PES MODERN COLLEGE OF PHARMACY (FOR LADIES), MOSHI

Lecture synopsis Sub: Pharmaceutical Analysis V Subject I/C: Dr. Tambe V.S.

Sr. No.	Proposed Topic	Proposed Syllabus
1.	HPLC	Theory
2.	HPLC	Isocratic and Gradient types of separation
3.	HPLC	Instrumentation, Pumps, columns
4.	HPLC	Sample handling
5.	HPLC	Instrumentation Detectors
6.	HPLC	Instrumentation, Detectors
7.	HPLC	Tubing's, Degassing techniques
8.	HPLC	Quantitation techniques
9.	HPLC	Trouble shooting
10.	HPLC	System suitability testing and applications
11.	UPLC	UPLC: Introduction and advantages over HPLC
12.	UPLC	UPLC: Introduction and advantages over HPLC
13.	Gas Chromatography	Theory,
14.	Gas Chromatography	Instrumentation, sample handling,
15.	Gas Chromatography	Instrumentation, sample handling,
16.	Gas Chromatography	Instrumentation, columns, Supports and stationary phases
17.	Gas Chromatography	Detectors
18.	Gas Chromatography	Detectors
19.	Gas Chromatography	Detectors
20.	Gas Chromatography	Derivatisation techniques
21.	Gas Chromatography	Quantitation (area normalization, percent area, Internal standard and External standard method)
22.	Gas Chromatography	Applications of gas chromatography
23.	IR Spectroscopy	Origin of IR spectra, Molecular vibrations,
24.	IR Spectroscopy	fundamental bands, Important spectral regions
25.	IR Spectroscopy	Vibrational frequency and Factors affecting it
26.	IR Spectroscopy	Vibrational frequency and Factors affecting it
27.	IR Spectroscopy	Vibrational frequency and Factors affecting it
28.	IR Spectroscopy	Instrumentation
29.	IR Spectroscopy	FTIR Theory, Instrumentation,
30.	IR Spectroscopy	Sample handling

31.	IR Spectroscopy	Different attachments used in recording FTIR, ATR
32.	IR Spectroscopy	Photo acoustic IR, FTIR Microscopy
33.	IR Spectroscopy	Applications of IR Spectroscopy
34.	IR Spectroscopy	FTIR Analysis and Interpretation of organic compounds based on FTIR Spectra
35.	IR Spectroscopy	FTIR Analysis and Interpretation of organic compounds based on FTIR Spectra
36.	IR Spectroscopy	FTIR Analysis and Interpretation of organic compounds based on FTIR Spectra
37.	NIR Spectroscopy	Introduction to Near Infrared (NIR)
38.	NIR Spectroscopy	Applications Near Infrared (NIR)
39.	Raman Spectroscopy	Theory Raman spectroscopy, Comparison with IR
40.	Raman Spectroscopy	Instrumentation and applications
41.	Scanning Electron Microscopy (SEM)	Principle, and Instrumentation of Scanning Electron Microscopy (SEM)
42.	Scanning Electron Microscopy (SEM)	Applications of Scanning Electron Microscopy (SEM)
43.	Transmission Electron Microscopy (TEM)	Principle and Instrumentation of Transmission Electron Microscopy (TEM)
44.	Transmission Electron Microscopy (TEM)	Applications of Transmission Electron Microscopy (TEM)
45.	SEM and TEM	Comparison of SEM and TEM

Lecture synopsis Sub: Pharmaceutical Analysis V Subject I/C: Dr. Tambe V.S.

Lecture No: 1

Name of topic/lesson – High Performance Liquid Chromatography (HPLC)

Subtopic: Introduction, Principle and Theory

Objective: To Study Principle and Theory of HPLC

Topic Outcomes: At the end of topic you will

1. Compare and know the advantages and disadvantages of HPLC
2. Know what makes HPLC a efficient separation tool

High performing technique due to minimised HETP value

Van Deempter equation

$$H = \frac{L}{N}$$

- *Height Equivalent to One Theoretical Plate (HETP)*

- $\bar{u}$: average linear velocity

$$H = A + \frac{B}{\bar{u}} + C_S\bar{u} + C_M\bar{u}$$

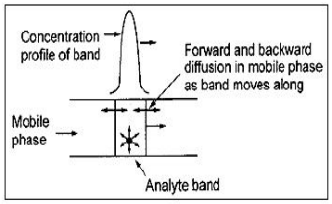
- *H:* as small as possible
- Some terms decrease, other increase with $\bar{u}$
  - There should be optimum $\bar{u}$
- There are other alternative models

A is

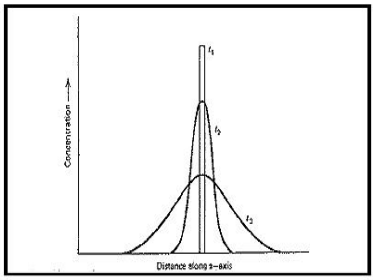
- **'Eddy diffusion' & unequal pathways**
- Molecules may travel unequal distances
- Particles (if present) cause eddies & turbulence
- A-Term is independent of $\bar{u}$
- A depends on size of stationary particles (want *small)* and their packing (want *uniform)* (or coating in TLC plate)
  - GC: 150 µm, HPLC: 5-10 µm

B is

- **Longitudinal Diffusion**
- Basically molecular diff., as is mobile phase was not moving $\alpha \sqrt{t_R}$



- Functional form of the term is $B/\bar{u}$



- Model for *B*: $B = 2\gamma D_M$
- γ is hindrance factor due to packing (0.7 in packed – 1 in open) and D_M is molecular diffusion coeff.
- *B* terms dominates at low *u*
- More important for GC than LC, since

Cs is resistance to mass transfer

$$C_s = \frac{d_f^2}{D_s}$$

df is film thickness of stationary phase

Ds is diffusion coefficient in to stationary phase

- C_M accounts for mass transfer on the mobile phase interface with the SP
- In packed columns:
  - d_p is particle diameter

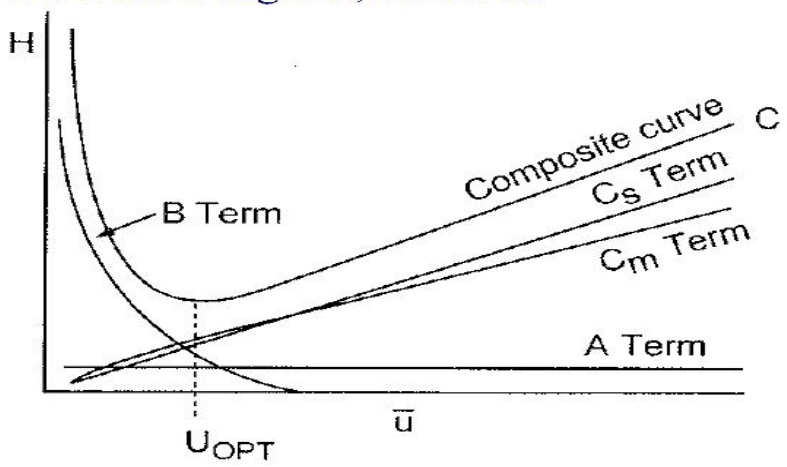
$$C_M = \frac{d_P^2}{D_M}$$

- In open columns
  - d_c is column diameter

$$C_M = \frac{d_C^2}{D_M}$$

- Diffusion is much faster in gas than liquid
  - GC: $C_M \ll C_s$
  - HPLC: $C_M \sim C_s$

- We want N highest, H lowest



References

1. Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed
2. Instrumental Methods of Analysis by Willard Merit, Dean Settle, 7th edition, CBS Publisher & Distributor
3. Instrumental Methods of Chemical Analysis by BK Sharma, Goel Publishing House.

Lecture 2

Name of topic/lesson – High Performance Liquid Chromatography (HPLC)

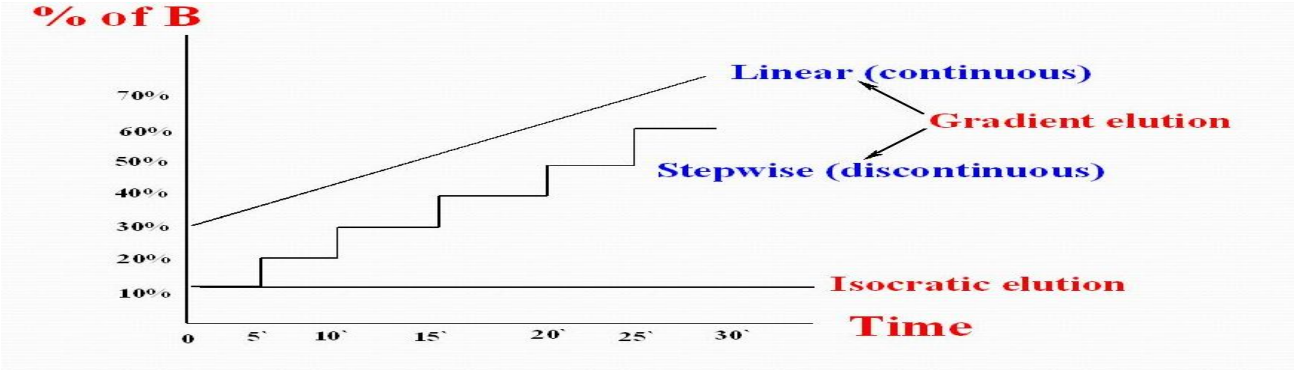
Subtopic: Types of elution

Objective: To Study types of elution used in HPLC

Topic Outcomes: At the end of topic you will

1. Know types of elution used in HPLC
2. Differentiate and understand the selection criteria

1. **Isocratic elution:** the composition of mobile phase remains constant throughout the HPLC separation.
2. **Gradient elution**: Often the only way to elute all of the compounds in the sample in a reasonable amount of time, while still maintaining peak resolution, is to change the ratio of polar to non-polar compounds in the mobile phase during the sample run. This is the technique of choice when a sample contains components of a wide range of polarities. For a **reverse phase gradient**, the solvent starts out relatively polar and slowly becomes more non-polar. The gradient elution offers the most complete separation of the peaks, without taking an inordinate amount of time. A sample containing compounds of a wide range of polarities can be separated by a gradient elution in a shorter time period without a loss of resolution in the earlier peaks or excessive broadening of later peaks. However, gradient elution requires more complex and expensive equipment and it is more difficult to maintain a constant flow rate while there are constant changes in mobile phase composition. Gradient elution, especially at high speeds, brings out the limitations of lower quality experimental apparatus, making the results obtained less reproducible in equipment already prone to variation. If the flow rate or mobile phase composition fluctuates, the results will not be reproducible.



Isocratic elution

- Can often use one pump
- Mix solvent together ahead of time
- Simple, no mixing chamber is required
- Limited flexibility, not used much in research
- Used mostly for process chemistry or routine analysis

Gradient elution

- Uses multiple pumps whose output is mixed together
- Often 2-4 pumps (binary to quaternary systems)
- Changing mobile phase components changes the polarity index
- Can be used to subsequently elute compounds that were previously (intentionally) stuck on the column
- Column has to reequilibrate to original conditions after each run.

References

1. Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed
2. Instrumental Methods of Analysis by Willard Merit, Dean Settle, 7th edition, CBS Publisher & Distributor
3. Instrumental Methods of Chemical Analysis by BK Sharma, Goel Publishing House.

Lecture 3

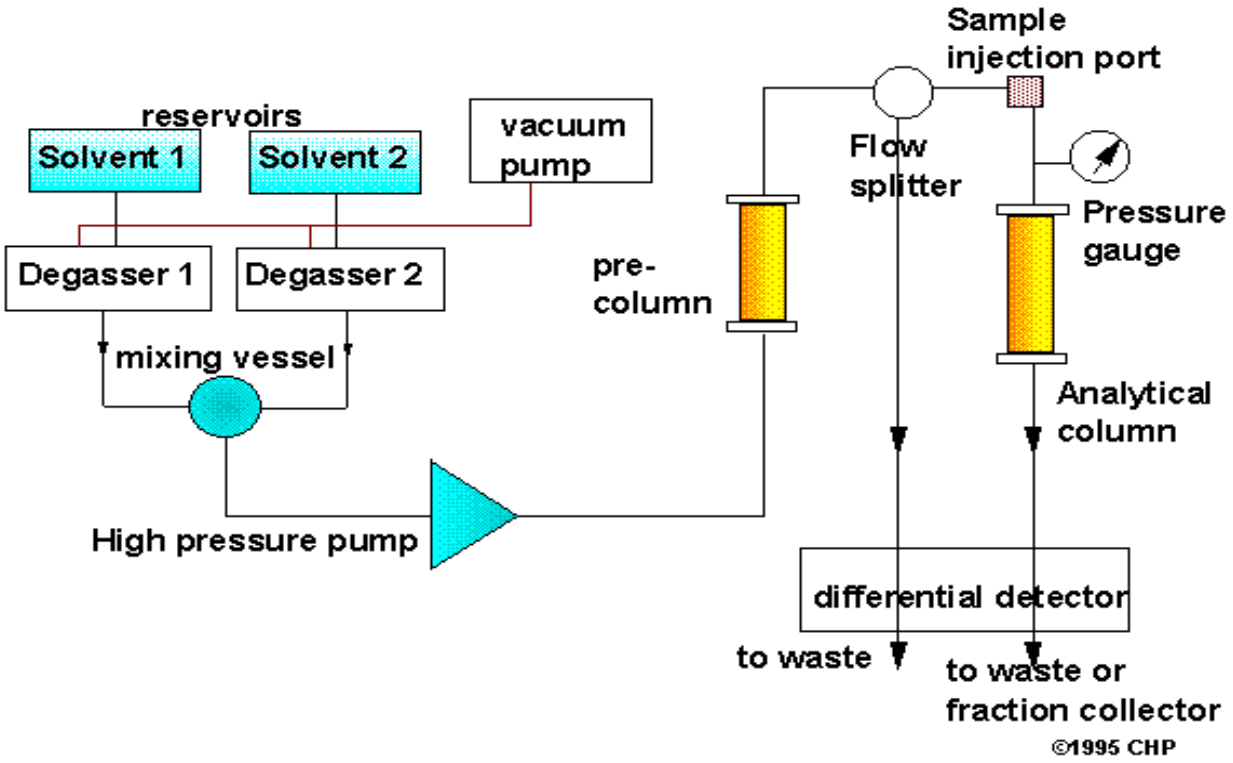
Name of topic/lesson – High Performance Liquid Chromatography (HPLC)

Subtopic: Instrumentation, Types of pumps, stationary phases

Objective: TO STUDY THE STATIONARY PHASES USED IN CHROMATOGRAPHY

Topic Outcomes: At the end of topic you will

1. **Selection of appropriates stationary phase**
2. **Know the parts of HPLC with function of each part**



Types of Pumps

1. **Reciprocating pump**
2. **Pneumatic pump**
3. **Syringe pump**

Modes of HPLC CHROMATOGRAPHIC separation

- Partition chromatography (liquid liquid, liquid bonded phase suitable for gradient elution), Most common
- Adsorption, or liquid-solid chromatography

- Ion exchange chromatography
- Size exclusion, or gel, chromatography

Stationary phases

- Particle based (silica and polymer based)
- Monolithic (silica and polymer based)
- HILIC and mixed beds (Hydrophilic interaction)
- Hybrid Packing
- Packing based on Zirconia or titania
- Fully porous packings

(large specific surface area (200-300 m^2 /g), larger retention, larger Loadability)

- These are less prone to exhibit broad peaks with increased injection.
- Pore size should give access to analyte
- Small molecules (m.wt-100-500) pore size 10nm
- Larger molecules – 30 nm (less retentive)

Advantages of silica

- Mechanical strength
- Availability of well established surface modification technique.
- Freedom to tailor surface area and pore size

Types

- Classical/ low purity silica (iron and alumina impurities) creates acidified surface silanol
- High purity silica : synthesised from very pure organic silanes(tetraethoxysilane) and by using carefully controlled manufacturing process

Polymer based packings

- Less commonly used in small molecule analysis dues to swelling and shrinking problems. And inferior mass transfer properties
- Used in SEC, HILIC , sample preparation techniques and biomolecule analysis
- Derivatisation of silanol with organosilane
- Reagent include chlorosilane, monofunctional, difunctional and trifunctional silanes are used. HCl is formed in the reaction hence base is required to scavenge the acid.

Hybrid organic/inorganic packings

- Adv- improved stability of silica to alkaline environment. (silica unstable above pH even at room temperature). C18 modification can partially solve the problem.
- Removed totally by using hybrid packing – a methyl (stable up to pH 11) or ethyl (stable upto pH 12)group in the matrix is introduced

Silanol activity is reduced

Zirconia based packings

- Stable from pH 1-14
- Stable at high temperatures.
- Bonding procedures are not available
- RP Packing preparation: coating of zirconia with polybutadiene or polystyrene with subsequent derivatisation with c 18 layer
- Large pore size (30 nm), low surface area
- BUFFERS ARE STRONGLY ADSORBED

References

1. Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed
2. Instrumental Methods of Analysis by Willard Merit, Dean Settle, 7th edition, CBS Publisher & Distributor
3. HPLC method development by Ahuja

Lecture 4

Name of topic/lesson – High Performance Liquid Chromatography (HPLC)

Subtopic: Sample handling

Objective: TO STUDY THE SAMPLE HANDLING TECHNIQUES USED IN HPLC

Topic Outcomes: At the end of topic you will

1. **Know the sample handling techniques used in HPLC**

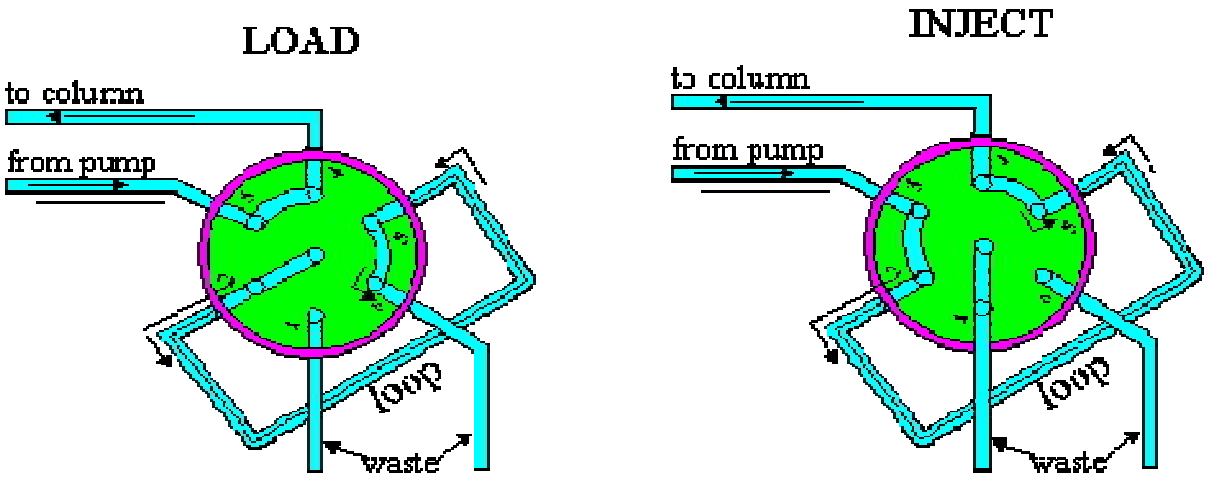
HPLC is one of the most common high-precision analytical methods. Its primary objective: deliver reproducible and specific results. A sample needs to be optimally prepared so it can be injected directly onto an HPLC column. To accomplish this, your sample not only needs to be dissolved in the appropriate solvent. Even more important, it must also be free of particles to rule out interference in the best possible way during detection and to prevent blockage of your column. This labor-intensive sample prep is often a tedious chore that is time-consuming.

