Unit 3 Instrumental Techniques of Chemical Analysis

Introduction

1. Qualitative and Quantitative analysis:

Qualitative data analysis is based on classification of objects (participants) according to properties and attributes whereas quantitative analysis is based on classification of data based on computable values. Qualitative analysis is subjective whereas quantitative is objective.

1.1. What is quantitative analysis?

Quantitative analysis is often associated with numerical analysis where data is collected, classified, and then computed for certain findings using a set of statistical methods. Data is chosen randomly in large samples and then analyzed. The advantage of quantitative analysis the findings can be applied in a general population using research patterns developed in the sample. This is a shortcoming of qualitative data analysis because of limited generalization of findings.

Quantitative analysis is more objective in nature. It seeks to understand the occurrence of events and then describe them using statistical methods. However, more clarity can be obtained by concurrently using qualitative and quantitative methods. Quantitative analysis normally leaves the random and scarce events in research results whereas qualitative analysis considers them.

Quantitative analysis is generally concerned with measurable quantities such as weight, length, temperature, speed, width, and many more. The data can be expressed in a tabular form or any diagrammatic representation using graphs or charts. Quantitative data can be classified as continuous or discrete, and it is often obtained using surveys, observations, experiments or interviews.

There are, however, limitations in quantitative analysis. For instance, it can be challenging to uncover relatively new concepts using quantitative analysis and that is where qualitative analysis comes into the equation to find out “why” a certain phenomenon occurs. That is why the methods are often used simultaneously.

1.2. What is a qualitative analysis?

Qualitative analysis is concerned with the analysis of data that cannot be quantified. This type of data is about the understanding and insights into the properties and attributes of objects (participants). Qualitative analysis can get a deeper understanding of "why" a certain phenomenon occurs. The analysis can be used in conjunction with quantitative analysis or precede it.

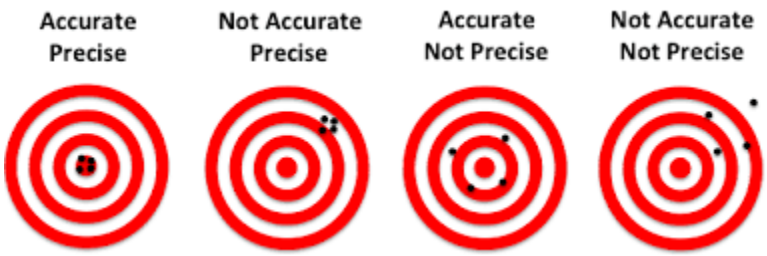
Unlike with quantitative analysis that is restricted by certain classification rules or numbers, qualitative data analysis can be wide ranged and multi-faceted. And it is subjective, descriptive, non-statistical and exploratory in nature.

Because qualitative analysis seeks to get a deeper understanding, the researcher must be well-rounded with whichever physical properties or attributes the study is based on. Oftentimes, the researcher may have a relationship with the participants where their characteristics are disclosed. In a quantitative analysis the characteristics of objects are often undisclosed. The typical data analyzed qualitatively include color, gender, nationality, taste, appearance, and many more as long as the data cannot be computed. Such data is obtained using interviews or observations.

There are limitations in qualitative analysis. For instance, it cannot be used to generalize the population. Small samples are used in an unstructured approach and they are non-representative of the general population hence the method cannot be used to generalize the entire population. That is where quantitative analysis into the factor.

2. **Accuracy and Precision:**

A good analogy for understanding accuracy and precision is to imagine a basketball player shooting baskets. If the player shoots with accuracy, his aim will always take the ball close to or into the basket. If the player shoots with precision, his aim will always take the ball to the same location which may or may not be close to the basket. A good player will be both accurate and precise by shooting the ball the same way each time and each time making it in the basket.



Accuracy refers to the closeness of a measured value to a standard or known value. For example, if in lab you obtain a weight measurement of 3.2 kg for a given substance, but the actual or known weight is 10 kg, then your measurement is not accurate. In this case, your measurement is not close to the known value.

Precision refers to the closeness of two or more measurements to each other. Using the example above, if you weigh a given substance five times, and get 3.2 kg each time, then your measurement is very precise. Precision is independent of accuracy. You can be very precise but inaccurate, as described above. You can also be accurate but imprecise.

For example, if on average, your measurements for a given substance are close to the known value, but the measurements are far from each other, then you have accuracy without precision.

3. **Reliability of Analytical data:**

So what is reliability? It is a key standard for the quality of a measure, because a measure must be reliable if it's to be valid. It refers to a measure's consistency and repeatability. We consider a measure reliable if, when we repeatedly measure the same person, plant, machine or system, along a particular dimension or variable, it produces approximately the same value. To illustrate, there is the simple example of a mechanical bathroom scale, and measurements recorded under the variable name "weight."

4. **Confidence limit:**

One of the strengths of statistics is that they quantify uncertainty about data. Confidence limits (sometimes called "confidence intervals") clearly illustrate that uncertainty, thus, regulators often require them. For example, confidence limits may be used to compare groundwater monitoring data to a fixed threshold, such as a compliance criterion, or for placing an upper limit on background. Confidence limits are the maximum and minimum values bracketing the statistic of

interest (usually the arithmetic or geometric mean) based on the distribution of the data (usually the normal or lognormal distribution) at a certain confidence level (usually 95%). In other words, confidence limits are the maximum or minimum values above or below which you are confident (at a selected confidence level) that the statistic will occur. Confidence limits can be parametric or nonparametric. For the calculation of parametric confidence limits, the underlying statistical distribution must be known in order to select the appropriate confidence limit. Certain more robust methods (e.g., calculation of robust confidence limits) may permit the calculation of confidence limits without removal of outliers within background data (USEPA 1999).

Confidence limits are calculated from experimental data and operate in tandem with a percentage confidence level.

For example, the average value of a set of pH measurements on a particular acid might be 1.4.

Statistical analysis of the data from which the average was calculated allows confidence limits to be placed on this average.

Let us assume statistical analysis indicates there is a 99 percent probability that the true value of the pH is 1.4 ± 0.2; then we can say that the confidence limits for the pH value are 1.2 and 1.6, and with 99% confidence that the true pH is between 1.2 and 1.6.

To calculate confidence limits, we need to know about the precision of our average value.

5. Conventional methods of Analysis:

Although modern analytical chemistry is dominated by sophisticated instrumentation, the roots of analytical chemistry and some of the principles used in modern instruments are from traditional techniques, many of which are still used today. These techniques also tend to form the backbone of most undergraduate analytical chemistry educational labs.

5.1. Gravimetric analysis:

Gravimetric analysis involves determining the amount of material present by weighing the sample before and/or after some transformation. A common example used in undergraduate education is the determination of the amount of water in a hydrate by heating the sample to remove the water such that the difference in weight is due to the loss of water.

5.1.1. Objectives:

- To precipitate nickel from the solution by adding dimethyl glyoxime.
- Filtration of precipitate using sintered glass crucible.
- Mass of nickel calculated from the mass of precipitate.

5.1.2. Theory:

Gravimetric analysis is one of the most accurate analytical methods available. It is concerned with the determination of a substance by the process of weighing. The element or radical to be determined is converted into a stable compound of definite composition and the mass of the compound is determined accurately. From this, the mass of element or radical is calculated.

The gravimetric analysis involves a) precipitation b) filtration c) washing of the precipitate and d) drying, ignition and weighing of the precipitate.

Following are the four fundamental types of gravimetric analysis:

1. Physical gravimetry
2. Thermogravimetry
3. Precipitative gravimetric analysis
4. Electrodeposition. These differ in the preparation of the sample before weighing of the analyte.

5.1.2a. Physical gravimetry: Physical gravimetry involves the physical separation and classification of matter in environmental samples based on volatility and particle size (e.g., total suspended solids). It is the most common type used in environmental engineering.

5.1.2b. Thermogravimetry: In this method the samples are heated and the changes in sample mass are recorded. Volatile solid analysis is an important example for this type of gravimetric analysis.

5.1.2c. Precipitative gravimetry: The chemical precipitation of an analyte occurs in the precipitative gravimetry. The most important application of this technique in the environmental field is the analysis of sulphite.

5.1.2d. Electrodeposition: It involves the electrochemical reduction of metal ions at a cathode and simultaneous deposition of the ions on the cathode.

The steps commonly followed in gravimetric analysis are;

(1) Preparation of a solution containing a known weight of the sample.
(2) Separation of the desired constituent.
(3) Weighing the isolated constituent.
(4) Computation of the amount of the particular constituent in the sample from the observed weight of the isolated substance.

5.1.3. Precipitative Gravimetric Analysis:

Precipitative gravimetric analysis requires that the substance to be weighed be readily removed by filtration. In order for a non-filterable precipitate to form, it must be supersaturated with respect to its solubility product constant. However, if it is too far above the saturation limit, crystal nucleation may occur at a rate faster than crystal growth (the addition of molecules to a crystal nucleus, eventually forming a non-filterable crystal). When this occurs, numerous tiny micro-crystals are formed rather than a few large ones. In the extreme case, micro-crystals may behave as colloids and pass through a fibrous filter. To avoid this, precipitating solutions may be heated. Because the solubility of most salts increases with increasing temperature, this treatment will lower the relative degree of super saturation and slow the rate of nucleation. Also, one might add the precipitant slowly with rapid mixing to avoid the occurrence of locally high concentrations.

Precipitative gravimetry is often practiced at high ionic strengths. This is to reduce the electric double layer thickness (salting-out effect) of the slowly forming crystals. When this occurs,

electrostatic repulsion between the crystal and its precipitating molecules is reduced. Crystal growth can then occur more rapidly. It is very important that the precipitate be pure and has the correct stoichiometry.

Co-precipitation:

For certain gravimetric analysis some other substances besides the desired substances also get precipitated in small amounts, the phenomenon is called co-precipitation. It occurs when an unwanted ion or molecule becomes trapped in the precipitate. This may be due to inclusion or occlusion. Inclusion is the term used for a single substitution in the crystal lattice by an ion of similar size. Occlusion refers to the physical trapping of a large pocket of impurities within the crystal. One technique for minimizing these problems is to remove the mother liquor, re-dissolve the precipitate and then re-precipitate. The second time the mother liquor will contain fewer unwanted ions capable of co-precipitation.

Conditions of Precipitation:

1. Precipitation should be carried out in dilute solutions.
2. The reagents should be mixed slowly and with constant stirring. This will assist the growth of large crystals.
3. Precipitation is effected in hot solutions, provided the solubility and stability of the precipitate permit it. Either one or both of the solutions should be heated to just below the boiling point. High temperature assists (a) coagulation and increase (b) velocity of crystallization.
4. Crystalline precipitates should be digested for as long as possible, preferably overnight.

Precipitating Reagents:

Ideally a gravimetric precipitating agent should react specifically or at least selectively with the analyte. Specific reagents which are rare, react only with a single chemical species. Selective reagents which are more common, react with a limited number of species. In addition to

Specificity and selectivity, the ideal precipitating reagent would react with analyte to give a product that is;

a) Easily filtered and washed free of contaminants;

b) Of sufficiently low solubility that no significant loss of the analyte occurs during filtration and washing;

c) Unreactive with constituents of atmosphere;

d) Of known chemical composition after it is dried or, if necessary, ignited.

Most of the inorganic ions have yielded to gravimetric analytical techniques, but one finds many interfering ions. The table below illustrates both the abundance of reagents available for use as well as the problems which can be encountered by interfering ions:

Analyte	**Precipitate**	**Measured form**	**Interferences**
K^+	$KB(C_6H_5)_4$	$KB(C_6H_5)_4$	NH_4^+, Ag^+, Hg^{2+}, Tl^+, Rb^+, Cs^+
Mg^{2+}	$Mg(NH_4)PO_4.6H_2O$	$Mg_2P_2O_7$	Many metals (none from Na^+ and K^+)
Ca^{2+}	$CaC_2O_4.H_2O$	$CaCO_3$ or CaO	Many metals (none from Mg^{2+}, Na^+ and K^+)
Ba^{2+}	$BaSO_4$	$BaSO_4$	Na^+,K^+,Li^+,Ca^{2+},Al^{3+} ,Cr^{3+},Fe^{3+},Sr^{2+}, Pb^{2+}
Ti^{4+}	TiO(5,7-dibromo-8-hydroxyquinoline)$_2$	TiO(5,7-dibromo-8-hydroxyquinoline)$_2$	Fe^{3+},Zr^{4+},Cu^{2+},$C_2O_4^{2-}$, citrate, HF
VO_4^{3-}	Hg_3VO_4	V_2O_5	Cl^-,Br^-,I^-,SO_4^{2-} , CrO_4^{2-},AsO_4^{3-},PO_4^{3-}
Cr^{3+}	$PbCrO_4$	$PbCrO_4$	NH_4^+,Ag^+
Mn^{2+}	$Mn(NH_4)PO_4.H_2O$	$Mn_2P_2O_7$	Interferences from numerous metals
Fe^{3+}	$Fe(HCO_2)_3$	Fe_2O_3	Interferences from numerous metals
Co^{2+}	Co(1-nitroso-2-naphtholate)$_3$	$CoSO_4$ (by reaction with H_2SO_4)	Fe^{3+},Zr^{4+},Pd^{2+}
Ni^{2+}	Ni(dimethylglyoximate)$_2$	Ni(dimethylglyoximate)$_2$	Pd^{2+},Pt^{2+},Bi^{3+},Au^{3+}
Cu^{2+}	CuSCN	CuSCN	NH_4^+,Pb^{2+},Hg^{2+},Ag^+
Zn^{2+}	$Zn(NH_4)PO_4.H_2O$	$Zn_2P_2O_7$	Interferences from numerous metals.

Ce^{4+}	$Ce(IO_3)_4$	CeO_2	Th^{4+},Ti^{4+},Zr^{4+}
Al^{3+}	Al(8-hydroxyquinolate)$_3$	Al(8-hydroxyquinolate)$_3$	Interferences from numerous metals.
Sn^{4+}	Sn(cupferron)$_4$	SnO_2	Cu^{2+},Pb^{2+},As(III)
Pb^{2+}	$PbSO_4$	$PbSO_4$	Ca^{2+},Sr^{2+},Ba^{2+},Hg^{2+}, Ag^{+},HCl, HNO_3
NH_4^+	$NH_4B(C_6H_5)_4$	$NH_4B(C_6H_5)_4$	K^+, Rb^+, Cs^+
Cl^-	AgCl	AgCl	Br^-, I^-, SCN^-, S^{2-}, $S_2O_3^{2-}$, CN^-
Br^-	AgBr	AgBr	Cl^-, I^-, SCN^-, S^{2-}, $S_2O_3^{2-}$, CN^-
I^-	AgI	AgI	Br^-, Cl^-, SCN^-, S^{2-}, $S_2O_3^{2-}$, CN^-
SCN^-	CuSCN	CuSCN	NH_4^+,Pb^{2+},Hg^{2+},Ag^+
CN^-	AgCN	AgCN	Cl^-, Br^-, I^- , SCN^-, S^{2-}, $S_2O_3^{2-}$
F^-	$(C_6H_5)_3SnF$	$(C_6H_5)_3SnF$	Except alkali metals, many interferences, and SiO_4^{4-} , CO_3^{2-}.
ClO_4^-	$KClO_4$	$KClO_4$	
SO_4^{2-}	$BaSO_4$	$BaSO_4$	Na^+,K^+,Li^+,Ca^{2+},Al^{3+} ,Cr^{3+},Fe^{3+},Sr^{2+}, Pb^{2+}
PO_4^{3-}	$Mg(NH_4)PO_4.6H_2O$	$Mg_2P_2O_7$	Many interferences except Na^+,K^+.
NO_3^-	Nitron nitrate	Nitron nitrate	ClO_4^-, I^-, SCN^-, CrO_4^{2-},ClO_3^-, NO_2^-, Br^-, $C_2O_42^-$
CO_3^{2-}	CO_2 (by addition of acid)	CO_2	CO_2 is trapped as Na_2CO_3 on Ascarite.

