NOTESKARTS

Premium Study Notes | B.Pharma 8th Semester

BP811ET — ADVANCED INSTRUMENTATION TECHNIQUES

UNIT II: Thermal Methods of Analysis & X-Ray Diffraction

PCI / AKTU Aligned

★ PREMIUM PAID NOTES ★

Topics Covered: TGA | DTA | DSC | X-Ray Diffraction | Crystal Systems | Powder Diffraction | PXRD | Drug Polymorphism

INTRODUCTION TO THERMAL ANALYSIS

Thermal analysis is a group of techniques in which a physical or chemical property of a substance is measured as a function of temperature, while the substance is subjected to a controlled temperature program.

General Principle of Thermal Analysis

Sample is heated/cooled at a controlled rate (typically 1–20°C/min) in a specified atmosphere (N_2, O_2, air, or inert). Changes in mass, heat flow, or dimension are recorded as a function of temperature or time.

Technique	Property Measured	Signal	Abbreviation
Thermogravimetric Analysis	Mass (weight)	Mass vs. Temperature	TGA (or TG)
Differential Thermal Analysis	Temperature difference (ΔT)	ΔT vs. Temperature	DTA
Differential Scanning Calorimetry	Heat flow (dH/dt)	mW or mW/mg vs. Temperature	DSC
Thermomechanical Analysis	Dimensional change	Deformation vs. Temperature	TMA
Dilatometry	Volume change	Volume vs. Temperature	DIL

THERMOGRAVIMETRIC ANALYSIS (TGA)

Principle of TGA

TGA measures the change in mass (weight) of a sample as it is heated, cooled, or held at constant temperature. The mass change occurs due to:

- Evaporation of moisture or volatile solvents
- Thermal decomposition (pyrolysis) of the sample
- Oxidation or reduction reactions with the atmosphere
- Dehydration of hydrates (release of water of crystallization)
- Sublimation of volatile components

The result is a **thermogram** (TGA curve): a plot of **% mass remaining (or mass in mg) on Y-axis vs. Temperature (°C) on X-axis**.

Parameters from TGA Thermogram

- **Onset Temperature (T_onset):** Temperature at which mass loss begins — start of thermal event
- **Inflection Temperature (T_i):** Point of maximum rate of mass change (steepest part of curve)
- **End Temperature (T_end):** Temperature at which mass loss is complete — plateau

- **Residue (%):** Mass remaining at end of heating — indicates inorganic content, ash, or stable product
- **Step height:** The % mass lost in each step — quantifies each thermal event

➡ **DIAGRAM TO DRAW — TGA Thermogram** Draw a graph: Y-axis = % Mass (100% at top, 0% at bottom); X-axis = Temperature (°C). Draw THREE plateau regions separated by TWO mass-loss steps. Label: First plateau = 100% (original mass). First step drop = 'Moisture loss (dehydration)'. Second plateau = e.g., 85%. Second step drop = 'Decomposition'. Third plateau = residue (e.g., 40%). Mark onset, inflection, and end temperatures on each step.

TGA Instrumentation

A TGA instrument consists of the following essential components:

➡ **BLOCK DIAGRAM TO DRAW** Draw connected boxes in sequence: Microbalance (center) → Sample Crucible (on microbalance) → Furnace (surrounds crucible) → Temperature Programmer/Controller → Gas Supply System (purge gas inlet). Then: Microbalance → Electronic Amplifier → Recorder/Computer. Add: Reference weights on other arm of microbalance. Label atmosphere: Nitrogen/Air/O_2.

Component	Function	Specification / Detail
Thermobalance (Microbalance)	Continuously measures sample mass during heating. Heart of TGA instrument.	Sensitivity: 0.001–1 µg. Types: Null-point or deflection type. Electromagnetic compensation used in modern instruments.
Furnace	Heats the sample at controlled rate.	Range: RT to 1500°C. Uniform heating with minimal thermal gradient. Platinum resistance winding.
Temperature Programmer	Controls heating/cooling rate precisely.	Rate: 0.1–150°C/min. Isothermal, dynamic, or step-wise programs possible.
Sample Crucible/Pan	Holds the sample. Must be chemically inert.	Materials: Platinum, alumina (Al_2O_3), aluminium, graphite. Platinum most common.
Gas System	Controls atmosphere around sample.	Gases: N_2 (inert), O_2, air, Ar. Flow rate: 20–100 mL/min. Purge gas prevents contamination.
Recorder / Data System	Plots TGA thermogram. Calculates derivative (DTG).	Outputs: TGA (mass vs T) and DTG (dm/dT vs T) curves simultaneously.

Derivative Thermogravimetry (DTG)

DTG is the first derivative of the TGA curve: plots **dm/dT or dm/dt vs. Temperature**. A DTG peak occurs at the inflection point of each TGA step, making it easier to:

- Resolve overlapping thermal events that appear as one broad step in TGA
- Precisely determine the temperature of maximum decomposition rate

- Identify the number of steps in a complex decomposition

Factors Affecting TGA Results

Factor	Effect on TGA	How to Control
Heating Rate	Faster rate → peaks shift to higher T, broader transitions	Use 5–10°C/min for resolution; higher rates for speed
Sample Mass	Larger mass → poorer resolution, thermal lag, incomplete reactions	Use 5–20 mg for routine analysis
Particle Size	Finer particles → faster reaction, lower onset T	Grind uniformly for reproducible results
Atmosphere	Oxidizing (air/O_2) promotes combustion; Inert (N_2) for pyrolysis	Choose based on purpose: oxidative or inert
Crucible material	Catalytic crucibles alter reaction onset T	Use platinum or alumina; avoid reactive metals
Gas Flow Rate	Low flow → accumulation of products → shifts equilibrium	Maintain constant flow 20–50 mL/min

Applications of TGA in Pharmacy

- **Moisture and volatile content determination:** Quantify water content, residual solvents in API — ICH Q3C compliance
- **Thermal stability assessment:** Identify decomposition onset temperature of drugs — accelerated stability studies
- **Hydrate and solvate characterization:** e.g., erythromycin exists as dihydrate — TGA confirms and quantifies water of crystallization
- **Drug-excipient compatibility:** Physical mixtures heated; new decomposition steps indicate incompatibility
- **Polymorphism studies:** Different polymorphs may show different thermal behavior (combined TGA-DSC)
- **Ash and residue determination:** Inorganic content, catalyst residues in API manufacturing
- **Oxidative stability:** Study oxidation threshold by switching from N_2 to O_2 atmosphere mid-run

Pharmaceutical Example Erythromycin Dihydrate: TGA shows first mass loss step ~80°C (loss of ~4.8% corresponding to 2 moles H_2O), confirming dihydrate form. Second step at ~250°C = decomposition of anhydrous API. This confirms the hydration state for quality control.

DIFFERENTIAL THERMAL ANALYSIS (DTA)

Principle of DTA

Differential Thermal Analysis (DTA) measures the **temperature difference (ΔT)** between a sample and an inert reference material, as both are heated/cooled under identical conditions at the same rate.

$$\Delta T = T_{sample} - T_{reference}$$

When no thermal event occurs: ΔT ≈ 0 (both sample and reference heat at same rate). When a thermal event occurs in the sample:

- **Endothermic process** (sample absorbs heat — melting, dehydration, phase transition): Sample temperature LAGS behind reference → ΔT becomes NEGATIVE → **peak below baseline** (by convention, some instruments show as positive — check sign convention!)
- **Exothermic process** (sample releases heat — crystallization, oxidation, decomposition): Sample temperature EXCEEDS reference → ΔT becomes POSITIVE → **peak above baseline**

⚡ **CRITICAL Exam Trick** DTA Convention: Endothermic = peak pointing DOWNWARD (negative ΔT). Exothermic = peak pointing UPWARD (positive ΔT). BUT many modern instruments and textbooks may show the reverse — always state your convention! Examiners commonly ask this.

DTA Instrumentation

Component	Function	Notes
Sample Holder (S)	Holds the test sample in an inert crucible	Al_2O_3 or Pt crucible; 5–50 mg sample
Reference Holder (R)	Holds inert reference material that undergoes no thermal events in the range studied	Al_2O_3 (alumina) powder most common reference
Thermocouples	Measure T_sample and T_reference independently	Type J, K, S, or R; positioned at base of holders for good thermal contact
Differential Amplifier	Amplifies and records ΔT = T_sample − T_reference	High sensitivity: can detect ΔT of 0.001°C
Furnace	Provides controlled heating/cooling around both holders	Single furnace with symmetric design for identical thermal environment
Temperature Programmer	Controls heating rate, isothermal holds, cooling	Rates: 1–50°C/min; Range: −150°C to 1600°C
Gas Supply	Controls atmosphere (inert, oxidizing)	N_2, Ar, O_2, or air; flow rate 10–100 mL/min

Component	Function	Notes
Recorder / Software	Plots ΔT vs. temperature; marks peak temperatures	Also computes peak onset, area (qualitative in DTA, not quantitative)

Applications of DTA

- **Phase transition studies:** Detection of melting, boiling, solid-solid phase transitions, sublimation
- **Polymorphism detection:** Different crystal polymorphs of same drug show different DTA peak temperatures
- **Glass transition:** Detection of Tg in amorphous pharmaceuticals (broad endothermic signal)
- **Drug-excipient compatibility:** Disappearance or shift of characteristic peaks indicates incompatibility
- **Purity assessment:** Impurities lower melting point and broaden DTA melting peak
- **Eutectic mixtures:** Detection of eutectic point in multi-component formulations

Limitations of DTA

- DTA is **qualitative** — peak areas are NOT directly proportional to enthalpy changes (heat capacity differences between sample and reference affect baseline)
- Cannot directly give enthalpy values (ΔH) — DSC is required for quantitative thermal analysis
- Resolution between overlapping transitions can be poor
- Sensitivity is lower than DSC for small samples

DIFFERENTIAL SCANNING CALORIMETRY (DSC)

Principle of DSC

Differential Scanning Calorimetry (DSC) measures the **heat flow (dH/dt, in mW or mW/mg)** into or out of a sample relative to an inert reference, as both are heated at the same controlled rate.

