BP704T · B.Pharm Semester VII · Theory Notes

Modern Analytical Techniques

Unit I — NMR and Mass Spectrometry

Unit I (10 hours)

Pills & Python Notes

From-zero teaching standard: every technical term is defined before use.

Syllabus coverage: Unit I (10 hours).

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CHAPTER 1: Nuclear Magnetic Resonance Spectroscopy

1.1 Learning Objectives

After studying this chapter, you will be able to:

1 Explain the basic principle of NMR spectroscopy in simple words.

2 State the principles of ^{1}H-NMR and ^{13}C-NMR spectroscopy.

3 Name the common NMR solvents and explain why deuterated solvents are used.

4 Define chemical shift, write its formula, and solve a numerical based on it.

5 List and explain the factors affecting chemical shift (electronegativity, anisotropy, hydrogen bonding, hybridization).

6 Explain coupling constant (J), spin–spin coupling, and the n+1 rule for splitting patterns.

7 Explain relaxation (T1 and T2) in simple terms.

8 Draw and label the block diagram of an FT-NMR instrument.

9 Describe special features of ^{13}C NMR (off-resonance decoupling, DEPT concept, weak signals).

10 List applications of NMR in pharmacy.

1.2 Definitions

- **Spectroscopy:** The study of how a substance absorbs or emits energy, and using that information to learn something about the substance.
- **Nuclear Magnetic Resonance (NMR) Spectroscopy:** An analytical technique that uses the magnetic properties of certain atomic nuclei (such as ^{1}H and ^{13}C) to find out the structure of a molecule.
- **Chemical Shift (δ):** The position of an NMR signal on the spectrum scale, measured in ppm (parts per million). It tells us about the environment of the nucleus in the molecule.
- **Coupling Constant (J):** The distance (in Hz) between two split peaks of a signal. It tells us about neighbouring protons.
- **Relaxation:** The process by which an excited nucleus returns to its normal (lower energy) state after absorbing energy.
- **TMS (Tetramethylsilane):** The standard reference compound used in NMR; its signal is taken as 0 ppm.
- **Fourier Transform (FT):** A mathematical method that converts a signal collected over time into a spectrum (signal vs. frequency).

1.3 Terminology Table

Term	Simple Meaning
Nucleus	The centre of an atom (contains protons and neutrons)
Spin	A nucleus behaves like a tiny spinning magnet
Magnetic field (B_0)	The strong external magnetic field inside the NMR machine
Resonance	When a nucleus absorbs energy exactly matching its "spin frequency"
ppm	Parts per million — the unit of chemical shift
Signal / Peak	A line on the NMR spectrum showing a group of nuclei
Integration	The area under a peak; tells how many nuclei give that signal
Shielding	Surrounding electrons protect (shield) the nucleus from the magnetic field
Deshielding	When shielding is reduced, the nucleus feels more magnetic field
Multiplicity	How a signal splits (singlet, doublet, triplet, quartet...)
Free Induction Decay (FID)	The raw time-signal collected by the FT-NMR detector
Probe	The part of the instrument that holds the sample tube
Deuterated solvent	A solvent in which hydrogen atoms are replaced by deuterium (^2H)
DEPT	Distortionless Enhancement by Polarization Transfer — a ^{13}C technique that tells CH_3, CH_2, CH, and C apart
Off-resonance decoupling	A ^{13}C NMR mode that shows split signals so we can identify carbon types

1.4 Simple Explanations

1.4.1 What is NMR? (The Simple Idea)

Think of every hydrogen nucleus (^1H) in a molecule as a tiny spinning bar magnet. Normally these tiny magnets point in random directions. When we place the molecule inside a very strong magnet (the NMR machine), the tiny magnets line up in two groups — most pointing with the big magnet (low energy), a few pointing against it (high energy).

Now we send a radio wave of exactly the right frequency. The nuclei in the low-energy group absorb this energy and flip to the high-energy state. This flipping is called **resonance**. After absorbing, they fall back and give out energy, which we detect. The exact frequency at which each nucleus absorbs depends on its surroundings in the molecule. So an NMR spectrum is like a **fingerprint of the molecule's structure**.

> **Key takeaway:** NMR tells us how atoms are connected in a molecule, because each nucleus reports on its own chemical environment.

1.4.2 Why only some nuclei show NMR

Only nuclei that have "spin" show NMR signals. A nucleus has spin when its proton number and neutron number are not both even.

- ^1H (hydrogen-1): spin — gives signals ✔

- ^{13}C (carbon-13): spin — gives signals ✔
- ^{12}C (common carbon): no spin — silent ✘
- ^{16}O (common oxygen): no spin — silent ✘

This is why ^{1}H-NMR and ^{13}C-NMR are the two most used types: hydrogen and carbon are present in almost all drug molecules.

1.4.3 ^{1}H-NMR — Principle

In ^{1}H-NMR, we study hydrogen nuclei. Protons in different parts of a molecule (e.g., a $-CH_3$ group vs. a –OH group) are surrounded by different electrons, so they absorb at slightly different frequencies. The spectrum shows:

1 **Number of signals** → how many different *types* of protons exist.

2 **Position (chemical shift)** → the chemical environment of each proton type.

3 **Integration (area)** → how many protons give each signal.

4 **Splitting pattern** → how many neighbouring protons exist.

Example: CH_3–CH_2–OH (ethanol) shows 3 signals — one for CH_3, one for CH_2, one for OH.

> **Key takeaway:** ^{1}H-NMR is like asking every hydrogen in the molecule "who are your neighbours?" and writing down the answers.

1.4.4 ^{13}C-NMR — Principle

In ^{13}C-NMR, we study carbon-13 nuclei instead of protons. It tells us how many different types of carbon atoms are in the molecule and their environments. Because ^{13}C is only about 1.1% of all carbon atoms in nature, its signals are weak — we need more sample and more scans (this is discussed in detail in section 1.4.13).

1.5 Chemical Shift

1.5.1 Concept

Chemical shift (δ) is the position of a signal on the NMR scale, measured in ppm (parts per million). It is measured relative to a reference compound, TMS (tetramethylsilane), whose signal is set at 0 ppm.

Why not just use frequency (Hz)? Because the frequency depends on the strength of the machine's magnet. A 400 MHz machine and a 600 MHz machine would give different Hz values for the same proton. Dividing by the operating frequency makes δ a **universal number** — the same on any machine.

1.5.2 Chemical Shift Formula

$$\delta\ (\text{ppm}) = \frac{\nu(\text{sample}) - \nu(\text{reference})}{\nu(\text{operating})} \times 10^{6}$$

- ν(sample) = frequency of the sample signal (Hz)
- ν(reference) = frequency of the TMS signal (Hz)

- ν(operating) = operating frequency of the instrument (Hz), e.g., 400 MHz = 400 × 10^6 Hz

1.5.3 Worked Numerical Example

Question: On a 400 MHz NMR instrument, a proton signal appears at 400,001,200 Hz and TMS appears at 400,000,000 Hz. Calculate the chemical shift in ppm.

