroscopy (Flame Photometry)

- **Principle:** excitation of ground-state atoms by propane-butane flame (2000-3000 °C), electron loss by analyte atom, when electron is recaptured, emission light of characteristic wavelength is emitted.
- **Elements:** Na and K; Li, Rb, Cs, Ca.
- **LOD**: about 0.1-0.5 mg/L.
- **Sample Preparation:** dry and wet digestion methods.
- **Advantages:** simple, quick and inexpensive analysis; wide dynamic range (0-100 mg/L); ideal for elements with low excitation potential (Na and K)
- **Disadvantages:** only some elements may be determined; elements analyzed one at a time.



Instrumentation

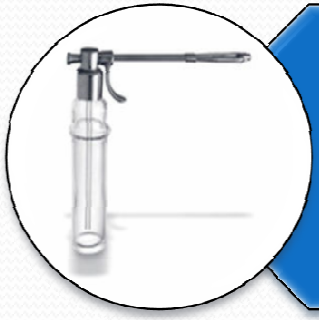
CONTENTS

NEBULIZER

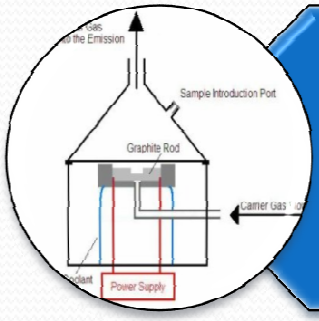
- This is the component of sample delivery system. which breaks up the bigger liquid droplet to smaller liquid droplets.

- The process of conversion of sample to a fine mist of finely divided droplets using a jet of compressed gas is known as Nebulization.

TYPES OF NEBULIZERS



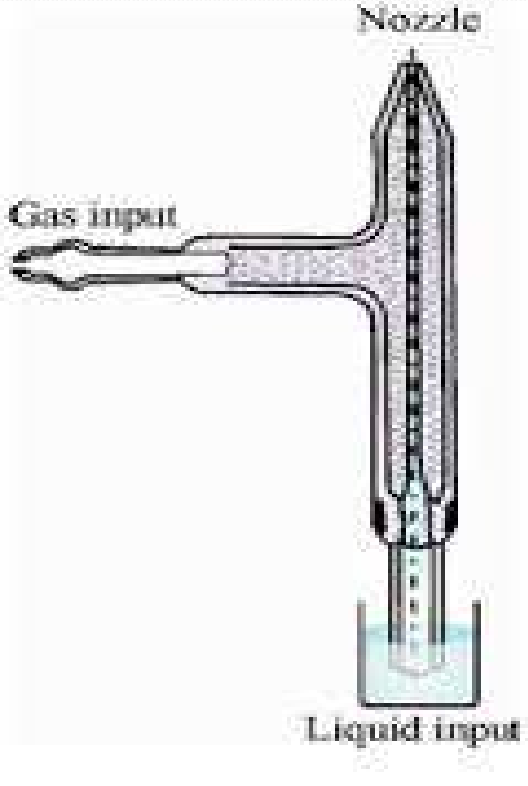
Pneumatic nebulizers



Electro thermal vaporizer

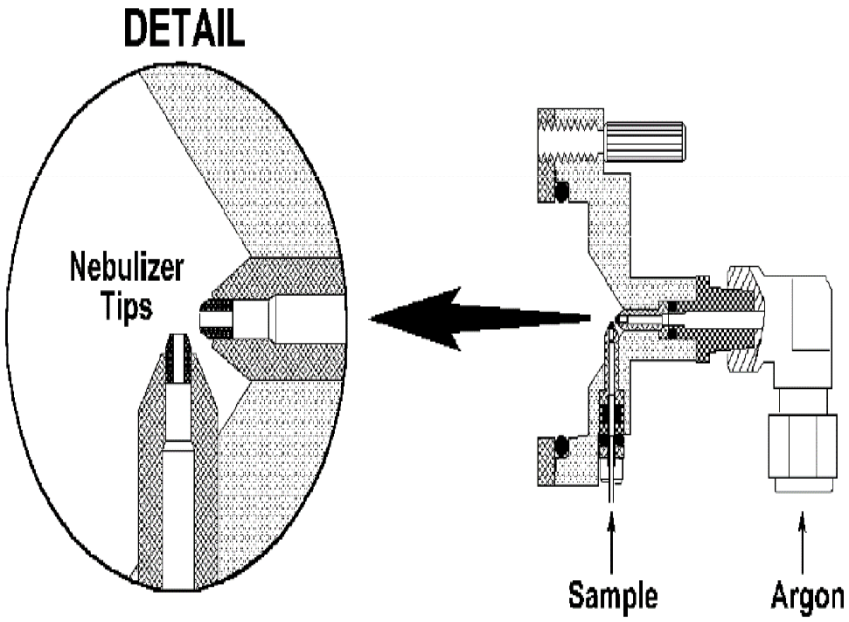
Ultrasound nebulizer

CONCENTRIC TUBES



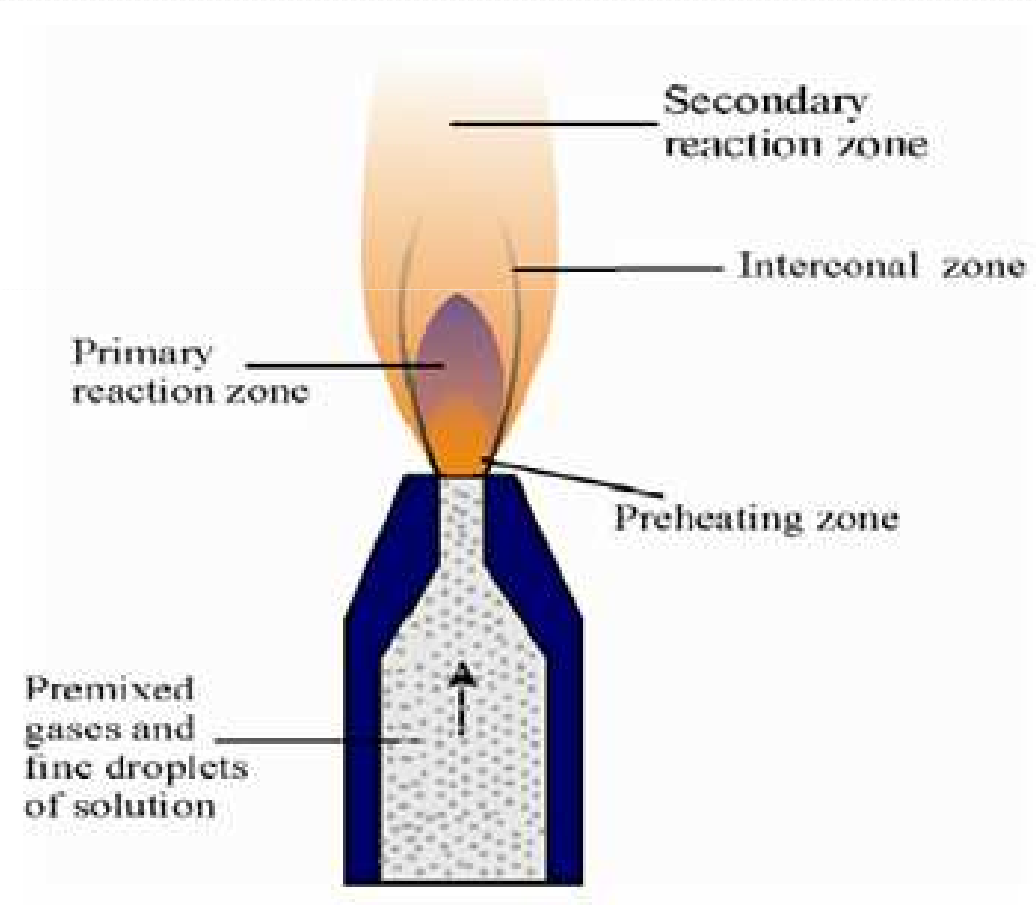
- The liquid sample is sucked through a capillary tube by a high pressure jet of gas flowing around the tip of the capillary.
- The high velocity breaks the sample into a mist and carries it to the atomization region.

CROSS FLOW



- The jet stream flows right angles to the capillary tip.
- It uses a high speed stream of gas perpendicular to the tip of the sample capillary

ZONES OF FLAME



REQUIREMENTS OF FLAME

- It should have proper temperature
- Temperature should remain constant throughout the operation
- There should not be any fluctuation during burning

FUNCTIONS OF FLAME

- To convert the analyte of the liquid sample into vapour state
- To decompose the analyte into atoms and simple molecules
- To excite the formed atoms/free atoms/simple molecules to emit radiant energy

BURNERS

Mecker burner

Nitrous Oxide-Acetylene Flames

Total consumption burner

Shielded Burner

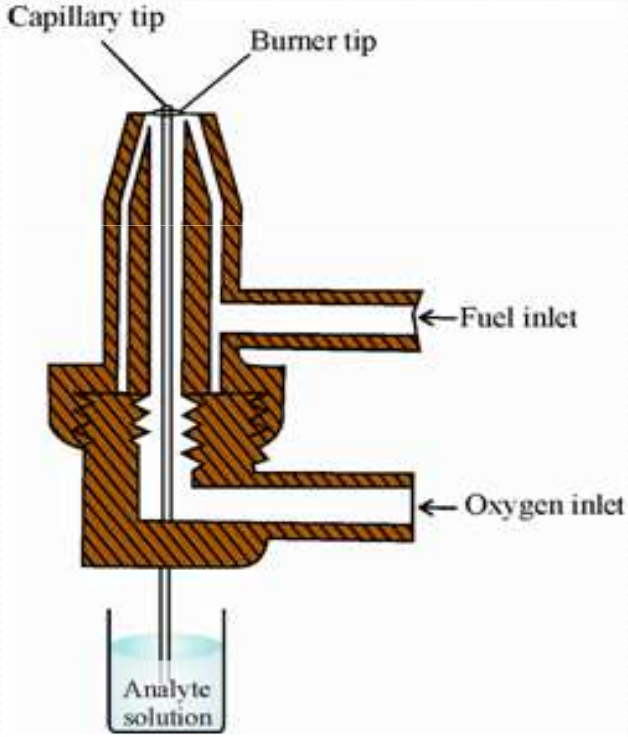
Premix of laminar flow burner

Lundergraph burner

MECKER BURNER

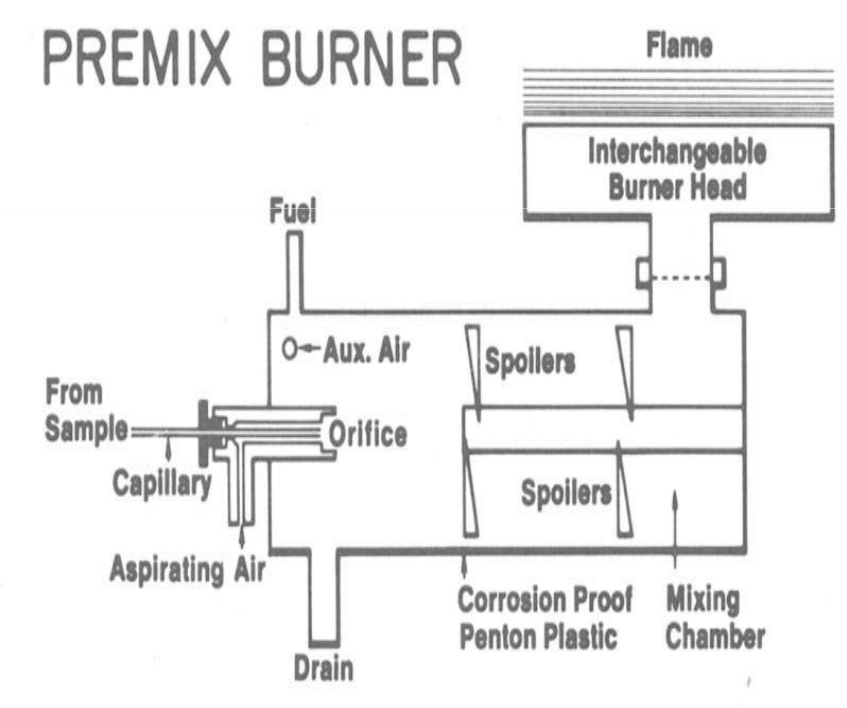
- This burner was used earlier and employed natural gas and oxygen. Produces relatively low temp. and low excitation energies. This are best used for ALKALI metals only. Now-a-days it is not used.

