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BIO & PHARMA ANALYTICAL TECHNIQUES

Chapter 3 Spectroscopy

by
Dr Siti Umairah Mokhtar
Faculty of Engineering Technology
umairah@ump.edu.my

Chapter Description

- Aims
  - Discuss theory, principles and application of analytical techniques used in material characterisation, pre-formulation development, manufacturing process and storage stability

- Expected Outcomes
  - Explain general facts of spectroscopy including application in other field
  - Illustrate theory and principle of spectroscopy: Raman & Near Infra-Red (NIR)
  - Discuss on the application of both instruments in pharmaceutical

- References

  - Gunzler H. & Williams A. (2002). Handbook of Analytical Techniques. Wiley-VCH, Weinheim, Germany.

Spectroscopy

Definition of spectroscopy:

Branch of science that deals with the study of interaction of **matter with light.**

OR

Branch of science that deals with the study of interaction of **electromagnetic radiation with matter.**

→ Spectroscopic data is often represented by **a spectrum**, a plot of the intensity of radiation as a function of wavelength or frequency.

History: Raman Spectroscopy

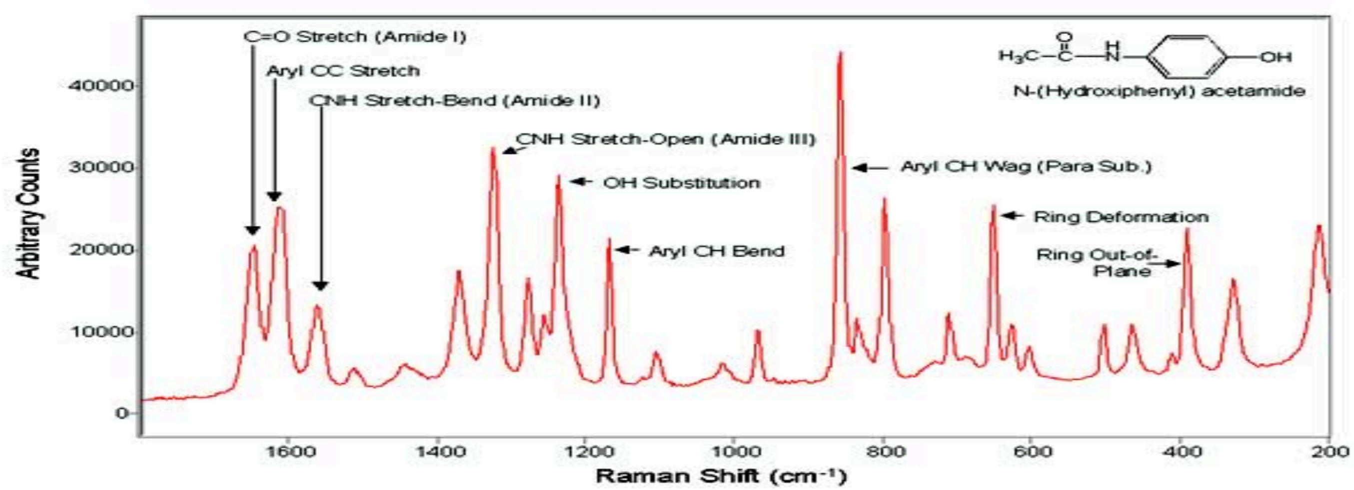
- Raman spectroscopy is named after Sir C. V. Raman (nobel prize in Physic 1930)
- Discover “Raman Effect”
- It relies on inelastic scattering, or Raman scattering, of monochromatic light, usually from a **laser in the visible, near infrared, or near ultraviolet range.**
- Raman spectroscopy is a non-destructive chemical analysis technique which provides detailed information about **chemical structure, polymorphic, crystallinity and molecular interactions.**

Raman Spectroscopy

- Raman is a **light scattering technique,** whereby a molecule scatters incident light from a high intensity laser light source.
- Most of the scattered light (**same wavelength** or color) as the laser source and does not provide useful information → **Rayleigh Scatter.**
- However, a small amount of light (typically 0.0000001%) is scattered at **different wavelengths** (or colors), which depend on **the chemical structure** of the analyte → **Raman Scatter.**

Raman Spectrum

- A Raman spectrum is a plot of the intensity of Raman scattered radiation as a function of its **frequency difference from the incident radiation** (usually in units of wavenumber, cm-1).
- This difference is called the **Raman shift.**



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Raman: Principles

- It relies on **inelastic scattering or Raman scattering**, of monochromatic light, usually from the laser in the visible, near infrared or near ultraviolet range.

