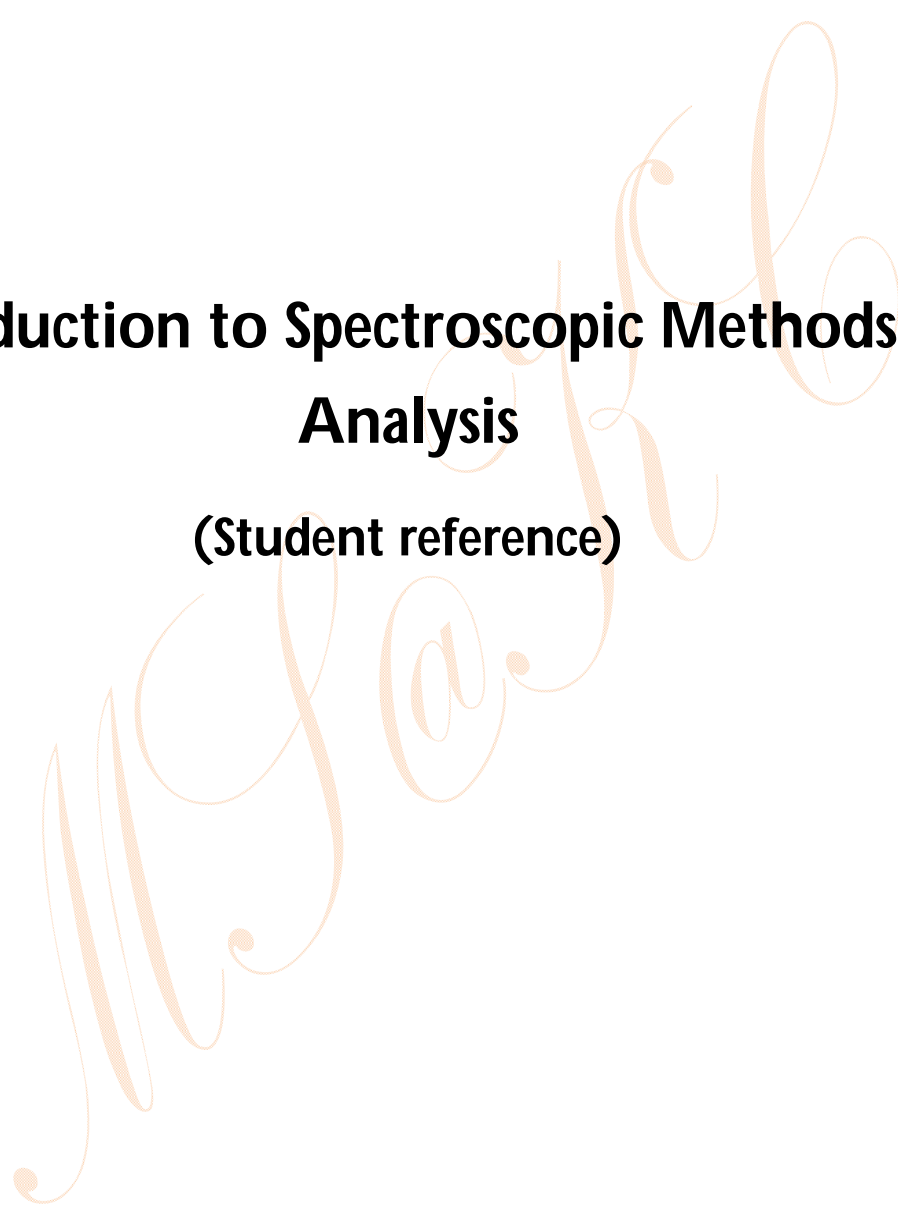
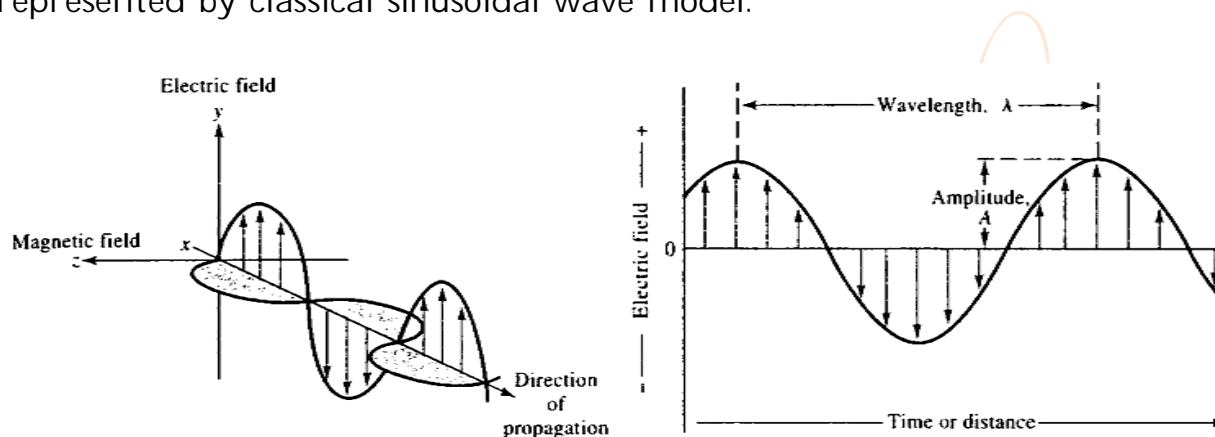


