# SOLUtions

# Q1

## (a) Enlist ideal properties of a detector. Describe various pumps and detectors used in High Performance Liquid Chromatography (HPLC).

### Ideal properties of an HPLC detector

An ideal HPLC detector should have:

1. **High sensitivity** — detect very small amounts of analyte.
2. **Good selectivity** — respond mainly to the analyte and not to baseline noise.
3. **Wide linear dynamic range** — signal proportional to concentration across wide range.
4. **Low noise and high signal-to-noise ratio (S/N)** — so low limits of detection (LOD).
5. **Fast response time** — to resolve narrow chromatographic peaks (good time resolution).
6. **Stable baseline** — minimal drift over time and different mobile phase conditions.
7. **Compatibility with mobile phases** — chemically stable when exposed to organic / aqueous solvents.
8. **Non-destructive or destructive depending on need** — some detectors allow sample collection after detection.
9. **Simple calibration & reproducibility** — easy to maintain linearity and reproducible response.
10. **Low dead volume** — minimal band broadening.
11. **Temperature independence and robustness** — operate reliably over typical lab temps.

---

### Pumps used in HPLC

A pump must deliver a stable, pulse-free flow at high pressure. Main types:

1. **Reciprocating piston pumps (binary or quaternary gradient pumps)**
   - Most common in modern HPLC.
   - Use one or more pistons and check valves to move solvent.
   - Can produce high pressure (up to several hundred bar / MPa).
   - Good for gradients (mixing solvents in precise ratios).
   - Have pulse dampers to reduce pulsation.
2. **Plunger (constant displacement) pumps**
   - Older type; deliver nearly constant flow but need good pulsation damping.
3. **Syringe pumps**
   - Very precise flow for micro-HPLC or infusion; limited volume and used for special applications.
4. **Isocratic pumps**
   - Single solvent or premixed solvent; simpler and cheaper.
5. **Gradient pumps (binary, ternary, quaternary)**
   - Allow automated mixing of two to four solvents to change mobile phase composition during run.
6. **Quaternary low-pressure mixing or high-pressure mixing systems**
   - Low-pressure mixing mixes solvents at low pressure then pumps; high-pressure mixing mixes after pressurization — gives better gradient accuracy.

**Important pump features**: flow accuracy, flow stability, ability to withstand backpressure, low pulsation, solvent compression compensation, leak protection.

---

### Detectors used in HPLC (common types, principle and uses)

1. **Ultraviolet–Visible (UV-Vis) Absorbance Detector**
   - **Principle**: measures light absorption by solutes at one or more wavelengths.
   - **Types**: single-wavelength, diode-array detector (DAD / PDA). DAD measures full spectrum (200–800 nm) for each peak.
   - **Advantages**: universal for chromophores, sensitive, inexpensive.
   - **Applications**: most organic drugs, impurities, assays.

2. **Fluorescence Detector (FLD)**
   - **Principle**: measures emitted light after excitation at a specific wavelength.
   - **High sensitivity and selectivity** for fluorescent compounds or derivatives.
   - **Applications**: trace analysis, derivatized amino acids, natural products.
3. **Refractive Index (RI) Detector**
   - **Principle**: measures change in refractive index of eluent vs reference.
   - **Non-destructive but low sensitivity**, and not compatible with gradient elution (baseline drifts).
   - **Applications**: sugars, lipids, polymers where no chromophores exist.
4. **Evaporative Light Scattering Detector (ELSD)**
   - **Principle**: mobile phase evaporated; non-volatile analyte particles scatter light; scattering measured.
   - **Works with non-volatile compounds**, gradient compatible, used for neutral compounds without chromophores.
   - **Limitations**: response not strictly linear.
5. **Charged Aerosol Detector (CAD)**
   - **Principle**: similar to ELSD but charges particles and measures current—more uniform response.
   - **Good sensitivity** and compatible with gradients.
6. **Mass Spectrometric Detector (LC–MS)**
   - **Principle**: ionizes eluted analyte and measures m/z.
   - **Advantages**: high sensitivity, structure information, high specificity, multiple reaction monitoring (MRM) in tandem MS.
   - **Applications**: trace analysis, metabolite ID, bioanalysis.
   - **Note**: requires compatible mobile phases and careful interface (electrospray, APCI).
7. **Electrochemical Detector**
   - **Principle**: measures current from oxidation/reduction of analyte at electrode.
   - **Very sensitive for electroactive species** (e.g., neurotransmitters).
   - **Limitations**: requires analyte be electroactive.
8. **Conductivity Detector**
   - **Principle**: measures electrical conductivity of eluate.
   - **Used for ionic species** (ion chromatography).
9. **Photodiode Array (PDA/DAD)** — variant of UV-Vis but provides spectral data for peak identification and purity checking.
10. **Flame Ionization Detector (rare in LC)** — mainly GC.

**Choosing a detector**: depends on analyte properties (chromophore, fluorescence, volatility, ionic nature), sensitivity required, compatibility with gradients and solvents, and whether structural info is needed.