- When monochromatic radiation is incident upon a sample then this light will **interact with the sample**.

↓

It may be reflected, absorbed or scattered in some manner.

For Raman, it is the scattering of the radiation that occurs.

↓

Information about **Molecular Structure (Raman Scatter)**

Raman: Components

1. LASER

Laser sources for Raman spectroscopy include **laser diodes, diode-pumped lasers and ion lasers.**

- Laser **wavelengths** ranging from ultra-violet through visible to near infra-red can be used for Raman spectroscopy.
- Laser are used as photon sources due to their **highly monochromatic nature**, and **high beam fluxes.**

2. **FILTER**

- It is essential to have **monochromatic radiations**.
- They may be made of **nickel oxide glass or quartz glass**.

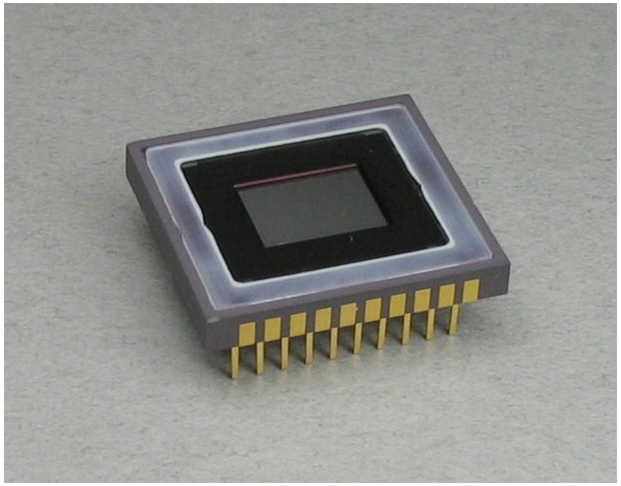
3. **GRATING DIFFRACTION**

- To determines the **wavelength range**
- Raman spectrometers typically use **halographic gratings,** to improve the performance of stray lights (reduce error).

4. **DETECTOR**

A sensitive, low noise detector is required to detect the Raman scattered light.

Charge Coupled Device (CCD) detector is the "camera" used to detect the Raman spectrum.



Applications in Pharmaceutical

- Raman spectroscopy is commonly used in chemistry, since information given is specific to the **chemical bonds and symmetry of molecules**. Therefore, it provides a **fingerprint by which the molecule can identified.**
- For example, in pharmaceuticals:

- Compound distribution in tablets
- Blends uniformity
- API concentration
- Powder content and purity
- Raw material verification
- Polymorphic forms
- Crystallinity
- Contaminant identification

FUNCTION OF SPECTROSCOPY

- Material verification
- Detection of counterfeit and Adulterated Pharmaceutical Products.
- Screening of polymorphism material:
  - Polymorphs may have **different properties** – solubility, dissolution rate, stability or bioavailability.
  - Different crystal forms provide **intensity and frequency shifts** in the Raman spectrum.

Raman: Advantages

- Widely applicable for various materials
- Samples can be solid or aqueous
- No sample preparation thus provides significant cost savings
- Raman spectra can be obtained non-invasively
- Raman analysis can be used to study small particles within homogenous sample matrices.
- Raman analysis time is short
- Raman experiments can be carried out easily
- Good reproducibility
- Highly specific like a chemical fingerprint of a material