Unlike DTA (which measures temperature difference), DSC maintains **the same temperature in both sample and reference** (for power-compensated DSC) OR measures the heat required to maintain this condition, giving a direct, quantitative measure of enthalpy.

Parameter	DTA	DSC
What is measured	Temperature difference $\Delta T = T_s - T_r$	Heat flow dH/dt (mW) or specific heat flow (mW/mg)
Nature of data	Qualitative (relative)	Quantitative (absolute enthalpy values)
Enthalpy calculation	NOT directly possible	Peak area = ΔH (J/g or kJ/mol)
Sensitivity	Lower	Higher — detects subtle transitions
Sample size	1–50 mg	1–10 mg (small samples OK)
Information given	Transition temperatures, peak direction	Tg, Tm, ΔHm, Tc, ΔHc, heat capacity Cp
Applications	Phase diagrams, broad screening	Purity, polymorphism, enthalpy — pharmaceutical QC

Types of DSC

Type	Principle	Key Feature
Power-Compensated DSC	Sample and reference have SEPARATE heaters. Power (energy/time) supplied to each is adjusted to KEEP both at the same temperature. Signal = difference in power (mW).	Measures power directly. True calorimeter. Perkin-Elmer design. More accurate for heat capacity.
Heat-Flux DSC	Sample and reference share a SINGLE furnace. Heat flows through a thermal resistance disk. Temperature difference measured and converted to heat flow using calibration.	Simpler, more robust design. Most commercial instruments (TA Instruments, Mettler-Toledo). Widely used in pharma QC.

DSC Instrumentation

➡ **BLOCK DIAGRAM (Power-Compensated DSC) TO DRAW** Draw two separate small furnace blocks side by side. Left = Sample furnace (with sample pan S and individual heater/thermocouple). Right = Reference furnace (with reference pan R and individual heater/thermocouple). Both connect to: (1) Differential Power Amplifier (measuring ΔP = power difference). (2) Temperature Programmer (controls both furnaces equally). Power Amplifier output → Y-axis of recorder (Heat flow mW). Temperature output → X-axis. Label: Nitrogen purge gas inlet at top.

Component	Function	Specification
Sample Pan/Cell	Holds sample. Must be impermeable, thermally conductive.	Aluminium pan (most common, up to 300°C); Platinum (to 700°C); Hermetic pans for volatile samples
Reference Pan	Identical empty pan as reference (or filled with inert material).	Must match sample pan mass and material exactly for accurate baseline
Furnace(s)	Heats sample and reference at programmed rate.	Temperature range: −180°C (with LN_2 cooling) to 700°C; Heating rate 0.01–500°C/min
Temperature Sensors	Monitor temperature at cell position.	Thermocouples or platinum resistance thermometers (PRT); Accuracy ±0.1°C
DSC Cell Lid	Provides thermal symmetry and protects sample.	Gold-plated for IR reflectance; hermetically sealable
Cooling System	Allows sub-ambient experiments.	LN_2 (liquid nitrogen): −180°C. Mechanical cooling: −90°C. Peltier: 0°C
Purge Gas System	Inert or reactive atmosphere control.	N_2 at 20–50 mL/min (standard); He for better thermal conductivity
Data Acquisition System	Records and processes DSC signal.	Software calculates Tg, Tm, ΔH, Tc; integration of peak areas

DSC Measurements and Thermogram Interpretation

➡ **DIAGRAM TO DRAW — DSC Thermogram** Draw Y-axis = Heat Flow (mW, Endo down / Exo up, OR Endo up — state convention). X-axis = Temperature (°C). Draw: (1) A baseline shift (step change) at Tg labeled 'Glass Transition (Tg)' — represents increase in heat capacity. (2) A small exothermic peak just above Tg = 'Cold Crystallization (Tc)'. (3) A large endothermic peak = 'Melting (Tm)'. Mark onset, peak, and end temperatures on melting peak. Draw peak area shaded = ΔHm. Label all events clearly.

Event	Symbol	Peak Type	What It Represents	Pharmaceutical Significance
Glass Transition	Tg	Baseline step (no peak)	Amorphous material changes from glassy → rubbery state. Increase in Cp.	Critical for amorphous drugs stability; Tg > storage temp needed
Cold Crystallization	Tc	Exothermic (Exo)	Amorphous material crystallizes upon heating above Tg.	Indicates amorphous content; quantify amorphous drug in formulation
Melting	Tm	Endothermic (Endo)	Crystalline material melts. Peak area = ΔHm (heat of fusion).	Identity test; purity (sharp Tm); polymorphism (different Tm)
Crystallization	Tc	Exothermic (Exo)	Liquid crystallizes on cooling. ΔHc = heat of crystallization.	Study of crystallization tendency of drug melts
Decomposition	Td	Often Exothermic	Chemical degradation, evaporation, or oxidation.	Thermal stability limit of API; processing temperature guide
Dehydration	—	Endothermic (Endo)	Loss of water of crystallization from hydrates.	Characterize hydrates vs anhydrous forms; quantify water

Quantitative DSC Analysis — Purity Determination

DSC can determine sample purity using the van't Hoff equation applied to melting point depression. Impurities lower the melting point (Tm) and broaden the melting peak. The relationship is:

$$1/F = (\Delta Hf / R \cdot T_0^2) \cdot (T_0 - Tm) + (K \cdot x_2)$$

Where: F = fraction melted, ΔHf = heat of fusion, R = gas constant, T_0 = melting point of pure compound, x_2 = mole fraction of impurity. Purity can be calculated from the shape of the melting endotherm.

📌 **Purity Rule of Thumb** A sharp, symmetrical DSC melting peak with high ΔHm indicates high purity. A broad, asymmetric peak with lower Tm indicates impurities. This is used for pharmacopoeial purity testing of drug substances.

Applications of DSC in Pharmacy

- **Polymorphism characterization:** Different polymorphs show different Tm, ΔHm (e.g., carbamazepine Form I vs III: Tm 175°C vs 192°C)
- **Purity testing:** DSC melting onset sharpness — van't Hoff analysis gives mole percent purity (USP method)

- **Drug-excipient compatibility:** Mixing drug + excipient; new exothermic peaks or disappearance of drug Tm indicates interaction
- **Amorphous content quantification:** Area of cold-crystallization peak / total melting peak area gives amorphous fraction
- **Glass transition temperature (Tg):** Critical for amorphous solid dispersions — Tg must be >50°C above storage temperature for physical stability
- **Eutectic composition:** Binary phase diagrams constructed from DSC data of drug-drug or drug-excipient mixtures
- **Lyophilized products:** DSC determines collapse temperature of freeze-dried formulations — critical for lyophilization cycle development
- **Lipid characterization:** Transition temperatures of lipid excipients in lipid-based drug delivery systems

Pharmaceutical Example — Carbamazepine Carbamazepine has 4 known polymorphs. DSC clearly distinguishes them: Form III (dihydrate) melts at 175°C, Form I (monoclinic) melts at 192°C. During heating, Form III converts to Form I (exothermic recrystallization peak) before final melting. This DSC fingerprint is used in QC to verify correct polymorph.

ORIGIN OF X-RAYS

What are X-rays?

X-rays are electromagnetic radiation with wavelengths in the range **0.01–10 Å (0.001–1 nm)**. The range useful for X-ray crystallography is **0.5–2.5 Å** — the same order of magnitude as interatomic distances in crystals (typically 1–3 Å). This wavelength match is the basis of diffraction by crystalline materials.

Generation of X-rays (X-ray Tube)

X-rays are generated when **high-energy electrons** bombard a metal target (anode). Two types of radiation are produced:

Type	Origin	Properties	Use in XRD
Bremsstrahlung (White radiation / Continuous)	Electrons decelerate in target material → broad spectrum of X-rays emitted	Continuous wavelength spectrum; intensity depends on kV and target	Generally NOT used in XRD (monochromatic needed)
Characteristic X-rays	Electrons eject inner shell electrons → outer shell electrons fill vacancy → emit photons of specific wavelength	Discrete wavelengths (Kα, Kβ lines) characteristic of target element	USED in XRD — Cu Kα (1.5418 Å) is standard for pharma XRD

X-ray Tube Components and Operation

Target Material	Kα Wavelength (Å)	Best Used For
Copper (Cu)	1.5418	Organic compounds, pharmaceuticals, polymers — MOST COMMON
Molybdenum (Mo)	0.7107	Larger unit cells, inorganic compounds, high absorption samples
Cobalt (Co)	1.7902	Iron-containing samples (avoids Fe fluorescence)
Chromium (Cr)	2.2909	Metals and alloys

📌 **Fact** Copper Kα radiation (λ = 1.5418 Å = 1.5418×10^{-10} m) is the most widely used X-ray source for pharmaceutical PXRD because organic drug molecules contain mainly C, H, N, O — all low atomic number elements that give good diffraction with Cu Kα.

BASIC ASPECTS OF CRYSTALS

What is a Crystal?