---

# (OR) Q1 (b) Describe various columns and stationary phases used in Gas Chromatography (GC). Add a note on Affinity Chromatography.

## Columns and stationary phases in Gas Chromatography

**Two main types of GC columns**:

1. **Packed columns**
   - Tubes filled with solid support coated with liquid stationary phase or solid adsorbent.
   - Larger diameter, shorter length, lower efficiency (fewer theoretical plates).
   - Mostly older use; used for preparative separations and high sample loading.
   - Stationary phases: polar solids (e.g., molecular sieves) or coated supports.
2. **Capillary (open tubular) columns** — most common today
   - Narrow fused silica capillary lined with stationary phase on inner wall.
   - Very high efficiency (many theoretical plates), good resolution, faster.
   - Two subtypes:
     - **Wall-coated open tubular (WCOT)** — stationary liquid film coated directly on glass/silica wall.
     - **Support-coated open tubular (SCOT)** — thin layer of support plus liquid phase.

**Stationary phases (liquid films) — categories by polarity**:

1. **Non-polar stationary phases**
   - Examples: polydimethylsiloxane (PDMS), dimethylpolysiloxane.
   - Best for nonpolar analytes; separate mainly by volatility (boiling point).
   - Low polarity → longer retention for nonpolar compounds.

2. **Mid-polarity phases**
   - Examples: 5% phenyl/95% methyl polysiloxane.
   - Moderate selectivity for aromatic and slightly polar compounds.
3. **Polar phases**
   - Examples: polyethylene glycol (Carbowax), cyanopropylphenyl polysiloxane, nitroterephthalic acid derivatives.
   - Good for polar analytes (alcohols, acids, amines); can separate isomers by polarity and hydrogen bonding.
4. **Specialty stationary phases**
   - Chiral selectors (cyclodextrin derivatives) for enantiomer separation.
   - Bonded phases (reduced bleed, higher temperature stability).
   - Porous layers for gas adsorption (molecular sieves for permanent gases).

**Choosing column/stationary phase**: depends on analyte polarity, thermal stability, column bleed tolerance (for MS detection), desired selectivity and temperature program.

---

## Note on Affinity Chromatography (short description)

- **Principle**: separation based on highly specific biological interactions — e.g., antigen–antibody, enzyme–substrate, receptor–ligand, lectin–carbohydrate.
- **Stationary phase**: inert support (agarose, sepharose) with an immobilized ligand that specifically binds target molecule.
- **Process**:
  1. Load sample → target binds to ligand; other components wash out.
  2. Elute target by changing pH, ionic strength, adding competitive ligand or denaturant.
- **Advantages**: extremely high selectivity, high purity in one step.
- **Applications**: protein purification, enzyme isolation, immunoassays, diagnostics, removing contaminants.

---

# Q2 (Attempt any TWO — I will answer all four)

## Q2 (a) Elucidate the structure of organic compound from the following data:

**Formula:** $C_8H_8O$
**IR:** 3048, 2922, 2733, 1703 $cm^{-1}$
**$^1$H NMR (PMR):** δ 9.9 (s, 1H), δ 7.3–7.8 (m, 4H), δ 2.4 (s, 3H)

## Step-by-step interpretation (simple)

1. **Degrees of unsaturation (DoU)**
   - Formula $C_8H_8O$ → maximum H for $C_8$ is 2n+2 = 18. Here H = 8 → DoU = (18 − 8)/2 = 5.
   - 5 degrees means combinations of rings and π bonds: likely one aromatic ring (4 DoU) + one more (carbonyl or double bond) → fits benzaldehyde derivative.
2. **IR analysis**
   - **1703 $cm^{-1}$**: strong carbonyl (C=O), typical of aldehyde/ketone.
   - **2733 $cm^{-1}$**: characteristic weak band for aldehyde C–H stretch (often around 2700–2830 $cm^{-1}$).
   - **3048 $cm^{-1}$**: aromatic C–H stretch.
   - **2922 $cm^{-1}$**: aliphatic C–H stretch (methyl).
     → IR strongly indicates an **aldehyde** function.
3. **$^1$H NMR analysis**
   - **δ 9.9 (s, 1H)** → classic aldehydic proton (–CHO).
   - **δ 7.3–7.8 (m, 4H)** → four aromatic protons (multiplet), suggests a **disubstituted benzene** with only four H (so two substituents on ring). The pattern of 4H often arises from a **para-disubstituted benzene** (two pairs of equivalent protons).
   - **δ 2.4 (s, 3H)** → a methyl group attached to an aromatic ring (p-methyl groups typically appear ~2.2–2.5 ppm) — singlet indicates no neighboring hydrogens (i.e., methyl directly on ring).
4. **Combine info**
   - Aldehyde present + aromatic ring with a methyl substituent → total carbons: benzene (6) + CHO (1 C) + $CH_3$ (1 C) = 8 C. Formula matches $C_8H_8O$.
   - Aromatic protons = 4 → two substituents on ring. The methyl and aldehyde likely are para to each other (para-methylbenzaldehyde, gives 4 aromatic H often in the δ7.3–7.8 region).