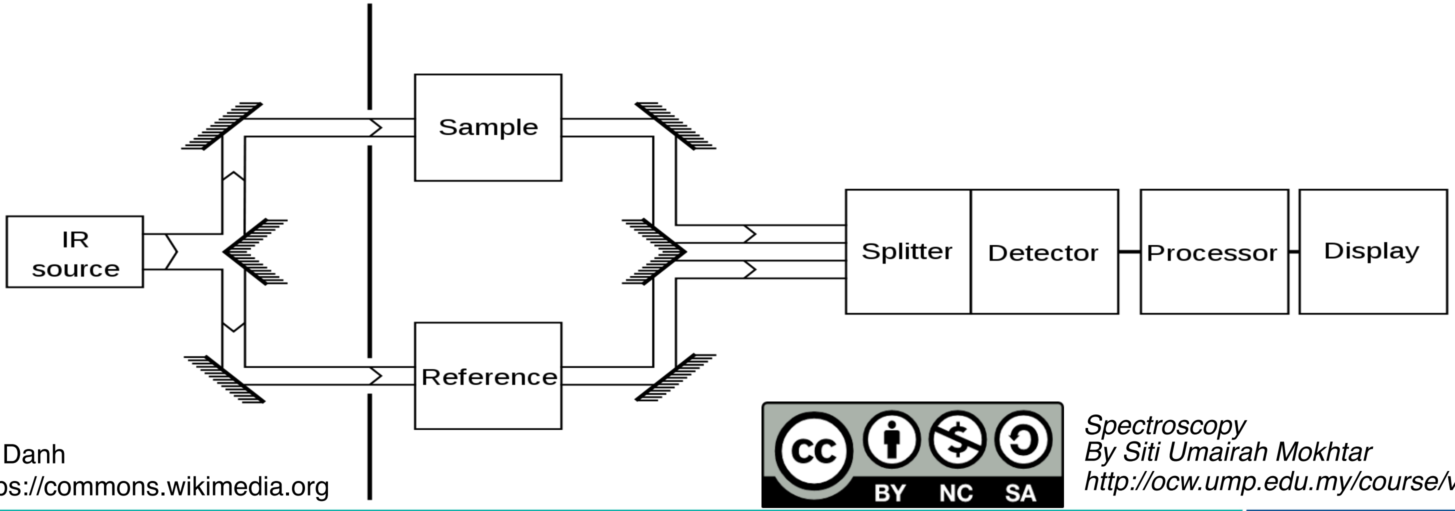
Limitations

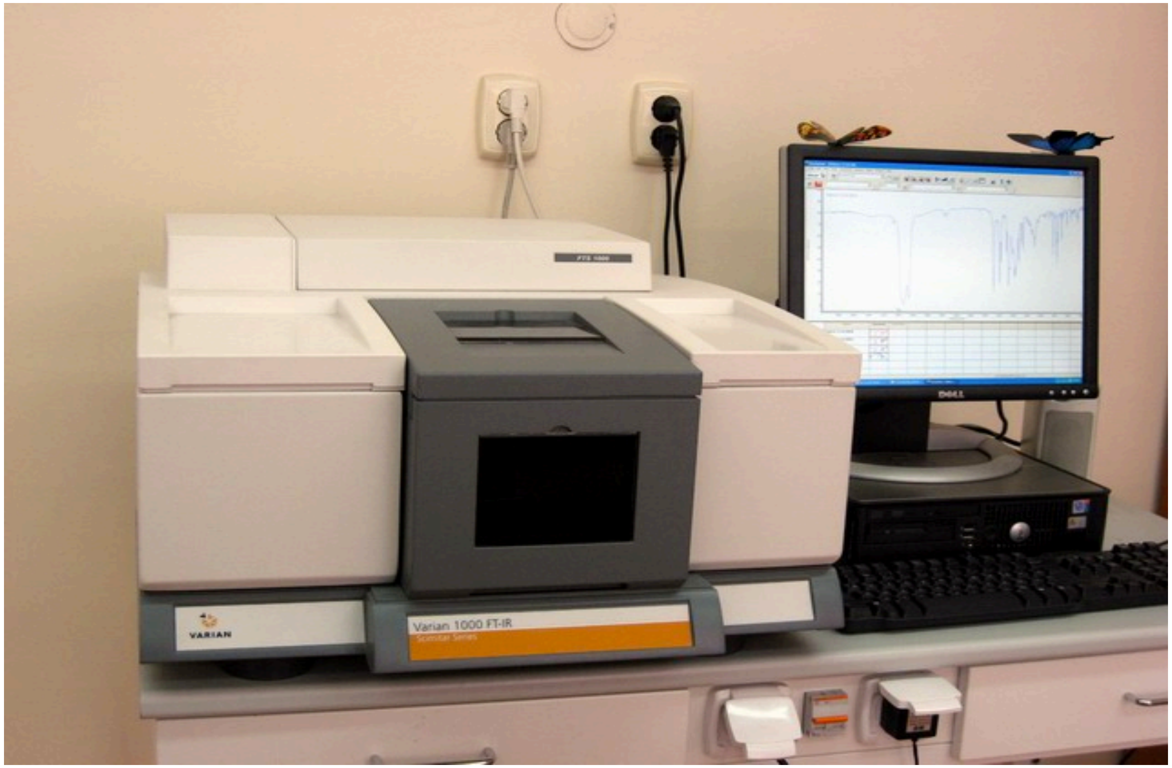
1. Fluorescence can often contaminate Raman spectra
2. More expensive (laser sources)
3. Some heating through the intense laser radiation can destroy the sample or cover the Raman spectrum

Infrared (IR) Spectroscopy

- IR spectroscopy: spectroscopy that **deals with the IR region** of the electromagnetic spectrum.