A **crystal** is a solid material in which the atoms, molecules, or ions are arranged in a **highly ordered, repeating 3D pattern** called the crystal lattice. This long-range order gives crystals their characteristic properties: fixed melting point, anisotropy, and ability to diffract X-rays.

Crystal Lattice and Unit Cell

- **Crystal Lattice:** The regular, repeating arrangement of points in 3D space, each point representing an identical chemical environment
- **Unit Cell:** The smallest repeating unit of the crystal lattice. The entire crystal is built by stacking unit cells in three dimensions.
- **Lattice Parameters:** Three unit cell edge lengths (a, b, c) and three angles (α, β, γ) — the 6 parameters that define unit cell geometry

Crystal System	Axes (a, b, c)	Angles (α, β, γ)	Example Compounds
Cubic	a = b = c	α = β = γ = 90°	NaCl, KCl, diamond, fluorite
Tetragonal	a = b ≠ c	α = β = γ = 90°	White tin, TiO_2 (rutile)
Orthorhombic	a ≠ b ≠ c	α = β = γ = 90°	Aspirin Form I, sulfur
Hexagonal	a = b ≠ c	α = β = 90°; γ = 120°	Graphite, quartz
Rhombohedral	a = b = c	α = β = γ ≠ 90°	Calcite ($CaCO_3$), quartz

Crystal System	Axes (a, b, c)	Angles (α, β, γ)	Example Compounds
Monoclinic	a ≠ b ≠ c	α = γ = 90°; β ≠ 90°	Sucrose, carbamazepine (Form III)
Triclinic	a ≠ b ≠ c	α ≠ β ≠ γ ≠ 90°	Copper(II) sulfate pentahydrate, many drug molecules

⚡ **Exam Trick** Memorize: Cubic has highest symmetry (all sides equal, all angles 90°). Triclinic has lowest symmetry (nothing equal). Most drug molecules crystallize in MONOCLINIC or TRICLINIC systems because they are complex, low-symmetry structures. This is a commonly tested MCQ!

Miller Indices (hkl)

Miller indices are a set of three integers (h, k, l) used to describe the orientation and spacing of crystal planes. They are essential for interpreting X-ray diffraction patterns.

How to Determine Miller Indices

1. Find where the plane intercepts the three crystallographic axes (a, b, c) in units of the lattice parameters
2. Take the reciprocals of these intercepts
3. Clear fractions to get the smallest set of integers (h, k, l)

Example: A plane intercepts a-axis at 1, b-axis at 2, c-axis at ∞ (parallel). Reciprocals: 1/1, 1/2, 1/∞ = 1, 0.5, 0. Smallest integers: **(2, 1, 0)** — this is the (210) plane.

Interplanar d-spacing

The **d-spacing (d_hkl)** is the perpendicular distance between adjacent parallel planes with Miller indices (hkl). For a cubic crystal:

$$d_{hkl} = a / \sqrt{(h^2 + k^2 + l^2)} \quad \text{[Cubic system]}$$

For other crystal systems, the formula is more complex but the principle is the same. d-spacings are the fundamental parameters measured by X-ray diffraction.

X-RAY CRYSTALLOGRAPHY AND BRAGG'S LAW

Bragg's Law — The Foundation of X-ray Diffraction

Bragg's Law (derived by W.H. Bragg and W.L. Bragg, 1913) describes the condition for constructive interference of X-rays reflected from crystal planes. It is the most important equation in X-ray crystallography.

$$n\lambda = 2d \cdot \sin\theta$$

Symbol	Quantity	Units	Meaning
n	Order of reflection	Integer (1, 2, 3...)	Order of diffraction; n=1 most common
λ	Wavelength of X-rays	Å or nm	For Cu Kα: λ = 1.5418 Å
d	Interplanar spacing (d-spacing)	Å	Distance between parallel crystal planes (hkl)
θ	Bragg angle (glancing angle)	degrees	Angle between incident X-ray beam and crystal plane surface
2θ	Diffraction angle	degrees	Angle between incident and diffracted beams — MEASURED in diffractometer

Physical Meaning of Bragg's Law

- When $n\lambda = 2d \cdot \sin\theta$: **constructive interference → diffraction peak observed** (bright spot or high count in detector)
- When $n\lambda \neq 2d \cdot \sin\theta$: **destructive interference** → no diffraction peak
- Each set of crystal planes (hkl) with specific d-spacing diffracts at a unique angle 2θ
- The collection of all diffraction peaks (their positions and intensities) forms the **diffraction pattern** — a unique fingerprint of the crystal structure

📌 **Critical Application** By measuring 2θ for each diffraction peak and knowing λ (fixed by X-ray tube target), we can calculate d-spacing using Bragg's law: $d = n\lambda/(2 \cdot \sin\theta)$. From d-spacings, unit cell dimensions and crystal structure can be determined.

X-RAY DIFFRACTION TECHNIQUES

Overview — Three Main Methods

Method	Sample Type	Output	Primary Use
Rotating Crystal Method	Single crystal rotated in X-ray beam	Spots on film/detector at different angles	Unit cell determination, systematic absences
Single Crystal Diffraction	Single crystal, stationary or goniometer-rotated	3D intensity data for all (hkl) reflections	Complete 3D structure determination (bond lengths, angles)

Method	Sample Type	Output	Primary Use
Powder X-ray Diffraction (PXRD)	Polycrystalline powder (millions of randomly oriented crystallites)	1D pattern — peaks at 2θ angles	Phase identification, polymorphism, unit cell, QC

Rotating Crystal Technique

Principle

A single crystal is mounted on a goniometer and **rotated about one of its crystallographic axes** while being irradiated with a monochromatic X-ray beam. As the crystal rotates, different sets of planes come into the Bragg condition successively, producing diffraction spots on a cylindrical film or area detector surrounding the crystal.

➡ **DIAGRAM TO DRAW — Rotating Crystal Method** Draw: Monochromatic X-ray beam (horizontal arrows from left). Center: Single crystal on rotation axis (vertical rod). Crystal rotates (curved arrow). Cylindrical film surrounds crystal. Diffraction spots appear as rows (layer lines) on the cylindrical film. Show: rotation axis vertical, diffracted beams going to film at various angles. Label: X-ray source, Crystal, Rotation axis, Layer lines on film, Film.

Features of Rotating Crystal Method

- **Layer lines** on the film correspond to reflections from planes containing the rotation axis
- **Systematic absences** (missing reflections) reveal the space group of the crystal
- **Oscillation photographs** use only a small rotation range (e.g., 1°–5°) to prevent spot overlap — now standard in modern protein crystallography
- From the layer line spacing, the unit cell dimension along the rotation axis is calculated: c = λ/sin(μ_n) where μ_n is the layer line angle

Single Crystal X-ray Diffraction (SCXRD)

Principle

Single Crystal X-ray Diffraction (SCXRD) is the gold standard for complete, unambiguous 3D structure determination. A single crystal (typically 0.1–0.5 mm) is mounted on a diffractometer. The crystal is rotated through all angles using a goniometer, and the intensity of every diffracted beam (all hkl reflections) is measured by an area detector.

Step	Process	Output
1. Crystal mounting	Single crystal mounted on glass fiber, loop, or pin; centered in X-ray beam	Crystal aligned for data collection
2. Data collection	Crystal rotated through all angles; detector records all reflections (hkl) and intensities I(hkl)	Complete set of structure factors F(hkl)
3. Data processing	Absorption correction, scaling, merging of equivalent reflections	Reduced data set with I(hkl) and σ(I)

Step	Process	Output
4. Structure solution	Phase problem solved by Direct Methods or Patterson function	Initial model — approximate atomic positions
5. Refinement	Least-squares refinement against all observed reflections	Final R-factor <5% for good structure
6. Validation	Check geometry, R-factors, difference electron density map	Crystal structure — bond lengths, angles, torsions

The Phase Problem in Crystallography

Diffraction data gives the **amplitude** of each structure factor F(hkl), but **NOT the phase**. The electron density distribution ρ(xyz) requires both amplitude AND phase (Fourier synthesis). This is the fundamental limitation of X-ray crystallography:

$$\rho(x,y,z) = (1/V) \Sigma F(hkl) \cdot e^{(-2\pi i(hx+ky+lz))} \quad \text{[Electron density equation]}$$

Solutions: Direct Methods (statistical relationships between phases for small molecules); Patterson function (uses F^2 to find heavy atom positions); Molecular Replacement (uses known similar structure as starting model — used for proteins).

Information from SCXRD

- **Complete 3D structure** — absolute positions of every atom in the unit cell
- **Bond lengths** — accurate to 0.001–0.005 Å
- **Bond angles** — accurate to 0.1–0.5°
- **Torsion angles** — conformation of drug molecule
- **Absolute configuration** — R/S assignment using anomalous dispersion (Flack parameter)
- **Hydrogen bonding** — positions of H atoms in crystal
- **Disorder** — partial occupancy of alternative conformations

Pharmaceutical Example SCXRD was used to determine the absolute configuration of the drug thalidomide — confirming R-enantiomer (teratogenic) vs S-enantiomer (therapeutic). This directly impacted drug regulations globally. SCXRD is now mandatory for NDA submission of chiral drug substances.

