SBSCHE- 02- ADVANCED ANALYTICAL TECHNIQUES

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Unit - 1 Statistical Analysis

Structure

1.0 Introduction

In this unit, you shall study the evaluation of analytical data obtained in quantitative analysis. In quantitative analysis, when numerical data and numerical results are measured with the greatest exactness then it has been found that the results of successive determination differ among themselves to a greater or lesser extent. Obviously not all, and perhaps none, of the values obtained are right within the possible limits of measurement. The average value of a series of measures is accepted as the most probable value. However, it should be noted that the average value may not always be the true value. In some cases, the difference may be small, and in others, it may be so large that the result is unacceptable. After a measurement or series of

measurements, the reliability of the result depends upon the magnitude of the difference between the average value and the true value.

Objectives

After studying this unit, you shall be able to understand the following:

- know about the factors that affect and control the reliability of the quantitative chemical analysis.
- how to evaluate analytical data obtained in quantitative analysis.
- study of types of errors in experimental data and know how these errors affect the result.
- understand the source of errors and their effects on the analytical results.
- know the method for reporting analytical data and statistical evaluation of data.
- know the uses of statistics.

1.1 Definition of Terms Mean and Median

Mean

- The mean is the numerical value obtained by dividing the sum of a set of measurements by the number of individual results in the set.
- Mean is the most useful measure of central tendency.
- Other words with a similar meaning are "arithmetic mean".
  Thus, the mean **m** is given by

$$m=\Sigma M_n/n$$

where **M** is the individual measurement
and **n** is the total number of measurements.

Median

- The median is a value about which all the others are equally distributed.
- Half of the values are smaller and the other half are larger than the median value.

- The median for an even number of results is the average of the two middle ones while for the odd number of results, the median is the middle value when the results are listed in order.
- The mean and the median are identical in a symmetrical distribution.
- The median is a less efficient measure of central tendency than the mean.

The **mean deviation** of a single measurement is the mean of the deviations of all the individual measurements. This can be calculated by determining the arithmetical mean of the results, then calculating the deviation of each individual measurement from the mean, and finally dividing the sum of the deviations, regardless of sign, by the number of measurements. The mean deviation or average deviation is calculated by

$$\overline{d} = \frac{\Sigma \mid Mn - m \mid}{n}$$

Where $\overline{d}$ = mean deviation.

$\mid Mn - m \mid$= Absolute value of the deviation of the M_nth number from the mean.

It may be made clear from the following examples.

(1) Suppose in a series of independent determinations of a given quantity, the errors are essentially random and suppose the following nine values are obtained.

46.62	46.76	46.60
46.47	46.71	46.60
46.64	46.53	46.71

The mean value **m** of the above results can be calculated by dividing the sum of the individual values by the number of measurements **n** made.
Thus

$$\text{m} = \frac{\Sigma\, Mn}{n} = \frac{46.62 + 46.47 + 46.64 + 46.76 + \ldots\ldots}{9} = 46.627$$

The difference between any one of the values and the mean (46.627) is the deviation X_i of that value from the mean. These deviations are:

0 .007	0.133	0.027
0.157	0.083	0.027
0.013	0.007	0.083

Now the average or mean deviation, $\overline{d}$ is given by

$$\overline{d} = \frac{\Sigma \mid Mn - m \mid}{n} = \frac{\Sigma Xi}{n}$$

$$= \frac{0.007 + 0.157 + 0.133 + 0.133 + \ldots\ldots\ldots\ldots}{9} = 0.070$$

where ΣXi is the sum of the individual deviation from the mean.

The **relative mean deviation** is the mean deviation divided by the mean. It is usually expressed in terms of percentage or parts per thousand. In the above example,

relative mean deviation = $\frac{0.070\ x\ 100}{46.627} = 0.15\ percent$

It should also be noted that the average deviation of the mean D is numerically equal to the average deviation $\overline{d}$ of the single measurement divided by the square root of the number of measurements made. Thus

$$D = \frac{\overline{d}}{\sqrt{n}} + \frac{0.070}{\sqrt{9}} = 0.023$$

SAQ-1

(i) Which of the following is the correct formula for the arithmetic mean?

a. The formula for arithmetic mean is the total sum of values of observations divided by the number of observations

b. The formula for arithmetic mean is the total sum of values of observations plus the number of observations

c. The formula for arithmetic mean is the total sum of values of observations minus the number of observations

d. The formula for arithmetic mean is the total sum of values of observations multiplied by the number of observations

(ii).In the set 10.2,10.4,10.8,11.0,11.2, the median is….

a. 10.4

b. 10.8

c. 11.0

d. 11.2

1.2.Precision

- The mean (or average) deviation or the relative mean deviation is a measure of precision.
- Precision is known as the concordance of a series of measurements of the same quantity.
- Precision is divided into two categories: **repeatability** and **reproducibility**.
- Precision obtained when all measurements are done by the same analyst during a single period of lab work, under similar experimental conditions is known as **Repeatability.**
- Precision obtained under different experimental conditions, including that between analysts, or between lab sessions for a single analyst is known as **Reproducibility**.
- The degree of agreement between two or more replicate measurements made on a sample in an identical manner is known as the precision of the measurement.

For example,

Let us suppose that the percentages of lead in a sample of brass determined by one analyst are 2.62, 2.60, and 2.63 while those determined by another analyst are 2.81, 2.75, and 2.70.

Obviously, the precision of the first set of results is better than that of the second.

If we make a large number of observations of a single quantity and then plot a graph between the number of times a given value occurs against the value of the quantity itself then we obtain a curve (Fig.1). This curve is known as the **error distribution curve.**

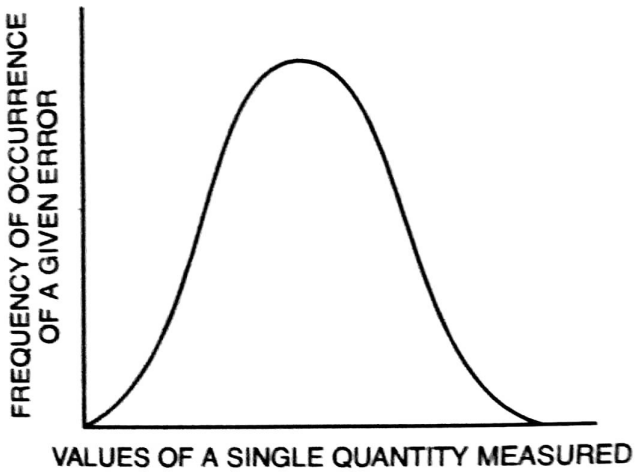


Fig. 1. The error distribution curve.

Source: Principal of Inorganic Chemistry by Puri Sharma Kalia

These curves have two qualitative features:
i) the spread of the distribution curve called **dispersion.**
ii) the height of the peak of the distribution curve.

Thus, the precision of a set of measurements is judged from the spread of the error distribution curve. The lesser the spread, the greater the precision of the measurements.

The precision of two sets of measurements of the same quantity can be compared with the help of their error distribution curves. (Fig.2)

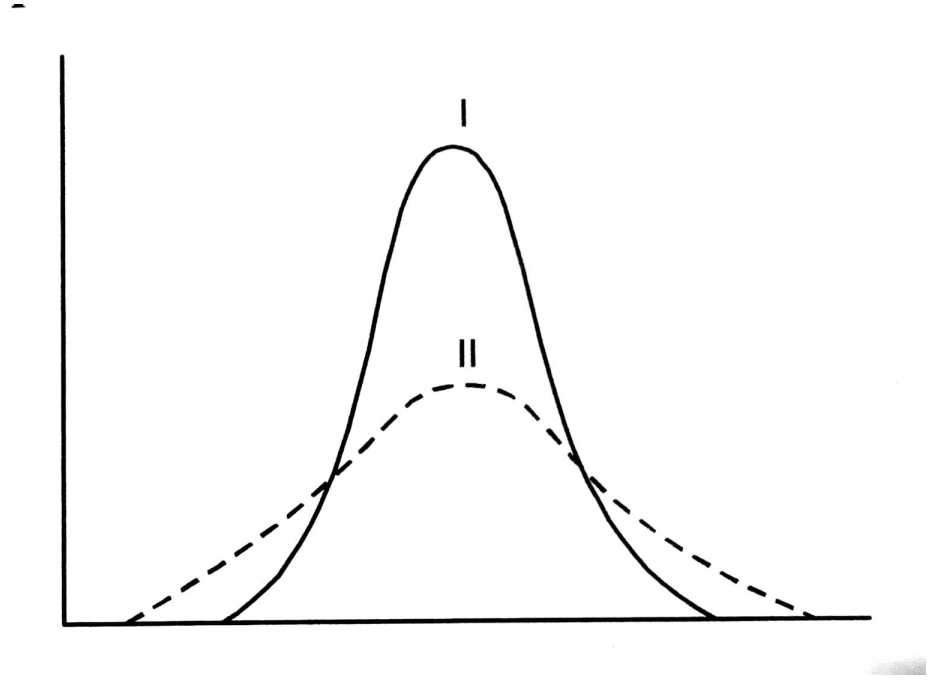


Fig. 2. Characteristics of less precise and more precise results.
Source: Principal of Inorganic Chemistry by Puri Sharma Kalia

Thus, from Fig. 2, it is clear that curve I with low spread and high peak is characterized by more precise measurements as compared to curve II shown by the dotted lines which represents less precise measurements.