TOTAL CONSUMPTION BURNER



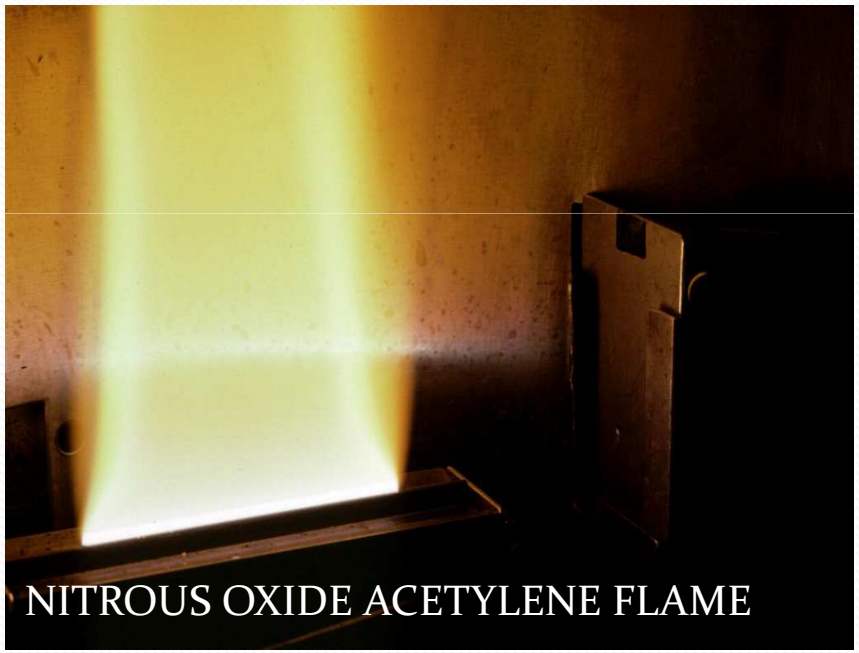
- In this burner fuel and oxidant are hydrogen and oxygen gases. Sample solution is aspirated through a capillary by high pressure of fuel and Oxidant and burnt at the tip of burner. Entire sample is consumed.

PREMIX OR LAMINAR FLOW BURNER



- In this type of the burner, aspirated sample, fuel and oxidant are thoroughly mixed before reaching the burner opening and then entering the flame. There is high loss of sample(95%) as large droplets which are drained out.

NITROUS OXIDE-ACETYLENE FLAME

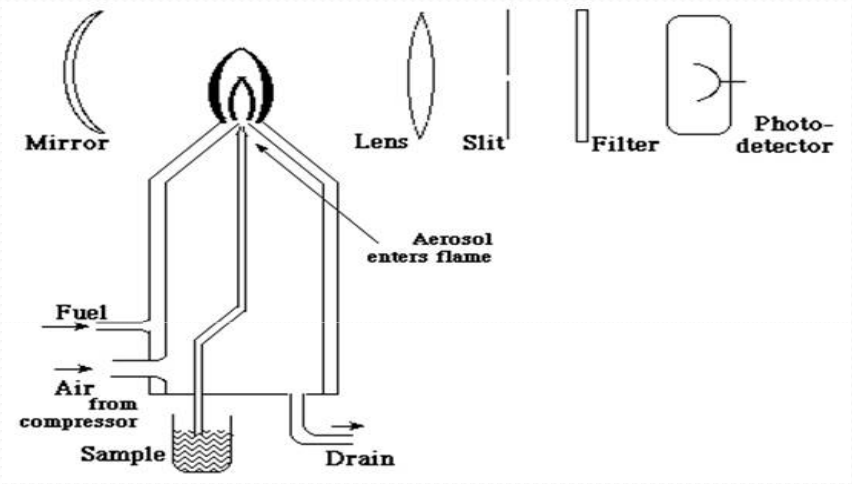


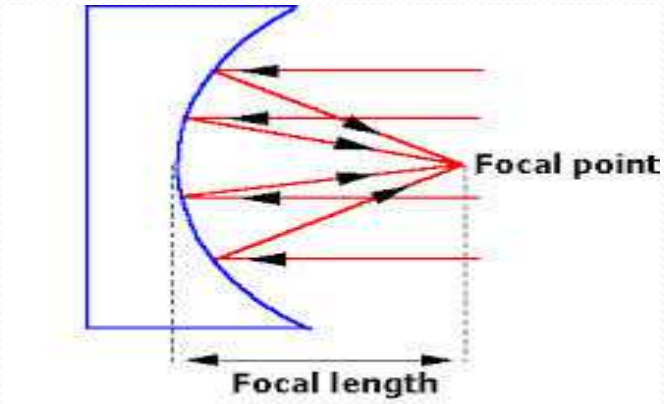
NITROUS OXIDE ACETYLENE FLAME

- These flames were superior to other flames for effectively producing free atoms. The drawback of it is the high temperature reduces its usefulness for the determination of alkali metals as they are easily ionized and Intense background emission, which makes the measurement of metal emission very difficult

Fuel	Oxidant	Temperature °C
Natural gas	Air	1700-1900
Natural gas	Oxygen	2700-2800
Hydrogen	Air	2000-2100
Hydrogen	Oxygen	2550-2700
Acetylene	Air	2100-2400
Acetylene	Oxygen	3050-3150
Acetylene	Nitrous oxide	2600-2800

MIRRORS

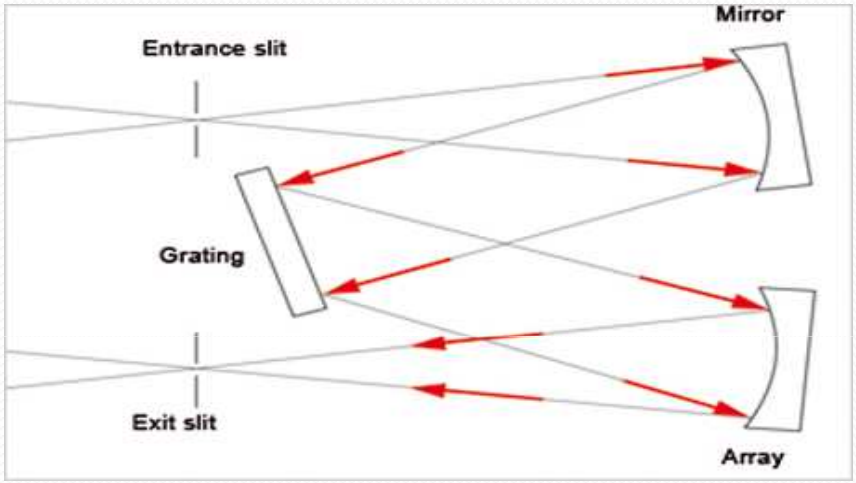


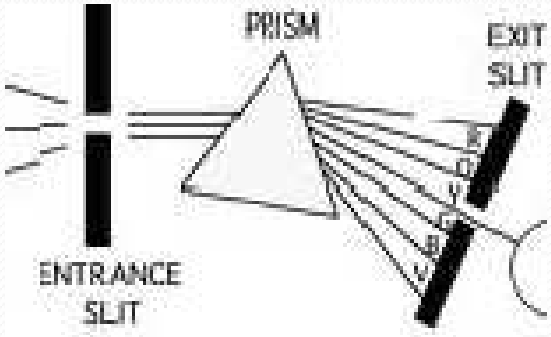


Reflection by a Concave Mirror

- The radiation from the flame is emitted in all the directions in space. Much of the radiation is lost and loss of signal results. A mirror is located behind the burner to reflect the radiation back to the entrance slit of the monochromator. The reflecting surface of the mirror is front-faced.

SLITS





- The entrance and exit slits are used before and after the dispersion elements.
- The entrance slit cuts off most if radiation from the surroundings and allows only the radiation from the flame and the mirror reflection of flame to enter the optical system.
- The exit slit is placed after the monochromator and allows only the selected wavelength range to pass through the detector

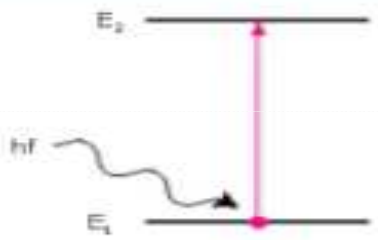
Difference between AAS&FES

Difference between AAS & FES

FES	AAS
Measurement of emitted radiation forms the basis of FES.	Measurement of intensity of absorbed radiation is basis of AAS.
Intensity of emitted radiation is directly proportional to the number of atoms in excited state.	Intensity of absorbed radiation is directly proportional to the number of atoms in ground state.
Here excitation process and signal response is influenced by flame temperature.	Here absorption intensity and signal response is independent to temperature.
Relationship between emission intensity Vs concentration in not that much linear.	Absorption intensity Vs concentration is very much linear.

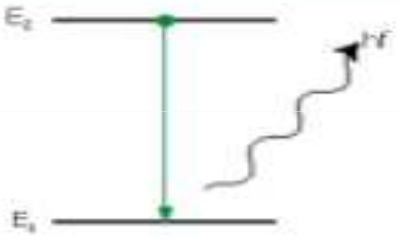
Difference

Absorption & Emission



Absorption

When atom absorbs energy of photon to promote electron to higher energy orbital



Emission

When atom emits energy as photon as electron falls from higher energy orbital to lower energy orbital.

When the electron falls back to the lower energy level the energy difference between the two levels is emitted as a unit of light (a 'photon') or some other form of radiation, such as infrared or ultra violet.

REFERENCES

Chatwal & Anand; Instrumental Methods of Chemical Analysis, 5/e 2013, page no- 2.370 to 2.375, Himalaya Publishing House.

B.K Sharma; Instrumental Methods of Chemical Analysis, 26/e 2007, page no- 430 to 437, GOEL Publishing House.


