

Instrumental Methods in Bioprospecting: Spectroscopy

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Introduction

Spectroscopy is a technique that uses the interaction of energy with a sample to perform an analysis. The data that is obtained from spectroscopy is called a spectrum. A spectrum is a plot of the intensity of energy detected versus the wavelength (or mass or momentum or frequency, etc.) of the energy. A spectrum can be used to obtain information about atomic and molecular energy levels, molecular geometries, chemical bonds, interactions of molecules, and related processes. Often, spectra are used to identify the components of a sample (qualitative analysis). Spectra may also be used to measure the amount of material in a sample (quantitative analysis). Because the response of a compound to electromagnetic (EM) radiation depends on its structure, spectroscopy can be used to elucidate the structure of unknown chemical products. EM radiation behaves both as a particle of light (called a photon) and as a wave moving at the speed of light (c ; $c = 3 \times 10^8 \text{ m/s}$).

Properties of EM particles and waves

1. **Wavelength (λ):** Distance between two peaks or troughs in a light wave.
2. **Frequency (ν):** Number of wave cycles that pass a given point per line. Usually measured in Hertz (Hz; 1 Hz = 1 cycle/second).
3. **Energy of a photon:** $E = h\nu = hc/\lambda$, where h = Planck's constant = 6.6×10^{-34} J/sec.

Types of Spectroscopy

There are several types of spectroscopy, and among all these three are important for bioprospecting.

1. **Nuclear magnetic resonance (NMR) spectroscopy:** Measures interaction of radio waves with atomic nuclei in a magnetic field.
2. **Infrared (IR) spectroscopy:** Measures absorption of infrared light by chemical bonds.
3. **Ultraviolet/Visible (UV/Vis) spectroscopy:** Measures absorption of ultraviolet or visible light by π bonds.

Nuclear magnetic resonance (NMR) spectroscopy

Atomic nuclei have a "spin" associated with them (i.e., they act as if they were spinning about an axis) due to the spin associated with their protons and neutrons. Because nuclei are positively

charged, their spin induces a magnetic field. When a magnetic field is applied to atomic nuclei, the magnetic fields of the nuclei align themselves either parallel or antiparallel to the applied magnetic field. The nuclei have a slight preference for the parallel alignment, as it has a slightly lower energy, but nuclei can flip between the two possible alignments. When EM radiation with energy equal to the energy difference between the two alignments is applied to the nuclei, it induces them to flip from parallel to antiparallel alignment. Rapid flipping between alignments occurs. The nuclei are said to be in resonance, and the energy they emit when flipping from the high to the low energy state can be measured. The energy at which a given nucleus achieves resonance depends on its chemical surroundings. NMR spectra are taken by applying a magnetic field to as ample, irradiating the sample with EM radiation whose energy is varied over a given range, and measuring the energy emitted by flipping nuclei at each energy.

1. The range of radiation energies is generally chosen such that emission from only one type of nucleus (e.g., ^1H) in a molecule is seen.
2. NMR spectroscopy does not work for nuclei that have an even number of protons and neutrons—these nuclei have no net spin.
3. NMR spectroscopy is most commonly done on ^1H and ^{13}C .

Features of an NMR Spectrum

The distinguished features and terms related to NMR spectrum are as follows:

1. NMR spectra are displayed as plots of intensity of energy emission (due to resonance) versus the energy of the radiation applied to the sample. Peaks in the spectrum represent resonance energies for nuclei in a molecule.
2. **Shielding:** An electron cloud circulates around each nucleus and creates a small magnetic field opposing the applied magnetic field. The electron cloud around each atom depends on the surrounding atoms. As a result, each nucleus experiences a slightly different magnetic field (the sum of the applied field and the field from the electron cloud). For this reason, the energy at which a nucleus achieves resonance depends on its surroundings.
3. **Chemical shift (δ):** The resonance energy for a given nucleus is reported in an NMR spectrum as the difference (in parts/million) between the resonance frequency for a given proton and the resonance frequency for protons in a reference compound, which is usually tetramethylsilane, $(\text{CH}_3)_4\text{Si}$. Chemical shifts give information about the atomic surroundings of a given nucleus.
4. **Peak intensity:** The area under a peak in an NMR spectrum is proportional to the number of nuclei in a given chemical environment in a molecule (e.g., if the area under a peak is two times the area under another peak, there are twice as many nuclei responsible for the larger peak than for the smaller one). The intensity of an NMR peak gives information about the relative number of a given type of nucleus in a molecule.
5. **Spin-spin splitting:** In ^1H NMR, a given hydrogen nucleus interacts with hydrogen nuclei on neighboring carbon atoms such that the peak from that nucleus is split into multiple peaks called a multiplet. Relative intensities of the peaks in a multiplet follow Pascal's triangle. Spin-spin splitting gives information about the hydrogen atoms neighboring a given hydrogen nucleus.