There are a number of organic functional groups which precipitate with metal ions by one of two routes: (1) chelating agents are organic compounds which "wrap around" a metal ion thanks to cationic side chains which form coordinate covalent bonds with the ion, and (2) a straightforward ion-ion bond which produces a new species that excludes water of solvation and thus precipitates. Good examples of chelating agents include Ethylene Diamine Tetraacetic Acid (EDTA), oxalic acid, glycine, 8-hydroxyquinoline and dimethylglyoxime. Some common organic precipitating agents:

Compound	**Ions precipitated**

Dimethylglyoxime	Ni^{2+},Pd^{2+},Pt^{2+}
EDTA (Ethylenediamine tetraacetic acid)	Zn^{2+}, Cu^{2+}, Pb^{2+}, Ca^{2+}, Ni^{2+}, Fe^{3+}
Cupferron	Fe^{3+},VO_2^+,Ti^{4+}, Zr^{4+},Ce^{4+},Ga^{3+},Sn^{4+}
8-Hydroxyquinoline	Fe^{3+},Al^{3+},Mg^{2+},Zn^{2+},Cu^{2+},Cd^{2+},Pb^{2+}, Bi^{3+}, Ga^{3+},Th^{4+}, Zr^{4+}, TiO^{2+}, UO_2^{2+}
Salicylaldoxime	Bi^{3+},Ni^{2+},Pd^{2+},Zn^{2+}, Cu^{2+},Pb^{2+}
1-Nitroso-2-naphthol	Fe^{3+},Co^{2+},Pd^{2+}, Zr^{4+}
Nitron ($C_{20}H_{16}N_4$)	NO_3^-, ClO_4^-, BF_4^-, WO_4^{2-}
Sodium tetraphenylborate	NH_4^+, organic ammonium, Ag^+, Cs^+, Rb^+, K^+
Tetraphenylarsonium chloride	$Cr_2O_7^{2-}$, MnO_4^-, ReO_4^-, MoO_4^{2-},WO_4^{2-}, ClO_4^-

The Gravimetric Estimation of Nickel:

The nickel is precipitated as nickel dimethyl glyoxime by adding alcoholic solution of dimethyl glyoxime $C_4H_6(NOH)_2$ and then adding a slight excess of aqueous ammonia solution.

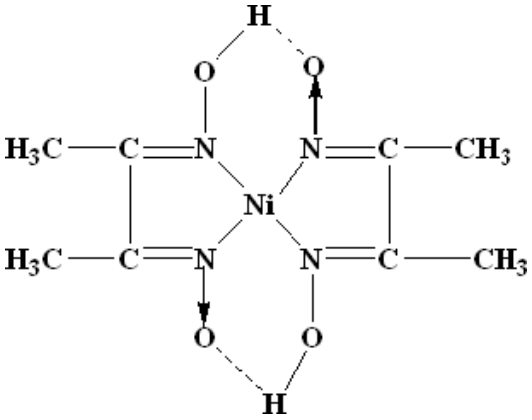
When the pH is buffered in the range of 5 to 9, the formation of the red chelate occurs quantitatively in a solution. The chelation reaction occurs due to donation of the electron pairs on the four nitrogen atoms, not by electrons on the oxygen atoms. The reaction is performed in a solution buffered by either an ammonia or citrate buffer to prevent the pH of the solution from falling below 5. If the pH does become too low the equilibrium of the above reaction favors the formation of the nickel (II) ion, causing the dissolution of Ni $(DMG)_2$ back into the mother liquor.

A slight excess of the reagent has no action on the precipitate, but a large excess should be avoided because of the possible precipitation of the reagent itself. The precipitate is soluble in the free mineral acids. It is therefore crucial to avoid the addition of too large and excess of the

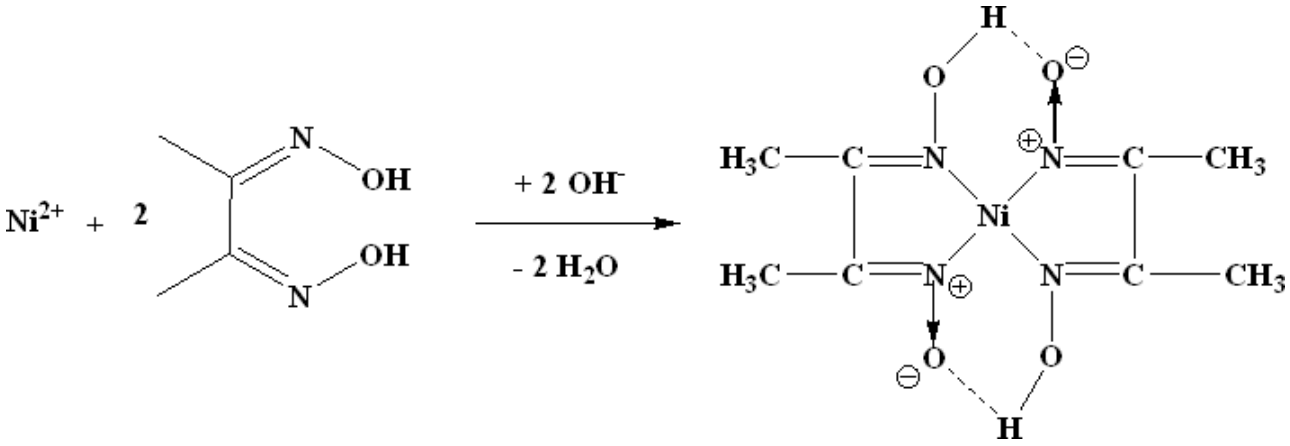
reagent because it may crystallize out with the chelate. It is also important to know that the complex itself is slightly soluble to some extent in alcoholic solutions. By adding small amount of chelating agents will minimize the errors from these sources. The amount of the reagent added is also governed by the presence of other metals such as cobalt, which form soluble complexes with the reagent. If a high quantity of these ions is present, a greater amount of DMG must be added. The nickel dimethylglyoximate is a very bulky precipitate. Therefore, the sample weight used in the analysis must be carefully controlled to allow more convenient handling of the precipitate during the transfer to the filtering crucible. The compactness of the precipitate is improved by adjusting the pH to 3 or 4, followed by the addition of ammonia solution.

A slow increase in the concentration of ammonia in the solution causes a slight increase in the pH gradually and results in the precipitation of the complex. The result is the formation of a denser precipitate. Once the filtrate has been collected and dried, the nickel content of the solution is calculated stoichiometrically from the weight of the precipitate.

The structure of DMG & the complex with nickel ions is given below;



Reaction:



5.2. Titrimetric analysis:

Titration involves the addition of a reactant to a solution being analyzed until some equivalence point is reached. Often the amount of material in the solution being analyzed may be determined. Most familiar to those who have taken chemistry during secondary education is the acid-base titration involving a color changing indicator. There are many other types of titrations, for example potentiometric titrations. These titrations may use different types of indicators to reach some equivalence point.

Acid-Base Titrations

Learning Objective

- Compute the concentration of an unknown acid or base given its volume and the volume and concentration of the standardized titrant.

Key Points

- An acid-base titration is a quantitative analysis of acids and bases; through this process, an acid or base of known concentration neutralizes an acid or base of unknown concentration.
- The titration progress can be monitored by visual indicators, pH electrodes, or both.

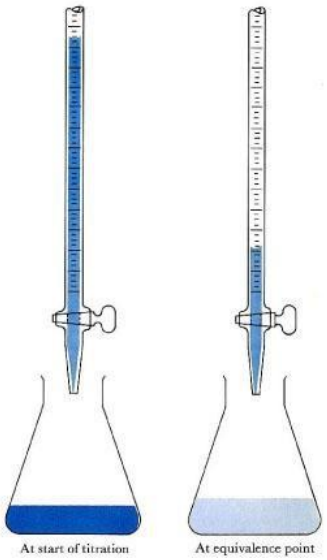
- The reaction's equivalence point is the point at which the titrant has exactly neutralized the acid or base in the unknown analyte; if you know the volume and concentration of the titrant at the equivalence point, you can calculate the concentration of a base or acid in the unknown solution.

Terms

- acid-base titration determines the concentration of an acid or base by exactly neutralizing it with an acid or base of known concentration
- equivalence point the point at which an added titrant's moles are stoichiometrically equal to the moles of acid/base in the sample; the smallest amount of titrant needed to fully neutralize or react with the analyte
- titrant the standardized (known) solution (either an acid or a base) that is added during titration
- analyte the unknown solution whose concentration is being determined in the titration

Setting up an Acid-Base Titration

An acid-base titration is an experimental procedure used to determined the unknown concentration of an acid or base by precisely neutralizing it with an acid or base of known concentration. This lets us quantitatively analyze the concentration of the unknown solution. Acid-base titrations can also be used to quantify the purity of chemicals.



Acid-base titration

The solution in the flask contains an unknown number of equivalents of base (or acid). The burette is calibrated to show volume to the nearest 0.001 cm^3. It is filled with a solution of strong acid (or base) of known concentration. Small increments are added from the burette until, at the end point, one drop changes the indicator color permanently. (An indication of the approaching equivalence point is the appearance, and disappearance after stirring, of the color that the indicator assumes beyond neutralization.) At the equivalence point, the total amount of acid (or base) is recorded from the burette readings. The number of equivalents of acid and base must be equal at the equivalence point.

Alkalimetry, or alkimetry, is the specialized analytic use of acid-base titration to determine the concentration of a basic (alkaline) substance; acidimetry, or acidometry, is the same concept applied to an acidic substance.

Materials for a Titration Procedure

- burette
- white tile (used to see a color change in the solution)
- pipette
- pH indicator (the type depends on the reactants)
- Erlenmeyer or conical flask
- titrant (a standard solution of known concentration; a common example is aqueous sodium carbonate)
- analyte, or titrand (the solution of unknown concentration)

Equivalence Point Indicators

Before you begin the titration, you must choose a suitable pH indicator, preferably one that will experience a color change (known as the “end point”) close to the reaction’s equivalence point; this is the point at which equivalent amounts of the reactants and products have reacted. Below are some common equivalence point indicators:

- strong acid-strong base titration: phenolphthalein indicator
- weak acid-weak base titration: bromthymol blue indicator

- strong acid-weak base titration: methyl orange indicator the base is off the scale (e.g., pH > 13.5) and the acid has pH > 5.5: alizarine yellow indicator
- the base is off the scale (e.g., pH > 13.5) and the acid has pH > 5.5: alizarine yellow indicator
- the base is off the scale (e.g., pH > 13.5) and the acid has pH > 5.5: alizarine yellow indicator
- the acid is off the scale (e.g., pH < 0.5) and the base has pH < 8.5: thymol blue indicator

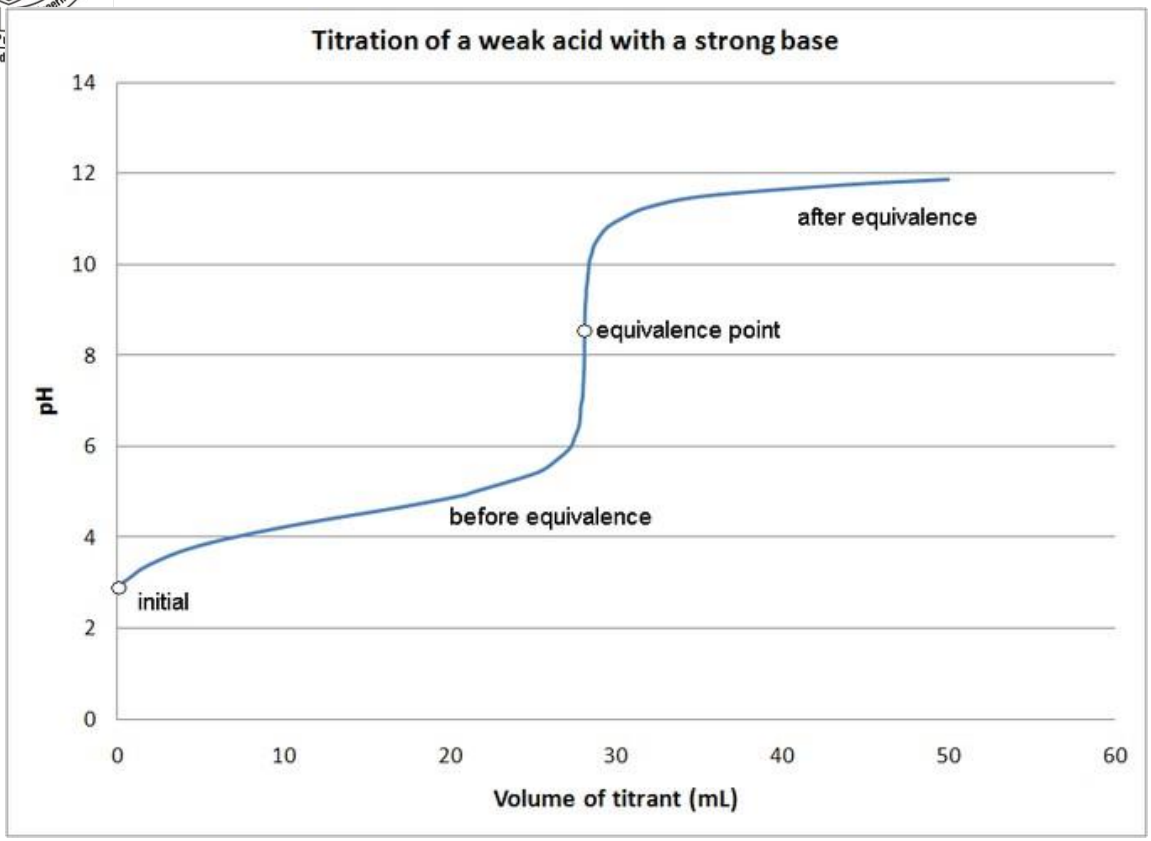
Estimating the Equivalence Point's pH

The resulting solution at the equivalence point will have a pH dependent on the acid and base's relative strengths. You can estimate the equivalence point's pH using the following rules:

- A strong acid will react with a weak base to form an acidic (pH < 7) solution.
- A strong acid will react with a strong base to form a neutral (pH = 7) solution.
- A weak acid will react with a strong base to form a basic (pH > 7) solution.