**Introduction to Spectroscopic Methods of
Analysis**
(Student reference)

A decorative graphic consisting of several overlapping, flowing orange calligraphic swirls that originate from the right side and curve downwards and to the left, partially framing the main title text.

Introduction to spectroscopic methods of analysis

A monochromatic electromagnetic wave of wavelength λ propagating along the x-axis of a suitably chosen laboratory coordinate system is schematically represented by classical sinusoidal wave model.



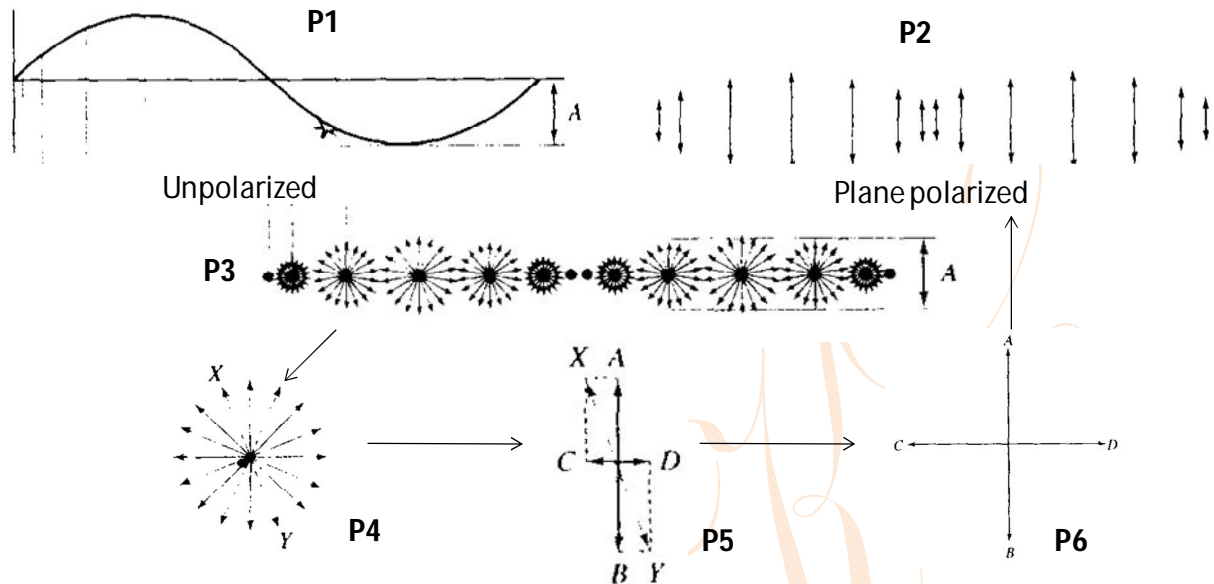
The electric field oscillates in particular direction of a plane perpendicular to the magnetic field. If the radiation is unpolarized, a component of the electric field would be seen in all planes. The electromagnetic radiation is viewed as a stream of discrete particles, or wave packets, of energy called **photons**. The energy of a photon is proportional to the frequency of the radiation. These wave particle duality of radiation are not mutually exclusive but, rather, **complementary**.

Visualization of Unpolarized and plane polarized monochromatic wave

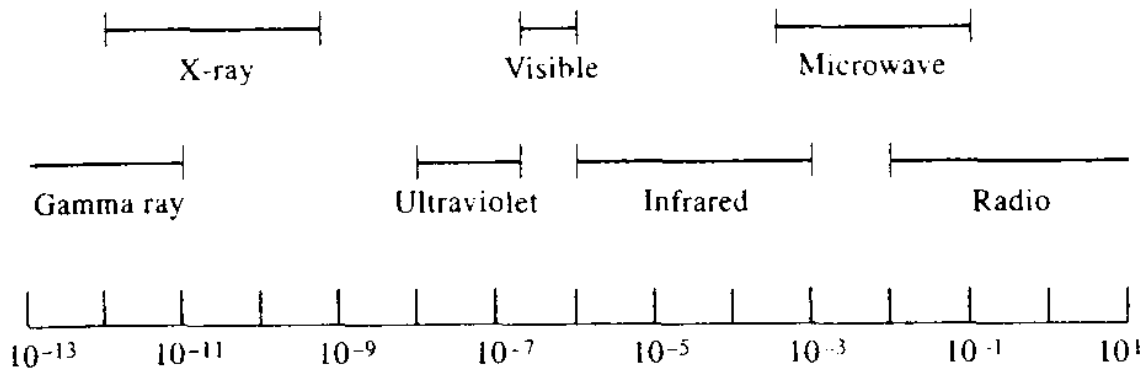
A beam of monochromatic radiation can be visualized (Fig. P3) as an infinite set of electric vectors that fluctuate in length from zero to maximum amplitude. The vector in XY plane (Fig. P4) can be resolved into two mutually perpendicular components AB and CD (Fig. P5). If two components of the planes (Fig. P4) are combined, the resultant is Fig. P6.

