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Unit V

IR and Raman Spectroscopy

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Infra-Red Spectroscopy

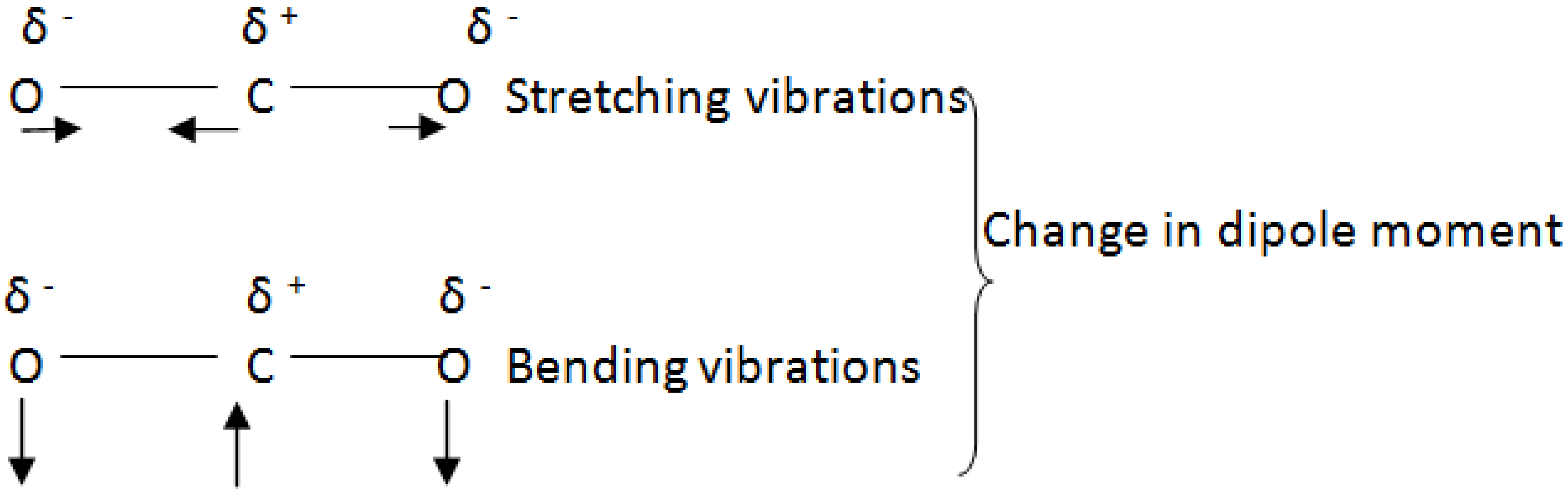
- The infra – red region of the electromagnetic spectrum is subdivided into near infra – red region, 1-2μm; infra – red region, 2-25μm and the far infra – red region, 25-250μm. The infra – red region between 2.5-20μm is used in analysis.

Principle

- The absorption of infra red region of electromagnetic spectrum results in vibrations of the bonding atoms in the molecule leading to alterations in dipole moment or displacement of charge. The fundamental vibrations are those which corresponds to $V_0 - V_1$ transitions. Non linear molecules may undergo vibrational motions like bond deformation and bond stretching. The energy requirement of stretching vibrations are higher and occur at higher frequencies than deforming or bending vibrations.

- The intensity of a particular absorption depends on the dipole moment of the molecule in the ground state and the vibrational excited state. The intensity of absorption depends on the difference in dipole moments. The infra red spectra is observed in heteronuclear molecules because homonuclear molecules have no dipole moment

- . The infra red spectrum of the functional groups in a molecule is highly specific and this facilitates the molecule to be identified and characterized so the term finger print is used to describe the characteristic infra red pattern.