- It is the result of **absorption of light by vibrating molecules** as compared to Raman in which there is scattering of light by the vibrating molecules.





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IR region:

0.8 µm (800 nm) to 100 µm (1 mm)

- **Near IR:** 0.8-2 µm
- **Middle IR:** 2-15 µm
- **Far IR:** 15 – 1000 µm

Most of the analytical applications are confined to the middle IR region because absorption of organic molecules are high in this region.

IR principle

- Atoms in molecules are linked by chemical bonds. The movement of atoms and the chemical bonds like spring and balls (**vibration**)

- This characteristic vibration are called Natural frequency of vibration.

IR: Principles

When energy in the form of infrared radiation is applied to the sample, cause the vibration between the atoms of the molecule.

When,

Applied infrared frequency = Natural frequency of vibration

Absorption of IR radiation takes place and a peak is observed (spectrum).

- Different functional groups absorb characteristic frequencies of IR radiation. Hence gives the characteristic peak value.
- Therefore, IR spectrum of a chemical substance is a finger print of a molecule for its identification.

IR Spectroscopy

There are two types of bond vibration:

- ***Stretch*** – Vibration or oscillation along the line of the bond

  (symmetric, assymmetric)
- ***Bend*** – Vibration or oscillation not along the line of the bond

  (in plane: scissor, rock; out of plane: twist, wag)

Applications of IR in pharmaceutical

Qualitative analysis (observation)
Identification of substances (compare spectrum, fingerprint)
Determination of molecular structure (absorption band)
Studying progress of reactions (rate of increasing/ disappearance bands)
Detection of impurities (in raw material-compare sample and reference)
Isomerism in organic chemistry (cis, trans isomers)
Functional group isomerism
Identification of functional group
Presence of water in sample

IR spectrum

Functional Group Region (4000 – 1600 cm-1)

Fingerprint Region (1600 – 625 cm-1)