The precision of a set of measurements is expressed in three ways:

1. Average deviation from the mean
2. Standard Deviation
3. Relative standard deviation.

1.3 Standard Deviation

- Standard Deviation is the most common statistical term employed in analytical chemistry.
- The term standard deviation is commonly used in statistics as a measure of precision.
- This quantity is obtained by the summation of the squares of the individual deviations from the mean, dividing the sum by n-1. This is also known as **root mean square deviation.**
  where **n** is the number of measurements and then takes the square root.
- The standard deviation is expressed by **S.**

If we consider a series of ***n*** observations which are arranged in ascending order of magnitude,

$$x_1+x_2+x_{3..} + \ldots +x_{n-1}+x_n$$

the arithmetic mean is given by the following relation:

$$\bar{x} = \frac{x1+x2+x3.. + \ldots +xn - l +xn}{n}$$

Spread of values is measured by standard deviation:

$$s = \sqrt{\frac{\sum (x-\bar{x})2}{n-1}}$$

Variance is defined as the square of standard deviation.

1.4 Relative Standard Deviation (RSD)

- The relative standard deviation is obtained by dividing the standard deviation by the mean of the set of measurements i.e.

$$RSD = \frac{S}{\bar{x}}$$

- It is expressed in terms of “parts per thousand”(ppt).

- The value of RSD is calculated by multiplying the quotient $\frac{S}{\bar{x}}$ by 1000.

Thus,

$$\text{RSD} = \frac{S}{\bar{x}} \text{ x } 1000.\text{ppt}$$

This measure is usually defined in the form of percentage by multiplying $S/\bar{x}$ x 100, called a **coefficient of variation (*CV*).**

Accordingly, $CV = S/\bar{x}$ x 100 percent

1.5 Accuracy

- The term accuracy is defined as the degree of agreement between a measured value and the most probable value or the true value.
- As we know, no measurement is completely accurate. Therefore the accuracy of value is never known except within certain limits.
- The highly accurate results are provided by accurate instruments.
- The accuracy of a result depends upon the instrument and the ability of an operator.
- A systematic error affects the accuracy of the measurement. e.g.

if the weight of a substance having a known standard mass equal to 100 g is measured by a physical balance and the result shows it as 79.5 g it means the result is not accurate. But if the result shows 99.9 g it means the result is accurate.

Thus, accuracy may also be defined as the "**nearness of a measurement to the standard or true value".**

Example:

The four different refrigerators were tested for accuracy and precision of temperature sensors by recording the temperature at different times. The results are given below along with the accuracy and precision of the temperature sensor. The refrigerator was kept at a constant temperature of 38.0 F.

Read.	Refrigerator 1	Refrigerator 2	Refrigerator 3	Refrigerator 4
1.	39.4.	37.8	39.3.	38.0
2.	38.1.	38.3	39.2	38.0
3.	39.3	38.1.	39.1.	38.0
4.	37.5	38.0.	39.0.	38.0
5.	38.3	37.6	39.1.	38.0
6.	39.1	38.2	39.3.	38.0
7.	37.7	38.0	39.2.	38.0
8.	37.1.	38.0	39.1.	38.0
9.	38.8.	37.4.	39.2.	38.0
10.	39.0	38.3	39.2.	38.0
Accuracy.	BAD.	GOOD	BAD	GOOD
Precision	BAD.	BAD.	GOOD.	GOOD

From the above example it is clear that **"It is not necessary that good precision means good accuracy or vice versa".**
Hence, Expression of accuracy can be expressed in terms of absolute error and relative error.

1.6 Absolute Error

The absolute error of measurement is the difference between the measured value and the true value.

$$E = x_i - x_t$$

where x_i is the measured value
and x_t is the true value for the given measurement.

- If the measurement value is more than the true value, the sign for absolute error is positive.
- If the measurement value is less than the true value, the sign for absolute error is negative.

Relative error (*Er*):

It is the absolute error divided by the true value. The relative error *Er* in a measurement is expressed as

$$Er = \frac{xi - xt}{xt}$$

where x_i is the measured value
and x_t is the true value for the given measurement.

It may be expressed in percent or parts per million or as parts per thousand (ppt) depending upon the magnitude of the result. It can be obtained using the following equation:

$$Er = \frac{xi - xt}{xt} \text{x } 100\%$$

$$Er = \frac{xi - xt}{xt} \text{x } 1000 \text{ ppt}$$

Difference between Precision and Accuracy

S.No.	**Accuracy**	**Precision**
1.	Accuracy is a measure of the agreement between an experimental result and the true value of a given quantity.	Precision, on the other hand, measures the agreement between several experimental results obtained for the same quantity under identical conditions.
2.	Accuracy, in contrast, can never be determined exactly because it involves the use of the absolute or true value of the quantity being measured which is never known. All that we use for measuring accuracy is the 'accepted' value of the quantity.	Precision can be determined almost exactly by replicating measurements of the same quantity.
3.	Accuracy is expressed in terms of absolute error or relative error	Precision is expressed in terms of various types of deviations from the mean.
4.	The error distribution curve for a less accurate set of measurements, however, differs from that of a more accurate set only with respect to the position of the mean value. The mean value for a more accurate set is in close proximity to the true value (curve I, Fig. 3.) whereas the mean value for a less accurate set of measurements is at a greater distance on either side (curves II and III, Fig. 3).	The error distribution curve for a less precise set of measurements differs from that of a more precise set with respect to its scatter or spread or dispersion; the spread being more for a less precise set of measurements, as already shown in Fig. 3.

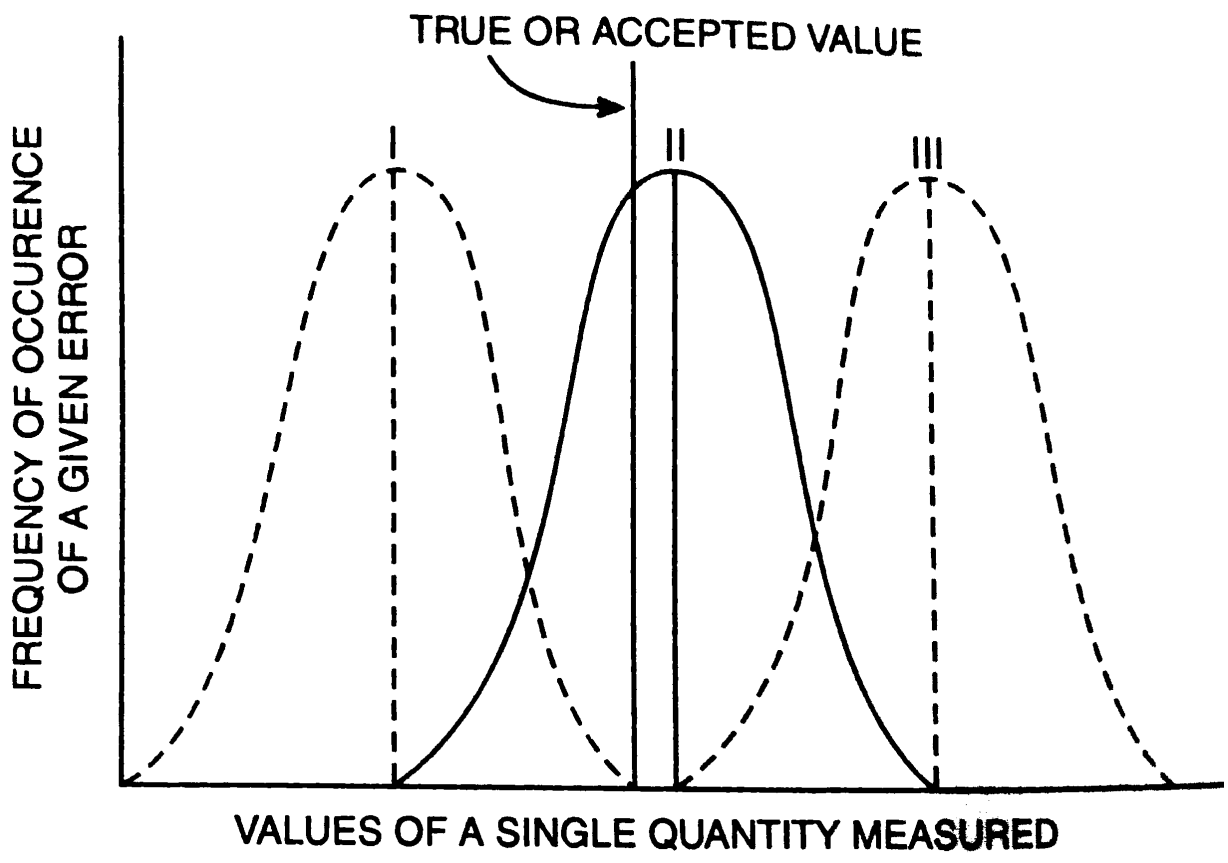


Fig. 3. Error distribution curves for less accurate and more accurate sets of results.
Source: Principal of Inorganic Chemistry by Puri Sharma Kalia

SAQ-2

(i). The standard deviation for the following set of results is 10.28,10.24,10.25,10.25.10.30
a. 0.0187
b. 10.26
c. 10.25
d. 0.223

(ii). in 5 experiments with the same objective, the values obtained are very near to each other. These values can be called ____
a) Invalid
b) Accurate
c) Average
d) precise

(iii). In an experiment, it is found that the experimental value is very close to the actual value, hence the experimental value can be called _____

a) Precise
b) Accurate
c) Suitable
d) Mean

1.7 Types of Error in experimental data

- Error is defined as the numerical difference between a measured value and the absolute or true value of an analytical determination. However, The absolute or true value of a quantity is never known.