POWDER X-RAY DIFFRACTION (PXRD)

Principle of Powder Diffraction

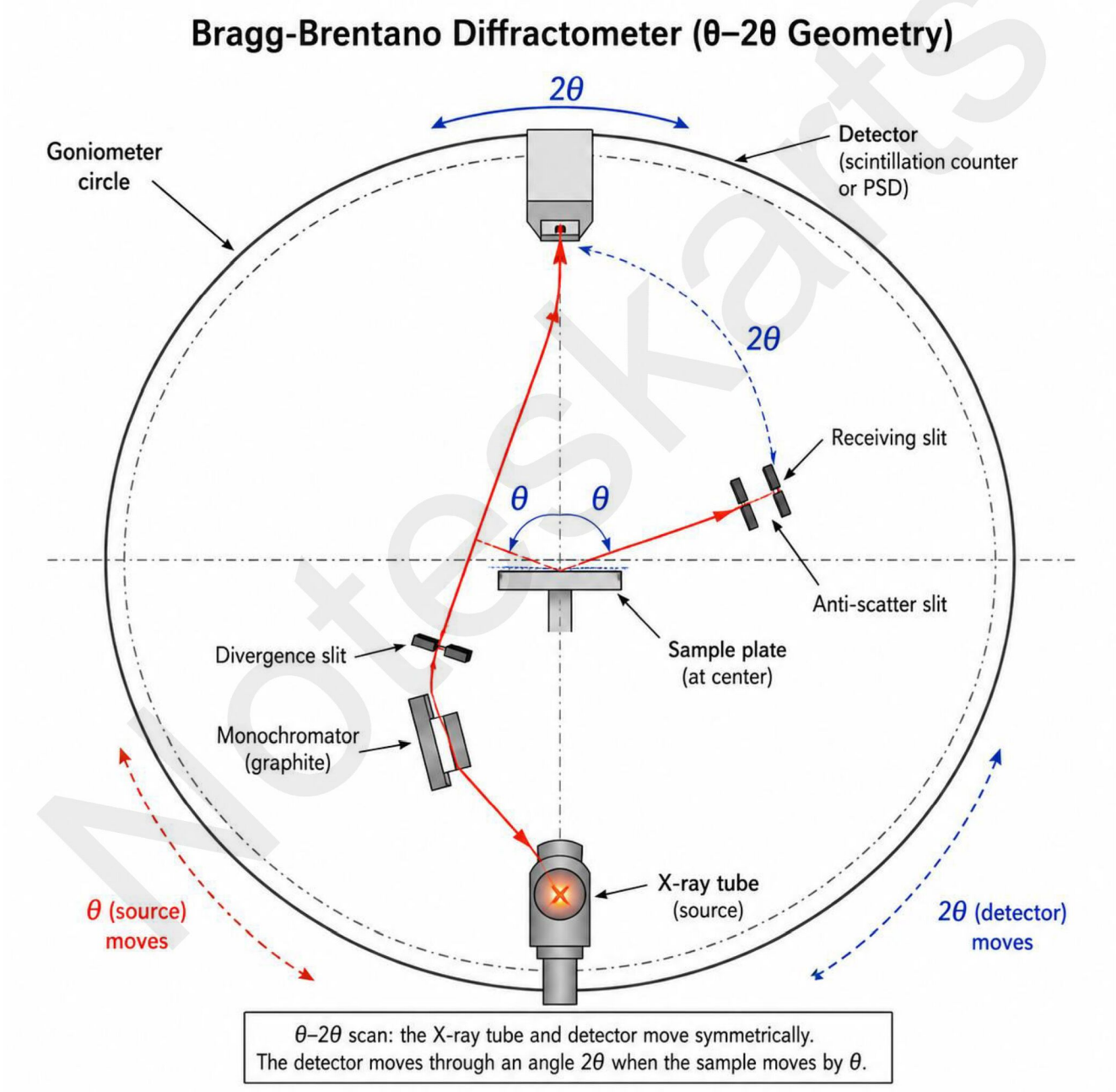
Powder X-ray Diffraction (PXRD) uses a finely ground polycrystalline sample containing **millions of randomly oriented crystallites**. Because of this random orientation, for any given d-spacing, there will always be some crystallites oriented at the correct Bragg angle θ, satisfying the Bragg condition.

The result is diffracted X-rays forming **cones** around the incident beam (Debye-Scherrer cones), one cone for each d-spacing. Intersecting a flat detector gives **Debye rings**; a diffractometer with a point detector records a **1D pattern** of intensity vs. 2θ.

➡ **DIAGRAM TO DRAW — Debye-Scherrer Method (Powder Diffraction)** Draw: X-ray beam (horizontal) entering a cylindrical camera from the left. Powder sample at center. Diffracted beams form CONES around the beam axis. Film strip (strip inside the cylinder) intersects cones → gives arcs/rings on film. Show: incident beam hole (left), beam stop (right), Debye-Scherrer rings on film. Label: Incident X-ray, Powder sample, 2θ angle, Diffraction cone, Debye-Scherrer rings, Film/detector.

PXRD Instrumentation — The X-ray Diffractometer

Modern PXRD uses a **Bragg-Brentano diffractometer** (reflection geometry) — the standard instrument for pharmaceutical powder diffraction.



Component	Function	Detail
X-ray Tube	Generates X-rays (Cu Kα, λ=1.5418 Å)	Sealed tube; 40 kV, 40 mA typical; rotating anode for higher intensity

Component	Function	Detail
Monochromator	Selects single wavelength (Kα) and removes Kβ	Graphite crystal or Kβ filter (Ni filter for Cu); reduces background
Divergence Slits	Control incident beam divergence	Typically 0.5° or 1°; smaller = better resolution, lower intensity
Sample Stage	Holds flat-plate powder sample; rotates at θ rate	Spinner stage for better statistics; capillary for transmission geometry
Anti-scatter Slit	Reduces diffuse scattering at low 2θ	Prevents background at very low angles
Receiving Slit	Defines angular acceptance of diffracted beam	Controls resolution vs. intensity trade-off
Detector	Measures intensity of diffracted X-rays	Scintillation counter, Si strip detector, or position-sensitive detector (PSD)
Goniometer	Rotates sample (θ) and detector (2θ) synchronously	Accuracy 0.001°; computer-controlled; 2–80° 2θ range for pharmaceuticals

9.3 PXRD Pattern and Interpretation

A PXRD pattern is a plot of **diffraction intensity (counts per second) vs. 2θ (degrees)**. Each peak corresponds to a specific set of crystal planes (hkl) satisfying Bragg's law.

Peak Parameter	Information Provided	Pharmaceutical Application
Peak Position (2θ)	Related to d-spacing via Bragg's law: $d = \lambda/(2\sin\theta)$. Unique for each crystal form.	Phase identification; polymorph discrimination
Peak Intensity	Depends on atom types and positions in unit cell (structure factor). Unique pattern for each compound.	Qualitative and quantitative phase analysis
Peak Width (FWHM)	Inversely proportional to crystallite size (Scherrer equation: $D = K\lambda/(\beta \cdot \cos\theta)$)	Crystallite size determination; amorphous content
d-spacing set	Complete set of d-spacings is a crystal fingerprint — unique for each phase.	Identity testing (compare with reference PXRD pattern)

Peak Parameter	Information Provided	Pharmaceutical Application
Peak presence/absence	Missing peaks indicate amorphous phase or wrong polymorph.	Amorphous vs crystalline; polymorph verification

Scherrer Equation — Crystallite Size from PXRD

$$D = K\lambda / (\beta \cdot \cos\theta)$$

Where: D = mean crystallite size (Å), K = Scherrer constant (≈0.9), λ = wavelength, β = peak width at half-maximum (FWHM, in radians), θ = Bragg angle. Broader peaks → smaller crystallites. Amorphous materials give very broad humps (no sharp peaks).

9.4 Applications of PXRD in Pharmacy

- **Polymorph identification and discrimination:** Each polymorph gives a unique PXRD pattern. Ranitidine Form 1 vs Form 2; Ritonavir Form I vs II (the famous recall case)
- **Identity testing:** BP/USP/IP pharmacopoeial PXRD identity tests — compare sample PXRD with reference pattern
- **Crystallinity determination:** Degree of crystallinity from ratio of crystalline peak area to total area (including amorphous hump)
- **Quantitative phase analysis:** Rietveld refinement — quantify mixtures of polymorphs or phases
- **Drug-excipient interactions:** New peaks in mixture PXRD indicate chemical interaction or co-crystal formation
- **Salt and co-crystal screening:** Each salt form of a drug has unique PXRD — used in solid form screening
- **Amorphous quantification:** Amorphous API gives broad halo; crystalline gives sharp peaks — ratio gives % amorphous
- **ICH Q6A compliance:** PXRD required for characterization of drug substance solid state form

Famous Case — Ritonavir Polymorphism Abbott's Ritonavir (HIV protease inhibitor) was withdrawn from market in 1998 when a new, more stable polymorph Form II spontaneously appeared in commercial batches. Form II was insoluble — drug failed dissolution. PXRD would have detected this polymorph transformation. This case made polymorph monitoring by PXRD a regulatory requirement.