Infrared (IR) spectroscopy: Theory

The principles and theory related to IR spectroscopy are detailed below:

1. Covalent bonds are similar to springs—bonded atoms vibrate (i.e., stretch and compress) and bend about their bonds. As a consequence of quantum mechanics, these bonded atoms can vibrate and bend only at frequencies that are integral multiples of a fundamental frequency that depends on the type of bond.
2. Bonds about which vibration and bending occur can absorb light if the frequency of the light wave is the same as the frequency of the movement about the bond. The frequency of light absorbed by these bonds is generally in the infrared region of the EM spectrum.
3. In IR spectroscopy, a chemical sample is irradiated with infrared light over a wide range of frequencies, and the light absorbed by the sample at each frequency is measured.
4. Bonds about which a molecule is symmetric cannot absorb IR light and therefore cannot be detected by IR spectroscopy.

Features of an IR spectrum

The distinguished features and terms related to IR spectrum are as follows:

- 1) IR spectra are displayed as plots of absorption versus wave number (cm^{-1} , $1/\lambda$), which is another measure of the energy of a light wave (similar to frequency). Peaks in an IR spectrum represent wavelengths at which light was absorbed by the molecules in the sample.
- 2) Because each functional group that is IR-active has a characteristic set of frequencies at which it absorbs IR light, IR spectroscopy is useful in detecting the presence of specific functional groups in a molecule.
- 3) Because nearly all organic molecules contain C-C and C-H bonds that absorb IR light in similar ways, IR spectroscopy is most useful in identifying functional groups that contain other bonds besides C-C and C-H bonds.
- 4) **Fingerprint region:** Region between 1200 cm^{-1} and 1700 cm^{-1} in an IR spectrum; contains complicated absorption peaks that are characteristic of a specific molecule. An unknown compound can be identified with reasonable certainty if its fingerprint region matches that of a known compound.

Ultraviolet/Visible (UV/Vis) spectroscopy : Theory

If a molecule has δ electrons, it can absorb UV or visible light to promote one of those electrons into a higher-energy orbital. UV/Vis spectroscopy generally involves the promotion of the δ electron in the highest-energy occupied orbital to the lowest-energy unoccupied orbital. A UV/Vis spectrum is taken by irradiating a sample with UV/Vis light over a range of wavelengths and measuring the amount of light absorbed at each wavelength.

Features of UV/Vis spectra

The distinguished features and terms related to UV/Vis spectrum are as follows:

1. UV/Vis spectra are displayed as plots of absorption versus wavelength. Peaks represent wavelengths at which light was absorbed by the molecules in the sample.

2. The energy of the UV/Vis light absorbed by a δ electron system in a molecule depends on the nature of the δ system. As a result, the presence of a particular type of δ system in a molecule can be identified by UV/Vis spectroscopy.
3. The energy gap between the highest-energy occupied orbital and the lowest-energy unoccupied orbital decreases as the size of the δ electron system increases. Consequently, the wavelength of UV/Vis light absorbed by a molecule increases as the size of its conjugated δ electron system increases (because the energy of a light wave decreases with increasing wavelength).
4. UV/Vis spectroscopy is generally used on conjugated hydrocarbon systems, but other molecules containing π electron systems, such as carbonyls, are also weak absorbers of UV/Vis light.

Suggested Reading

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