INJECTORS

- Should provide the possibility of injecting the liquid sample within the range of 0.1 to 100 ml of volume with high reproducibility and under high pressure (up to the 4000 psi).
- Should also produce minimum band broadening and minimize possible flow disturbances.

Rheodyne injector (six-port Rheodyne valve)

With these sampling valves, samples can be introduced reproducibly into pressurized columns without significant interruption of flow, even at elevated temperatures.



Sample fills an external loop. Compared to shorter, wider i.d. sample loops, long, narrow loops are preferred when large sample volumes are required, because of lesser band-broadening effects. Alternatively, a specially designed syringe may be used to inject a small volume (e.g., <10 ,ul) into the loop when required, although in this case the precision in the sample introduction is dependent on the precision of syringe delivery.

A clockwise rotation of the valve rotor places the sample-filled loop into the mobile-phase stream, with subsequent injection of the sample onto the top of the column through a low-volume, cleanly swept channel.

Automatic Injectors

With commercially available automatic sampling devices, large numbers of samples can be routinely analyzed by LC without operator intervention.

References

1. Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed
2. Instrumental Methods of Analysis by Willard Merit, Dean Settle, 7th edition, CBS Publisher & Distributor

Lecture 5

Name of topic/lesson – High Performance Liquid Chromatography (HPLC)

Subtopic: Instrumentation, Detectors

Objective: TO STUDY VARIOUS TYPES OF DETECTORS USED IN HPLC

Topic Outcomes: At the end of topic you will

1. **Understand various types of detectors used in HPLC**
2. **Select correct detector by studying different parameters**

Detectors for HPLC are designed to take advantage of some physical or chemical attribute of either the solute or mobile phase in the chromatographic process in one of four ways:

. A bulk property or differential measurement

. Analyte specific properties

. Mobile phase modification

. Hyphenated techniques

Desired detectors characteristic

- High sensitivity and reproducible, predictable response
- Respond to all solutes, or have predictable specificity
- Wide linear dynamic range; Response that increases linearly with the amount of solute
- Response unaffected by changes in temperature and mobile phase flow
- Respond independently of the mobile phase
- Not contribute to extra-column band broadening
- Reliable and convenient to use
- Nondestructive of the solute
- Provide qualitative and quantitative information on the detected peak
- Fast response

References

1. Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed
2. Instrumental Methods of Analysis by Willard Merit, Dean Settle, 7th edition, CBS Publisher & Distributor

Lecture 6

Name of topic/lesson – High Performance Liquid Chromatography (HPLC)

Subtopic: Instrumentation, Detectors
Objective: TO STUDY VARIOUS TYPES OF DETECTORS USED IN HPLC

Topic Outcomes: At the end of topic you will

1. **Understand various types of detectors used in HPLC**
2. **Select correct detector by studying different parameters**

Detector	Key Attributes	Limitations
UV/Vis/PDA	Most widely used and accepted; Near "universal" at low UV; Gradient compatible Qualitative and Quantitative; PDA peak purity/ homogeneity, spectral library searches/ID, contour maps and 3D spectral display; Nondestructive Cost; Very Reliable; Easy to use	Must have a chromophore; Solvents must be transparent; Widely varying response for different solutes
Light Scattering	Detects most non volatile analytes; Works well with gradient HPLC; Better sensitivity than RI detection	Requires the use of volatile buffers, optimization; Limited dynamic range; Reproducibility of methods
Corona discharge	Highest sensitivity of "universal" type detector; Wide dynamic range; Detects any non volatile or semi-volatile; Consistent response; Ease of use	Requires the use of volatile buffers
FL	Very selective and sensitive; Works well with gradients	Not all compounds fluoresce; Often requires derivative formation; Quenching; Cost for performance
Radioactivity	Gradient compatible; can determine distribution and mass balance for drug metabolite studies, wide response range	Large flow cell volumes increase peak broadening and decrease resolution
EC	Very selective and sensitive; Modern ECs are reliable and easy to use	Mobile phase must be conductive; susceptible to background noise and electrode fouling; only applicable to compounds that can be oxidized or reduced
Conductivity	Detector of choice for ion chromatography-inorganic ions and organic acids; Very selective; Low cost	Requires suppression of mobile phase background conductivity; Not all compounds are detected; Requires special HPLC systems and columns
RI	Original detector for HPLC in many methods; Excellent versatility/ Universal detection; Solvent compatibility; Nondestructive; Cost; reliable and easy to operate	Sensitivity; Gradient incompatible; Stability (Temperature and Flow)

References

1. Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed

Lecture 7

Name of topic/lesson – High Performance Liquid Chromatography (HPLC)

Subtopic: Tubings and Mobile phase preparation Techniques

Objective: TO STUDY TUBINGS AND SAMPLE PREPARATION TECHNIQUES USED IN HPLC

Topic Outcomes: At the end of topic you will

1. **Know the materials compatible for making HPLC tubings**
2. **Understand importance and types of Mobile phase preparation Techniques**

PEEK (polyetheretherketone) polymer tubing

- Biocompatible,
- chemically inert to most solvents,
- can be used to replace stainless steel tubing in most liquid analytical systems.
- Unlike stainless steel and titanium tubing, PEEK tubing is flexible and can be easily cut to desired lengths. PEEK tubing can be used with stainless steel or polymer fittings.
- The benefits of PEEK polymer tubing include a high pressure rating (up to 7,000 psi in most cases)
- A high temperature rating (maximum continuous use temperature of 100ºC). Additionally, PEEK tubing has a very smooth internal surface, which causes less turbulance than similar sized metal tubing. Turbulence can cause remixing of separated sample bands and dilution of bands by the mobile phase.
- PEEK is the least permeable to gas.

Stainless steel tubing

Titanium tubing

References

1. Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed
2. Instrumental Methods of Analysis by Willard Merit, Dean Settle, 7th edition, CBS Publisher & Distributor

Lecture 8

Name of topic/lesson – High Performance Liquid Chromatography (HPLC)

Subtopic: Quantitation Techniques used HPLC

Objective: TO STUDY VARIOUS QUANTITATION TECHNIQUES USED HPLC

Topic Outcomes: At the end of topic you will

3. **Be able to apply these techniques in chromatographic problems**

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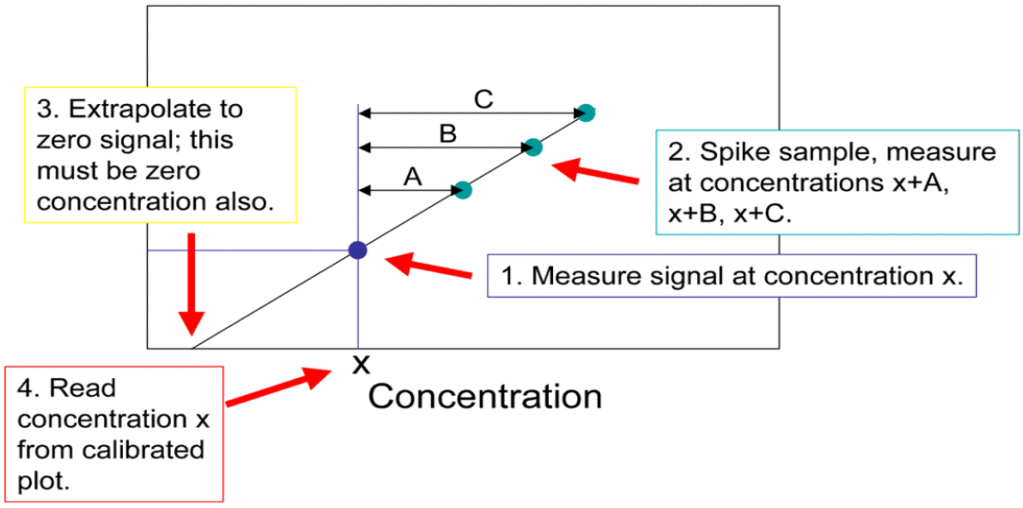
1. External Standard method

2. **Standard addition method**: The method of **standard addition** is a type of quantitative analysis approach often used in analytical chemistry whereby the standard is added directly to the aliquots of analyzed sample. This method is used in situations where sample matrix also contributes to the analytical signal, a situation known as the matrix effect, thus making it impossible to compare the analytical signal between sample and standard using the traditional calibration curve approach



3. Internal Standard method

The method of internal standards is used to improve the precision of quantitative analysis. An *internal standard* is a known concentration of a substance that is present in every sample that is analyzed. Internal standards can be used with either the calibration curve or standard addition methods. The purpose of the internal standard is to behave similarly to the analyte but to provide a signal that can be distinguished from that of the analyte. Ideally, any factor that affects the analyte signal will also affect the signal of the internal standard to the same degree. Thus, the ratio of the two signals will exhibit less variability than the analyte signal.

4. Area normalization method

The normalization method is the easiest and most straightforward and requires no reference standards or calibration solutions to be prepared. However, the detector must have the same response to all the components of the sample.

References

1. Instrumental Methods of Analysis by Willard Merit, Dean Settle, 7th edition, CBS Publisher

Lecture 9

Name of topic/lesson – High Performance Liquid Chromatography (HPLC)

Subtopic: Troubleshooting

Objective: TO STUDY THE PROBLEMS IN HPLC WITH ITS CAUSES AND REMEDY

Topic Outcomes: At the end of topic you will

1. **Identify the problem in chromatogram with reason**
2. **Be able to suggest the remedies for chromatographic problems**

TROUBLESHOOTING IN HPLC

- No Peaks/Very Small Peaks
- No Flow, No Pressure/Pressure Lower Than Usual, Pressure Higher Than Usual
- Loss of Resolution
- Variable Retention Times
- Peaks Tail on Initial and Later Injections
- Split Peaks
- Tailing Peaks
- Fronting Peaks
- Rounded Peaks
- Baseline Drift
- Baseline Noise (regular)
- Baseline Noise (Irregular)
- Broad Peaks
- Change in Peak Height (one or more peaks)
- Change in Selectivity
- Negative peaks
- Ghost peaks

References

1. Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed
2. Instrumental Methods of Analysis by Willard Merit, Dean Settle, 7th edition, CBS Publisher & Distributor

Lecture 10

Name of topic/lesson – High Performance Liquid Chromatography (HPLC)

Subtopic: System Suitability testing (SST)

Objective: TO STUDY IMPORTANCE AND PARAMETERS OF SST

Topic Outcomes: At the end of topic you will

1. **Know the importance of SST**
2. **Understand how to perform SST**

System suitability test (SST) is a test to determine the suitability and effectiveness of chromatographic system prior to use. The performance of any chromatographic system may continuously change during their regular use, which can affect the reliability of the analytical results. The operation parameters of the whole chromatographic system can be checked with properly selected SST mixtures. These mixtures are used to establish characteristic chromatographic parameters, such as the number of effective theoretical plates, resolution, asymmetry, detection limit and selectivity. The system is then only declared suitable if the responses are within given limits.

References

1. Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed
2. Instrumental Methods of Analysis by Willard Merit, Dean Settle, 7th edition, CBS Publisher & Distributor

Lecture 11

Name of topic/lesson – Ultra High Performance Liquid Chromatography (UPLC)

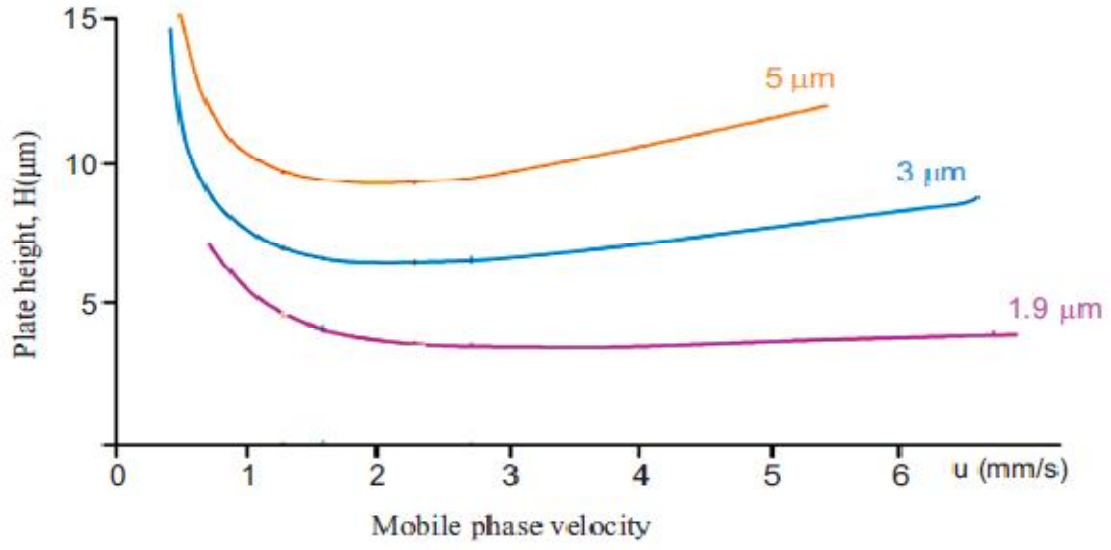
Subtopic: Introduction

Objective: To study Theory of UPLC

Topic Outcomes: At the end of topic you will

1. **Know Principle and working of UPLC**

To further achieve the dramatic increase in resolution, speed and sensitivity in LC, a significant advancement in the instrumentation and column technology (column particle size and column dimension) were made. To achieve the above targets, Waters in 2004, launched and trademarked Ultra Performance Liquid Chromatography (UPLC) which is based upon small, porous particles (sub 2micron particles).



Van deempter Equation

$$H = A + \frac{B}{v} + Cv$$

References:

1. Gita Chawla and Chanda Ranjan, Principle, Instrumentation, and Applications of UPLC: A Novel Technique of Liquid Chromatography, *Open Chemistry Journal*, 2016, *3*, 1-16.
2. Michael E. Swartz, UPLC™: An Introduction and Review, Journal of Liquid Chromatography & Related Technologies*w*, 28: 1253–1263, 2005

Lecture 12

Name of topic/lesson – Ultra High Performance Liquid Chromatography (UPLC)

Subtopic: Comparison of HPLC and UPLC

Objective: To study and compare HPLC and UPLC

Topic Outcomes: At the end of topic you will

1. **Compare and contrast between HPLC and UPLC**

Advantages of UPLC

- More selective and sensitive with high resolution performance
- Faster resolving power.
- Reduces process cycle time and assures end-product quality with reduced cost of operation and decreased run time.
- It increases sensitivity and provides quick analysis through the use of a novel column material of very small particle size.
- It decreases the consumption of solvent and increases sample throughput and also provides real-time analysis in step with manufacturing processes.

Disadvantages

The higher back pressures compared to conventional HPLC which decreases the life of the columns. Increasing the column temperature reduces the back pressure problem in UPLC. Moreover, the particles of less than 2 µm are mostly non-regenerable and, therefore, have a narrow use.

Characteristics	HPLC	UPLC
Particle size	3 to 5µm	Less than 2µm
Maximum backpressure	300-400 bars	1000 bars
Analytical column	C18	UPLC BEH C18
Column dimensions	150 X 3.2 mm	50 X 2.1 mm
Injection volume	5µL	2µL
Column temperature	30 ° C	65 °C
Total run time	10 min.	1.5 min
USP resolution	3.2	3.4
Plate count	2000	7500
Flow rate	3.0 ml/min	0.6ml/min

References:

1. Gita Chawla and Chanda Ranjan, Principle, Instrumentation, and Applications of UPLC: A Novel Technique of Liquid Chromatography, *Open Chemistry Journal*, 2016, *3*, 1-16.