δ^- δ^+ δ^-

O — C — O Stretching vibrations

Change in dipole moment

δ^- δ^+ δ^-

O — C — O Bending vibrations

Fundamental vibrations of carbon dioxide

Application

- It is useful for structural analysis, to distinguish between C – H vibrations in methylene ($-CH_2$) and methyl ($-CH_3$).
- It is used in the elucidation of structure of purified biological molecules of intermediate sizes.
- It is employed in the examination of secondary structure of proteins.
- The nature and the intensity of bands at 3290, 1650 and 1535 cm^{-1} provides information about the secondary structure.
- It is used to measure different gases such as carbon dioxide for photosynthesis and respiration and acetylene for biological nitrogen fixation.

Raman Spectroscopy

- It is a well known phenomenon that if a substance is irradiated with monochromatic light, scattered light is observed in a direction at right angles to that of the incident beam. An examination of the scattered beam of light with the help of a spectroscope reveals that the frequenct of the scattered radiations is generally the same as that of the incident radiations. This is known as **Rayleigh scattering**.

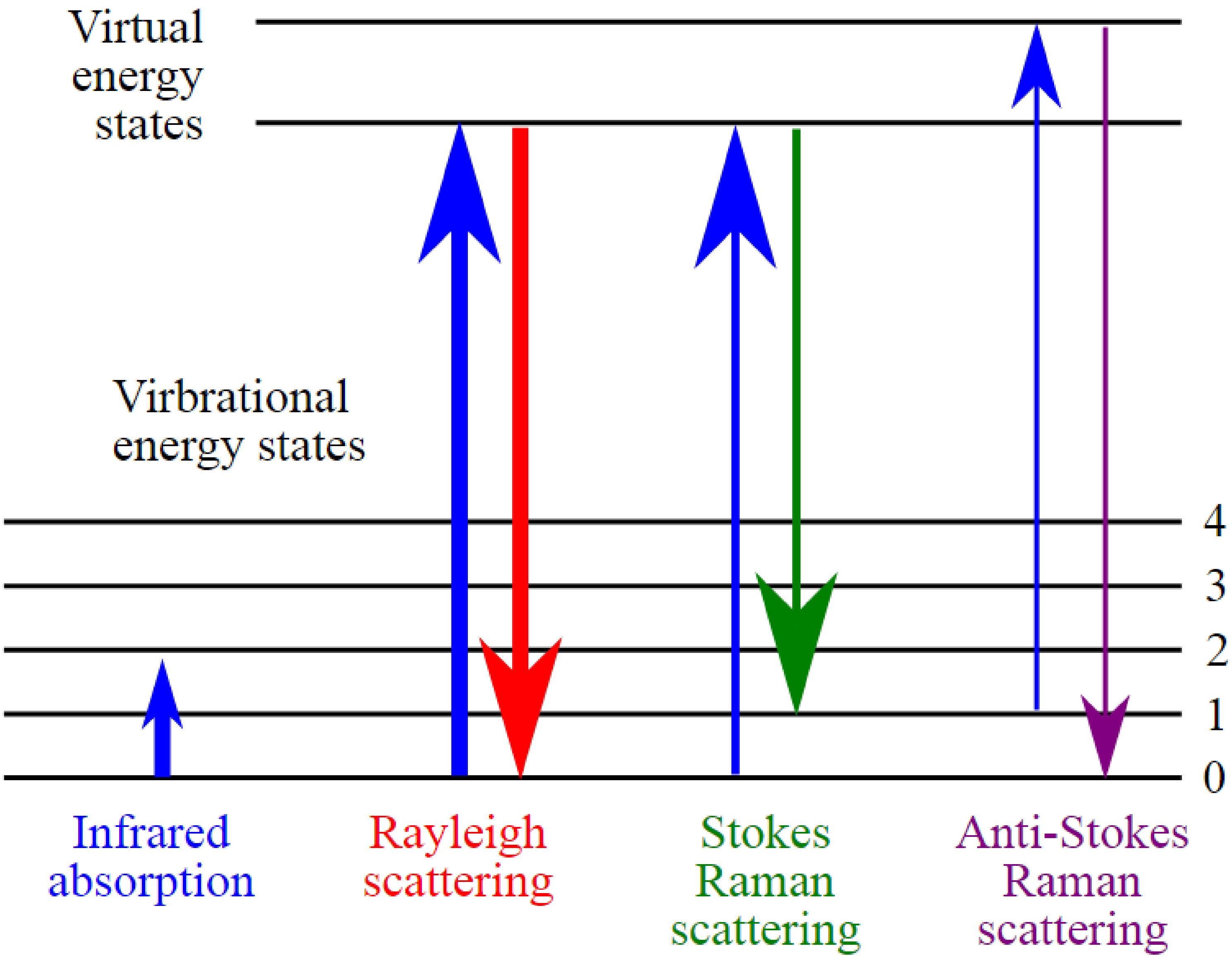
Principle

- If any substance, gaseous, liquid or even solid is exposed to radiation of definite frequency, then the light scattered at right angles contain frequency different from the incident radiation, and is characteristic of the substance under examination.
- Thus the phenomenon due to which the scattering light has a slightly different frequency from that of the incident light and there is a change in the atomic oscillations within the molecules, is called **Raman effect**.

- Raman also observed that difference Δν between the incident and any given scattered line was constant and characteristic of the substance irradiated, and quite independent of the frequency of incident radiation.
- If Δi and Δs are the frequencies of incident and that of scattered light, respectively, then the difference $\Delta \nu = \Delta i - \Delta s$ is known as **Raman frequency**.

- When the scattering of light is observed with the help of a spectrometer it is found that spectrum is made of lines of the incident light together with a series of lines on either side of them.
- This series of lines in the scattering of light is known as **Raman spectra**.
- In the spectra of scattered radiation, the Raman lines appear in the spectrum on either side of the line of incident radiation with higher as well as lower frequencies.