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Removal of one of the two resultant planes of vibration in Fig. P6 produces a beam that is **plane polarized** (Fig. P2).



Region of electromagnetic spectrum



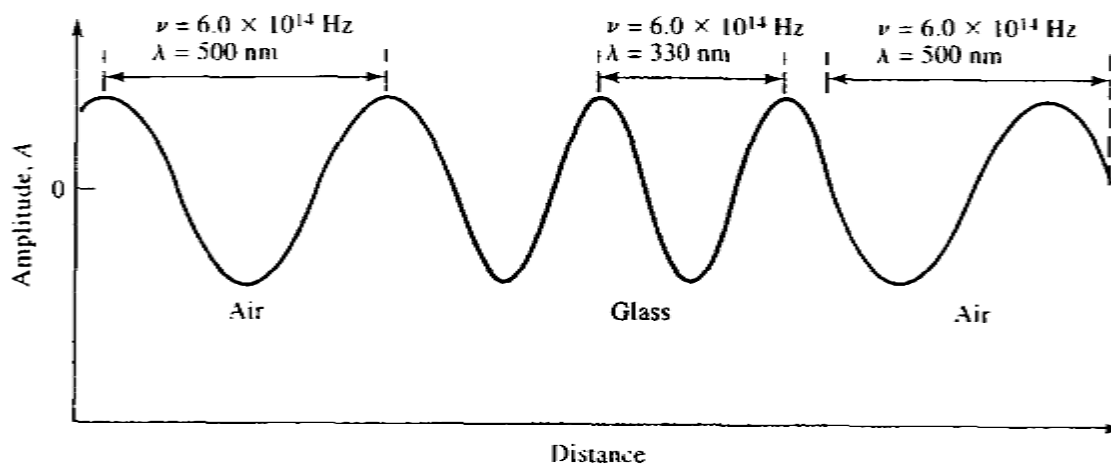
Change in wavelength as function of medium

In a vacuum, the velocity of electromagnetic radiation is independent of wavelength rather it depends on the medium ($v = c/\lambda$). The propagation of radiation is slowed by the interaction between the electromagnetic field of the radiation and the bound electrons in the matter. For example, if a radiation passes through a dense glass from air and then back to air, the

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wavelength shortens by nearly 200 nm (> 30%), into glass. It retains the same wavelength as air after coming out from the glass.



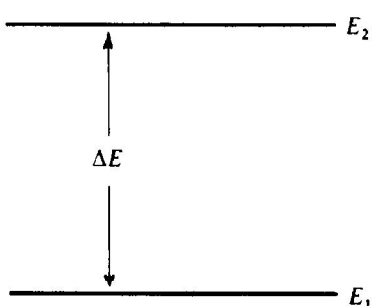
The interaction of a molecular system with an electromagnetic wave can be described by the interaction of the electric and magnetic dipole moments (quadrupole moments) with the electric and magnetic fields (gradients) respectively (neglecting all interactions except between the dipoles and the fields). Thus, **molecular spectroscopy** is the study of interaction of electromagnetic radiation with matter to understand the fundamental property associated with matter.

A molecule in space can have either rotational energy by virtue of rotation about its centre of gravity or vibrational energy due to the periodic displacement of its atoms from their equilibrium positions or electronic energy since the electrons associated with each atom or bond are in unceasing motion. Thus, an electron can exist in one of several discrete energy levels. The energy is quantized.

The suffixes 1 and 2 are quantum numbers which is used to distinguish the energy levels. Transitions can take place between the levels E_1 and E_2 provided the appropriate amount of energy, $\Delta E = E_2 - E_1$, can be either

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absorbed or emitted by the system. Planck suggested that such absorbed or emitted energy can take the form of electromagnetic radiation. ($\Delta E = h\nu$; Planck constant $h = 6.63 \times 10^{-34}$ joules s). Spectroscopist measures the various characteristics of the absorbed or emitted radiation during transitions between energy states.

What happens to the energy absorbed?

Possibility 1: A finite number of sample molecules appear to be capable of absorbing an infinite amount of energy. An energized molecule under thermally collision with its neighbours gradually loses its excess energy to them as kinetic energy—the sample as a whole becomes warm.

