

PharmacoLearn

MPC201T Advanced Spectral Analysis, M.Pharm Semester 2

Unit 1: UV and Infrared Spectroscopy: Woodward-Fieser Rules, ATR-IR and IR Interpretation

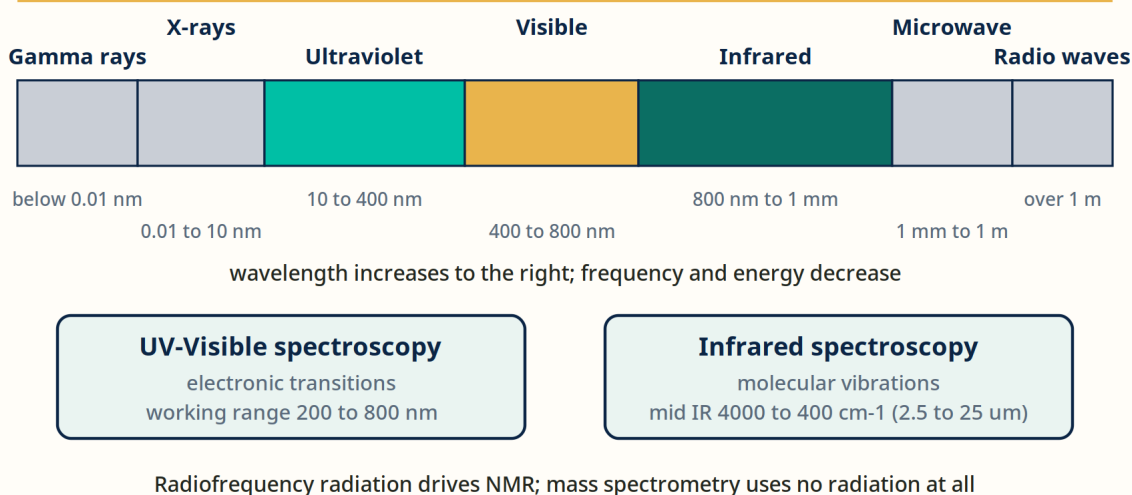
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Advanced Spectral Analysis, subject code MPC201T, is a core Semester 2 subject of M.Pharm Pharmaceutical Chemistry. It carries 4 credits and 60 hours of theory, taught at 4 hours per week, for 100 marks. Semester 1's Modern Pharmaceutical Analytical Techniques (MPC101T) taught how each instrument works. This subject teaches the skill the instruments exist for: **interpretation**. Unit 1 turns UV and IR spectroscopy into a predictive and reading discipline, the Woodward-Fieser rules for calculating the absorption maximum of dienes and enones, ATR-IR, and a systematic routine for reading an infrared spectrum. Unit 2 moves NMR into two dimensions: COSY, NOESY, HETCOR and INADEQUATE, the experiments that trace which proton couples to which, which proton sits on which carbon, and how the carbon skeleton itself is connected. Unit 3 treats the mass spectrum as a fragmentation puzzle governed by rules: alpha cleavage, the McLafferty rearrangement, metastable ions, the ring rule and isotopic patterns. Unit 4 couples separation to identification in the hyphenated techniques, GC-MS, LC-MS, LC-NMR, CE-MS and their companions, with HPTLC, supercritical fluid, ion and flash chromatography. Unit 5 covers the supporting physical and biological methods: DSC, DTA and TGA, Raman spectroscopy, and the immunoassay family, ELISA and the radioimmunoassays of insulin and digitalis. A student who completes this subject should be able to take an unknown compound's UV, IR, NMR and mass spectra and argue, spectrum by spectrum, to a structure that fits all four.

1.1 Where this subject begins: from instruments to interpretation

Every technique in this unit measures the same event from a different side: a molecule absorbing energy of a particular wavelength and changing state. UV and visible light drive electronic transitions, infrared light drives vibrations, radiofrequency radiation flips nuclear spins in NMR, and mass spectrometry, the exception, uses no radiation at all and instead weighs ions. Interpretation is the reverse journey: from the positions and shapes of absorptions back to the structural features that caused them.