**Final structure**: **p-Tolualdehyde** (4-methylbenzaldehyde).
(Also acceptable name: 4-methylbenzaldehyde).
Representation: a benzene ring with –CHO at one position and $–CH_3$ at the para position.

---

# Q2 (b) Explain various types of ions formed in Mass Spectrometry with suitable examples.

## Overview

Mass spectrometry ion types depend on the ionization method. In electron ionization (EI), chemical ionization (CI), electrospray (ESI), etc., different ions appear. Key ion types:

1. **Molecular ion ($M^{+\bullet}$)** — radical cation
   - Formed often in EI (hard ionization). It corresponds to parent molecule minus an electron.
   - Example: benzene ($C_6H_6$) → $M^{+\bullet}$ at m/z 78.
   - Gives molecular weight information (may be weak if fragmenting).
2. **Protonated molecule $(M+H)^+$**
   - Common in soft ionization methods like ESI and APCI. Proton attaches, no radical.
   - Example: in ESI positive mode, caffeine (M = 194) gives $[M+H]^+$ at m/z 195.
3. **Deprotonated molecule $(M-H)^-$**
   - In negative ion mode (ESI−), analyte loses a proton.
   - Example: carboxylic acids give $[M-H]^-$.
4. **Adduct ions**
   - **$[M+Na]^+$, $[M+K]^+$, $[M+NH_4]^+$** — metal or ammonium attach in ESI or APCI.
   - Example: sugars often show $[M+Na]^+$.
5. **Fragment ions**
   - Result from breaking molecular bonds (common in EI). Give structural information.
   - Types of fragmentations:
     - **α-cleavage** (carbonyl compounds): cleavage next to functional group.
       - Example: in acetophenone, cleavage next to carbonyl gives benzyl cation.
     - **McLafferty rearrangement**: typical for carbonyls with γ-hydrogen → characteristic neutral loss and radical ion.
       - Example: ketones with γ-H show a fragment due to transfer of γ-H and bond cleavage.
     - **Retro-Diels–Alder** cleavage (in cyclics) etc.
6. **Base peak**
   - The most intense peak in the spectrum (relative intensity 100%). Not always the molecular ion. Example: in many aromatic compounds, a tropylium ion (m/z 91) is base peak.
7. **Radical ions vs even-electron ions**
   - **Radical cation ($M^{+\bullet}$)** has odd electron (EI).
   - **Even-electron ions** (e.g., $[M+H]^+$) are more stable and common in soft ionization.
8. **Isotopic peaks**
   - Peaks from isotopes (e.g., $^{13}C$, $^{37}Cl$, $^{34}S$). Help determine presence of Cl (3:1 pattern), Br (1:1), S, etc.
9. **Multiple charge states**
   - In ESI, large biomolecules may carry multiple charges (z>1), producing peaks at m/z = (M + nH)/n. Deconvolution gives molecular mass.

## Examples (simple)

- **EI of toluene**: molecular ion at m/z 92 ($C_7H_8^{+\bullet}$), prominent fragment m/z 91 (tropylium ion).
- **ESI of a peptide**: shows $[M+nH]^{n+}$ (multiply charged) peaks.
- **CI (using methane)**: gives $[M+H]^+$ more prominently and less fragmentation than EI.

---

# Q2 (c) Elaborate the principle and instrumentation of Differential Thermal Analysis (DTA).

## Principle (simple)

- **DTA compares temperature changes** between a **sample** and an **inert reference** while both are subjected to a controlled temperature program (heating or cooling at constant rate).
- **If the sample undergoes an endothermic or exothermic event** (melting, crystallization, phase change, decomposition), its temperature will differ slightly from the reference. That **temperature difference (ΔT)** is recorded versus time or temperature.
- The DTA trace shows **peaks**: **endothermic peaks** (down or up depending on instrument convention) correspond to heat absorbed (melting, evaporation), **exothermic peaks** correspond to heat released (crystallization, oxidation).

## Instrumentation (components)

1. **Furnace / Heating System**
   - Provides controlled heating or cooling at a set rate (e.g., 5–20 °C/min).
2. **Sample and reference holders (pans)**
   - Two inert pans: one contains sample; the other contains an inert reference (e.g., alumina). Both placed close together.
3. **Thermocouples (pair)**
   - Two thermocouples measure temperature of sample and reference separately; a differential thermocouple measures ΔT (difference).
4. **Temperature controller / programmer**
   - Controls heating rate and program.
5. **Atmosphere control / gas purge**
   - Inert gas ($N_2$, Ar) or reactive gas ($O_2$) depending on analysis.
6. **Data acquisition and recorder**
   - Records ΔT vs T (DTA curve). Peak temperatures and shapes interpreted qualitatively and quantitatively (with calibration).

## Uses / Applications

- Detect phase transitions (melting, crystallization), glass transition (with care), polymorphic transitions, decomposition, oxidation, compatibility studies, thermal stability screening.