9.5 Comparison: Rotating Crystal vs Single Crystal vs Powder Diffraction

Parameter	Rotating Crystal	Single Crystal (SCXRD)	Powder (PXRD)
Sample required	Single crystal (rotated)	Single crystal (0.1–0.5 mm)	Polycrystalline powder
Crystal quality	Good single crystal needed	High quality single crystal needed	No single crystal needed — easiest
Data output	Layer line photograph	3D intensity data (all hkl)	1D pattern (intensity vs 2θ)
Information	Unit cell, systematic absences	Complete 3D structure	Phase ID, polymorphism, unit cell (Rietveld)
Structural detail	Limited	Complete — all bond lengths/angles	Limited — no absolute 3D structure
Instrument	Rotation camera/goniometer	4-circle diffractometer + area det.	Bragg-Brentano diffractometer
Pharma use	Historical — now combined with SCXRD	NDA structure proof, absolute config.	Routine QC, polymorph, identity
Time required	Hours	Hours to days	Minutes to hours
Complexity	Moderate	High	Low — routine analysis

STRUCTURAL ELUCIDATION BY X-RAY METHODS

Steps in Crystal Structure Determination (SCXRD)

1. **Crystal growth:** Drug dissolved in suitable solvent; slow evaporation or vapor diffusion → single crystal formation
2. **Data collection:** Crystal on diffractometer; measure intensities I(hkl) for all reflections up to resolution limit
3. **Structure solution:** Direct Methods / Patterson function → find approximate atomic positions
4. **Structure refinement:** Least-squares fit → refine all atomic parameters; R-factor < 5% for publishable structure
5. **Structure validation:** Check ORTEP diagram (thermal ellipsoids), difference Fourier map (no residual electron density)
6. **Deposition:** Deposit to Cambridge Crystallographic Data Centre (CCDC) with CSD refcode

Parameter	Good Structure	Poor Structure
R-factor (R_1)	< 5% (< 0.05)	> 10% — poor fit to data
wR_2	< 10%	> 20% — problematic
GooF (S)	Close to 1.0 (0.9–1.1)	Very far from 1 — weighting problem
Difference density	< 0.3 e/Å³ max peak	> 1 e/Å³ — unmodeled atoms/disorder
Flack parameter	~0 (correct abs. config.)	~1 (inverted structure) or 0.5 (racemic)

Cambridge Structural Database (CSD)

The **Cambridge Structural Database (CSD)** maintained by the Cambridge Crystallographic Data Centre (CCDC) contains over **1.2 million** crystal structures of organic and organometallic compounds. For pharmaceutical scientists:

- Search for crystal structures of known drugs and excipients
- Compare predicted vs experimental crystal structures
- Identify known polymorphs and solvate structures
- Study intermolecular interactions (H-bonding, π-π stacking) in drug crystals

IMPORTANT QUESTION BANK

A. 2-Mark Questions with Model Answers

Q1. Define TGA and state what it measures.

Ans: Thermogravimetric Analysis (TGA) is a thermal analysis technique that measures the change in mass (weight) of a sample as a function of temperature (or time), under a controlled atmosphere and temperature program. The output is a thermogram: % mass remaining (Y-axis) vs. temperature °C (X-axis). Units of mass change: mg or % initial mass.

Q2. What is the difference between DTA and DSC?

Ans: DTA (Differential Thermal Analysis) measures the temperature difference ($\Delta T = T_{sample} - T_{reference}$) between sample and inert reference — qualitative technique; cannot give enthalpy values. DSC (Differential Scanning Calorimetry) measures heat flow (dH/dt, mW) directly — quantitative technique; gives enthalpy values (ΔH) for transitions (melting, Tg, crystallization).

Q3. State Bragg's law with all terms defined.

Ans: Bragg's Law: $n\lambda = 2d \cdot \sin\theta$. Where: n = order of reflection (integer: 1, 2, 3...). λ = wavelength of X-rays (in Å; for Cu Kα: 1.5418 Å). d = interplanar spacing (distance between parallel crystal planes, in Å). θ = Bragg angle (glancing angle between X-ray beam and crystal plane surface, in degrees). Condition for constructive interference (diffraction).

Q4. What is glass transition temperature (Tg)?

Ans: Glass transition temperature (Tg) is the temperature at which an amorphous material transforms reversibly from a hard, rigid, glassy state to a soft, rubbery, mobile state. In DSC, Tg appears as a step-change (shift) in baseline heat flow — NOT a peak. Tg is critical for amorphous drug stability: Tg must be at least 50°C above storage temperature to prevent recrystallization.

Q5. Define polymorphism and give a pharmaceutical example.

Ans: Polymorphism is the ability of a solid substance to exist in more than one distinct crystalline form (polymorph) with the same chemical composition but different crystal lattice arrangements. Different polymorphs have different physical properties: melting point, solubility, bioavailability, stability. Example: Carbamazepine has 4 polymorphs (Forms I–IV) with different melting points. Ritonavir — Form II caused market withdrawal due to low solubility.

Q6. What is a unit cell? Name the 7 crystal systems.

Ans: A unit cell is the smallest repeating unit of a crystal lattice that, when stacked in three dimensions, generates the entire crystal. It is defined by 6 parameters: a, b, c (edge lengths) and α, β, γ (angles). The 7 crystal systems in order of decreasing symmetry: Cubic, Tetragonal, Orthorhombic, Hexagonal, Rhombohedral, Monoclinic, Triclinic.

Q7. What is PXRD and what is its pharmaceutical significance?

Ans: Powder X-ray Diffraction (PXRD) is an X-ray diffraction technique using finely powdered polycrystalline samples. Millions of randomly oriented crystallites ensure all d-spacings simultaneously satisfy Bragg's law, giving a 1D pattern (intensity vs 2θ). Pharmaceutical significance: (i) Crystal phase identification; (ii) Polymorph discrimination — each polymorph has unique PXRD pattern; (iii) ICH Q6A compliance; (iv) Amorphous content measurement.

Q8. What is DTG and how does it relate to TGA?

Ans: DTG (Derivative Thermogravimetry) is the first derivative of the TGA curve — it plots dm/dT (or dm/dt) vs. temperature. Significance: (i) DTG peak occurs at the inflection point of each TGA step (point of maximum rate of mass loss); (ii) Resolves overlapping thermal events that appear as one broad step in TGA; (iii) Helps precisely identify the temperature of maximum decomposition rate. DTG is typically displayed simultaneously with TGA.

B. 5-Mark Questions with Structured Answers

Q1. Describe the instrumentation of DSC. Explain the difference between power-compensated and heat-flux DSC.

Q2. Discuss the applications of DSC in pharmaceutical analysis.

Q3. Explain the principle and applications of Thermogravimetric Analysis (TGA) in pharmacy.

Q4. Explain the powder X-ray diffraction technique with instrumentation and pharmaceutical applications.

Q5. Explain Bragg's law. Derive the condition for diffraction from crystal planes.

C. 10-Mark Question Skeletons

Q1. Describe in detail the instrumentation of TGA, DTA, and DSC. Compare their principles, instruments, and pharmaceutical applications. (10 marks)

Q2. Write a detailed note on X-ray crystallography. Explain Bragg's law, crystal systems, X-ray diffraction techniques (rotating crystal, single crystal, powder), and applications. (10 marks)

Q3. Write a comprehensive note on DSC: principle, types, instrumentation, thermogram interpretation, and pharmaceutical applications. (10 marks)

PREVIOUS YEAR-STYLE QUESTIONS WITH FULL SOLUTIONS

PYQ 1. Explain the principle, instrumentation and applications of DSC in pharmacy. [AKTU-style, 7 marks]

PYQ 2. Differentiate between TGA, DTA, and DSC. (5 marks)

PYQ 3. What is Bragg's law? Explain powder X-ray diffraction with diagram and applications in pharmacy. (7 marks)

PYQ 4. Explain the factors affecting TGA results with appropriate examples. (5 marks)

PYQ 5. Compare single crystal X-ray diffraction and powder X-ray diffraction. Mention applications of each in pharmaceutical analysis. (5 marks)

50 MCQs — CATEGORIZED BY DIFFICULTY

1. TGA measures which property of a sample?

A. Temperature difference

B. Heat flow (mW)

C. Change in mass ✓

D. Electrical conductivity

Explanation: TGA (Thermogravimetric Analysis) measures the CHANGE IN MASS (weight) of a sample as a function of temperature or time. Output = % mass remaining vs temperature (thermogram). DTA measures ΔT; DSC measures heat flow (mW).

2. In DTA, the temperature difference is measured between:

A. Sample and furnace wall

B. Sample and an inert reference material ✓

C. Two different samples

D. Sample at two different heating rates

Explanation: DTA (Differential Thermal Analysis) measures ΔT = T_sample − T_reference, where the reference is an inert material (usually α-alumina, Al_2O_3) that undergoes no thermal events in the studied temperature range.

3. DSC is characterized as a quantitative technique because it measures:

A. Temperature of phase transitions only

B. Mass changes during heating

C. Heat flow (dH/dt) — gives enthalpy values directly ✓

D. Electrical resistance changes

Explanation: DSC measures heat flow (dH/dt, mW or mW/mg) directly. The area under a DSC peak = enthalpy change (ΔH) for that transition. This makes DSC quantitative — unlike DTA which can only give temperatures, not ΔH values.

4. The glass transition temperature (Tg) appears in a DSC thermogram as:

A. A sharp endothermic peak

B. A sharp exothermic peak

C. A step change in the baseline (no peak) ✓

D. A gradual linear slope change

Explanation: Tg appears as a STEP CHANGE (shift) in the baseline heat flow — it represents an increase in heat capacity (Cp) of the material, NOT a latent heat event. Therefore, it has no peak area and cannot give an enthalpy value. This distinguishes it from melting (sharp endo peak) and crystallization (exo peak).

5. The most common inert reference material used in DTA is:

A. Calcium carbonate ($CaCO_3$)

B. α-Alumina (Al_2O_3) ✓

C. Silicon carbide

D. Quartz (SiO_2)

Explanation: α-Alumina (Al_2O_3, corundum) is the standard reference material for DTA and DSC because it is thermally stable, inert, has no phase transitions up to ~2000°C, and has heat capacity similar to many organic samples. It is used in the range −150°C to 1600°C without any thermal events.