Thank You!

High Performance Liquid Chromatography

JAGDALE D B

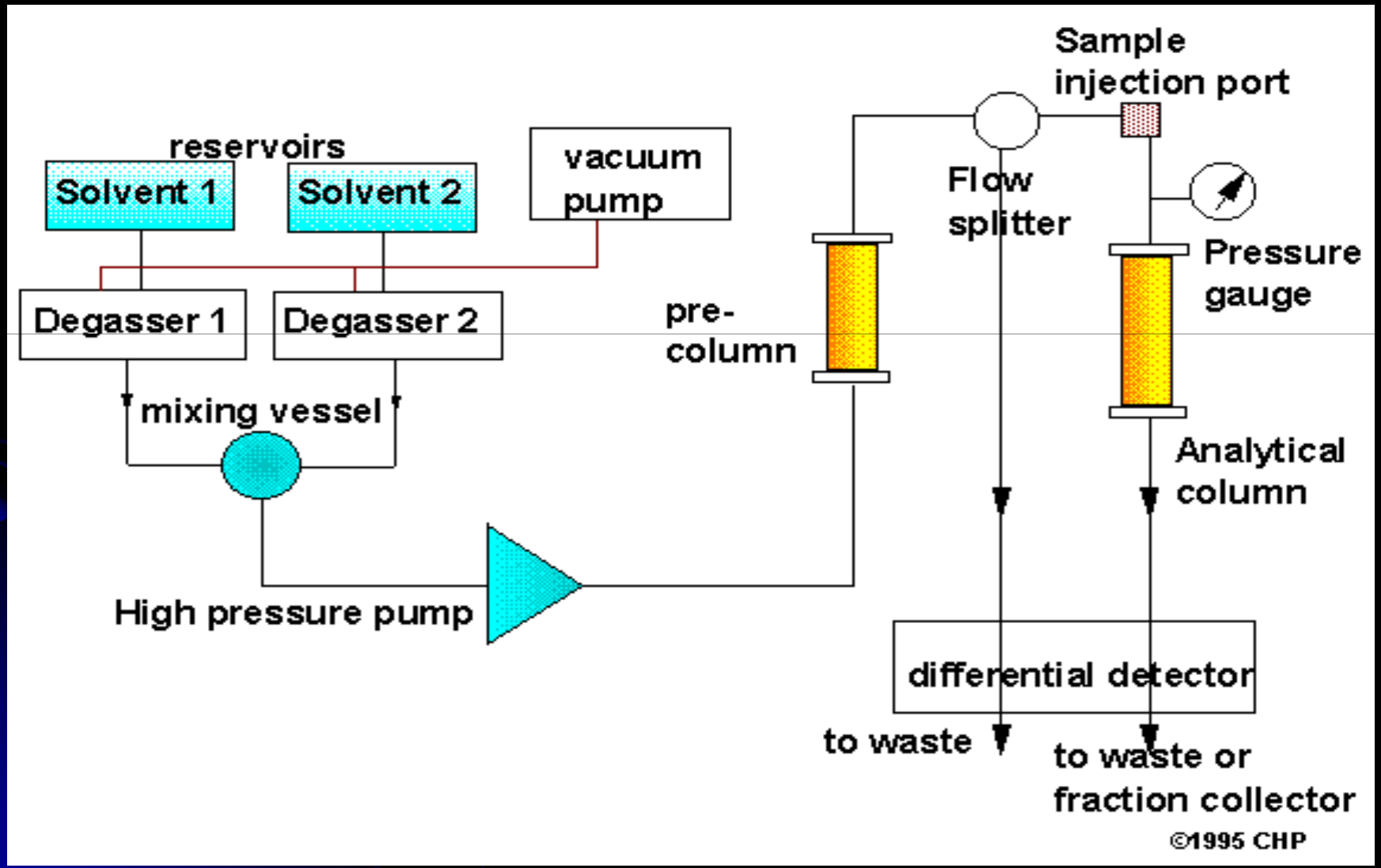
Chem. 344

Introduction

- 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. 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.

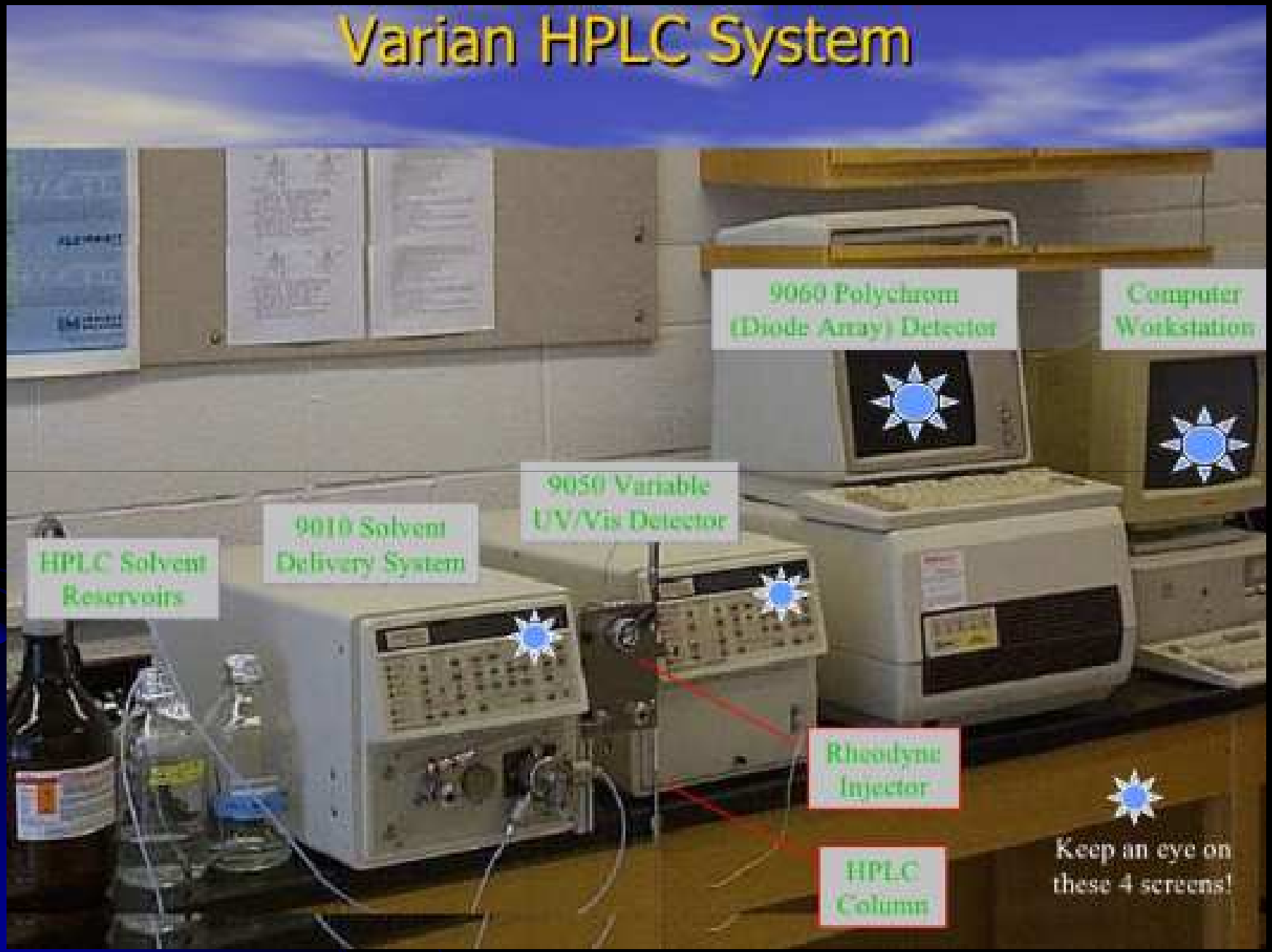
HPLC system


reservoirs
Solvent 1
Solvent 2
vacuum pump
Degasser 1
Degasser 2
mixing vessel
High pressure pump
pre-column
Sample injection port
Flow splitter
Pressure gauge
Analytical column
differential detector
to waste
to waste or fraction collector
©1995 CHP

Instrumentation

- Solvent Reservoirs
- Pump
- Sample Injector
- Column(s)
- Detector
- Data System

Picture of HPLC instrument


Varian HPLC System
9060 Polychrom (Diode Array) Detector
Computer Workstation
9050 Variable UV/Vis Detector
9010 Solvent Delivery System
HPLC Solvent Reservoirs
Rheodyne Injector
HPLC Column
Keep an eye on these 4 screens!