The electromagnetic spectrum and the spectroscopic regions



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Figure: The spectroscopic regions. UV and IR interpretation dominate this unit; NMR and MS complete the evidence in Units 2 and 3.

The working discipline of the whole subject can be stated now. No single spectrum proves a structure. UV speaks about conjugation, IR about functional groups, NMR about the proton and carbon framework, and MS about molecular weight and fragmentable subunits. A structure is accepted when it is the only reasonable one consistent with all the evidence, and the exam problems in this subject are built exactly that way.

1.2 UV-Visible spectroscopy at the advanced level

The electronic transitions were introduced in MPC101T; what matters for interpretation is their ranking. In decreasing order of energy the transitions run sigma to sigma star (far UV, below the working range), n to sigma star (saturated compounds with heteroatoms, weak), pi to pi star (alkenes, dienes, aromatics and carbonyls, strong, molar absorptivity typically in the thousands to tens of thousands) and n to pi star (carbonyls and other unsaturated heteroatom groups, weak and symmetry forbidden, absorptivity of the order of 10 to 100). Two consequences follow.

First, the intense pi to pi star band of a conjugated system is the analyst's handle: its position moves predictably with the length and substitution of the conjugated system, which is exactly what the Woodward-Fieser rules of the next sections quantify. Second, solvent polarity moves the two band types in opposite directions. A polar solvent stabilises the n orbital of a carbonyl, so n to pi star bands shift to shorter wavelength (a blue or hypsochromic shift) in polar solvents, while pi to pi star bands, whose excited state is more polar than the ground state, shift to longer wavelength (a red or bathochromic shift). Watching a band move when the solvent is changed is therefore a quick experimental test of which transition it is.

The vocabulary of shifts is used constantly in interpretation and should be exact: bathochromic (to longer wavelength), hypsochromic (to shorter), hyperchromic (more intense) and hypochromic (less intense). A fifth practical point: UV evidence is corroborative, never decisive on its own. A band near 254 nm with high intensity says "conjugated or aromatic system present"; it cannot say which one. That is why this subject always pairs UV with IR, NMR and MS.

1.3 The Woodward-Fieser rules for dienes

Woodward, and later Fieser, noticed that the pi to pi star maximum of a conjugated diene can be predicted by addition. Start from a base value that depends on the diene's geometry, add a fixed increment for every structural feature present, then correct for the solvent. Two base values cover dienes:

- **214 nm** for an acyclic diene, or a **heteroannular** diene, one whose two double bonds lie in different rings.
- **253 nm** for a **homoannular** diene, one whose two double bonds lie in the same ring (a 1,3-cyclohexadiene type system). The higher base value reflects the fixed cisoid geometry, which conjugates more effectively.

The increments (each counted once per feature) are: alkyl substituent or ring residue on the diene system, +5 nm; an exocyclic double bond (a double bond of the diene sticking out of a ring, one carbon in the ring and one outside), +5 nm; a further double bond extending the conjugation, +30 nm; and auxochromes attached directly to the diene carbons: alkoxy (-OR) +6 nm, thioether (-SR) +30 nm, chloro or bromo +5 nm, and dialkylamino (-NR₂) +60 nm. Solvent corrections are made relative to ethanol: water +8 nm, methanol 0, chloroform -1 nm, dioxane -5 nm, ether -7 nm and cyclohexane or hexane -11 nm. Predictions within about 5 nm of the observed value count as agreement.