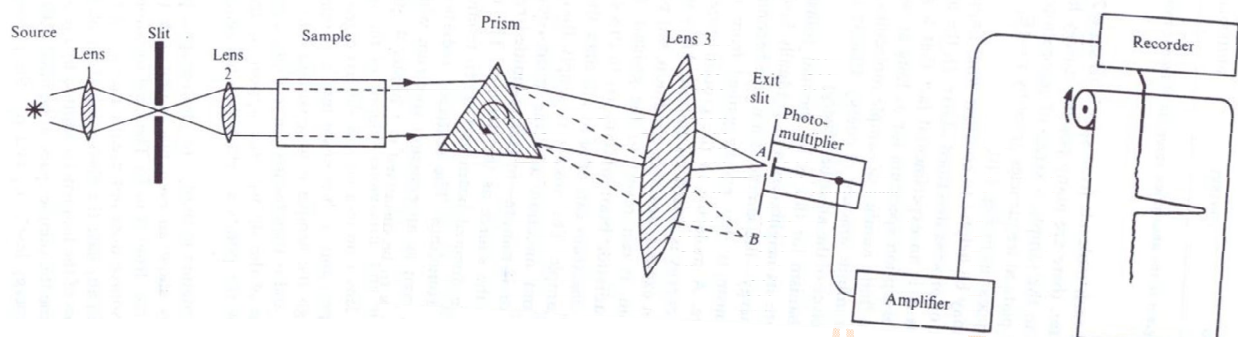
Possibility 2: A molecule in the ground state absorbs energy at frequency ν and raise to an excited, unstable, condition. But by emitting radiation of frequency ν , it can revert to the ground state and is able to reabsorb radiation from the source beam once more.

Then the question arises that how does it produce an absorption spectrum if the absorbed energy is re-emitted by the sample?

The answer is simply that the radiation is re-emitted in a random direction and the proportion of such radiation reaching the detector is tiny—in fact re-emitted radiation has the same chance of reaching the source as the detector. The net effect, then, is an absorption from the directed beam.

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Schematic diagram of spectrophotometer



The Intensity of Spectral Lines

- 1) **Transition probability:** Whether the transition is allowed or forbidden is essentially reliant of selection rules.
- 2) **Population of states:** The most intense spectral line will arise from the level which initially has the greater population.

$$\frac{N_{\text{upper}}}{N_{\text{lower}}} = \exp(-\Delta E/kT)$$

The Width of Spectral Lines

- 1) **Collision broadening:** Collisions inevitably cause some deformation of the particles and hence perturb, to some extent, the energies of at least the outer electrons in each.
- 2) **Doppler broadening:** Liquids and gases the motion of the particles causes their absorption and emission frequencies to show a Doppler shift; since the motion is random in a given sample, shifts to both high and low frequencies occur and hence the spectral line is broadened.

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3) **Heisenberg uncertainty principle**: A system exists in an energy state for a limited time δt seconds, then the energy of that state will be uncertain (fuzzy) to an extent δE where;

$$\delta E \times \delta t \approx h/2\pi \approx 10^{-34} \text{ J s}$$

#Signal-to-Noise Ratio

Modern spectrometers use some form of electronic amplification to magnify the signal produced by the detector, every recorded spectrum has a background of random fluctuations (referred as noise) caused by spurious electronic signals produced by the source or detector, or generated in the amplifying equipment.

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$$E = \frac{1}{2}mV^2$$

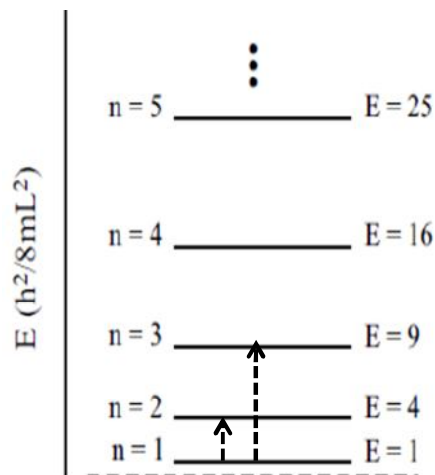
$$E = \frac{p^2}{2m}$$

$$E = \frac{h^2}{2m\lambda^2}$$

$$E = \frac{n^2h^2}{8mL^2}$$

where
 $p^2 = m^2V^2$
 $\lambda = 2L/n$

V = velocity
 p = momentum of the particle
 m = mass of the particle
 L = length of the box
 n = quantum number



