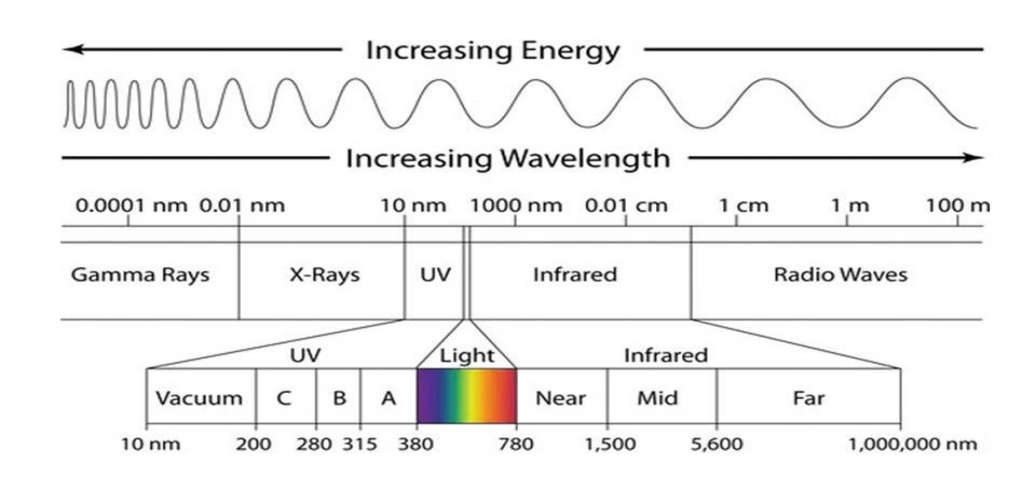
SPECTROSCOPY

Spectroscopy is the measurement and interpretation of electromagnetic radiation (EMR) that molecules, atoms and ions in a sample absorb and emit as they move from one energy level to another energy level.

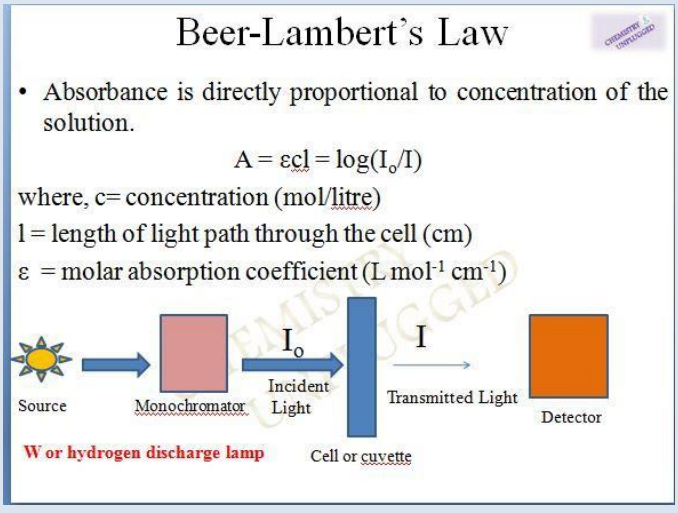


UV-VISIBLE SPECTROSCOPY

Ultraviolet and visible spectroscopy, also known as electronic spectroscopy, is used to measure the number of double bonds and aromatic conjugation in a molecule. Spectroscopy is the measurement and interpretation of electromagnetic radiation that is absorbed or emitted when molecules or atoms or ions in a sample move from one energy state to another. UV spectroscopy is a type of absorption spectroscopy in which a molecule absorbs light in the ultraviolet range (200-400 nm), as a result of which electrons are excited from the ground state to a higher energy state. The ultraviolet range corresponds to ~200-400 nm and the visible range to ~400-800 nm

UV-visible spectroscopy based on the Beer-Lambert law

Beer Lambert law: It is the relationship between the absorbance of light is directly proportional to the thickness of the media through which the light is being transmitted multiplied by the concentration of absorbing medium.



Also,

Absorbance= $-logT$ (T is transmittance)

A= $-log\frac{\%T}{100}$

A= $-(log\%T - log100)$

$A = -(log\%T - 2)$; $A = 2 - log\%T$ ⇨ Relationship between absorbance and transmittance

PRINCIPLES OF UV-VISIBLE SPECTROPHOTOMETER

1. Basically, spectroscopy deals with the interaction of light with matter.

2. When a substance absorbs light, the result is an increase in the energy content of the atoms or molecules.

3. Absorption of ultraviolet radiation leads to the excitation of electrons from the ground state to a higher energy state.

4. Molecules that contain π-electrons or non-bonded electrons (n-electrons) can absorb energy in the form of ultraviolet light to excite those electrons to higher non-bonded molecular orbitals.