Solution — step by step:

Step 1: Find the difference between sample and reference frequencies. ν(sample) − ν(reference) = 400,001,200 − 400,000,000 = 1,200 Hz

Step 2: Write the operating frequency in Hz. ν(operating) = 400 MHz = 400 × 10^6 Hz = 400,000,000 Hz

Step 3: Apply the formula. δ = (1,200 / 400,000,000) × 10^6

Step 4: Simplify. 1,200 / 400,000,000 = 0.000003 = 3 × 10^{-6}

Step 5: Multiply by 10^6. δ = 3 × 10^{-6} × 10^6 = **3.0 ppm**

Answer: δ = 3.0 ppm.

Key takeaway: The chemical shift formula converts a machine-dependent frequency into a universal ppm value.

1.6 Solvents Used in NMR

1.6.1 Why Deuterated Solvents?

The solvent is the liquid in which the drug sample is dissolved. In ^{1}H-NMR, we must use **deuterated solvents** — solvents in which hydrogen atoms are replaced by deuterium (^{2}H, "heavy hydrogen"). Why?

1. Ordinary solvents like $CHCl_3$ contain ^{1}H, which would give a huge signal and hide the sample's signals. Deuterium gives signals at a very different frequency, so it does not disturb the ^{1}H spectrum.
2. The deuterium signal is used by the instrument to "lock" the magnetic field steady during the experiment.

1.6.2 Common NMR Solvents

Solvent	Short Form	Used For
Deuterated chloroform	$CDCl_3$	Most organic compounds, first choice
Deuterated dimethyl sulfoxide	DMSO-d_6	Polar drugs, compounds not soluble in $CDCl_3$
Deuterated water	D_2O	Water-soluble drugs, salts
Deuterated methanol	CD_3OD	Polar compounds, alcohols
Deuterated acetone	$(CD_3)_2CO$	General polar organics

Key takeaway: Choose a solvent that dissolves the sample, has no ^{1}H signals, and does not react with the sample.

1.7 Factors Affecting Chemical Shift

1.7.1 Electronegativity

Electronegativity means the power of an atom to pull electrons towards itself. When an electronegative atom (like F, O, Cl, N) is near a proton, it pulls electrons away from the proton. The proton becomes **deshielded** (less protected), feels more magnetic field, and its signal moves to higher δ (downfield).

Example: CH_3–F appears at higher δ (~4.3 ppm) than CH_3–H (methane, ~0.2 ppm), because fluorine pulls electrons away.

Analogy: Imagine a person (the proton) standing under an umbrella (electrons). An electronegative atom is like a strong wind that snatches the umbrella away — the person now feels the full rain (magnetic field).

1.7.2 Anisotropy (Magnetic Anisotropy)

Some groups (like C=C double bonds, C≡C triple bonds, aromatic benzene rings, C=O) create their own small magnetic fields when placed in the big magnet. These small fields add to or subtract from the main field depending on the direction. This is called **anisotropy** ("different in different directions").

Example: Protons on a benzene ring appear around 7–8 ppm (very downfield) because of the ring current effect — the circulating electrons of the ring create a field that adds to the main field.

Analogy: Like a ceiling fan that blows air down in the centre but pulls air up at the sides — the effect depends on where you stand.

1.7.3 Hydrogen Bonding

When a proton forms a hydrogen bond (e.g., –OH, –NH groups), electrons are pulled away from it, so it becomes deshielded and its signal moves downfield (higher δ). Stronger hydrogen bonding = larger shift.

Example: The –OH proton of phenol appears at higher δ than the –OH of a simple alcohol, because phenol forms stronger hydrogen bonds.

1.7.4 Hybridization

The type of carbon the proton is attached to matters:

Attached carbon	Hybridization	Typical δ range
–CH_3 (alkane)	sp^3	0.9–1.5 ppm
–CH_2– next to C=C	sp^3 (allylic)	~1.6–2.5 ppm
=CH– (alkene)	sp^2	4.5–6.5 ppm
≡C–H (alkyne)	sp	2.0–3.0 ppm
Ar–H (aromatic)	sp^2 (ring)	6.5–8.5 ppm
–CHO (aldehyde)	sp^2 (C=O)	9–10 ppm

More "s-character" ($sp > sp^2 > sp^3$) pulls electrons closer to carbon, deshielding attached protons.

Key takeaway: Chemical shift is decided by how much electron protection (shielding) the nucleus gets — anything that removes electrons pushes the signal downfield.

1.8 Coupling Constant and Spin–Spin Coupling

1.8.1 Spin–Spin Coupling (Simple Idea)

Neighbouring magnetic nuclei "talk" to each other through the chemical bonds between them. A proton feels not only the big magnet but also the tiny magnetic fields of its neighbouring protons. This neighbour effect **splits** its signal into two or more peaks. This is called **spin–spin coupling** (or scalar coupling).

Analogy: Imagine a person trying to listen to music while neighbours are talking. Depending on whether each neighbour is talking loudly or softly, the person hears the music slightly differently. Similarly, a proton's signal splits depending on the spin states (up or down) of its neighbours.

1.8.2 The n+1 Rule and Multiplicity

If a proton has **n equivalent neighbouring protons**, its signal splits into **n + 1 peaks**.

Neighbours (n)	Peaks (n+1)	Name	Intensity pattern
0	1	Singlet	1
1	2	Doublet	1 : 1
2	3	Triplet	1 : 2 : 1
3	4	Quartet	1 : 3 : 3 : 1

Example (ethanol, CH_3–CH_2–OH): the CH_3 protons have 2 neighbours (the CH_2) → triplet; the CH_2 protons have 3 neighbours (the CH_3) → quartet.

1.8.3 Coupling Constant (J)

The **coupling constant J** is the distance between two adjacent split peaks, measured in Hz. Important points:

- J is measured in **Hz** (not ppm), because it does not depend on the magnet strength — it is the same on every instrument.
- Typical ^{1}H–^{1}H coupling constants: 0–15 Hz (for protons on neighbouring carbons, usually 6–8 Hz).
- Typical ^{13}C–^{1}H coupling constants: 120–250 Hz (much larger).
- In routine ^{13}C NMR, proton decoupling removes ^{13}C–^{1}H splitting, so each carbon gives a single peak (singlet) — this simplifies the spectrum.

Key takeaway: Splitting tells us the number of neighbours (n+1 rule); J tells us the strength of the magnetic conversation between them.

1.9 Relaxation

1.9.1 What is Relaxation? (Simple Idea)

After a nucleus absorbs energy and jumps to the high-energy state, it does not stay there forever — it falls back to the low-energy state, giving out energy. This "coming back to normal" is called **relaxation**.

Analogy: Like a stretched rubber band — when you release it, it snaps back to its original shape. Relaxation is the nucleus snapping back to its normal state.

1.9.2 Two Types of Relaxation

Type	Name	Simple Meaning
T1	Spin–lattice relaxation	The nucleus gives its extra energy to the surroundings ("lattice") and returns to the low-energy state. T1 is the time for this.
T2	Spin–spin relaxation	Neighbouring nuclei exchange energy with each other, causing the signal to fade. T2 decides how sharp or broad a peak is — short T2 = broad peak.