- All we can use is just an accepted value.

- The value for any quantity is accepted when the uncertainty in this value is less than the uncertainty in some other quantity with which the given quantity is to be compared.

- The values which we may like to 'accept' can be obtained by a variety of methods that have their own limitations.

- It is very rare that two different methods may yield identical accepted values.

The error in a measured quantity may be represented either as an absolute error or as a relative error.

Absolute Error. The absolute error E in a measurement is expressed as

$$E = x_i - x_t$$

where x_i is the measured value
and x_t is the true value for the given measurement.

Relative Error. The relative error Er in a measurement is expressed as

$$Er = \frac{xi - xt}{xt}$$

where x_i is the measured value
and x_t is the true value for the given measurement.

Errors affecting experimental data are categorized into

(i) Determinate or Systematic Errors and (ii) Indeterminate or Random Errors.

1.8 Determinate (Systematic) Error

Determinate errors have a definite source that can be identified. A given determinate error is generally unidirectional with respect to the true (accepted) value. This makes the measured value either lower or higher than the true value. These errors are often reproducible. They can be easily predicted by an expert analyst. Thus, these errors can either be avoided or corrected.

These are of **three** types-

a. Instrument errors and reagent errors
b.Operational and personal errors
c.Method errors
d.Additive and proportional errors

a. Instrument and reagent errors

These errors arise from imperfections in measuring devices. For example, glassware, attack of reagents upon glassware, faulty construction of balances, use of uncalibrated weights, volatilization of platinum at very high temperatures, and use of reagents consisting of impurities are sources of reagent errors.

b. Operational and personal errors

The analyst is responsible for these types of errors and these are not connected with method or procedure. These errors occur when the correct method is not followed. These errors may arise from the inability of analysts to make certain observations accurately. Such as the judgment of the colour of solution at the end point in a titration, the judgment of the level of a liquid with respect to graduation on a burette or a pipette, and the estimation of the position of a pointer

between two scale divisions. Such errors would vary from person to person and can be reduced to a minimum by experience and careful physical manipulation.

c. method Errors

These errors originate:

- due to incorrect sampling and incompleteness of a reaction.
- Due to solubility of precipitates, co-precipitation, and post-precipitation.
- decomposition of weighing forms on the ignition, and precipitation of substances other than the desired ones.

These types of errors are encountered in gravimetric analysis.

d. Additive and proportional errors

- Absolute value of proportional error is dependent on the quantity of desired substance.
- The Absolute value of an additive error is not dependent on the quantity of analyte present in determination.

1.9 Indeterminate (Random) and Gross Error

Indeterminate (Random) Error

These errors occur with slight variations in successive measurements made by the same analyst under similar experimental conditions. When a large number of observations are received and shown in Fig. 4. we get an error curve. From the error curve, it is clear that

(i) the chances of occurrence of small errors are more than large ones, and

(ii) positive and negative errors of similar magnitude have an equal probability to occur.

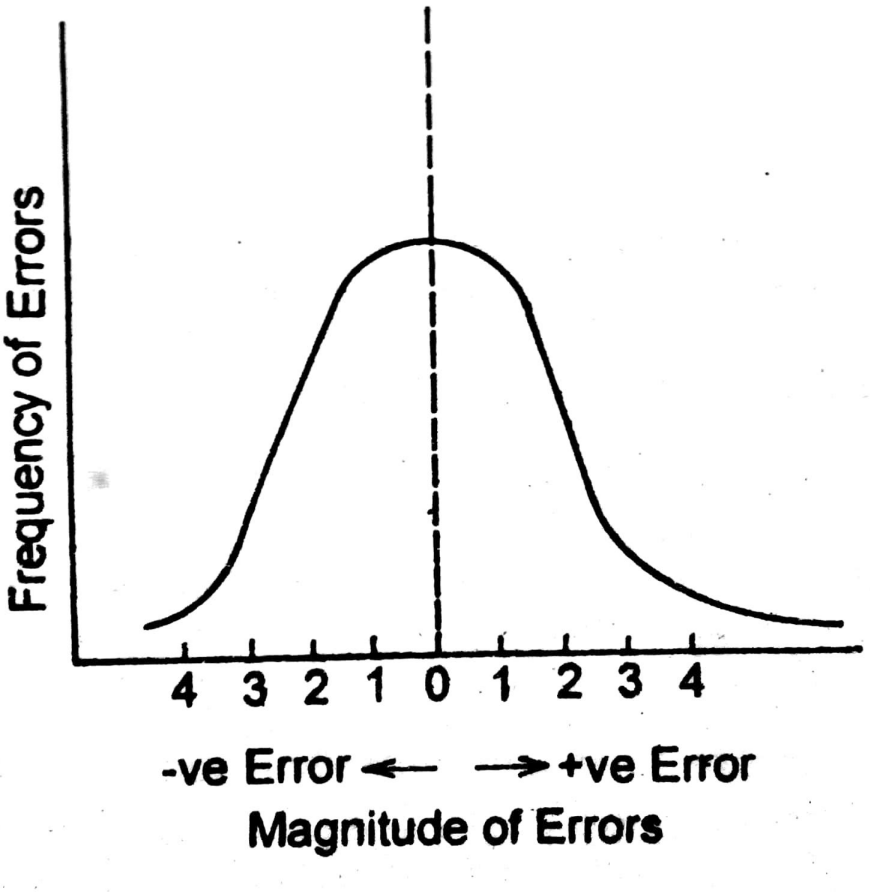


Fig. 4. Error curve

Source: Instrumental Methods of Chemical Analysis by B.K.Sharma

Indeterminate errors can be divided into **two** classes.

(a) Variation within determinate errors

(i)Temperature changes and humidity variations in a balance room give determinate errors, but if they are not controlled, they become indeterminate.

(ii) As we know, if the knife edge of the balance is worn out, there may be a determinate error, but when the error varies, due to weight (or other reasons), it becomes an indeterminate error.

(b) Erratic Errors

These types of errors are very difficult to pinpoint and in fact, the analyst may be unaware of their presence.

examples:

(i) Fluctuation in the balance room can cause erratic errors in weighing.
(ii) Accidental loss of materials during an analysis.

Gross Error

- Gross errors are those that arise as a result of the observer's lack of experience when taking measurement results.
- The notion of gross error is entirely based on the observer or the specific experiment as such.
- These errors are caused by the observer using the capturing of the data, instruments, and computing certain types of experiment measurement findings. As a result, it is classified as human error.
- Gross errors are physical errors in analytical equipment or computing measurement results.

We have taken the vernier caliper as an example to explain the causes of gross errors. These are:

1. Improper handling: This can include:
- holding the caliper at an incorrect angle.
- not properly aligning the jaws of the vernier caliper with the object being measured.

2. Poor judgment: This can include:
- not selecting the appropriate jaws or scale for the measurement being taken.
- irregularities in the object being measured.

3. Inexperience: Inexperienced users may make errors:
- in using the jaws properly.
- sliding them into the correct position.
- interpreting the readings correctly.

4. Equipment failure: This can include problems:
- with the caliper itself, such as damaged jaws or a misaligned scale.
- with the object being measured, such as an irregular surface or deformity.

5. Human factors: This can include:
- physiological factors such as fatigue or stress which can affect a user's ability:
- to properly operate the caliper.
- read the scale accurately.

Minimisation of Gross errors

All of the observations show that the user's judgment and carefulness are entirely responsible for the gross error. As a result, when the observer is diligent and mentally alert, gross error can be reduced. It's more of a user-dependent error-reduction procedure. Increasing the number of experiments will also be beneficial.

If an experimenter takes different readings at different times, the average of all the readings can help to reduce the total gross error. A good degree of patience, as well as a free mind with sufficient care, are necessary from the individual for these proper experimenting facilities.

Difference Between Gross Error and Systematic Error

S.No.	Gross Error	Systematic Error
1.	Gross errors usually arise during the study, when the experimenter may read or record a value that is different from the true one.	In physics, systematic error is normal when the measuring instrument has zero error.
2.	Gross errors are caused by reading instruments or manual errors in recording and computing measurement findings.	Systematic errors develop as a result of changes in environmental conditions, secondary causes, or erroneous observations.
3.	Gross errors can be reduced.	Systematic errors can also be reduced.

2.0 Sources of errors and effects upon the analytical results

Analytical methods used for the measurement and/or analysis purposes involve optimization of several parameters using specific equipment operated by expert persons.

In addition to this calculative data such as amounts required for the experiment, yields, detection limits, etc. is directly related to the purity of the chemicals and reagents. The involvement of all these parameters directly or indirectly (equipment, experimentation procedures, and operational expertise) may alternate the final result by adding some errors. For example, to prepare a N/20 NaOH solution, the following factors are responsible for the addition of errors at each step.

The procedure for the preparation of N/20 NaOH solution involves:

- The weight of NaOH required to prepare its N/20 solution is calculated.
- Weighed and dissolved in distilled water in a 100 mL measuring flask.
- The total volume is made up to the mark by adding extra distilled water.