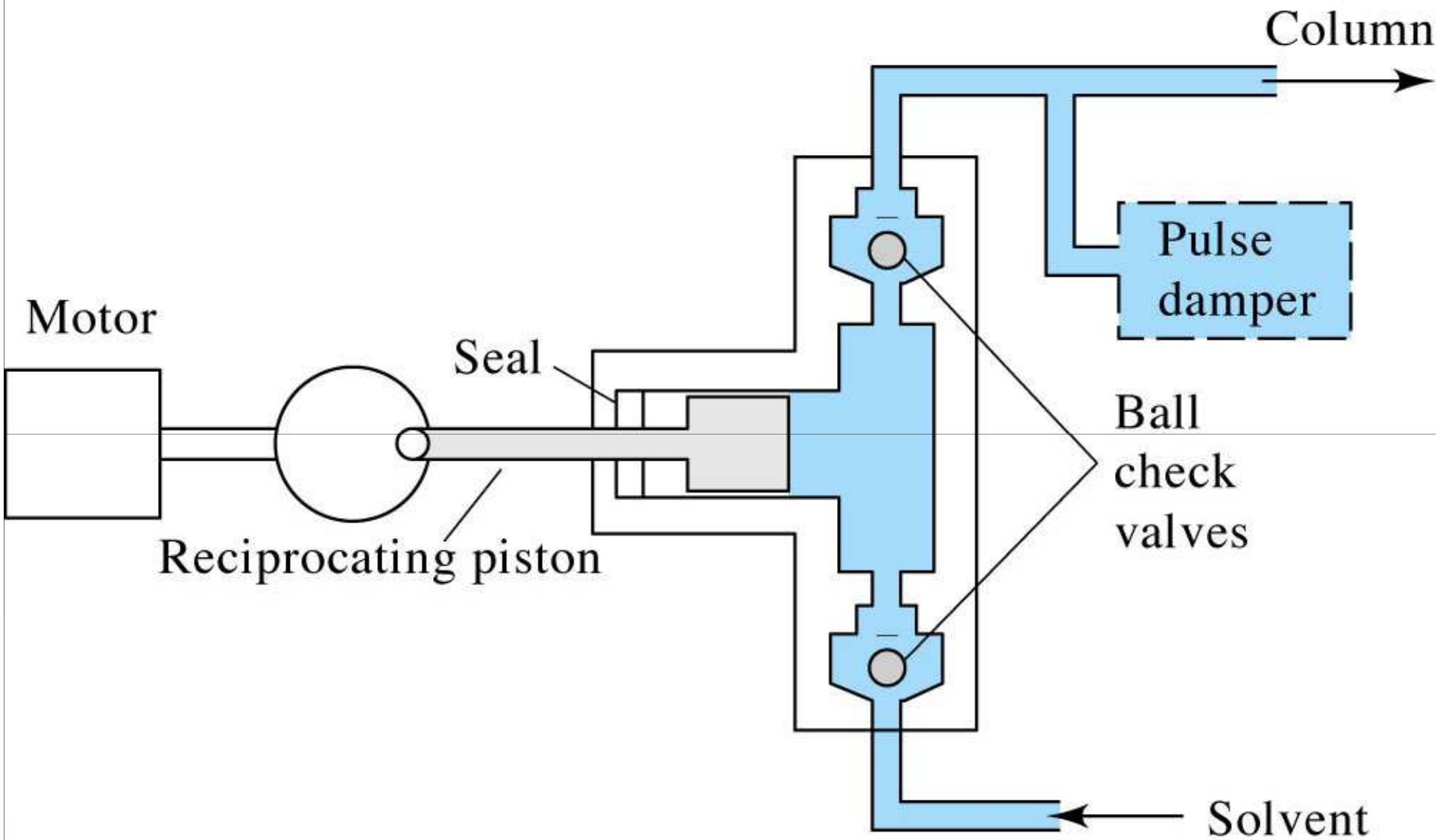
HPLC Pumps: Types

- Reciprocating pumps
- Gas displacement pumps
- Pneumatic pumps

Reciprocating Pumps

- One, two, or three pump heads

  - more heads, less pulse
- Small head volumes (50 to 250 mL)
- Short piston stroke
- Inert pistons (generally sapphire)
- Continuous use (no refill time)
- Pulse dampeners



Gas displacement pumps

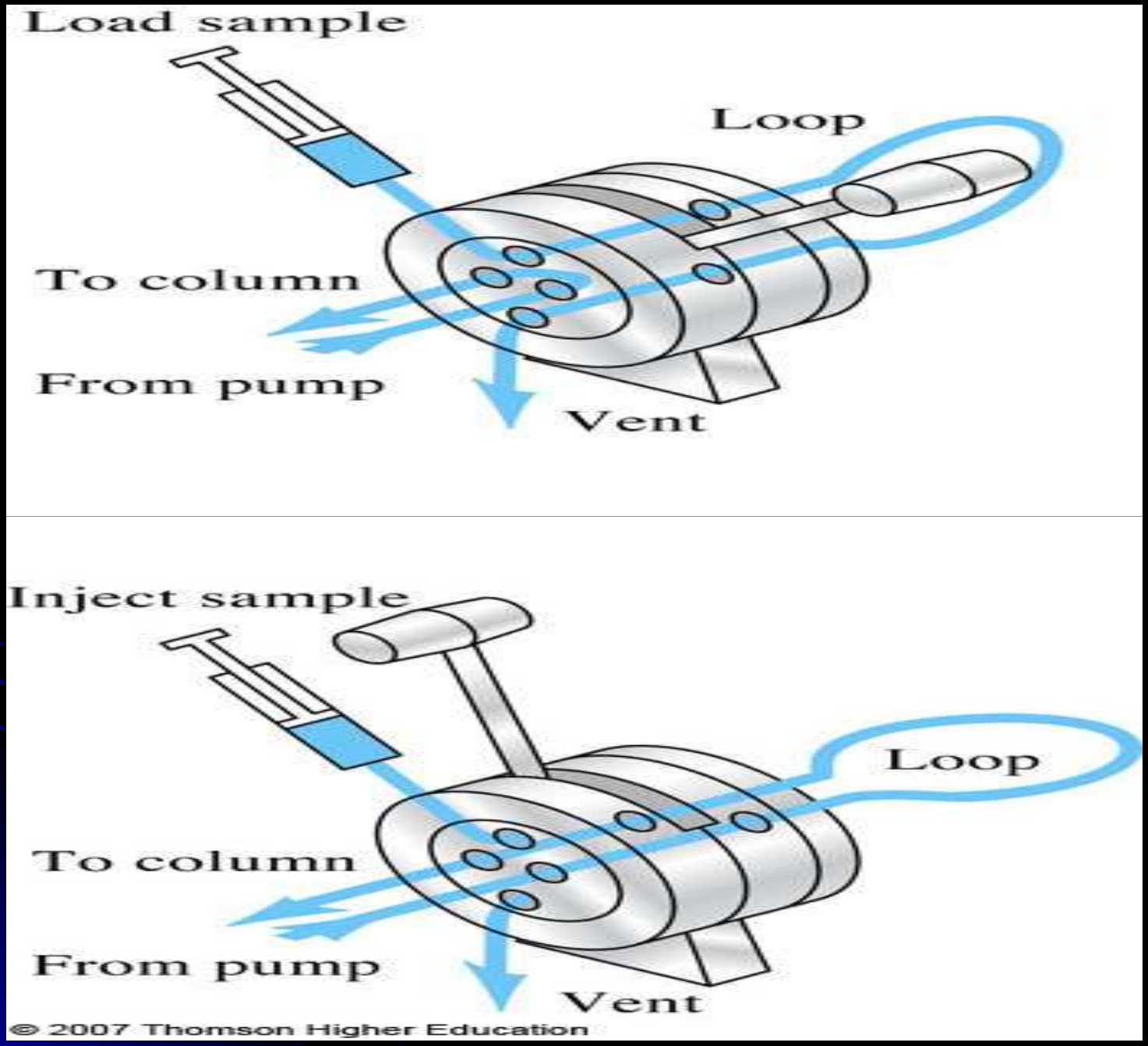
- Constant flow rate pump
- Non-pulsating flow
- Low flow rates (1 to 100 mL/min)
- Isocratic flow only
- Refill required when reservoir (~50mL) expended

Pneumatic pumps

- Constant pressure pump, not constant flow
- Can deliver high pressures
- Stable flow during delivery stroke
- Stop flow on refill stroke
- Low cost

Sample Introduction

- Valve-type injectors
  - Six port fixed volume Rheodyne
    - reproducible injection volumes
    - variable loop size
    - easy to use, reliable
  - Six port variable volume Waters
    - variable injection volumes without loop change increased maintenance, operator skill required more expensive



HPLC Chromatograph 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.

- 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.

Picture of an HPLC column

Several column types (can be classified as)

- ***Normal phase***
- ***Reverse phase***
- ***Size exclusion***
- ***Ion exchange***

Normal phase

- In this column type, the retention is governed by the interaction of the polar parts of the stationary phase and solute. For retention to occur in normal phase, the packing must be more polar than the mobile phase with respect to the sample

Reverse phase

- In this column the packing material is relatively nonpolar and the solvent is polar with respect to the sample. Retention is the result of the interaction of the nonpolar components of the solutes and the nonpolar stationary phase. Typical stationary phases are nonpolar hydrocarbons, waxy liquids, or bonded hydrocarbons (such as C18, C8, etc.) and the solvents are polar aqueous-organic mixtures such as methanol-water or acetonitrile-water.

Types of Liquid Column Chromatography (LCC)

- LLC (Liquid Liquid)
- LSC (Liquid Solid - adsorption)
- SEC (Size Exclusion)
- GLC GSC
- SFC (Supercritical Fluid)

Types of Detectors

- Absorbance (UV with Filters, UV with Monochromators)
- IR Absorbance
- Fluorescence
- Refractive-Index
- Evaporative Light Scattering Detector (ELSD)
- Electrochemical
- Mass-Spectrometric
- Photo-Diode Array

- **Ultraviolet Absorbance Detectors with Filters:** The simplest UV absorption detectors are filter photometers with a mercury lamp as the source. Most commonly the intense line at 254 nm is isolated by filters. Deuterium or tungsten filament sources with interference filters also provide a simple means of detecting absorbing species.

- **Refractometer detector : it is universal detector.** Refractometer detectors have the significant advantage of responding to nearly all solutes. In addition, they are reliable and unaffected by flow rate. They are, however, highly temperature sensitive and must be maintained at a constant temperature to a few thousandths of a degree centigrade. Furthermore, they are not as sensitive as most other types of detectors.

Uses of HPLC

- **This technique is used for chemistry and biochemistry research analyzing complex mixtures, purifying chemical compounds, developing processes for synthesizing chemical compounds, isolating natural products, or predicting physical properties. It is also used in quality control to ensure the purity of raw materials, to control and improve process yields, to quantify assays of final products, or to evaluate product stability and monitor degradation.**

- **In addition, it is used for analyzing air and water pollutants, for monitoring materials that may jeopardize occupational safety or health, and for monitoring pesticide levels in the environment. Federal and state regulatory agencies use HPLC to survey food and drug products, for identifying confiscated narcotics or to check for adherence to label claims.**

Application of HPLC

1. Pharmaceuticals industry

- To control the drug stability
- Quantity of drug determination from pharmaceutical dosage forms, ex. Paracetamol determination in panadol tablet
- Quantity of drug determination from biological fluids, ex: blood glucose level

2. Analysis of natural contamination

- Phenol & Mercury from sea water

3. Forensic test

- Determination of steroid in blood, urine & sweat.
- Detection of psychotropic drug in plasma