When a weak acid reacts with a weak base, the equivalence point solution will be basic if the base is stronger and acidic if the acid is stronger; if both are of equal strength, then the equivalence pH will be neutral. Weak acids are not often titrated against weak bases, however, because the color change is brief and therefore very difficult to observe.

You can determine the pH of a weak acid solution being titrated with a strong base solution at various points; these fall into four different categories: (1) initial pH; (2) pH before the equivalence point; (3) pH at the equivalence point; and (4) pH after the equivalence point.



Titration of a weak acid by a strong base The pH of a weak acid solution being titrated with a strong base solution can be found at each indicated point.

Titration Procedure

1. Rinse the burette with the standard solution, the pipette with the unknown solution, and the conical flask with distilled water.

2. Place an accurately measured volume of the analyte into the Erlenmeyer flask using the pipette, along with a few drops of indicator. Place the standardized solution into the burette, and indicate its initial volume in a lab notebook. At this stage, we want a rough estimate of the amount of known solution necessary to neutralize the unknown solution. Let the solution out of the burette until the indicator changes color, and record the value on the burette. This is the first titration and it is not very precise; it should be excluded from any calculations.

3. Perform at least three more titrations, this time more accurately, taking into account where the end point will roughly occur. Record the initial and final readings on the

burette, prior to starting the titration and at the end point, respectively. (Subtracting the initial volume from the final volume will yield the amount of titrant used to reach the endpoint.)

4. The end point is reached when the indicator permanently changes color.

6. Advantages of Instrumental Methods Modern analytical techniques: an overview,

All the Instrumental Methods have many advantages, which are listed below:

a. Instruments have very high sensitivity & samples with less concentrations (10-3 N) can be tested. In AAS samples with concentrations in ppm or ppb can be tested with high accuracy.
b. The results obtained are very accurate & can be reproducible.
c. The reliability of the Results is very high.
d. Large number of samples can be analyzed with equal sensitivity and high accuracy.
e. Estimations can be carried out on molecular, atomic or even sub-atomic levels.
f. Poisonous, Carcinogenic and Explosives can also be handled safely.
g. Samples which are Costly or scarce or which have Historic importance can also be analyzed using non-destructive testing methods like Neutron Activation Analysis (NAA) or X-ray powder diffraction methods. The sample remains unaffected after testing.
h. Instrumental methods are Fast comparable to Classical Methods. So, the results are obtained in less time.
i. These methods require very less amount of sample. For example in Gas Chromatography, about 10µl of sample is needed.
j. Classical Methods require large number of reagents and involve frequent weighing, drying, filtration types of operations whereas, Instrumental methods very little time and no sample preparation is required.
k. Instrumental Methods can be fully Automatic and hence requires less human attention decreasing the personal errors.
l. Very slow or very fast reactions (for example: reactions like Radio-active decay) can be studied using Instrumental methods.

Limitations Of Instrumental Methods Of Analysis :-

a. Frequent calibration and standardization of the Instruments is necessary,
b. Cost of Instruments is high, in some cases very high,
c. Specialized training is required for handling these Instruments,
d. Some Instruments occupy very large space,
e. For most of the Instruments, undisturbed Power Supply is necessary.

7. Electro-analytical technique:

Electroanalytical methods are a class of techniques in analytical chemistry, which study an analyte by measuring the potential (volts) and/or current (amperes) in an electrochemical cell containing the analyte.

The three main categories are potentiometry (the difference in electrode potentials is measured), coulometry (the cell's current is measured over time), and voltammetry (the cell's current is measured while actively altering the cell's potential).

Potentiometry passively measures the potential of a solution between two electrodes, affecting the solution very little in the process. The potential is then related to the concentration of one or more analytes.

Examples: pH measurement, ion selective electrodes, gas sensing electrodes

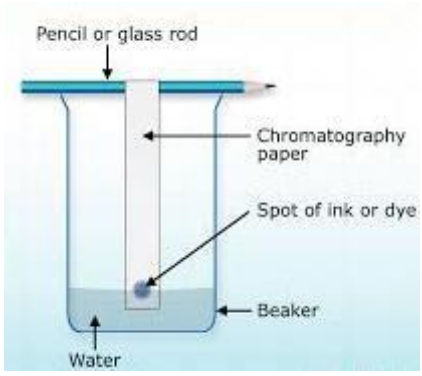
Coulometry uses applied current or potential to completely convert an analyte from one oxidation state to another. In these experiments, the total current passed is measured directly or indirectly to determine the number of electrons passed.

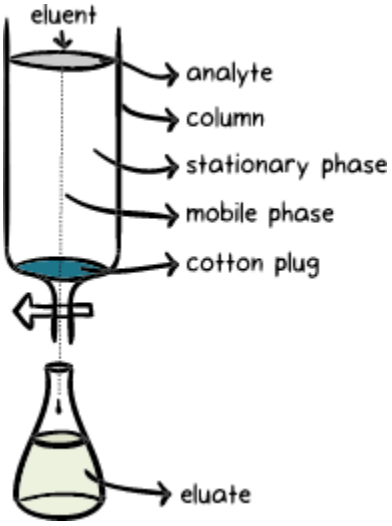
Voltammetry applies a constant and/or varying potential at an electrode's surface and measures the resulting current with a three electrode system. This method can reveal the reduction potential of an analyte and its electrochemical reactivity. This method in practical terms is nondestructive since only a very small amount of the analyte is consumed at the two-dimensional surface of the working and counter electrodes.

Examples: Voltammetric sensors, oxygen electrode, Analysis of concentration of organic and inorganic samples

8. Chromatography:

Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus affect the separation.





'Chromatography' is an analytical technique commonly used for separating a mixture of chemical substances into its individual components, so that the individual components can be thoroughly analyzed. There are many types of chromatography e.g., liquid chromatography, gas chromatography, ion-exchange chromatography, affinity chromatography, but all of these employ the same basic principles.

Chromatography, technique for separating the components, or solutes, of a mixture on the basis of the relative amounts of each solute distributed between a moving fluid stream, called the mobile phase, and a contiguous stationary phase. The mobile phase may be either a liquid or a gas, while the stationary phase is either a solid or a liquid.

The basis of the chromatography technique is,

- **Stationary phase:** This phase is always composed of a "solid" phase or "a layer of a liquid adsorbed on the surface a solid support".
- **Mobile phase:** This phase is always composed of "liquid" or a "gaseous component."
- Separated molecules

Types and uses of chromatography

- Column chromatography
  - Purifying individual chemical compounds from a mixture of compounds
  - Preparative applications .
- Ion-exchange chromatography
  - Separation of vitamins and other biological compounds
  - Water purification
  - To determine the base competition of nucleic acids
  - For analysis of amino acids
- Gel-permeation (molecular sieve) chromatography
  - To determine the relative molecular weight of polymer samples
  - To determine the distribution of molecular weights.
- Affinity chromatography
  - The purification and concentration of substances from a mixture in to a buffering solution
  - To reduce the amount of substance in a given mixture
  - To purify and concentrate enzyme solutions
  - For discerning the type of biological compounds that bind a given substrate
- Paper chromatography
  - Qualitative method to identify components of a mixture
  - Crime scene investigation and DNA/RNA sequencing
  - In analytical chemistry to identify and separate coloured mixtures.
  - In scientific studies to identify unknown organic and inorganic compounds from a mixture.
- Thin-layer chromatography

- Determine the number of components in a given mixture
- To monitor reaction progress
- To compare compounds
- To determine the effectiveness of a separation achieved on a column
- To determine the appropriate solvent for column chromatography).

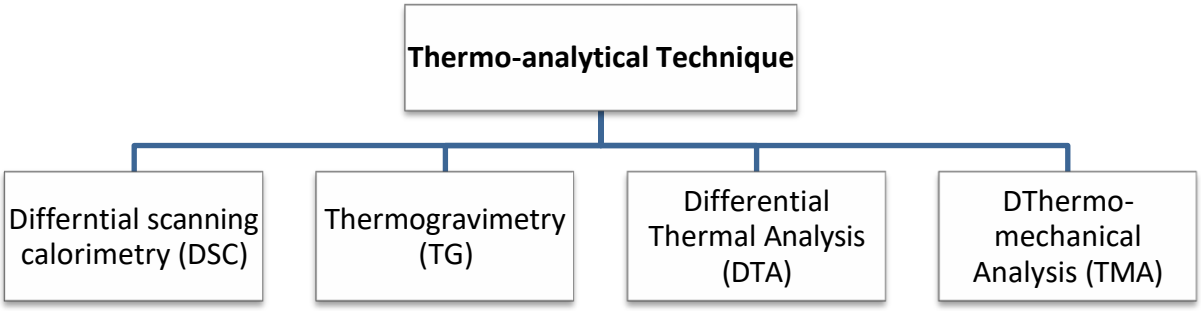
➢ Gas chromatography

- Analysis of various body fluids and secretions containing large amounts of organic volatiles
- Analysis of air samples
- To determine the components of certain mixtures using retention time and abundance samples in pharmaceuticals

➢ High-pressure liquid chromatography (HPLC)

- DNA fingerprinting
- Bioinformatics.

9. Thermo-analytical Technique:



Thermal analysis is a branch of materials science where the properties of materials are studied as they change with temperature. Several methods are commonly used – these are distinguished from one another by the property which is measured:

➢ Dielectric thermal analysis (DEA): dielectric permittivity and loss factor

➢ Differential thermal analysis (DTA): temperature difference versus temperature or time

➢ Differential scanning calorimetry (DSC): heat flow changes versus temperature or time

➢ Dilatometry (DIL): volume changes with temperature change

- Dynamic mechanical analysis (DMA or DMTA) : measures storage modulus (stiffness) and loss modulus (damping) versus temperature, time and frequency
- Evolved gas analysis (EGA): analysis of gases evolved during heating of a material, usually decomposition products
- Laser flash analysis (LFA): thermal diffusivity and thermal conductivity
- Thermogravimetric analysis (TGA): mass change versus temperature or time
- Thermomechanical analysis (TMA): dimensional changes versus temperature or time
- Thermo-optical analysis (TOA): optical properties
- Derivatography: A complex method in thermal analysis

Example: For calcium and strontium binary mixture, the decomposition temperature is for $CaCO_3$ is 65-85^oC whereas for strontium carbonate the decomposition temperature is 95-115^oC.

10. Spectroscopy:

Spectroscopy, study of the absorption and emission of light and other radiation by matter, as related to the dependence of these processes on the wavelength of the radiation. More recently, the definition has been expanded to include the study of the interactions between particles such as electrons, protons, and ions, as well as their interaction with other particles as a function of their collision energy. Spectroscopic analysis has been crucial in the development of the most fundamental theories in physics, including quantum mechanics, the special and general theories of relativity, and quantum electrodynamics. Spectroscopy, as applied to high-energy collisions, has been a key tool in developing scientific understanding not only of the electromagnetic force but also of the strong and weak nuclear forces.

11. X-ray powder diffraction (XRD):

X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analyzed material is finely ground, homogenized, and average bulk composition is determined.

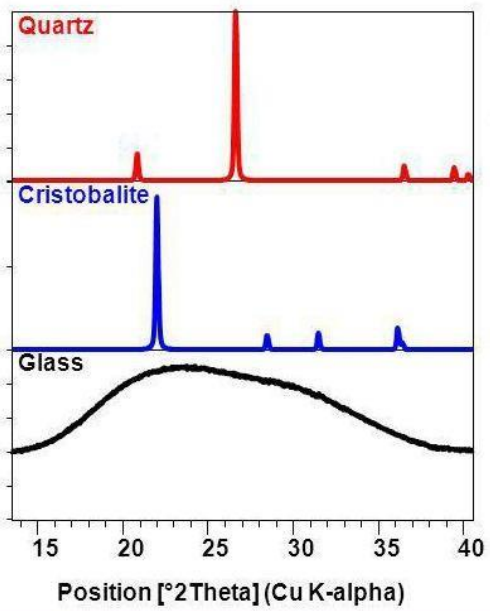
Max von Laue, in 1912, discovered that crystalline substances act as three-dimensional diffraction gratings for X-ray wavelengths similar to the spacing of planes in a crystal lattice. X-ray diffraction is now a common technique for the study of crystal structures and atomic spacing.

X-ray diffraction is based on constructive interference of monochromatic X-rays and a crystalline sample. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample. The interaction of the incident rays with the sample produces constructive interference (and a diffracted ray) when conditions satisfy Bragg's Law ($n\lambda=2d \sin \theta$). This law relates the wavelength of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample. These diffracted X-rays are then detected, processed and counted. By scanning the sample through a range of 2θangles, all possible diffraction directions of the lattice should be attained due to the random orientation of the powdered material. Conversion of the diffraction peaks to d-spacings allows identification of the mineral because each mineral has a set of unique d-spacings. Typically, this is achieved by comparison of d-spacings with standard reference patterns.

All diffraction methods are based on generation of X-rays in an X-ray tube. These X-rays are directed at the sample, and the diffracted rays are collected. A key component of all diffraction is the angle between the incident and diffracted rays. Powder and single crystal diffraction vary in instrumentation.

Example:

Each "phase" produces a unique diffraction pattern



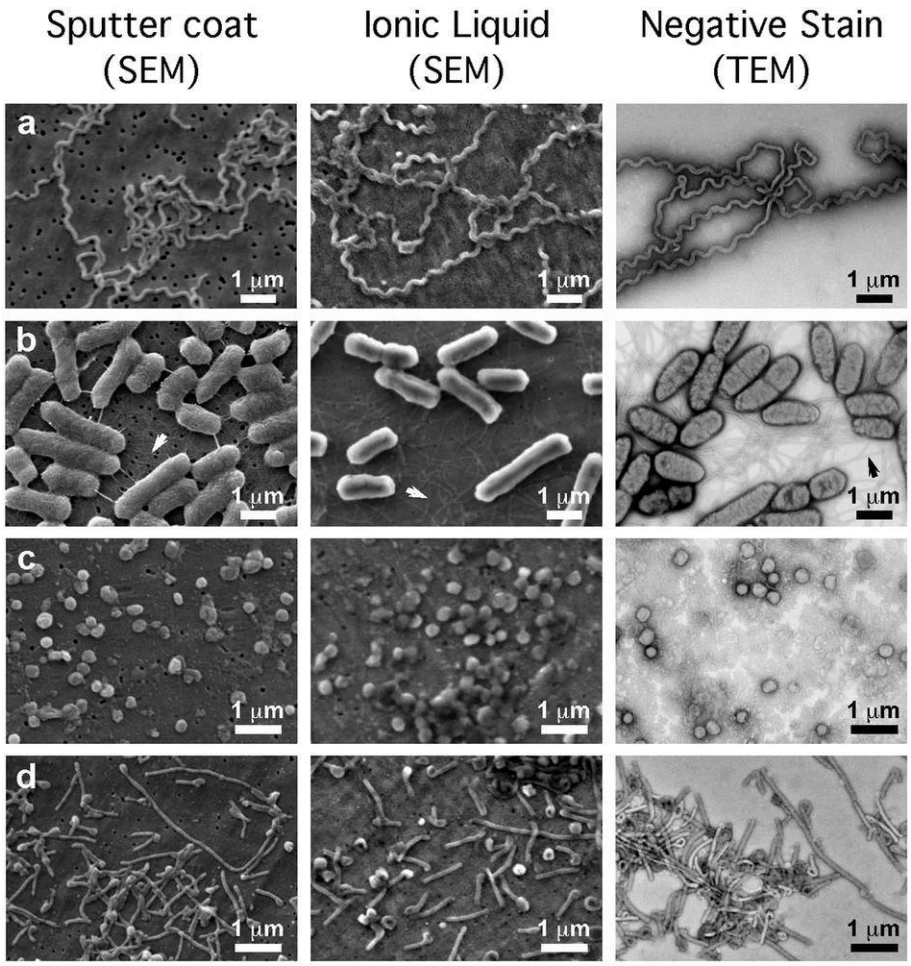
- A phase is a specific chemistry and atomic arrangement.
- Quartz, cristobalite, and glass are all different phases of SiO_2
  - They are chemically identical, but the atoms are arranged differently.
  - As shown, the X-ray diffraction pattern is distinct for each different phase.
  - Amorphous materials, like glass, do not produce sharp diffraction peaks.