There is discrete set of energy level for a given mass m and a given box length L . It is assumed that the potential energy between the wall is constant ($0 \leq x \leq L$). If the quantum numbers $n = 1, 2, 3, 4, \dots$ then by solving the Schrödinger equation for simple one-dimensional particle in a box system, the allowed energies are

$$E = \frac{h^2}{8mL^2}, \quad \frac{4h^2}{8mL^2}, \quad \frac{9h^2}{8mL^2}, \quad \frac{16h^2}{8mL^2}, \dots$$

For particle in box energy level, the energy (E) represented as unit of $h^2/8mL^2$ is sharply increased as a square function of quantum number. The absorption of photon (indicated by arrows) that can bring an electron from lowest to higher energy level must be equal to the difference of energy level. **So, the energy is quantized.** It is the primary process for spectroscopy where electromagnetic radiation interacts with matter (molecules). The lowest possible energy is NOT zero; meaning the particle can never be at rest owing to always has some kinetic energy. This is called the **zero-point energy**. This is also consistent with the Heisenberg Uncertainty Principle.

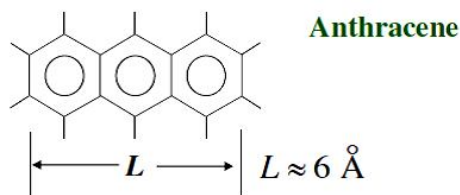
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Analogy of the concept of spectroscopy from particle in box

The "particle in a box" quantum-mechanical model can be applied to predict the energy levels of electrons responsible for UV or visible wavelength transitions. Thus, absorption spectra can be explained using particle in box model. Absorbance of photon by a molecule results in transition from one electronic energy state (HOMO) to another (LUMO).

Plank constant $h = 6.6 \times 10^{-34}$ Js ($\text{Kg m}^2 \text{s}^{-1}$)

Electron mass $m = 9.1 \times 10^{-31}$ Kg



Consider a simple model of molecular energy level in particle in box for the anthracene molecule. Two assumptions are made: The π -electrons of anthracene are free in the box of length $L = 6 \text{ \AA}$ and the Coulombic interactions are ignored. If anthracene is excited, the energy required for the transition is:

$$\Delta E = \frac{3 (6.626 \times 10^{-34})^2}{8(9.1 \times 10^{-31}) (6 \times 10^{-10})^2}$$
$$= 5 \times 10^{-19} \text{ J}$$

The energy corresponds to the wavelength is $\Delta E = h\nu = hc / \lambda$

Or, $\lambda = hc / \Delta E = (6.626 \times 10^{-34} \text{ Js}) \times (3 \times 10^8 \text{ ms}^{-1}) / (5 \times 10^{-19} \text{ J})$
 $= 3.97 \times 10^{-7} \text{ m} = 397 \text{ nm}$. So, it absorbs at far UV or nearly visible region
(Note: experimental value 376 nm).

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Q. Calculate the wavelength of transition of unknown molecules in 1D particle in box model with length $L = 5\text{\AA}$, $L = 7\text{\AA}$ and $L = 8\text{\AA}$ respectively and mention which one will absorb at UV region and which at visible region?

Ans: In one dimensional particle in box model of $L = 5\text{\AA}$, the minimum energy required for transition from $n= 1$ to $n= 3$

$$\Delta E = \frac{3 (6.626 \times 10^{-34})^2}{8(9.1 \times 10^{-31}) (5 \times 10^{-10})^2}$$
$$= 7.2 \times 10^{-19} \text{ J}$$

$\lambda = hc / \Delta E = (6.626 \times 10^{-34} \text{ Js}) \times (3 \times 10^8 \text{ ms}^{-1}) / (7.2 \times 10^{-19} \text{ J})$
 $= 2.76 \times 10^{-7} \text{ m} = 276 \text{ nm}$. So, it absorbs at UV region.

For $L = 7\text{\AA}$,

$$\Delta E = \frac{3 (6.626 \times 10^{-34})^2}{8(9.1 \times 10^{-31}) (7 \times 10^{-10})^2}$$
$$= 3.7 \times 10^{-19} \text{ J}$$

$\lambda = hc / \Delta E = (6.626 \times 10^{-34} \text{ Js}) \times (3 \times 10^8 \text{ ms}^{-1}) / (3.7 \times 10^{-19} \text{ J})$
 $= 5.37 \times 10^{-7} \text{ m} = 537 \text{ nm}$. So, it absorbs at visible range (green colour).

For $L = 8\text{\AA}$,

$$\Delta E = \frac{3 (6.626 \times 10^{-34})^2}{8(9.1 \times 10^{-31}) (8 \times 10^{-10})^2}$$
$$= 2.8 \times 10^{-19} \text{ J}$$

$\lambda = hc / \Delta E = (6.626 \times 10^{-34} \text{ Js}) \times (3 \times 10^8 \text{ ms}^{-1}) / (2.8 \times 10^{-19} \text{ J})$
 $= 7.09 \times 10^{-7} \text{ m} = 709 \text{ nm}$. So, it absorbs at visible range (red colour).