5. The more easily electrons are excited, the longer wavelengths of light they can absorb. There are four transitions (π–π*, n–π*, σ–σ* and n–σ*) and they can be ordered in terms of energy as follows: σ–σ* > n–σ* > π– π* > n – π *.

Instrumentation

There are two types of UV-Vis spectrophotometers

1. Single beam UV-Vis spectrometer 2. Two-beam or double beam UV-Vis spectrometer

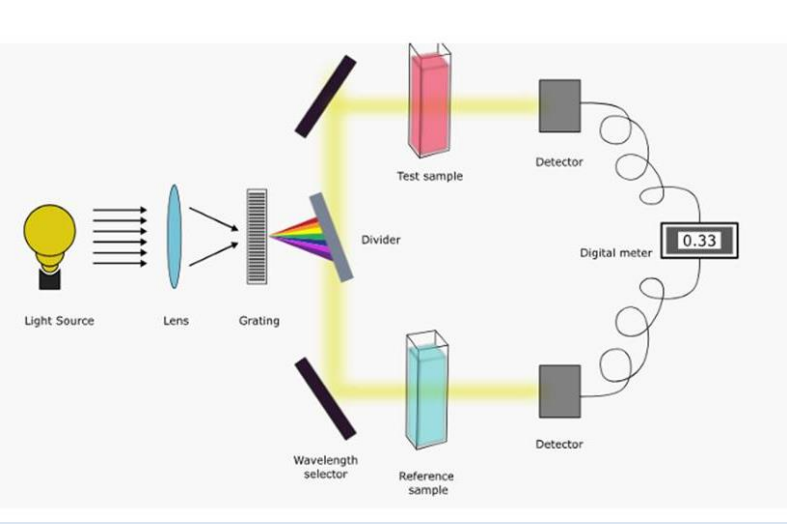
The instrumentation of single- and double -beam spectrophotometers is almost the same. The main difference with a single-beam UV-Vis spectrophotometer is that the incoming light beam passes through the sample and reference cells simultaneously. The incoming light is split and directed into both reference and sample cuvettes in double beam spectrophotometer.

Detectors detect a refracted or transmitted beam. A two-ray UV-vis spectrophotometer requires two detectors that detect the ratio of electrons to measure or calculate the absorbance of the sample being studied. It also requires a stabilized voltage supply.

The Main equipment of the UV-visible spectrometer consists of
1. Light source
2. Monochromator (Diffraction grating)
3. Sample container or cells or cuvette
4. Detector

LIGHT SOURCE - tungsten Incandescent lamps and hydrogen deuterium lamps are the most used and suitable light sources because they cover the entire UV range.

MONOCHROMATOR - Monochromators usually consist of prisms and slits



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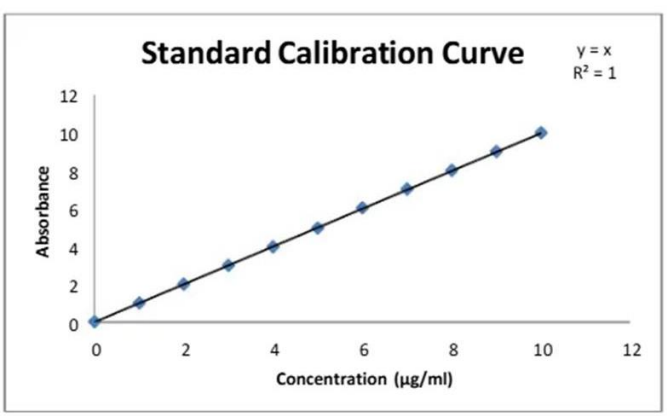
CELLS - One of the two split beams is passed through the sample solution and the other beam through the reference solution. The cells contain both sample and reference solution. These cells are made of either silica or quartz. Glass cannot be used for cells because it also absorbs UV light.

DETECTOR - Usually two photocells serve the purpose of a detector in UV spectroscopy. One of the photocells receives the sample cell beam and the other detector receives the reference beam.

Before any quantitative measurement of sample, construction of calibration curve is very important. Calibration curve is a general method for determining the concentration of a substance in an unknown sample by comparing the unknown to a set of standard samples of known concentration. In spectroscopic analysis, a series of standard solutions of known concentrations are prepared and absorbance is measured.