## Limitations vs DSC

- DTA gives **temperature difference**, not direct heat flow; **DSC** (differential scanning calorimetry) measures heat flow (more quantitative). DTA is simpler and often cheaper but less quantitative.

---

# Q2 (d) Describe FT-IR. Enlist its advantages.

## FT-IR principle (simple)

- **FT-IR** (Fourier Transform Infrared Spectroscopy) uses an **interferometer** (usually Michelson interferometer) to collect spectral information over a broad IR range at once. Instead of scanning one wavelength at a time (dispersive IR), FT-IR measures an **interferogram** (intensity vs optical path difference) and then applies a **Fourier transform** to convert the interferogram into the conventional IR spectrum (intensity vs wavenumber).

**Basic steps**:

1. Broadband IR source emits all frequencies.
2. Light passes through a Michelson interferometer — one mirror fixed, one moving — creating an interference pattern (interferogram).
3. Light passes through sample; detector measures resulting interferogram.
4. Computer performs Fourier transform → spectrum.
5. Result: absorbance (or transmittance) vs wavenumber ($cm^{-1}$).

## Main advantages of FT-IR

1. **Fellgett's (multiplex) advantage** — collects all frequencies simultaneously → faster and better S/N for same scanning time.
2. **Jacquinot's (throughput) advantage** — no slit means higher light throughput → improved sensitivity.
3. **High speed** — rapid data collection, useful for time-resolved studies.
4. **High spectral resolution and accuracy** — precise wavenumber calibration using laser reference (He–Ne).
5. **Good signal-to-noise** — especially when co-adding scans.
6. **Wider spectral range per scan** — collect whole mid-IR at once.
7. **Flexible sampling** — can use transmission, ATR (attenuated total reflectance), diffuse reflectance, gas cell, etc. ATR allows easy analysis of solids/liquids with minimal prep.

8. **Digital data handling** — easy baseline correction, smoothing, library searching.
9. **Stable and reproducible** — low instrumental drift.

### Typical applications

- Functional group identification, qualitative analysis, monitoring reactions, polymer analysis, pharmaceuticals (identifying APIs and excipients), quantitative analysis with calibration.

---

# Q3 (Attempt any THREE — I will answer all five)

## Q3 (a) Discuss about choice of solvent and solvent effect in UV-Visible Spectroscopy.

### Choosing a solvent (rules, simple)

1. **Transparency in wavelength region** — solvent should not absorb where analyte is measured (no strong absorbance at λmax). For UV (200–400 nm) choose solvents transparent in UV (e.g., hexane, cyclohexane, acetonitrile, methanol; avoid solvents with strong UV cutoffs like water below ~190 nm is okay but above that check). For visible, most solvents OK.
2. **Polarity matching** — solvent polarity affects solubility and spectral shifts; choose solvent that dissolves analyte and gives stable spectrum.
3. **Protic vs aprotic** — protic solvents can hydrogen bond with analyte and change spectral properties.
4. **Chemical inertness** — solvent should not react with analyte (no chemical reaction or degradation).
5. **Refractive index / viscosity** — may affect baseline and instrument optics but usually minor.
6. **Volatility and toxicity** — practical lab concerns.
7. **Compatibility with cuvette material** — e.g., quartz cuvette needed for far UV; glass/plastic cuvettes absorb in UV.

### Solvent effects on UV-Vis spectra (solvatochromism)

- **Solvent polarity** can shift the position (λmax) and intensity of absorption bands:
  - **Bathochromic shift (red shift)** — λmax moves to longer wavelength (lower energy) when solvent stabilizes excited state more than ground state.
  - **Hypsochromic shift (blue shift)** — λmax moves to shorter wavelength when ground state stabilized more than excited state.
  - **Hyperchromic / hypochromic effects** — change in molar absorptivity (intensity) due to solvent interactions.
- **Protic solvents** can hydrogen bond to solute groups (e.g., –OH, –NH), often changing electronic distribution and shifting λmax.
- **Specific interactions** (H-bonding, charge transfer complexes) can produce new bands or broaden peaks.
- **Solvent polarity scale** (e.g., dielectric constant, Reichardt's ET(30) parameter) correlates with extent of solvatochromic shifts.

### Practical tips

- For accurate λmax and molar absorptivity, use **same solvent for calibration standards and samples**.
- If studying intrinsic chromophores, use **non-interacting solvent** (aprotic, low polarity) to minimize specific solute-solvent interactions unless you are studying solvatochromism.

---

## Q3 (b) Explain Pharmaceutical Applications and types of Differential Scanning Calorimetry (DSC).

### DSC principle (short)

- Measures **heat flow** into a **sample** relative to a **reference** as temperature is programmed. Endothermic/exothermic events are recorded as heat flow vs temperature. Unlike DTA, DSC gives direct quantitative heat flow (enthalpy).