6. In a DSC thermogram, the melting of a crystalline drug appears as:

A. Exothermic peak (upward)

B. Endothermic peak (downward) for Endo-down convention ✓

C. Step change in baseline

D. Gradual exothermic slope

Explanation: Melting is an ENDOTHERMIC process — the sample ABSORBS heat to break crystal lattice forces. In standard DSC with endo-down convention, this appears as a downward (negative) peak. The peak area = ΔHm (heat of fusion, J/g). Sharp, narrow peak = high purity sample.

7. X-rays used in crystallography have wavelengths in the range:

A. 100–1000 nm (UV range)

B. 0.5–2.5 Å (comparable to interatomic distances) ✓

C. 1–100 mm (microwave range)

D. 0.001–0.01 nm (gamma ray range)

Explanation: Useful X-rays for crystallography: 0.5–2.5 Å (0.05–0.25 nm). This range is essential because crystal interplanar spacings (d-values) are typically 1–5 Å — the same order of magnitude as X-ray wavelengths, enabling diffraction (Bragg's law).

8. In Bragg's law nλ = 2d·sinθ, the symbol 'd' represents:

A. Diameter of the crystal

B. Interplanar spacing between crystal planes ✓

C. Distance from sample to detector

D. Depth of penetration of X-rays

Explanation: In Bragg's law nλ = 2d·sinθ, 'd' is the interplanar d-spacing — the perpendicular distance between adjacent parallel crystal planes with the same Miller index (hkl). This is the fundamental parameter calculated from measured 2θ diffraction angles.

9. The wavelength of Cu Kα X-rays used in pharmaceutical PXRD is:

A. 0.7107 Å (Mo Kα)

B. 1.5418 Å (Cu Kα) ✓

C. 2.2909 Å (Cr Kα)

D. 1.7902 Å (Co Kα)

Explanation: Cu Kα = 1.5418 Å is the standard X-ray wavelength for pharmaceutical PXRD because organic drug molecules contain mainly C, H, N, O (low atomic number elements) that give excellent diffraction with Cu Kα. Mo Kα (0.7107 Å) is used for heavier atoms and larger unit cells.

10. The crystal system with highest symmetry is:

A. Tetragonal

B. Triclinic

C. Cubic ✓

D. Monoclinic

Explanation: Cubic crystal system has the HIGHEST symmetry: all three unit cell edges equal (a=b=c) and all angles are 90° (α=β=γ=90°). It has 23 symmetry elements. Triclinic has the LOWEST symmetry (a≠b≠c, α≠β≠γ≠90°). Most drug molecules crystallize in monoclinic or triclinic due to low molecular symmetry.

11. In PXRD, all d-spacings are simultaneously in Bragg condition because:

A. Only one crystal plane diffracts at a time

B. Millions of randomly oriented crystallites ensure all planes are represented ✓

C. The X-ray beam has multiple wavelengths

D. The sample rotates at high speed during measurement

Explanation: Polycrystalline powder contains millions of randomly oriented crystallites. For any given d-spacing (hkl plane set), some crystallites will always be oriented at exactly the Bragg angle θ — satisfying nλ = 2d·sinθ and producing diffraction. This is why a powder gives all reflections simultaneously.

12. A DTG (derivative thermogravimetry) curve shows peaks at:

A. The onset of each TGA step

B. The residue level at end of heating

C. The inflection point of each TGA step (maximum rate of mass loss) ✓

D. The melting point of the sample

Explanation: DTG = dm/dT or dm/dt vs temperature. A DTG PEAK occurs at the INFLECTION POINT of the corresponding TGA step — where the rate of mass change is at maximum. The DTG peak temperature is the temperature of maximum decomposition rate, useful for kinetic analysis.

13. In DTA, an endothermic peak indicates:

A. Sample releases heat to surroundings

B. Sample absorbs heat from surroundings (T_sample < T_reference) ✓

C. No thermal event is occurring

D. Sample mass is decreasing

Explanation: In DTA, ΔT = T_sample − T_reference. Endothermic process (melting, dehydration): sample ABSORBS heat → sample temperature LAGS behind reference → ΔT becomes NEGATIVE → peak appears BELOW the baseline (downward peak by convention). Endothermic = sample cooler than reference.

14. Power-compensated DSC was developed by:

A. TA Instruments
B. Mettler-Toledo
C. Perkin-Elmer ✓
D. Shimadzu

Explanation: Power-compensated DSC was developed and commercialized by Perkin-Elmer. In this design, sample and reference have SEPARATE, individual heaters. The power supplied to each is adjusted to maintain IDENTICAL temperatures. Signal = power difference (mW). Most accurate for absolute heat capacity measurement.

15. The 'Scherrer equation' in PXRD is used to determine:
A. d-spacing of crystal planes
B. Molecular weight from diffraction data
C. Mean crystallite size from peak width ✓
D. Absolute configuration of drug molecules

Explanation: Scherrer Equation: $D = K\lambda/(\beta \cdot \cos\theta)$. D = mean crystallite size (Å), K = Scherrer constant ≈ 0.9, λ = X-ray wavelength, β = peak width at half maximum (FWHM in radians), θ = Bragg angle. Broader PXRD peaks → smaller crystallites. Amorphous materials give very broad humps.

16. Polymorphism is best defined as:
A. The ability of a substance to exist in more than one molecular formula
B. The ability of a substance to exist in more than one crystalline form with same composition ✓
C. The decomposition of a drug at multiple temperatures
D. The presence of multiple impurities in a drug

Explanation: Polymorphism: same chemical composition but different crystal packing arrangements (different unit cell, space group, molecular conformation in crystal). Different polymorphs have different: melting point, solubility, dissolution rate, bioavailability, stability. Classic pharma example: ritonavir, carbamazepine, ranitidine.

17. The standard atmospheric condition for most pharmaceutical TGA runs is:
A. Oxygen atmosphere (promotes oxidation)
B. Air atmosphere (normal)
C. Nitrogen atmosphere (inert, prevents oxidation) ✓
D. Carbon dioxide atmosphere

Explanation: Most pharmaceutical TGA is run under NITROGEN (N_2) purge — an inert atmosphere that prevents oxidative degradation of the drug sample during heating. This gives the 'true' thermal decomposition profile without confounding oxidation. Oxygen/air TGA is used specifically to study oxidative stability.

18. Miller indices (hkl) describe:
A. The molecular weight of a crystal
B. The orientation and spacing of crystal planes ✓
C. The heating rate in TGA
D. The detector angle in PXRD

Explanation: Miller indices (h, k, l) are three integers that describe a specific set of parallel planes in a crystal lattice. They are determined from the reciprocals of the plane's intercepts on the a, b, c axes. Different (hkl) planes have different d-spacings → different Bragg angles → different 2θ peaks in PXRD.

19. In DSC, the area under a melting endotherm represents:
A. Melting temperature (Tm)
B. Heat of fusion (ΔHm) in J/g ✓
C. Glass transition temperature (Tg)
D. Crystallite size

Explanation: Area under DSC melting endotherm = enthalpy of fusion (ΔHm, J/g or kJ/mol). This is the heat energy absorbed per unit mass during melting. Higher ΔHm indicates more stable crystal (more energy to break lattice). This quantitative measurement is unique to DSC — DTA cannot give ΔHm.

20. The TGA residue (%) at the end of a pharmaceutical run represents:
A. Moisture content of the drug
B. Amount decomposed
C. Inorganic content / ash / thermally stable residue ✓
D. The amount of drug that melted

Explanation: TGA residue = the mass % remaining at the end of the heating program. For a completely organic drug, residue ≈ 0% (complete volatilization/decomposition). Non-zero residue indicates inorganic content (catalyst residue, inorganic excipient, ash). Useful for determining inorganic impurities or verifying complete decomposition.

21. The Cambridge Structural Database (CSD) contains:

A. DNA and protein sequences

B. ^{1}H-NMR spectra of organic molecules

C. Crystal structures of organic and organometallic compounds ✓

D. IR spectra of pharmaceutical compounds

Explanation: The Cambridge Structural Database (CSD), maintained by CCDC, contains >1.2 million crystal structures of organic and organometallic compounds determined by X-ray and neutron diffraction. Pharmaceutical scientists use it to find crystal structures of drugs, identify polymorphs, and study crystal packing interactions.

22. In the rotating crystal method of X-ray diffraction, what is varied to satisfy Bragg's condition?

A. X-ray wavelength

B. Crystal orientation (by rotation) ✓

C. Sample temperature

D. Detector distance

Explanation: In rotating crystal method, a single crystal is rotated about one axis while being irradiated with MONOCHROMATIC X-rays. As the crystal rotates, different sets of planes come into the Bragg condition (correct θ angle) at different rotation positions, producing diffraction spots (layer lines) on the cylindrical film.

23. Which DSC technique is used to study amorphous drug stability?

A. TGA

B. Glass transition temperature (Tg) measurement by DSC ✓

C. DTA

D. Rotating crystal XRD

Explanation: Tg measurement by DSC is critical for amorphous drug stability. Rule: Tg must be at least 50°C above storage temperature to ensure the amorphous drug remains physically stable (below Tg, molecular mobility is too low for crystallization). DSC detects Tg as a baseline step and Tg can be elevated by using polymeric carriers (amorphous solid dispersions).

24. In a DSC thermogram of a polymorphic drug, two melting endotherms indicate:

A. Two different drugs in the sample

B. Presence of two polymorphs — one converts to other before melting ✓

C. Double melting due to instrument error

D. Decomposition and melting at same temperature

Explanation: Two melting endotherms in DSC indicate the presence of TWO POLYMORPHS. The less stable polymorph melts first (lower Tm, smaller endotherm), then recrystallizes to the more stable form (exothermic peak), which then melts at higher Tm. Example: Carbamazepine dihydrate (Form III) → anhydrous Form I → melting. This pattern is diagnostic of polymorphic conversion.

