RAMA UNIVERSITY

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DEPARTMENT OF BIOTECHNOLOGY
FACULTY OF ENGINEERING & TECHNOLOGY

LT. 18 General principle of electromagnetic radiation

Content Outline

1. General Principle of electromagnetic radiation
2. Types of spectra
3. Biological application of spectra

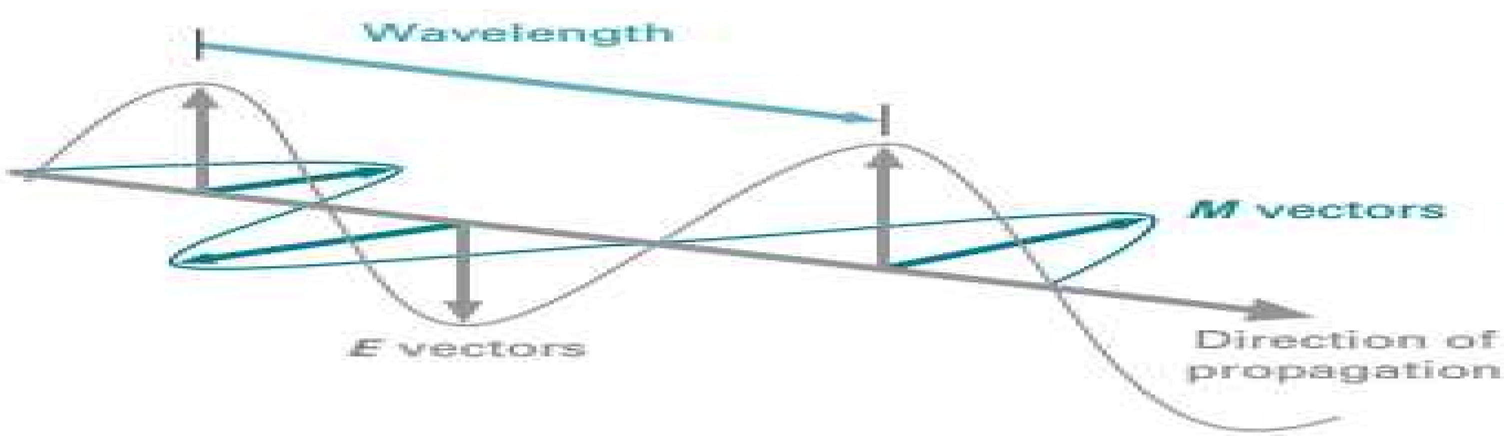
General principles of electromagnetic radiation

General principles of electromagnetic radiation

Spectroscopic techniques employ light to interact with matter and thus probe certain features of a sample to learn about its consistency or structure. Light is electromagnetic radiation, a phenomenon exhibiting different energies, and dependent on that energy, different molecular features can be probed.

Principle

Electromagnetic radiation is composed of an electric and a perpendicular magnetic vector, each one oscillating in plane at right angles to the direction of propagation. When electromagnetic radiation interacts with matter, matters undergoes transition which is quantum phenomena within matter and the spectra which arise from such transitions are principally predictable.



•Electromagnetic phenomena are explained in terms of quantum mechanics. The photon is the elementary particle responsible for electromagnetic phenomena. It carries the electromagnetic radiation and has properties of a wave, as well as of a particle, albeit having a mass of zero. As a particle, it interacts with matter by transferring its energy E:

$$E = \frac{hc}{\lambda} = h\nu$$

•Most biologically relevant molecules possess more than two atoms and, therefore, the energy diagrams become more complex. Different orbitals combine to yield molecular orbitals that generally fall into one of five different classes (Fig. 4.2): s orbitals combine to the binding s and the antibinding σ* orbitals. Some p orbitals combine to the binding π and the anti-binding π*orbitals. Other p orbitals combine to form non-binding n orbitals. The population of binding orbitals strengthens a chemical bond, and, vice versa, the population of anti-binding orbitals weakens a chemical bond.

Types of Spectra & their biological applications

Emission Spectrum

An emission spectra occurs when the atoms and molecules in a hot gas emit extra light at certain wavelengths, causing bright lines to appear in a spectra. As with absorption spectra, the pattern of these lines are unique for each element.