The X-ray diffraction pattern is a fingerprint that lets you figure out what is in your sample.

12. Transmission electron microscope (TEM):

The transmission electron microscope is a very powerful tool for material science. A high energy beam of electrons is shone through a very thin sample, and the interactions between the electrons and the atoms can be used to observe features such as the crystal structure and features in the structure like dislocations and grain boundaries. Chemical analysis can also be performed. TEM can be used to study the growth of layers, their composition and defects in semiconductors. High resolution can be used to analyze the quality, shape, size and density of quantum wells, wires and dots.

The TEM operates on the same basic principles as the light microscope but uses electrons instead of light. Because the wavelength of electrons is much smaller than that of light, the optimal resolution attainable for TEM images is many orders of magnitude better than that from a light microscope. Thus, TEMs can reveal the finest details of internal structure - in some cases as small as individual atoms.



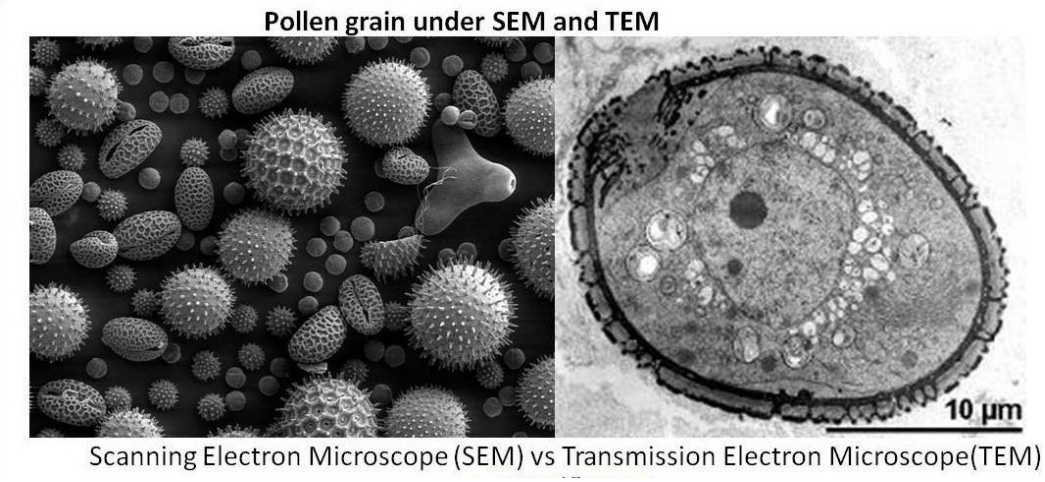
13. Scanning electron microscope (SEM):

SEM stands for scanning electron microscope. Electron microscopes use electrons for imaging, in a similar way that light microscopes use visible light. SEMs use a specific set of coils to scan the beam in a raster-like pattern and use the electrons that are reflected or knocked off the near-surface region of a sample to form an image. Since the wavelength of electrons is much smaller than the wavelength of light, the resolution of SEMs is superior to that of a light microscope.

A scanning electron microscope (SEM) scans a focused electron beam over a surface to create an image. The electrons in the beam interact with the sample, producing various signals that can be used to obtain information about the surface topography and composition.

The SEM is routinely used to generate high-resolution images of shapes of objects (SEI) and to show spatial variations in chemical compositions: 1) acquiring elemental maps or spot chemical

analyses using EDS, 2) discrimination of phases based on mean atomic number (commonly related to relative density) using BSE, and 3) compositional maps based on differences in trace element "activitors" (typically transition metal and Rare Earth elements) using CL. The SEM is also widely used to identify phases based on qualitative chemical analysis and/or crystalline structure. Precise measurement of very small features and objects down to 50 nm in size is also accomplished using the SEM. Backescattered electron images (BSE) can be used for rapid discrimination of phases in multiphase samples. SEMs equipped with diffracted backscattered electron detectors (EBSD) can be used to examine microfabric and crystallographic orientation in many materials.



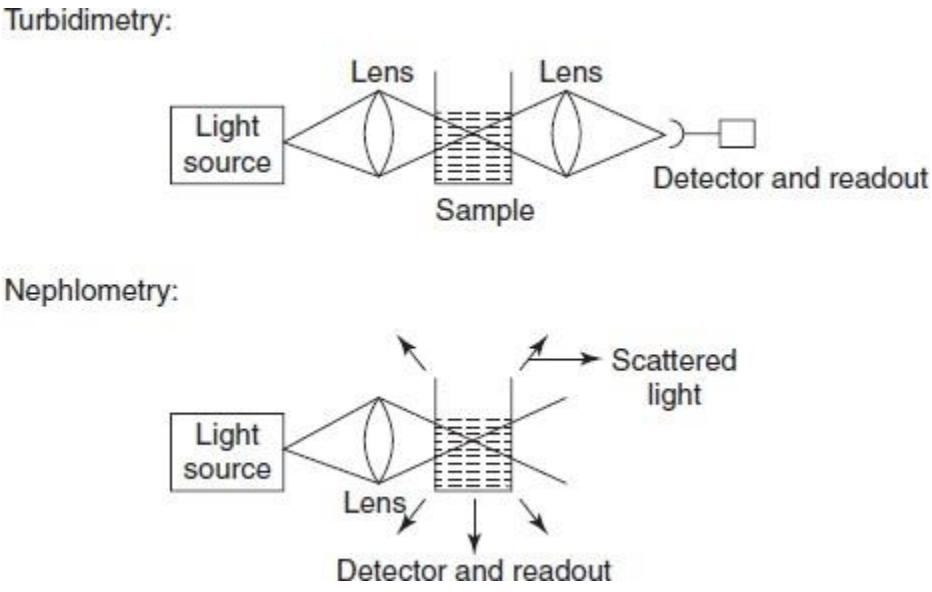
Scanning Electron Microscope (SEM) vs Transmission Electron Microscope(TEM)

14. Nephlometry & Turbidimetry:

Nephelometry and turbidimetry, in analytical chemistry, methods for determining the amount of cloudiness, or turbidity, in a solution based upon measurement of the effect of this turbidity upon the transmission and scattering of light. Turbidity in a liquid is caused by the presence of finely divided suspended particles. If a beam of light is passed through a turbid sample, its intensity is reduced by scattering, and the quantity of light scattered is dependent upon the concentration and size distribution of the particles. In nephelometry the intensity of the scattered light is measured, while, in turbidimetry, the intensity of light transmitted through the sample is measured. Nephelometric and turbidimetric measurements are used in the determination of suspended material in natural waters and in processing streams.

Example: The technique is also used for determination of sulphur in coal, oil, and other organic materials; the sulphur is precipitated as barium sulphate.

Schematic representation of Nephelometry and Turbidimetry



Spectroscopy

Spectroscopy, study of the absorption and emission of light and other radiation by matter, as related to the dependence of these processes on the wavelength of the radiation.

Following Table will give you the idea of Analytical Property under study & different Instrumental methods used.

Sr No	ANALYTICAL PROPERTY UNDER STUDY	INSTRUMENTAL METHODS USED FOR IT
1	Absorption of Radiation	X-ray, Ultra-Violet, Visible, Infra-Red Spectrometry, Nuclear Magnetic Resonance (NMR), electron spin resonance (ESR).

2	Emission of radiation	X-ray, UV (Ultra-Violet), Visible, IR (Infra-Red) spectroscopy, Fluorescence, Phosphorescence etc.
3	Scattering of radiation	Raman Spectroscopy, Turbidimetry, Nephelometry (scattering of light from cuvette).
4	Diffraction of radiation	X-ray, electron, neutron diffraction analysis.
5	Rotation of radiation	Polarimetry, circular dichroism.
6	Electrical Potential	Potentiometry, Chromo-potentiometry.
7	Electrical charge	Coulometry, dielectric measurements.
8	Electric current / Resistance	Polarography, Amperometry, Conductrometry, Volume resistivity.
9	Mass-charge ratio	Mass spectrometry.
10	Rate of reaction	Chemical kinetics.
11	Thermal Properties	Thermogravimetry (TG), Differential Thermal Analysis (DTA), Differential Scanning Calorimetry (DSC), Thermal Conductivity measurements
12	Radioactivity	Neutron Activation Analysis (NAA), Dosimetry.
13	Adsorption on a stationary phase & desorption	Gas Chromatography
14	Partition or distribution of sample in various solvents	Liquid Chromatography (LC), High Performance Liquid Chromatography (HPLC)

15	Surface Analysis	Electron Spectroscopy for Chemical analysis (ESCA)
16	Hydrogen ion concentration	pH-metry

- From all the different methods mentioned above, we are going to learn about:
  1) UV-Visible Spectroscopy,
  2) Infra-Red (IR) Spectroscopy and

ADVANTAGES OF INSTRUMENTAL METHODS OF ANALYSIS:-

All the Instrumental Methods have many advantages, which are listed below:

a) Instruments have very high sensitivity & samples with less concentrations (10^{-3} N) can be tested. In AAS samples with concentrations in ppm or ppb can be tested with high accuracy.
b) The results obtained are very accurate & can be reproducible.
c) The reliability of the Results is very high.
d) Large number of samples can be analyzed with equal sensitivity and high accuracy.
e) Estimations can be carried out on molecular, atomic or even sub-atomic levels.
f) Poisonous, Carcinogenic and Explosives can also be handled safely.
g) Samples which are Costly or scarce or which have Historic importance can also be analyzed using non-destructive testing methods like Neutron Activation Analysis (NAA) or X-ray powder diffraction methods. The sample remains unaffected after testing.
h) Instrumental methods are Fast comparable to Classical Methods. So, the results are obtained in less time.
i) These methods require very less amount of sample. For example in Gas Chromatography, about 10 μl of sample is needed.
j) Classical Methods require large number of reagents and involve frequent weighing, drying, filtration types of operations whereas, Instrumental methods very little time and no sample preparation is required.
k) Instrumental Methods can be fully Automatic and hence requires less human attention decreasing the personal errors.
l) Very slow or very fast reactions (for example: reactions like Radio-active decay) can be studied using Instrumental methods.

LIMITATIONS OF INSTRUMENTAL METHODS OF ANALYSIS :-

a) Frequent calibration and standardization of the Instruments is necessary,
b) Cost of Instruments is high, in some cases very high,
c) Specialized training is required for handling these Instruments,

d) Some Instruments occupy very large space,
e) For most of the Instruments, undisturbed Power Supply is necessary.

CRITERIA FOR SELECTION OF A METHOD :-

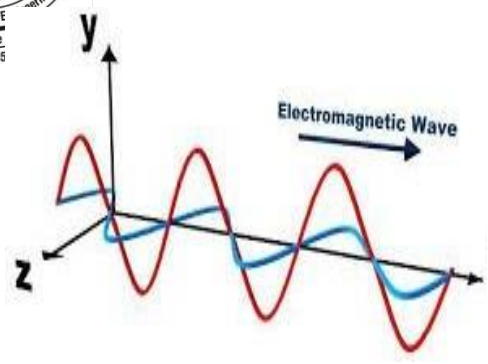
- When any sample comes to a Chemist, selection of proper method for Analysis of that sample is very important.
- Selection of a proper method is very important. There are many methods which are available, as seen on the previous page in the table given.
- Following factors should be taken into consideration before selecting the most appropriate method for the analysis of a given sample.
- These Factors are as under:-

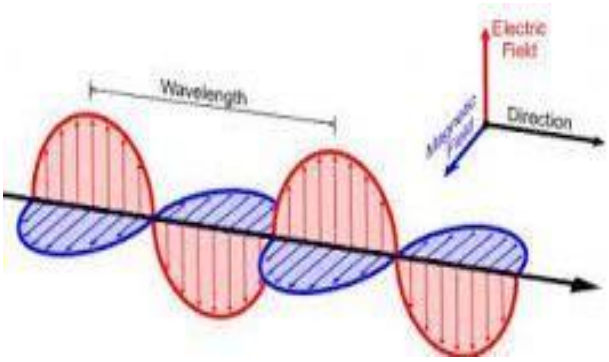
a) Quality of the sample available,
b) Cost of the sample,
c) Time available for Analysis,
d) Level of accuracy needed by the results,
e) Number of samples to be analyzed,
f) Types of impurities present in the sample and their effect on the analytical results,
g) Availability of the Instrument needed and the Expertise of the Analyst etc.