- Long T1 means we must wait longer between scans, otherwise the signal becomes weak.
- ^{13}C nuclei have long T1 values, which is one reason ^{13}C NMR needs more time.

Key takeaway: Relaxation is simply the nucleus returning to normal after being excited; T1 is energy loss to surroundings, T2 is energy exchange between neighbours.

1.10 Instrumentation of FT-NMR

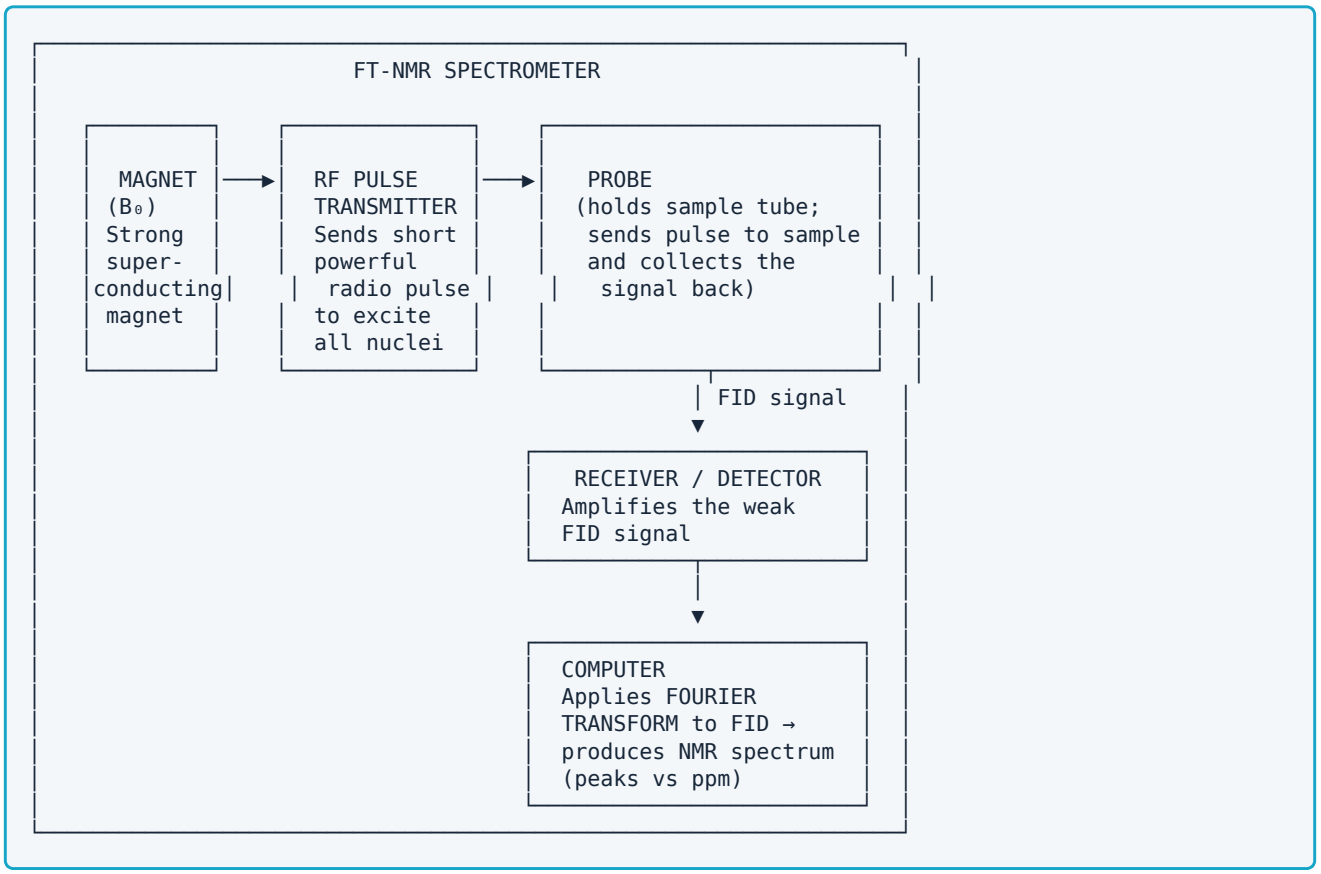
1.10.1 Why "FT"? (Simple Explanation of Fourier Transform)

Old NMR machines scanned one frequency at a time — very slow. **FT-NMR** sends a short, powerful radio pulse that excites ALL nuclei at once. The detector records all their signals together as a wiggly signal over time — this is the **Free Induction Decay (FID)**.

The FID is a mixture — like hearing a whole orchestra playing at once. The **Fourier Transform** is a mathematical tool (done by computer) that separates this mixture into individual frequencies — like identifying each instrument in the orchestra. The result is the familiar spectrum: peaks vs. ppm.

Analogy: FID is like a mixed fruit juice; Fourier Transform is the machine that tells you exactly how much mango, orange, and apple went into it.

1.10.2 Block Diagram of FT-NMR (All Parts Labelled)



Parts and their jobs:

Part	Job
Magnet (B_0)	Provides the strong, steady magnetic field; sample sits inside it
RF transmitter	Sends the short radio-frequency pulse that excites nuclei
Probe	Holds the spinning sample tube; has coils that send and receive signals
Receiver / Detector	Collects and amplifies the weak FID signal from the sample
Computer	Applies Fourier Transform to convert FID into the final spectrum

1.11 ^{13}C NMR — Special Points

1 **Weaker signals:** Only ~1.1% of carbon atoms are ^{13}C, so signals are weak. We run many scans and add them up to see clear peaks.

2 **Broad chemical shift range:** ^{13}C signals spread over 0–220 ppm (much wider than ^{1}H's 0–12 ppm), so peaks rarely overlap — easier to count carbons.

3 **Proton decoupling:** Normally each carbon signal would split due to attached protons (large J of 120–250 Hz). We apply "broadband decoupling" — a continuous radio signal that removes this splitting — so every carbon appears as a single sharp peak (singlet).

4 **Off-resonance decoupling:** A partial decoupling mode where signals still split a little: CH_3 appears as quartet, CH_2 as triplet, CH as doublet, and quaternary carbon (C with no H) as singlet. This helps identify the carbon type.

5 **DEPT (Distortionless Enhancement by Polarization Transfer):** A special experiment that gives separate spectra showing only CH_3, only CH_2, only CH groups — quaternary carbons disappear. By comparing DEPT spectra with the normal spectrum, we can label every carbon.

Key takeaway: ^{13}C NMR counts the different carbons; decoupling keeps the spectrum simple; DEPT tells us whether each carbon is CH_3, CH_2, CH, or C.