Lecture 13

Name of topic/lesson – Gas Chromatography (GC)

Subtopic: Theory

Objective: To study Theory of Gas Chromatography

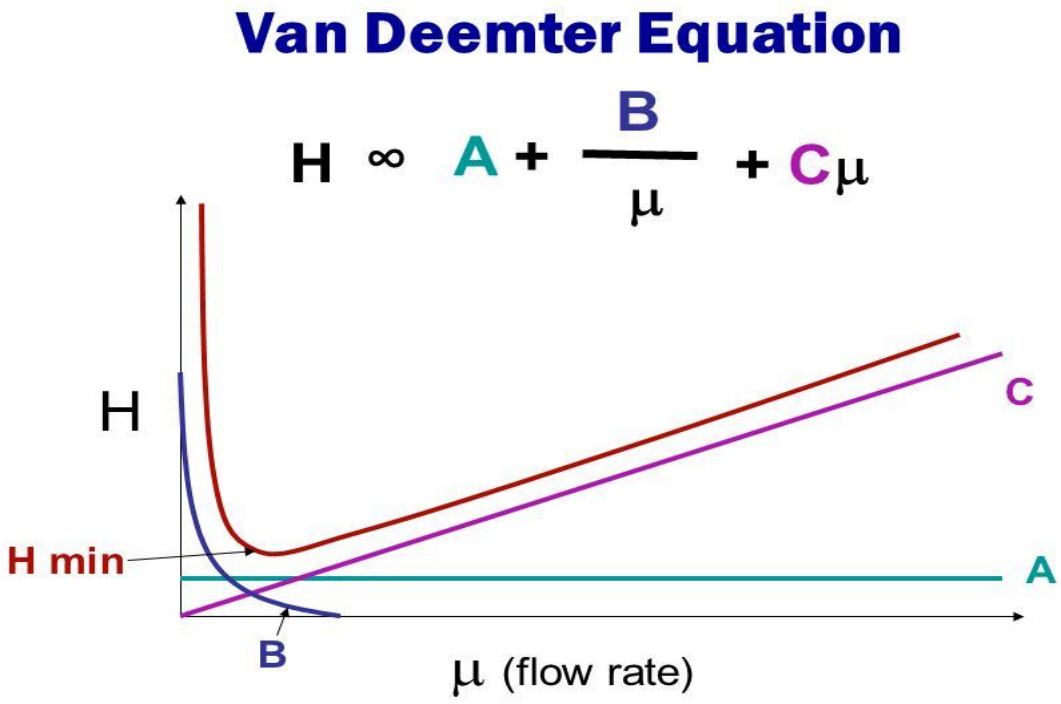
Topic Outcomes: At the end of topic you will

1. Explain Theory of GC

•In gas chromatography (GC), the sample is vaporized and injected onto the head of a chromatographic column. Elution is brought about by the flow of an inert gaseous mobile phase.

•The mobile phase does not interact with molecule of the analyte; its only function is to transport the analyte through the column.

•Gas-liquid chromatography is based upon the partition of the analyte between a gaseous mobile phase and a liquid phase immobilized on the surface of an inert solid.



H: HEIGHT EQUIVALENT TO THEORETICAL PLATE, A: EDDYS DIFFUSION, B: LONGITUDINAL MASS TRANSFER, C: RESISTANCE TO MASS TRANSFER, U: AVERAGE LINEAR VELOCITY

ADVANTAGES OF GAS CHROMATOGRAPHY

- Fast analysis (Typically minutes or even sec.)
- High Resolution
- Sensitive detectors (easy ppm, often ppb)
- Highly accurate quantification (1-5 % RSD)
- Automated systems
- Non-destructive
- Small sample (mL)
- Reliable and relatively simple
- Low cost (~€20,000)

Disadvantages of gas chromatography

- Limited to volatile samples, limited to ~ 380 °C, Need P_{vap} ~ 60 Torr at that temperature
- Not suitable for thermally labile samples
- Some samples may require extensive preparation
- Requires spectroscopy (usually MS) to confirm peak identify

References:

1. Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed
2. Practical Pharmaceutical Chemistry Part-I & II by Beckett A H & Stanlake J B, 4/Ed., CBS Publisher & Distributors.
3. Instrumental Methods of Analysis by Willard Merit, Dean Settle, 7th edition, CBS Publisher & Distributor
4. Instrumental Methods of Chemical Analysis by BK Sharma, Goel Publishing House.

Lecture 14

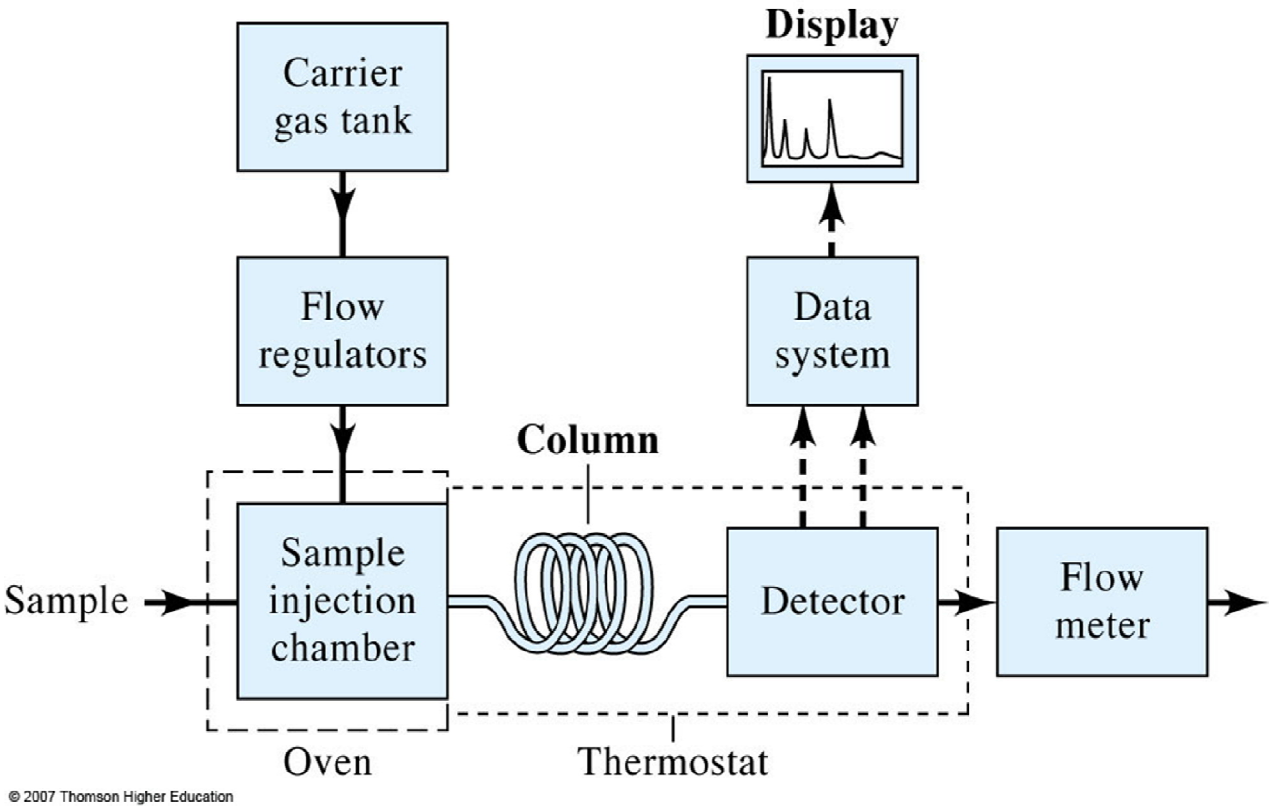
Name of topic/lesson – Gas Chromatography

Subtopic: Instrumentation

Objective: To study block diagram of GC

Topic Outcomes: At the end of topic you will

1. **Be able to explain the function of each part of GC**
2. **Draw block diagram of GC**



References:

1. Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed
2. Practical Pharmaceutical Chemistry Part-I & II by Beckett A H & Stanlake J B, 4/Ed., CBS Publisher & Distributors.
3. Instrumental Methods of Analysis by Willard Merit, Dean Settle, 7th edition, CBS Publisher & Distributor
4. Instrumental Methods of Chemical Analysis by BK Sharma, Goel Publishing House.

Lecture 15

Name of topic/lesson – Gas Chromatography

Subtopic: Sample handling, Carrier gases used in GC

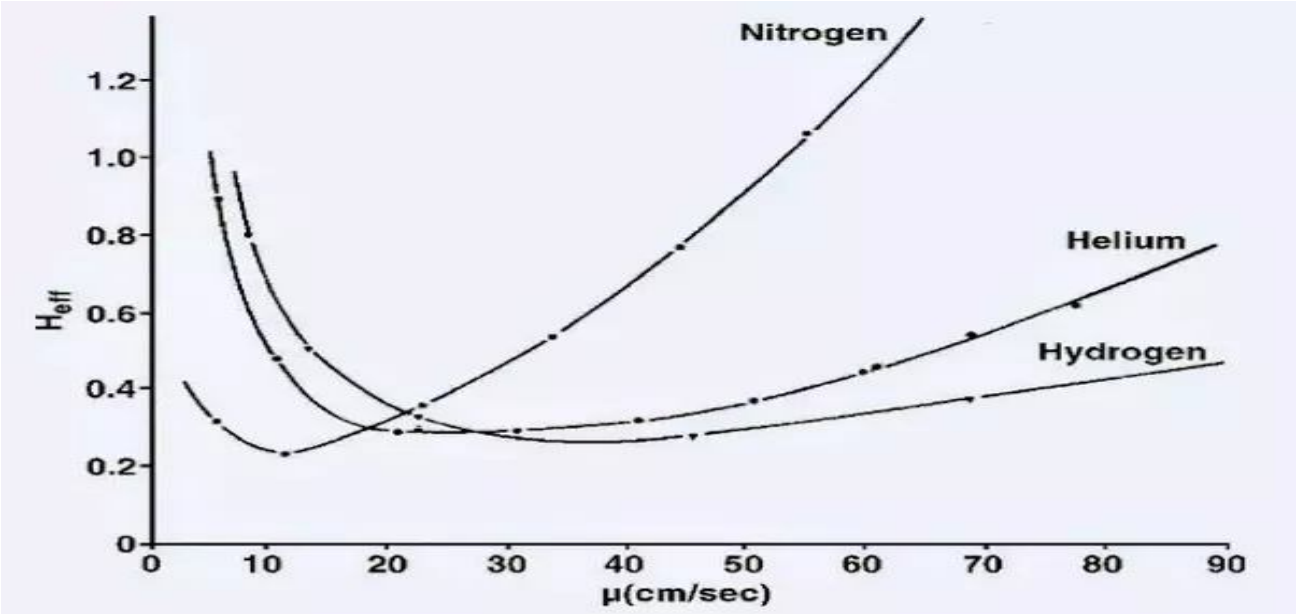
Objective: To study and compare Sample handling, Carrier gases used in GC

Topic Outcomes: At the end of topic you will

1. **Know various Sample handling Techniques**
2. **Understand advantages and disadvantages of various carrier gases used in GC**

Carrier gases, which must be chemically inert, include helium, nitrogen, carbon dioxide, helium and hydrogen (explosive).

Carrier gases are compressible gases that expand with increasing temperature. This results in a change in the gas viscosity. The selection and linear velocity of the carrier gas will affect resolution and retention times. Carrier gases should be inert to the stationary phase and free of detectable contaminants.



Safety concerns with nitrogen and helium are minimal. Both are compressed gases and can cause asphyxiation if rapidly released in a small confined area. Hydrogen is combustible over a concentration range of 4% to 74.2% by volume. Combustion can occur due to rapid expansion of the gas from a high pressure cylinder. Hydrogen is a highly diffusive gas in air. Hydrogen generators and EPC typically have automatic built-in shut down devices when a leak is detected.

SAMPLE HANDLING IN GAS CHROMATOGRAPHY

Column efficiency requires that the sample be of suitable size and be introduced as a "plug" of vapor; slow injection of oversized samples causes band spreading and poor resolution.

- **FLASH VAPORISER**
- **SAMPLE VALVE INJECTION**
- **SPLIT – SPLITLESS INJECTION**
- **GROBS INJECTOR**
- **HEAD SPACE ANALYSIS**
- **PURGE AND TRAP**
- **SOLID PHASE MICROEXTRACTION**
- **PYROLYSIS GAS CHROMATOGRAPHY (PGC)**
- **DIRECT THERMAL EXTRACTION**

References:

1. Practical Pharmaceutical Chemistry Part-I & II by Beckett A H & Stanlake J B, 4/Ed., CBS Publisher & Distributors.
2. Instrumental Methods of Analysis by Willard Merit, Dean Settle, 7th edition, CBS Publisher & Distributor
3. Instrumental Methods of Chemical Analysis by Munson.
3. https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/.../ 1/t411126h.pdf

Lecture 16

Name of topic/lesson – Gas Chromatography

Subtopic: Columns

Objective: To study various types of columns used in GC

Topic Outcomes: At the end of topic you will

1. **Know types of columns used in GC**

Types of columns

1. Capillary column
2. Megabore column
3. Packed column

TABLE 27-2 Properties and Characteristics of Typical GC Columns

	Type of Column			
	FSWC*	**WCOT†**	**SCOT‡**	**Packed**
Length, m	10–100	10–100	10–100	1–6
Inside diameter, mm	0.1–0.3	0.25–0.75	0.5	2–4
Efficiency, plates/m	2000–4000	1000–4000	600–1200	500–1000
Sample size, ng	10–75	10–1000	10–1000	$10–10^6$
Relative pressure	Low	Low	Low	High
Relative speed	Fast	Fast	Fast	Slow
Flexibility?	Yes	No	No	No
Chemical inertness	Best →			Poorest

*Fused silica, wall-coated open tubular column.

†Wall-coated open tubular metal, plastic, or glass columns.

‡Support-coated open tubular column (also called porous-layer open tubular, or PLOT).

References: 1. Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed

Lecture 17

Name of topic/lesson – Gas Chromatography

Subtopic: Supports and stationary phases

Objective: To study various types of Supports and stationary phases

Topic Outcomes: At the end of topic you will

1. **Know and select Supports and stationary phases for GC analysis**

The most widely used **support** material is prepared from naturally occurring **diatomaceous earth,** which is made up of the skeletons of thousands of species of single-celled plants (diatoms) that inhabited ancient lakes and seas. Such plants received their nutrients and disposed of their wastes via molecular diffusion through their pores. As a consequence, their remains are well-suited as support materials because gas chromatography is also based upon the same kind of molecular diffusion.

TABLE 27-3 Some Common Liquid Stationary Phases for GLC

Stationary Phase	Common Trade Name	Maximum Temperature, °C	Common Applications
Polydimethyl siloxane	OV-1, SE-30	350	General-purpose nonpolar phase, hydrocarbons, polynuclear aromatics, steroids, PCBs
5% Phenyl-polydimethyl siloxane	OV-3, SE-52	350	Fatty acid methyl esters, alkaloids, drugs, halogenated compounds
50% Phenyl-polydimethyl siloxane	OV-17	250	Drugs, steroids, pesticides, glycols
50% Trifluoropropyl-polydimethyl siloxane	OV-210	200	Chlorinated aromatics, nitroaromatics, alkyl substituted benzenes
Polyethylene glycol	Carbowax 20M	250	Free acids, alcohols, ethers, essential oils, glycols
50% Cyanopropyl-polydimethyl siloxane	OV-275	240	Polyunsaturated fatty acids, rosin acids, free acids, alcohols

References:

1. Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed
2. Instrumental Methods of Analysis by Willard Merit, Dean Settle, 7th edition, CBS Publisher & Distributor

Lecture 18

Name of topic/lesson – Gas Chromatography

Subtopic: Detectors

Objective: To study ideal characteristic of detectors

Topic Outcomes: At the end of topic you will

1. **Know ideal characteristic of detectors**

Characteristics of the Ideal Detector: The ideal detector for gas chromatography has the following characteristics:

1. Adequate sensitivity
2. Good stability and reproducibility.
3. A linear response to solutes that extends over several orders of magnitude.
4. A temperature range from room temperature to at least 400^{o}C.
5. A short response time that is independent of flow rate.
6. High reliability and ease of use.
7. Similarity in response toward all solutes or a highly selective response toward one or more classes of solutes.
8. Nondestructive of sample.

No one detector exhibits all of these characteristics

References:

1. Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed

Lecture 19

Name of topic/lesson – Gas Chromatography

Subtopic: Detectors

Objective: To study various types of detectors used in GC

Topic Outcomes: At the end of topic you will

1. **Be able to select Detector depending upon the application**

TABLE 27-1 Typical Gas Chromatographic Detectors

Type	Applicable Samples	Typical Detection Limit
Flame ionization	Hydrocarbons	1 pg/s
Thermal conductivity	Universal detector	500 pg/mL
Electron capture	Halogenated compounds	5 fg/s
Mass spectrometer (MS)	Tunable for any species	0.25 to 100 pg
Thermionic	Nitrogen and phosphorous compounds	0.1 pg/s (P), 1 pg/s (N)
Electrolytic conductivity (Hall)	Compounds containing halogens, sulfur, or nitrogen	0.5 pg Cl/s, 2 pg S/s, 4 pg N/s
Photoionization	Compounds ionized by UV radiation	2 pg C/s
Fourier transform IR (FTIR)	Organic compounds	0.2 to 40 ng

References:

1. Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed

Lecture 20

Name of topic/lesson – Gas Chromatography
Subtopic: Derivatisation techniques
Objective: To study Derivatisation techniques used in GC
Topic Outcomes: At the end of topic you will

1. **Understand need and types of Derivatisation techniques in GC**

Derivatization reactions are meant to transform an analyte for detectability in Gas Chromatography (GC) or other instrumental analytical methods. Derivatization in GC analysis can be defined as a procedural technique that primarily modifies an analyte's functionality in order to enable chromatographic separations. A modified analyte in this case will be the product, which is known as the derivative. Volatility of sample is a requirement for GC analysis. Derivatization will render highly polar materials to be sufficiently volatile so that they can be eluted at reasonable temperatures without thermal decomposition or molecular re-arrangement

For GC analysis, compounds containing functional groups with active hydrogens such as -SH, -OH, -NH and -COOH are of primary concern because of the tendency of these functional groups to form intermolecular hydrogen bonds. These intermolecular hydrogen bonds affect the inherent volatility of compounds containing them, their tendency to interact with column packing materials and their thermal stability. Derivatization is aimed towards:

i. **Suitability:** is the form of compounds that is amenable to the analytical technique. For GC, it is a requirement that the compound to be analyzed should be volatile with respect to gas chromatographic analysis conditions, as compared to liquid chromatography (LC), where the compound of interest should be soluble in the mobile phase. Therefore, derivatization procedure modifies the chemical structure of the compounds so that they can be analyzed by the desired technique.

ii. **Efficiency** is the ability of the compound of interest to produce good peak resolution and symmetry for easy identification and practicability in GC analysis. Interactions between the compounds themselves or between the compounds and the GC column may reduce the separation efficiency of many compounds s and mixtures. Derivatization of analyte molecules can reduce these interactions that interfere with analysis. Also, compounds that co-elute or have poor resolution from other sample components during separation in GC can frequently be resolved by an appropriate derivative.

iii. **Detectability** is the outcome signal that emanates from the interaction between the analyte and the GC detector. Increasing the amounts of materials will impact the range at which they can be detected in Gas chromatography. This can be achieved either by increasing the bulk of the compound or by introducing onto the analyte compound, atoms or functional groups that interact strongly with the detector and hence improve signal identification.