Whenever electromagnetic radiation interacts with atoms and molecules of matter, the electrons in these atoms may absorb energy and jump to a higher energy state, losing their stability.

In order to regain their stability, they need to move from the higher energy state to the previous lower energy state.

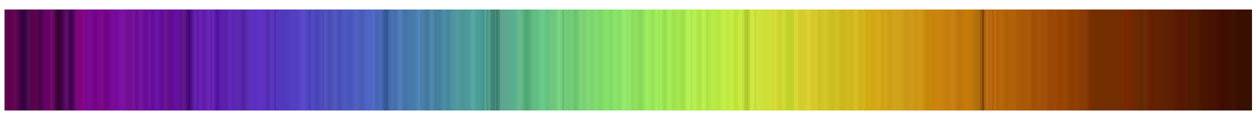
To accomplish this job, these atoms and molecules emit radiation in various regions of the electromagnetic spectrum.

This spectrum of radiation emitted by electrons in the excited atoms or molecules is known as an ***emission spectrum.***

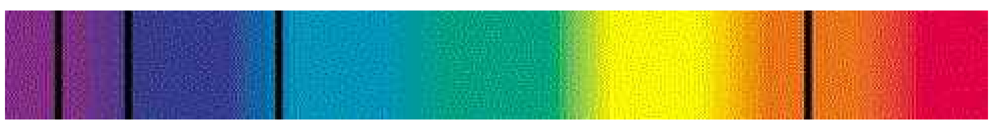
Absorption spectrum

A spectrum of electromagnetic radiation transmitted through a substance or gases, showing dark lines or bands due to absorption at specific wavelengths. These lines are caused by the Sun's atmosphere absorbing light at certain wavelengths, causing the intensity of the light at this wavelength to drop and appear dark. The atoms and molecules in a gas will absorb only certain wavelengths of light. The pattern of these lines is unique to each element and tells us what elements make up the analyte.

The most common **types** of waves measured by **absorption spectroscopy** are infrared, atomic, visible, ultraviolet (UV), and x-ray



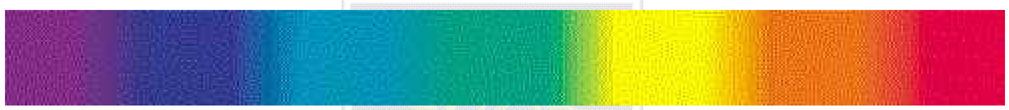
Detailed image of our Sun's visible light spectra



The absorption spectra of hydrogen - can you see this pattern in the solar spectrum above this image? Hint: hydrogen is the most abundant element in the sun - look at the darkest lines

Continuous spectrum

Continuous spectra (also called a thermal or blackbody spectra) are emitted by any object that radiates heat (has a temperature). The light is spread out into a continuous band with every wavelength having some amount of radiation.