25. What is the primary use of TGA in pharmaceutical hydrate characterization?

A. Determines melting point of hydrate

B. Quantifies water of crystallization from mass loss step ✓

C. Identifies crystal system of hydrate

D. Measures heat of dehydration

Explanation: TGA quantifies water of crystallization (water of hydration) from the mass loss step. At ~50–150°C, the hydrate loses water → TGA step. % mass loss corresponds to moles of water: e.g., monohydrate MW 200, water MW 18, expected mass loss = 18/200 × 100 = 9%. Compare calculated vs observed mass loss to confirm hydrate stoichiometry. Example: Theophylline monohydrate shows 9.1% mass loss.

26. A DSC trace shows: endotherm at 80°C, exotherm at 120°C, large endotherm at 195°C. This pattern most likely represents:

A. Decomposition, recrystallization, melting of impurity

B. Dehydration, cold crystallization of amorphous form, melting of crystalline drug ✓

C. Two impurities melting and one drug melting

D. Three successive polymorphic transitions

Explanation: Classic amorphous drug DSC pattern: (1) Endotherm ~80°C = dehydration (loss of surface-adsorbed or loosely bound water). (2) Exotherm ~120°C = cold crystallization (amorphous drug crystallizes upon heating above Tg —

releases latent heat). (3) Large endotherm ~195°C = melting of freshly crystallized drug. Recognizing this pattern is essential for amorphous solid dispersion characterization.

27. In PXRD, an amorphous pharmaceutical material produces:

A. Sharp, well-resolved diffraction peaks at specific 2θ positions

B. No signal at all

C. Broad, diffuse hump (amorphous halo) at characteristic 2θ range ✓

D. A flat baseline with no features

Explanation: Amorphous materials have NO long-range crystalline order, so they do NOT produce sharp Bragg diffraction peaks. Instead, they show a broad, diffuse hump (amorphous halo) centered around the average nearest-neighbor distance (typically 15–25° 2θ for organics). % amorphous = amorphous halo area / (amorphous halo + crystalline peak areas).

28. The 'Flack parameter' in single crystal X-ray structure refinement is used to determine:

A. Crystal system

B. R-factor quality

C. Absolute configuration of chiral molecules ✓

D. Unit cell volume

Explanation: The Flack parameter (x) is used in SCXRD to determine the ABSOLUTE CONFIGURATION of chiral molecules using anomalous X-ray scattering. x ≈ 0: correct absolute structure assigned. x ≈ 1: inverted (wrong enantiomer). x ≈ 0.5: racemic mixture. Essential for chiral drugs where R/S configuration determines pharmacological activity and safety.

29. The key advantage of DSC over DTA is:

A. DSC can detect mass changes; DTA cannot

B. DSC gives quantitative enthalpy (ΔH) values; DTA only gives transition temperatures ✓

C. DSC operates at higher temperatures than DTA

D. DSC requires less sample than DTA

Explanation: The fundamental advantage of DSC over DTA: DSC is QUANTITATIVE — measures heat flow (dH/dt) directly, so peak area = enthalpy ΔH (J/g). DTA measures only temperature difference ΔT — it can detect WHEN events occur (transition T) but NOT how much energy is involved (ΔH). This makes DSC essential for purity, amorphous content, and enthalpy-based characterization.

30. In the Bragg-Brentano diffractometer geometry, when the sample rotates by angle θ, the detector rotates by:

A. θ

B. 2θ ✓

C. θ/2

D. 3θ

Explanation: In the Bragg-Brentano (θ/2θ) geometry: the sample rotates by angle θ (from horizontal), while the detector simultaneously rotates by 2θ from the incident beam direction. This maintains the geometry required for Bragg diffraction from flat-plate samples and ensures the detector always tracks the diffracted beam. Hence the name 'θ-2θ scan'.

31. The famous Ritonavir drug recall case illustrated the importance of:

A. DSC for purity testing

B. PXRD monitoring for polymorph stability during storage ✓

C. TGA for moisture content

D. DTA for melting point

Explanation: Ritonavir (HIV protease inhibitor, Abbott) was recalled in 1998 when Form II polymorph spontaneously appeared in commercial batches. Form II had much lower solubility — drug failed dissolution, reducing bioavailability. This case established that routine PXRD monitoring of drug substance and product is essential to detect polymorphic conversion during manufacturing and storage.

32. When using TGA to study drug-excipient compatibility, a NEW decomposition step in the physical mixture (not seen in either component alone) indicates:

A. Perfect compatibility

B. Chemical interaction or incompatibility between drug and excipient ✓

C. Improved thermal stability

D. Moisture contamination during mixing

Explanation: In TGA drug-excipient compatibility: if a PHYSICAL MIXTURE shows a new decomposition step or an existing step shifts to significantly lower temperature (compared to individual components), this indicates CHEMICAL INTERACTION (incompatibility). Compatible systems show TGA profiles that are mathematical combinations of the individual components' profiles — no new steps.

33. For a monoclinic crystal system, which statement is correct?

A. a = b = c and α = β = γ = 90°

B. a ≠ b ≠ c and α = γ = 90°, β ≠ 90° ✓

C. a = b ≠ c and all angles = 90°

D. a ≠ b ≠ c and all angles ≠ 90°

Explanation: Monoclinic crystal system: a ≠ b ≠ c (all three axes different length), BUT α = γ = 90° while β ≠ 90° (only one angle tilted). It has one 2-fold symmetry axis. Triclinic is even lower: a ≠ b ≠ c AND α ≠ β ≠ γ ≠ 90° — no right angles at all. Most drug molecules crystallize in monoclinic ($P2_1/c$ most common space group for organics).

34. In DSC purity determination by van't Hoff analysis, impurities cause:

A. Higher melting point and sharper peak

B. Lower melting point (Tm depression) and broader melting peak ✓

C. Additional exothermic peaks

D. No change in melting peak

Explanation: Impurities in a crystalline drug depress the melting point (Tm) and broaden the melting endotherm — directly analogous to freezing point depression (colligative property). Van't Hoff equation applied to DSC: $\Delta Tm = (R \cdot T_0^2/\Delta Hf) \times x_2$, where x_2 = mole fraction of impurity. Plotting 1/F (fraction melted) vs Tm gives a line whose slope yields x_2 (impurity content).

35. Simultaneous TGA-DSC (STA) instruments are preferred because they:

A. Use less sample than separate TGA and DSC

B. Allow mass change and heat flow to be measured in exactly the same run on same sample, enabling direct correlation of events ✓

C. Are cheaper than separate instruments

D. Give better resolution than separate instruments

Explanation: Simultaneous TGA-DSC (STA) measures BOTH mass change (TGA) and heat flow (DSC) on the SAME sample, at the same time, in the same thermal environment. This directly correlates thermal events: e.g., if an endothermic DSC peak coincides with a TGA mass loss step → dehydration. If DSC shows endotherm with no TGA mass loss → melting. Eliminates sample-to-sample variability.

36. Using Bragg's law (n=1, Cu Kα λ=1.5418 Å), a diffraction peak at 2θ = 20° corresponds to a d-spacing of:

A. 4.44 Å ✓

B. 3.54 Å

C. 2.22 Å

D. 7.08 Å

Explanation: Using Bragg's law: $d = \lambda/(2 \cdot \sin\theta)$. $\theta = 20°/2 = 10°$. $\sin(10°) = 0.1736$. $d = 1.5418/(2 \times 0.1736) = 1.5418/0.3472 = 4.44$ Å. This is a typical d-spacing for the most intense low-angle peak of many organic pharmaceuticals. Higher d-spacing = lower 2θ angle (large planes diffract at small angles).

37. An amorphous drug has Tg = 75°C. For physical stability at room temperature (25°C) storage:

A. Drug is physically stable — Tg is 50°C above storage T ✓

B. Drug is unstable — Tg should be below storage T

C. Need more information; Tg alone does not determine stability

D. Drug will always crystallize regardless of Tg

Explanation: Rule of Thumb: Tg must be ≥ 50°C above storage temperature for physical stability of amorphous drug. Here: Tg = 75°C, storage T = 25°C, ΔT = 50°C. Exactly at the 50°C threshold — borderline stable. Below Tg, molecular mobility is restricted → slow crystallization. Rule derived from Williams-Landel-Ferry (WLF) theory of glass dynamics. In practice, Tg ≥ 50°C + storage T is required for commercial products.

38. In SCXRD structure refinement, an R-factor (R_1) of 15% suggests:

A. Excellent structure — publishable

B. Good structure — meets regulatory standards

C. Poor structure — likely incorrect or disordered crystal ✓

D. Acceptable for quick screening purposes

Explanation: $R_1 = \Sigma||Fo|-|Fc|| / \Sigma|Fo|$ — measures agreement between observed (Fo) and calculated (Fc) structure factors. $R_1 < 5\%$ = excellent, publishable. R_1 5–8% = acceptable for complex/disordered structures. $R_1 > 10\%$ = poor — indicates structural problems (wrong space group, disorder, wrong formula, systematic errors). For regulatory NDA submission, $R_1 < 5\%$ is expected for drug absolute configuration proof.