References:

1. Instrumental Methods of Analysis by Willard Merit, Dean Settle, 7th edition, CBS Publisher & Distributor

2.Instrumental Methods of Chemical Analysis by BK Sharma, Goel Publishing House.

Lecture 21

Name of topic/lesson – Gas Chromatography

Subtopic: Quantitation techniques

Objective: To study Quantitation techniques in GC

Topic Outcomes: At the end of topic you will

1. **Know Quantitation techniques in GC**

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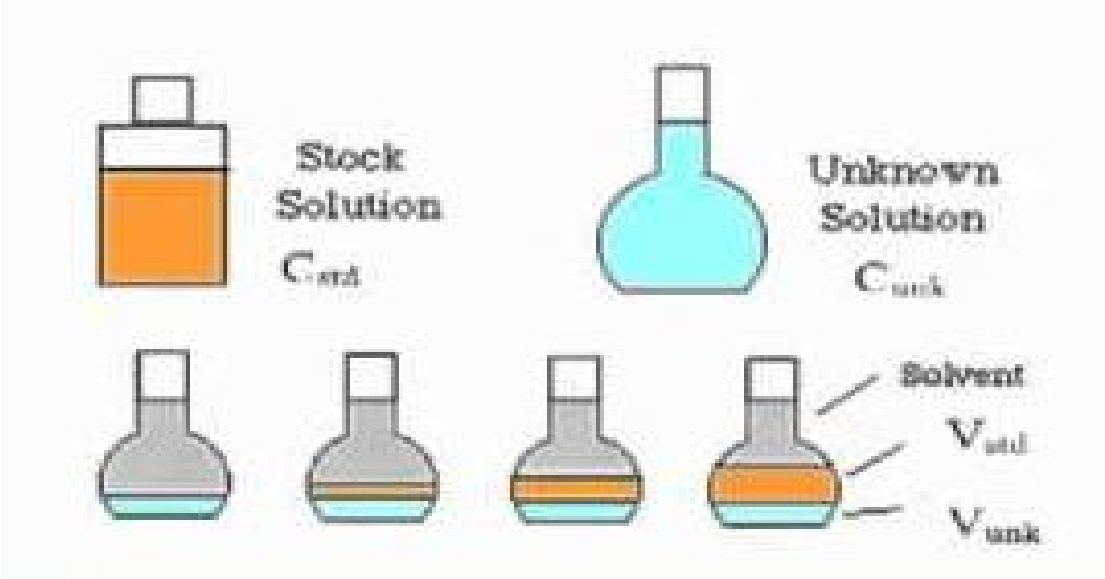
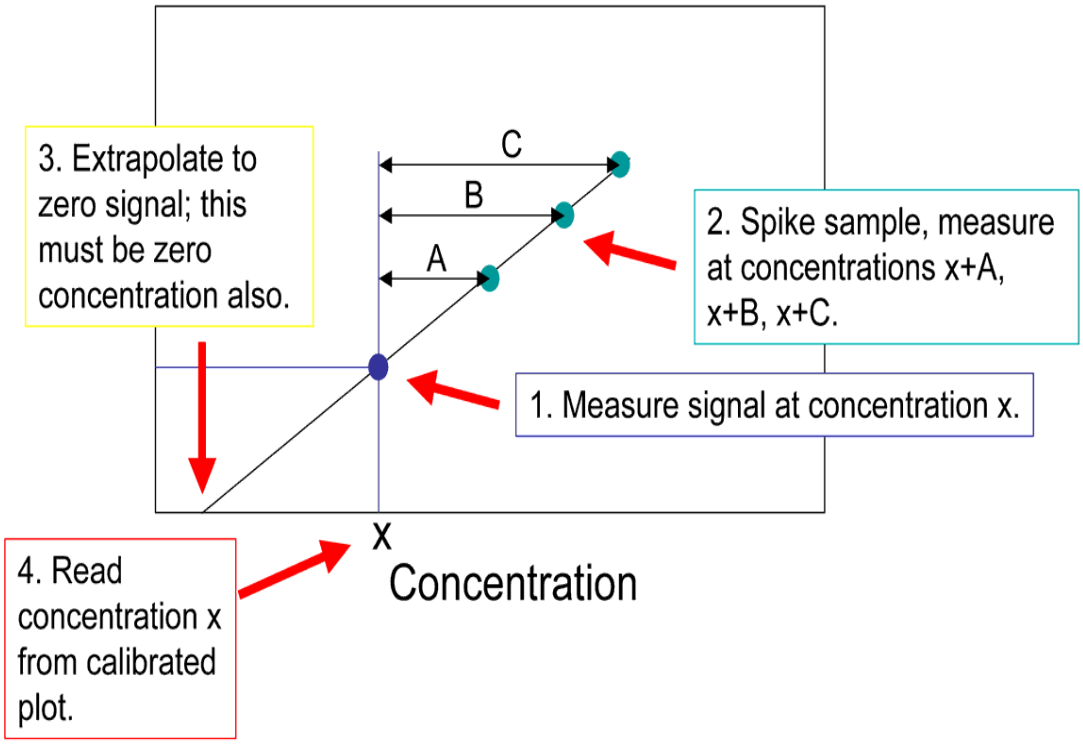
1. **Standard addition method**
2. **External standard method**
3. **Internal standard method**
4. **Area normalisation method**

Standard addition: If only a few samples are to be chromatographed, it is possible to employ the method of standard addition. The chromatogram of the unknown is recorded. Then a known amount of the analyte(s) is added, and the chromatogram is repeated using the same reagents, instrument parameters, and procedures. From the increase in the peak area (or peak height), the original concentration can be computed by interpolation. The **detector response** must be a linear function of analyte concentration and yield no signal (other than background) at zero concentration of the analyte. Sufficient time must elapse between addition of the standard and actual analysis to allow equilibrium of added standard with any matrix interferant.

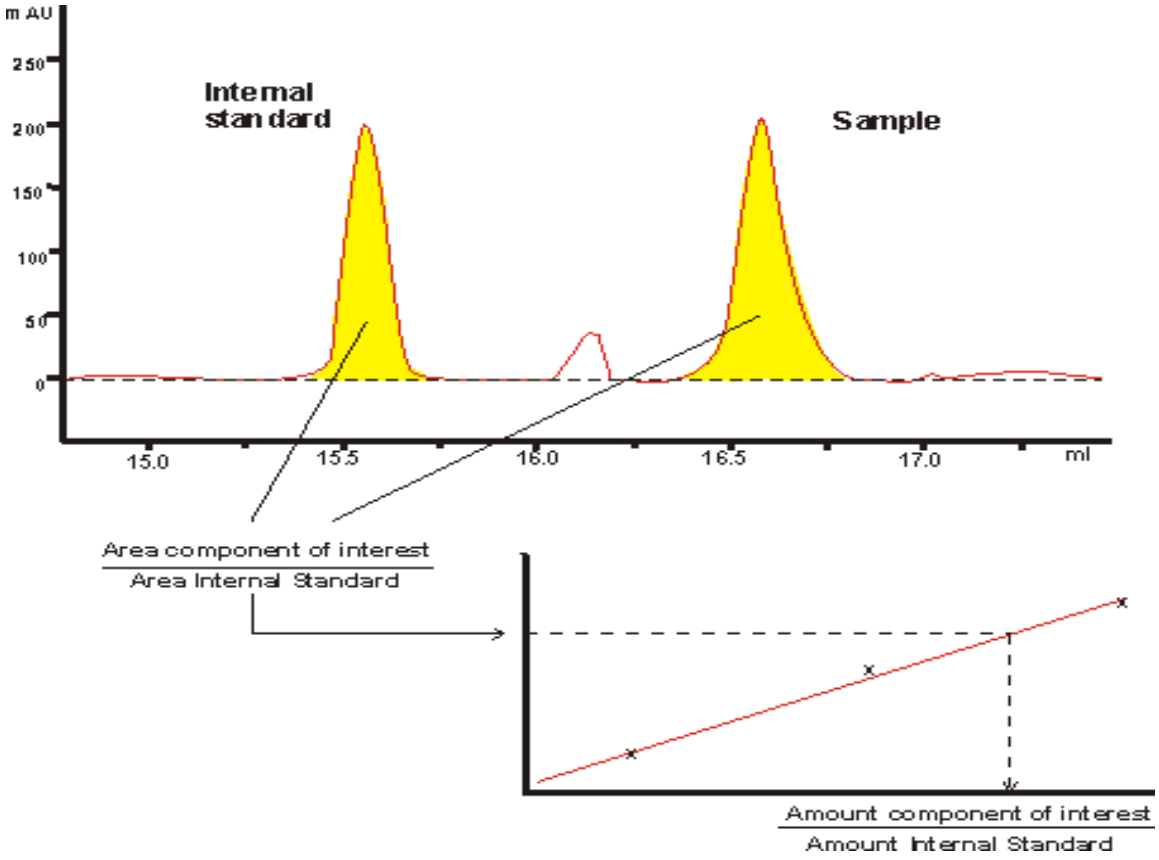




An internal standard is a standard whose identity is different from the analyte's, that is added to all samples and standards (calibrants) containing the analyte. Since the **analyte** and **internal standard** in any sample or standard receive the same treatment, the ratio of their signals will be unaffected by any lack of reproducibility in the procedure. With this method, an equal amount of an **internal standard (IS)** is added to both the sample and calibrator solutions. The IS selected should be chemically similar to the analyte and have a similar retention time and similar derivatization. It is also important to ensure that the IS is stable and does not interfere with any of the sample components. The IS should be added before any preparation of the sample so that extraction efficiency can be evaluated. **Quantitation** is achieved by using **ratios of peak areas** of the component to the **internal standard.**

Uncertainties in sample injection can be overcome by use of an internal standard. Any inconsistency in injection of the sample will affect both the analyte and internal standard. The retention times of internal standard and analyte should be different and the two peaks

must be well separated, R >1.25.The detector response factor for the analyte and the internal standard should be the same.Using internal standards can significantly improve precision to better than 1%.



An external standard method

The most common method of standardization uses one or more external standards, each containing a known concentration of analyte. We call them “external” because we prepare and analyze the standards separate from the samples.

1. **Single-Point Standardization**

The simplest way to determine the value of kA in equation by a single-point standardization in which we measure the signal for a standard, Sstd, containing a known concentration of analyte, Cstd. Substituting these values into equation

Ka= Sstd/ Cstd. gives the value for kA.

Having determined the value for kA, we can calculate the concentration of analyte in any sample by measuring its signal, Ssamp, and calculating Cstd using equation. CA= Ssamp/kA.

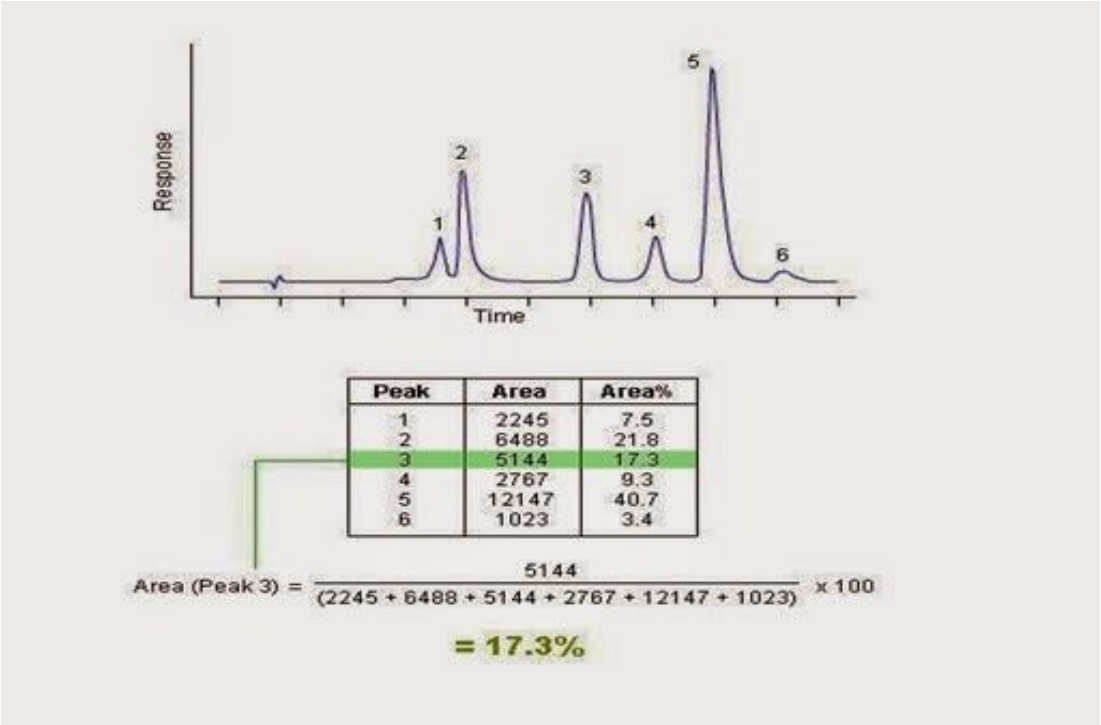
A single-point standardization is the least desirable method for standardizing a method. There are at least two reasons for this. First, any error in our determination of kA carries over into our calculation of CA. Second, our experimental value for kA is for a single concentration of analyte. Extending this value of kA to other concentrations of analyte requires us to assume a linear relationship between the signal and the analyte's concentration, an assumption that often is not true.

2. **Double point standardization**
3. **Multiple-Point Standardizations**

The preferred approach to standardizing a method is to prepare a series of standards, each containing the analyte at a different concentration. Standards are chosen such that they bracket the expected range for the analyte's concentration. A multiple-point standardization should include at least three standards, although more are preferable. A plot of Sstd versus Cstd is known as a calibration curve. The exact standardization, or calibration relationship is determined by an appropriate curve-fitting algorithm. There are at least two advantages to a multiple-point standardization. First, although a determinate error in one standard introduces a determinate error into the analysis, its effect is minimized by the remaining standards. Second, by measuring the signal for several concentrations of analyte we no longer must assume that the value of kA is independent of the analyte's concentration. Constructing a calibration curve similar to the "actual relationship" in Figure is possible.

Area normalization method

The normalization method is the easiest and most straightforward and requires no reference standards or calibration solutions to be prepared. However, the detector must have the same response to all the components of the sample. In GC the response of the flame ionization detector (FID) depends largely on the carbon content of the solute. Thus, the technique can be used in GC when employing the FID sensing compounds of similar types (e.g. high molecular weight paraffins). An exceptional example in LC, where the normalization procedure is often used, is in the analysis of polymers by exclusion chromatography using the refractive index detector.



References

1. Instrumental Methods of Analysis by Willard Merit, Dean Settle, 7th edition, CBS Publisher & Distributor
2. Instrumental Methods of Chemical Analysis by BK Sharma, Goel Publishing House.

Lecture 22

Name of topic/lesson – Gas Chromatography

Subtopic: Applications of GC

Objective: To study Applications of GC

Topic Outcomes: At the end of topic you will

1. **Know Applications of GC**

Applications

- **Identification of the oil** elements by GC/MS
- Skin samples analysis
- **Residual solvent analysis** (methanol, dichloromethane, acetonitrile, chloroform, toluene, isopropanol, dioxin)
- Freon and freon substitute impurities (Q.C. of propellants)
- An interesting combination of GC with another spectral technique is GC/FT-IR. Infra red spectroscopy is widely used as a routine **control in chemical synthesis**. Both at the chemical production plant, as well as during the manufacture of the final drug product, both drug substances and excipients are routinely checked by infra re d spectroscopy
- **Pharmacokinetic Assay:** Occasionally, gas chromatography coupled to mass spectrometry can still be found useful for pharmacokinetic assays, when the drug substance and major metabolites are both sufficiently volatile.