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Q. What is the expected value of the energy of the particle in a three dimensional box, if $L_x = 10 \text{ \AA}$, $L_y = 5 \text{ \AA}$ and $L_z = 1 \text{ \AA}$. when $n_x = 2$, $n_y = 2$ and $n_z = 1$? Comment on the degeneracy of the energy levels of a three dimensional box resulting from the three sides of the box becoming equal.

Ans:

$$\begin{aligned} E(n_x, n_y, n_z) &= E(2, 2, 1) = \frac{2^2 h^2}{8mL_x^2} + \frac{2^2 h^2}{8mL_y^2} + \frac{1^2 h^2}{8mL_z^2} \\ &= \frac{h^2}{8m} \left(\frac{2^2}{L_x^2} + \frac{2^2}{L_y^2} + \frac{1^2}{L_z^2} \right) \\ &= \frac{h^2}{8m \cdot 10^{-18}} \left(\frac{2^2}{10^2} + \frac{2^2}{5^2} + \frac{1^2}{1^2} \right) \\ &= \frac{(6.626 \times 10^{-34})^2}{8(9.1 \times 10^{-31}) \cdot 10^{-18}} (0.04 + 0.16 + 1) \\ &= 7.2 \times 10^{-20} \end{aligned}$$

$$\begin{aligned} \lambda &= hc / \Delta E = (6.626 \times 10^{-34} \text{ Js}) \times (3 \times 10^8 \text{ ms}^{-1}) / (0.72 \times 10^{-19} \text{ J}) \\ &= 27.60 \times 10^{-7} \text{ m} = 2760 \text{ nm. It absorbs at mid IR region.} \end{aligned}$$

If $L = L_x = L_y = L_z$, then the following number of states with quantum numbers can have the same energy.

(i) 1, 2, 3, (ii) 2, 1, 3, (iii) 1, 3, 2, (iv) 2, 3, 1, (v) 3, 1, 2 and (vi) 3, 2, 1

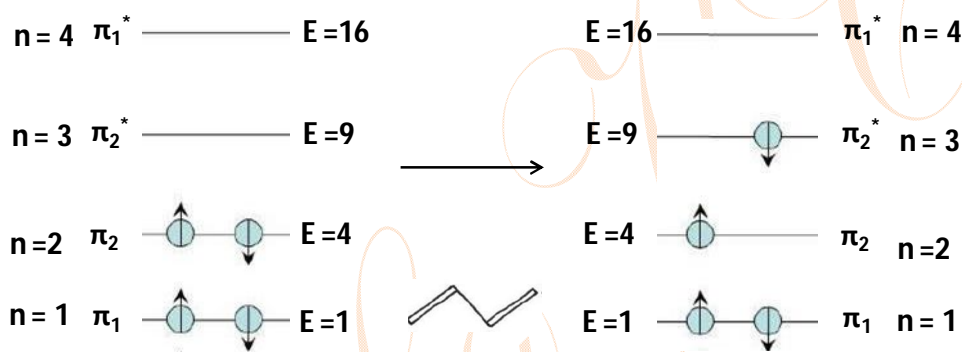
The energy of these states is same and are said to be degenerate. The value is $(1^2 + 2^2 + 3^2) h^2 / 8 mL^2 = 14 h^2 / 8 mL^2$. For example, the 3 p orbitals of the hydrogen atom are degenerate.

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Q. Assuming butadiene a linear molecule and the electrons move in a line, electrons do not interact with one another, calculate the minimum energy required to excite its outermost electron into a higher level by treating it as a particle in a box problem with four pi electrons confined to this length.

Ans: Single bond length = 1.54 Å, Double bond length = 1.34 Å, Each carbon end is considered to be added ≈ 0.8 Å.

The estimated bond length = $0.8 + 1.34 + 1.54 + 1.34 + 0.8 = 5.82$ Å.



The minimum energy required to excite its outermost electron into a higher level is the transition from level $n=2$ (HOMO) to $n=3$ (LUMO) energy state.

The difference in energy is $\Delta E = 9 - 4 = 5E$.

$$E = \frac{h^2}{8mL^2} = \frac{(6.626 \times 10^{-34})^2}{8(9.1 \times 10^{-31})(5.82 \times 10^{-10})^2} = 1.78 \times 10^{-19} \text{ J}$$

$$\Delta E = 5 \times (1.78 \times 10^{-19}) \text{ J} = 8.90 \times 10^{-19} \text{ J}$$

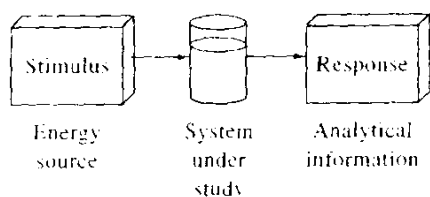
$\lambda = hc / \Delta E = (6.626 \times 10^{-34} \text{ Js}) \times (3 \times 10^8 \text{ ms}^{-1}) / (8.90 \times 10^{-19} \text{ J})$
 $= 2.23 \times 10^{-7} \text{ m} = 223 \text{ nm}$. It absorbs at UV region. (Note: the experimental value for $\pi-\pi^*$ transition was found to be 217 nm). It is also noted that several approximations are implicit to calculate the E and λ .

