Decarboxylation

A Decarboxylation Reaction is a chemical reaction in which a carboxyl group is removed and carbon dioxide is released (CO_2). Decarboxylation is a process in which carboxylic acids remove a carbon atom from a carbon chain. Carboxylation is the split chemical step in photosynthesis, where CO_2 is added to the substance. It is a completely reversible reaction. Decarboxylases, on the other hand, are enzymes that catalyze decarboxylation.

- The decarboxylation process is important since the products of decarboxylation reactions give rise to physiologically active amines.
- The enzymes, amino acid decarboxylases are pyridoxal phosphatedependent enzymes.
- Pyridoxal phosphate forms a Schiff's base with the amino acid so as to stabilise the alph-carbanion formed by the cleavage of bond between carboxyl and alph- carbon atom.
- The physiologically active amines epinephrine, nor-epinephrine, dopamine, serotonin, gamma-amino butyrate and histamine are formed through decarboxylation of the corresponding precursor amino acids.

UREA CYCLE

Urea is the end product of protein metabolism (amino acid metabolism). The nitrogen of amino acids, converted to ammonia is toxic to the body. It is converted to urea and detoxified. As such, urea accounts for 80-90% of the nitrogen containing substances excreted in urine. Urea is synthesized in liver and transported to kidneys for excretion in urine. Urea cycle is the first metabolic cycle that was elucidated by Hans Krebs and Kurt Henseleit (1932), hence it is known as Krebs-Henseleit cycle.
Urea has two amino (NH_2) groups, one derived from NH_3 and the other from aspartate. Carbon atom is supplied by CO_2. Urea synthesis is a five-step cyclic process, with five distinct enzymes. The first two enzymes are present in mitochondria while the rest are localized in cytosol. The details of urea cycle are described.

1. Synthesis of carbamoyl phosphate

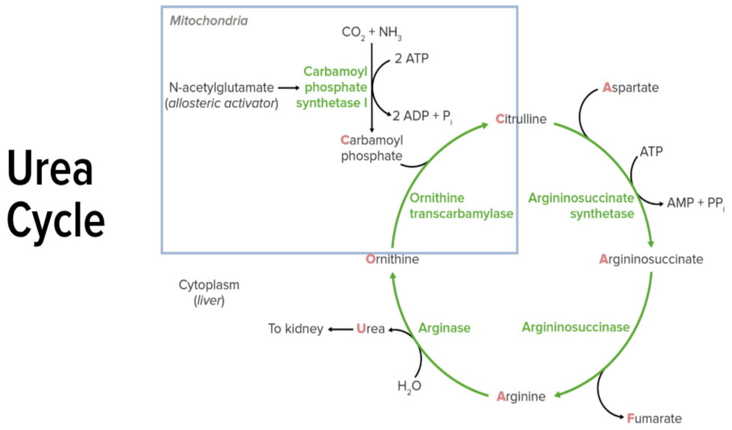
Carbamoyl phosphate synthase I (CPS I) of mitochondria catalyses the condensation of NH_4^+ ions with CO_2 to form carbamoyl phosphate. This step consumes two ATP and is irreversible, and rate-limiting. CPS I requires N-acetylglutamate for its activity. Another enzyme, carbamoyl phosphate synthase II (CPS II) involved in pyrimidine synthesis is present in cytosol. It accepts amino group from glutamine and does not require N-acetylglutamate for its activity.

2. Formation of citrulline

Citrulline is synthesized from carbamoyl phosphate and ornithine by ornithine transcarbamoylase. Ornithine is regenerated and used in urea cycle. Therefore, its role is comparable to that of oxaloacetate in citric acid cycle. Ornithine and citrulline are basic amino acids. (They are never found in protein structure due to lack of codons). Citrulline produced in this reaction is transported to cytosol by a transporter system.

3. Synthesis of arginosuccinate

Arginosuccinate synthase condenses citrulline with aspartate to produce arginosuccinate. The second amino group of urea is incorporated in this reaction. This step requires ATP which is cleaved to AMP and pyrophosphate (PPi). The latter is immediately broken down to inorganic phosphate (Pi).



Outline of urea cycle

4. Cleavage of arginosuccinate

Arginosuccinase cleaves arginosuccinate to give arginine and fumarate. Arginine is the immediate precursor for urea. Fumarate liberated here provides a connecting link with TCA cycle, gluconeogenesis etc.