Application of HPLC

4. Clinical test
   - Monitoring of hepatic chirosis patient through aquaporin 2 in the urine.

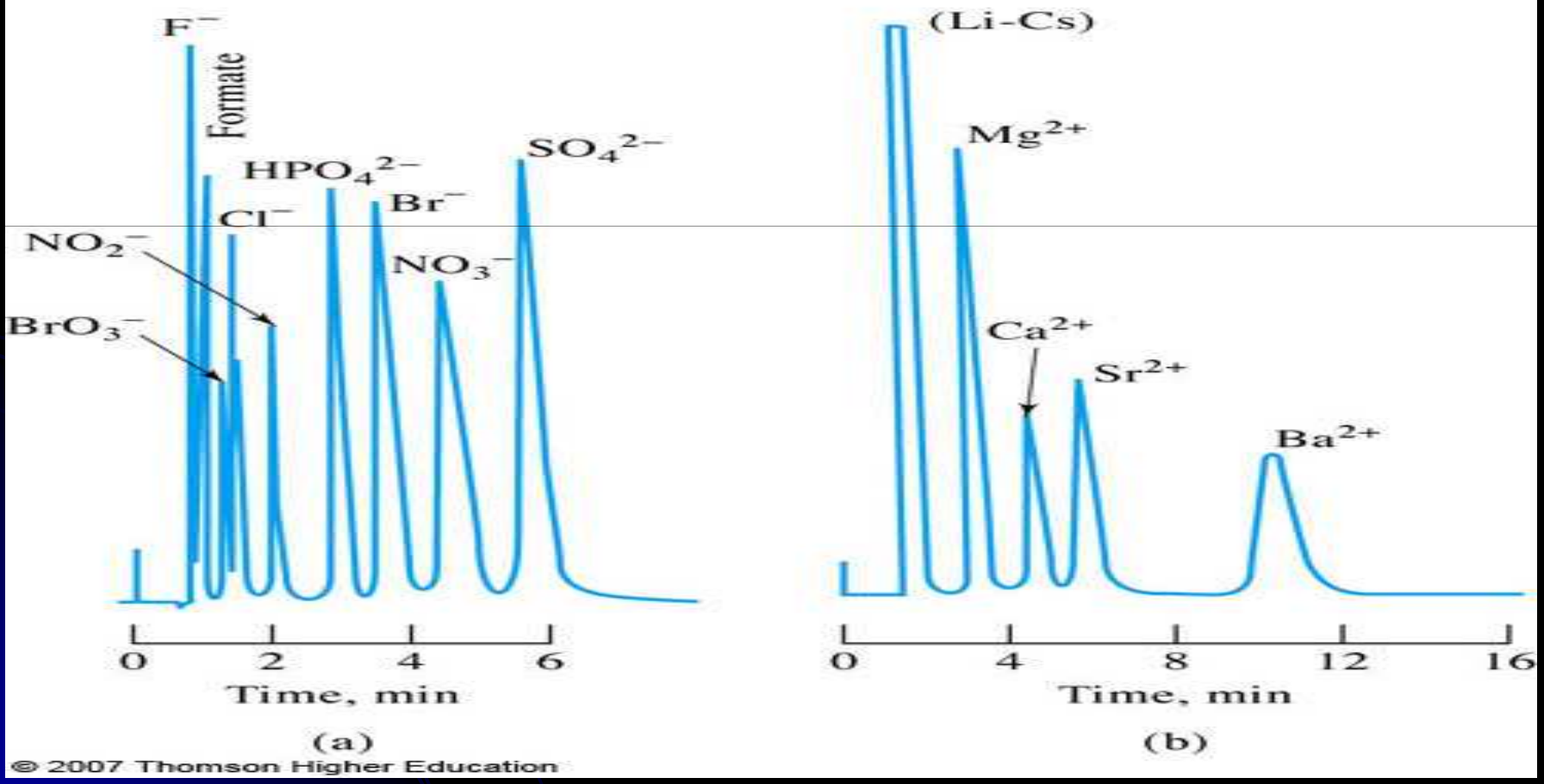
5. Food and essence manufacture
   - sweetener analysis in the fruit juice
   - preservative analysis in sausage.

advantages

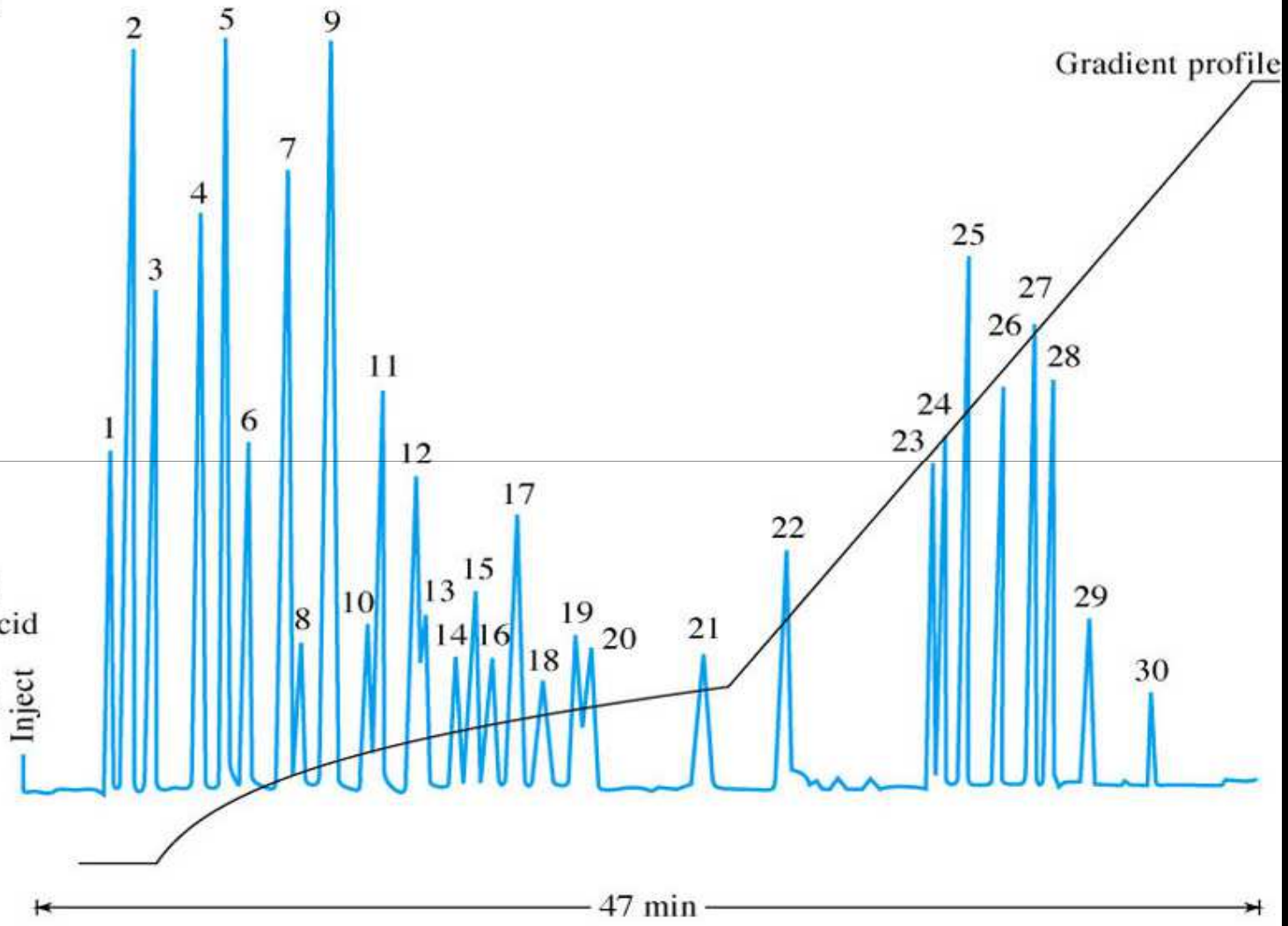
1. Needs a small sample with a high accuracy and precis
2. Non-destructed sample during operation compared to GC.

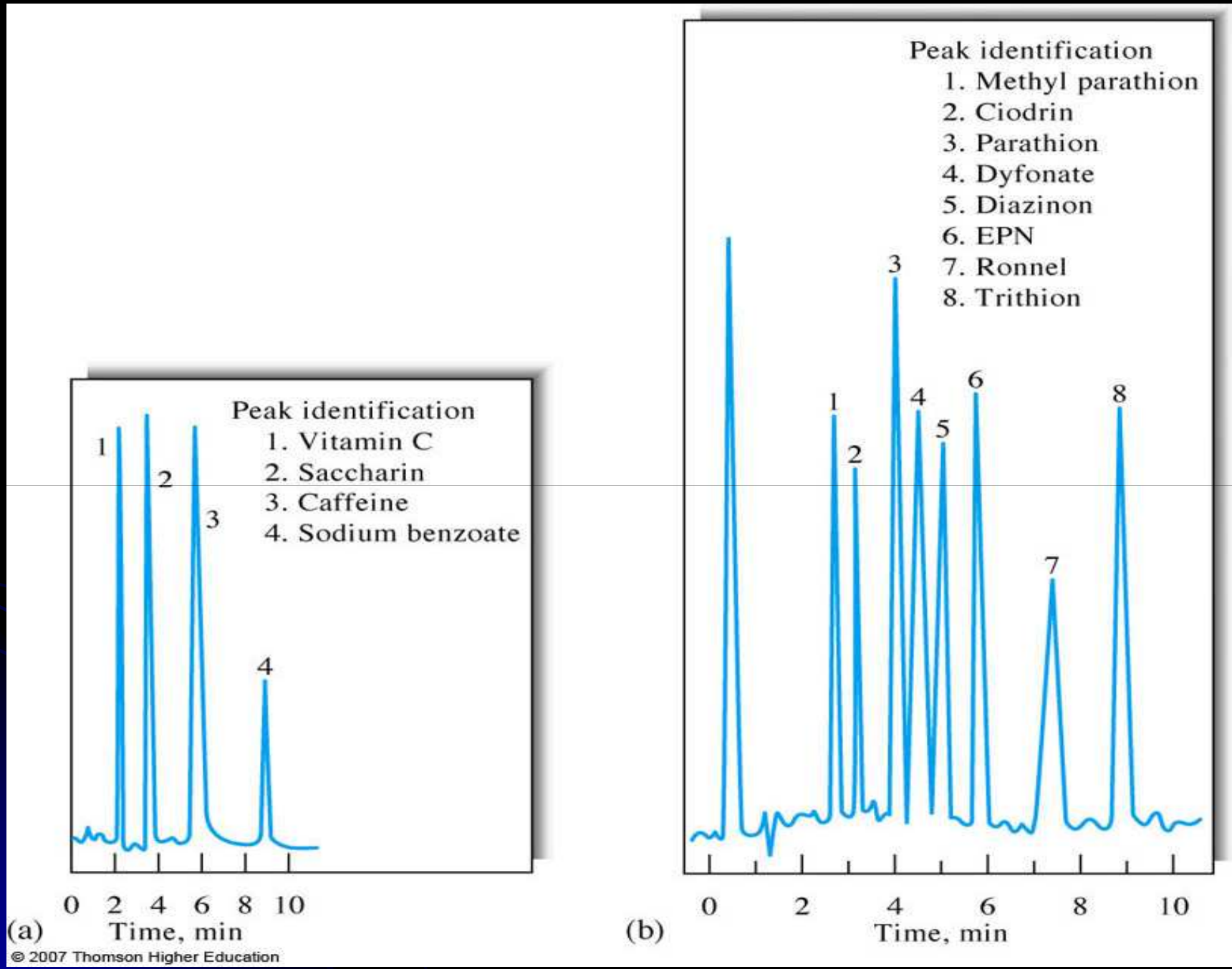
Concentrations, ppm	
F^-	3
Formate	8
BrO_3^-	10
Cl^-	4
NO_2^-	10
HPO_4^{2-}	30
Br^-	30
NO_3^-	30
SO_4^{2-}	25

Concentrations, ppm	
Ca^{2+}	3
Mg^{2+}	3
Sr^{2+}	10
Ba^{2+}	25



1. Phosphoserine
2. Aspartic acid
3. Glutamic acid
4. α-Amino adipic acid
5. Asparagine
6. Serine
7. Glutamine
8. Histidine
9. Glycine
10. Threonine
11. Citrulline
12. 1-Methylhistidine
13. 3-Methylhistidine
14. Arginine
15. β-Alanine
16. Alanine
17. Taurine
18. Anserine
19. β-Aminobutyric acid
20. β-Aminoisobutyric acid
21. Tyrosine
22. α-Aminobutyric acid
23. Methionine
24. Valine
25. Tryptophan
26. Phenylalanine
27. Isoleucine
28. Leucine
29. δ-Hydroxylysine
30. Lysine





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Thank
you

SPECTOSCOPY

Prof. JAGDALE D B

Properties of ElectroMagnetic Radiation (Light)