39. A pharmaceutical company measures DSC of Drug A + Excipient B mixture. The drug's Tm peak disappears completely in the mixture. This most likely indicates:

A. The excipient improved drug stability

B. Eutectic formation or chemical interaction causing complete melting point suppression ✓

C. The DSC instrument malfunctioned

D. Drug was diluted too much to detect

Explanation: Complete DISAPPEARANCE of drug Tm peak in a DSC mixture scan indicates either: (1) Eutectic formation — the drug and excipient form a eutectic mixture with a melting point BELOW both individual Tm values (may appear as single broad endotherm at lower T). OR (2) Complete chemical interaction/reaction that consumes the crystalline drug. This is a RED FLAG for incompatibility in pharmaceutical formulation development.

40. In a TGA thermogram of aspirin (acetylsalicylic acid, MW=180), if a 10 mg sample loses 0.5 mg of mass at 60–80°C, this most likely represents:

A. Decomposition of acetyl group

B. Loss of 5% moisture — adsorbed surface water ✓

C. Sublimation of aspirin

D. Loss of acetic acid (MW=60) indicating 8.3% decomposition

Explanation: 5% mass loss (0.5 mg from 10 mg) at 60–80°C = moisture or adsorbed solvent loss — typical for pharmaceutical raw materials. Aspirin's decomposition to acetic acid + salicylic acid occurs at much higher temperatures (>150°C in TGA). The 5% at low T is most consistent with surface-adsorbed moisture. If it were acetic acid loss (MW=60), that would be 60/180 = 33% mass loss — not 5%.

41. Rietveld refinement of PXRD data is used for:

A. Obtaining single crystal structure at atomic resolution

B. Quantitative phase analysis and crystal structure refinement from powder data ✓

C. Measuring TGA of polycrystalline samples

D. Identifying molecular formula from diffraction data

Explanation: Rietveld refinement fits a calculated PXRD pattern (based on crystal structure model) to the observed powder pattern by least-squares minimization. Uses the FULL PATTERN — not just peak positions. Applications: (1) Quantitative phase analysis — % of each polymorph or phase in mixture; (2) Unit cell parameter refinement; (3) Crystal structure refinement from powder data when single crystals unavailable. Essential for polymorphic API quantification in drug products.

42. Which statement correctly describes the relationship between d-spacing and 2θ in PXRD?

A. Larger d-spacing → larger 2θ angle

B. Larger d-spacing → smaller 2θ angle (inversely related via Bragg's law) ✓

C. d-spacing and 2θ are linearly proportional

D. d-spacing does not change with 2θ

Explanation: From Bragg's law: $\sin\theta = n\lambda/(2d)$. LARGER d → SMALLER $\sin\theta$ → SMALLER θ → SMALLER 2θ. Large crystal planes (large d-spacing) diffract at SMALL angles (low 2θ). Small planes diffract at HIGH angles (high 2θ). This is why the most prominent low-angle peaks (2θ <15°) in pharmaceutical PXRD correspond to large d-spacings — often the unit cell dimensions.

43. In drug-excipient DSC compatibility screening, which observation is MOST INDICATIVE of compatibility (no interaction)?

A. New peaks appearing in the mixture thermogram

B. Drug Tm shifts significantly to lower temperature

C. The mixture thermogram is additive superposition of individual component thermograms ✓

D. Complete disappearance of drug Tm peak

Explanation: COMPATIBLE drug-excipient pairs show DSC thermograms that are the MATHEMATICAL SUPERPOSITION (weighted average) of the individual component thermograms — no new peaks, no significant Tm shifts. INCOMPATIBLE pairs show: new peaks, large Tm depression, peak disappearance, or new exotherms. This is the basis of high-throughput DSC compatibility screening used in early formulation development.

44. The 'direct methods' approach in single crystal X-ray structure determination is used to:

A. Directly measure bond lengths without refinement

B. Solve the phase problem using statistical relationships between structure factor amplitudes ✓

C. Directly measure d-spacings without Bragg's law

D. Determine molecular weight from diffraction intensities

Explanation: The Phase Problem in crystallography: diffraction data gives |F(hkl)| amplitudes but NOT phases φ(hkl). Direct Methods use statistical relationships (Sayre equation, triplet phase relationships) to ESTIMATE phases from the amplitudes. Works best for small molecules (<100 non-H atoms). Gives approximate atomic positions as starting model for least-squares refinement. Alternative methods: Patterson function (for heavy atoms), Molecular Replacement (for proteins).

45. A TGA curve shows a mass loss of 18% at 110°C. The molecular weight of the compound is 180 g/mol. The number of water molecules lost per molecule is approximately:

A. 0.5 (half-hydrate)

B. 1 (monohydrate)

C. 2 (dihydrate) ✓

D. 3 (trihydrate)

Explanation: Moles of H_2O per mole of compound: % mass loss = (n × MW_H_2O / MW_compound) × 100. 18% = (n × 18 / 180) × 100 = n × 10%. Therefore n = 18/10 = 1.8 ≈ 2. This is a DIHYDRATE. Confirmation: expected mass loss for dihydrate = (2×18/180)×100 = 20% (close to 18% — slight discrepancy due to anhydrous MW being 180−36=144, so true calc: 36/180×100=20%). This type of calculation is very common in AKTU exams!

46. Single crystal X-ray diffraction is MOST COMMONLY used in pharmaceutical industry for:

A. Routine batch-to-batch polymorph monitoring in QC

B. Absolute configuration determination and complete 3D structure for NDA submission ✓

C. Moisture content measurement during manufacturing

D. Quantitative assay of API in final dosage form

Explanation: SCXRD is used for: (1) Complete 3D structure determination of new drug molecules — atomic coordinates, bond lengths/angles; (2) Absolute configuration (R/S) by Flack parameter — required for chiral drug NDAs; (3) Proof of polymorph structure. SCXRD is NOT used for routine QC (requires single crystal growth — too slow). PXRD is used for routine QC; TGA for moisture; HPLC/UV for assay.

47. If a pharmaceutical monoclinic crystal has unit cell parameters a=7Å, b=8Å, c=10Å, β=95°, what is the volume of the unit cell approximately?

A. 560 ų

B. 559 ų (a·b·c·sinβ) ✓

C. 400 ų

D. 700 ų

Explanation: For monoclinic system, V = a·b·c·sinβ (because only β ≠ 90°). V = 7 × 8 × 10 × sin(95°) = 560 × 0.9962 = 557.9 ≈ 559 ų. Compare to rectangular parallelepiped (V=a·b·c=560): monoclinic volume is slightly less because one angle is tilted. This type of calculation tests understanding of crystal geometry.

48. In DSC, the cold crystallization of an amorphous drug appears:

A. Before Tg as endotherm

B. After Tg as exotherm, before Tm ✓

C. After Tm as endotherm

D. Simultaneously with Tg

Explanation: Cold crystallization occurs in this SEQUENCE in DSC: Tg (baseline step) → Cold Crystallization Tc (EXOTHERM — amorphous crystallizes spontaneously when heated above Tg, releasing latent heat of crystallization) → Tm (ENDOTHERM — crystalline drug melts). So Tc always appears BETWEEN Tg and Tm, always EXOTHERMIC (energy released as disordered amorphous → ordered crystal). Key: amorphous must be heated above Tg to have molecular mobility for crystallization.

49. Which X-ray source is preferred for pharmaceutical PXRD analysis of organic drug molecules and WHY?

A. Mo Kα (0.71Å) — shorter wavelength gives higher resolution

B. Cu Kα (1.54Å) — wavelength matched to d-spacings of organics; C,H,N,O give low absorption ✓

C. Cr Kα (2.29Å) — longest wavelength for best penetration

D. Synchrotron — always gives best results for pharma

Explanation: Cu Kα (1.5418 Å) is preferred for pharmaceutical organic drug molecules because: (1) Wavelength 1.54 Å is well-matched to typical d-spacings of organic crystals (2–10 Å range); (2) C, H, N, O have low X-ray absorption at Cu Kα

energy — good penetration and signal. Mo Kα (0.71 Å) is better for inorganic compounds and larger unit cells. Synchrotron is used for special studies (very small crystals, time-resolved) — not routine pharma QC.

50. A drug manufacturer finds that after 6 months of storage at 40°C/75% RH, the PXRD pattern of their amorphous API has new sharp peaks that were absent in the initial release material. This indicates:

A. Instrument calibration drift

B. Crystallization of the amorphous API during storage — polymorph formation ✓

C. Moisture absorption without structural change

D. Chemical degradation (hydrolysis)

Explanation: New sharp PXRD peaks in previously amorphous API = CRYSTALLIZATION has occurred. Amorphous → crystalline phase transition is driven by heat (above Tg) and moisture (plasticizer, lowers Tg). At 40°C/75% RH (ICH stress conditions), if Tg is lowered below 40°C by moisture uptake, amorphous API crystallizes. This is a critical stability failure — may alter bioavailability and dissolution. Solution: reformulate as amorphous solid dispersion with polymer to raise Tg > 90°C.

EXAM STRATEGY — HOW TO ATTEMPT UNIT II IN A 70-MARK PAPER

Guaranteed Mark-Scoring Tips for Unit II:

- **ALWAYS draw labelled block diagrams** — DSC, TGA, PXRD diffractometer, Bragg's law derivation — each worth 1–2 marks
- **State Bragg's law correctly** in EVERY XRD answer: $n\lambda = 2d \cdot \sin\theta$ — examiners check this
- **Compare DSC and DTA in a table** when asked about DSC — gets full marks + shows depth
- **Use the Ritonavir example** for PXRD applications — shows clinical awareness
- **Name crystal systems** in order (Cubic to Triclinic) — common MCQ
- **Tg vs Tm distinction** — Tg = step (no latent heat, no peak area), Tm = sharp endo peak (area = ΔHm)
- **Mention ICH Q6A** for solid state characterization in any DSC/PXRD application answer