5. Formation of urea

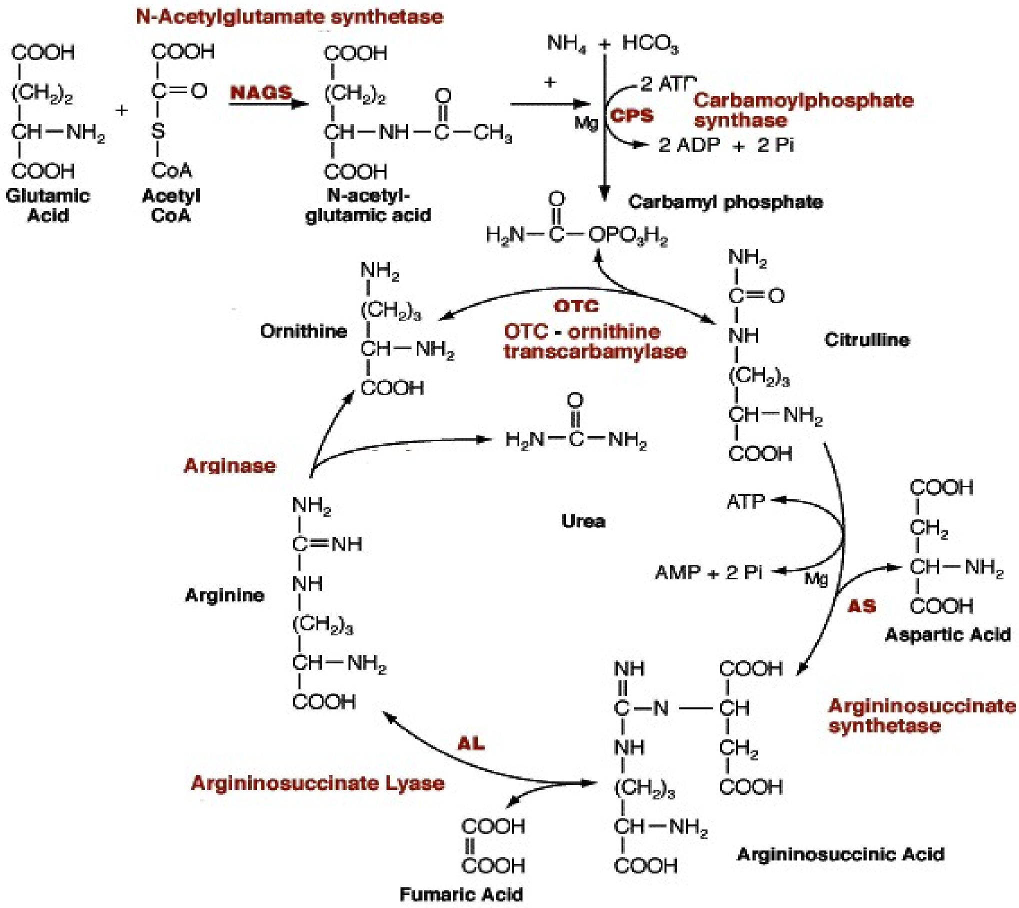
Arginase is the fifth and final enzyme that cleaves arginine to yield urea and ornithine. Ornithine, so regenerated, enters mitochondria for its reuse in the urea cycle. Arginase is activated by Co2 + and Mn2 + . Arginase is mostly found in the liver, while the rest of the enzymes (four) of urea cycle are also present in other tissues. For this reason, arginine synthesis may occur to varying degrees in many tissues. But only the liver can ultimately produce urea.

Overall reaction and energetics

The urea cycle is irreversible and consumes 4 ATP. Two ATP are utilized for the synthesis of carbamoyl phosphate. One ATP is converted to AMP and PPi to produce arginosuccinate which equals to 2 ATP. Hence 4 ATP are actually consumed. NH_4^+ + CO_2 + Aspartate + 3ATP →Urea+ Fumarate + 2 ADP + 2 Pi + AMP + PPi.

Regulation of urea cycle

The first reaction catalysed by carbamoyl phosphate synthase I (CPS I) is rate-limiting reaction or committed step in urea synthesis. CPS I is allosterically activated by Nacetylglutamate (NAG). The rate of urea synthesis in liver is correlated with the concentration of N-acetylglutamate. High concentrations of arginine increase NAG. The consumption of a protein-rich meal increases the level of NAG in liver, leading to enhanced urea synthesis. Carbamoyl phosphate synthase I and glutamate dehydrogenase are localized in the mitochondria. They coordinate with each other in the formation of NH_3, and its utilization for the synthesis of carbamoyl phosphate. The remaining four enzymes of urea cycle are mostly controlled by the concentration of their respective substrates.



Reactions of urea cycle (NAG—N-acetylglutamate; in the formation of urea, one amino group is derived from free ammonium ion while the other is from aspartate; carbon is obtained from CO2; * mitochondrial enzymes, the rest of the enzymes are cytosomal).(REFER CLASS NOTES ALSO)

Metabolic disorders of urea cycle

Metabolic defects associated with each of the five enzymes of urea cycle have been reported are given below. All the disorders invariably lead to a build-up in blood ammonia (hyperammonemia), leading to toxicity. Other metabolites of urea cycle also accumulate which, however, depends on the specific enzyme defect. The clinical symptoms associated with defect in urea cycle enzymes include vomiting, lethargy, irritability, ataxia and mental re tardation.

SPECTROSCOPY

Introduction Spectroscopy is the branch of science dealing with the study of interaction of electromagnetic radiation with matter like atoms and molecules. The interaction of EMR with matter gives rise to two types of spectra namely atomic spectra and molecular spectra.

Atomic spectrum arises from the transition of electrons from one energy level to another due to changes of energy in the atom.

Molecular spectrum involves transition of electrons between rotational and vibrational energy levels in addition to electronic transition. Therefore molecular spectrum is much more complicated than the atomic spectrum.

Molecular Spectroscopy provides a clear image of how diatomic and polyatomic molecules interact by looking at the Frequency, Wavelength, Wave number, Energy, and molecular process also provides most useful information regarding the shape and size of molecules, the bond angles, bond lengths, strength of bonds and bond dissociation energies.

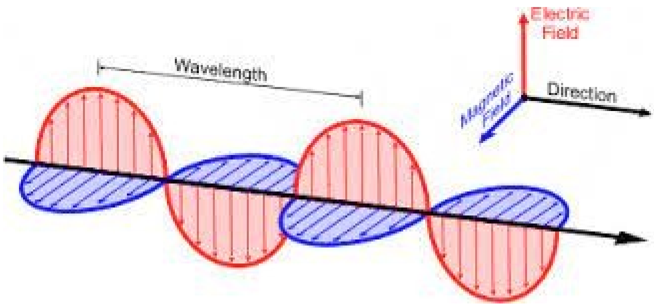
Hence molecular spectroscopy is of great use in determining the structure and constitution of compounds.

Electromagnetic Radiations (EMR)

EM radiation is created when a subatomic particle, such as an electron, is accelerated by an electric field. The movement produces oscillating electric and magnetic fields, which travel at right angles to each other in a bundle of light energy called a photon. Photons travel as harmonic waves at the fastest speed possible in the universe: 186,282 miles per second (299,792,458 meters per second) in a vacuum, also known as the speed of light.

The EM waves are characterized by frequency, wavelength, wave number and energy. Electromagnetic (EM) radiation is a form of energy that is all around us and takes many forms, such as radio waves, microwaves, X-rays and gamma rays.

Visible light is only a small portion of the EM spectrum, which contains a broad range of electromagnetic wavelengths.



Electromagnetic waves are formed when an electric field (shown in red arrows) couples with a magnetic field (shown in blue arrows). Magnetic and electric fields of an electromagnetic wave are perpendicular to each other and to the direction of the wave.