Environmental monitoring

- Food, beverage, flavor and fragrance analysis
- Forensic and criminal cases
- Biological and pesticides detections
- Security and chemical warfare agent detection
- Astro chemistry and Geo chemical Research
- RNA isolation

References: 1. Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed

2. Instrumental Methods of Chemical Analysis by BK Sharma, Goel Publishing House.

Lecture 23

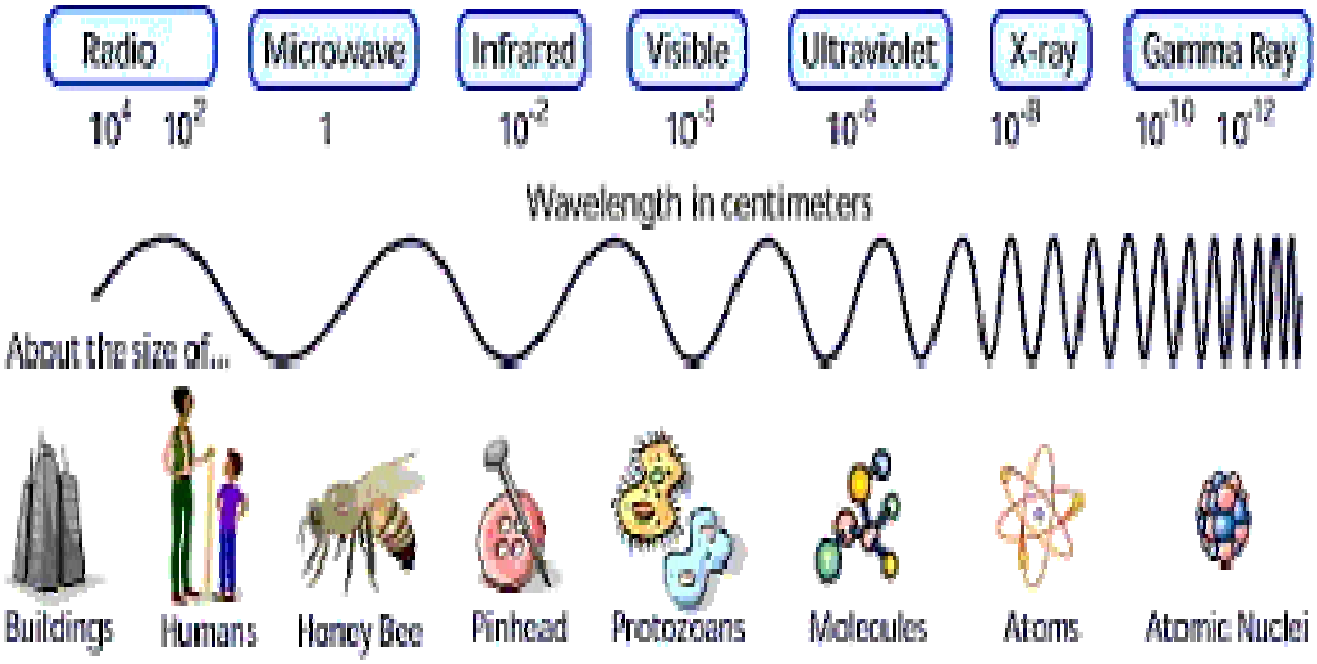
Name of topic/lesson – TO REVISE AND DISCUSS EMR SPECTRA

Subtopic: Origin of IR spectra
Objective: To understand origin of IR spectra, Molecular Vibrations

Topic Outcomes: At the end of topic you will

1. **Identify the problem in chromatogram with reason**
2. **Be able to suggest the remedies for chromatographic problems**

EMR SPECTRA

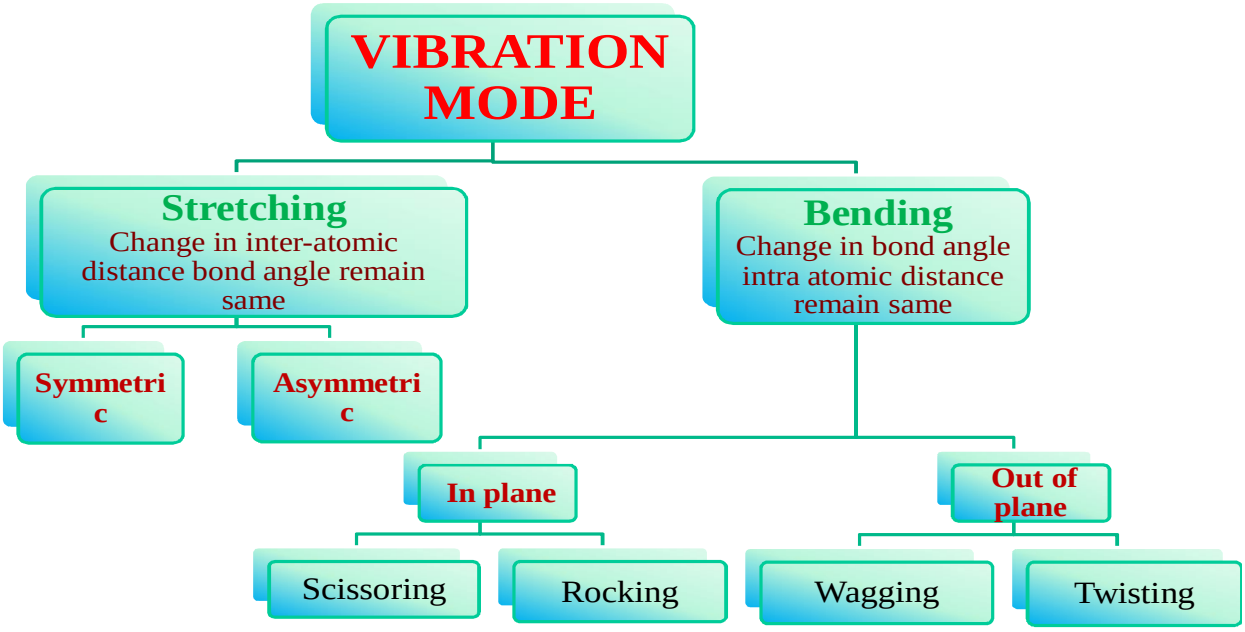


What is Vibration?

Any change in **shape of the molecule- stretching of bonds, bending of bonds,** or internal rotation around single bonds

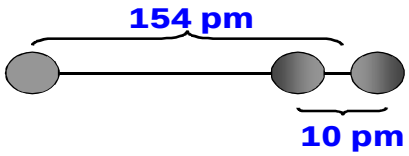
Asymmetrical stretching/bending and internal rotation **change the dipole moment of a molecule**. Asymmetrical stretching/bending are **IR active**.

Symmetrical stretching/bending **does not change the dipole moment of a molecule.** Not IR active



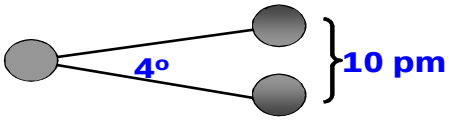
How much movement occurs in the vibration of a C-C bond?

stretching vibration



For a C-C bond with a bond length of 154 pm, the variation is about 10 pm.

bending vibration



For C-C-C bond angle a change of 4° is typical. This moves a carbon atom about 10 pm.

References:

1. Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed
2. Instrumental Methods of Analysis by Willard Merit, Dean Settle, 7th edition, CBS Publisher & Distributor
3. Instrumental Methods of Chemical Analysis by BK Sharma, Goel Publishing House.

Lecture 24

Name of topic/lesson – IR Spectroscopy

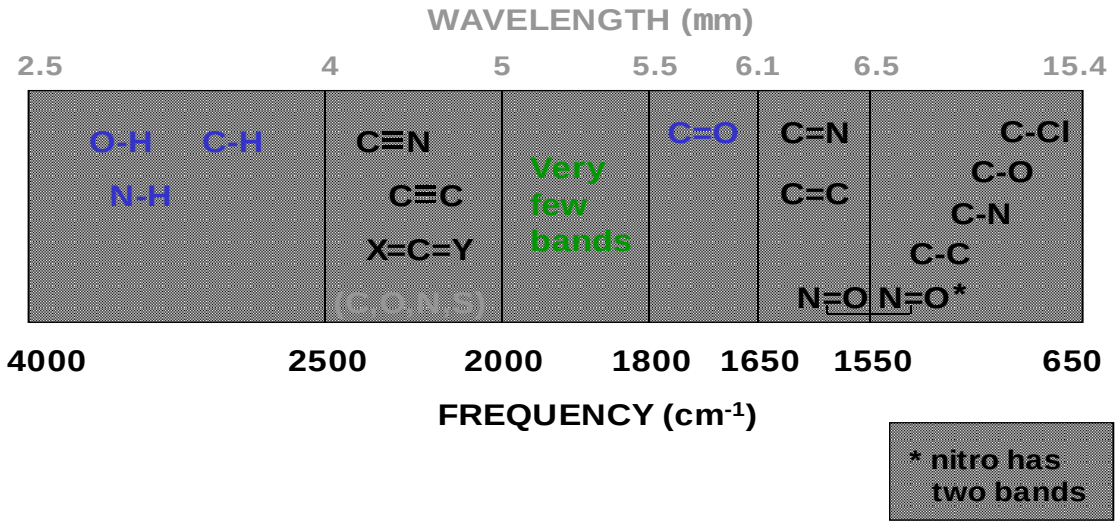
Subtopic: Fundamental bands, Important spectral regions

Objective: To understand origin of IR spectra, Molecular Vibrations

Topic Outcomes: At the end of topic you will

1. **Know IR spectral regions and functional group analysis based on it**

Typical Infrared Absorption Regions
(stretching vibrations)



POSITION	**REDUCED MASS** **BOND STRENGTH (STIFFNESS)**	**LIGHT ATOMS HIGH FREQUENCY** **STRONG BONDS HIGH FREQUENCY**
STRENGTH	**CHANGE IN 'POLARITY'**	**STRONGLY POLAR BONDS GIVE INTENSE BANDS**
WIDTH	**HYDROGEN BONDING**	**STRONG HYDROGEN BONDING GIVES BROAD BANDS**

References:

1. Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed
2. Instrumental Methods of Analysis by Willard Merit, Dean Settle, 7th edition, CBS Publisher & Distributor
3. Instrumental Methods of Chemical Analysis by BK Sharma, Goel Publishing House.

Lecture 25

Name of topic/lesson – IR Spectroscopy

Subtopic: Vibrational frequency and Factors affecting it

Objective: To Study Factors Affecting Vibrational Frequency.

Topic Outcomes: At the end of topic you will

1.Identify the factor which has affected vibrational frequency

---

Vibration freq. calculated by hooks low is never same in practical spectrum. Diff due to the factor influence due to structure of neighboring atom Force constant of bond changes with electronic structure Factor

- **ELECTRONIC EFFECTS**
- **COUPLED VIBRATION/ VIBRATIONAL COUPLING**
- **FERMI RESONANCE**
- **HYDROGEN BONDING**
- **BOND ANGLE/ RING SIZE**
- **ATOMIC MASS**
- **PHYSICAL STATE OF COMPOUND DURING MEASUREMENT**
- **HYDRIDIZATION**
- **CONJUGATION**
- **FIELD EFFECT / STEARIC FACTOR**

THE EQUATION OF A SIMPLE HARMONIC OSCILLATOR

$$\bar{\nu} = \frac{1}{2\pi c}\sqrt{\frac{K}{\mu}}$$

where

$$\mu = \frac{m_1 m_2}{m_1 + m_2}$$

This equation describes the vibrations of a bond.

$\bar{\nu}$ = frequency in cm^{-1}

c = velocity of light (3×10^{10} cm/sec)

K = force constant in dynes/cm

C≡C > C=C > C—C
multiple bonds have higher K's

m = atomic masses

μ = reduced mass

Reference: 1. Organic Spectroscopy by P.S.Kalsi

Lecture 26

Name of topic/lesson – IR Spectroscopy

Subtopic: Vibrational frequency and Factors affecting it

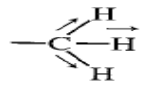
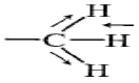
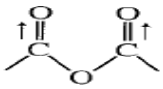
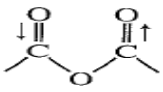
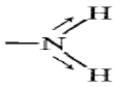
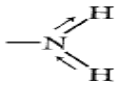
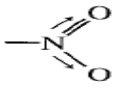
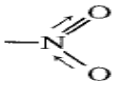
Objective: TO STUDY FACTORS AFFECTING VIBRATIONAL FREQUENCY.

Topic Outcomes: At the end of topic you will

1. **Identify the factor which has affected vibrational frequency**

Vibrational coupling

- Interaction between vibrations can occur (*coupling*) if the vibrating bonds are joined to a single, central atom. Vibrational coupling is influenced by a number of factors.
- Strong coupling of stretching vibrations occurs when there is a common atom between the two vibrating bonds
- Coupling of bending vibrations occurs when there is a common bond between vibrating groups
- Coupling between a stretching vibration and a bending vibration occurs if the stretching bond is one side of an angle varied by bending vibration
- Coupling is greatest when the **coupled groups have approximately equal energies**
- No coupling is seen between groups separated by two or more bonds

	Symmetric Stretch	Asymmetric Stretch
Methyl	 ~2872 cm^{-1}	 ~2962 cm^{-1}
Anhydride	 ~1760 cm^{-1}	 ~1800 cm^{-1}
Amino	 ~3300 cm^{-1}	 ~3400 cm^{-1}
Nitro	 ~1350 cm^{-1}	 ~1550 cm^{-1}

-CH2-	3000	2900
-SO2-	1350	1150
COO-	1600	1400

Reference: 1. Organic Spectroscopy by P.S.Kalsi

Lecture 27

Name of topic/lesson – IR Spectroscopy

Subtopic: Vibrational frequency and Factors affecting it

Objective: To Study Factors Affecting Vibrational Frequency.

Topic Outcomes: At the end of topic you will

1. **Identify the factor which has affected vibrational frequency**

Overtone:

- Excitation from ground state to higher energy state which is correspond to integral multiple of frequency of fundamental vibration
- The transitions from v_0 TO v_2 and v_0 TO v_3 are the first and second overtones of the fundamental and require radiation of twice and thrice times its frequency.
- For eg. The first overtone for the carbonyl fundamental at 1700 cm^{-1} will be 3400 cm^{-1}.
- Most overtones are found in the near infrared region beyond 4000 $cm^{-1.}$
- Such absorptions are much weaker.
- The intensity of overtone decreases as the order of the overtone increases.
- Aromatic compounds exhibit overtone absorptions in 2000 – 1667 cm^{-1}region which are characteristic of the aromatic substitution.

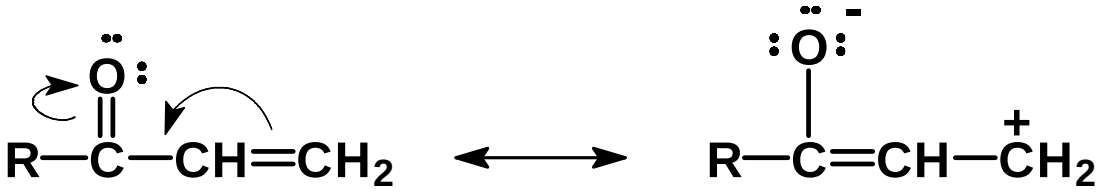
Fermi resonance

- 1st study by Enrico Fermi
- When an overtone or combination band falls near a strong fundamental vibration, it causes a decrease in the intensity of the fundamental vibration and a large increase in the intensity of the overtone or combination vibration
- Eg. Co2
- Fundamental vibration =4

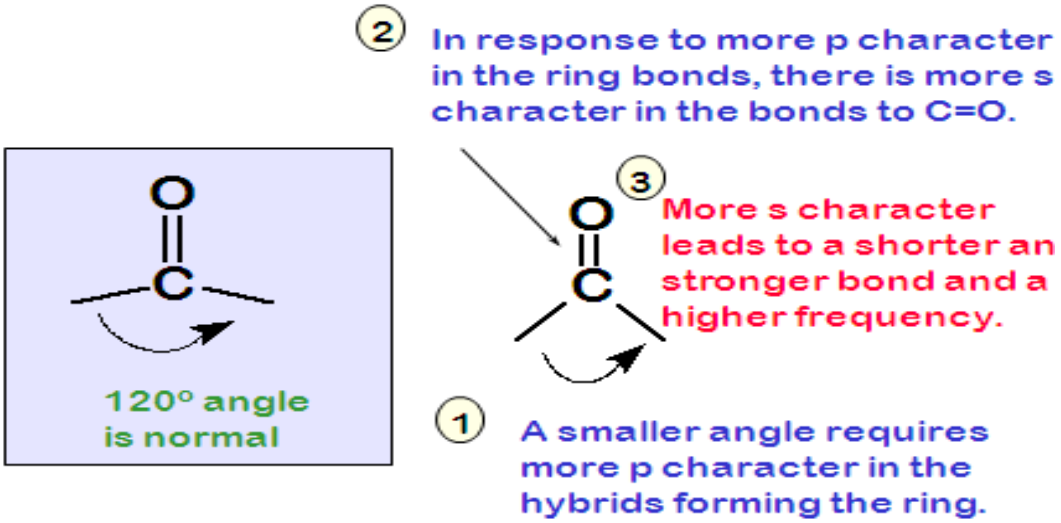
2 stretching	2 bending
Asymmetric stretching	both (667.3)
(1337)	667.3× 2 = 1334.6

2 band at 1285.5 and 1388.3 cm-1

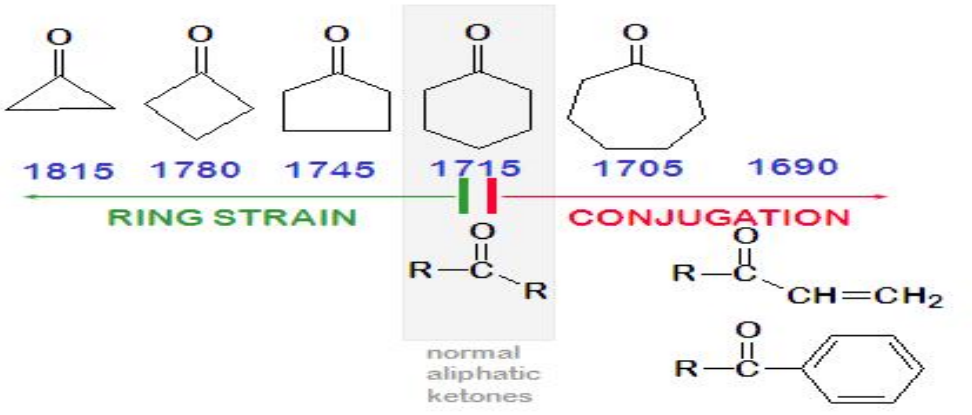
Conjugation: Conjugation weaken the double bond. Hence, reduces the absorption frequency.