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Introduction to spectroscopic methods of analysis

Types of instrumental methods: Special reference to spectroscopic methods

The physical and chemical properties of the compounds are qualitatively or quantitatively analyzed by various instrumental methods. Applying newer methods for separating and determining chemical species are known collectively as **instrumental methods of analysis**.



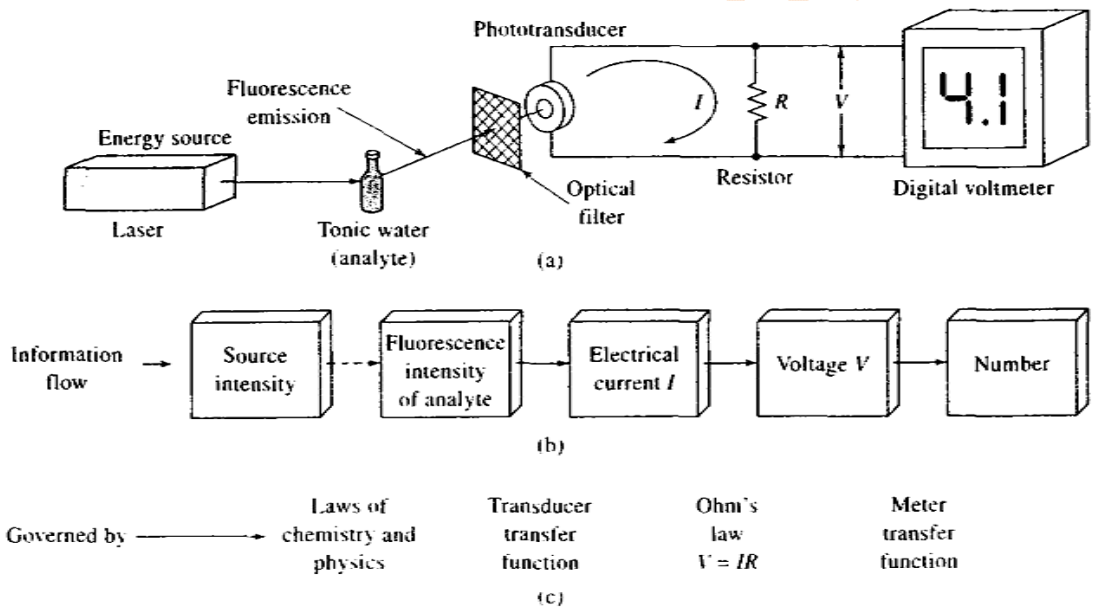
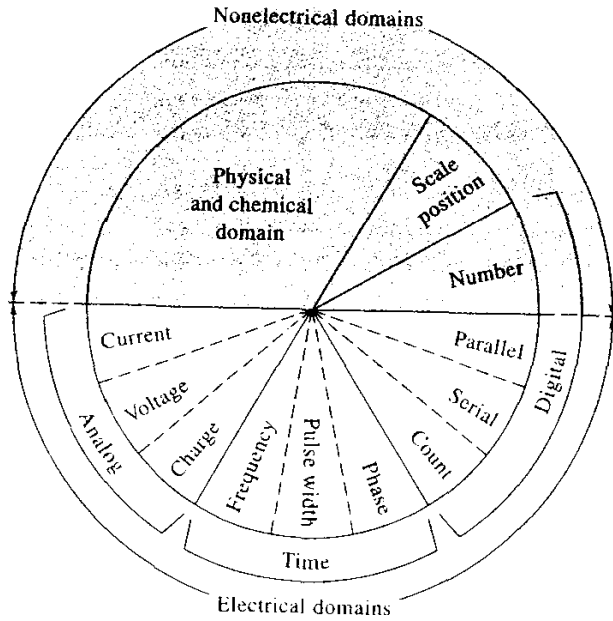
Those Instrumental methods include spectroscopic, chromatographic, non-spectroscopic analytical techniques. However, the majority of analytical characteristics as listed in the table involve spectroscopic methods that include a source of energy to stimulate a measurable response from the analyte. The process brings about a change in quantized energy states corresponding to a particular electromagnetic radiation that is measured by spectroscopic tool. The spectroscopic techniques are advantageous over classical techniques such as precipitation, extraction, distillation, gravimetric/ volumetric titration.