A calibration curve, where concentration is plotted against absorbance, then a straight line is obtained. This line is used to convert absorbance of unknown sample into concentration. (Refer to class notes)



Colorimetric analysis

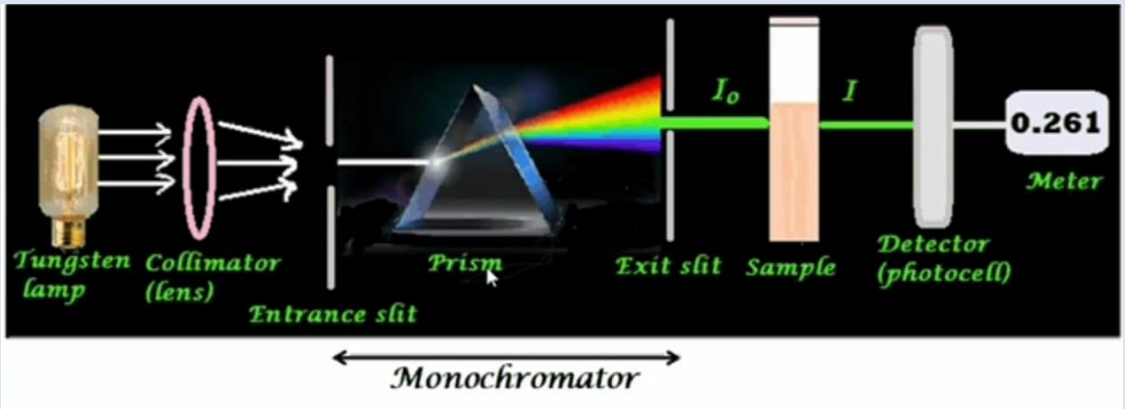
Colorimeter is works on principle of photometry A colorimeter is a device used to test the concentration of a solution by measuring its absorbance of a specific wavelength of light. The basic function of a colorimeter is to determine what quality of color is emitted from solution.

In colorimetric determinations A specific reagents are used which react with the specific component and form a colored complex. The concentration of the colored complex is directly proportional to the concentration of the component in the specimen. That colour density absorbed specific spectum of light and rest of light get transmitted from speciment. That transmitted light is detected by colorimeter detector.

According to following formula, Optical density (O. D.) or absorbance is calculated. O.D. = 2 – log %T

O.D. is directly proportional to concentration of substance.

Basic components of colorimeter



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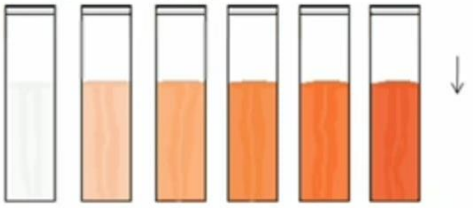
DETERMINATION OF THE UNKNOWN CONCENTRATION OF THE GIVEN SAMPLE (Fe^{3+})

- A blank sample is prepared with the complexing agent added to the solvent.

$$\underset{\text{Ferric chloride}}{Fe^{3+}} + \underset{\text{Potassium thiocyanate}}{SCN^-} \rightarrow \underset{\text{Ferric thiocyanate}}{[FeSCN]^{2+}}$$

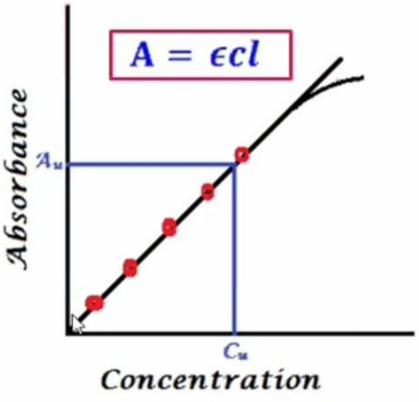
Blood red colour

- A series of $FeCl_3$ solution of known concentration is prepared to which the complexing agent is added.



- The wavelength is fixed at 480nm using the monochromator.
- The blank sample is placed in the cell holder and the absorbance is set to zero.
- Now the series of samples are placed one by one in the same cell holder and the absorbance values are noted.

Concentration	Absorbance
Blank (C_o)	o
C_1	A_1
C_2	A_2
C_3	A_3
C_4	A_4
C_5	A_5



- The calibration graph is plotted with the concentration against absorbance.
- The sample of unknown concentration (C_u) is placed in the same cell and the absorbance value (A_u) is noted.
- From the calibration graph the unknown concentration (C_u) can be found.

APPLICATIONS

To determine the

- *concentration of iron, chloride, fluoride, manganese, zinc etc. in water samples.*
- *nitrates, phosphorous, ammonia in fertilizers, soil samples etc.*
- *haemoglobin in blood samples.*
- *colour in beverage, food products, etc.*
- *monitors the growth of bacterial and yeast growth.*
- *colours used in printer toners.*

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ADVANTAGES OF COLORIMETER

- It is very easy to operate.
- For the photometric reading of unstable colored complexes, the single cell photometer can be very useful

INFRA-RED (IR) SPECTROSCOPY

IR spectroscopy has been used for examination and quantification of even trace evidences found at crime scene.