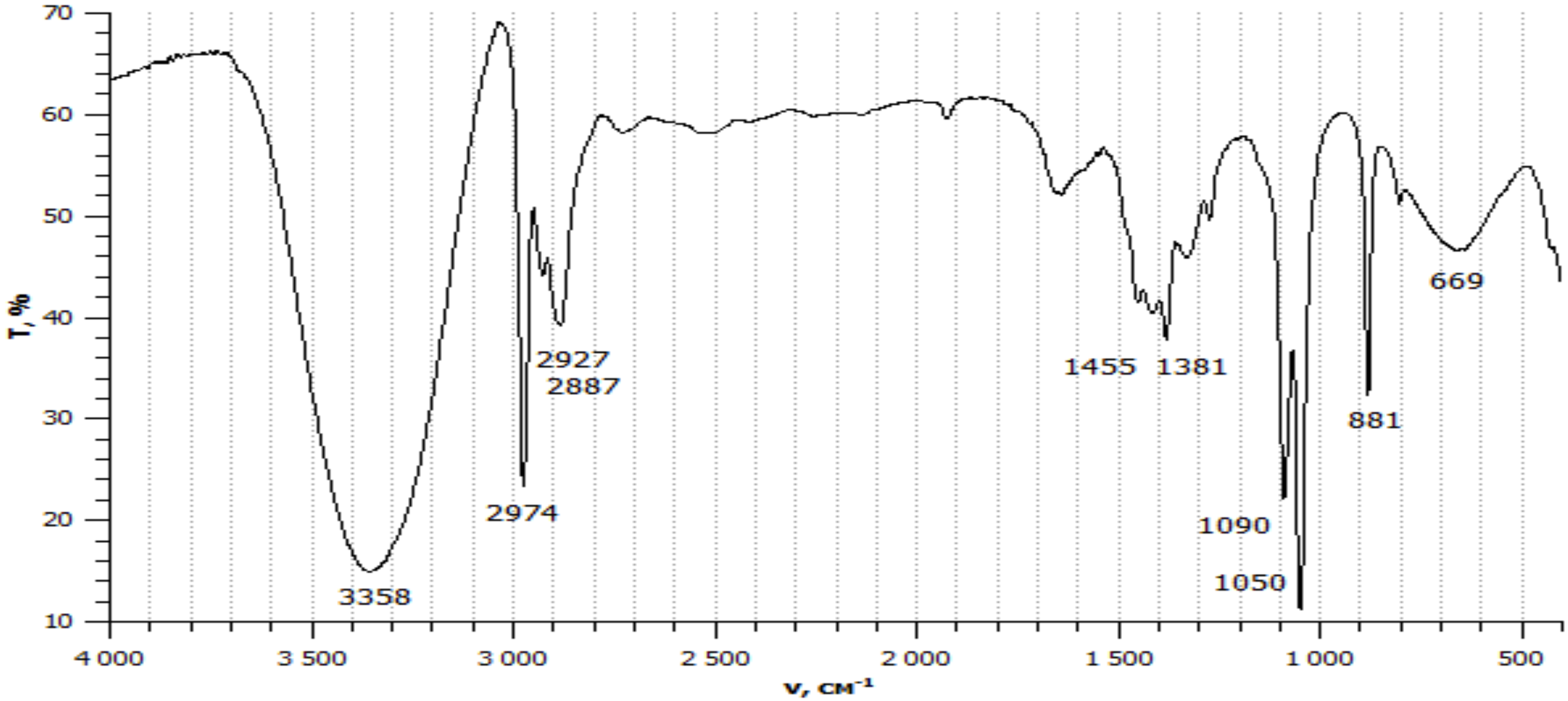

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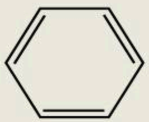
Table 13.4 Important IR Stretching Frequencies

Type of bond	Wavenumber (cm^{-1})	Intensity
C≡N	2260–2220	medium
C≡C	2260–2100	medium to weak
C=C	1680–1600	medium
C=N	1650–1550	medium
	~1600 and ~1500–1430	strong to weak
C=O	1780–1650	strong
C—O	1250–1050	strong
C—N	1230–1020	medium
O—H (alcohol)	3650–3200	strong, broad
O—H (carboxylic acid)	3300–2500	strong, very broad
N—H	3500–3300	medium, broad
C—H	3300–2700	medium

IR: Advantages

Advantages of NIR-spectroscopy:

- ❖ Simple: Almost no sample preparation required
- ❖ Easy to operate: No special operator training required
- ❖ Low operating costs: No solvent, No toxic waste
- ❖ Non-destructive: Sample used for other purposes
- ❖ Fast: Results with in minute
- ❖ Versatile: Determination of several parameters

Comparing RAMAN and IR

RAMAN	IR
It is due to the **scattering of light** by the vibrating molecules	It is the result of **absorption of light** by vibrating molecules
The vibration is Raman active if it causes a **change in polarisability**	Vibration is IR active if there is **change in dipole moment**
Sample preparation is not very elaborate, it can be in any state	Sample preparation is elaborate gaseous samples can rarely be used
Water can be used as a solvent	Water cannot be used due to its intense absorption of IR
Gives an indication of **covalent character** in the molecule	Gives an indication of **ionic character** in the molecule
Cost of instrumentation is very high – high stability of laser source	Comparatively inexpensive

RAMAN: Variants

Several variations of Raman spectroscopy:

- ✧ Surface Enhanced Raman Spectroscopy (SERS)
- ✧ Resonance Raman Spectroscopy
- ✧ Surface-Enhanced Resonance Raman Spectroscopy (SERRS)
- ✧ Angle Resolved Raman Spectroscopy
- ✧ Hyper Raman
- ✧ Spontaneous Raman Spectroscopy (SRS)
- ✧ Optical Tweezers Raman Spectroscopy (OTRS)
- ✧ Stimulated Raman Spectroscopy

CONCLUSION OF THE CHAPTER

1. Raman is a vibrational spectroscopy which is capable of providing chemical and component information with high spatial resolution.
2. Raman spectroscopy is routinely used as a PAT tool for the development of drug substance (reaction analysis and polymorph identification) and for formulation applications (Tablet content uniformity and coating quality).
3. IR spectroscopy is a qualitative analytical technique that helps to indicate mainly the functional group of a molecule.

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Any Question?

Please refer to:

Dr. Siti Umairah Mokhtar

umairah@ump.edu.my