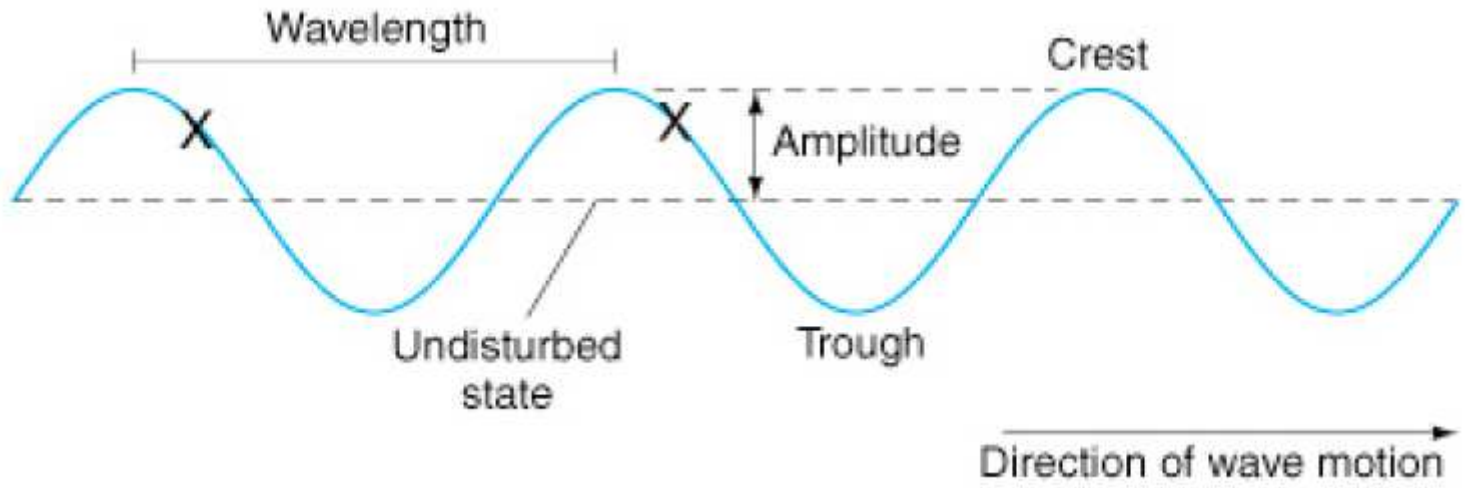
Introduction

A.) Spectroscopy: A method of analysis based on the interaction, absorption or production of light by matter. (also may include the interaction of electrons, ions or acoustics with matter)

B.) Light: Electromagnetic radiation

Two different views of light:

1.) *Wave Model*

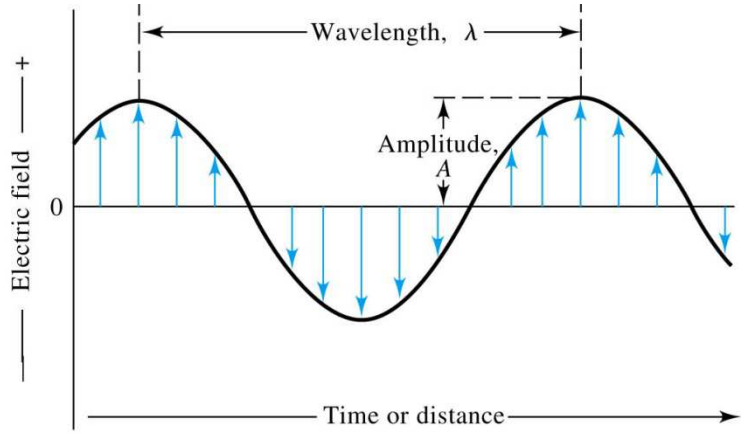


1. *Wave Model*

i.) represented by a sinusoidal wave traveling in space with an oscillating electric field and perpendicular magnetic field. (electric field is what is considered or used in most spectroscopic methods – except NMR)

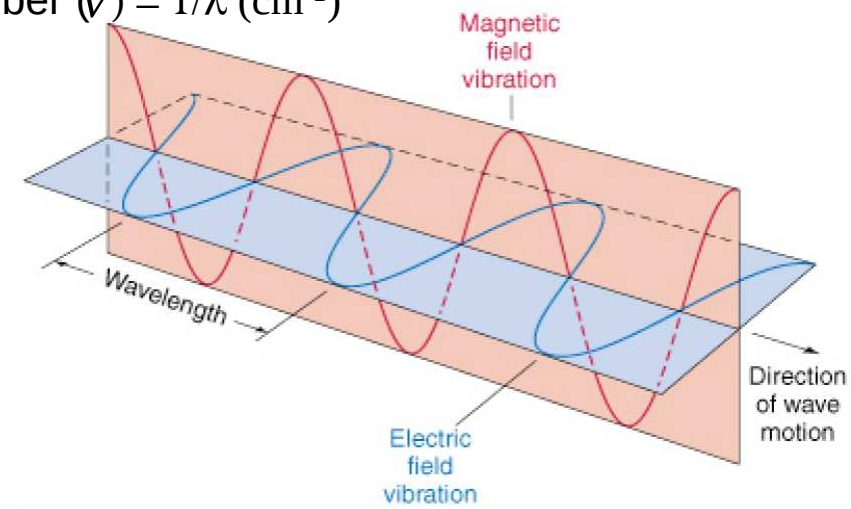
ii.) description of wave model

1) amplitude (A) – height of wave's electric vector



2) wavelength (λ) – distance (nm, cm, m) from peak to peak

a) wave number $(\bar{\nu}) = 1/\lambda\ (\mathrm{cm}^{-1})$

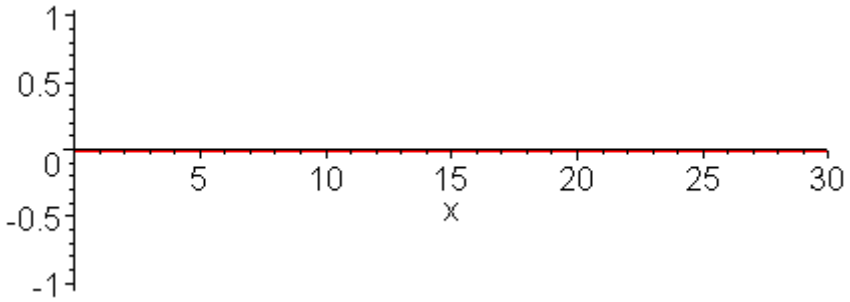


1. *Wave Model*

ii.) description of wave model

3) frequency (ν) – number of cycles or oscillations per second

a) hertz (Hz) or s^{-1}.

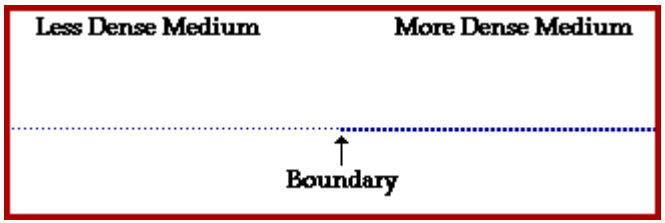


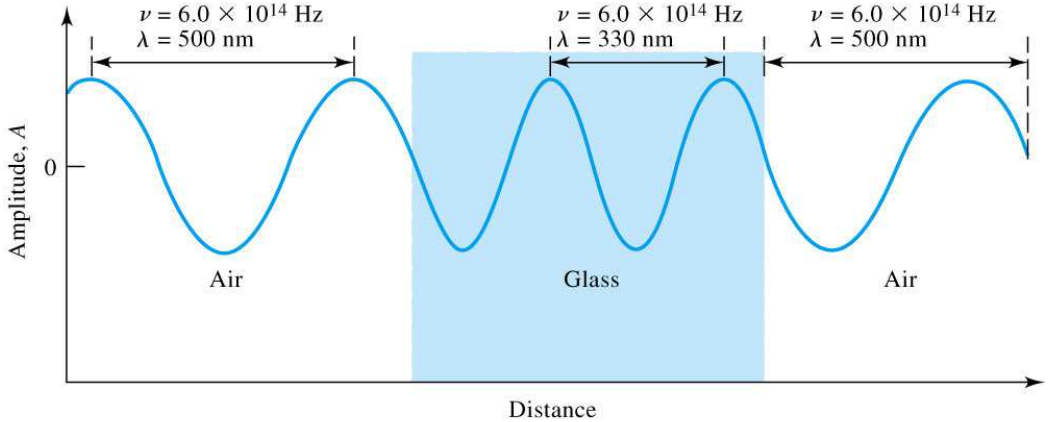
4) velocity of propagation (v_i) – rate of travel through space, dependent on composition of medium

a) $v_i = \nu\lambda_i$

b) maximum velocity (c) – speed of light in a vacuum (3.00 x10^8 m/s)

c) slower in other media (~ 0.03% slower in air)

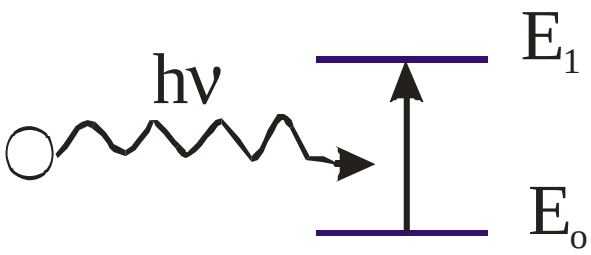




2. *Particle Model*

i.) light viewed as discrete particles of energy called photons

a) like other particles, light can be scattered, counted (quantized) , etc



Energy required of photon to give this transition:

$$\Delta E = E_1 - E_o$$

ii.) Energy of wave/particle:

$$E = h\nu = hc/\lambda = hc\bar{\nu}$$

h = Plank's constant (6.63×10^{-34} J·S)