Principle of IR: IR spectroscopy is based on the absorption of infrared radiation, which causes vibrational transition in the molecule. Infrared spectroscopy is the technique in which the measurements of different IR frequencies by a sample are done, positioned in the path of IR beam. IR spectrometer can accept wide range of sample types such as gases, liquid and solids.

The infrared region of the electromagnetic spectrum extends from the red end of the visible spectrum to the microwave region. The region includes radiation at wave-lengths between 0.7 and 500 μm or, in wave numbers, between 14,000 and 20 cm^{-1}. IR spectrometry involves examination of the twisting, bending, rotating, and vibrational motions of atoms in a molecule. Atoms or atomic groups in molecules are in continuous motion with respect to one another. For qualitative analysis, one of the most excellent features of an infrared spectrum is that the absorption or the lack of absorption in specific frequency regions can be correlated with specific stretching and bending motions and, in some cases, with the relationship of these groups to the rest of the molecule.

Table: Some of the common functional groups and their IR frequency regions

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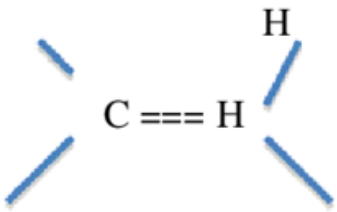
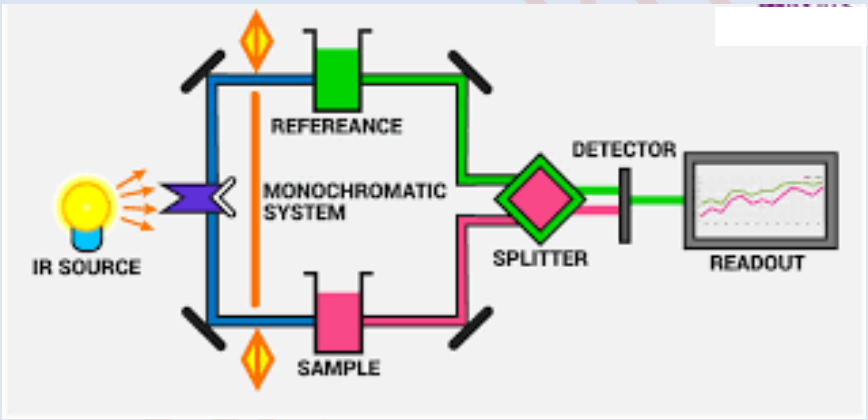
Bond	Compound Types	Regional Frequency
C – H	Alkanes (Alkana)	2850 - 2970 1340 - 1470
C – H	Alkenes (Alkena) 	3010 – 3095 675 - 995
C – H	Alkyne (Alkuna)	3300
C – H	Aromatic Ring	3010 – 3100 690 – 900
O – H	Phenol, Monomer Alcohol, Alcohol Hydrogen Bond, Phenol	3590 – 3650 3200 – 3600
	Monomer Carboxylic Acid, Hydrogen Bond Acid, Carboxylic	3500 – 3650 2500 - 2700
N – H	Amine, Amide	3300 – 3500
C ==== C	Alkenes	1610 – 1680
C ==== C	Aromatic Ring	1500 – 1600
C ΞΞΞ C	Alkyne	2100 – 2260
C – N	Amine, Amide	1180 – 1360
C ΞΞΞ N	Nitrile	2210 – 2280
C – O	Alcohol, Ether, Carboxylic Acid, Ester	1050 – 1300
C ==== O	Aldehydes, Ketone, Carboxylic Acid, Ester	1690 - 1760
NO_2	Nitro Compound	1500 – 1570 1300 – 1370

Infrared spectroscopy is characterized by fast, low sample volume (a few micrograms to a few milligrams), strong characterization (various substances have their own specific infrared spectrum.

Instrumentation

The main parts of an IR spectrometer are as follows:

1. The IR Radiation Sources: The various popular sources of IR radiations are (a) Incandescent Lamp (b) Nernst Glower (c) Globar Source (d) Mercury Arc
2. Monochromators: a) Prism Monochromator b) Grating Monochromator
3. Sample Cells and Sampling of Substances: As infrared spectroscopy has been used for the characterization of solid, liquid or gas samples, it is evident that samples of different phases have to be handled. But these samples have to be treated differently. However, the only common point to the sampling of different phases is that the material containing the sample must be transparent to IR radiation.
4. Detectors: The various types of detectors used in IR spectroscopy are (a) Bolometers (b) Thermocouple (c) Thermistors (d) Golay Cell (e) Photoconductivity Cell (f) Semiconductor Detectors (g) Pyroelectric Detectors (h) Fourier Transform Systems



Applications of Infrared Spectroscopy

- Identification of Organic Compounds
- Determination of Molecular Structure
- Studying the Progress of Reactions
- Detection of Impurities
- Examination of Old Paintings and Artifacts

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