Woodward-Fieser rules: base values and increments for dienes

Heteroannular or acyclic diene
base value 214 nm

Homoannular diene
base value 253 nm

Add for each feature present:

Alkyl substituent or ring residue	+5 nm
Exocyclic double bond	+5 nm
Double bond extending conjugation	+30 nm
Auxochrome -O-R (ether oxygen)	+6 nm
Auxochrome -S-R (thioether)	+30 nm
Auxochrome -Cl or -Br	+5 nm
Auxochrome -N(R) ₂ (amino)	+60 nm

Then correct for the solvent, relative to ethanol: water +8, chloroform -1, ether -7, cyclohexane -11 nm

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Figure: The diene rules at a glance. Base value, plus increments, plus solvent correction, gives the predicted absorption maximum.

1.4 Worked diene calculations

Example 1, an acyclic diene: 2,4-hexadiene. Base value for an acyclic diene 214 nm; two alkyl substituents (the two terminal methyl groups), $2 \times 5 = 10$ nm; total calculated 224 nm. Observed 227 nm. Agreement is good, and the calculation confirms the conjugated diene rather than two isolated double bonds, whose pi to pi star bands would lie below the working range.

Example 2, a homoannular steroidal diene: ergosterol. The conjugated diene of ergosterol (double bonds at 5-6 and 7-8) lies within ring B, so it is homoannular: base 253 nm. Ring residues on the diene carbons: C-5 carries two ring residues and C-8 carries two, total $4 \times 5 = 20$ nm. Both double bonds are exocyclic to a neighbouring ring (the 5-6 bond to ring A, the 7-8 bond to ring C), $2 \times 5 = 10$ nm. Total calculated $253 + 20 + 10 = 283$ nm. Observed 282 nm. This is the classic demonstration that the rules work on real drug like molecules: a student given only the structure predicts the spectrum of a sterol within 1 nm.

Two habits make these calculations reliable in examinations. First, count ring residues carefully: a ring residue is any carbon substituent on a diene carbon that is part of a ring, and ring junction carbons often carry two. Second, an exocyclic double bond is counted once for each ring to which it is exocyclic. Steroidal dienes fail more students on counting than on chemistry.

1.5 The Woodward-Fieser rules for alpha,beta-unsaturated carbonyl compounds

Enones have their own base values and increments, and the same additive logic. The base value depends on the carbonyl class and ring size:

Parent chromophore	Base value (nm)
Acyclic alpha,beta-unsaturated ketone	215
Six membered cyclic enone	215
Five membered cyclic enone	202

Parent chromophore	Base value (nm)
Alpha,beta-unsaturated aldehyde	210
Alpha,beta-unsaturated carboxylic acid or ester	195

The increments are position dependent, which is what makes enone calculations a genuine test of structure reading:

Feature	Increment (nm)
Alkyl group or ring residue at the alpha carbon	+10
Alkyl group or ring residue at the beta carbon	+12
Alkyl group or ring residue at the gamma or delta carbon	+18
Exocyclic double bond	+5
Double bond extending conjugation	+30
Homoannular diene component within the enone	+39
Hydroxyl at alpha / beta	+35 / +30
Alkoxy at alpha / beta / delta	+35 / +30 / +31
Chloro at alpha / beta	+15 / +12
Bromo at alpha / beta	+25 / +30
Dialkylamino at beta	+95

Solvent corrections for enones, again relative to ethanol or methanol (0): water +8, chloroform -1, dioxane -5, ether -7, hexane or cyclohexane -11 nm. The alpha and beta positions are counted from the carbonyl carbon: the carbon adjacent to C=O is alpha, the next is beta, and an extended system continues gamma, delta.

1.6 Worked enone calculations, and reading enones from spectra

Example 1: mesityl oxide (4-methylpent-3-en-2-one). An acyclic enone: base 215 nm. The beta carbon carries two methyl groups, $2 \times 12 = 24$ nm. Calculated 239 nm; observed 235 nm. The beta disubstitution is read directly off the size of the shift from base value.

Example 2: cholest-4-en-3-one. The enone sits in a six membered ring (ring A of the steroid): base 215 nm. The beta carbon (C-5, the ring junction) carries two ring residues, $2 \times 12 = 24$ nm. Calculated 239 nm; observed 241 nm. Note how the same arithmetic distinguishes this common steroidal enone pattern from a five membered ring enone, whose base value is 13 nm lower: ring size is encoded in the spectrum.