### Types of DSC

1. **Heat-flux DSC**
   - Measures temperature difference between sample and reference; heat flow calculated using calibration. Common lab DSC instruments (e.g., PerkinElmer DSC).
2. **Power-compensated DSC**

- Keeps sample and reference at same temperature by varying power to each; measures power difference. Better for fast heating rates and high sensitivity.

3. **Modulated DSC (MDSC)**
   - A small sinusoidal temperature modulation is superimposed on linear heating. Allows separation of reversible (heat capacity related) and non-reversible events (kinetic events like crystallization).
4. **Fast/Chip DSC**
   - Very fast heating/cooling rates for small samples (useful for rapid kinetics and small sample mass).

### Pharmaceutical applications (clear, simple)

1. **Melting point and melting enthalpy** — identify drug and purity assessment (impurities lower and broaden melting point).
2. **Polymorphism and polymorph screening** — detect different crystalline forms; different polymorphs have different melting points and heats of fusion.
3. **Glass transition temperature (Tg)** — important for amorphous drugs, stability, and formulation (e.g., solid dispersions).
4. **Compatibility studies (drug–excipient)** — mixing drug with excipients and observing new thermal events (e.g., new peaks, disappearance of melting) indicates interaction.
5. **Crystallinity determination** — estimate % crystallinity from enthalpy of fusion compared to pure crystalline standard.
6. **Stability studies / degradation** — detect exothermic decomposition events.
7. **Curing and polymerization studies** — for controlled-release matrices and polymer excipients.
8. **Quality control** — batch-to-batch consistency of API and formulation.
9. **Kinetics of thermal events** — activation energy of decomposition via advanced DSC methods.

---

## Q3 (c) Describe Capillary Electrophoresis (CE).

### Principle (simple)

- **CE separates analytes by their electrophoretic mobility** in a narrow capillary under a high electric field. Mobility depends on **charge to size ratio (q/r)**. Under an electric field, cations migrate toward cathode, anions toward anode; neutral molecules migrate with electroosmotic flow (EOF).

### Basic instrumentation

1. **Fused silica capillary** (typically 25–100 μm i.d., lengths 20–100 cm).
2. **High-voltage power supply** (several kV up to tens of kV).
3. **Buffer solution (background electrolyte, BGE)** — controls pH, ionic strength.
4. **Sample injector** — hydrodynamic (pressure) or electrokinetic injection.
5. **Detector** — UV-Vis, fluorescence, mass spectrometry (CE–MS).
6. **Data system** — records electropherogram (signal vs time).
7. **Electrodes and reservoirs** — at each capillary end.

### Modes of CE

1. **Capillary Zone Electrophoresis (CZE)** — simplest; separation by intrinsic electrophoretic mobility.
2. **Micellar Electrokinetic Chromatography (MEKC)** — uses micelles (surfactant in BGE) to separate neutral molecules by partitioning between micelle and aqueous phase (like pseudo-stationary phase).
3. **Capillary Isoelectric Focusing (CIEF)** — separates ampholytes by isoelectric point (pI) using pH gradient.
4. **Capillary Gel Electrophoresis (CGE)** — sieving matrix inside capillary; used for DNA, proteins, peptides by size.
5. **Capillary Electrochromatography (CEC)** — hybrid of CE and LC (stationary phase coated in capillary).

### Advantages and applications in pharmaceuticals

- **High efficiency (sharp peaks), rapid separation, low sample volume**, and versatility.
- **Applications**: separation of small ions, drug enantiomers (with chiral selectors), peptides, proteins, degradation products, purity analysis, charge heterogeneity of biologics, pharmaceutical formulation analysis.
- **CE–MS** enables high sensitivity and structural information.

### Limitations

- Detection sensitivity sometimes lower than HPLC (small path length), though laser-induced fluorescence and CE–MS mitigate that.
- Reproducibility can be affected by EOF variability and capillary surface conditions; needs careful control.

## Q3 (d) Give difference between $^{13}C$ NMR and $^{1}H$ NMR.

| Feature | $^{1}H$ NMR | $^{13}C$ NMR |
|---|---|---|
| **Nucleus abundance** | $^{1}H$ ~99.98% (high) | $^{13}C$ ~1.1% (low natural abundance) |
| **Sensitivity** | High | Much lower (requires more scans) |
| **Chemical shift range** | ~0–12 ppm | Much wider: ~0–220 ppm (better dispersion) |
| **Multiplicity** | Complex coupling (JHH), splitting patterns reveal neighbors (n+1 rule) | Often run with **broadband $^{1}H$ decoupling**, so usually singlets for each carbon — simplifies spectrum |
| **Signal intensity** | Proportional to number of protons (integration simple) | Integration not straightforward (signal size influenced by relaxation, NOE) — not reliable for quantitation without special methods |
| **Coupling constants** | JHH well observed | One-bond $^{1}JCH$ large (~125–250 Hz) if observed; long-range couplings small |
| **NOE effects** | Present | $^{13}C$ shows strong NOE enhancement when proton decoupled (alters intensities) |
| **Applications** | Proton environment, connectivity via coupling, integration gives proton counts | Carbon skeleton identification, distinguishing carbon types (quaternary vs CH/$CH_2$/$CH_3$ via DEPT), better for complex molecules due to wide chemical shift spread |
| **Acquisition time** | Shorter | Longer (more scans to improve S/N) |

**Additional notes**:

- **DEPT (Distortionless Enhancement by Polarization Transfer)** experiments are used in $^{13}C$ NMR to classify carbons: $CH_3$, $CH_2$, CH, and quaternary (no signal in DEPT).
- $^{13}C$ is powerful for establishing backbone carbon types and observing carbonyls, aromatic carbons, etc., because of large chemical shift window.