ELECTRO-MAGNETIC RADIATIONS AND ELECTRO-MAGNETIC SPECTRUM:-

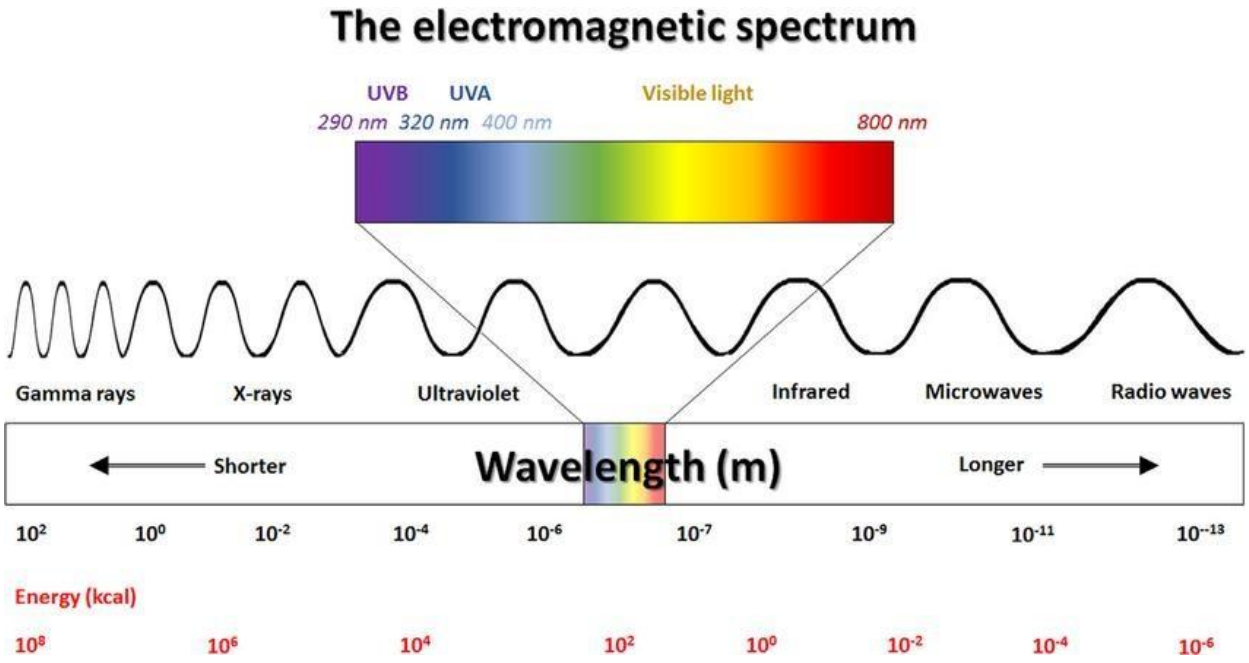
We receive Energy mainly from Sun. This Energy that we receive is in the form of Electro-Magnetic radiations. Electro-Magnetic radiations are the Transverse Waves travelling at very high speed and they are associated with Electric field as well as magnetic Field.

If a wave is imagined to be travelling along X-Axis (which is assumed in the plane of this paper) then Electric Field associated with it is along Y-Axis (which is also in the plane of paper) (XY plane) and the Magnetic Field associated with it, is along Z-Axis (XZ plane), which perpendicular to the plane of the paper.





- All the three, i) Direction of the Transmitting Wave, ii) Electric Field and iii) magnetic Field are perpendicular to each other.
- The Electro-Magnetic radiations can have the values of the wave-lengths ranging from 10^{-1} nm to 10^{8} nm.
- All Electromagnetic radiations travel with the velocity which is approximately = 3×10^{8} m/sec.
- They are manly originated from Changes involved in :- Nuclear Region of Atoms, Inner shell electrons of the Atoms, Ionization of toms or Molecules, Valance electrons, Vibrations of the different bonds present in the Molecule or ion, Spin Orientation of the Electrons or Nuclei etc.
- Electro-Magnetic Spectrum is Graphical representation of all these kinds of waves on one axis, in increasing order of their wavelengths.



ABSORPTION SPECTROSCOPY

IN TRODUCTION TO SPECTROSCOPY:- "In Spectroscopy, the sample containing molecules or ions is exposed to electromagnetic radiations. As a result of the interaction between the molecules or ions or atoms present in the sample, some part of radiations are Absorbed, some are scattered, some are transmitted, some of them are refracted and rest are Transmitted. These responses are recorded in the form of a graph (mainly Absorbed & Transmitted) which is called a Spectrum".

- Any Spectroscopy technique depending measurement of an Absorption Spectrum is called "Absorption Spectroscopy".

ORIGIN OR CAUSE OF ABSORPTION SPECTROSCOPY:-

In any given sample (the molecules, atoms or ions or bonds present in them) can assume a only certain discrete values, corresponding to various permitted energy levels for the molecules.

- When a beam of certain monochromatic radiations are made to fall on the sample, its molecules or atoms or ions or electrons in the bonds, gain energy from the quanta of electro-magnetic radiations and get excited. However, it should be remembered that the molecules or their parts can absorb only those quanta (photons) having radiations exactly equal to the energy difference between two permitted energy levels in the molecule.
- The wave-length of the radiations absorbed depends upon the molecular substance under the examination. In other words, a molecule of a given substance can absorb radiations of certain definite wave-lengths only.
- The radiant energy absorbed by a molecule can bring about following transitions mainly:-
  a) Electronic Transitions (This leads to Absorption in UV or Visible Region [d-d transitions]),
  b) Vibrational Transitions (of the polar covalent bonds, leads to Absorption in IR region),
  c) Rotational Transitions (this leads to Absorption in the Micro-waves Region).

UV – VISIBLE SPECTROSCOPY

INTRODUCTION TO UV & VISIBLE RADIATIONS:-

a) UV (Ultra- Violet) Radiations:- In general the electro-magnetic radiations having the wavelength in the range of 10 nm to 400 nm are called UV Radiations.

i) The radiations having wave-length in the range 10 to 200 nm are called "Vacuum UV".
ii) The radiations having wave-length in the range 200 to 400 nm are called "Near UV".

b) Visible radiations:- The electro-magnetic radiations having wavelength in the range of about 400 nm to 800 nm are called "Visible radiations"

c) The electronic transitions of different types of electrons in different types of MOs lead to absorption of radiations in UV-Visible region.

THE COMMON TERMS USED TO DESCRIB E DIFFERENT T YPES OF ELECTRONS IN UV- VISIBLE SPECTROSCOPY:-

A) "n"- ELECTRONS :- The non-bonded electron or electron pair present in the last shell or valance shell are called "n"- ELECTRONS. The electrons present in the valance shell & are non-bonded are these electrons. They are usually present in saturated compounds.

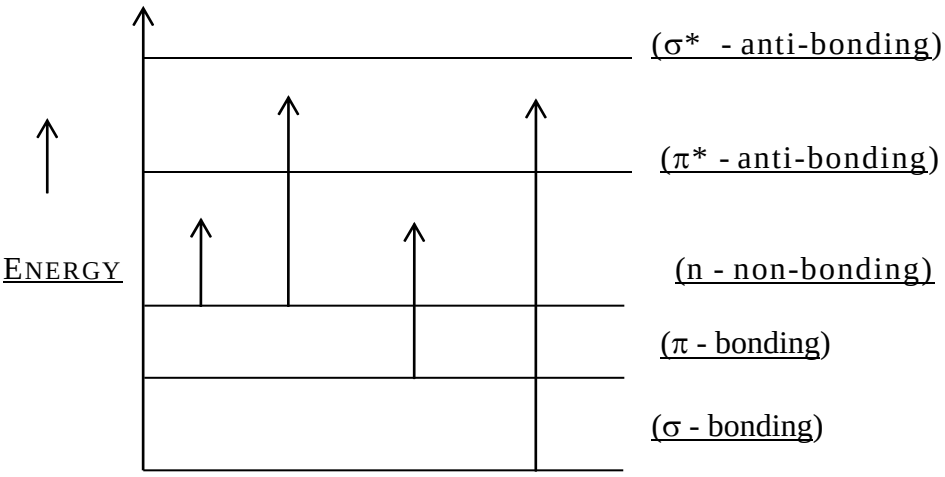
B) "σ - ELECTRONS":- All the electrons which are present in the "σ - MO" are called "σ - ELECTRONS". Or The electrons which are involved in the formation of " σ -bond are called " σ - ELECTRONS".

C) " π - ELECTRONS ":- All the electrons which are present in the " π - MO" are called " π - ELECTRONS". Or The electrons which are involved in the formation of " π -bond are called " π - ELECTRONS".

D) "σ* - ELECTRONS ":- All the electrons which are present in the "σ - MO" are called " σ - ELECTRONS". Or The electrons which are involved in the formation of " σ -bond are called "σ - ELECTRONS".

E) " π* - ELECTRONS ":- All the electrons which are present in the " π* - MO" are called " π* - ELECTRONS".

DIFFERENT TYPES OF ELECTRONIC T RANSITIONS & ENERGY ASSOCIATED WIT H THEM:-



ORDER OF ENERGY ASSOCIATED WITH THESE TRANSITIONS IS AS UNDER

$\sigma \rightarrow \sigma^* > \sigma \rightarrow \pi^* \sim \pi \rightarrow \sigma^* > \pi \rightarrow \pi^* \sim n \rightarrow \sigma^* > n \rightarrow \pi^*$

$\leftarrow$ Vacuum UV (10 to 200 nm) $\rightarrow$ $|\leftarrow$ Near UV & Visible (200 to 800 nm) $\rightarrow$

A) σ to σ* - transition:- In this, the electron in the bonding "σ" orbital is excited to corresponding anti-bonding "σ*" orbital.

- For example, in Methane (CH_4), all C—H bonds are "σ" bonds, so it can undergo "σ to σ* transition" only. It shows maximum absorption at 125 nm in Vacuum UV range. It is not observed in general UV-Visible Spectrum as the general UV-Visible shows absorptions in the range of about 200 to 800 nm.

B) n to σ* - transition:- In this, when UV rays fall on the Organic compound containing Hetero atoms like N, P, S, O, X (Halogens) etc, the 'n' (non-bonded) electron in them absorb UV rays and undergoes $n \rightarrow \sigma^*$ transition.

- Due to these Transitions, the radiations in the region 180 to 208 nm get absorbed.

C) π to π* or π to σ* transitions:- In unsaturated compounds double and/ or triple bonds is/are present. These unsaturated compounds contain 'π' electrons. When UV rays in the region 190 to 300 nm fall on them, π to π* or π to σ* transitions take place.

- electrons give Transitions in the range of 10 to 200 nm (Vacuum UV region).
- n & π electrons give Transitions in the range of 200 to 800 nm (Near UV and Visible region).
- The Energy associated with these transitions is given above diagram.

IT SHOULD BE REMEMBERED THAT, IF HIGHER ENERGY IS NECESSARY FOR THE TRANSITION, THE RADIATIONS OF SM ALLER WAVE- LENGTH ARE ABSORBED & VISA-VERSE.

DIFFERENT TERMS USED FOR ABSORPTION IN UV – VISIBLE SPECTROSCOPY:-

- To explain the changes which are observed in the Wave-length of Absorption or Intensity of Absorption peaks in UV-Visible Spectroscopy are as Under:-

A) CHROMOPHORE:- The molecule as a whole does not absorb radiations , but the part of the molecule or ion which is responsible for Absorption in the UV-Visible region is called "Chromophore".

B) BATHOCHROMIC SHIFT (OR) RED SHIFT :- When the value of λ_{max} increases, (i.e. Absorption takes place at longer wave-length) than its normal value, it is called Bathochromic Shift or Red Shift. It is called Red Shift as Absorption shifts towards Red region.

- This is usually caused by substitution or change in the solvent.
- Example:- p-nitro phenol (λ_{max} = 255 nm) shows Red Shift in alkaline medium (λ_{max} = 265 nm).

C) HYPSO-CHROMIC SHIFT (OR) BLUE SHIFT:- When the value of λ_{max} decreases, (i.e. Absorption takes place at shorter wave-length) than its normal value, it is called Hypsochromic Shift or Blue Shift. It is called Blue Shift as Absorption shifts towards Blue region.

- This is also caused by substitution or change in the solvent.
- Example:- Aniline (λ_{max} = 230 nm) shows Blue Shift in acidic medium (λ_{max} = 203 nm).

D) HYPERCHROMIC SHIFT :- In this case, Intensity of Absorption increases. (Abs intensity ↑).

E) HYPOCHROMIC SHIFT:- In this case, Intensity of Absorption decreases. (Abs intensity ↓).

BEER AND LAMBERT' S LAW

Transmittance (T) :- It is a measure of how much portion of the incident light passes through the transparent medium.

- It can be Defined as – "The ratio of intensity of the Transmitted light to the intensity of the incident light". Mathematically, ,

  where It = Intensity of Transmitted Light & Io = Intensity of the Incident light.

ABSORBANCE (A) :- The reciprocal of Transmittance is called Absorbance (A).

- It can be defined as - "The ratio of intensity of the Absorbed light to the intensity of the incident light". Mathematically, ,

  where It = Intensity of Transmitted Light & Io = Intensity of the Incident light.

Both, Transmittance and Absorbance are related to each other mathematically as under:-

$$A = \log \quad - \log T$$

LAMBERT'S LAW:- When electro- magnetic radiation is passed through a transparent medium, it can be (in parts) Transmitted, Absorbed, Refracted or Scattered, respectively.

$$I_0 = I_t + I_a + I_r + I_s \text{ ---------- (a)}$$

Where, I_0 = Intensity of the Incident radiation, I_t = Intensity of the Transmitted radiation,

I_a = Intensity of the Absorbed radiation, I_r = Intensity of the Refracted radiation

and I_s = Intensity of the Scattered radiation.

- The values of I_r & I_s being very small are neglected.
- The above equation now can be written as: $I_0 = I_t + I_a$ ----------(b)
- LAMBERT (1760) studied the decrease in the intensity of the incident radiations (I_0) when passed through the transparent medium & I_t. where, I_t = intensity of light after passing through the transparent medium.
- STATEMENT:- "When a beam of mono-chromatic Light is allowed to pass through a transparent medium (solution), the Rate of Decrease in the Intensity of the Incident Radiations (I_0) is directly proportional to the thickness (or) the path-length of the transparent medium".

Mathematically, — □(or) —

i.e. — ----------(c)

where, (-) dI_0 = small decrease in the in the intensity of light.

x = thickness of the transparent medium,

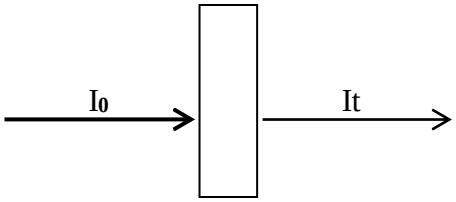
I_t = intensity of transmitted light and

K_1 = Proportionality constant.

- The proportionality constant "K" is called "*Absorption co-efficient*" & its value depends upon the absorbing medium.

If I_0 is the intensity of light before entering transparent medium, when (x = 0), then the Intensity of light I_t after passing through any finite thickness 'x' of the medium can be obtained by integrating the equation (c) within the limits x = 0 to x = x and I_0 and I_t.

- Integrating above equation (c), putting ,
  I = I_0 & when, x = 0, we get,



—

- Therefore,

→| " x" |←

i.e.

So, ln — Therefore, —

- - - - - - - - - - (d) Or - - - - - - - - - - - - (e)

Where, —

Thus, Lambart's Law can also be stated as:- "As the thickness of the absorbing medium increases, the intensity of the transmitted light (I_t) decreases exponentially, for a fixed concentration of the absorbing medium (solution)".

'BEER'S LAW:- When electro- magnetic radiation is passed through a transparent medium, it can be (in parts) Transmitted, Absorbed, Refracted or Scattered, respectively.

$$I_0 = I_t + I_a + I_r + I_s \text{ ---------- (a)}$$

Where, I_0 = Intensity of the Incident radiation, I_t = Intensity of the Transmitted radiation,

I_a = Intensity of the Absorbed radiation, I_r = Intensity of the Refracted radiation

and I_s = Intensity of the Scattered radiation.