1.12 Applications of NMR in Pharmacy

Application	Simple Explanation
Structure confirmation	Confirm that a synthesized drug has the expected structure
Purity checking	Extra peaks reveal impurities in a drug sample
Quantitative NMR (qNMR)	Peak area is proportional to number of nuclei — used to find the amount (assay) of a drug without a reference standard of the same drug
Isomer distinction	Distinguish isomers that have the same formula but different arrangement
Metabolite identification	Identify how a drug changes inside the body
Natural product structure	Determine structures of compounds isolated from plants

1.13 Infographic Concept Box

INFOGRAPHIC CONCEPT: "The NMR Story in One Picture" — A central magnet (horseshoe style) labelled "Strong magnet B_0 lines up tiny nuclear magnets". Around it, four panels: (1) "TMS = 0 ppm" with a small molecule icon and a flag at zero on a scale; (2) "Chemical shift scale 0–12 ppm" with example markers — CH_3 at ~1, benzene ~7, aldehyde ~10; (3) "n+1 rule" showing a triplet labelled "2 neighbours → 3 peaks"; (4) "FT step" showing a wiggly FID line entering a computer icon and a clean peak spectrum coming out. Bottom strip: "NMR answers: HOW MANY types? WHERE (environment)? HOW MANY neighbours?"

1.14 Flowchart — How an FT-NMR Experiment Works

```
Sample dissolved in deuterated solvent
        |
        ▼
Tube placed in probe inside strong magnet (B₀)
        |
        ▼
Nuclei line up (low-energy and high-energy states)
        |
        ▼
Short RF pulse excites ALL nuclei at once
        |
        ▼
Nuclei relax, giving out energy → FID recorded
        |
        ▼
Computer applies FOURIER TRANSFORM
        |
        ▼
Spectrum obtained: peaks vs chemical shift (ppm)
        |
        ▼
Interpret: count signals → read δ → measure integration → read splitting
```

1.15 Tables

Table 1: Typical ^{1}H Chemical Shift Ranges

Proton type	δ (ppm)
Alkane CH_3, CH_2	0.9–1.5
CH next to electronegative atom	3–4.5
Alkene =CH–	4.5–6.5
Aromatic Ar–H	6.5–8.5
Aldehyde –CHO	9–10
Carboxylic acid –COOH	10–12

Table 2: ^{1}H-NMR vs ^{13}C-NMR

Feature	^{1}H-NMR	^{13}C-NMR
Nucleus studied	Hydrogen-1	Carbon-13
Natural amount	~99.98% (strong signals)	~1.1% (weak signals)
Chemical shift range	0–12 ppm	0–220 ppm
Splitting	Usually shown (n+1 rule)	Removed by decoupling (singlets)
Main use	Proton environments and neighbours	Carbon skeleton of molecule

1.16 Examples

Example 1 — Ethanol (CH_3CH_2OH): ^{1}H-NMR shows 3 signals. CH_3 (triplet, ~1.2 ppm, integration 3), CH_2 (quartet, ~3.6 ppm, integration 2 — shifted downfield because it is next to oxygen), OH (singlet, variable position).

Example 2 — Toluene ($C_6H_5CH_3$): Aromatic protons appear ~7 ppm (anisotropy of benzene ring), CH_3 ~2.3 ppm.

Example 3 — Aspirin structure confirmation: ^{1}H-NMR shows aromatic protons (4 H, ~7–8 ppm), a CH_3 of the acetyl group (~2.3 ppm), and the COOH proton (~11 ppm) — matching the expected structure.

1.17 Analogies

- **Chemical shift:** Like the pitch of voices in a choir — each singer (nucleus) sings at a slightly different pitch depending on where they stand (their environment).
- **TMS reference:** Like the zero mark on a ruler — every measurement starts from it.
- **Spin–spin coupling:** Like ripples from two stones thrown close together in water — the waves interfere and create a pattern.
- **FT (Fourier Transform):** Like a prism splitting white light into rainbow colours — FT splits the mixed FID into separate frequencies.
- **Relaxation:** Like a stretched rubber band snapping back.

1.18 Pharmacy Relevance

- Every new drug molecule synthesized in a medicinal chemistry lab is confirmed by ^{1}H and ^{13}C NMR before further testing — NMR is the "identity card" of a molecule.
- In quality control, NMR detects impurities that other methods may miss.
- qNMR is accepted as a method for assay (purity determination) of drug substances.
- For herbal drugs, NMR helps identify the active constituents in complex plant extracts.

1.19 India Context

- Indian pharmaceutical companies and national laboratories routinely use 400 MHz and higher FT-NMR instruments for drug structure confirmation and impurity profiling.
- NMR is a prescribed spectroscopic tool in B.Pharm and M.Pharm curricula across Indian universities, and skill in spectral interpretation is expected in pharmaceutical analysis jobs in Hyderabad, Mumbai, and Ahmedabad industrial clusters.
- The Indian Pharmacopoeia includes spectroscopic identification tests for drug substances, for which NMR data supports structural identity.

1.20 Key Points

1 NMR studies magnetic nuclei (^{1}H, ^{13}C) placed in a strong magnetic field.

2 Chemical shift δ (ppm) = (ν_sample − ν_reference)/ν_operating × 10^6; TMS = 0 ppm.

3 Deuterated solvents ($CDCl_3$, DMSO-d_6, D_2O, CD_3OD) avoid solvent ^{1}H signals.

4 Factors affecting δ: electronegativity, anisotropy, hydrogen bonding, hybridization.

5 n+1 rule: n equivalent neighbours split a signal into n+1 peaks.

6 J (coupling constant) is measured in Hz and is magnet-independent.

7 Relaxation: T1 (spin–lattice, energy to surroundings), T2 (spin–spin, between neighbours).

8 FT-NMR: one RF pulse excites all nuclei → FID recorded → Fourier Transform gives spectrum.

9 ^{13}C NMR: weak signals (1.1% abundance), wide range (0–220 ppm), proton decoupling gives singlets; DEPT identifies CH_3/CH_2/CH/C.

10 Applications: structure confirmation, purity, qNMR assay, metabolite ID.

1.21 Common Confusions

- **Chemical shift (ppm) vs coupling constant (Hz):** Chemical shift changes with magnet strength if measured in Hz, so we use ppm; J is always in Hz and never changes with the magnet.
- **Shielding vs deshielding:** Shielded = more electrons = signal moves upfield (low δ). Deshielded = fewer electrons = signal moves downfield (high δ). Students often reverse these.
- **^{13}C weak signals:** Not because ^{13}C is "bad at NMR" but because only 1.1% of carbons are ^{13}C.
- **Integration in ^{13}C NMR:** Peak areas in routine ^{13}C NMR are NOT reliable for counting carbons (unlike ^{1}H NMR) because of decoupling and relaxation effects.

1.22 Common Mistakes

1 Writing the chemical shift formula without × 10^6 — the answer then comes out as a tiny decimal instead of ppm.

2 Using ordinary (non-deuterated) solvents for ^{1}H-NMR — the solvent's own protons hide the sample signals.

3 Counting the TMS peak as part of the sample's signals.

4 Applying the n+1 rule to non-equivalent neighbours — the rule works simply only for equivalent neighbours.

5 Confusing "upfield" (low δ, right side) with "downfield" (high δ, left side).

6 Expecting ^{13}C–^{1}H splitting in a routine decoupled ^{13}C spectrum — decoupling removes it.

1.23 Mnemonics

- **Factors affecting chemical shift — "EAHH": E**lectronegativity, **A**nisotropy, **H**ydrogen bonding, **H**ybridization.
- **n+1 rule:** "**N**eighbours **p**lus **o**ne **p**eaks" — count neighbours, add one.
- **Relaxation types:** "**T1** = **T**o surroundings (**1** = first, energy leaves first); **T2** = **T**wo nuclei talking (**2** = pair exchange)."
- **FT steps — "PEF": P**ulse → **E**mit (FID) → **F**ourier transform.