---

## Q3 (e) Write types of vibrations in IR Spectrometry and Hooke's Law.

### Types of molecular vibrations (simple)

1. **Stretching vibrations** (change in bond length)
   - **Symmetric stretching**: two bonds stretch in phase (e.g., in $CO_2$ symmetric stretch).
   - **Asymmetric stretching**: bonds stretch out of phase.
2. **Bending (deformation) vibrations** (change in bond angle)
   - **Scissoring**: in-plane angle decrease/increase (like two H on $CH_2$).
   - **Rocking**: in-plane movement together.
   - **Wagging**: out-of-plane up/down movement.
   - **Twisting**: out-of-plane twisting motion.
3. **Out-of-plane vibrations** — especially for substituted benzene rings (C–H oop bending gives substitution pattern clues).

**Note**: For polyatomic molecules, vibrations are collective; number of fundamental vibrations = 3N − 6 (nonlinear) or 3N − 5 (linear), where N = number of atoms.

---

### Hooke's Law as applied to vibrational frequency

- Treat a chemical bond as a simple **harmonic oscillator** (two masses connected by a spring). The vibrational frequency (in wavenumbers, $\tilde{\nu}$, $cm^{-1}$) is given by the relation derived from Hooke's law:

$$\tilde{\nu} = \frac{1}{2\pi c}\sqrt{\frac{k}{\mu}}$$

Where:

- $\tilde{\nu}$ = vibrational frequency in $cm^{-1}$ (wavenumber)
- $c$ = speed of light (cm $s^{-1}$)
- $k$ = force constant of the bond (N $m^{-1}$) — represents bond strength; larger $k$ → higher frequency.
- $\mu$ = reduced mass of the two atoms (kg), defined as:

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

Where $m_1$ and $m_2$ are masses of the two atoms.

**Interpretation**:

- **Stronger bonds (larger k)** → higher stretching frequencies (e.g., C≡C or C≡N higher than C=C higher than C–C).
- **Lighter atoms** (smaller μ) → higher frequency (e.g., C–H stretch ~3000 $cm^{-1}$ higher than C–D).

---

# Q4

## (a) Give block diagram of Mass Spectrometer with function of each port. Describe various types of Mass Analyzers.

### Simple block diagram (text form)

1. **Sample inlet** → 2. **Ion source** → 3. **Mass analyzer** → 4. **Detector** → 5. **Vacuum system** → 6. **Data system / processor**

### Function of each part (simple)

1. **Sample inlet / introduction**
   - Introduce sample (gas, liquid, solid) into ion source. In GC–MS sample comes from GC; in LC–MS via interface (ESI/APCI).
2. **Ion source (ionization)**
   - Converts neutral molecules into ions (molecular and fragment ions). Common sources: EI (electron ionization), CI (chemical ionization), ESI (electrospray), APCI, MALDI. Choice affects fragmentation and sensitivity.
3. **Mass analyzer**
   - Separates ions by mass-to-charge ratio (m/z). Types vary in resolving power, mass range, speed, and MS/MS capability.
4. **Detector**
   - Detects separated ions (current, electron multiplier, microchannel plate, Faraday cup) and converts to electrical signal. Detector sensitivity and linearity matter.
5. **Vacuum system**
   - Pumps (rotary, turbomolecular) maintain high vacuum in analyzer/detector regions to allow free ion travel and avoid collisions.
6. **Data system / electronics**
   - Processes signals, records mass spectrum, controls instrument, performs calibration, and data analysis.