Sources of errors

- Purity of NaOH provided by the manufacturer.
- Accuracy of the weighing machine used for weighing NaOH or the physical balance and the weights used for weighing.
- Accuracy of the graduated apparatus used in the measurement such as a cylinder, or measuring flask.
- Purity of the distilled water.
- Environmental parameters such as NaOH are hygroscopic so the weight may vary.
- Experimental hand of the person who is performing the experiment.
- Judgment of the meniscus in the measuring flask to make it 100 mL.

  From the example, It is clear that a single step may add a large number of errors and deviate results from the actual value. These deviations go on increasing with each step and may vary the results beyond acceptable limits. Therefore, it is important to know the sources of errors that affect the results-

(1) Errors in reading a burette.
(2) Difference between the observed end point and the stoichiometric end points of a reaction.
(3) Failure to wash and ignite a precipitate properly.
(4) Insufficient cooling of the crucible before weighing.
(5) Coprecipitation and post precipitation.
(6) Solubility of a precipitate in the solution in which it is precipitated, and in the wash liquid. For example, in gravimetric analysis, it has been observed that all precipitates are soluble to some extent.
(7) Balance arms of unequal length
(8) Hygroscopicity of the weighing forms.
(9) Using reagents containing harmful impurities etc.
(10) Failure of a reaction to proceed to quantitative completion.
(11) Occurrence of induced or side reactions.
(12) Mechanical loss of material in various steps of analysis.
(13) The temperature changes and variations in humidity in a balance room also give rise to errors.
(14) Accidental loss of materials during an analysis.
(15) Decomposition or volatilization of weighing forms of precipitates on ignition or on heating.

SAQ-3

(i). Which of the following is the type of determinate error..

a. Error of method
b. Operative error
c. Instrumental error
d. All of the above

(ii). An error may be defined as the difference in

a. Measured value and observed value
b. The measured value and true value
c. Both a and b
d. None of these

2.1 Methods for reporting analytical data

While reporting analytical data, it is absolutely essential to indicate the extent of its accuracy or reliability. Without this, a numerical result is of little value. One of the best ways to indicate reliability is to give the absolute standard deviation or the coefficient of variation of the data. The number of data used in obtaining the standard deviation should also be indicated.

The probable uncertainty associated with a given experimental measurement can be represented informatively by indicating the limits of accuracy as the observed number ± uncertainty. For example,

If the temperature of a body is represented as 25.0 ± 0.1 °C, it means that the actual temperature lies somewhere between 24.9 and 25.1°C.

Uncertainty in results involving addition and subtraction

The maximum uncertainty in a result involving addition and subtraction is obtained by adding the absolute values of uncertainties in various measurements. For example,

If the absolute uncertainties in the given two data are ± 0.2 and ±0 .3, then the uncertainty in the summation of the data would be ± (0.2 + 0.3) = ± 0.5. Example:

If calculate the maximum uncertainty in each of the following expressions then we get -

(a) (17.3 ± 0.2) ml - (9.7 ± 0.3) ml + (11. 6 ± 0.1) ml
(b) (16. 6 ± 0.1) g - 2 (16.7 ± 0.2) g + 3 (7.3 ± 0.1) g

(a)	(b)
17.3 ± 0.2	16.6 ± 0.1
-9.7 ± 0.3	-33.4 ± 0.4
11.6 ± 0.1	21.9 ± 0.3
—----------	—----------
19.2 ± 0.6 ml	5.1 ± 0.8 g

(Uncertainties are always added)

Uncertainty in results involving multiplication and division

The maximum uncertainty in a result invoking multiplication and division is obtained by following the procedure:

- From the given data, calculate the percentage uncertainties.
- Add up these percentage uncertainties.
- Convert the percentage uncertainty to uncertainty.

Example:

If calculate the result of the following expression: (23.4 ± 0.1) (17·7± 0.05)

then we get -

Step 1. (23.4 ± 0.1) (17.7± 0.05) = 414.18 ±?

Step 2. Percentage uncertainties of the two multipliers are $\pm\frac{0.1}{23.4}$ x 100 and $\frac{0.05}{17.7}$ x 100

Step 3. Summation of percentage uncertainties = ± 0.71

Therefore, the results may be written as 414.18 ± 0.71 %

Step 4. Uncertainty of the result = $\pm\frac{0.71}{100}$ x 414.18 = ±2.94

The final result is 414.18 ± 2.94

2.2 Statistical Treatment of Analytical data

The statistical treatment of analytical data is based on the assumption that a set of the finite number of replicate measurements actually made experimentally is a very small fraction of the set of an infinite number of measurements that could be made if infinite time was available for such measurements. The limited set of data of a finite number of values is called '**the sample'** which is regarded as a subset of a universe of data that exists only in imagination and is called **the population.**

The sample mean is the mean of the 'sample' of measurements, i.e., a finite number of measurements drawn from a 'population' of data.It is denoted by the symbol $\overline{x}$ and is mathematically represented as

$$\overline{x} = \sum_{i=1}^{i=N} x_i /N$$

where N is usually a relatively small number.

The population mean is the mean of the entire population of data. It is denoted by the symbol μ and is mathematically represented as

$$\mu = \sum_{i=1}^{i=N} x_i /N$$

where N approaches infinity.

usually, the value $\overline{x}$ differs from μ. because a 'sample' of data for small values of N cannot be truly representative of a 'population' of data for infinite values of N.

Standard Deviation for Sample and Population of Data

The standard deviation for a sample of measurements is called the sample standard deviation. It is denoted by S. The standard deviation for a population of data, is referred to as the population standard deviation. It is denoted by σ.

Confidence Limits

With the help of statistical theory, it is possible to set limits around experimentally derived mean within which the sample mean $\overline{x}$ is likely to be in agreement with the population mean μ with a given degree of probability. These limits are referred to as the **confidence limits** and the range defined by these limits is known as the **confidence interval.**

Mathematically, the confidence limit is given by

Confidence limit = $\overline{x} \pm \frac{tS}{\sqrt{N}}$

where $\overline{x}$ is the sample mean,
N is the number of measurements (N< 25),

S is the sample standard deviation and
't' is a factor whose magnitude depends upon the number of measurements. The confidence level signifies the degree of probability (99%, 95%, 90%, 50%, etc.) acceptable in the results. The factor t is often called the **Student's *t* .** The values of *t* corresponding to various levels of probability and varying degrees of freedom are available in Statistical Tables.

Student's **'t'** values for Various Levels of Probability and Varying Degrees of Freedom

Number of Observations N	Number of the degree of freedom N-1	Probability Labels:			
		50%.	90%.	95%.	99%
2	1	1.000.	6.314.	12.706.	27.320
3	2	0.816.	2.920.	4.303.	9.925
4	3	0.765.	2.353.	3.182.	5.841
5	4	0.741.	2.132.	2.776.	4.604
6	5	0.727.	2.015.	2.571.	4.032
7	6	0.718.	1.943.	2.447.	3.707
8	7	0.711.	1.895.	2.365.	3.500
9	8	0.706.	1.860.	2.306.	3.355
10	9	0.703.	1.833.	2.262.	3.250
11	10	0.700.	1.812.	2.228.	3.169
21	20	0.687.	1.725.	2.086.	2.845
∞	∞	0.674.	1.645.	1.960.	2.576

Rejection of a Result

When we perform a large number of replicate analyses, one of the results may differ excessively from the average value, ie, the particular result may be too high or too low. So we have to take a decision whether we should retain or reject that suspect result. Several statistical tests have been proposed for this purpose but these have to be applied with utmost care.

The simplest test which has been widely used by analysts is called the ***Q test*** (Quotient test). In this test

- The results of various observations in a given set are arranged in the decreasing order of their numerical values.
- The difference between the suspected result x_s and its nearest neighbor x_n (ignoring the signs + or-) is divided by the spread w of the entire set to give a quantity Q_{exp}. Thus,

$$Q_{exp} = \frac{Xs - Xn}{w}$$

3. The ratio *Qexp* is then compared with critical values of the rejection ratios obtained statistically at appropriate confidence levels and tabulated as a function of the number of observations. These ratios are termed *Qcritical.*

The critical values for the rejection quotient at a 90% confidence level are given in Table.

Critical Values for Rejection Quotient at 90% Confidence Level

Number of Observations, N	$Q_{critical}$	Number of Observations, N	$Q_{critical}$
3	0.94	7	0.51
4	0.76	8	0.47
5	0.64	9	0.44
6	0.56	10	0.41

If Q_{exp} is greater than $Q_{critical}$ the suspect result is rejected.

SAQ-4

(i). For rejection of the result, the test used is…..
a. Q-Test
b. Testing of significance
c. F-test
d. T-test

(ii).For rejection of the result of which of the following rules is used…..
a. 2.5d rule
b. 4.0d rule
c. Both a and b
d. None of these

2.3 Uses of statistics

Statistics is an indispensable tool. The use of statistics can inhibit wrong judgments based on insufficient information.