1.24 Exam Summary

NMR spectroscopy studies magnetic nuclei (^{1}H, ^{13}C) in a strong magnetic field; resonance occurs when nuclei absorb matching radio frequency. Chemical shift δ (ppm) measures signal position relative to TMS (0 ppm); formula δ = (ν_sample − ν_reference)/ν_operating × 10^{6}. Deuterated solvents ($CDCl_3$, DMSO-d_6, D_2O, CD_3OD) are used to avoid solvent proton signals. δ is affected by electronegativity, anisotropy, hydrogen bonding, and hybridization. Spin–spin coupling splits signals per the n+1 rule; J (Hz) is magnet-independent. Relaxation (T1 spin–lattice, T2 spin–spin) returns nuclei to normal. FT-NMR uses one RF pulse for all nuclei, records the FID, and the computer's Fourier Transform produces the spectrum; block diagram: magnet → RF transmitter → probe → receiver → computer. ^{13}C NMR has weak signals (1.1% abundance), a 0–220 ppm range, proton decoupling (singlets), off-resonance decoupling, and DEPT for carbon-type identification. Applications: structure confirmation, purity, qNMR assay, isomer distinction, metabolite identification.

1.25 One-Minute Revision

- NMR = tiny nuclear magnets + big magnet + radio pulse → spectrum.
- δ (ppm) = (ν_s − ν_r)/ν_op × 10^{6}; TMS = 0.
- Deuterated solvents: $CDCl_3$, DMSO-d_6, D_2O, CD_3OD.
- EAHH affects δ: Electronegativity, Anisotropy, H-bonding, Hybridization.
- n+1: neighbours + 1 = peaks; J in Hz.
- T1 = to surroundings; T2 = between neighbours.
- FT: pulse → FID → Fourier Transform → spectrum.
- ^{13}C: weak, 0–220 ppm, decoupled singlets, DEPT = CH_3/CH_2/CH/C.

1.26 Short Answer Questions

1 Define chemical shift and write its formula.

2 Why are deuterated solvents used in ^{1}H-NMR?

3 What is TMS and why is it chosen as the reference?

4 State the n+1 rule with one example.

5 Define coupling constant. Why is it measured in Hz and not ppm?

6 What is relaxation? Name its two types.

7 What is FID in FT-NMR?

8 Why are ^{13}C NMR signals weaker than ^{1}H NMR signals?

9 What is the purpose of proton decoupling in ^{13}C NMR?

10 Give two pharmaceutical applications of NMR.

1.27 Long Answer Questions

1 Explain the principle of ^{1}H-NMR spectroscopy. Describe how the number of signals, chemical shift, integration, and splitting pattern help in structure elucidation, with the example of ethanol.

2 Define chemical shift. Derive/explain its formula and solve: on a 400 MHz instrument a signal appears at 400,001,200 Hz and TMS at 400,000,000 Hz. Calculate δ showing all steps.

3 Discuss any four factors affecting chemical shift with suitable examples.

4 Explain spin–spin coupling and the coupling constant. Describe the splitting patterns (singlet to quartet) using the n+1 rule.

5 Draw a labelled block diagram of an FT-NMR spectrometer and explain the function of each part. Explain the role of the Fourier Transform in simple terms.

6 Write a note on ^{13}C NMR covering its principle, why signals are weak, proton decoupling, off-resonance decoupling, and DEPT.

1.28 MCQs with Answer Keys

Q1. The reference compound used in NMR, assigned 0 ppm, is: a) Chloroform b) TMS c) D_2O d) Benzene **Answer: b) TMS**

Q2. Chemical shift is measured in: a) Hz b) ppm c) cm^{-1} d) nm **Answer: b) ppm**

Q3. Which solvent is most commonly used as a first choice in ^{1}H-NMR? a) H_2O b) $CHCl_3$ c) $CDCl_3$ d) Ethanol **Answer: c) $CDCl_3$**

Q4. A proton with 2 equivalent neighbouring protons will appear as a: a) Singlet b) Doublet c) Triplet d) Quartet **Answer: c) Triplet**

Q5. The coupling constant J is measured in: a) ppm b) Hz c) Tesla d) Joules **Answer: b) Hz**

Q6. T1 relaxation is also called: a) Spin–spin relaxation b) Spin–lattice relaxation c) Free induction decay d) Decoupling **Answer: b) Spin–lattice relaxation**

Q7. In FT-NMR, the raw time-domain signal is called: a) FID b) TMS c) DEPT d) J-value **Answer: a) FID**

Q8. The natural abundance of ^{13}C is approximately: a) 50% b) 12% c) 1.1% d) 99% **Answer: c) 1.1%**

Q9. The chemical shift range of ^{13}C NMR is approximately: a) 0–12 ppm b) 0–220 ppm c) 0–10 ppm d) 100–200 ppm only **Answer: b) 0–220 ppm**

Q10. DEPT experiment helps to identify: a) Molecular weight b) CH_3, CH_2, CH and quaternary carbons c) Melting point d) Optical rotation **Answer: b) CH_3, CH_2, CH and quaternary carbons**

1.29 Viva Questions

1 What does NMR stand for?

2 Which nuclei are most commonly studied in NMR?

3 Why is TMS used as a reference in NMR?

4 What is the unit of chemical shift?

5 Why do we use deuterated solvents?

6 State the n+1 rule.

7 What is the difference between T1 and T2 relaxation?

8 What is the function of the Fourier Transform in FT-NMR?

9 Why is ^{13}C NMR less sensitive than ^{1}H NMR?

10 Name any two applications of NMR in pharmacy.

1.30 Application-Based Questions

1 A drug sample's ^{1}H-NMR shows an unexpected extra singlet at 2.1 ppm integrating for 3 protons. What might this indicate, and what action would you take in quality control?

2 Two isomers have the same molecular formula. How would you use ^{1}H-NMR splitting patterns to distinguish them?

3 A chemist records a proton signal at 3.0 ppm on a 400 MHz instrument. What frequency difference (in Hz) from TMS does this correspond to? Show the calculation.