---

## Types of Mass Analyzers (with simple descriptions)

1. **Quadrupole mass filter (Q)**
   - Four parallel rods with DC + RF voltages. Only ions with specific m/z have stable trajectories and pass to detector.
   - **Pros**: robust, inexpensive, fast scanning, good for quantitative work.
   - **Cons**: moderate resolving power (~1000).
2. **Time-of-Flight (TOF)**
   - Ions accelerated into field-free region; time to detector depends on m/z (lighter ions arrive first).
   - **Pros**: very high mass range, fast, high resolution with reflectron.
   - **Cons**: needs pulsed ion source or orthogonal acceleration.
3. **Magnetic sector / Double focusing (magnetic + electrostatic)**
   - Ions bent in magnetic field; separation by momentum (m/z). Double focusing (electric + magnetic) improves resolution and mass accuracy.
   - **Pros**: high resolution and mass accuracy.
   - **Cons**: bulky, expensive, slower scanning.
4. **Ion trap (3D Paul trap / linear ion trap)**
   - Traps ions using RF fields; can isolate and eject ions sequentially to detector. Useful for $MS^n$ experiments (multiple stages of fragmentation).
   - **Pros**: $MS^n$ capability, high sensitivity.
   - **Cons**: limited mass range, space charge issues.
5. **Orbitrap**
   - Traps ions in electrostatic field; ions orbit and axial oscillation frequency is measured — gives very high resolving power and mass accuracy.
   - **Pros**: high resolution and mass accuracy.
   - **Cons**: more complex and costly.
6. **Fourier Transform Ion Cyclotron Resonance (FT-ICR)**
   - Uses high magnetic field; ions orbit and induce image current; Fourier transform gives high resolution and mass accuracy.
   - **Pros**: highest resolution and mass accuracy.
   - **Cons**: expensive, needs superconducting magnet.
7. **Hybrid analyzers**
   - Combine analyzers for capabilities (e.g., Q-TOF, Q-Exactive (Q + Orbitrap), triple quadrupole QqQ).
   - **Triple quadrupole (QqQ)**: Q1 selects precursor m/z, Q2 collision cell (CID) fragments, Q3 analyzes fragments → excellent for quantitation (MRM).
   - **Q-TOF**: quadrupole for selection + TOF for high-res measurement.

**Choosing an analyzer**: depends on required resolution, mass accuracy, speed, MS/MS capability, and application (quantitation vs identification).

---

# (OR) Q4 (b) Define Chemical Shift. Write its significance and formulas. Describe the factors affecting chemical shift in NMR.

## Definition (simple)

- **Chemical shift (δ)** is a measure of how much the resonance frequency of a nucleus differs from a reference (commonly TMS for $^1H$ and $^{13}C$) due to its electronic environment. It is reported in **parts per million (ppm)**.

**Formula**

$$\delta(\text{ppm}) = \frac{\nu_{\text{sample}} - \nu_{\text{ref}}}{\nu_{\text{ref}}} \times 10^6$$

Where:

- $\nu_{\text{sample}}$ = resonance frequency of nucleus in sample
- $\nu_{\text{ref}}$ = resonance frequency of reference (e.g., tetramethylsilane, TMS)

Also, resonance frequency relates to magnetic field:

$$\nu = \frac{\gamma}{2\pi} B_0 (1 - \sigma)$$

Where:

- $\gamma$ = gyromagnetic ratio
- $B_0$ = external magnetic field
- $\sigma$ = shielding constant (electrons shield nucleus from $B_0$)

**Chemical shift arises because** electrons create a local magnetic field opposing $B_0$; different environments change shielding (σ), shifting resonance.

## Significance

- Chemical shift tells you about **electronic environment**: type of functional group, hybridization, electronegativity of neighboring atoms, aromaticity, hydrogen bonding. It is a primary tool for structural assignment.

## Factors affecting chemical shift

1. **Electronegativity of neighboring atoms**
   - Electronegative atoms (O, N, halogens) withdraw electron density → **deshielding** → **downfield shift** (higher δ). Example: $CH_3$–Cl proton appears more downfield than $CH_3$–$CH_3$.
2. **Hybridization**
   - sp² carbons (alkenes, aromatics) cause protons to be more deshielded vs sp³. E.g., aromatic protons ~7 ppm, vinyl protons ~4.5–6.5 ppm.
3. **Aromatic ring currents / anisotropy**
   - In aromatic rings, circulating π electrons create induced magnetic fields; protons in different positions experience upfield or downfield shifts (e.g., benzene protons ~7.2 ppm; anisotropic effects near carbonyl or triple bond shift nearby protons).
4. **Hydrogen bonding**
   - H-bond donors (OH, NH) often appear downfield and broadened; extent depends on solvent, concentration, temperature.
5. **Magnetic anisotropy of functional groups**
   - Carbonyls, nitriles, alkynes have anisotropic fields that shift nearby protons.
6. **Substituent effects and electronegativity via inductive/resonance**
   - Electron-withdrawing substituents shift neighboring nuclei downfield; electron-donating groups shift upfield sometimes.
7. **Solvent and temperature**
   - Solvent polarity and hydrogen bonding ability change chemical shifts; temperature affects exchangeable protons.
8. **Conformational effects and steric crowding**
   - Different conformers expose protons to different electronic environments → shift differences.
9. **Reference and field strength**
   - Chemical shift in ppm is independent of magnetic field (advantage), but absolute frequencies vary with $B_0$.

---

- Moisture and solvent content, thermal stability, decomposition temperatures, compositional analysis, polymer degradation, ash content, kinetics of weight loss, compatibility testing.

---

## Q5a) Principle and Instrumentation of Thermogravimetric Analysis (TGA)

**Principle:**
Thermogravimetric Analysis (TGA) is a technique used to measure the change in weight of a sample as a function of temperature or time in a controlled environment. When a substance is heated, cooled, or held at a constant temperature, physical or chemical changes occur which result in weight loss (like evaporation of solvent, decomposition) or weight gain (oxidation). By monitoring these changes, we can study the thermal stability, composition, and purity of a substance.