- The Raman lines on the lower frequency side are called **Stokes lines** that where $\Delta i > \Delta s$ and those on the higher frequency side are termed **anti stokes lines** that is when $\Delta s > \Delta i$.

- **Mechanism of Raman effect**: Raman effect arises from the absorption of energy by a molecule from the light radiation falling on it. This causes transitions from lower to higher vibrational or rotational levels. As a result of removal of energy from the incident beam, the energy of the latter falls and so does the frequency. The scattered radiation therefore has a lower frequency than the incident radiation. This explains the occurence of Stokes lines.
- It is evident that the difference in energy of the two vibrational or rotational levels is given by hc $(\bar{\upsilon}_i - \bar{\upsilon}_s)$ where h is Plancks constant and c is the velocity of light.


Virtual energy states
Virbrational energy states
4
3
2
1
0
Infrared absorption
Rayleigh scattering
Stokes Raman scattering
Anti-Stokes Raman scattering

- Regarding anti-Stokes line, it has been suggested that the molecule, instead of absorbing energy, emits energy which is added on to that of the incident light so that the frequency of the latter becomes higher. The scattered radiation, therefore has a higher frequency than the incident radiation.
- The magnitude of Raman effect corresponds to the vibrational and rotational regions of electromagnetic spectrum. Raman effect is mainly due to vibrations. In Raman spectrum there must be change in the polarisability of the molecule during vibration rather than a change in dipole movement.

Applications

- Molecular structure of a compound can be studied. Raman spectra yield the information about the structure of a molecule.
- Intermediate sized molecules such as drugs, metabolic intermediates and substrates. Examples are the identification of substances such as penicillin and its derivatives, small peptides and environmental pollutants.

Application

- It is a rapid method for measuring certain contaminants in food stuffs and can be coupled to Gas liquid chromatography when it is also frequently used for the analysis of drug metabolites.
- Gas analysis is rapid particularly for measuring different concentrations of gases such as CO_2, CO and acetylene in biological samples.
- Used to study CO_2 metabolism in photosynthesis and respiration in plants.

References

- Principles and Techniques of Biochemistry and Molecular Biology 7th edition.Eds Keith Wilson and John Walker, 2010.
- Introductory Practical Biochemistry. Eds Sawhney SK and Randhir Singh, 2000.