ANGLE STRAIN RAISES THE CARBONYL FREQUENCY



CONJUGATION AND RING SIZE EFFECTS



Reference: Organic Spectroscopy by P.S.Kalsi

Lecture 28

Name of topic/lesson – IR Spectroscopy

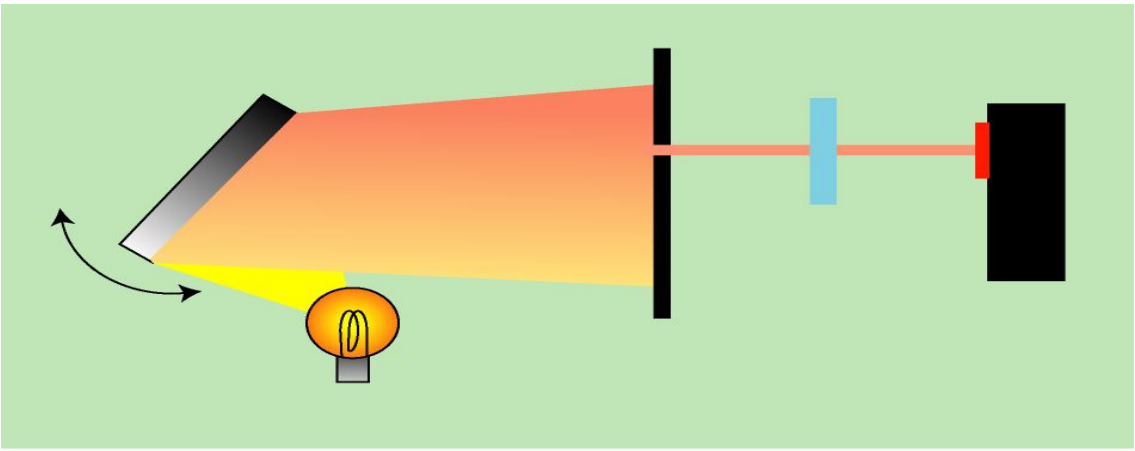
Subtopic: Instrumentation

Objective: To Study Instrumentation (Comparison Between Ftir And Dispersive Spectroscopy)

Topic Outcomes: At the end of topic you will

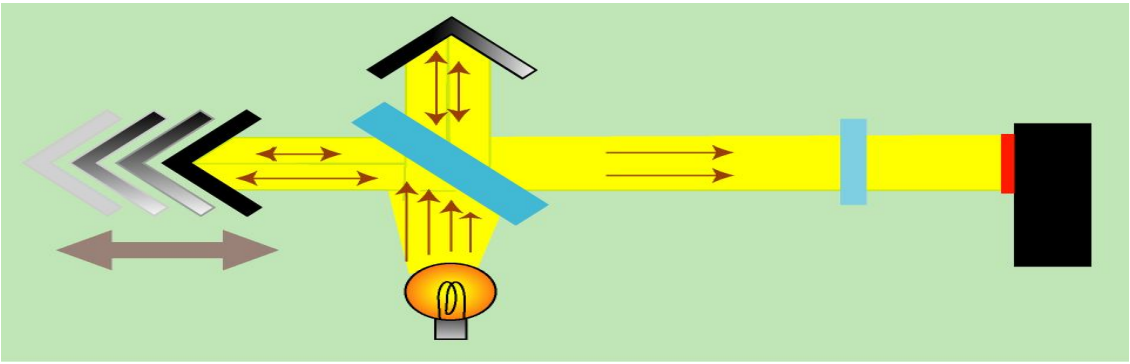
1. **Know the advantages of FT-IR over Dispersive IR**

Dispersion Spectrometer



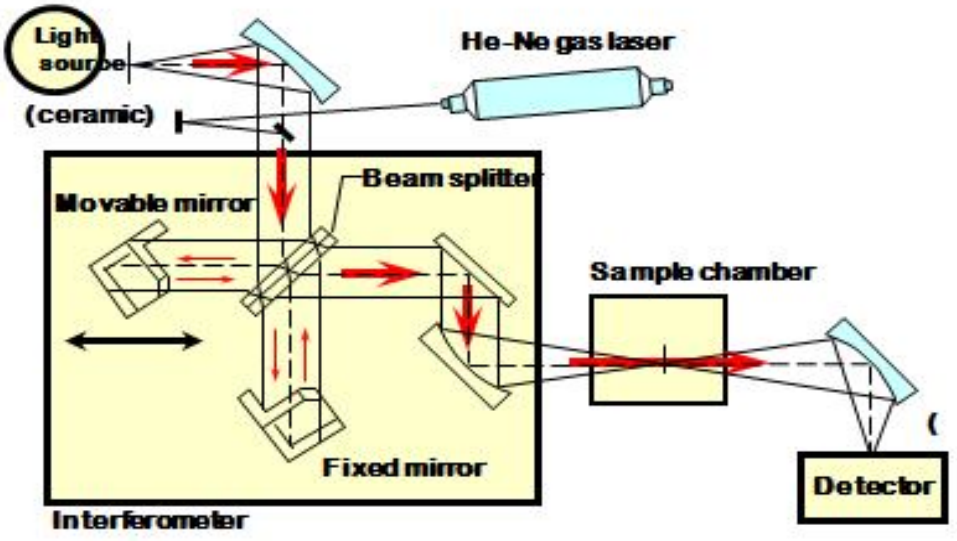
In order to measure an IR spectrum, the dispersion Spectrometer takes several minutes. Also the detector receives only a few % of the energy of original light source.

FT-IR



In order to measure an IR spectrum, FTIR takes only a few seconds. Moreover, the detector receives up to 50% of the energy of original light source.(much larger than the dispersion spectrometer.)

FT Optical System Diagram



ADVNTAGES OF FT-IR

- **FELLGETT'S (MULTIPLEX) ADVANTAGE**
- **CONNES ADVANTAGE**
- **JACQUINOT ADVANTAGE**

Source	Composition	Temp.	Range	Other Characteristics
Nernst Glower	Rare earth oxides, Yttrium, thorium, zirconium(Cylinder with 1-2mm diameter & 20mm length)	1200 -2200 K	Mid IR	Negative temp. coefficient, requires external heating
Globar cell	Silicon carbide rod (5 mm in dia, 50mm length)	1300-1500 K	Mid IR	Positive coefficient of resistance, water cooling req.
Incandescent wire lamp	Nichrome / Rhodium	1100 K	Mid IR	Longer life
Mercury arc	Quartz jacketed tube containing mercury vapour at a pressure greater than one atm.		Far IR	Internal plasma formed, produces continuum radiation.
Tungsten filament lamp	A tungsten filament heated to incandescence by an electric current. Sometimes small amounts of a halogen, such as iodine, are added to improve the int ensity (tungsten-halogen lamp)	2,000 to 3,300 K	Near IR	The glass bulb enclosing the filament contains a low pressure of inert gas, usually argon.
Carbon dioxide laser source			Mid IR	

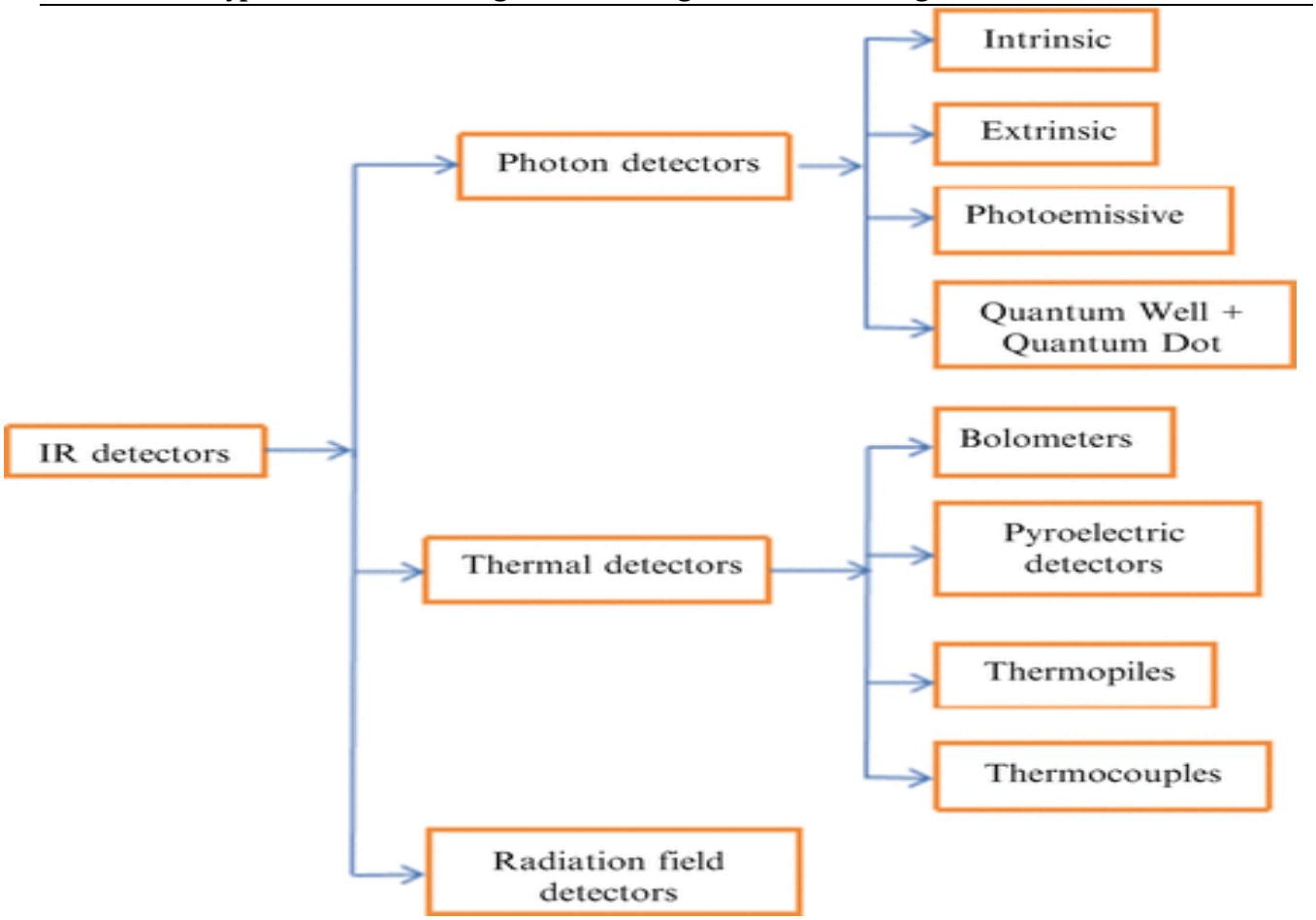
References

1. Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed

Lecture synopsis Sub: Pharmaceutical Analysis V Subject I/C: Dr. Tambe V.S.

Lecture 29

Name of topic/lesson – IR Spectroscopy
Subtopic: Instrumentation **(Detectors)**
Objective: To study classification of detectors
Topic Outcomes: At the end of topic you will
1. **Know the types of detectors along with advantages and disadvantages**



Detectors used in IR

1) PYROELECTRIC
2) PHOTOCONDUCTING
3) THERMAL TRANSDUCER
   1. Thermocouple 2. Bolometer 3. Thermistor 4. Golay cell

References :

1. Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed
2. Practical Pharmaceutical Chemistry Part-I & II by Beckett A H & Stanlake J B, 4/Ed., CBS Publisher & Distributors.
3. Instrumental Methods of Analysis by Willard Merit, Dean Settle, 7th edition, CBS Publisher & Distributor
4. Instrumental Methods of Chemical Analysis by BK Sharma, Goel Publishing House.

Lecture 30
Name of topic/lesson – IR Spectroscopy
Subtopic: Sample handling
Objective: To study various types of sample handling techniques used in IR
Topic Outcomes: At the end of topic you will
1. Know Sample Preparation Techniques used in IR Spectroscopy

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TYPES OF SAMPLES

1. **Gases**
2. **Liquids (Liquid film techniques, solution technique)**
3. **Solid samples (KBR pelleting, Mull Techniques, Thin film technique, solution technique)**

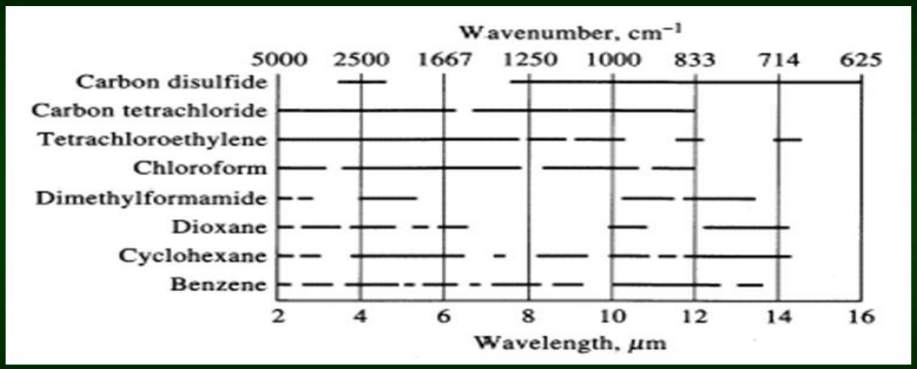
Advanced technique

1. **ATTENEUATED TOTAL REFECTANCE (ATR)**
2. **FT-IR MICROSCOPY**
3. **PHOTOACOUSTIC SPECTROSCOPY**

Materials that transmit IR

Calcium Fluoride (CaF_2)	Potassium Bromide (KBr)
Fused Silica (FS)	Sapphire
Germanium (Ge)	Silicon (Si)
Magnesium Fluoride (MgF_2)	Sodium Chloride (NaCl)
N-BK7	Zinc Selenide (ZnSe)
	Zinc Sulfide (ZnS)

Regions of transparency for common infrared solvents.



The horizontal lines indicate regions where solvent transmits at least 25% of the incident radiation in a 1-mm cell.

References Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed

Lecture 31

Name of topic/lesson – IR Spectroscopy

Subtopic: Sample handling

Objective: To understand various sample handling techniques used in IR

Topic Outcomes: At the end of topic you will

1. Know sampling requirement and Be able to select appropriate sampling techniques of IR

IR spectroscopy is used for the characterization of solid, liquid or gas samples. Material containing sample must be transparent to the IR radiation. So, the salts like NaCl, KBr are only used.

1. Sampling of solids

Various techniques used for preparing solid samples are as follows

a) **Mull technique:** In this technique, the finely crushed sample is mixed with Nujol (mulling agent) in n a marble or agate mortar, with a pestle to make a thick paste. A thin film is applied onto the salt plates. This is then mounted in a path of IR beam and the spectrum is recorded.

b) **Solid run in Solution –** In this technique, solid sample may be dissolved in a non-aqueous solvent provided that there is no chemical interaction with the solvent and the solvent is not absorbed in the range to be studied. A drop of solution is placed on the surface of alkali metal disc and solvent is evaporated to dryness leaving a thin film of the solute.

c) **Case film technique –** If the solid is amorphous in nature then the sample is deposited on the surface of a KBr or NaCl cell by evaporation of a solution of the solid and ensured that the film is not too thick to pass the radiation.

d) **Pressed pellet technique –** In this technique, a small amount of finely ground solid sample is mixed with 100 times its weight of potassium bromide and compressed into a thin transparent pellet using a hydraulic press. These pellets are transparent to IR radiation and it is used for analysis.

2. Sampling of liquids

Liquid sample cells can be sandwiched using liquid sample cells of highly purified alkali halides, normally NaCl. Other salts such as KBr and CaF_2can also be used. Aqueous solvents cannot be used because they cannot dissolve alkali halides. Organic solvents like chloroform can be used. The sample thickness should be selected so that the transmittance lies between 15-20%. For most liquids, the sample cell thickness is 0.01-0.05 mm. Some salt plates are highly soluble in water, so the sample and washing reagents must be anhydrous

3. Sampling of gases

The sample cell is made up of NaCl, KBr etc. and it is similar to the liquid sample cell. A sample cell with a long path length (5 – 10 cm) is needed because the gases show relatively weak absorbance.

Reference: Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed

Lecture 32

Name of topic/lesson – IR Spectroscopy

Subtopic: Different attachments used in recording FTIR, ATR

Objective: To study Different attachments used in recording FTIR, ATR

Topic Outcomes: At the end of topic you will
1. Know sampling requirement and Be able to select appropriate sampling techniques of IR

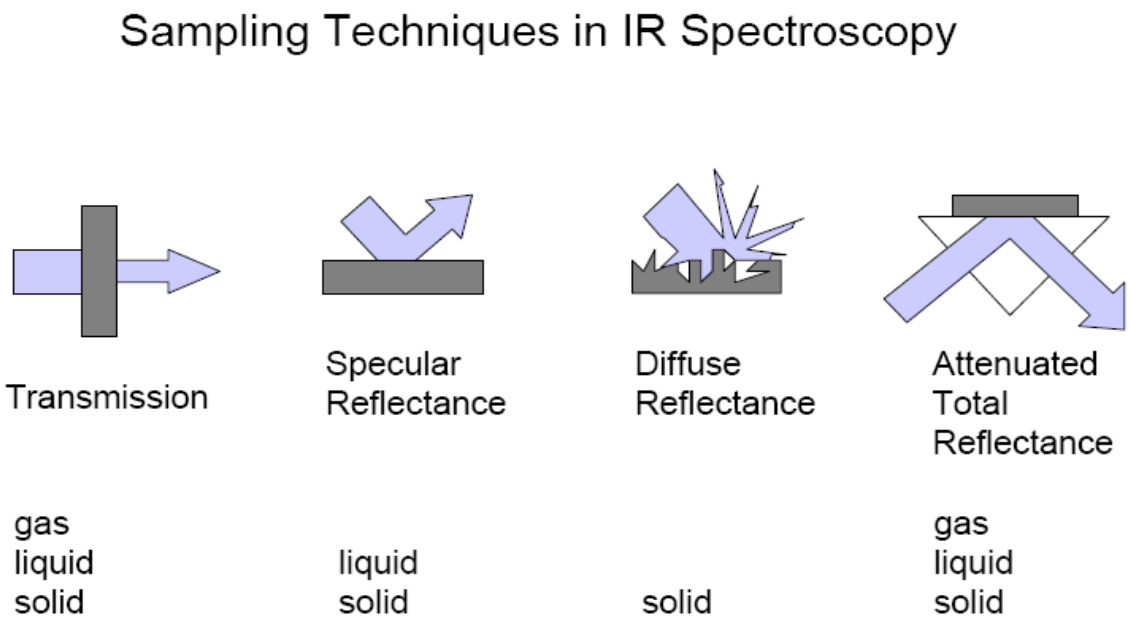
Reflectance spectroscopy

A set of technique for obtaining IR spectra of problematic compounds such as solids of limited solubility, films, threads, pastes, adhesives and powders

Noninvasive

Types of relectance spectroscopy

- **Specular reflectance**
- **Diffuse reflection**
- **Attenuated total relection**



Reference: Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed

Lecture 33

Name of topic/lesson – IR Spectroscopy

Subtopic: Photo acoustic IR, FTIR Microscopy

Objective: To understand principle and instrumentation of Photo acoustic IR, FTIR Microscopy

Topic Outcomes: At the end of topic you will

1.Know principle and instrumentation of Photo acoustic IR, FTIR Microscopy

Photoacoustic spectroscopy is the measurement of the effect of absorbed electromagnetic energy (particularly of light) on matter by means of acoustic detection. The discovery of the photoacoustic effect dates to 1880 when Alexander Graham Bell showed that thin discs emitted sound when exposed to a beam of sunlight that was rapidly interrupted with a rotating slotted disk. The absorbed energy from the light causes local heating, generating a thermal expansion which creates a pressure wave or sound. Later Bell showed that materials exposed to the non-visible portions of the solar spectrum (i.e., the infrared and the ultraviolet) can also produce sounds.

A **photoacoustic spectrum** of a sample can be recorded by measuring the sound at different wavelengths of the light. This spectrum can be used to identify the absorbing components of the sample. The photoacoustic effect can be used to study solids, liquids and gases.