Continuous visible light spectra of Sun

Biological usefulness of various spectra

Absorption spectra

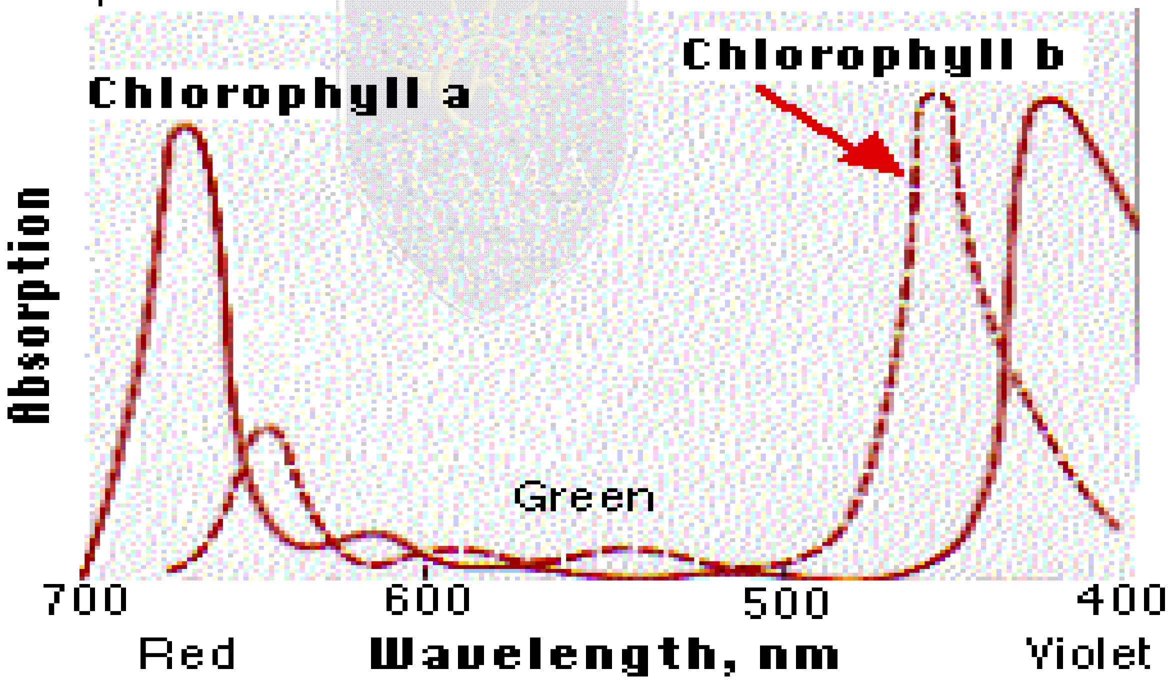
UV-Visible spectra can be used for qualitative, quantitative, enzymatic, and structural studies. Some of the biological applications of UV-VIS spectroscopy are listed below.

Visible or UV light to probe consistency and conformational structure of biological molecules. Usually, these methods are the first analytical procedures used by a biochemical scientist.

It can be used for measuring the concentration of unknown biological analyte with the help of standard graph

Identification of unknown biological sample: UV-visible spectrophotometry may be used to identify various classes of compounds in both pure state and as well as in biological preparations. This is done by plotting the absorption spectrum curves. These curves represent specific class of compounds and knowledge of these curves will help in identification of any substance.

The graph on the right shows the absorption spectrum of a mixture of chlorophyll a and chlorophyll b in the range of visible light. It can be seen clearly that chlorophylla and chlorophyll b gives absorption maxima at different wavelength which is attributed to presence of different functional group. As we can see each of the pigment has its own peculiar absorption spectrum which will help it identify in a mixture of compounds.



Enzyme Assay: The enzyme activity can be easily, quickly and conveniently be calculated when the substrate or the product is colored or absorbs light in the UV range. The rate of appearance or disappearance of light absorbing product or substrate can be measured with the help of spectrophotometer

Control of Purification: This is one of the most important application of UV-visible spectrophotometry. Impurities can be detected very easily by testing if the compound is not showing its characteristic absorption spectrum. Example: Benzene impurity in absolute alcohol can be detected by this method. This can be detected by measuring the absorbance at 280nm. As at 280nm, benzene will absorb, whereas alcohol (210nm) will not absorb.

Study of Cis-Trans Isomerism: The trans-isomer is more elongated as compared to its counterpart cis-isomer. Hence, this structural difference will be reflected in absorbance spectrum. The trans-isomer will have a higher wavelength of maximum absorption. The graph at the adjacent shows the absorption spectrum of the azobenzene dye, 4-n-butyl-4'-methoxyazobenzene (BMAB) where both cis-BMAB and trans-BMAB have different absorption spectrum

Turbidimetry: Any particulate matter (or even bacteria) makes the solution look turbid. This is due to Tyndall effect which is because of the light scattering by the colloidal particles.The particles in this solution will absorb at a particular wavelength and these particles will scatter the incident light. If this happens, then the radiation of a wavelength which is not absorbed by the solution is made to pass through the suspension and the apparent absorption will be solely because of the scattering by the particles. So, the transmitted light will have lower intensity as compared to that of the incident light. As a result, if the intensity of the transmitted light is measured, it will give an idea of the number of particles in the suspension. This technique is turbidimetry. By using this technique, we can find out an approximate number of particles in a given suspension.