- The values of I_r & I_s being very small are neglected.
- The above equation now can be written as: $I_0 = I_t + I_a$ ----------(b)

- BEER (1760) studied the decrease in the intensity of the incident radiations (I_0) when passed through the transparent medium & I_t. where, I_t = intensity of light after passing through the transparent medium.
- STATEMENT:- "When a beam of mono-chromatic Light is allowed to pass through a transparent medium (solution) having a certain concentration, the Rate of Decrease in the Intensity of the Incident Radiations (I_0) is directly proportional to the concentration of the transparent medium (solution)".

Mathematically, — (or) —

So, —– ----------(c)

where, (-) dI_0 = small decrease in the in the intensity of light.

C = Concentration of the transparent medium (solution) in Moles/Lit i.e. Molarity,

I_t = intensity of transmitted light and

K_2 = Proportionality constant.

- If I_0 is the intensity of light before entering transparent medium, when (C = 0), then the Intensity of light I_t after passing through any fixed concentration 'C' of the medium can be obtained by integrating the equation (c) within the limits C = 0 to C = C and I_0 and I_t.
- Integrating above equation (c), putting ,

I = I_0 & when, x = 0, we get, —–

- Therefore, i.e.
- So, ln – Therefore, –
- Rearranging,
- Thus, Beer's Law can also be stated as:- "As the concentration of the absorbing medium (solution) increases, the intensity of the transmitted light (I_t) decreases exponentially, for a fixed thickness of the absorbing medium (solution)".

LAMBART – BEER'S LAW :- Later on Scientists combined both the Lambart's Law & Beer's Law, which is now called Lambart – Beer's Law.

- STATEMENT:- When a beam of mono-chromatic light is passed through a transparent medium (solution), the Rate of Decrease in the Intensity of the Incident radiation is directly proportional to the path length (x) as well as concentration (C) of the solution".
- Mathematically, Lambart's Law is:- —
- Similarly, Beer's Law is :- —
- Combining the two we get:- —
- So, — i.e. — - - - - - - - - - - - - -(i)
- Integrating within the limits, we get,

—

,

So,

i.e. —

So, —

- Therefore, — i.e. - - - -(ii)

In above equation both 2.303 & K_3 are constant. So can be written as Under:-

—

i.e. ----------(iii)

(where, A = Absorbance, but log (I_t/I_0) = Transmittance.)

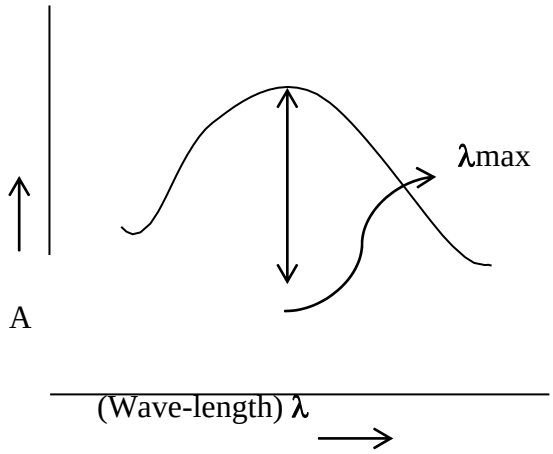
—

Where, A = Absorbance which is also called, "D" = optical density or Opticity or E = Extention.

MOLAR EXTINCTION CO-EFFICIENT:- *It can be defined as, "Extinction coefficient (E), when the thickness of the transparent medium is 1 cm & the concentration of the solution is 1M".*

EXPERIMENT :- TO VERIFY BEER'S LAW USING COLOURIMETER:- This experiment is carried out in two parts. In Part 1, we find out λmax for a given coloured solution.

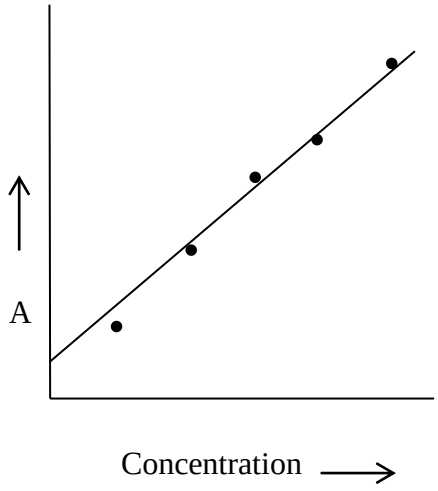
- Each coloured solution shows maximum Absorption of a certain fixed wavelength of light.
- IN PART 1:- We find out λmax for a given coloured solution with the help of Colourimeter.
- For this, we take the given coloured solution having a certain fixed (dilute) concentration in a cuvette and pass a monochromatic beam of light through it. With the help of colourimeter, we find its absorption at different λ values. We do so for increasing wave-lengths in visible region.
- By observing the values of Absorption at different wave-lengths, we can see at what λ value, the Absorption is maximum.



- The graph/plot of wavelength (λ) on X – axis and Absorption (A) on Y-axis, is drawn in such a way that, it is a smooth curve as shown in the diagram.
- From graph, λmax for a given coloured solution is found out and this λmax is used for all the Observations (Readings) of Part 2.

- IN PART 2:- We prepare solutions of different (dilute) concentrations of the substance under the experiment. For example we prepare 0.01M, 0.02M, 0.03M, 0.04 M 0.05M etc.
- Then find absorption for all these solutions at λmax obtained from Part 1.
- According to Beer's Law, "When a beam of mono-chromatic Light is allowed to pass through a transparent medium (solution) having a certain concentration, the Rate of Decrease in the

Intensity of the Incident Radiations (I_0) is directly proportional to the concentration of the transparent medium (solution)". In other words, Absorption goes on increasing with the increase in the concentration of the solution or Absorption is directly proportional to the concentration of the solution. Mathematically, A C (where, A = Absorption & C = concentration of the solution).



- The graph/plot of concentration of the solution on X – axis and Absorption (A) on Y-axis, is drawn, we get a straight line passing through Origin.
- From graph, λmax for a given coloured solution is found out and this λmax is used for all the Observations (Readings) of Part 2.
- It is possible to find concentration of a solution of the same substance, if it is not known.

DEVIATIONS FROM BEER' S LAW:- According to Beer's Law, a straight line should be obtained, when a graph of Absorption (A) (on Y-axis) is drawn against different Concentrations (C) of the same solution (on X-axis). (As shown in above diagram)

- But, many times, there is a deviation of the graph and is non-linear. The graph sometimes show the (+ve) Positive deviation or sometimes (-ve) Negative deviation from the graph with the increase in concentration of the solution. At very high concentrations the Beer's Law is not applicable.
- The Deviation from Beer's Law is mainly due to following factors:-

a) Interaction between Solute and Solvent molecules or
b) Instrumental error.

- FOLLOWING ARE THE MAIN FACTORS FOR THE DEVIA TION OF BEER'S LAW:-

i. The Law fails when the coloured substances undergo, Ionization, Association or Dissociation of the solute molecules.
ii. When the pH value of a solution changes due to dilution.
iii. When colour of the solution changes due to dilution.

For example, di-chromate ions which are Orange in colour, change their colour to Yellow when the dichromate solution is diluted in Acidic medium.

$$\underset{\text{(Orange)}}{Cr_2O_7^{2-}} + H_2O \longleftrightarrow \underset{\text{(Yellow)}}{CrO_4^{2-}} + 2H^+.$$

iv. Presence of impurities which are fluorescent or scatter light or absorb the given wave-length also brings about deviation in the Beer's law.

v. If the slit width of Colourimeter is not proper, deviations are expected. Because of improper width radiations, having undesirable wave-length fall on the on the sample.

vi. With the increase in the concentration of the solution, the Refractive Index of the goes on increasing, this also causes the deviation from the normal behavior.

vii. Beer's Law is not applicable for suspensions.

- FOLLOWING ARE THE MAIN FACTORS/CAUSES FOR THE DEVIATION FROM BEER' S LAW:-

a) Environmental factors like Temperature, Pressure nature of Solvent etc,
b) Instrumental Errors such as: Stray Radiations, Stability of the Radiation Source, wave-length selector, slit control, reliability and the quality of the optical parts and the instrument,
c) Chemical deviations such as dissociation, association of the solute molecules, interaction between solute & solvent molecules, equilibrium position, change in the pH value, concentration of the solution etc,
d) Refractive index of the sample,
e) Non-monochromatic radiations etc.

SPECTROPHOTOMETERS

There are types of spectrophotometers.

- They are:- **A)** UV-Visible Spectrophotometers,
  B) Visible Spectrophotometer (Colourimeter),
  C) Infra-Red Spectrophotometer and

- UV-Visible Spectrophotometer is an instrument which shows absorption of electro-magnetic radiations usually in the range of 200 to 800 nm, (Vacuum UV λ = 10 to 200 nm and Near UV, λ = 200 to 400 nm) and In Visible range (λ = 400 to 800 nm).
- Colourimeter (Visible Spectrophotometer) shows absorption in the range of 400 to 800 nm.

- Visible colours absorb in different regions. Following list gives you the idea of that:-

ULTRA – VIOLET RADIATIONS (UV)

UV Radiations	Absorb in the region
Vacuum UV	10 to 200 nm
Near UV	400 to 800 nm

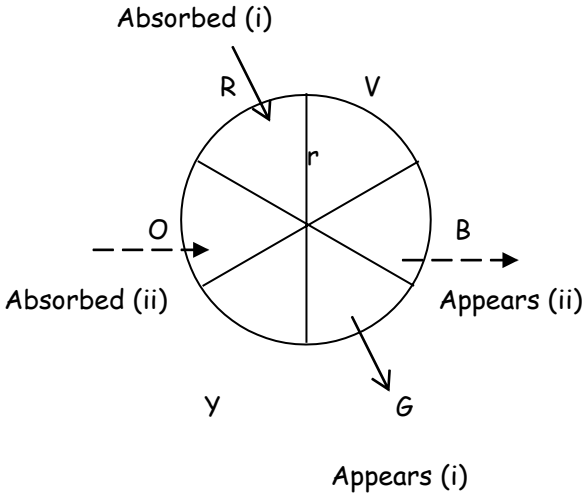
IN FRA-RED RADIATIONS (IR)

IR Radiations	Absorb in the region
Near IR	800 to 2500 nm
Fundamental IR	2500 nm 25000 nm
Far IR	25000 to 10^5 nm

VISIBLE RADIATIONS

Visible Colours	Absorb in the region
Violet	400 to 420 nm
Indigo	420 to 440 nm
Blue	440 to 490 nm
Green	490 to 570 nm
Yellow	570 to 585 nm
Orange	585 to 620 nm
Red	620 to 780 nm

ABSORPTION AND APPEARANCE O F A COLOU R



- The diagram given here, gives us the idea of the colour absorbed by a substance and how it appears to our eyes.
- **THE DIAGRAM GIVEN HERE IS NOT AT ALL A RULE OR A LAW, BUT IS AN EMPIRICAL OBSERVATION ONLY.**
- IT IS FOUND TO TRUE IN MANY CASES AND WRONG IN SOME CASES. SO DON' T TREAT IT AS A RULE OR A LAW.

- For example, if a substance absorbs in the region of Red (R) colour, that substance appears to be Greenish (G) coloured to our eyes.

INSTRUMENTATION OF UV- VISIBLE SPECTROMETRY

- PRINCIPLE:- In both UV-Visible spectrophotometers and Visible Spectrophotometers (Colourimeter), it is found that, the absorption in this region occurs due to excitation of electrons from one MO to other MO.
- UV radiations have smaller value of λ and therefore are more energetic than Visible radiations having greater value of the λ.
- So, it can be said that, the Energy associated with quanta (photons) of UV radiations is higher than Energy associated with quanta (Photons) of Visible radiations.

a) Energy required for the excitation of electrons from $\sigma_{MO} \rightarrow \sigma^*_{MO}$ & $n_{MO} \rightarrow \sigma^*_{MO}$ are very high. UV radiations can provide higher energy. Therefore, during these transitions, Radiations in UV region are absorbed & the Absorption is seen in UV region.
For example,

Absorption due to $\sigma_{MO} \rightarrow \sigma^*_{MO}$ transition are seen in Vacuum UV Range at 125 nm (in CH_4),

Absorption due to $n_{MO} \rightarrow \sigma^*_{MO}$ transitions are seen in Vacuum UV range at 150 to 200 nm.

- Similarly, less Energy is needed for the excitation of electrons from $n_{MO} \rightarrow \pi^*_{MO}$ & $\pi_{MO} \rightarrow \pi^*_{MO}$. So, they are absorbed in Near UV (200 to 400 nm) and Visible Range (400 to 800 nm).

INSTRUMENTATION OF UV – VISIBLE SPECTROMETER:-

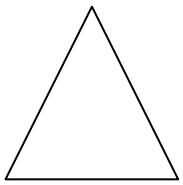
- PRINCIPLE:- Absorption is seen in UV-Visible region mainly due to excitation of electrons from
  i. $\sigma_{MO} \rightarrow \sigma^*_{MO}$ (Vacuum UV),
  ii. $n_{MO} \rightarrow \sigma^*_{MO}$ (Near UV),
  iii. $n_{MO} \rightarrow \pi^*_{MO}$ (Near UV) &
  iv. $\pi_{MO} \rightarrow \pi^*_{MO}$ (Visible).

- UV-Visible Spectrophotometers mainly consists of following parts:-

a) Radiation Source,
b) Monochromator and Filter Wheel,
c) (*) Chopper
d) Sample Compartment/s,
e) (*) Chopper
f) Signal Amplifier (or) Detector and
g) Read Out Device.

- RADIATION SOURCE:- It produces light in the desired wave-length (λ) in the range of 200 to 800 nm.
- For UV region, Hydrogen (H_2) or Duterium (D_2) discharge lamp is used. This produces radiations in the range of 160 to 360 nm.
- For UV and Visible Region, Incandescent filament lamps of Tungsten or Tungsten halogen lamps are used. These give radiations in the range of 200 to 1000 nm.

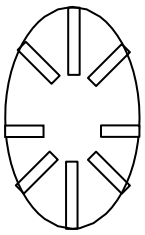
MONOCHROMATOR & FILTER WHEEL:-



(Monochromator)

- In Spectroscopy, a good Monochromator should provide much narrower band of wave-length (λ),preferably, Mono-chromatic light.
- In this, Quartz Prisms are used for UV Region,
- A Glass Prism is used in Visible Region,
- Monochromator disperses the light falling on it and radiations of desired wave-length (λ) fall on the sample, through the Filter wheel.

[Alkali Halide Prisms are used in IR Region.]