4 Why would you choose DMSO-d_6 instead of $CDCl_3$ for a highly polar drug salt?

1.31 References

- Brown
- Friebolin
- Silverstein / Webster / Kiemle / Bryce
- Skoog / Holler / Crouch

CHAPTER 2: Mass Spectrometry

2.1 Learning Objectives

After studying this chapter, you will be able to:

1 Explain the basic principle of mass spectrometry in simple words.

2 Describe the steps: ionization → separation by m/z → detection.

3 State the rules of fragmentation (nitrogen rule, Stevenson rule, McLafferty rearrangement, loss of small neutrals).

4 Compare ionization techniques: EI, CI, MALDI, FAB, API/ESI (hard vs soft).

5 Compare analyzers: time of flight (TOF), quadrupole, ion trap.

6 Name common detectors used in mass spectrometry.

7 List pharmaceutical applications of mass spectrometry.

2.2 Definitions

- **Mass Spectrometry (MS):** An analytical technique that converts molecules into charged particles (ions), separates them by their mass-to-charge ratio (m/z), and detects them to find molecular weight and structure.
- **Ionization:** The process of giving a molecule an electric charge (usually by adding or removing an electron or proton).
- **m/z:** Mass-to-charge ratio — the quantity actually measured in MS. For singly charged ions, m/z equals the mass.
- **Molecular ion ($M^{+\bullet}$):** The ion formed from the whole molecule without breaking — its m/z gives the molecular weight.
- **Fragment ion:** A smaller ion formed when the molecular ion breaks apart.
- **Base peak:** The tallest peak in a mass spectrum (assigned 100% intensity); all other peaks are measured relative to it.
- **Fragmentation:** The breaking of the molecular ion into smaller ions and neutral pieces inside the instrument.
- **Hard ionization:** Ionization that breaks the molecule into many fragments (e.g., EI).
- **Soft ionization:** Ionization that keeps the molecule mostly whole, giving mainly the molecular ion (e.g., ESI, MALDI).
- **Analyzer:** The part of the mass spectrometer that separates ions by m/z.
- **Detector:** The part that counts the ions and measures how many arrive.

2.3 Terminology Table

Term	Simple Meaning
Ion	An atom or molecule carrying an electric charge
Cation	Positively charged ion
Electron impact (EI)	Ionization by hitting molecules with high-energy electrons
Chemical ionization (CI)	Ionization by reaction with pre-made reagent ions; gentler than EI
MALDI	Matrix-Assisted Laser Desorption/Ionization — laser + matrix for large molecules
FAB	Fast Atom Bombardment — high-speed atoms knock ions out of a liquid matrix
API	Atmospheric Pressure Ionization — ionization at normal air pressure (family name)
ESI	Electrospray Ionization — a type of API; sprays charged droplets

Term	Simple Meaning
TOF	Time of Flight — ions separated by how fast they fly down a tube
Quadrupole	Four rods using electric fields to filter ions by m/z
Ion trap	A device that traps ions and releases them one m/z at a time
Vacuum	Near-empty space inside the MS; ions must travel without hitting air

2.4 Simple Explanations

2.4.1 The Basic Principle (Simple Idea)

Mass spectrometry answers one question: **"How heavy are the pieces?"** The steps are:

1 **Ionize:** Give the molecule an electric charge (make ions).

2 **Separate:** Sort the ions by their mass-to-charge ratio (m/z) — light ions behave differently from heavy ions.

3 **Detect:** Count how many ions of each m/z arrive.

Analogy: Like a post office sorting parcels — first every parcel gets a barcode sticker (ionization), then parcels travel on belts that separate light from heavy ones (analyzer), and finally each parcel is counted at the exit (detector).

2.4.2 Why Must Molecules Become Ions?

Only charged particles can be pushed, pulled, and sorted by electric and magnetic fields. A neutral molecule would just fly straight and cannot be separated by m/z. So the first step is always to give the molecule a charge.

2.4.3 Reading a Mass Spectrum

- The **x-axis** is m/z (mass-to-charge ratio).
- The **y-axis** is relative intensity (how many ions).
- The peak at the highest m/z is usually the **molecular ion ($M^{+\bullet}$)** — it tells the molecular weight.
- The tallest peak is the **base peak** (100%).
- All other peaks are **fragment ions** — broken pieces that reveal the structure.

Key takeaway: Molecular ion = molecular weight; fragment ions = structural clues; base peak = reference for all heights.

2.5 Fragmentation and Its Rules

2.5.1 What is Fragmentation?

In hard ionization (EI), the molecular ion gets excess energy and breaks into smaller pieces — like a clay pot shattering when dropped. The pattern of pieces is characteristic of the molecule's structure, so

fragmentation helps identify unknown compounds.

2.5.2 Rules of Fragmentation

Rule 1 — Nitrogen rule: If a molecule has an odd number of nitrogen atoms, its molecular ion appears at an **odd** m/z value. With zero or an even number of nitrogens, the molecular ion appears at an **even** m/z.

Example: A drug with formula $C_8H_{10}N_4O_2$ (caffeine, 4 nitrogens — even) has its molecular ion at m/z 194 (even). A molecule with 1 nitrogen would show an odd molecular ion.

Rule 2 — Stevenson rule: When a molecular ion breaks into two pieces, the positive charge stays on the fragment that can handle it best (the one with the lower ionization energy — usually the more stable piece). In simple words: **the charge goes to the stronger, more stable fragment.**

Rule 3 — McLafferty rearrangement: A special, common rearrangement in molecules containing a C=O group (ketones, acids, esters). A hydrogen atom from the gamma (third) carbon jumps to the oxygen through a six-membered ring arrangement, and the molecule splits — typically losing an alkene piece. It is a favourite exam topic because it is predictable.

Rule 4 — Loss of small neutral molecules: Molecular ions commonly lose small, stable neutral pieces. Learning these losses helps read spectra:

Loss (mass)	Piece lost	Hint
15	CH_3• (methyl)	Methyl group present
18	H_2O (water)	Alcohol present
28	CO or C_2H_4	Carbonyl or ethyl group
29	C_2H_5• (ethyl)	Ethyl group present
31	OCH_3	Methoxy group
43	CH_3CO•	Acetyl group
44	CO_2	Carboxylic acid

Example: If the molecular ion is at m/z 150 and a strong peak appears at m/z 132, the loss is 18 → water was lost → the molecule likely contains an –OH group.

Key takeaway: Fragmentation follows rules; each common mass loss is a clue about a functional group.

2.6 Ionization Techniques

2.6.1 Hard vs Soft Ionization (Simple Idea)

- **Hard ionization (EI):** Hits the molecule hard — lots of fragments, rich structural information, but the molecular ion may be weak or missing.
- **Soft ionization (CI, ESI/API, MALDI, FAB):** Handles the molecule gently — mainly the whole molecular ion, little fragmentation; best for finding molecular weight, especially of large or fragile molecules.

Analogy: Hard ionization is like smashing a clay pot to study its pieces; soft ionization is like weighing the whole pot intact.