ν = frequency, λ = wavelength, $\bar{\nu}$=wave number

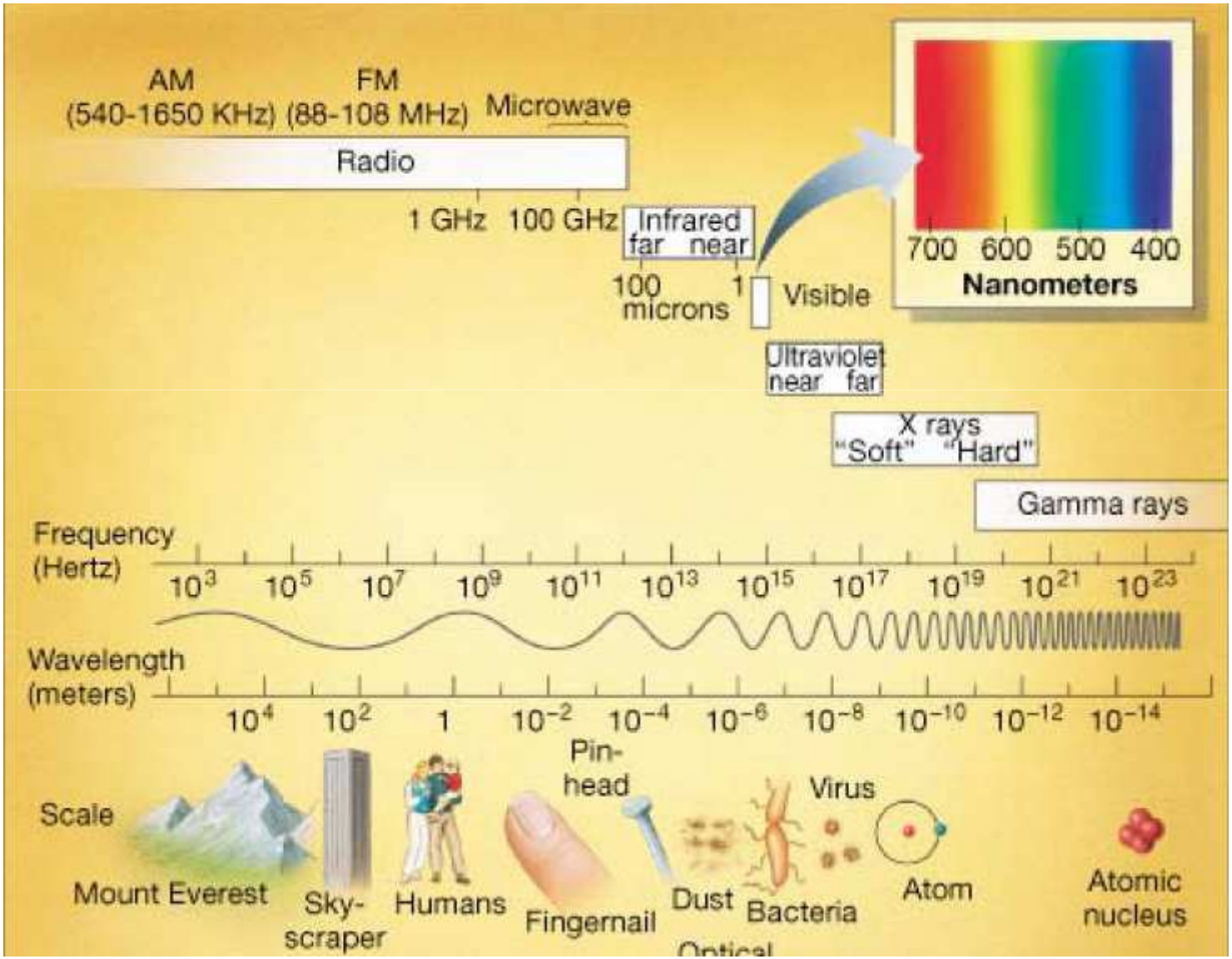
note:

energy is proportional to frequency and wave number ($\uparrow\nu \Leftrightarrow \uparrow E$)

energy is inversely proportional to wavelength ($\uparrow\lambda \Leftrightarrow \downarrow E$)

Wide Range of Types of Electromagnetic Radiation in nature.

1. Only a small fraction (350-780 nM is visible light).
2. The complete variety of electromagnetic radiation is used throughout spectroscopy.
3. Different energies allow monitoring of different types of interactions with matter.



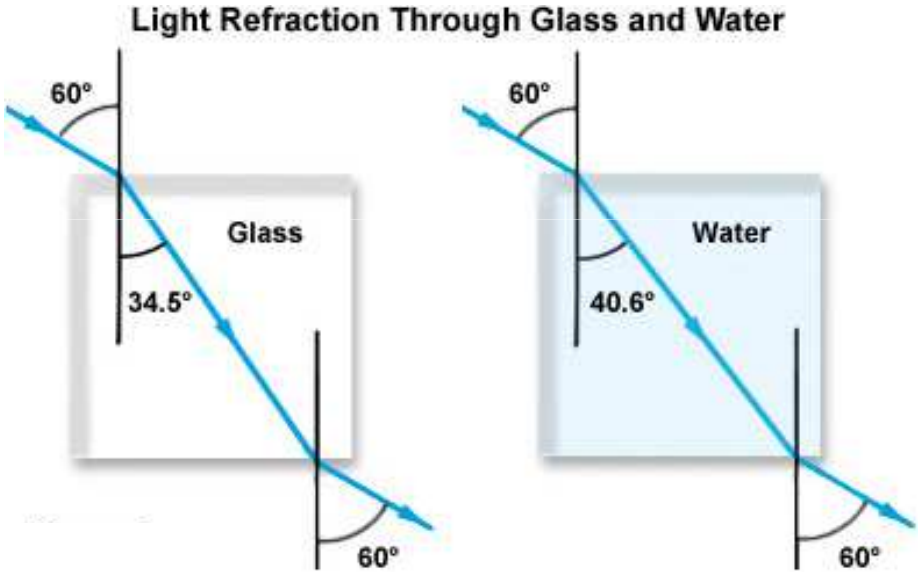
Common Spectroscopic Methods Based on Electromagnetic Radiation

Type of Spectroscopy	Usual Wavelength Range	Usual Wave number Range, cm^{-1}	Type of Quantum Transition
Gamma-ray emission	0.005-1.4 Å	_	Nuclear
X-ray absorption, emission, fluorescence, and diffraction	0.1-100 Å	_	Inner electron
Vacuum ultraviolet absorption	10-180 nm	$1x10^{6}$ to $5x10^{4}$	Bonding electrons
Ultraviolet visible absorption, emission, fluorescence	180 -780 nm	$5x10^{4}$ to $1.3x10^{4}$	Bonding electrons
Infrared absorption and Raman scattering	0.78-300 μm	$1.3x10^{4}$ to $3.3x10^{1}$	Rotation/vibration of molecules
Microwave absorption	0.75-3.75 mm	13-27	Rotation of molecules
Electron spin resonance	3 cm	0.33	Spin of electrons in a magnetic field
Nuclear magnetic resonance	0.6-10 m	$1.7x10^{-2}$ to $1x10^{3}$	Spin of nuclei in a magnetic field

Properties of Light

1.) Refraction: change in direction in the travel of a light beam when it comes at an angle to a boundary (interface) between two transparent media with different densities.

Pencil appears to bend at water/air interface due to refraction of light



a.) Refraction Index (η_i): medium/substance specific

$\eta_i = c/v_i$

c = speed of light in a vacuum

v_i = speed of light in medium of interest at the specified frequency

$\eta_i \geq 1$ since $v_i \leq c$

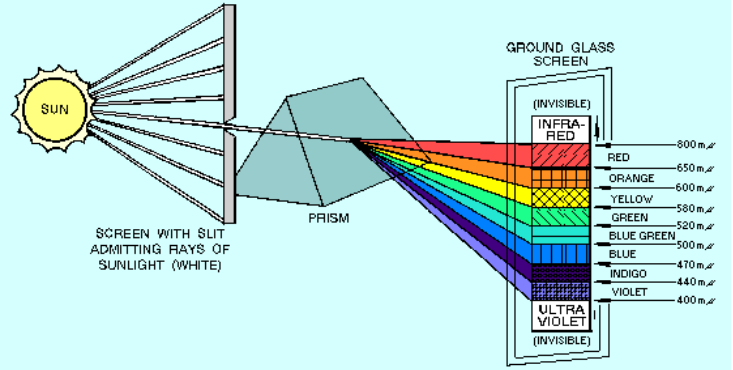
Typical values for η:

Material	Refractive Index
Air	1.0003
Water	1.33
Glycerin	1.47
Immersion Oil	1.515
Glass	1.52
Flint	1.66
Zircon	1.92
Diamond	2.42
Lead Sulfide	3.91

Values of η are wavelength dependent

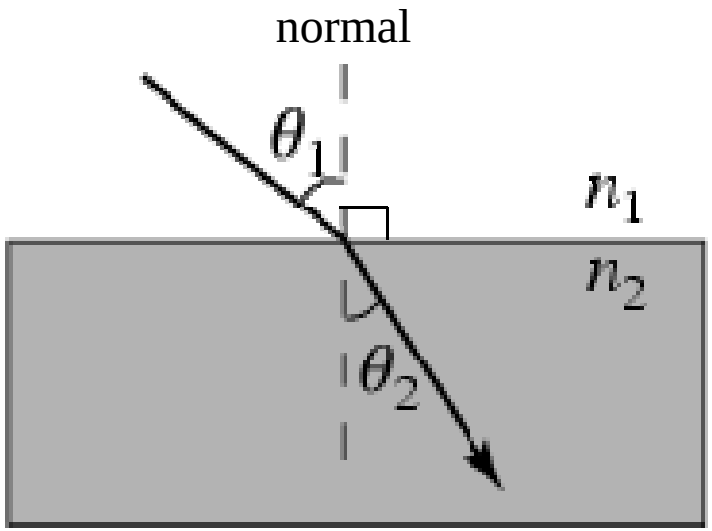
(useful for design of prisms)

values of η in table (if no frequency given) are usually for sodium double (D) line at 590 nm.



b.) Snell's Law: process of refraction

$$\eta_1 \sin\theta_1 = \eta_2 \sin\theta_2$$

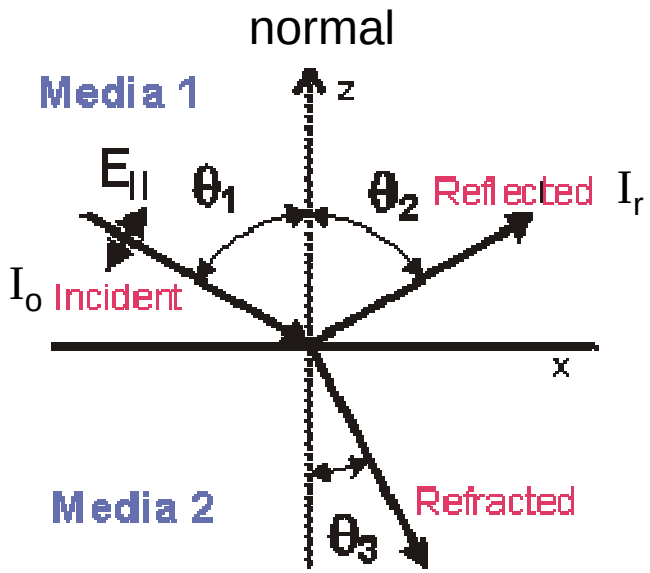


Change in direction of light after it encounters the interface. Change in interface is given by Snell's Law

- If $\eta_1 = \eta_2$, no change in direction, no refraction occurs
- The bigger the difference in η_1 and η_2, the more bending or refraction that occurs
- When light comes in at a right angle ($\theta_1 = 0$), no refraction occurs.

Properties of Light

2.) Reflection: when radiation crosses an interface between media that differ in refractive index, some or all of the light travels back into the medium from where it travel



Ansel Adams Mono Lake

- Reflected light comes out at same angle as incident beam, but on other side of normal.
  - Reflection occurs at each interface (when enters and exit)

- Always occurs along with refraction, reflection increases with bigger difference in η_1 and η_2.