Infrared spectrum (Measurement is known as spectroscopy)

Infrared spectroscopy is an analytical technique for studying the structure of organic molecules and is being used increasingly in the biological and biochemical fields. It can be used to determine the structures of proteins and enzymes and has forensic applications such as the analysis of blood for drugs.

•This spectroscopic approach enables such molecules to be identified and changes to their chemical structures to be characterized.

•It can be utilized to identify various functional group present in the biological sample.

•Infrared spectroscopy provides a valuable tool for the examination of protein and peptide structures, particularly for studies of conformation and reactions

•Lipids, including fatty acids, phospholipids, cholesterol esters, and glycerides, can be readily characterized using infrared spectroscopy.The technique is also very useful for studying phase transitions in lipid molecules.

•The nucleic acids, DNA and RNA, may be studied using infrared spectroscopy. The technique may be used to identify bases and conformational changes and to study base pairing and DNA–ligand interactions. The spectra of nucleic acids may be divided into the modes depending non the constituent base, sugar, and phosphate groups.

•FTIR spectroscopy is useful for probing the structure of membrane proteins. The technique can be used to study the secondary structure of proteins both in their native environment, as well as after reconstitution into model membranes. Information about lipid conformation and protein secondary structure from the same sample can be obtained in a single experiment.

•Infrared spectroscopy is suitable for monitoring the structural changes associated with diseased tissues and can be used for diagnostic purposes The technique has the advantage of examining specimens without the need for staining or enhancing procedures. Both mid-infrared and near-infrared spectroscopies have been employed for medical diagnosis applications.

•Infrared spectroscopy has proved to be a valuable tool for characterizing and differentiating microbial cells. The FTIR spectra of bacteria show modes predominantly due to the protein component. The ability to identify individual strains of bacteria has been permitted by the use of multivariate statistical analysis and pattern recognition techniques. The technique can be used to monitor the biochemical heterogeneity of microcolonies. By examining the individual spectra and calculating the difference spectra, it is possible to gain a better understanding of the source of the clustering.

X-rays are a form of electromagnetic radiation that can pass through solid objects, including the body. **X-rays** penetrate different objects more or less according to their density. In medicine, **X-rays** are used to view images of the bones and other structures in the body. X-rays were first discovered by Wilhelm Conrad Roentgen, a German physics professor. Roentgen also studied X-rays and their ability to pass through human tissues to produce images of the bones and metals visible on developed film.

The most common form of X-ray used is X-ray radiography, which can be used to help detect or diagnose:

- Bone fractures
- Infections (such as pneumonia)
- Calcifications (like kidney stones or vascular calcifications)
- Some tumors
- Arthritis in joints
- Bone loss (such as osteoporosis)
- Dental issues
- Heart problems (such as congestive heart failure)
- Blood vessel blockages