2.6.2 Comparison Table — Ionization Techniques

Technique	Full Name	Hard / Soft	How It Works (Simply)	Best For
EI	Electron Impact	Hard	High-energy electrons knock an electron out of the molecule	Small, stable, volatile organics; rich fragmentation
CI	Chemical Ionization	Soft	Reagent gas ions gently pass a proton to the molecule	Molecular weight of small organics when EI destroys $M^{+\bullet}$
FAB	Fast Atom Bombardment	Soft	Fast atoms knock ions out of sample dissolved in liquid matrix	Polar, non-volatile compounds
MALDI	Matrix-Assisted Laser Desorption/Ionization	Soft	Laser fires at sample mixed in a light-absorbing matrix	Large biomolecules: proteins, peptides, polymers
API (ESI)	Atmospheric Pressure Ionization (Electrospray)	Soft	Liquid sprayed into fine charged droplets at normal pressure; solvent evaporates leaving ions	Drug molecules, LC-MS coupling, polar compounds

2.6.3 EI vs CI — Key Difference

In EI, a 70 eV electron beam hits the molecule directly — violent, many fragments. In CI, the electron beam first ionizes a reagent gas (like methane), and these reagent ions gently transfer a proton to the sample molecule — the molecule survives mostly whole, giving a strong $(M+H)^+$ peak.

2.7 Analyzers

2.7.1 What Does an Analyzer Do?

The analyzer separates the ions by m/z so the detector can count each type one by one.

2.7.2 Comparison Table — Analyzers

Analyzer	Principle (One Simple Line)	Strengths	Limits
Time of Flight (TOF)	All ions get the same energy; lighter ions fly faster down a tube and arrive first	Very fast, wide mass range, good for large molecules	Needs precise timing electronics
Quadrupole	Four rods with electric fields let only one m/z pass through at a time (mass filter)	Compact, cheap, ideal for routine LC-MS/GC-MS	Limited mass range, lower resolution
Ion trap	Ions are trapped in a small space by electric fields, then released one m/z at a time	MS/MS capability (fragment-of-fragment studies), compact	Limited ion capacity, lower mass range

Simple one-line memory: TOF = race down a tube; Quadrupole = gate that opens for one m/z; Ion trap = cage that releases ions one by one.

2.7.3 Flowchart — How a Mass Spectrometer Works

```
Sample inlet (gas, liquid, or solid sample introduced)
        |
        ▼
Ion source — molecules become ions (EI / CI / MALDI / FAB / ESI)
        |
        ▼
Analyzer — ions separated by m/z (TOF / quadrupole / ion trap)
   (under high vacuum so ions don't hit air molecules)
        |
        ▼
Detector — ions counted; signal amplified
        |
        ▼
Computer — mass spectrum drawn (m/z on x-axis, intensity on y-axis)
```

2.8 Detectors (Briefly)

Detector	Simple Working
Electron multiplier	Each arriving ion knocks out electrons, creating a cascade — like a snowball growing as it rolls
Faraday cup	Ions hit a metal cup and their charge is measured directly — simple and sturdy
Microchannel plate	A plate with millions of tiny tubes; each ion triggers an electron cascade — used with TOF

Key takeaway: Detectors simply count arriving ions and amplify the tiny signal so the computer can draw the spectrum.

2.9 Infographic Concept Box

INFOGRAPHIC CONCEPT: "Mass Spec in 4 Steps" — Four connected panels with icons: (1) "IONIZE" — molecule struck by lightning bolt, becoming $M^{+\bullet}$; (2) "SEPARATE" — ions of different sizes flying through a tube, small/fast ahead, big/slow behind, labelled "sorted by m/z"; (3) "DETECT" — ions hitting a target with a counter clicking; (4) "READ" — a spectrum with peaks labelled "$M^{+\bullet}$ = molecular weight", "fragments = structure clues", "tallest = base peak". Side strip: "Hard (EI) = many pieces; Soft (ESI/MALDI) = whole molecule".

2.10 Tables

Table 1: Hard vs Soft Ionization

Feature	Hard (EI)	Soft (ESI, MALDI, CI, FAB)
Fragmentation	Extensive	Little
Molecular ion	Often weak/absent	Strong
Best information	Structure from fragments	Molecular weight
Molecule type	Small, volatile, stable	Large, fragile, polar

Table 2: Ionization Technique Selection Guide

If your sample is...	Choose
Small volatile organic, need fragments	EI
Small organic but $M^{+\bullet}$ missing in EI	CI
Protein / peptide / polymer (large)	MALDI
Drug in liquid, coupled to LC	ESI (API)
Polar, non-volatile, thermally fragile	FAB

2.11 Examples

Example 1 — Molecular weight confirmation: A newly synthesized drug is expected to be $C_9H_8O_4$ (aspirin, MW 180). Its ESI mass spectrum shows $(M+H)^+$ at m/z 181 → confirms molecular weight 180.

Example 2 — Using the nitrogen rule: An unknown shows a molecular ion at m/z 194 (even). By the nitrogen rule, it has zero or an even number of nitrogens — consistent with caffeine (4 nitrogens).

Example 3 — Reading a loss: Molecular ion at m/z 150, strong fragment at m/z 132 → loss of 18 (H_2O) → alcohol group likely present.

Example 4 — Choosing ionization: For a large peptide drug that would be destroyed by EI, MALDI or ESI is chosen (soft ionization keeps it whole).

2.12 Analogies

- **m/z separation:** Like a running race where light runners finish first (TOF).
- **Quadrupole:** Like a security gate that opens only for people with a specific ticket number.
- **Fragmentation:** Like breaking a clay pot — the pieces tell you the pot's shape.
- **Base peak:** Like the highest mountain in a range — all other heights are compared to it.

2.13 Pharmacy Relevance

- **Molecular weight confirmation:** Every new drug candidate's molecular weight is confirmed by MS.
- **Impurity profiling:** MS detects and identifies impurities at trace levels in drug substances.
- **Metabolite identification:** LC-MS/MS identifies how a drug is broken down in the body during drug development.
- **Quality control:** MS-based methods check for adulterants and degradation products in formulations.

2.14 India Context

- Indian pharmaceutical R&D centres and quality-control laboratories widely use LC-MS/MS for impurity profiling and bioanalytical studies supporting generic drug development.
- Skill in interpreting mass spectra is routinely expected in pharmaceutical analysis roles in India's major pharma hubs (Hyderabad, Mumbai, Ahmedabad, Bengaluru).

- Regulatory expectations for impurity identification in drug substances make MS an essential technique in Indian pharmaceutical quality systems.

2.15 Key Points

1. MS: ionize → separate by m/z → detect.
2. Molecular ion ($M^{+\bullet}$) gives molecular weight; base peak is the tallest peak (100%).
3. Nitrogen rule: odd nitrogens → odd m/z molecular ion.
4. Stevenson rule: charge stays on the more stable fragment.
5. McLafferty rearrangement: gamma-hydrogen transfer in carbonyl compounds via a six-membered arrangement.
6. Common losses: 15 (CH_3), 18 (H_2O), 28 (CO), 44 (CO_2).
7. EI = hard (fragments); CI, FAB, MALDI, ESI/API = soft (molecular ion).
8. Analyzers: TOF (speed race), quadrupole (m/z filter), ion trap (trap and release).
9. Detectors: electron multiplier, Faraday cup, microchannel plate.
10. Applications: MW confirmation, impurity profiling, metabolite ID.