- The analyst makes use of statistical methods in describing quantitatively the accuracy as well as the precision of the analytical results and methods.
- Statistical methods also play a key role in deciding upon the adequacy of sampling procedures and in planning experiments.
- It is interesting to note that by proper design of experiments, guided by a statistical approach, the effect of experimental variables can be determined more accurately and more efficiently than by making use of a classical approach in which all variables but one are maintained constant.
- Accuracy, precision, significant figures, mean, medium, absolute and relative error, average deviation, standard deviation, etc. are the various terms that have been employed in the evaluation of the data.
- The term "measurement" in statistics applies to all experimentally determined data.
- In addition, some other methods, such as the "Q Test", the "t distribution" etc. also have been used.
- In addition, there is a rapidly growing field of chemometrics, which is interpreted as the application of mathematical and statistical procedures to design and/or optimize analysis methods and to present chemical information by analysis of associated data.
- It represents complex data in graphical form, tabular form, and in diagrammatic representation for easy understanding.
- It provides an accurate description and better understanding.
- It helps in preparing an effective and proper plan of statistical investigation in any field.

2.4 Summary

- The mean is the numerical value obtained by dividing the sum of a set of measurements by the number of individual results in the set.
- The median is a value about which all the others are equally distributed. Half of the values are smaller and the other half are larger than the median value. The median for an even number of results is the average of the two middle ones while for the odd number of results, the median is the middle value when the results are listed in order.
- The mean (or average) deviation or the relative mean deviation is a measure of precision. Precision is known as the concordance of a series of measurements of the same quantity.
- The term accuracy is defined as the degree of agreement between a measured value and the most probable value or the true value.
- The absolute error of measurement is the difference between the measured value and the true value.

- Error is defined as the numerical difference between a measured value and the absolute or true value of an analytical determination.
- Determinate errors have a definite source that can be identified. A given determinate error is generally unidirectional with respect to the true (accepted) value. This makes the measured value either lower or higher than the true value.
- Indeterminate errors occur with slight variations in successive measurements made by the same analyst under similar experimental conditions.
- Gross errors are those that arise as a result of the observer's lack of experience when taking measurement results.
- While reporting analytical data, it is absolutely essential to indicate the extent of its accuracy or reliability. Without this, a numerical result is of little value. One of the best ways to indicate reliability is to give the absolute standard deviation or the coefficient of variation of the data.
- The statistical treatment of analytical data is based on the assumption that a set of the finite number of replicate measurements actually made experimentally is a very small fraction of the set of an infinite number of measurements that could be made if infinite time was available for such measurements.
- Statistics is an indispensable tool. The use of statistics can inhibit wrong judgments based on insufficient information.
- The analyst makes use of statistical methods in describing quantitatively the accuracy as well as the precision of the analytical results and methods.

2.5 Terminal Questions

Q1.What is meant by measurement error?
Answer._____
_____
_____

Q2. What are the types of errors?
Answer._____
_____
_____

Q3 The error seen due to the effect of the external conditions on the measurement is known as?
Answer._____
_____
_____

Q4. Define absolute error.
Answer._____
_____
_____

Q5. A length was calculated to be 10.1 feet, but the absolute length was 10.5 feet. Calculate the absolute error.
Answer._____
_____
_____

Q6.What is a gross error?
Answer._____
_____
_____

Q7. Explain methods for reporting analytical data.
Answer._____
_____
_____

Q8. Differentiate between the terms precision and accuracy.
Answer._____
_____
_____

Q 9. What is the difference between gross error and systematic error?
Answer._____
_____
_____

Q 10. The normality of a solution is determined by four separate titrations, the results being 0.2039,0.2041,0.2049 and 0.2043. Calculate the mean and median.
Answer._____
_____
_____

Q11. Explain the terms mean, median and standard deviations.

Answer._____
_____
_____

Q12. What kind of errors are most likely to be made when applying the Q-test?
Answer._____
_____
_____

Q13.Write notes on precision.
Answer._____
_____
_____

Q14.What are the causes of gross error?
Answer._____
_____
_____

Q15.How to avoid gross error?
Answer._____
_____
_____

_____

2.6 Answers

SAQ-1 (i) a. (ii) b.
SAQ-2 (i) a. (ii) d (iii) b
SAQ-3 (i) d (ii) b.
SAQ-4 (i) a. (ii) c.

Suggested readings

1. Principles of Physical Chemistry by Puri and Sharma
2. VOGEL'S Textbook of Quantitative Chemical Analysis by J Mendham, R.C. Denney, J.D.Baranes, M.J.K.Thomas

3. Quantitative Analysis by R.A.Day, Jr.A.L.Underwood
4. Basic Concepts of Analytical Chemistry by S.M.Khopkar
5.Instrumental Method of Chemical Analysis by B.K.Sharma
6. Statistical Methods in Analytical Chemistry by Peter C. Meier and Richard E. Zund
7. Modern Analytical Chemistry by David T Harvey

Unit -2 Volumetric Analysis

Structure

2.0 Introduction

Quantitative analysis is concerned with the determination of the amount of a chemical substance present either alone or in a sample or complex mixture of other substances. There are two main types of quantitative analytical methods –
(i) Gravimetric method
(ii) Volumetric method

In this unit, we study volumetric analysis. The gravimetric analysis has been dealt with in Unit III. "Volumetric analysis" is also known as "titrimetric analysis". A reaction suitable for titrimetric analysis must satisfy the following conditions:

- The reaction must go to completion by mixing chemically equivalent amounts of the reacting substances.
- The reaction should be instantaneous.
- The endpoint must be sharply defined by some change in the physical or chemical properties of the solution.

Titration methods generally prefer quantitative analysis of substances in comparison to gravimetric methods because of the following reasons :

(1) Titrimetric methods require simpler apparatus and are generally quickly performed While Gravimetric methods often involve tedious and difficult separations and time-taking operations.

(i) Titrimetric methods are more susceptible to high accuracy than gravimetric methods when the Gravimetric methods are subjected to errors that cannot be easily avoided.
The accuracy of quantitative analysis of a substance depends upon a number of factors:

- Cleaning of glass apparatus
- Calibration of volumetric apparatus
- Calibration of weights

Objectives

After studying this unit you shall be able to:

- understand volumetric analysis and its uses.
- calculate the concentration of acid from the volume of a base that neutralizes it and vice versa.
- calculate a solution's unknown concentration using the results from a titration experiment.
- understand different types of titration.
- learn about different types of EDTA titration.
- learn to determine the hardness of water.
- learn how a metal ion indicator works.

2.1 General Principles of acid-base Titration

Volumetric Analysis

The titrimetric analysis involves the determination of the volume of a solution of accurately known concentration which is required to react quantitatively with the solution of the substance being determined. Depending upon the nature of the chemical reactions, there are many types of titrations but the most common are given below:

a. Acid–base or neutralisation titration
b. Precipitation titration
c. Oxidation–reduction titration
d. Complexometric titration

Important Terms

1. Titration: Titration is the process of determining the volume of the known strength. (concentration) solution required to react with a known volume of unknown strength solution.

2. Titrant and Titrate: The solution taken in a burette used in titration is called the **titrant** and the solution taken in a conical flask is called **titrate.**

3. Equivalence Point : The volume at which the reaction actually completed by the addition of a stoichiometric amount of titrant is known as the **equivalence point.**

4. Indicator: A substance that is used for the visual detection of the completion of a particular reaction to determine the end point of a titration is called an **indicator.**

5. End Point: The volume at which the completion of the reaction is observed by using an indicator is called the **endpoint**, the visual observation is colour change or fluorescence or turbidity formation.

6. Titration error: In practice, there is a difference between the equivalence point and the endpoint. This difference is known as **titration error.**

7. Standard solution: The solution of accurately known strength is called the **standard solution.** The weight of the substance to be determined is then calculated from the volume of the Standard solution and the known laws of chemical equivalence. These are of two types -
(i) Primary Standard solution
(ii) Secondary Standard solution

(i) Primary Standard solution

It is prepared by dissolving an accurately weighed sample of the dry reagent in the known volume of the solvent.
Commonly used Primary Standards are Anhydrous sodium carbonate, Oxalic acid, Succinic acid, Sodium Chloride, Ferrous ammonium sulphate, etc.

(ii) Secondary Standard solutions

Those substances whose Standard solutions can not be prepared directly by weighing . These types of substances are called Secondary standard substances.
Commonly used Secondary Standards are Alkali hydroxides and inorganic acids etc.

8. Standardization

The approximate weight of the Secondary Standards is dissolved in a known volume and the exact strength of the solution is found by titrating it against the standard solution of the suitable reagent. This process is called '**Standardization'**.

Indicators. The reagent used to locate the exact completion stage i.e. end point of the reaction by showing a change in its colour, is called an indicator. On adding even the smallest excess of the titrant, beyond what is necessary for the exact completion of the reaction, the indicator changes colour.
The following are certain types of indicators that will be dealt with when considering different divisions of volumetric analysis.

(a) External indicator. It is placed outside on a reference porcelain plate, e.g., $K_3Fe(CN)_6$ dilute solution in the titration of ferrous sulphate or ferrous ammonium sulphate against $K_2Cr_2O_7$.

(b) Self-indicator. When colour of one of the titrants acts as an indicator, e.g., the pink colour of permanganate ion in the titration of oxalic acid or ferrous ions against $KMnO_4$

(c) Internal indicator. It is added to the solution taken in the conical flask or beaker. The most common internal indicators are:

(i) Phenolphthalein
(ii) Methyl orange
(iii) Starch solution
(iv) Methyl red.
(v) Internal indicators used in the titration of ferrous ion against $K_2Cr_2O_7$
(a) Diphenyl amine or symmetrical diphenyl benzidine.
(b) Barium or sodium salt of diphenylamine-p-sulfonic acid.
(c) N-phenyl anthranilic acid.