**Instrumentation:**

1. **Furnace/Heating Unit:** Provides a precisely controlled temperature environment for the sample. Heating can be linear (ramp) or stepwise.
2. **Sample Holder/Pan:** Usually made of inert materials such as platinum, alumina, or ceramic. The pan holds the sample during heating.
3. **Balance:** Ultra-sensitive microbalance capable of detecting minute changes in weight.
4. **Temperature Controller:** Ensures accurate and reproducible heating rates.
5. **Recording System:** Produces a TGA curve (weight % vs. temperature/time) for analysis.

**Applications in Pharmaceuticals:**

- Studying thermal stability of drugs and excipients.
- Detecting residual solvents in powders and tablets.
- Characterizing polymers, co-crystals, and hydrates.
- Predicting shelf-life and storage conditions of pharmaceuticals.
- Investigating degradation patterns for formulation development.

---

## Q5b) Production of X-rays and Different X-ray Diffraction (XRD) Methods

**Production of X-rays:**
X-rays are generated when high-energy electrons collide with a metal target (typically copper, molybdenum, or tungsten). The sudden deceleration of electrons produces **bremsstrahlung radiation** (continuous spectrum), and transitions of electrons between inner-shell orbitals of the target produce **characteristic X-rays** of specific wavelengths.

**X-ray Diffraction (XRD) Methods:**

1. **Powder XRD:** Used for powdered crystalline samples. Produces diffraction pattern that identifies phases and crystal structure.
2. **Single Crystal XRD:** Applied to a single crystal to determine detailed 3D atomic structure and bonding.
3. **Grazing Incidence XRD (GIXRD):** Surface-sensitive method for thin films and coatings.
4. **High-Resolution XRD:** Used for detailed lattice parameter analysis and strain measurements.

**Applications:**

- Identification of polymorphs in drugs.
- Characterization of crystal lattice, unit cell dimensions.
- Detection of impurities and amorphous content in formulations.
- Quality control in pharmaceutical manufacturing.

---

## Q5c) Principle and Applications of Flame Emission Spectroscopy (FES)

**Principle:**
Flame Emission Spectroscopy relies on the fact that when atoms are excited in a flame, their electrons jump to higher energy levels. As electrons return to their ground state, they emit light of a specific wavelength. The intensity of this emitted light is proportional to the concentration of the element in the sample.

**Instrumentation:**

1. **Nebulizer:** Converts liquid sample into fine mist for introduction into the flame.
2. **Flame:** Air-acetylene or nitrous oxide-acetylene flame excites the atoms.
3. **Monochromator:** Selects the wavelength of light corresponding to the element of interest.
4. **Detector:** Usually a photomultiplier tube that measures light intensity.
5. **Data System:** Converts light intensity into concentration using calibration curves.

**Applications in Pharmaceuticals:**

- Determination of alkali and alkaline earth metals ($Na^+$, $K^+$, $Ca^{2+}$) in raw materials and formulations.
- Trace element analysis in drugs and nutraceuticals.
- Monitoring and quality control during manufacturing.
- Studying elemental impurities in excipients.

---

## Q5d) Instrumentation of X-ray Diffraction Techniques

**Components and Function:**

1. **X-ray Source:** Typically Cu-Kα radiation. Provides a consistent and monochromatic beam of X-rays.
2. **Sample Holder:** Holds powder, single crystal, or thin film sample in correct orientation.
3. **Goniometer:** Rotates sample and detector to measure diffraction angles (2θ).
4. **Detector:** Records diffracted X-rays. Older instruments use scintillation counters, modern systems use CCD detectors.
5. **Data System:** Analyzes diffraction pattern to determine crystal structure, lattice parameters, and phase composition.

**Applications:**

- Determination of crystal structure of pharmaceutical solids.
- Identification of polymorphic forms.
- Characterization of excipients, APIs, and inorganic compounds.
- Quality control and impurity detection.
- Research in drug formulation and development.

---

## Q5e) Moving Boundary Electrophoresis (MBE)

**Principle:**
Moving Boundary Electrophoresis is one of the earliest techniques used to separate charged particles in a solution under the influence of an electric field. The ions move at different rates depending on their charge-to-mass ratio, producing visible moving boundaries.

**Instrumentation:**

1. **Electrophoresis Tube:** Vertical tube filled with buffer solution.
2. **Electrodes:** Positioned at top and bottom to apply electric field.
3. **Sample Introduction:** Sample introduced at top; ions migrate down the tube.
4. **Observation:** Movement of boundaries can be visually observed or detected optically.

**Applications:**

- Early separation and analysis of proteins, amino acids, and peptides.
- Basis for modern techniques like capillary electrophoresis.
- Educational tool for demonstrating electrophoretic mobility.
- Studying ion transport properties and mobility in solutions.

---