2.16 Common Confusions

- **m/z vs mass:** For singly charged ions they are equal, but for multiply charged ions (common in ESI of proteins) m/z is smaller than the mass.
- **Base peak vs molecular ion peak:** The base peak is the tallest; the molecular ion peak is at the highest m/z. They are often different peaks.
- **EI and CI:** Both use electrons, but in CI the electrons hit a reagent gas first — CI is the gentler one.
- **MALDI vs ESI:** MALDI uses a laser + solid matrix (great for large biomolecules); ESI sprays liquid at atmospheric pressure (great for LC coupling).

2.17 Common Mistakes

1. Calling the tallest peak the molecular ion — check the highest m/z, not the tallest peak.
2. Forgetting that EI often destroys the molecular ion — switch to CI or a soft technique for MW.
3. Ignoring the nitrogen rule when proposing a molecular formula.
4. Trying to analyze a non-volatile, heat-sensitive drug by EI — choose ESI or MALDI.
5. Forgetting that the MS analyzer works under vacuum — air molecules would scatter the ions.

2.18 Mnemonics

- **MS steps — "ISD": I**onize → **S**eparate → **D**etect.

- **Common losses:** "**15-18-28-44**" — "**M**ethyl **15**, **W**ater **18**, **CO 28**, **CO_2 44**" (MWCC).
- **Analyzers:** "**T**ortoise? No — **T**OF = **T**rack race; **Q**uadrupole = **Q**ueue filter; **I**on trap = **I**mprison then release" (TQI).
- **Soft techniques — "C FEM": C**I, **F**AB, **E**SI, **M**ALDI are soft (EI is the hard one).

2.19 Exam Summary

Mass spectrometry converts molecules to ions, separates them by m/z, and detects them. The molecular ion gives molecular weight; fragment ions give structural clues; the base peak is the tallest peak. Fragmentation rules: nitrogen rule (odd N → odd m/z M^{+}•), Stevenson rule (charge stays on the most stable fragment), McLafferty rearrangement (gamma-H transfer in carbonyls), and characteristic losses of small neutrals (15 CH_3, 18 H_2O, 28 CO, 44 CO_2). Ionization: EI is hard (rich fragments); CI, FAB, MALDI, and API/ESI are soft (strong molecular ion). EI suits small volatiles; CI when M^{+}• is lost; FAB for polar non-volatiles; MALDI for large biomolecules; ESI/API for LC-MS of drugs. Analyzers: TOF separates by flight speed, quadrupole filters one m/z at a time, ion trap traps and releases ions sequentially. Detectors: electron multiplier, Faraday cup, microchannel plate. Applications: molecular weight confirmation, impurity profiling, metabolite identification.

2.20 One-Minute Revision

- MS = Ionize → Separate (m/z) → Detect.
- M^{+}• (highest m/z) = molecular weight; tallest = base peak.
- Nitrogen rule: odd N → odd M^{+}•.
- Losses: 15, 18, 28, 44.
- EI = hard; CI/FAB/MALDI/ESI = soft.
- TOF = race; quadrupole = filter; ion trap = cage.
- Uses: MW check, impurities, metabolites.

2.21 Short Answer Questions

1 State the basic principle of mass spectrometry.

2 Define m/z, molecular ion, and base peak.

3 State the nitrogen rule with an example.

4 What is the Stevenson rule?

5 What is the McLafferty rearrangement?

6 Differentiate hard and soft ionization with one example each.

7 Why is EI unsuitable for large biomolecules? Which technique would you use instead?

8 Explain the principle of a TOF analyzer in one line.

9 Name two detectors used in MS.

10 Give two pharmaceutical applications of MS.

2.22 Long Answer Questions

1 Explain the principle of mass spectrometry with a labelled block diagram. Describe how a mass spectrum is read (molecular ion, base peak, fragment ions).

2 Discuss the rules of fragmentation: nitrogen rule, Stevenson rule, McLafferty rearrangement, and loss of small neutral molecules, with examples.

3 Compare the ionization techniques EI, CI, MALDI, FAB, and API/ESI under the heads: hard/soft, working principle, and suitable analyte type.

4 Describe the three analyzers — time of flight, quadrupole, and ion trap — giving the separation principle, strengths, and limitations of each.

5 Write a note on the applications of mass spectrometry in pharmaceutical analysis.

2.23 MCQs with Answer Keys

Q1. In MS, ions are separated on the basis of: a) Colour b) m/z c) Solubility d) Boiling point **Answer: b) m/z**

Q2. The tallest peak in a mass spectrum is called the: a) Molecular ion peak b) Base peak c) Fragment peak d) Parent peak **Answer: b) Base peak**

Q3. Which ionization technique is classified as "hard" ionization? a) ESI b) MALDI c) EI d) CI **Answer: c) EI**

Q4. According to the nitrogen rule, a molecule with an odd number of nitrogen atoms shows its molecular ion at: a) Even m/z b) Odd m/z c) m/z 100 d) Cannot be predicted **Answer: b) Odd m/z**

Q5. A loss of 18 mass units in a mass spectrum suggests the loss of: a) CH_3 b) H_2O c) CO d) CO_2 **Answer: b) H_2O**

Q6. Which ionization technique is best suited for large biomolecules like proteins? a) EI b) CI c) MALDI d) None of these **Answer: c) MALDI**

Q7. In a TOF analyzer, ions are separated by: a) Their colour b) Their speed of flight c) Their charge only d) Magnetic deflection **Answer: b) Their speed of flight**

Q8. An ion trap analyzer works by: a) Racing ions down a tube b) Filtering one m/z through rods c) Trapping ions and releasing them one m/z at a time d) Burning the sample **Answer: c) Trapping ions and releasing them one m/z at a time**

Q9. The McLafferty rearrangement is commonly seen in compounds containing: a) Only alkanes b) C=O groups c) Noble gases d) Metals **Answer: b) C=O groups**

Q10. ESI is a type of: a) Hard ionization b) Atmospheric pressure (soft) ionization c) Thermal analysis d) Chromatography **Answer: b) Atmospheric pressure (soft) ionization**

2.24 Viva Questions

1 What is the basic principle of mass spectrometry?

2 What is m/z?

3 Which peak gives the molecular weight?

4 What is the base peak?

5 Name one hard and one soft ionization technique.

6 State the nitrogen rule.

7 What is the McLafferty rearrangement?

8 How does a quadrupole analyzer separate ions?

9 Why does the MS analyzer operate under vacuum?

10 Name two applications of MS in pharmacy.

2.25 Application-Based Questions

1 A new drug candidate has molecular formula $C_9H_8O_4$. Its EI spectrum shows no clear molecular ion. Which ionization technique would you switch to, and what m/z would you expect for the protonated molecule?

2 An impurity in a drug shows a molecular ion at m/z 167 (odd). What does the nitrogen rule tell you about this impurity?

3 A mass spectrum shows $M^{+\bullet}$ at m/z 150 and a strong fragment at m/z 105. Calculate the mass lost and suggest what neutral piece was lost.

4 You need to couple a liquid chromatography system to a mass spectrometer for analyzing a polar drug in plasma. Which ionization technique and analyzer combination would you choose, and why?

2.26 References

- Brown
- Dong
- Niessen
- Silverstein / Webster / Kiemle / Bryce
- Skoog / Holler / Crouch