Terms to Express Concentration:

Normality: The number of gram equivalent of solute (substance) dissolved in one litre (1000 ml) of the solution is called **normality.**
It is indicated by N.

$$\text{Normality} = \frac{\text{Gram equivalent weight of solute}}{\text{Number of replaceable } H^+ \text{and } OH^-}$$

Molarity: Number of moles of solute (substance) dissolved in one litre (1000 ml) of the solution is called **molarity**.
It is indicated by M.

$$\text{Molarity} = \frac{\text{Number of moles of solute}}{\text{1000 ml of solution}}$$

Molality: A molal solution contains one mole of solute per one kg of the solution is called **molality**.
It is indicated by m.

$$\text{Molality} = \frac{\text{Number of molecular weight of substance}}{\text{1000 gm of solution (1 kg)}}$$

Formality: The number of gram formula weight of the solute (substance) dissolved per litre (1000 ml) of the solution is called **formality.**

$$\text{Formality} = \frac{\text{Number of gram formula weight of masses of solute}}{\text{volume of solution in litres}}$$

Percentage solution:

i) Percent by weight: The percent composition by weight is the number of grams of solute present in 100 gm of solution.

ii) Percent by volume: The percent composition by volume is the number of grams of solute present in 100 c.c. of solution.

iii) Percent by weight per volume: It is the number of solutes present in 100 c.c of solvent.

Mole Fraction: It is defined as the number of moles of the component divided by the total number of moles present in the solution.

$$x_a = \frac{n_a}{n_a + n_b}$$

SAQ-1

(i). What is the primary objective of titration?
a.To find the concentration of an unknown acid or base
b.To find the volume of an unknown acid or base
c.To find the pH of an unknown acid or base
d.To find the pressure of an unknown acid or base

(ii). A solution of known concentration is the definition of a
a. Buffer solution b. Standard solution
c. Neutral solutions d. Saturated solution

a. Acid–base or Neutralization titration

- In acid-base titrations, the amount of acid becomes exactly equivalent chemically to the amount of base present.
- If the acid and base are both strong, the resultant solution will be neutral (pH = 7).

- If either the acid or alkali is a weak electrolyte, the solution at the endpoint will be either roughly alkaline or slightly acidic because the salt will be hydrolyzed to a certain degree.
- The correct endpoint is characterized by a definite value of the hydrogen ion concentration of the solution.
- Choosing proper acid-base indicators

Acid-base indicators

- Acid-base indicators possess different colours depending upon the hydrogen ion concentration of the solution.
- In these indicators, the change from a predominantly "alkaline" colour is not sudden but takes place within a small interval of the pH range, usually of about two units.
- Hence, in acid-base titrations, one selects a proper indicator which may exhibit a distinct colour change at a pH close to the endpoint.
- In the case of strong acid and strong base (for 0.1N solution or stronger), any indicator may be used which has a pH range between 4.5 and 9.5.

  Some of the proper acid-base indicators used in different acid-base titrations are given in Table.

Acid-base titration	pH range	Acid-base indicator	Colour of acid form	Colour of basic form
Weak acid and strong base	8.3 - 10.0	Phenolphthalein	Colourless	Red
Weak base and strong acid	4.2- 6.3 3.1-4.4	Methyl red Methyl orange	Red Red	Yellow Orange
Anions of Weak acid and strong base	8.6 - 9.6 2.9- 4.0 3.1- 4.4	Thymol blue Methyl yellow Methyl orange	Yellow Red Red	Blue Yellow Orange

The first useful theory of indicator action was suggested by W. Ostwald. All acid-base indicators in general are very weak organic acids or bases. According to him, the undissociated indicator acid (HIn) or base (InOH) had a different colour from that of its ion. The equilibria in aqueous solution may be written as

$$HIn \rightleftarrows H^+ + In^-$$

$$InOH \rightleftarrows OH^- + In^+$$

unionized colour ionized colour

The ionization of an acid indicator in the presence of an acid solution (in the presence of excess H^+ ions) will be depressed due to the common ion effect and the concentration of the ionized

form of indicator In^- will be very small, and the colour will be that of unionized form. If the medium is alkaline, the decrease in H^+ ion concentration will result in the further ionization of the indicator. In this way, the concentration of the ionized form of indicator In increases and the colour of the ionized form becomes apparent.

Phenolphthalein is an acid form of indicator. Its dissociation in the presence of an acid is very very small and so it remains colourless in the presence of an acid.

$$HPh \rightleftarrows H^+ + Ph^- \text{ (}\textit{very very small}\text{)}$$

(Phenolphthalein in acid medium)

In addition to a base, the sodium salt of phenolphthalein has a greater degree of dissociation with the result that the concentration of the ionized form of indicator (Ph^-, phenolphthalate ions) increases which give pink colour to the solution.

Thus in a basic medium,

$$NaOH \rightarrow Na^+ + OH^-$$

$$Na^+ + Ph^- \rightarrow NaPh$$

$$NaPh \rightarrow Na^+ + Ph^- \text{ (}\textit{coloured pink}\text{)}$$

OH^- combines with H^+ ions to give water.

SAQ-2

(i). Which of the following is used as an indicator in titrating a strong acid with a weak base?

a. Methyl orange. b. Methyl red
c .Sodium hydroxide d. Phenolphthalein

(ii). What is the principle of acid-base titration?

a. Neutralisation b. Alkalimetry
c. Both a and b d. None of the above

2.2 Precipitation titration

The titrations which are based on the formation of insoluble precipitates when the solutions of two reacting substances are brought into contact with each other, are called Precipitation Titrations. For example, when a solution of silver nitrate is added to a solution of sodium chloride, a white precipitate of silver chloride is formed.

$$AgNO_3 + NaCl \longrightarrow AgCl\downarrow + + NaNO_3$$

Similarly, when $AgNO_3$ is added to ammonium thiocyanate a white precipitate of silver thiocyanate is formed.

$$AgNO_3 + NH_4CNS \longrightarrow AgCNS\downarrow + NH_4NO_3$$

Both of these reactions may be used for the volumetric estimation of silver if the completion of the precipitation is judged with the help of suitable indicators. Such volumetric estimations involving silver nitrate are called **Argentometric titrations.**

According to the end point detection method, these are of three types:

1. Volhard's Method 2.Fajans Method 3. Mohr's Method

1. Volhard's Method:

In this titration potassium or ammonium thiocyanate is titrated against silver nitrate using ferric alum or ferric nitrate as an indicator.

2. Fajan's Method:

This method was given by American chemist Kazimierz Fajan. This method is also known as the indicator absorption method . Here, dichlorofluorescein works as an indicator and methyl chloride ions that are present in excess are absorbed on the silver chloride surface. At the endpoint green suspension of AgCl is obtained which turns pink.

3. Mohr's Method:

This method was given by German Chemist Karl Friedrich Mohr. This is a direct titration method in which silver nitrate is used as a titrant and a solution of chloride ion is used as an analyte. The indicator used in the titration is Potassium chromate. At the end of the reaction, the reddish-brown precipitate is formed after the consumption of all the chloride ions.

Applications of Precipitation Titration: precipitation titration is used as follows:

- to determine the halide ions in the solution.
- to measure salt content in various food and beverages.
- to analyse various drugs.
- for analysing the concentration of the anion in the analyte.

Limitations of Precipitation Titration:

Precipitation titration also has many limitations:

- Only very few halide ions can be titrated by using the precipitation titration method.
- Coprecipitation also occurs.

- The endpoint is very difficult to analyse.

SAQ-3

(i). In the Volhard method, the solution filled in the burette is–

a. Silver nitrate b. Ferric ammonium sulphate
c. Potassium thiocyanate d. Potassium chromate

(ii). Which of the following is/are indicators for precipitation reaction?
a. Potassium chromate b. Silver nitrate
c. Fluorescein ions d. All of the above

2.3 Oxidation-Reduction(Redox) Titrations

- Redox titrations include all those varieties of titrations where one reactant is oxidised and the other is reduced.
- Oxidation is defined as the process of loss of one or more electrons and reduction is the gain of electrons by atoms or ions. Both oxidation and reduction are complementary to one another and take place simultaneously.
- The reagent undergoing reduction is called an oxidising agent (*oxidant*), i.e.an oxidising agent gains electrons and is reduced to lower valency state. eg. Potassium permanganate, Potassium dichromate, Iodine solution etc.
- The reagent which undergoes oxidation is called a reducing agent (reductant) i.e., the reducing agent loses electrons and is oxidised to a higher-valency state. eg. sodium oxalate, ferrous sulphate, ferrous ammonium sulphate, oxalic acid etc.