The four main electromagnetic interactions:

The force of attraction or repulsion between electric charges is inversely proportional to the square of the distance between them.

Magnetic poles come in pairs that attract and repel each other, similar to that of electrical charges.

An electric current in a wire produces a magnetic field, the direction of which depends on the direction of the current.

A moving electric field produces a magnetic field, and vice versa.

Characterization of EMR

Wavelength (λ) : It is the distance between two consecutive peaks of a wave (crests). Unit = m.

Frequency ($\surd$): It is the number of waves that are formed in a given length of time. Unit = number of wave cycles per second or hertz (Hz).

A short wavelength means that the frequency will be higher and a longer wavelength has a lower frequency.

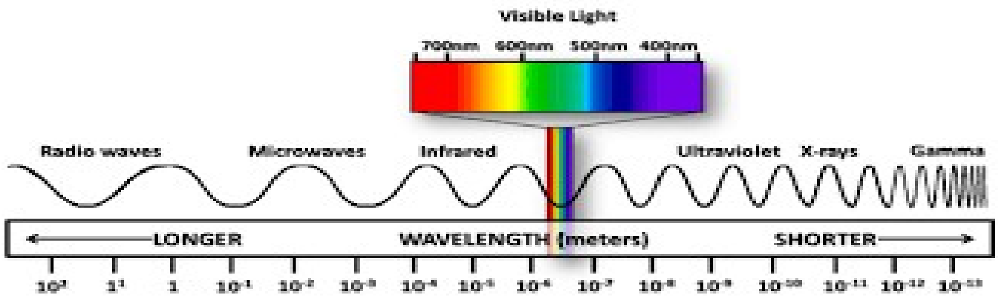
Wavenumber ($\bar{\upsilon}$): It is the number of waves per unit distance. Unit = cm-1 $\bar{\upsilon} = 1/\lambda$

Energy of EMR (E):

Electromagnetic radiations consists of particles having small packets of energies called quanta or photons. Photons possess the characteristic of wave and travel with the speed of light. The amount of energy corresponding to one photon is expressed by Planck's equation as

$E = h\nu$ or $E = h/\lambda$

where h = Planck's constant (6.62x10-34Js)
ν - frequency in Hz
λ - wavelength in cm/m.



Energy (E) = hc/λ
E = h√
E = $hc\bar{\upsilon}$

Interaction of EMR with matter

EMR interacts with matter only when the matter has some electric and magnetic effect and are influenced by the electric and magnetic components of the EM radiation.

The net change in the electric/magnetic dipole moment in the molecule or nuclear spin, interact with the magnetic/electrical component of the EMR by either absorption or emission of the EMR.

Total energy of molecules = Translational + rotational + vibrational + electronic Absorption or emission of EMR causes a change in any of these types of energies.

In molecular spectroscopy, we measure the change in these energy states.

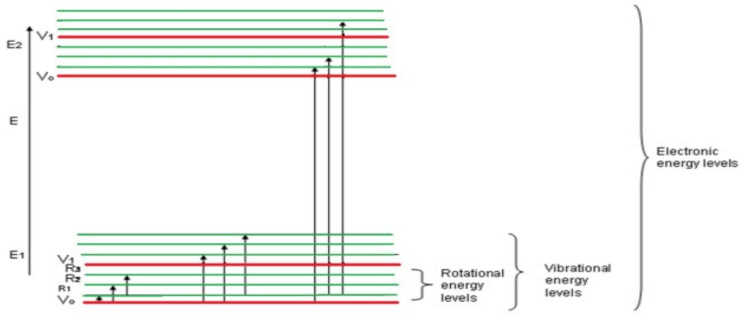
Translational energy – It is due to the overall movement of the molecule. Energy levels are not quantized . Hence no spectroscopy.

Rotational energy – It is due to spinning of molecules about the axis passing through the centre of gravity - Rotational Levels are quantized – Rotational spectroscopy (Microwave spectroscopy).

Vibrational energy – It is due to vibrations in molecules – Vibrational Levels are Quantized – IR Spectroscopy (Vibrational spectroscopy)

Electronic energy – Consists of electronic levels which are quantized – UV/visible spectroscopy (Electronic spectroscopy)

If E is the total energy of a molecule, it can be expressed as the sum of translational, rotational, vibrational and electronic contributions. E = Etrans +Erot + Evib + Eelec



Spectra

Molecular spectra result from either the absorption or the emission of electromagnetic radiation as molecules undergo changes from one quantized energy state to another.

• The electrons in a molecule possess kinetic energy due to their motions and potential energy arising from their attraction by the positive nuclei and their mutual repulsion. These two energy factors, along with the potential energy due to the mutual electrostatic repulsion of the positive nuclei, constitute the electronic energy of a molecule.

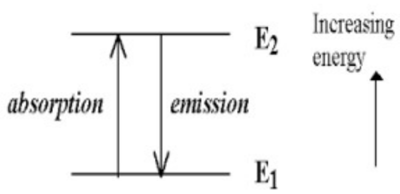
• Molecules are not rigid structures, and the motion of the nuclei within the molecular framework gives rise to **vibrational energy levels.**

• In the gas phase, where they are widely separated relative to their size, molecules can undergo free rotation and as a result possess quantized amounts of **rotational energy**.

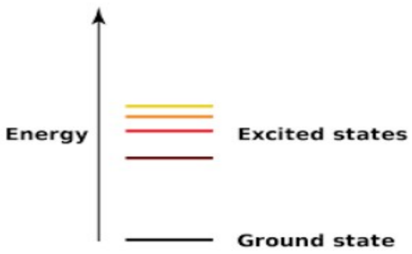
• In theory, the translational energy of molecules through space is also quantized, but in practice the quantum effects are so small that they are not observable, and the motion appears continuous.

• The interaction of electromagnetic radiation with these molecular energy levels constitutes the basis for Electronic, IR and Microwave spectroscopy.

Absorption and Emission Spectra



Absorption of electromagnetic radiation by compounds gives absorption spectrum and spectrum obtained by the emission of absorbed radiation is called emission spectrum.