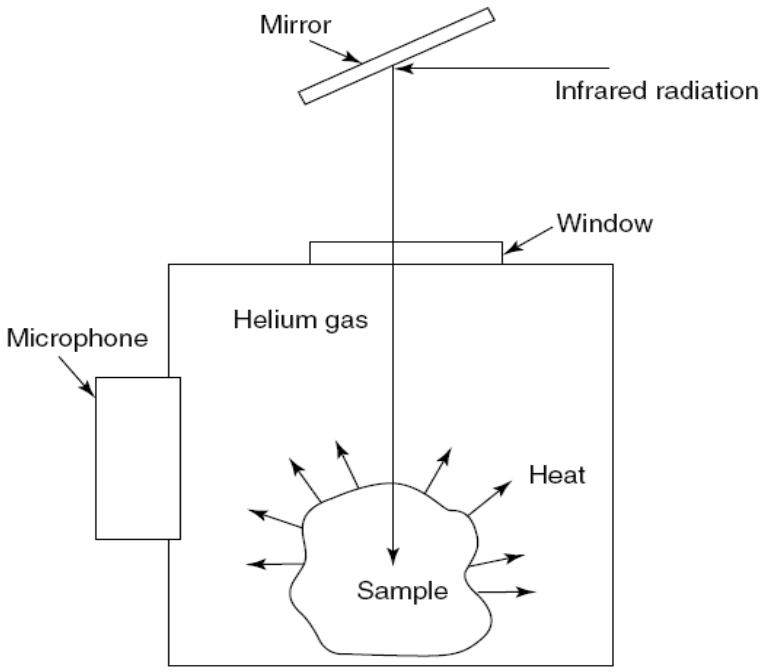


Figure 2.18 Schematic of a typical photoacoustic spectroscopy cell. From Stuart, B., *Modern Infrared Spectroscopy*, ACOL Series, Wiley, Chichester, UK, 1996. © University of Greenwich, and reproduced by permission of the University of Greenwich.

Reference: Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed

Lecture 34

Name of topic/lesson – IR Spectroscopy
Subtopic: Applications of IR Spectroscopy
Objective: To study applications of IR Spectroscopy
Topic Outcomes: At the end of topic you will
1. Know applications of IR spectroscopy

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.

Some of the major applications of IR spectroscopy are as follows:

1. Identification of functional group and structure elucidation

Entire IR region is divided into group frequency region and fingerprint region. Range of group frequency is 4000-1500 cm^{-1} while that of finger print region is 1500-400 cm^{-1}.

In group frequency region, the peaks corresponding to different functional groups can be observed. According to corresponding peaks, functional group can be determined.

2. Identification of substances

IR spectroscopy is used to establish whether a given sample of an organic substance is identical with another or not. This is because large number of absorption bands is observed in the IR spectra of organic molecules and the probability that any two compounds will produce identical spectra is almost zero. So if two compounds have identical IR spectra then both of them must be samples of the same substances.

3. Studying the progress of the reaction

Progress of chemical reaction can be determined by examining the small portion of the reaction mixure withdrawn from time to time. The rate of disappearance of a characteristic absorption band of the reactant group and/or the rate of appearance of the characteristic absorption band of the product group due to formation of product is observed.

4. Detection of impurities

IR spectrum of the test sample to be determined is compared with the standard compound. If any additional peaks are observed in the IR spectrum, then it is due to impurities present in the compound.

5. Quantitative analysis

The quantity of the substance can be determined either in pure form or as a mixure of two or more compounds. In this, characteristic peak corresponding to the drug substance is chosen and log I0/It of peaks for standard and test sample is compared. This is called base line technique to determine the quantity of the substance.

Reference: Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed

Lecture 35, 36, 37

Name of topic/lesson – IR Spectroscopy

Subtopic: FTIR Analysis and Interpretation of organic compounds based on FTIR Spectra

Objective: To study use of IR in structural elucidation

Topic Outcomes: At the end of topic you will

1.Be able to identify functional groups present in a compound

---

Bond	Type of Compound	Frequency Range, cm^{-1}	Intensity
C-H	Alkanes	2850-2970	Strong
C-H	Alkenes	3010-3095 675-995	Medium strong
C-H	Alkynes	3300	Strong
C-H	Aromatic rings	3010-3100 690-900	Medium strong
0-H	Monomeric alcohols, phenols Hydrogen-bonded alchohols, phenols Monomeric carboxylic acids Hydrogen-bonded carboxylic acids	3590-3650 3200-3600 3500-3650 2500-2700	Variable Variable, sometimes broad Medium broad
N-H	Amines, amides	3300-3500	medium
C=C	Alkenes	1610-1680	Variable
C=C	Aromatic rings	1500-1600	Variable
	Alkynes	2100-2260	Variable
C-N	Amines, amides	1180-1360	Strong
	Nitriles	2210-2280	Strong
C-O	Alcohols, ethers,carboxylic acids, esters	1050-1300	Strong
C=O	Aldehydes, ketones, carboxylic acids, esters	1690-1760	Strong
NO_2	Nitro compounds	1500-1570 1300-1370	Strong

Lecture 38

Name of topic/lesson – IR Spectroscopy

Subtopic: FTIR Analysis and Interpretation of organic compounds based on FTIR Spectra
Objective: To study use of IR in structural elucidation
Topic Outcomes: At the end of topic you will

1.Be able to identify functional groups present in a compound

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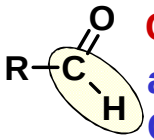
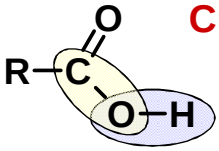
CONFIRMATION OF FUNCTIONAL GROUP

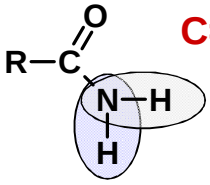
Every type of carbonyl compound has other places you can look to confirm your conclusion based on frequency alone.



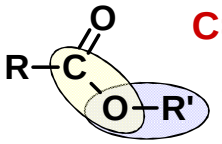
C=O at 1725 cm^{-1}
also look for aldehyde CH 2850 and 2750 cm^{-1}



C=O at 1710 cm^{-1}
also look for OH (H-bonded) and C-O ~1200 cm^{-1}



C=O at 1690 cm^{-1}
also look for two NH peaks at 3400 cm^{-1}



C=O at 1735 cm^{-1}
also look for two C-O at 1200 and 1000 cm^{-1}

Ketones have C=O at 1715 cm^{-1} and no NH, OH, C-O or -CHO

Anhydrides have two C=O peaks near 1800 cm^{-1} and two C-O

BASE VALUES
(+/- 10 cm^{-1})

O-H	3600
N-H	3400
C-H	3000
C≡N	2250
C≡C	2150
C=O	1715
C=C	1650
C-O	~1100

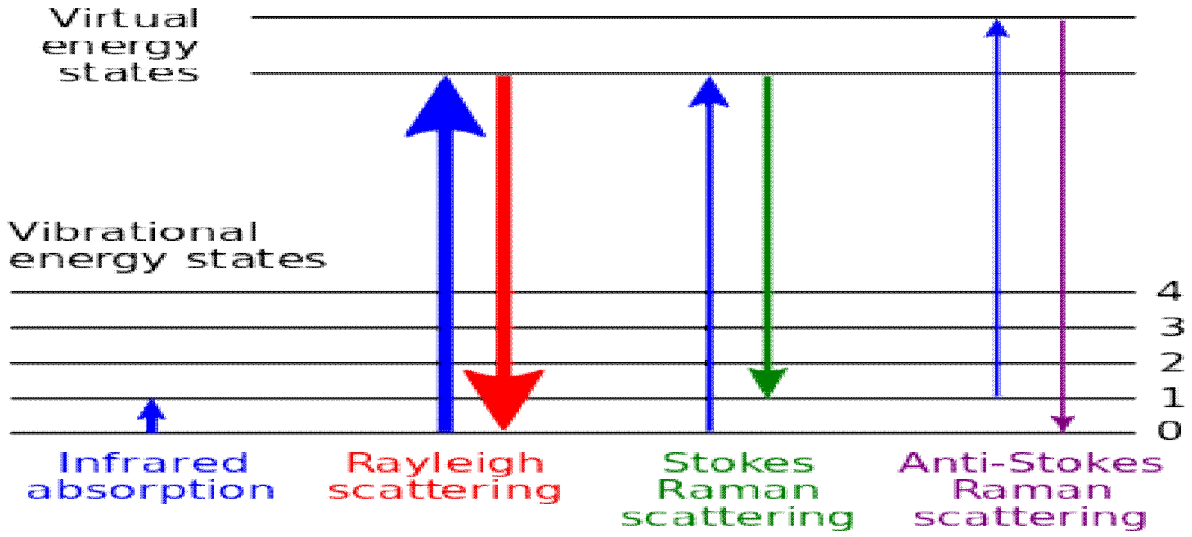
These are the minimum number of values to memorize.

large range

Lecture 39

Name of topic/lesson – Raman Spectroscopy
Subtopic: Theory Raman spectroscopy, Comparison with IR
Objective: To study principle of Raman Spectroscopy
Topic Outcomes: At the end of topic you will

1.Know the principle of Raman Spectroscopy and differences from IR



	Raman	IR
1	It is due to the scattering of light by the vibrating molecules.	It is the result of absorption of light by vibrating molecules.
2	The vibration is Raman active if it causes a change in polarisability.	The vibration is IR active if there is a change in dipole moment during the vibration.
3	The molecule need not possess a permanent dipole moment.	The vibration concerned should have a change in dipole moment due to that vibration.
4	Water can be used as a solvent.	Water cannot be used due to its intense absorption.
5	Sample preparation is not very elaborate sample can be almost in any state.	Sample preparation is elaborate Gaseous samples can rarely be used.
6	Gives an indication of covalent character in the molecule.	Gives an indication of ionic character in the molecule.
7	Cost of instrumentation is very high	Comparatively inexpensive.

References: 1. Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed

Lecture 40

Name of topic/lesson – Raman Spectroscopy

Subtopic: Instrumentation and applications
Objective: To study instrumentation and applications of Raman Spectroscopy
Topic Outcomes: At the end of topic you will

1. Know the components of Raman Spectrophotometer with function of each part

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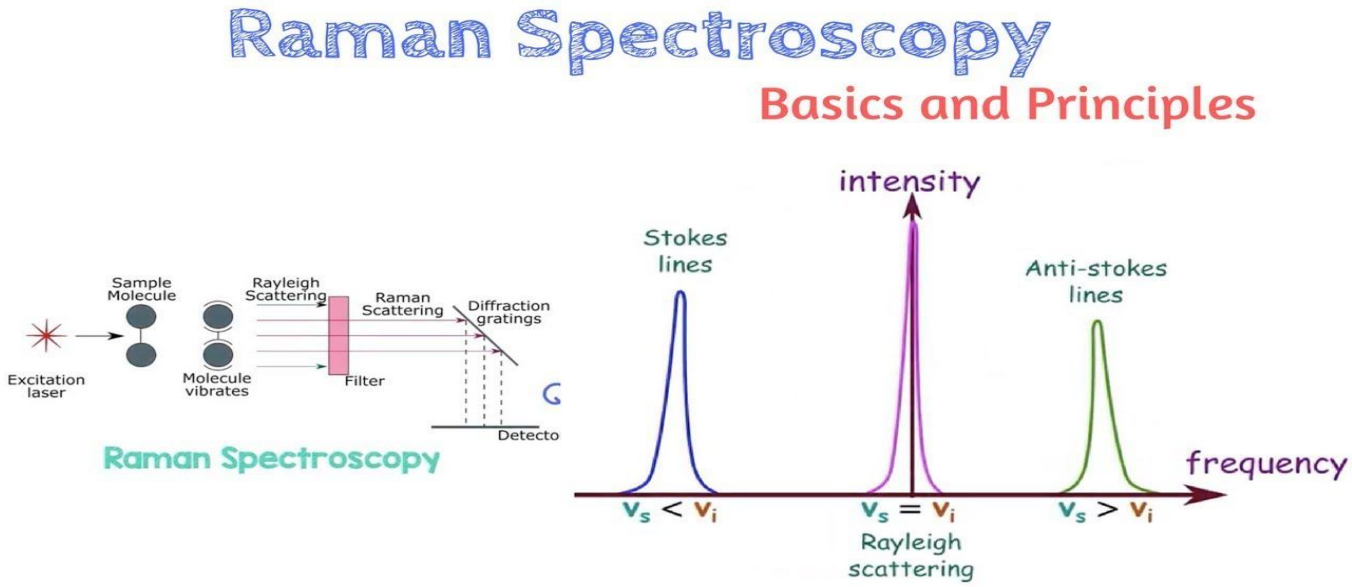
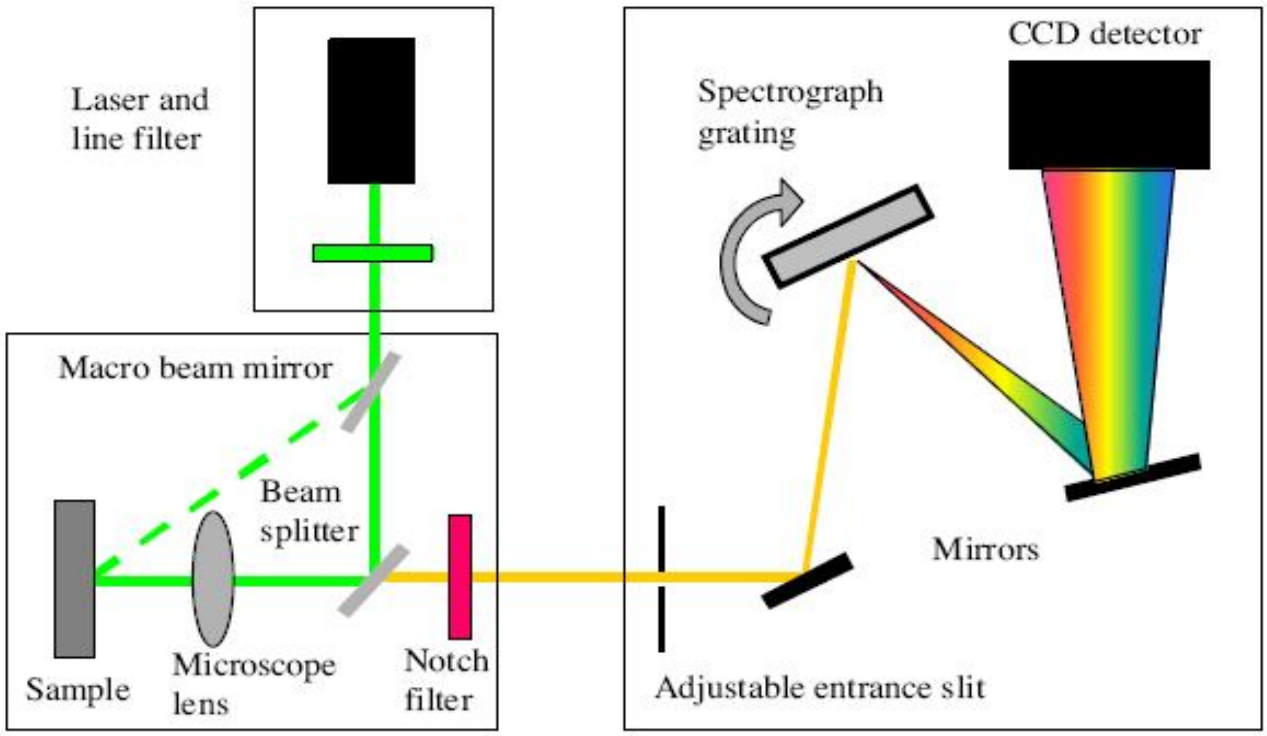




References: 1. Fundamentals of Analytical Chemistry by Skoog, West, Holler, Harvest, 8/Ed

Lecture synopsis Sub: Pharmaceutical Analysis V Subject I/C: Dr. Tambe V.S.

Lecture 41

Name of topic/lesson – Scanning Electron Microscopy (SEM)

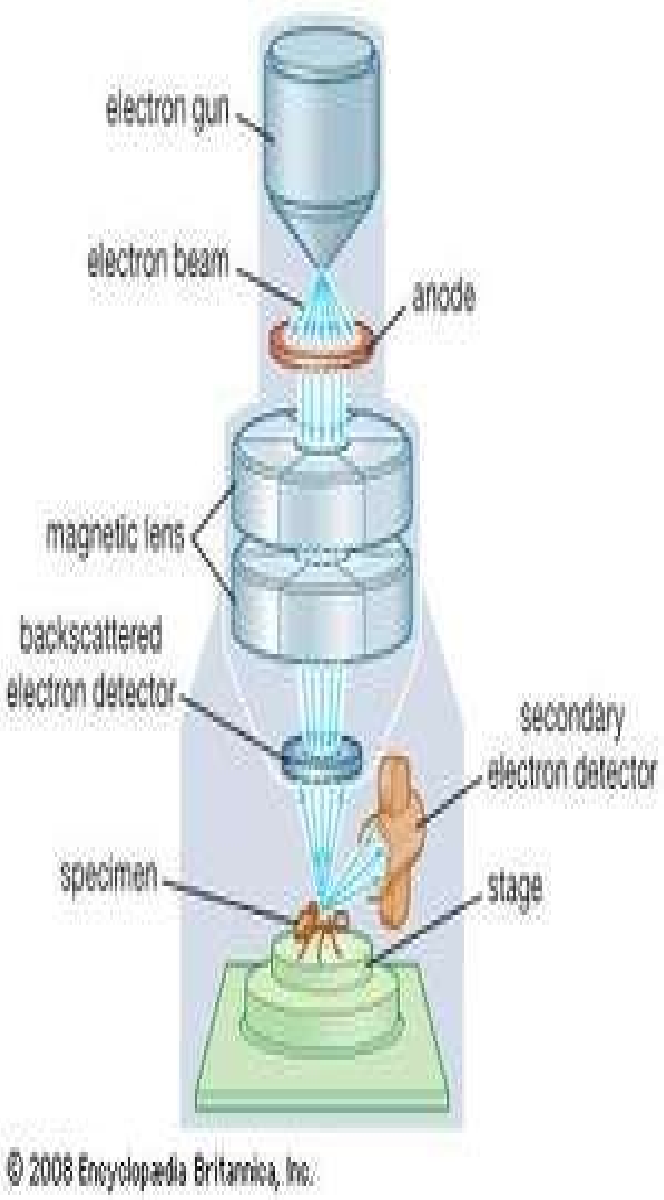
Subtopic: Principle and Instrumentation of Scanning Electron Microscopy (SEM)

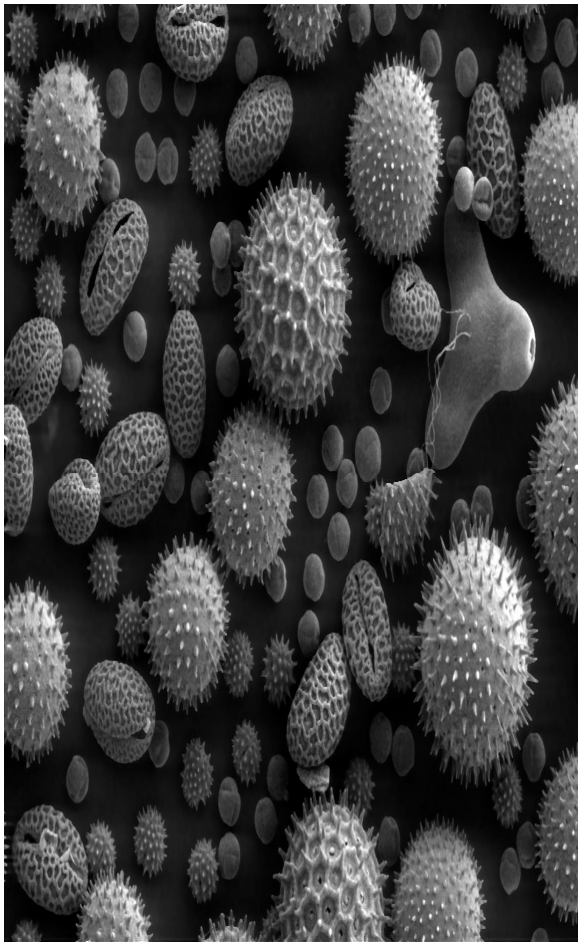
Objective: To understand the working principles of electron microscopes. To study Common applications of electron microscopes.