As we know, the volumetric titration correlation for redox titrationsis represented as

Volume of oxidising agent x Concentration of oxidising agent
= (Volume of reducing agent x Concentration of reducing agent) x (Stoichiometric ratio)

Where,

$$\text{Stoichiometric ratio} = \frac{\text{Number of mols of oxidising agent}}{\text{Number of mols of reducing agent}}$$

If the redox titration involving $KMnO_4$ and $FeSO_4$, the chemical reaction is:

$MnO_4^- + 5Fe^{2+} + 8H^+ \rightarrow Mn^{2+} + 5Fe^{2+} + 4H_2$
(1 mole) (5 moles)

$$\text{Stoichiometric ratio} = \frac{\text{Number of mols of } KMnO_4}{\text{Number of mols of } FeSO_4} = \frac{1}{5}$$

Some typical redox titrations

Titration of Mohr Salt against $KMnO_4$

- Mohr salt is titrated against $KMnO_4$ in the presence of dilute sulphuric acid when $FeSO_4$ of the Mohr salt is oxidised to $Fe_2(SO_4)_3$.
- $KMnO_4$ solution is added to a known volume of Mohr salt solution containing dilute H_2SO_4 gradually in small amounts.
- Rapid addition of $KMnO_4$ results in the formation of hydrated manganese dioxide, $MnO_2.H_2O$, which is brown in colour.

$$KMnO_4 + 3MnSO_4 + 7H_2O \rightarrow K_2SO_4 + 5MnO_2.H_2O + 2H_2SO_4$$
Brown colour ppt

- In these titrations, $KMnO_4$ itself acts as the indicator.

Titration of Oxalic Acid against $KMnO_4$.

- When Oxalic acid, (or an alkali oxalate) is titrated against $KMnO_4$ in the presence of dilute H_2SO_4, keeping the temperature of the oxalic acid solution between 60-70°C.
- Oxalic acid on oxidation with acidified $KMnO_4$ produces CO_2 according to the reaction

$2MnO_4^- + 5C_2O_4^{2-} + 16H \rightarrow 2Mn^{2+} + 10CO_2 + 8H_2O$
(2 mol) (5 mol)

- The solution is kept hot to expel the CO_2 produced so as to avoid the back reaction.
- The stoichiometric ratio in this titration is 2/5.

Titration of $FeSO_4$ against $K_2Cr_2O_7$

- When Ferrous sulphate is titrated against $K_2Cr_2O_7$ in the presence of dilute sulphuric acid using an internal or an external indicator.
- The redox reaction involved in titration is

$$6Fe^{2+} + Cr_2O_7^{2-} + 14H^+ \rightarrow Cr^{3+} + 6Fe + 7H_2O$$

6 mol 1 mol

$$\text{the stoichiometric ratio} = \frac{\text{Number of mols of } K_2Cr_2O_7}{\text{Number of mols of } FeSO_4} = \frac{1}{6}$$

- The internal indicator is diphenylamine which is blue in colour.
- The external indicator used in the above titration is freshly prepared potassium hexacyanoferrate(III), which gives a blue colour with Fe^{2+} ion due to the formation of the complex $Fe_3[Fe(CN)_6]_2$.
- During the titration, drops of the reaction mixture are taken out with the help of a glass rod at intervals and tested with drops of $K_3[Fe(CN)_6]$ placed on a tile.
- The absence of a colour change indicates the absence of Fe^{2+} ions and hence the completion of titration. This is, of course, a rough titration.
- An almost exact end point is obtained in a subsequent titration in which the number of drops taken out from the titration solution is minimum.

2.4 Iodometry and Iodimetry

The reduction of free iodine to iodide ion and the oxidation of iodide ion to free iodine form the basis of iodimetry and iodometric titrations. These are widely used titrations. The advantages of iodine processes are in the exact quantitative nature of the reaction of titration and the sharp endpoint which is obtained.

Iodometric method

- It is an indirect method.
- In this method, iodine is just used as such.
- The oxidizing agent is allowed to react in the acidic medium in the presence of excess soluble iodide. The reaction is as follows.

  iodide ion + oxidizing agent → free iodine

- In such reactions, an equivalent amount of iodine is liberated.
- The liberated iodine is then titrated against a standard reducing agent solution (sodium thiosulphate solution).
- This method is used for estimating cupric ions, dichromates, oxy-halogen compounds etc.

The equation for some of the reactions is as follows:

(i) $2CuSO_4 + 4KI \rightarrow Cu_2I_2 + 2K_2SO_4 + I_2$

(ii) $2KMnO_4 + 3H_2SO_4 \rightarrow K_2SO_4 + 2MnSO_4 + 3H_2O + 5O$

$10KI + 5H_2SO_4 + 5O \rightarrow 5K_2SO_4 + 5H_2O + 5I_2$

(iii) $K_2Cr_2O_7 + 4H_2SO_4 \rightarrow K_2SO_4 + Cr_2(SO_4)_3 + H_2O + 3O$

$6KI + 3H_2SO_4 + 3O \rightarrow 3K_2SO_4 + 3H_2O + 3I_2$

In the above reactions the liberated iodine is titrated against standard sodium thiosulphate solution.

$$2Na_2S_2O_3 + I_2 \rightarrow Na_2S_4O_6 + 2NaI$$

Iodimetric method

- In this method, iodine is directly used as an oxidizing agent either in an acid or in a neutral medium
- This method is used for estimating sulphates, arsenites, ferrocyanide, thiosulphate etc.

iodine + reducing agent → iodide ion

In some cases the oxidation-reduction reactions are given as

(i) $2Na_2S_2O_3 + I_2 \rightarrow Na_2S_4O_6 + 2NaI$

(ii) $Na_2SO_3 + I_2 + H_2O \rightarrow Na_2SO_4 + 2HI$

(iii) $Na_3AsO_3 + I_2 + H_2O \rightarrow Na_2AsO_4 + 2HI$

SAQ-4

(i) Excess of KI reacts with the $CuSO_4$ solution and then $Na_2S_2O_3$ solution is added to it. Which of the statements is incorrect for this reaction?

a) $Na_2S_2O_3$ is oxidised b) CuI_2 is formed

c) Cu_2I_2 is formed d) Evolved I_2 is reduced

(ii) Dichromate ion reduces to _____ ion in an acidified solution of potassium dichromate (VI).

a.) Chromate (V) ions b.) Chromium (III) ions

c.) Chromium (II) ions d.) Chromium (IV) ions

2.5 Complexometric Titration

A complexometric titration is one in which a soluble, undissociated stoichiometric complex is formed when a titrant is added to the sample solutions. The general complexometric titration method involves:

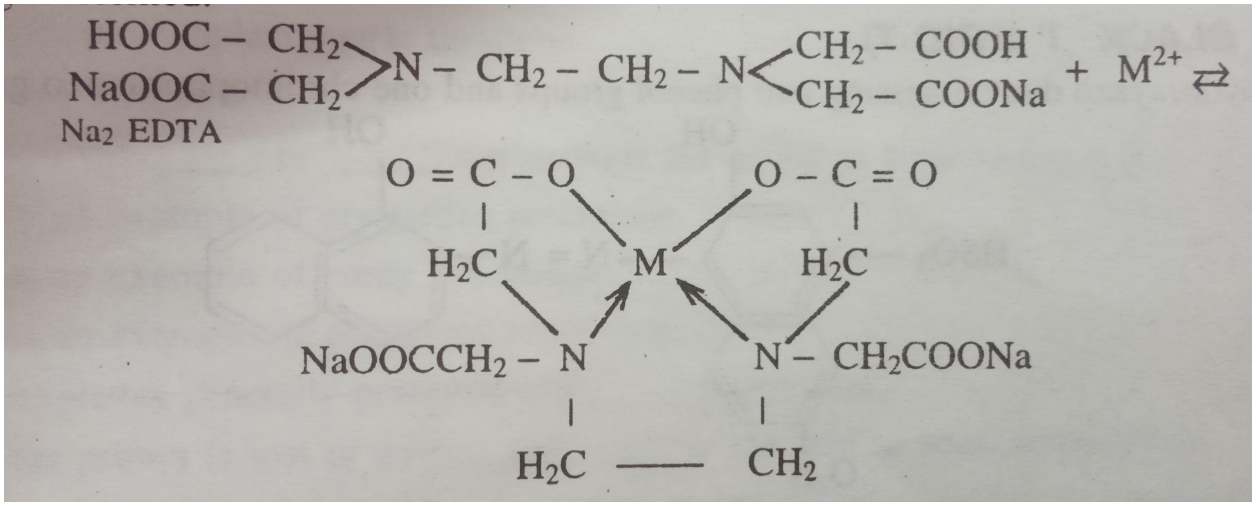
- Choosing a suitable titrant having chelating properties.
- Choosing experiment conditions, such as pH, presence of complexing ligands etc. at which optimum titration is obtained.
- Choosing a suitable method of detection of the endpoint.

Limitations of Complexometric Titration

- The complex is undissociated, it does not suffer from co-precipitation errors.
- Complexing agents coordinate with only certain metals. Hence the method is selective.
- Stoichiometry is not always as clearly defined as in other types of titrations, such as redox, neutralization or precipitation titrations.
- In the case of an organic complexing reagent, solubility properties are taken into consideration.

Reasons for the versatility of EDTA.