Ground level/state is the lowest energy state. Higher energy levels/states are called excited states.

What is Colorimeter?

A colorimeter is a device that is used in Colorimetry. It refers to a device which helps specific solutions to absorb a particular wavelength of light. The colorimeter is usually used to measure the concentration of a known solute in a given solution with the help of the Beer-Lambert law. The colorimeter was invented in the year 1870 by Louis J Duboscq.

Principle of Colorimeter

It is a photometric technique which states that when a beam of incident light of intensity Io passes through a solution, the following occur:

- A part of it is reflected which is denoted as Ir
- A part of it is absorbed which is denoted as Ia
- Rest of the light is transmitted and is denoted as It

Therefore, Io = Ir + Ia + It

To determine Ia the measurement of Io and It is sufficient therefore, Ir is eliminated. The amount of light reflected is kept constant to measure Io and It.

Colorimeter is based on two fundamental laws of photometry. We have discussed them below:

Beer's law:

According to this law the amount of light absorbed is proportional to the solute concentration present in solution.

Log10 Io/It = asc

where,

as is absorbency index

c is the concentration of solution

Lambert's law:

According to this law the amount of light absorbed is proportional to the length as well as thickness of the solution taken for analysis.

A = log10 Io/It = asb

Where,

A is the test absorbance of test

as is the standard absorbance

b is the length / thickness of the solution

Uses of Colorimeter

- It is used in laboratories and hospitals to estimate biochemical samples such as urine, cerebrospinal fluid, plasma, serum, etc.
- It is used in the manufacturing of paints.
- It is used in textile and food industry.

- It is used in the quantitative analysis of proteins, glucose, and other biochemical compounds.
- It is used to test water quality.
- It is used to determine the concentration of haemoglobin in the blood.

Advantages and disadvantages of Colorimeter

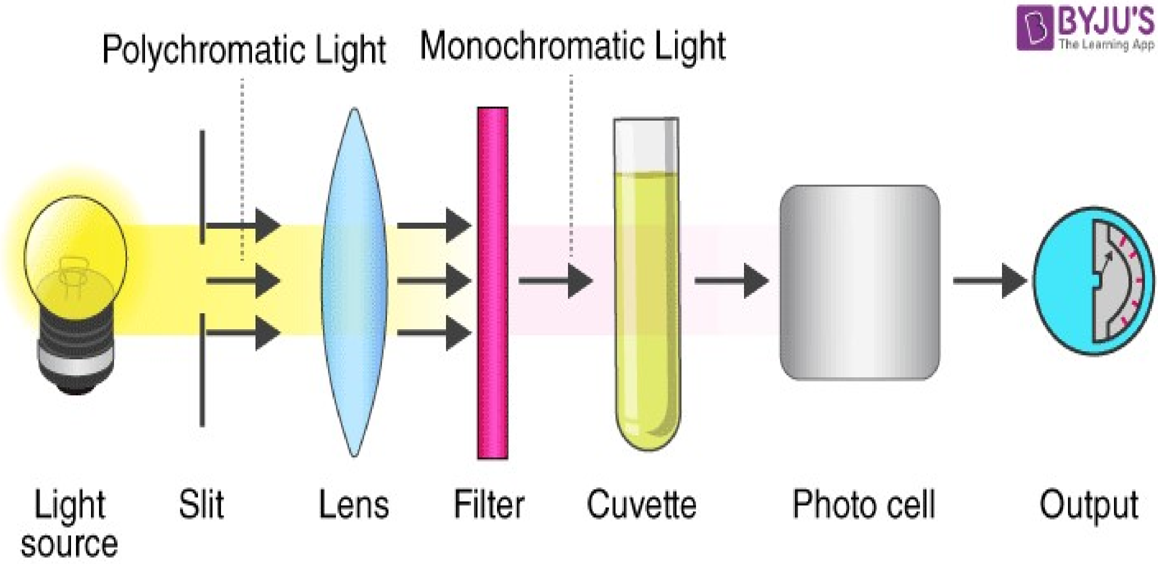
Some benefits are as follows:

It is an inexpensive method, widely used in the quantitative analysis of coloured samples, easy to carry, and transport.

Some disadvantages are as follows:

Analysis of colourless compounds is not possible, does not work in IR and UV regions.

Diagram of Colorimeter



UV VISIBLE SPECTROSCOPY

What is UV Visible Spectroscopy?

UV-visible spectroscopy is a technique that measures the amount of light absorbed by a chemical substance. It is absorption spectroscopy or reflectance spectroscopy technique within the ultraviolet and visible regions of the electromagnetic spectrum. When continuous radiation is passed through a compound a portion of that compound is absorbed by the compound. The residual radiation after passing through the compound yields a spectrum with gaps in it due to absorption by the compound, this spectrum is called the absorption spectrum.

Absorption of UV-Visible radiation results in the electronic transition of the compound, i.e., an electron in the ground state (occupied orbital) is promoted to the excited state (unoccupied orbital), and the amount of radiation absorbed corresponds to the energy difference between the ground state and the excited state.

UV Visible Spectroscopy Principle

UV-visible spectroscopy is a quantitative technique used in analytical chemistry to measure the amount of light absorbed by a substance. When light falls upon a substance it absorbs and reflects a certain amount of radiation. As the light passes through the sample, the amount of radiation absorbed by the substance is the difference between the incident radiation (Io) and the transmitted radiation (I). The amount of radiation absorbed is called absorbance (A) and transmittance (T), which is a fraction (I/ Io) indicating the amount of light that has passed through the sample.

Transmittance, $T = I/ Io$

Absorbance, $A = \log10(Io /I) = \log10 (1/T) = - \log10 (T\}$

According to Beer-Lambert's Law, the absorbance of a solution (containing the compound) is directly proportional to the concentration of the absorbing species (the compound) and the path length. This translates to, as the number of molecules (concentration) capable of absorbing the radiation at a given wavelength increases, the extent of absorption is increased. Also, the efficiency of the molecule (recorded by its molar absorptivity) in absorbing the radiation contributes to greater absorption.