Interpretation runs the calculation backwards. An unknown showing an intense band near 240 nm whose Woodward-Fieser estimate for a proposed enone structure agrees within a few nm supports the enone; if the estimate for the proposed structure is 215 nm but the band sits at 250 nm, the structure is wrong somewhere, usually in the substitution pattern at the beta carbon. Two cautions complete the topic. Strong steric twisting of the conjugated system out of planarity (heavily hindered enones) lowers intensity and can shift the band, because conjugation needs coplanarity. And the weak n to pi star band of the carbonyl, near 300 to 350 nm with absorptivity under 100, should still be visible for an enone and moves to shorter wavelength in polar solvents, a useful confirmation that the chromophore really contains C=O.

1.7 Reading an infrared spectrum: the correlation chart

An infrared spectrum is read in two zones. The **functional group region**, roughly 4000 to 1500 cm^{-1} , holds the stretching vibrations whose frequencies are characteristic of particular bonds and groups: this is where O-H, N-H, C-H, triple bonds, C=O and C=C announce themselves. The **fingerprint region**, roughly 1500 to 600 cm^{-1} , holds coupled skeletal vibrations and bending modes that are unique to the whole molecule: useless for naming groups, decisive for confirming identity against a reference spectrum.

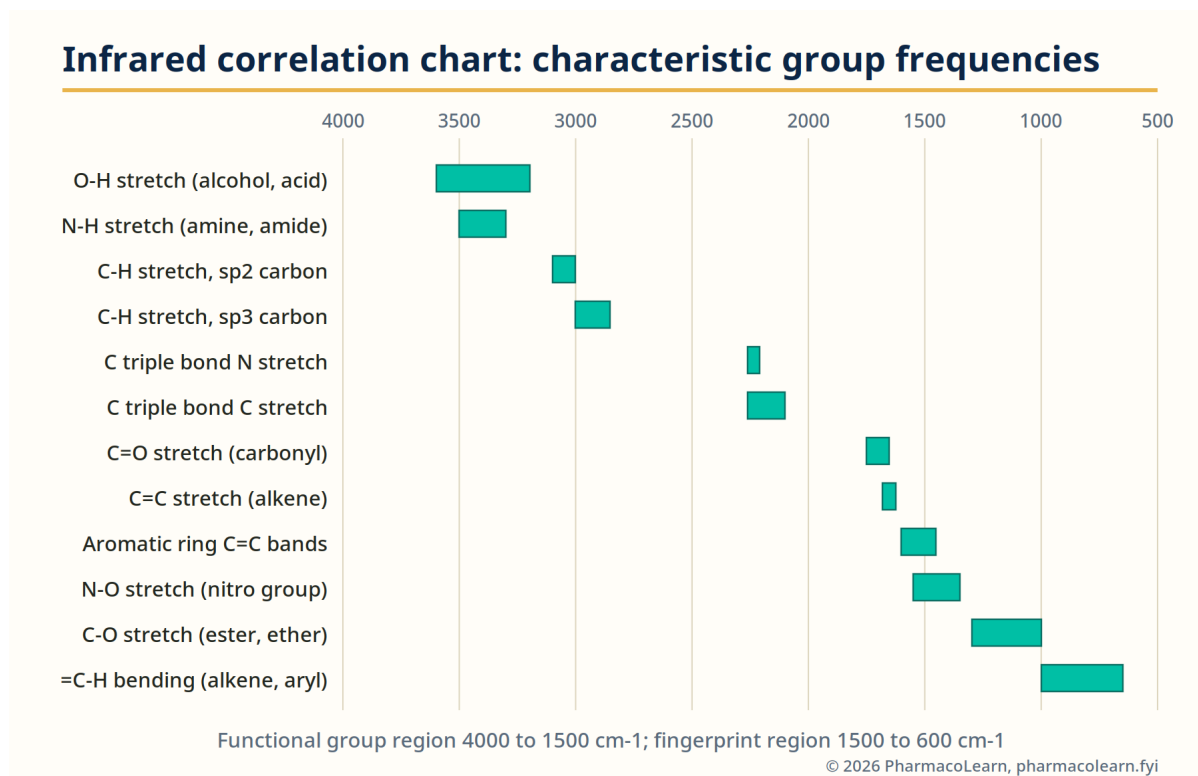


Figure: The correlation chart. Every band in the functional group region should be assigned to a row of this chart before any structure is proposed.

The chart is used with three refinements that separate interpretation from mere band spotting.