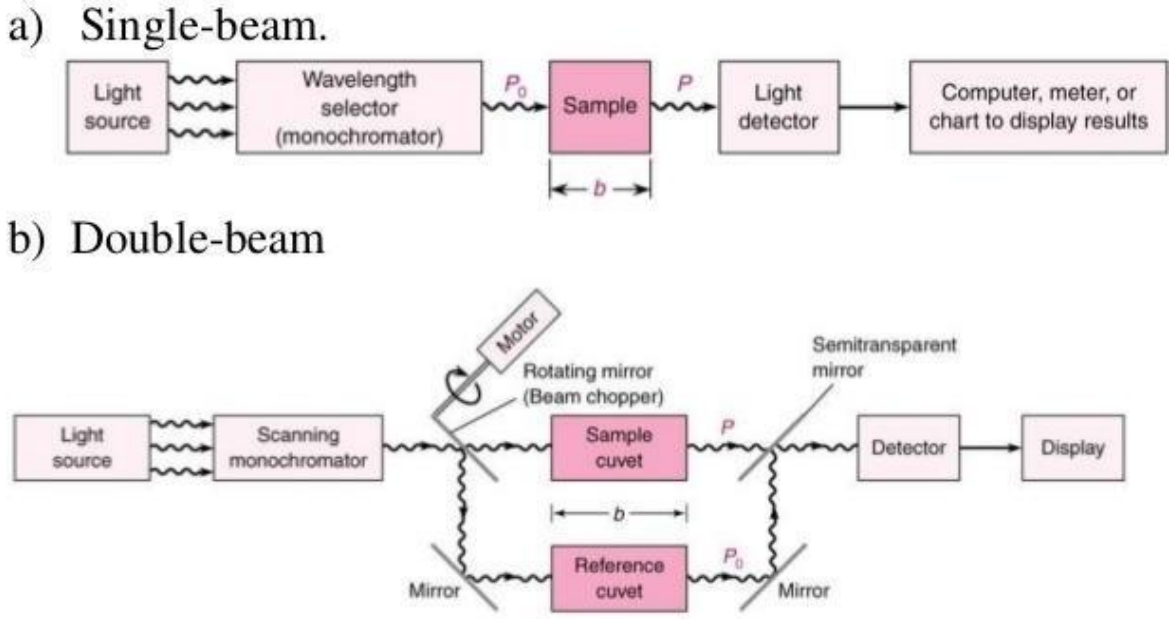
(Filter wheel)

- In Spectroscopy, it is necessary that, a very small band-width of light (preferably, Mono-chromatic light) should fall on the sample. As a result, accurate measurement of the Absorbed radiation can be taken.
- The different slits present in the Filter wheel allows the light band of desired λ, falls on the sample. (± 20 nm) band width falls on the sample.
- A Filter wheel is not considered to be a real Monochromator.
- A proper slit is chosen for the desired value of wave-length (λ_{max}).

- Other types (than Filter Wheels) Filters are available. They are mainly :- 1) Wedge Type Filters, 2) Multilayered Filters, 3) Long wave pass Filters, 4) Interference Filters (also called Fabry-Perot Filters etc.

CHOPPER*:- In a Single Beam Spectrophotometers, Choppers are not present. Choppers are used only in Double Beam Spectrophotometers.

- In a double beam spectrophotometer, the monochromatic light coming out from Monochromator & Filter Wheel, is divided into two identical/equal beams, with the help of corner mirrors or rotating sector mirrors.
- One beam passes through the reference solution (usually the solvent in which coloured substance is dissolved) and other beam passes through the sample holder.



SAMPLE HOLDERS:- In a Single beam spectrophotometer, only beam of monochromatic light passes, therefore there is only one slot for sample holder.

- In a Double beam spectrophotometer, two beams of monochromatic light passes, therefore there are two slot for sample holder. In one slot the reference sample (usually the solvent) is kept and in other slot, the sample being analyzed (Analyte) is kept.
- The Sample Holder usually look like mini-test tube and is called a "Cuvette".

- In Visible Region, ordinary glass cuvettes are used.
- In UV region, Quartz cuvettes are used.

[In IR Region, Alkali Halide (NaCl, KCl, NaBr, KBr) Crystals or Teflon, Polyehtylene also can be used.]

CHOPPER*:- As mentioned earlier, Choppers are not present in a Single beam spectrometer but are present I a double beam Spectrophotometer.

- In a double beam spectrophotometer, one beam passes through the Reference material (solvent) and major portion gets Transmitted. While, other beam passes through the sample being analyzed (Analyte) & some portion (depending upon the conc. & width) gets absorbed.
- The double beam instrument consists of a rotating sector mirror & corner mirrors. It cuts off any one beam of the light and allows to pass through it alternately.

SIGNAL AMPLIFIER S (OR) DETECTORS:- The Transmitted which come out of the Chopper (if present), need enhancement or Amplification in order to make it recognizable by the Read Out Device (or) Recorder.

- Different types of Signal Amplifiers (or) Detectors are used:- They are,

i) Photo-Voltaic Cells (which can detect only one element),
ii) Solid-state Photo diodes,
iii) Photo-emissive tubes,
iv) Multiple element detectors (Solid arry detectors) etc.

- This Signal Amplifier or a detector converts electro-magnetic radiations into flow of electrons which goes into Read Out Device or Recorder.

READ OUT DEVICE (OR) RECORDER:- In this, the amplified signal passes into a dynode voltage regulator. The Dynode Voltage is varied to maintain a constant Reference Signal.

- The Signals obtained from Sample Amplifiers are compared with the Reference Signals, they are compared and is output is displayed on the Read Out Device (in the form of Absorbance or Transmittance) or are Recorded in the form of a graph.

APPLICATIONS OF UV-VISIBLE SPECTROSCOPY:-

Structures of Organic compounds which are colourless or pale yellow or greenish coloured, can be determined using UV Spectroscopy.

ii. Transition metal salts which are coloured (such as : $CuSO_4$ (blue), $KMnO_4$ (purple), $FeSO_4$ (blue-green) etc can be estimated Qualitatively as well as Quantitatively by Visible Spectrophotometry or Colourimetry.

iii. It is helpful in differentiating between para-substituted or ortho & meta-substitute isomers of a compound.

iv. It is very useful for predicting wave-length of maximum absorption (λ max).

v. It is also useful in differentiating between cis- and trans- isomers of a compound.

vi. It is also possible to predict the presence of a particular group in an Organic compound. For example: Organic Nitro compounds show strong Absorption at 210 nm. Similarly, Organic compounds containing Cyanide ($-C \equiv N$) group show strong absorption at 160 nm.

vii. It is possible to find out the concentration of a coloured solution by plotting the Graph of Absorption against concentration of that substance using λ max.

viii. With its help it is also possible to carry out analysis of mixtures of two different substance if their absorption is takes place in separate regions.

IN FRA–RED (IR) SPECTROSCOPY

IN TRODUCTION:- Radiations in the range of 780 nm to 25000 to 10^6 nm are called Infra Red Rays. They are mainly classified into 3 major part. They are :-

a) NEAR IR RADIATIONS :- They absorb electro-magnetic radiations in the range of 800 to 2500 nm. (0.78 μm to 1 μm).

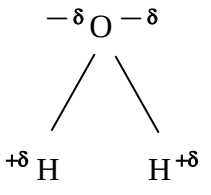
b) FUNDAMENTAL IR RADIATIONS:- They absorb electro-magnetic radiations in the range of 2500 nm 25000 nm. (1μm to 25 μm).

c) FAR IR RADIATIONS:- They absorb electro-magnetic radiations in the range of 25000 nm 10^6 nm. (25 μm to 1 mm).

PRINCIPLE OF IR SPECTROMETRY:- In IR region wavelength of the radiations is higher than in UV-Visible range. Energy of photons (quanta) of IR is therefore less than that of UV-Visible radiations.

- As a result of lesser Energy possessed by them (IR) than (UV-Visible), they are not able to excite electrons.

- IR radiations are usually absorbed by Polar covalent bond having certain non-zero dipole moment. (Dipole moment is equal to the product of charge separation and bond length.)
- As a result, a water molecule has large value of dipole moment.

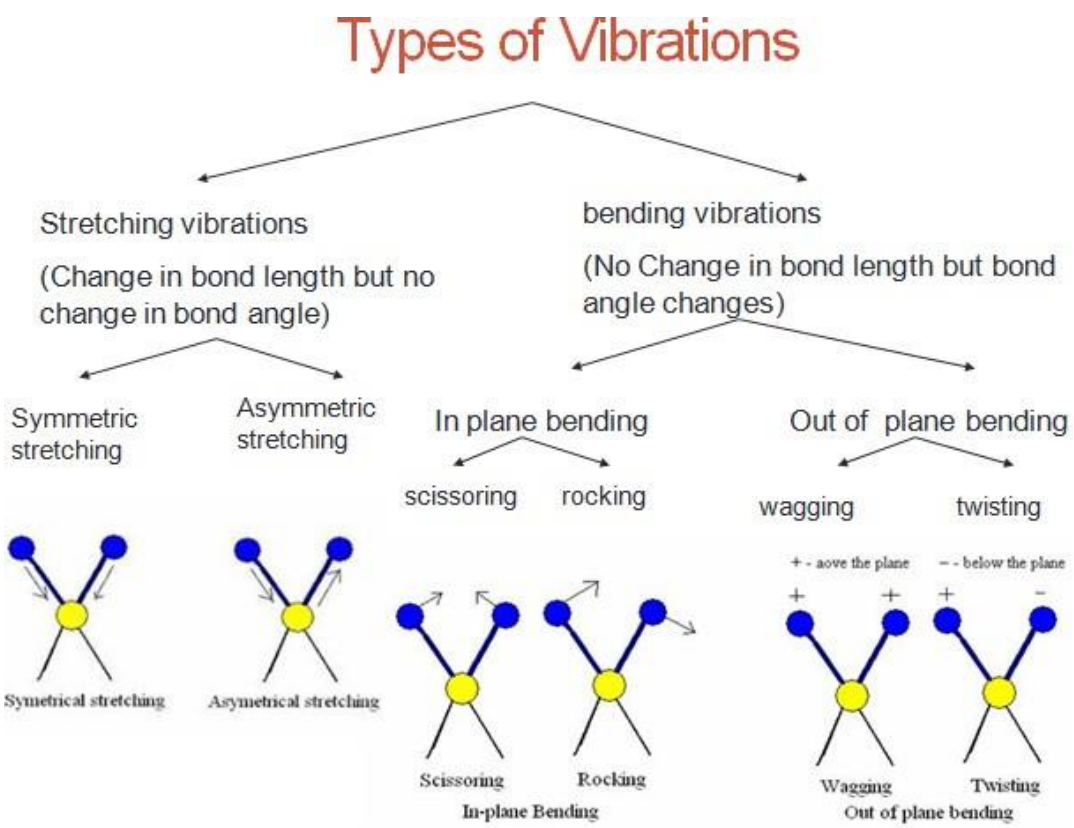


- For example in a water molecule there are two O – H covalent bonds & each O – H bond is a polar bond, in which is "O" end of the bond is partially negatively charged & "H" end of the bond is partially positively charged.
- This is because; "O" atom has higher electronegativity than "H" atom.

- Because of dipole moment possessed by bonds present in water, its bonds are continuously vibrating. As a result, it sets up an electric field of its own around itself.
- If, such a Polar molecule interacts with the IR radiations, the electric field set up by Polar molecules (like water molecules in this case) interact with the field set by IR radiations.
- If their frequencies match with each other, the polar molecules absorb energy from the IR radiations which results into increase in the amplitude & the rate of vibrations.
- These IR radiations which get absorbed get recorded during IR Spectroscopy.
- In general, a molecule with "N" number of atoms in it, has (3N – 6) number of modes of Normal Vibrations. But, if a molecule is linear (like CO_2), it shows (3N – 5) number of vibrations. This is because it does not show rotation around its bond-axis.
- For example, H_2O molecule (in which no of atoms are 3), show (3n – 6) = (9 – 6) = 3 types of vibrations. They are:- i) Symmetric stretching, ii) Asymmetric Stretching & iii) Scissoring.

DIFFERENT FUNDAMENTAL MODES OF VIBRATIONS:- There are mainly 2 types of fundamental vibrations of the bonds.

- They are :- a) Stretching Vibrations and b) Bending Vibrations.



A) **STRETCHING VIBRATIONS** :- In this type, the bonded atoms move either towards each other or move away from each other. In other words, their bond length either decreases or increases. During this process, the atoms remain on the Bond-axis only and their Bond Angle also remains same. It involves two types of Stretching as given under.

Stretching Vibrations are of 2 types, a) Symmetric Stretching and b) Asymmetric Stretching.

SYMMETRIC STRETCHING VIBRATIONS:-

- Due to this, Dipole moment of the molecule does not change. Therefore such a vibration does not absorb Energy in the IR region.

ASYMMETRIC STRETCHING VIBRATIONS:- In this type, one bond length increases while other decreases simultaneously. This process takes place alternately with two bonds simultaneously. The atoms remain on the Bond-axis only and their Bond Angle also remains same.

- During asymmetric stretching, there is a change in the dipole moment of the molecule & so it shows absorption in the IR region.

B) BENDING VIBRATIONS:- In this type, the position of the atoms deviates from the bond-axis. There are mainly 4 types of Bending Vibrations. They are :-

a) Scissoring, b) Rocking, c) Wagging d) Twisting.

a) SCISSORING:- During this, the bond angle decreases and then increases alternately. This type of change in bond angle continuously takes place & a sort of simple harmonic motion (SHM) is established between the bonds. The Bond length remains same during Scissoring.

- In other words, the two bonds which attached to a central atom move towards and away from each other. All the atoms remain in the same plane during.
- If this brings about the change in the bond polarity of the molecule it absorption in IR region.

b) ROCKING:- It involves, bending of the bonds in the same plane as that of molecule. Both the bonds which are attached to the central atom bend in same direction at a time and then in opposite direction next time. This goes on & a kind of SHM takes place here too.

- In Rocking also, all the atoms always remain in the same plane.

c) WAGGING:- In this type of vibration, the bonds attached to the central atom, move towards and away from the plane of the molecules. So, this is an "out of phase" type of bending vibration.

d) TWISTING:- The structural unit rotates about the bond which is joined to the rest of the molecule. (So, such vibration might not show absorption in IR Region but it might show absorption in Micro-waves region.)

- It is not necessary that each type of Fundamental Vibration of the bonds, show absorption in IR Region.
- Once again, it should be noted that, if a given vibration is changing the dipole moment of the molecule, then only, it will show absorption in the IR Region.