The formulation for Beer-Lambert's law is given by

$A= \varepsilon cl$

for a given wavelength

Where ε = molar absorptivity (also known as molar extinction coefficient)

c = molar concentration of the absorber (solute)

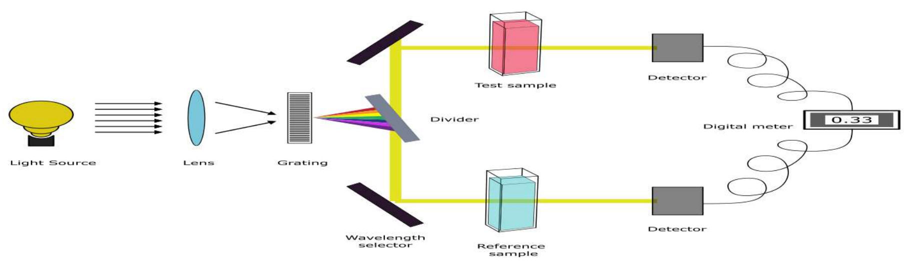
l = path length (length of the sample cell or cuvette; in cm)

The mathematical relation between absorbance and concentration established by the Beer-Lambert law allows direct measurement of the concentration of the absorber in a solution from absorbance for a fixed path length.

Instrumentation of UV Visible Spectroscopy

The instrumentation of UV-Visible spectroscopy is called a UV-Visible spectrophotometer. The spectrophotometer has a few key components, namely:

- The light source emits broadband electromagnetic radiation across the UV-visible spectrum.
- The dispersion device or monochromator - it's a diffraction grating that separates the radiation into its component wavelength.
- A sample area - the radiation interacts with the sample as it passes through or reflects off.
- Detector - measures the reflected or transmitted radiation intensity.



Double beam spectrophotometer

The measurement is done by placing the sample in the samplcompartment. Liquid samples are held in a rectangular holder made of glass, quartz or plastic, called a cuvette. Standard cuvettes have a 10mm path length and allow easy transmittance of ultraviolet radiation. The sample is placed in the path of the radiation as the beam from the monochromator passes through the sample to the detector.

The spectrophotometer compares the light intensity of the incident radiation (Io) before passing through the sample and the intensity of the transmitted radiation (I) that has passed through the sample and presents the absorbance value (A or Abs).

Application of UV Visible Spectroscopy

UV-Visible spectroscopy is widely used to determine the chemical and physical properties of substances. It can be used for:

- Identification of molecules in a sample.
- Determining the concentration of a compound in a sampl
- Determine the purity or concentration of biological samples containing DNA or RNA.
- It finds application in characterising the rate of a chemical reaction.

Advantages and Disadvantages of UV Visible Spectroscopy

Advantages

- The method is non-destructive so that the sample can be reused.
- The technique is fairly simple and can be used easily. No prior training is necessary.
- Measurement can be done in a short span of time, helping easy integration into experiments.
- Data analysis is simple and requires less processing.
- Instrumentation is relatively inexpensive and can be procured easily by laboratories.

Disadvantages

- Real instruments are not always perfect; hence stray light may interfere with the measurements.
- Scattering of light due to bubbles or undissolved solid particles in the sample solution causes measurement error.
- Beer-Lambert Law is only obeyed when a single absorbing species is present in the solution. A sample containing multiple absorbing species cannot be used to determine concentration using absorbance.
- Improper orientation of the sample holder or misalignment can imbibe errors in the measurement.

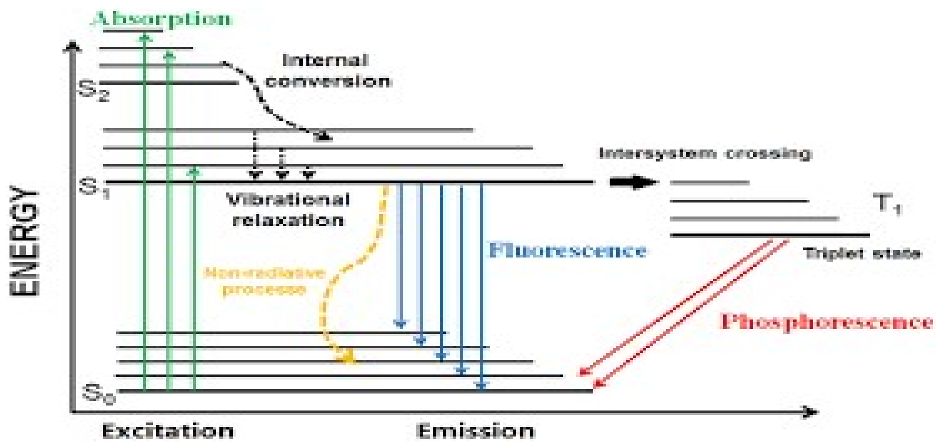
SPECTROFLUORIMETRY

Fluorescence spectroscopy or fluorimetry or spectrofluorimetry is a techniqiue to detect and analyze the fluorescence in the sample. Fluorescence is the emission of light by a substance (fluor) that has absorbed light or other electromagnetic radiation. In this emission phenomenon, a beam of light (usually UV light) excites the electron in a molecule which moves from ground state to higher energy excited state. When the electron falls back to the ground state, it emits fluorescence. Fluorescence spectroscopy is mainly concerned with electronic (ground state and excited state) and vibrational states.

Principle of fluorescence spectroscopy

Absorption of UV or visible radiation causes transition of electrons from singlet ground state to the singlet excited state. As this state is not stable, it emits energy in the form of UV or visible radiation and returns to singlet ground state. Fluorescence emission occurs as the fluorophore decay from the singlet electronic excited states to an allowable vibrational level in the electronic ground state.

The fluorescence excitation and emission spectra reflect the vibrational level structures in the ground and the excited electronic states respectively.