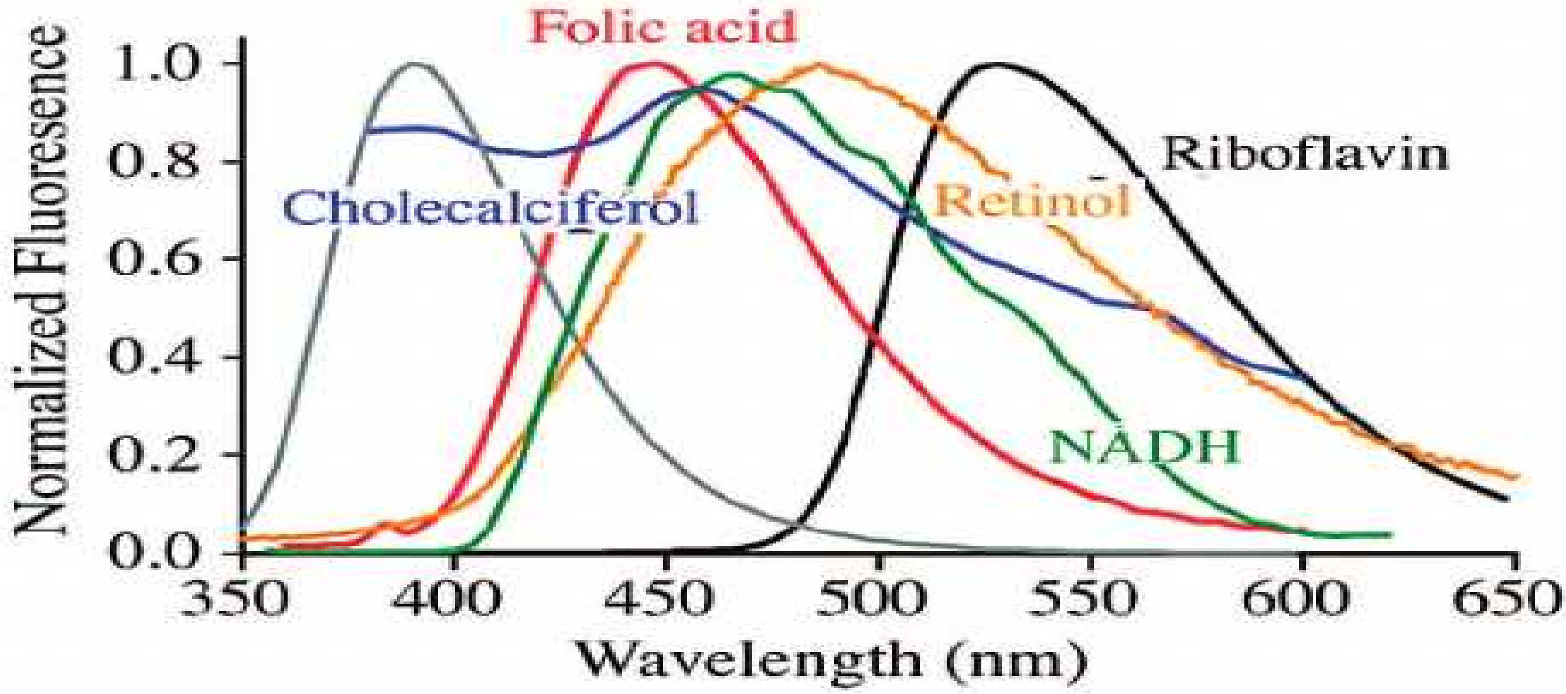
Emission Spectra

•Atomic emission spectroscopy is one of the most useful and commonly used techniques for analyses of metals and nonmetals providing rapid, sensitive results for analytes in a wide variety of sample matrices. It can be used for both quantitative and qualitative analysis and it is a single element method. Atoms or molecules that are excited to high energy levels can decay to lower levels by emitting radiation (emission or luminescence). For atoms excited by a high-temperature energy source this light emission is commonly called atomic or optical emission (atomic-emission spectroscopy,) and for atoms excited with light it is called atomic fluorescence (atomic-fluorescence spectroscopy.)

•It's most important **uses** are in the determination of sodium, potassium, lithium and calcium in **biological** fluids and tissues.

•Biomaging of cells using various fluorescent dyes.

•Determination of cellular constituents, biological intermediates by coupling it with fluorescent dyes and measuring the emission wavelength or fluorescent intensity.


Folic acid
Riboflavin
Cholecalciferol
Retinol
NADH
Normalized Fluoresence
1.0
0.8
0.6
0.4
0.2
0.0
350
400
450
500
550
600
650
Wavelength (nm)

Test your understanding

Electromagnetic radiation is composed of
a. Vibratiuonal and rotational field
b. electric and magnetic field
c. Electric field only
d. Magnetic field only

What happens when electromagnetic radiation interacts with matter
a. Matters undergoes transition according to principle of quantum mechanics
b. Electromagnetic radiation passes through the matter witrhout causing any effect
c. It destroys the matter
d. None of the above

Electromagnetic phenomena are explained in terms of
a. Quantum Mechanics
b. Statistical Mechanics
c. Fluid Mechanics
d. None of the above

The energy change needed by interacting matter is defined by
a. ΔE = Efinal – Estart = hv
b. c = v.λ
c. c = v
d. None of the above

For non-fluorescent compound, the transition of electron from excited state to ground state is accompanied by release of
a. Light
b. Sound
c. Heat
d. α-rays