- **Position is evidence about environment, not just identity.** A carbonyl at 1715 cm^{-1} is an ordinary saturated ketone; conjugation with a double bond or ring lowers it to about 1685 cm^{-1} ; a strained four membered ring ketone rises towards 1780 cm^{-1} ; an amide, whose carbonyl is weakened by nitrogen lone pair donation, falls to about 1650 cm^{-1} . Hydrogen bonding lowers and broadens O-H (sharp free O-H near 3600 cm^{-1} , broad associated O-H 3200 to 3400 cm^{-1} , the very broad envelope of a carboxylic acid dimer spanning 3300 to 2500 cm^{-1}).
- **Intensity and shape carry information.** Carbonyl bands are strong; alkene C=C bands are weak to medium and vanish for symmetrically substituted double bonds; the aldehyde C-H pair near 2820 and 2720 cm^{-1} is weak but diagnostic; aromatic rings declare themselves by the pair of bands near 1600 and 1500 cm^{-1} plus the substitution pattern bands between 900 and 650 cm^{-1} .
- **Absence is evidence too.** No band in the 1850 to 1650 cm^{-1} region means no carbonyl of any kind, which can eliminate half the candidate structures at a stroke. No broad band above 3100 cm^{-1} argues against O-H and N-H. A disciplined interpreter lists the absences beside the presences.

1.8 ATR-IR: infrared without sample preparation

In attenuated total reflectance, the infrared beam does not pass through the sample at all. It travels inside a crystal of high refractive index (diamond, zinc selenide or germanium) and is totally internally reflected at the crystal surface. At each reflection an **evanescent wave** extends a short distance, of the order of 0.5 to

2 micrometres depending on wavelength, crystal and angle, into the sample pressed against the surface. Where the sample absorbs, the reflected beam is attenuated at that wavenumber, and the detector records an absorption like spectrum after as many reflections as the crystal geometry provides.

ATR-IR: the evanescent wave samples the surface

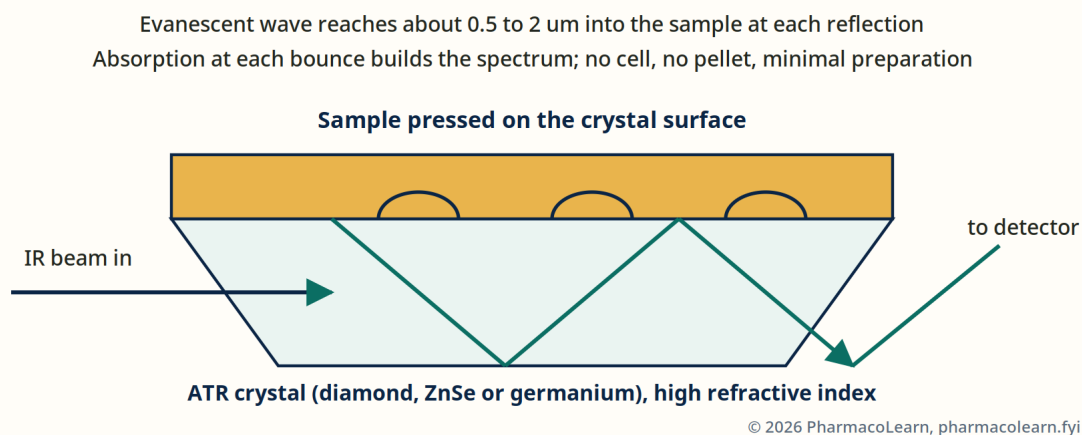


Figure: ATR geometry. The beam never crosses the bulk sample; the spectrum comes from a surface layer about a micrometre deep.