Characteristic Properties	Instrumental Methods
Emission of radiation	Emission spectroscopy (X-ray, UV, visible, electron, Auger); fluorescence, phosphorescence, and luminescence (X-ray, UV, and visible)
Absorption of radiation	Spectrophotometry and photometry (X-ray, UV, visible, IR); photoacoustic spectroscopy; nuclear magnetic resonance and electron spin resonance spectroscopy
Scattering of radiation	Turbidimetry; nephelometry; Raman spectroscopy
Refraction of radiation	Refractometry; interferometry
Diffraction of radiation	X-ray and electron diffraction methods
Rotation of radiation	Polarimetry; optical rotary dispersion; circular dichroism
Electrical potential	Potentiometry; chronopotentiometry
Electrical charge	Coulometry
Electrical current	Amperometry; polarography
Electrical resistance	Conductometry
Mass	Gravimetry (quartz crystal microbalance)
Mass-to-charge ratio	Mass spectrometry
Rate of reaction	Kinetic methods
Thermal characteristics	Thermal gravimetry and titrimetry; differential scanning calorimetry; differential thermal analyses; thermal conductometric methods
Radioactivity	Activation and isotope dilution methods

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Data analysis:

Data domain is connectivity between nonelectrical and electrical domains of how various modes of information being encoded by physical and chemical characteristics and particularly by electrical signals, such as current, voltage, and charge.

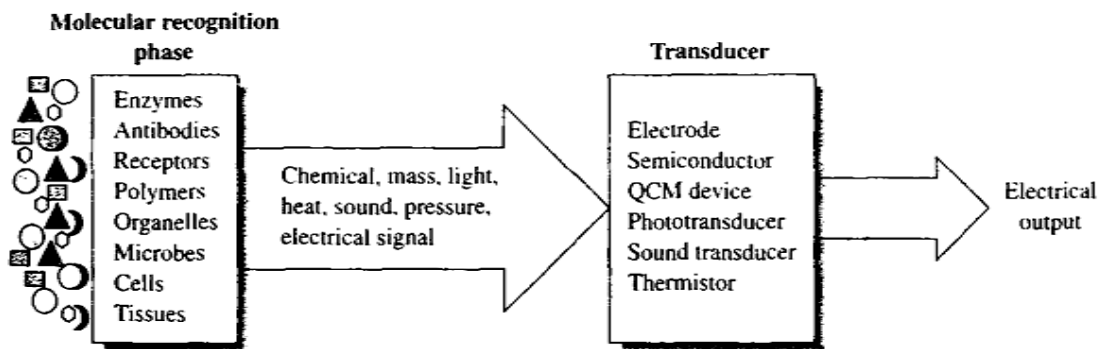


a) a general diagram of the instrument, (b) a diagrammatic representation of the flow of information through various data domains in the instrument, and (c) the rules governing the data-domain transformations during the measurement process.

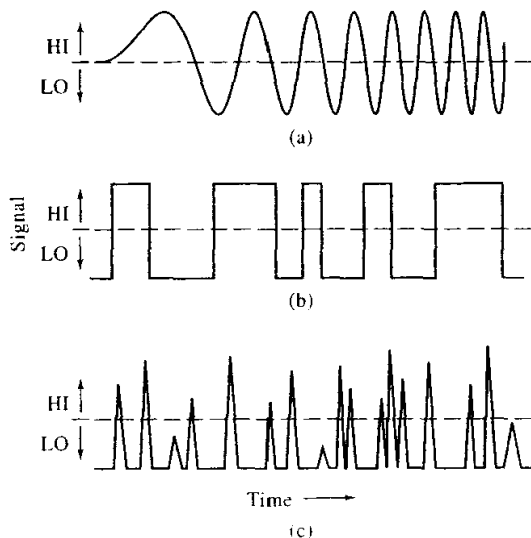
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Detector-transducer-sensors :

A molecular recognition is arbitrated by molecular recognition elements; called sensors (Chemo/biosensors). The recognition phase as considered to be nonelectrical domain (input), converts the information of interest into electrical domain (output) via a detectable characteristic (chemical, mass, light, or heat) with the help of transducer (device).



Signals:



Information is stored in the time domain as the time relationship of signal fluctuations, rather than in the amplitudes of the signals. The time relationships between transitions of the signal from HI to LO or from LO to HI contain the information. The time between successive LO to HI transitions is called the **period** and the time

between a LO to HI and a HI to LO transition is called the **pulse width**.

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Readout device:

A readout device is a transducer that converts information from an electrical domain to a form that is understandable by a human observer. Usually, the transduced signal takes the form of the alphanumeric or graphical output.

Computers in instruments

Most modern analytical instruments containing data-domain converters, such as operational amplifiers, integrated circuits, analog-to-digital and digital-to-analog converters, counters, microprocessors are attached to computers for data analysis using software.

In view of analytical methods analysis, spectrometric methods are versatile and are referred to the measure of the intensity of radiation with a photoelectric transducer or other type of electronic device.

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