Examples of UV spectra:

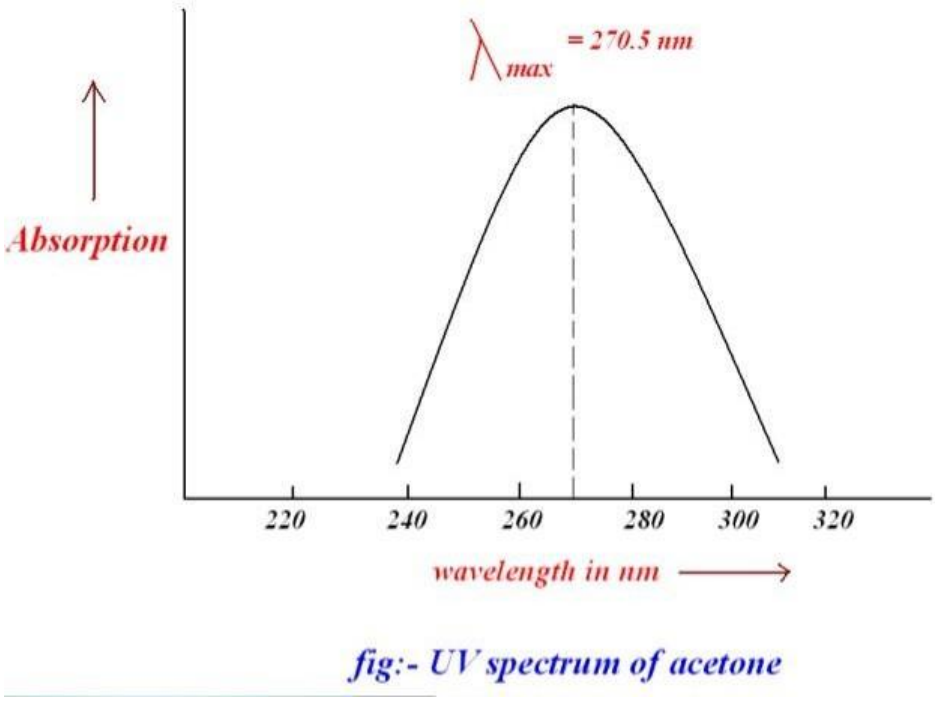


fig:- UV spectrum of acetone

INSTRUMENTATION OF IN FRA–RED (IR) SPECTROMETRY

INSTRUMENTATION OF IN FRA RED (IR) SPECTROMETER:-

- PRINCIPLE:- IR radiations have lesser energy involved (compared to UV-Visible) with their photons (quanta). So, IR radiations cannot excite electrons, but, the bonds present in the molecules vibrate in different ways due to which the dipole moment of the non-polar molecules changes. Whenever the dipole moment of the molecules change, they absorb in the IR Region.
- The most frequently used region of IR in structural identification is Mid-IR region having wave-length in the Mid IR region (2500 nm to 25000 nm).
- In principle, instrumentation in IR Spectrophotometers is similar to UV-Visible spectrophotometers. The main parts remain same but there are internal changes in the materials used in those parts.

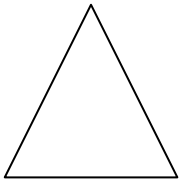
- IR Spectrophotometers mainly consists of following parts:-

a) Radiation Source, b) Monochromator and Filter Wheel,
c) (*) Chopper d) Sample Compartment/s,
e) (*) Chopper f) Signal Amplifier (or) Detector and
g) Read Out Device.

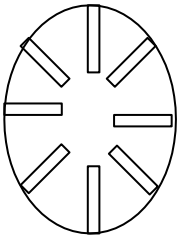
A) RADIATION SOURCE:- It produces light in the desired wave-length (λ) in the range of 2500 nm to 25000 nm. For IR region, the radiation source used are one of the following: **i)** Nernst's Glower, **ii)** Glober, **iii)** Nichrome coil etc.

- *Nernst's Glower consists of a mixture of rare earth oxides ($ZrO_2 + Y_2O_3$) in hollow rods of about 1 to 3 mm in diameter and about 2 to 5 cm long. When it is heated to about 1500^0c, it Glows and emits radiations in the Mid IR Region having wave-length from 10^3 to 10^4 nm. (1 to 10 μm).
- This is the brightest source of IR radiations. Proper insulation is needed to around the source (which is heated to about 1500^0c) to stop the spreading of the heat to other parts of Spectrophotometer.

b) MONOCHROMATOR & FILTER WHEEL:-



(Monochromator)



(Filter wheel)

- In any Spectroscopy, a good Monochromator should provide much narrower band of wave-length (λ),preferably, Mono-chromatic light.
- In this, Quartz Prisms are used for Near IR Region,
- Prisms made up of Alkali Halides like NaCl, NaBr, KCl, Kbr etc are used for Mid IR Region,

- The prisms made up of Teflon, Polyethylene also can be used.
- The samples in IR spectroscopy are in the form of solid tablets or pellets. (In UV-Visible solutions of the samples are used.)
- Monochromator disperses the light falling on it and radiations of desired wave-length (λ) fall on the sample, through the Filter wheel.

- In Spectroscopy, it is necessary that, a very small band-width of light (preferably, Mono-chromatic light) should fall on the sample. As a result, accurate measurement of the Absorbed radiation can be taken.
- The different slits present in the Filter wheel allows the light band of desired λ, falls on the sample. ($\pm$ 20 nm) band width falls on the sample.
- A Filter wheel is not considered to be a real Monochromator.
- A proper slit is chosen for the desired value of wave-length (λ_{max}).
- Other types (than Filter Wheels) Filters are available. They are mainly:- 1) Wedge Type Filters, 2) Multilayered Filters, 3) Long wave pass Filters, 4) Interference Filters (also called Fabry-Perot Filters etc.

c) CHOPPER*:- In a Single Beam Spectrophotometers, Choppers are not present. Choppers are used only in Double Beam Spectrophotometers.

- In a double beam spectrophotometer, the monochromatic light coming out from Monochromator & Filter Wheel, is divided into two identical/equal beams, with the help of corner mirrors or rotating sector mirrors.
- One beam passes through the reference solution (usually the solvent in which coloured substance is dissolved) and other beam passes through the sample holder.

❖ (*) FOR THE DIAGRAMS OF SINGLE BEAM IR SPECTROMETER) AND DOUBLE BEAM SPECTROPHOTOMETER REFER THE DIAGRAMS OF UV-VISIBLE SPECTROPHOTOMETERS.

d) SAMPLE HOLDERS:- In a Single beam spectrophotometer, only beam of monochromatic light passes, therefore there is only one slot for sample holder.

- In a Double beam spectrophotometer, two beams of monochromatic light passes, therefore there are two slot for sample holder. In one slot the reference sample in the form of solid tablet or palette is kept and in other slot, the sample being analyzed (Analyte) is kept.
- The Sample Holder usually look like mini-test tube and is called a "Cuvette".
- In IR Region, Alkali Halide (NaCl, KCl, NaBr, KBr) Crystals or Teflon, Polyehtylene are used.

e) CHOPPER*:- As mentioned earlier, Choppers are not present in a Single beam spectrometer but are present in a double beam Spectrophotometer.

- In a double beam spectrophotometer, one beam passes through the Reference material (solvent) and major portion gets Transmitted. While, other beam passes through the sample being analyzed (Analyte) & some portion (depending upon the conc. & width) gets absorbed.
- The double beam instrument consists of a rotating sector mirror & corner mirrors. It cuts off one beam of the light and allows to pass through it alternately.

f) SIGNAL AMPLIFIERS (OR) DETECTORS:- The Transmitted which come out of the Chopper (if present), need enhancement or Amplification in order to make it recognizable by the Read Out Device (or) Recorder.

- Detectors are used for Mid IR region are mainly are two types:

i) Thermal Detectors (which change physical properties) and

ii) Photon Detectors which change electrical property of semi-conductors.

- This Signal Amplifier or a detector converts electro-magnetic radiations into flow of electrons which goes into Read Out Device or Recorder.

g) READ OUT DEVICE (OR) RECORDER:- In this, the amplified signal passes into a dynode voltage regulator. The Dynode Voltage is varied to maintain a constant Reference Signal.

- The Signals obtained from Sample Amplifiers are compared with the Reference Signals, they are compared and is output is displayed on the Read Out Device (in the form of Absorbance or Transmittance) or are Recorded in the form of a graph.

APPLICATIONS OF IN FRA RED SPECTROSCOPY:- The use of IR radiations is not limited to identification of organic molecules present in the functional groups.

- The uses of IR Spectroscopy are done in large number of fields such as: Defense/Military, Various Industries, medical field etc.
- Infra Red applications can also be classified on the basis of information provided by different measurement in it. Following are the few examples of it;-

i) A set of applications such as: search, track and range related with tracing of the moving or a stationary object,

ii) Related with the heat radiated by the body,

iii) Spectroradiometry – used for gas analysis,

iv) Thermal Imaging – applications related to temperature of bodies,

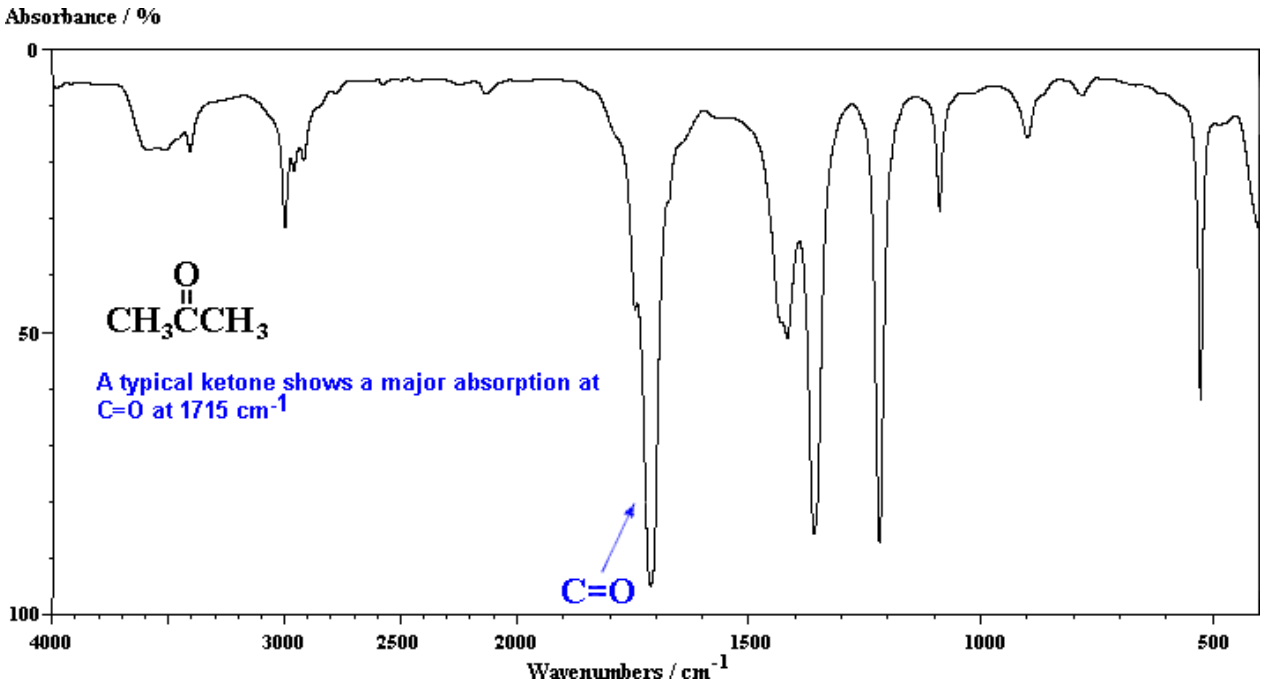
v) Applications related to reflected portion of the IR radiations, Communication & remote Sensing.

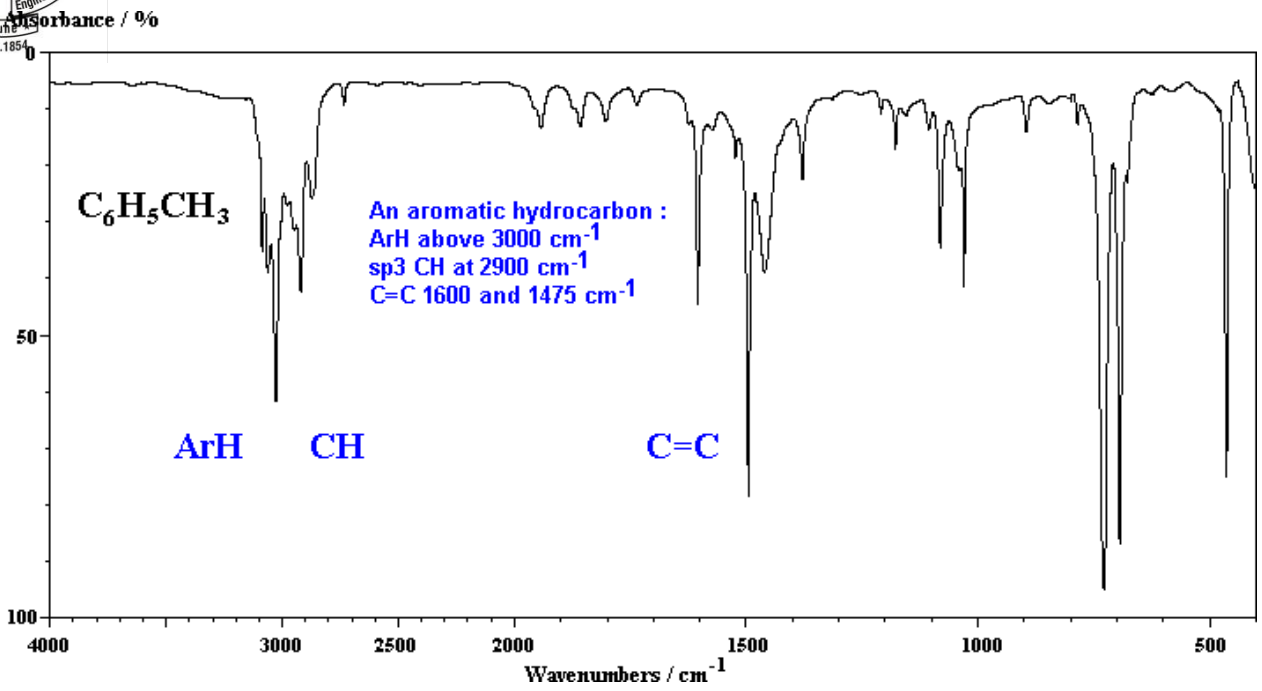
- Few more applications of IR Spectroscopy are as under:-

a) Identification of Functional Groups present in the compound,
b) Detection of impurities I the sample,
c) Detection of force constant from Vibrational spectrum,
d) Identification of Unknown Compound,
e) To indentify whether a molecule is cis or trans isomer,
f) To detect Intra- and Inter- molecular Hydrogen Bonding in a molecule,

For Analysis of mixture of aromatic Hydrocarbons etc.

Examples of IR spectra:





Use of spectroscopy as an analytical tool

- Spectroscopy is used as a tool for studying the structures of atoms and molecules. The large number of wavelengths emitted by these systems makes it possible to investigate their structures in detail, including the electron configurations of ground and various excited states.
- Cure monitoring of composites using optical fibers.
- Estimate weathered wood exposure times using near infrared spectroscopy.
- Measurement of different compounds in food samples by absorption spectroscopy both in visible and infrared spectrum.
- Measurement of toxic compounds in blood samples
- Non-destructive elemental analysis by X-ray fluorescence.
- Electronic structure research with various spectroscopes.

- Spectroscopy also finds uses in astronomy to obtain information about the composition, density, temperature, and other principal physical processes of a certain astronomical object.
- By measuring red-shift (recession speed), scientists can use spectroscopy to calculate the relative velocities of supernovae and galaxies.
- Raman spectroscopy can result in the vibrational spectrum of a certain analyte (often referred to as its “fingerprint”), which then allows straightforward identification and interpretation. Its potential applications range from archaeology to modern nanotechnology.