Three practical consequences explain why ATR has displaced the KBr pellet for routine work. There is almost no sample preparation: a solid, powder, paste, film or liquid is simply pressed into contact. Water, the enemy of transmission IR, is tolerable because the effective path length is micrometres, so aqueous and biological samples become measurable. And the spectrum is a surface spectrum, which is a limitation to remember with coated tablets or oxidised surfaces: ATR reports the outermost micrometre, not the bulk. Two honest caveats complete the picture: band intensities in ATR grow relatively stronger at low wavenumber than in transmission spectra (penetration depth rises with wavelength), so library matching works best against ATR libraries, and poor crystal contact gives weak spectra, which is why ATR accessories carry a pressure clamp.

1.9 A systematic routine for IR interpretation

Guessing from one familiar band is the commonest cause of wrong identifications. The routine below, applied in order, converts IR interpretation into a procedure.

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START: note the physical state and how the spectrum was recorded
|
v
1. Carbonyl present? Look at 1850 to 1650 cm-1
  - strong band: assign the class by position
    (anhydride pair, ester 1735, aldehyde 1725, ketone 1715,
     acid 1710, amide 1650 cm-1, adjusted for conjugation/strain)
  - no band: no C=O; rule out acids, esters, ketones, amides
|
v
2. Above 3000 cm-1: broad 3200 to 3400 = associated O-H;
   very broad 2500 to 3300 = acid O-H; sharp 3600 = free O-H;
   3300 to 3500 pattern = N-H (one band 2 deg, two bands 1 deg amine)
|
v
3. C-H pattern: above 3000 = unsaturated or aromatic C-H;
   below 3000 = aliphatic; 2820 and 2720 pair = aldehyde C-H
|
v
4. Triple bonds and cumulenes: 2100 to 2260 cm-1 (nitrile, alkyne)
|
v
5. C=C and ring bands: 1620 to 1680 alkene; 1600 and 1500 aromatic;
   nitro pair near 1550 and 1370; C-O region 1000 to 1300
|
v
6. Fingerprint: substitution pattern 650 to 900 cm-1; compare
   the whole region against reference spectra for final identity
|
v
STATE: groups present, groups absent, and what remains undecided

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Worked example. An unknown liquid shows: strong band at 1700 cm⁻¹; two weak bands at 2820 and 2720 cm⁻¹; medium bands at 1600 and 1500 cm⁻¹; bands at 760 and 700 cm⁻¹; nothing broad above 3100 cm⁻¹. Reading in order: a carbonyl is present at the conjugated position (1700, lowered from 1715 by conjugation); the 2820/2720 pair identifies an aldehyde; 1600 and 1500 say aromatic ring; 760 and 700 cm⁻¹ are the classic pair for a monosubstituted benzene; no O-H. Conclusion: benzaldehyde, C₆H₅CHO, consistent with every observation. Note that the answer was assembled from five independent observations, no one of which would have sufficed alone.

1.10 What UV and IR together settle

Close the unit by fixing the division of labour. UV answers one question well: **how far does the conjugated system extend, and how is it substituted** (quantitatively, through Woodward-Fieser). IR answers a different one: **which functional groups are present, and in what environment**. Neither sees the carbon skeleton's connectivity or the molecular weight; those are the business of NMR and MS. When an exam problem gives all four spectra, the intended order of attack is usually molecular formula and unsaturation count from MS, functional groups from IR, chromophore check from UV, and the framework from NMR. Units 2 and 3 now supply the second half of that method.

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