Applications of Fluorimetry:

- Determination of uranium in salts used extensively in the field of nuclear research.
- Estimation of traces of boron in steel by means of the complex formed with benzene.
- Estimation of calcium by fluorimetry with a calcium solution.
- Determination of Vitamin B (B1 thiamine and B2 riboflavin) in the food samples like meat, cereals, etc.
- Fluorimetry is employed to carry out both qualitative and quantitative analyses for various aromatic compounds present in cigarette smoke, air-pollutant, concentrates, and automobiles exhaust.

Advantages

- It's one of the newer methods and its potentialities are still largely unexplored.
- It also affects precision. Up to 1% can be achieved easily in Flourimetric.
- The method is very sensitive and also possesses specificity because there is a choice of wavelength not only for the radiation emitted, but also for the light which excites it.

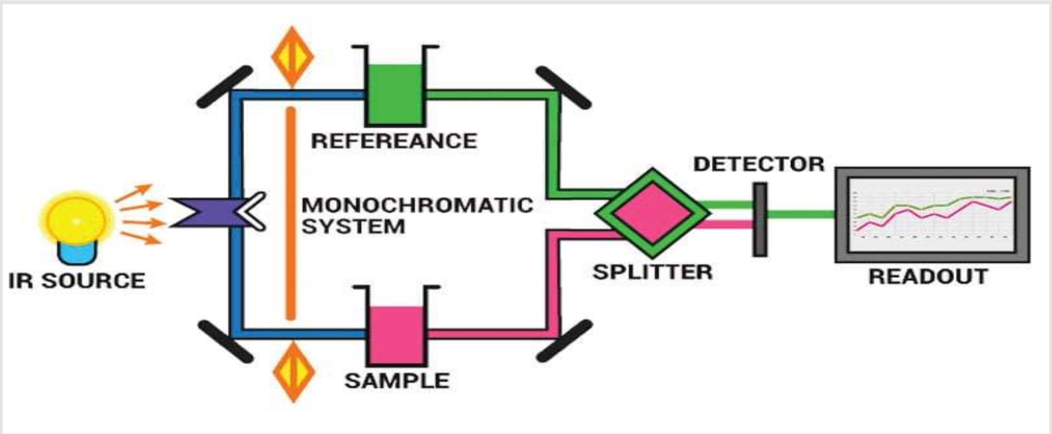
Infrared spectreoscopy

What is Infrared (IR) Spectroscopy?

Infrared (IR) spectroscopy or vibrational spectroscopy is an analytical technique that takes advantage of the vibrational transitions of a molecule.

It is one of the most common and widely used spectroscopic techniques employed mainly by inorganic and organic chemists due to its usefulness in determining the structures of compounds and identifying them.

The method or technique of infrared spectroscopy is conducted with an instrument called an infrared spectrometer (or **spectrophotometer**) to produce an infrared spectrum.



Principle of Infrared (IR) Spectroscopy

1. Infrared Spectroscopy is the analysis of infrared light interacting with a molecule.
2. The portion of the infrared region most useful for analysis of organic compounds have a wavelength range from 2,500 to 16,000 nm, with a corresponding frequency range from 1.9*1013 to 1.2*1014 Hz.
3. Photon energies associated with this part of the infrared (from 1 to 15 kcal/mole) are not large enough to excite electrons, but may induce vibrational excitation of covalently bonded atoms and groups.
4. It is known that in addition to the facile rotation of groups about single bonds, molecules experience a wide variety of vibrational motions, characteristic of their component atoms.
5. Consequently, virtually all organic compounds will absorb infrared radiation that corresponds in energy to these vibrations.

6. Infrared spectrometers, similar in principle to other spectrometer, permit chemists to obtain absorption spectra of compounds that are a unique reflection of their molecular structure.
7. The fundamental measurement obtained in infrared spectroscopy is an infrared spectrum, which is a plot of measured infrared intensity versus wavelength (or frequency) of light.

- IR Spectroscopy measures the vibrations of atoms, and based on this it is possible to determine the functional groups.
- Generally, stronger bonds and light atoms will vibrate at a high stretching frequency (wavenumber).

Applications of Infrared (IR) Spectroscopy

It has been of great significance to scientific researchers in many fields such as:

- Protein characterization
- Nanoscale semiconductor analysis and
- Space exploration.
- Analysis of gaseous, liquid or solid samples
- Identification of compounds
- Quantitative analysis
- Information regarding functional groups of molecules and constitution of molecules can be deduced from IR spectrum
- To know about interaction among molecules

NMR SPECTROSCOPY

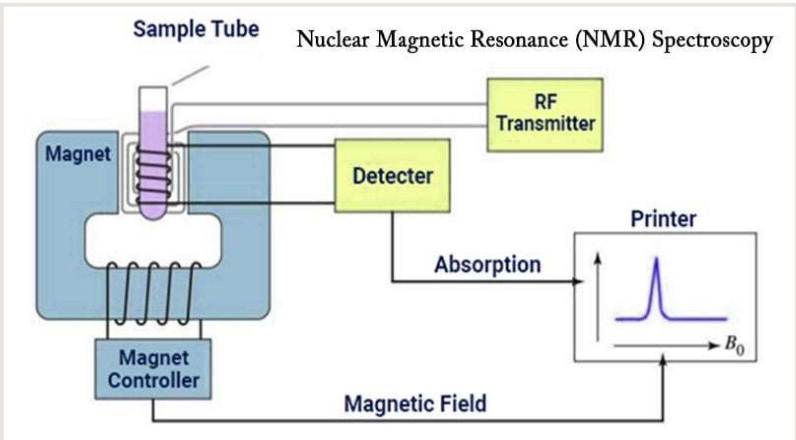
What is NMR?

Nuclear magnetic resonance spectroscopy, most commonly known as NMR spectroscopy or magnetic resonance spectroscopy (MRS), is a spectroscopic technique to observe local magnetic fields around atomic nuclei.

It is a spectroscopy technique that is based on the absorption of electromagnetic radiation in the radiofrequency region 4 to 900 MHz by nuclei of the atoms.

Over the past fifty years, NMR has become the preeminent technique for determining the structure of organic compounds.

Of all the spectroscopic methods, it is the only one for which a complete analysis and interpretation of the entire spectrum is normally expected.