- Occurs at all angles. At 90º to boundary (on normal) fraction reflected is given by:

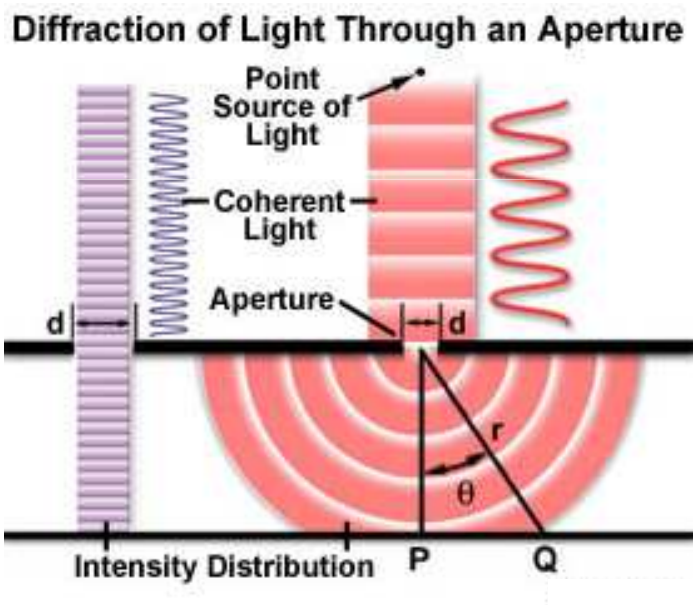
$$I_r/I_o = (\eta_2-\eta_1)^2/(\eta_2+\eta_1)^2$$

↑ I_r/I_o at values of $\theta_1 > 0$ approaches 1 at large angles (basis of fiber optics)

Properties of Light

3.) Diffraction: the bending of a parallel beam of light (or other electromagnetic radiation) as it passes a sharp barrier or through a narrow opening.

a.) most pronounced when size of slit or opening is approximately the same size as the frequency of light.

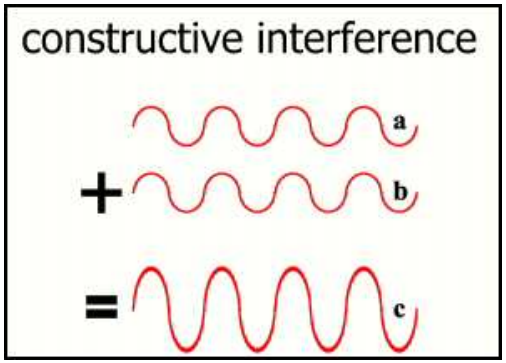


Radiation of a point source of light in all directions on other side of slit.

b.) *Interference – diffraction is a consequence of interference*

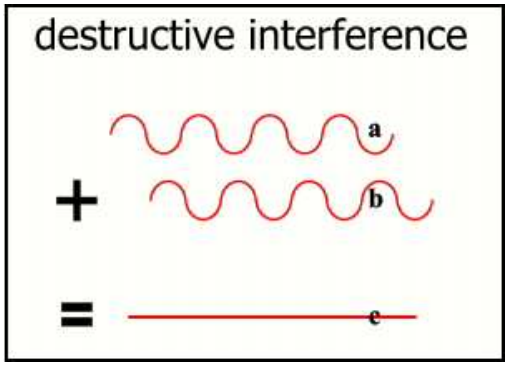
i.) Two types of interference.

1) constructive – waves “in-phase” electric fields are additive



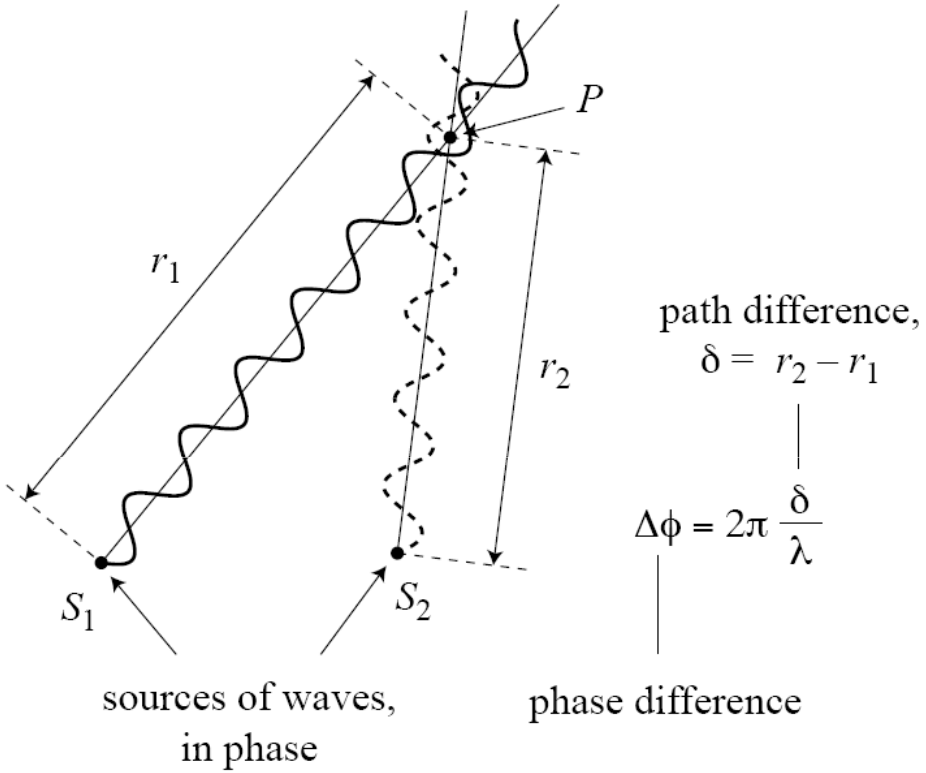
When two light waves of the same wavelength (color) combine exactly in phase (in step) their amplitudes add to produce a large (brighter) wave of maximum intensity.

2) destructive – waves “out of phase” electric fields subtract



If the light waves combine out of phase (out of step) their combined amplitudes are less, and may even totally cancel each other!

c.) *Destructive Interference* *can be created when two waves from the same source travel different paths to get to a point.*



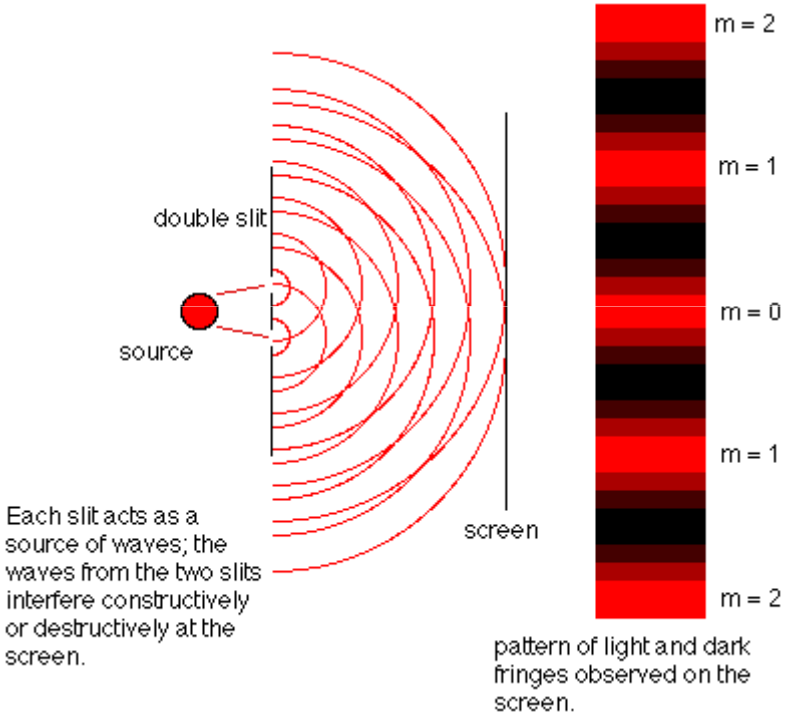
This may cause a difference in the phase between the two waves.

- *If the paths differ by an integer multiple of a wavelength, the waves will also be in phase.*
- *If the waves differ by an odd multiple of half a wave then the waves will be 180 degrees out of phase and cancel out.*

d.) *More then One Slit:* series of constructive and destructive interference that produces a series of high and low intensity regions – ***Interference Pattern***

Multiple rainbows – Interference Pattern

Thomas Young double slit experiment

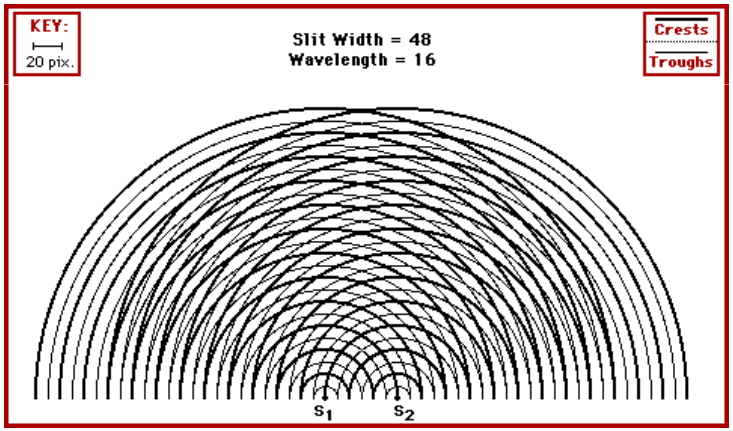


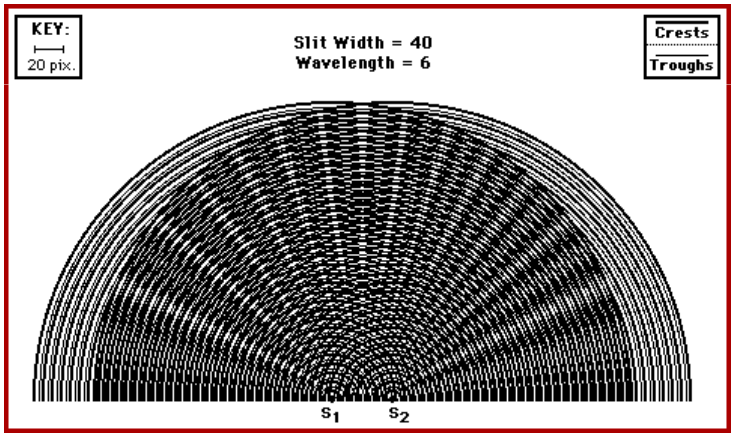
Patterns on screen depend on frequency (ν) or wavelength (λ), distance between two slits (d) and angle from normal (θ)

d.) *Order of Interference (n): $n\lambda = d \sin\theta$*

n=0 if two waves travel exactly the same distance, n=1 if differ by exactly λ

Note: equation is frequency (ν) or wavelength (λ) dependent, so it is useful in separating different λ for use in spectroscopy (different λ's at different points in space)





Change slit distance

Change frequency (ν)

Thomas Young double slit experiment

Example 2: What is the wavelength of a photon that has three times as much energy as that of a photon whose wavelength is 779 nm?

Example 3: Calculate the output of a ruby laser at 694.3 nm when it is passing through a piece of quartz, which has a refractive index of 1.55

Thank You