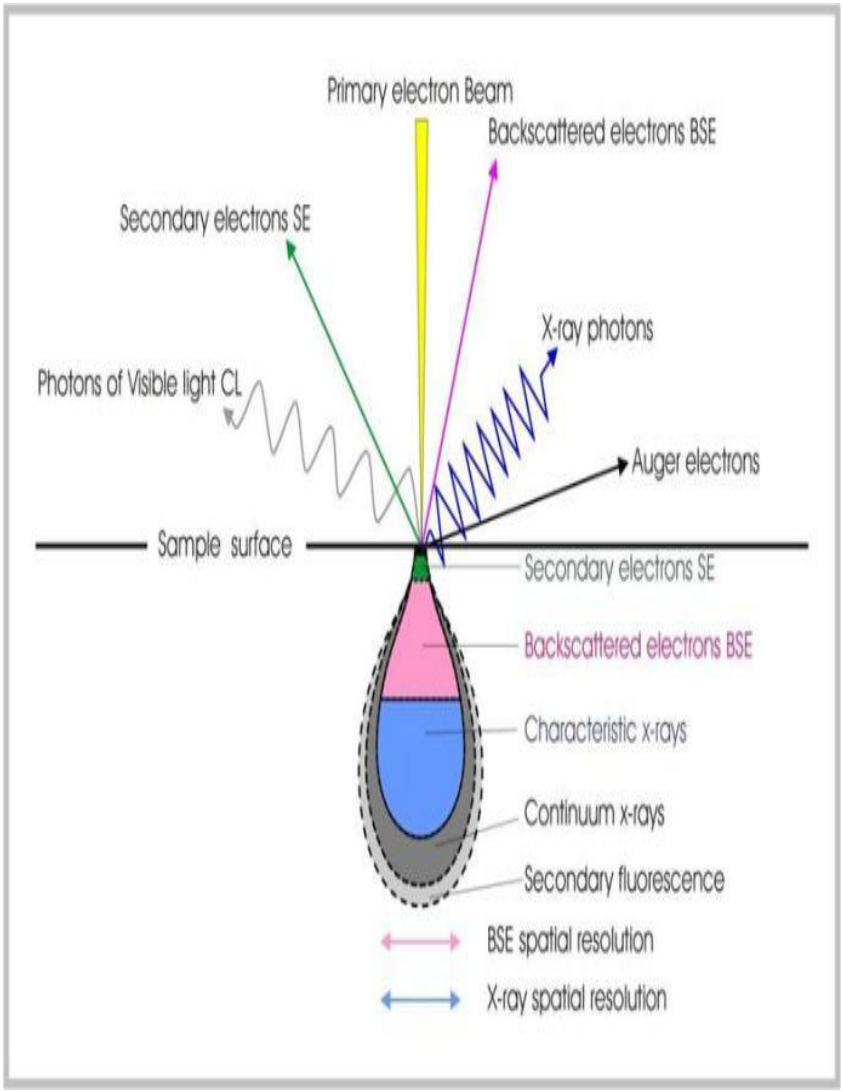
Topic Outcomes: At the end of topic you will

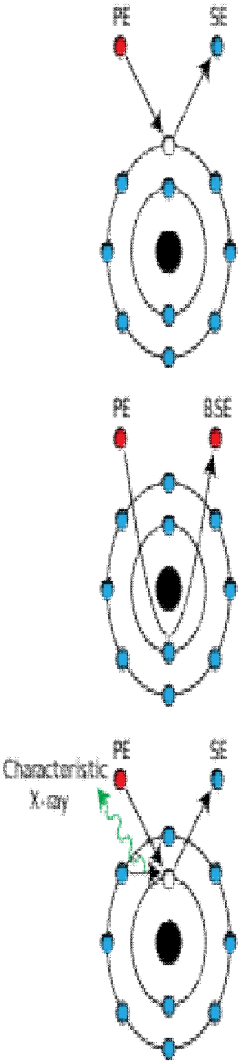
1. Know the mechanism involved in image formation using SEM

A **scanning electron microscope** (**SEM**) is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that contain information about the surface topography and composition of the sample.









Reference: https://en.wikipedia.org/wiki/Scanning_electron_microscope

Lecture 42

Name of topic/lesson – Scanning Electron Microscopy (SEM)

Subtopic: Applications of Scanning Electron Microscopy (SEM)
Objective: To study uses of SEM
Topic Outcomes: At the end of topic you will

1. Know applications of applications of SEM

- In pharmaceutical research, SEMs are used for powder imaging and analysis, to gain insights into cellular interactions with new drugs, and for applications in the most complicated cancer treatments.
- The surface structure and porosity of the dried beads using a SEM.
- For a successful cancer research, the morphology of tissues needs to be analyzed and understood. At present, this can be achieved using the correlated light and electron microscopy technique.
- A study revealed that pathogens present on polymer medical appliances can be very efficiently destroyed when ZnO and Ag-ZnO crystals are added to antibiotics. Here, a SEM was used to analyze the elemental composition and morphology of the crystals before using them for further experiments.
- To learn more about the composition and topography of man-made and naturally occurring materials. For instance, scanning electron microscopy has allowed biologists to learn much more about microscopic organisms, like bacteria and viruses. Geologists often use scanning electron microscopy to learn more about crystalline structures.
- Industries including microelectronics, semiconductors, medical devices, general manufacturing, insurance and litigation support, and food processing, all use scanning electron microscopy as a way to examine the surface composition of components and products.
- SEM can help businesses involved in the **development or manufacturing of products** learn more about the composition and topography of products and components. For instance, some products, like stainless steel, must be evenly coated with special chemicals for optimal performance. Scanning electron microscopy can help identify cracks, imperfections, or contaminants on the surfaces of coated products.
- **Industries, like cosmetics, that work with tiny particles** can also use scanning electron microscopy to learn more about the shape and size of the small particles they work with. For instance, particles that are too large or jagged might not flow or mix as well as particles that are small and round. Particles that are the wrong size or shape may have an impact on the consistency or performance of the product. Scanning electron microscopy can be used to identify problems with particle size or shape before products reach the consumer.
- Finally, **industries that use small or microscopic components** to create their products often use scanning electron microscopy to examine small components like fine filaments and thin films. If there is

a problem occurring at a microscopic level, scanning electron microscopy can be used to pinpoint the problem and help find a solution.

Due to its superior performance the SEM is used in an increasing number of various applications and provides valuable results for instance in the following applications:

- Gunshot residue analysis
- Firearms identification (bullet markings comparison)
- Investigation of gemstones and jewellery
- Examination of paint particles and fibres
- Filament bulb investigations at traffic accidents
- Handwriting and print examination / forgery
- Counterfeit bank notes
- Trace comparison
- Examination of non-conducting materials
- High resolution surface imaging

Reference: https://www.innovatechlabs.com/newsroom/742/scanning-electron-microscopy/

Lecture 43

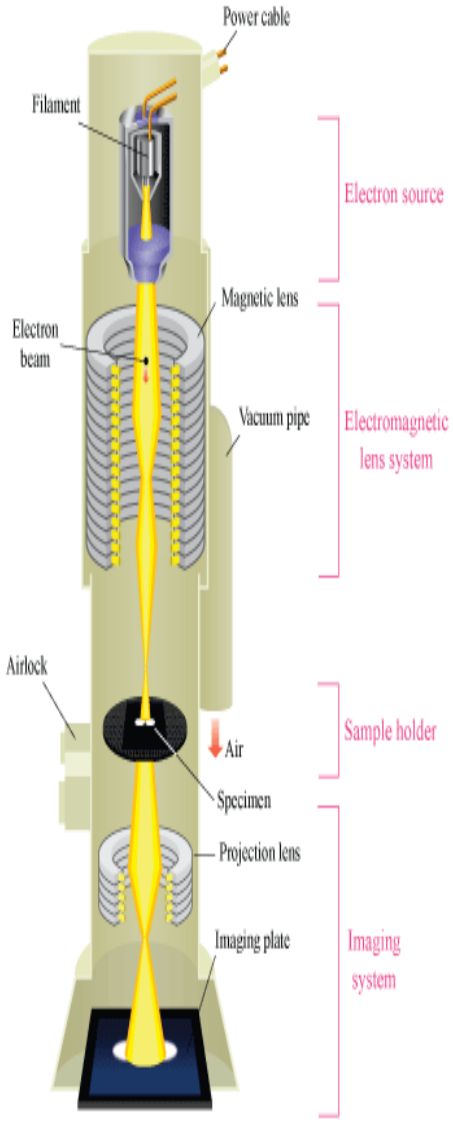
Name of topic/lesson – Transmission Electron Microscopy (TEM)

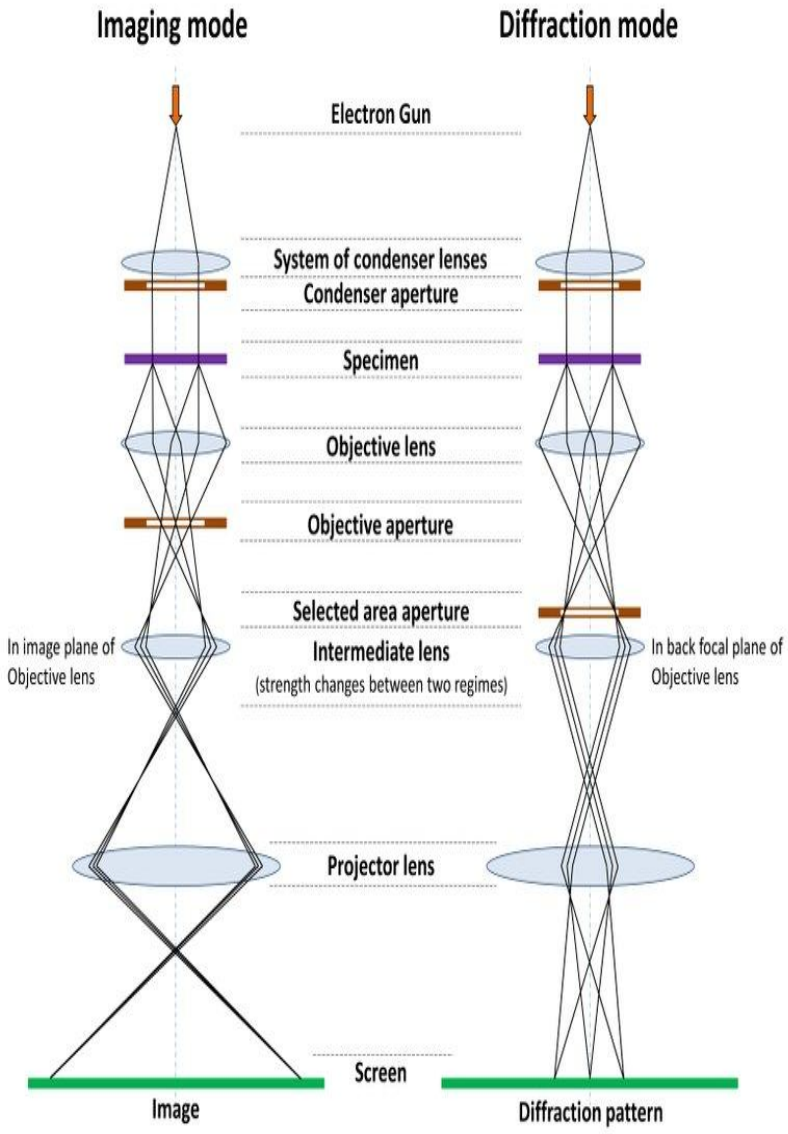
Subtopic: Principle and Instrumentation of Transmission Electron Microscopy (TEM)

Objective: To study Principle and Instrumentation of TEM

Topic Outcomes: At the end of topic you will

1. Know construction of TEM with function of each part





Reference: https://en.wikipedia.org/wiki/Transmission_electron_microscopy

Lecture 44

Name of topic/lesson – Transmission Electron Microscopy (TEM)

Subtopic: Applications of Transmission Electron Microscopy (TEM)

Objective: To study applications of TEM

Topic Outcomes: At the end of topic you will

1. Know applications of applications of TEM

- Provides high magnification images of the internal structure of a sample. Being able to obtain an internal image of a sample opens new possibilities for what sort of information can be gathered from it.
- A TEM operator can investigate the crystalline structure of an object, see the stress or internal fractures of a sample, or even view contamination within a sample through the use of diffraction patterns.
- Characterize a variety of pharmaceutical compounds, pharmaceutical salts and cocrystals.
- Morphology, polymorph identification, mapping of crystal habit to crystal structure and crystal defect characterization.
- Distinguishing between the different polymorphs of pharmaceutical compounds
- TEM can aid the study of multiphasic materials and solid dispersions, where a drug is held in a polymer matrix.
- Ideal technique for characterising nano and microcrystalline materials that result from milling and micronisation. Defects in crystals of several pharmaceutical compounds have been observed and characterised and the dislocations that were identified in crystals of Form II of theophylline have been shown to be responsible for the fracturing of the crystals

Reference: MARK D. EDDLESTON, ERICA G. BITHELL, WILLIAM JONES, TRANSMISSION ELECTRON MICROSCOPY OF PHARMACEUTICAL MATERIALS, JOURNAL OF PHARMACEUTICAL SCIENCES, VOL. 99, NO. 9, SEPTEMBER 2010

Lecture synopsis Sub: Pharmaceutical Analysis V Subject I/C: Dr. Tambe V.S.

Lecture 45

Name of topic/lesson – Transmission Electron Microscopy (TEM)

Subtopic: Comparison of SEM and TEM

Objective: To compare and contrast between SEM and TEM

Topic Outcomes: At the end of topic you will

1. Be able to select the correct technique as per application

2. Know Advantages and disadvantages of each technique

Sr. No.	SCANNING ELECTRON MICROSCOPY (SEM)	TRANSMISSION ELECTRON MICROSCOPY (TEM)
1.	SEMs use a specific set of coils to scan the beam in a raster-like pattern and collect the scattered electrons. SEM provides information on the sample's surface and its composition.	TEM use the transmitted electrons; the electrons which are passing through the sample before they are collected. So, TEM offers invaluable information on the inner structure of the sample, such as crystal structure, morphology and stress state information.
2.	SEM resolution is limited to ~0.5 nm.	TEM has resolution of even less than 50 pm.
3.	If you want to get information on the surface of your sample, like roughness or contamination detection, then you should choose a SEM.	If you would like to know what the crystal structure of your sample is, or if you want to look for possible structural defects or impurities, then using a TEM is the only way to do so.
4.	SEMs provide a 3D image of the surface of the sample.	TEM images are 2D projections of the sample, which in some cases makes the interpretation of the results more difficult for the operator.
5.	SEM imaging there is no specific requirement of sample preparation.	Due to the requirement for transmitted electrons, TEM samples must be very thin, generally below 150 nm, and in cases that high-resolution imaging is required, even below 30 nm.
6.	SEM samples require little or no effort for sample preparation and can be directly imaged by mounting them on an aluminum stub.	TEM sample preparation is a quite complex and tedious procedure that only trained and experienced users can follow successfully. The samples need to be very thin, as flat as possible, and the preparation technique should not induce any artefacts (such as precipitates or amorphisation) to the sample. Many methods

		have been developed, including electro polishing, mechanical polishing and focused ion beam milling. Dedicated grids and holders are used to mount the TEM samples.
7.	SEMs usually use acceleration voltages up to 30 kV.	TEM uses it in the range of 60 – 300 kV.
8.	The magnifications for the SEM is limited up to 1-2 million times.	The magnifications that TEMs is much higher compared to SEMs. TEM users can magnify their samples by more than 50 million times,
9.	However, the maximum Field of View (FOV) that SEMs can achieve is far larger than TEMs, Similarly, the depth of field of SEM systems are much higher than in TEM systems.	Users can only use to image a very small part of their sample.
10.	In addition, the way images are created are different in the two systems. In SEMs, samples are positioned at the bottom of the electron column and the scattered electrons (back-scattered or secondary) are captured by electron detectors. Photomultipliers are then used to convert this signal into a voltage signal, which is amplified and gives rise to the image on a PC screen.	In a TEM microscope, the sample is located in the middle of the column. The transmitted electrons pass through it, and through a series of lenses below the sample (intermediate and projector lenses). An image is directly shown on a fluorescent screen or via a charge-coupled device (CCD) camera, onto a PC screen.
11.	Relatively simple to operate	Generally, TEMs are more complex to operate. TEM users require intensive training before being able to operate them. Special procedures need to be performed before every use, with several steps included that ensure that the electron beam is perfectly aligned.
12.	Relatively simple	TEMs may enable much more resolving power and versatility to the user, but they are much more expensive and larger than SEMs and require more effort in order to acquire and interpret results.

Reference: https://www.lambdaphoto.co.uk/news/2018/02/21/sem-and-tem-whats-the-difference/