Principle of Nuclear Magnetic Resonance (NMR) Spectroscopy

1. The principle behind NMR is that many nuclei have spin and all nuclei are electrically charged. If an external magnetic field is applied, an energy transfer is possible between the base energy to a higher energy level (generally a single energy gap).
2. The energy transfer takes place at a wavelength that corresponds to radio frequencies and when the spin returns to its base level, energy is emitted at the same frequency.
3. The signal that matches this transfer is measured in many ways and processed in order to yield an NMR spectrum for the nucleus concerned.

Working of Nuclear Magnetic Resonance (NMR) Spectroscopy

- The sample is placed in a magnetic field and the NMR signal is produced by excitation of the nuclei sample with radio waves into nuclear magnetic resonance, which is detected with sensitive radio receivers.
- The intramolecular magnetic field around an atom in a molecule changes the resonance frequency, thus giving access to details of the electronic structure of a molecule and its individual functional groups.
- As the fields are unique or highly characteristic to individual compounds, NMR spectroscopy is the definitive method to identify monomolecular organic compounds.
- Besides identification, NMR spectroscopy provides detailed information about the structure, dynamics, reaction state, and chemical environment of molecules.
- The most common types of NMR are proton and carbon-13 NMR spectroscopy, but it is applicable to any kind of sample that contains nuclei possessing spin.

Applications of Nuclear Magnetic Resonance (NMR) Spectroscopy

- Spectroscopy is the study of the interaction of electromagnetic radiation with matter. NMR spectroscopy is the use of the NMR phenomenon to study the physical, chemical, and biological properties of matter.
- It is an analytical chemistry technique used in quality control.
- It is used in research for determining the content and purity of a sample as well as its molecular structure. For example, NMR can quantitatively analyze mixtures containing known compounds.
- NMR spectroscopy is routinely used by chemists to study chemical structure using simple one-dimensional techniques. Two-dimensional techniques are used to determine the structure of more complicated molecules.
- These techniques are replacing x-ray crystallography for the determination of protein structure.
- Time domain NMR spectroscopy techniques are used to probe molecular dynamics in solution.
- Solid state NMR spectroscopy is used to determine the molecular structure of solids.
- Other scientists have developed NMR methods-of measuring diffusion coefficients.

ATOMIC ABSORPTION SPECTROSCOPY

Atomic absorption spectroscopy (AAS) is an absorption spectroscopic method that uses the absorption of light by free atoms in a gaseous state to determine the quantitative composition of chemical components. It is used to determine the concentration of metals present in a sample to be analyzed.

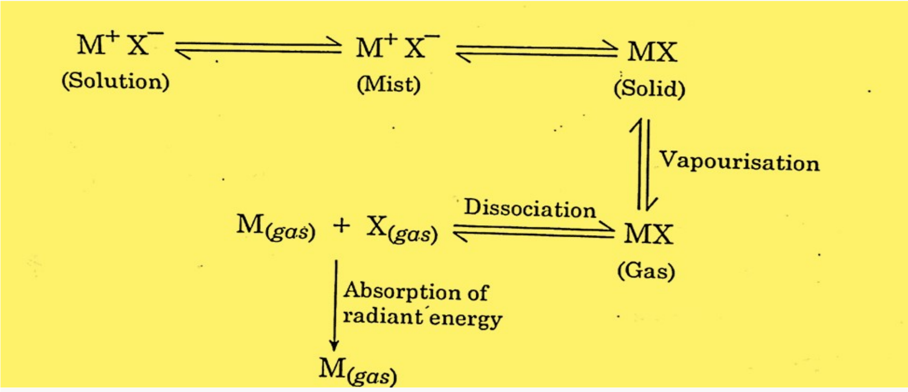
AAS can be used to quantify more than 70 different elements either in solution or solid form and possesses wider applications in clinical analysis, food analysis, the pharmaceutical industry, the mining sector, and so on. Because the atomic absorption method is largely free of interference and the set of electronic energy levels is specific to that element, it is a highly good analytical technique with great sensitivity.

The modern form of **AAS** was developed by Australian Chemist, Sir Alan Walsh in the 1950s.

Principle of atomic absorption spectroscopy

If a solution containing metal salt (M+X–) is aspirated to the flame, a vapor that contains atoms of metal may be formed. A large number of the gaseous metal atom remains in the ground state, and are capable of absorbing radiant energy of their specific wavelength. If the light of resonance wavelength is passed through the flame containing the atoms which are analyte, the part of the light will be absorbed and the extent of absorption will be directly proportional to the number of ground state atoms present in the flame.

The process by which gaseous metal atoms are produced into the flame can be illustrated as:



When a metal atom is changed into gas and light is passed from the sources, the ground state of the atom gets excited by absorbing the radiation of a particular wavelength. The absorbance is given by **Beer-Lambert's law**; the logarithmic ratio of the intensity of incident light to the intensity of absorbing species.

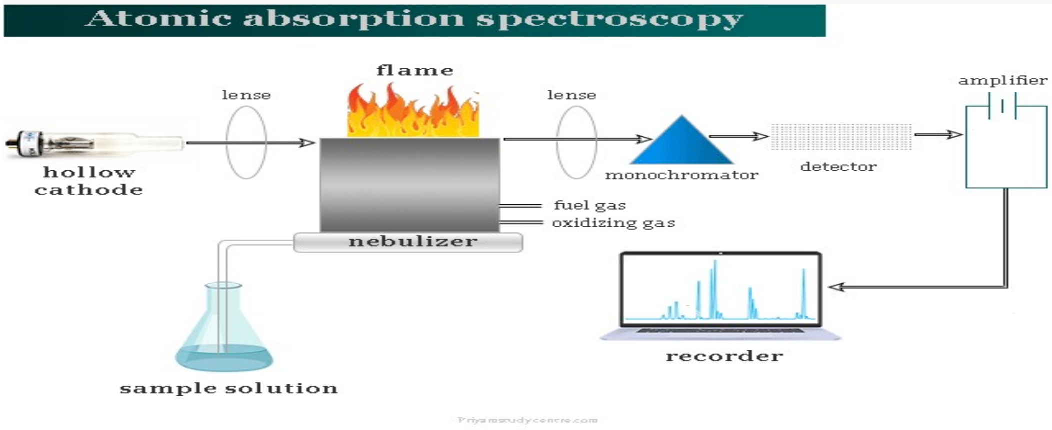
$$A = \log\frac{I_o}{I_t} = KLN_o$$

Where,

N_o = concentration of atoms in the flame

L= path length through the flame

K = constant related to absorption coefficient



Application of atomic absorption spectroscopy

Some of the major applications of atomic absorption spectroscopy are:

- Analysis of magnesium and calcium in tap water (water analysis).
- Determination of amount of trace elements in contaminated soil (soil analysis).
- Clinical analysis: Estimation of metals in biological fluids such as blood, urine, serum, etc.
- Environmental analysis
- Trace elements analysis in foods, cosmetics, hair, etc.
- Mining: The amount of metal such as gold can be determined in rocks.
- Pharmaceuticals: The amount of catalyst used may be present in trace amounts in final pharmaceuticals. Thus, AAS is used to determine the amount of catalyst present.

Advantages of atomic absorption spectroscopy

- Easy to use
- Cheap
- Rapid
- Greater sensitivity
- Efficient atomization
- Even small quantities of the sample can be analyzed (5-50μL).
- Samples either in solid or slurry or solution form can be analyzed.

Disadvantages of atomic absorption spectroscopy

- Fails to detect non-metals
- Simultaneous analysis of elements is not possible
- Only able to detect 70 elements excluding earth metals

MASS SPECTROMETRY

What is Mass Spectrometry?

Mass spectrometry is an analytical method useful for calculating the mass-to-charge ratio (m / z) of one or more molecules in the sample. Such measurements may also often be used to determine the precise molecular weight of the sample components. Mass spectrometry is an analytical method to find the molecular mass of a compound and indirectly helped to prove the identity of isotopes.

1. Principle of Mass Spectrometry

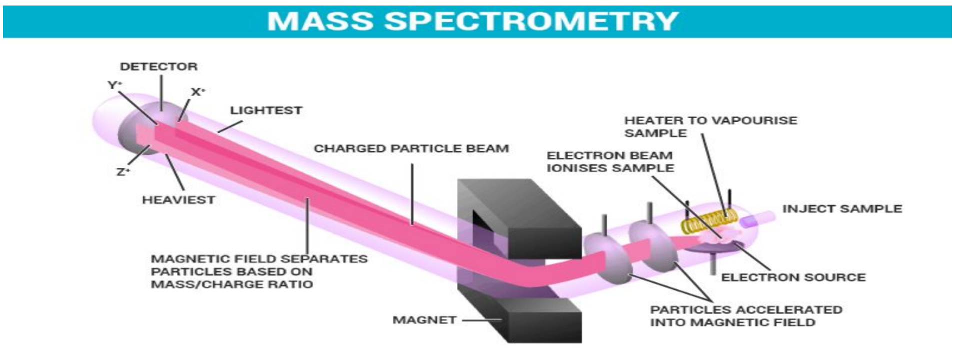
Based on Newton's second law of motion and momentum, a mass spectrometer uses this property of matter to plot ions of varying masses on a mass spectrum. From the law, we infer how much mass is relevant to the inertia and acceleration of a body. This principle is applied to the aspect where ions with different mass to charge ratios are deflected by different angles in an electric or magnetic field.

2. Mass Spectrum

A mass spectrum is a graph obtained by performing mass spectrometry. It is a relation between the mass to charge ratio and ion signal.

3. Mass Spectrometry Diagram

- Inlet system
- Ionization
- Deflector
- Ion detector



Mass Spectrometry Instrumentation

Mass Spectrometry Detectors

At different deflections a detector counts the number of ions. The data are plotted as a graph or continuum of various masses. Detectors function by recording the induced charge or current generated by an ion hitting or passing through a surface. Since the signal is very small it is possible to use an electron amplifier, Faraday cup, or ion-to-photon detector. To generate a spectrum the signal is greatly amplified.

The Mass Analyzer

When ionized, the ions are sorted and divided according to the mass-to – charge (m / z) ratio. A variety of mass analyzers are currently available, each of which has trade-offs related to speed of operation, separation resolution and other technical criteria. The different forms in use at the Broad Institute are described in the following section. The mass analyzer often works in concert with the ion detection system.

What is a Quadrupole?

Mass spectrometry determines the chemical by calculating the typical mass fragments formed by the ionization of the material. Test molecules are ionized by an electron beam, and the resulting molecular ion and component ions travel into a mass analyzer where their masses are measured.

Mass spectrometry is generally considered the benchmark for identification of unknown organic chemicals because it is highly sensitive and selective, and mass spectra are easily searchable against vast reference databases.

How does Mass Spectrometry Work?

In a regular mass spectrometer, we initially have the material to be analyzed, but we need it to be ionized to pass through the spectrometer with enough energy. Thus, the sample is bombarded by electrons to ionize it.

This ionized beam is now passed through a series of electric or magnetic fields depending on the type of the sample and its properties. The ions are deflected by the field through which they are passed through in such a way that the ions with the same mass to signal ratio will follow the same path to the detector.

These charged and deflected ions are now incident onto a detector which is capable of distinguishing the charged particles falling on it. Based on the mass spectrum produced by the charged ions, we can identify the atoms or molecules constituting the sample by comparing them with known masses or through a characteristic fragmentation pattern.

Applications of Mass Spectrometry

Mass spectrometry is an efficient method to elucidate the chemical composition of a sample or molecule. More recently, it has been used to classify biological products, in particular proteins and protein complexes, in a number of species. Usually, mass spectrometers can be used to classify unknown substances by molecular weight measurement, to measure known compounds, and to determine the structure and chemical properties of molecules.

- Due to its capability to distinguish between substances, Mass spectrometry is used to determine unknown substances.
- To identify the isotopes of a substance.
- In analytical laboratories that study the chemical, physical and biological properties of substances. It is favored over several other analytical techniques as it has less background interference since it is performed in a vacuum.