- It forms soluble, stoichiometric 1:1 complexes with metal ions.
- Monosodium salt of EDTA is insoluble in water and so disodium salt as the dihydrate is used as a primary standard.
- All the metal EDTA complexes are soluble and form rapidly.
- The end point is readily detectable by wet methods or instrumental methods.
- The titration is suitable for a semi-micro to macro concentration range.
- EDTA is also called ethylenediamine tetraacetic acid.
- The doubly changed metal ion replaces two hydrogen atoms of the carboxyl groups in Na_2EDTA and is simultaneously coordinately bonded to the nitrogen of the amino groups. As a result, a stable five-membered ring is formed.



source: Instrumental Methods of Chemical Analysis by B.K.Sharma

2.6 Use of EDTA for the determination of Ca^{2+} and Mg^{2+}

Determination of calcium with EDTA

Preparation of solutions:

1. 0.1 M calcium solution: Molecular weight of $CaCO_3$= 100.09
   Hence for 0.1M solution 10 gm salt is needed per litre.
2. 0.1 M EDTA solution: Molecular weight of disodium salt of EDTA= 372.25
   Hence for 0.1M solution 9.3 gm of salt is needed for 250 ml solution.
3. Ammonia Buffer Solution: Dissolve 70 gm of ammonium chloride in 570 ml concentrated ammonia and dilute to one litre of distilled water.
4. Indicator Solution: Dissolve 0.4 gm Eriochrome - Black T in 100 ml methanol.

Procedure:

In a 250 ml conical flask, pipette out 10 ml of prepared calcium carbonate solution. Add 20 ml distilled water, 19 ml buffer solution, and 5-6 drops of the indicator solution. Add EDTA solution from the Burette dropwise till the red colour of the solution changes to permanent blue. Repeat the titration to get two concordant values.

Calculation: Calculate as follows:

1 ml of 0.1M EDTA = 4.008 mg of Ca

Determination of Magnesium with EDTA

Preparation of solutions:

1. 0.01 M Magnesium sulphate Solution: Molecular weight of $MgSO_4.7H_2O$ = 246.5
   Hence for 0.01M solution 2.46 gm salt is needed per litre.
2. 0.01M EDTA solution: Weigh 0.93 gm disodium salt of EDTA and prepare 250 ml solution in distilled water.
3. Ammonia Buffer Solution: Dissolve 70 gm of ammonium chloride in 570 ml concentrated ammonia and dilute to one litre of distilled water.
4. Indicator Solution: Dissolve 0.4 gm Eriochrome - Black T in 100 ml methanol.

Procedure:

Take 10 ml. of prepared magnesium solution in a conical flask. To this add 10 ml. buffer solution and 4-5 drops of the indicator. Now run in EDTA solution from the burette gradually till the colour of the solution changes from red to blue and there remains not even a red tinge. Repeat the titration to get two concordant values.

Calculation:

1 ml 0.01 M EDTA = 0.243 mg of magnesium

2.7 Determination of the Hardness of Water

- Water contaminated with Ca or Mg salt is called hard water.
- When impurities are in the form of bicarbonate salts, easily removed by boiling, they are called temporary hard.
- When impurities are in the form of chloride or nitrates etc. , called permanent hardness of water.

Expression for hardness

Since these salts are in trace amounts, hardness is expressed in parts per million (PPM). Although both Ca and Mg may be present, hardness is expressed in the form of only calcium carbonate. This means the number of grams of $CaCO_3$ present in 10^6 grams of water. This is equivalent to the number of milligrams of this salt present in a litre of the sample water (in 1000 gm of water).

2.8 Types of EDTA Titrations

Direct titration:

- It is employed to determine Bi, Zr and other multi-charged cations in an acidic medium, Zn, Ni, Pb and other doubly charged cations in a weak acid medium and calcium and magnesium in a basic medium.
- In a direct titration, the analyte solution containing the cation is placed in a titration flask and a definite amount of ammonia buffer (Ammonia-Ammonium chloride buffer) and an indicator are added.
- Titration is then carried out with Na_2 EDTA until the indicator changes colour.

Back titration

- It is suitable when complex-forming reactions are slow, in the absence of a suitable indicator for the determination, in the analysis of substances that are slightly soluble in water, but soluble in Na_2 EDTA.
- In back titration, an excess of standardized Na_2 EDTA solution, a buffer and an indicator are added to the analyte solution.
- The unreacted Na_2 EDTA is titrated against a standard magnesium or zinc chloride solution or magnesium or zinc sulphate solution.

Indirect titration

- It is based on the formation of the Mg- Na_2 EDTA complex, which is less stable than most of the other cations and can be readily destroyed by adding other ions.
- An exchange reaction takes place with the release of Mg ions, which are then titrated with standard Na_2 EDTA solution.
- The influence of interfering ions can be suppressed by bonding into more stable complexes with additional complex-formation ligands.
- The equivalence point is estimated by using metallo chromic indicators, which are organic dyes.

2.9 Metal Ion Indicators

- Metal ion indicator or metallochromic indicator is a dye that can act as a complexing agent to the metal being titrated.

- The endpoint for an EDTA titration is usually found by using a metallochromic indicator.
- The colour of the indicator depends on whether it is bound or free from the metal ion.
- In favourable conditions the metal-indicator complex formed has an intense colour which is distinctly different from the free indicator.
- While using the metallochromic indicators one must be careful about the pH of the reaction solution because most visual metallochromic indicators, in addition to being complexing agents, are also acid-base indicators. In other words, they are capable of undergoing a colour change with a corresponding change in the pH of the solution.
- A number of metallochromic indicators are available. However, for any such indicator to be used for the visual detection of endpoints in complexometric titration must meet the following requirements. The colour reaction must occur at the end point when nearly all the metal ions are complexed with EDTA.

The indicator must be very sensitive to metal ions so that the colour change occurs as near the equivalence point as possible.

1. The colour reaction should be specific or selective.
2. The colour contrast between the free and the metal-bound indicator complex should be readily observable.
3. The metal-indicator complex must possess sufficient stability else it would not display a sharp colour change.
4. It is desirable that the change in equilibrium from the metal indicator complex to the metal-EDTA complex should be sharp and rapid.

Metal Ion Indicators

Metal ion indicators	**Colour change**	**pH range**	Metals detected
Eriochrome Black T SoloChrome Black T	Red to Blue	6 - 7	Ca, Ba, Mg, Zn, Cd, Mn, Pb, Hg
Murexide	Violet to Blue	12	Ca, Cu, Co
Catechol- Violet	Violet to Red	8 - 10	Mg, Cd, Mn, Pb,
Thymol Blue	Blue to Grey	10-12	Zn, Cd, Pb, Hg
Sodium alizarin sulphonate	Blue to Red	4	Al, Thorium
Alizarin	Red to yellow	4.3	Zn, Co, Pb, Mg' Cu

SAQ-5

(i) Complexometric titrations are useful for the determination?
a. Non-metal ions b. Basic drug
c.Metal ions d. None of the above

(ii) EDTA is a
a. Tetradentate ligand b.Hexadentate ligand
c.Octadentate ligand d.Pentadentate ligand

2.10 Summary

- Quantitative analysis is concerned with the determination of the amount of a chemical substance present either alone or in a sample or complex mixture of other substances. There are two main types of quantitative analytical methods –
  (i) Gravimetric method (ii) Volumetric method

- The volumetric analysis involves the determination of the volume of a solution of accurately known concentration which is required to react quantitatively with the solution of the substance being determined.

- In acid-base titrations, the amount of acid becomes exactly equivalent chemically to the amount of base present. If the acid and base are both strong, the resultant solution will be neutral (pH = 7).

- The titrations which are based on the formation of insoluble precipitates when the solutions of two reacting substances are brought into contact with each other, are called Precipitation Titrations.

- Redox titrations include all those varieties of titrations where one reactant is oxidised and the other is reduced.Oxidation is defined as the process of loss of one or more electrons and reduction is the gain of electrons by atoms or ions. Both oxidation and reduction are complementary to one another and take place simultaneously.

- The reduction of free iodine to iodide ion and the oxidation of iodide ion to free iodine form the basis of iodimetry and iodometric titrations.

- Complexometric titration is one in which a soluble, undissociated stoichiometric complex is formed when a titrant is added to the sample solutions.

- Metal ion indicator is a dye that can act as a complexing agent to the metal being titrated.

2.11 Terminal Questions

1 Why is acid base titration important?
Ans._____
_____
_____
.
2 What are the applications of acid-base titration?

Answer:_____
_____
_____
_____

3 Which indicator is used in acid-base titration?
Answer:_____
_____
_____

4 What are two acid-base indicators?
Answer:_____
_____
_____

5 Define the Equivalence point.
Answer:_____
_____
_____

6 What are the types of complexometric titration?
Answer:
_____
_____

_____
_____

7 What are the applications of complexometric titration?
Answer:
_____
_____
_____

8 What is a complex?
Answer:
_____
_____
_____

9 What are the different types of indicators used in complexometric titrations?
Answer:_____
_____
_____

.10 What is the end point in titration?
Answer:_____
_____
_____

11 What are acid-base indicators?
Answer:_____
_____
_____

12 Discuss the use of Eriochrome Black T (EBT) in EDTA titration.
Answer:_____
_____
_____

13 What is the titration principle of precipitation?
Answer:_____
_____
_____
.
14 What is the significance of precipitation titration?
Answer:_____
_____
_____

15 Which indicators are used in precipitation titration?

Answer:_____
_____
_____
.
16 What is the difference between Mohr Volhard and Fajans methods?
Answer:_____
_____
_____

2.12 ANSWERS

SAQ- 1 (i) a (ii) b
SAQ- 2 (i) a (ii) a
SAQ- 3 (i) c (ii) d
SAQ- 4 (i) b (ii) b
SAQ- 5 (i) c (ii) b

Suggested readings

1. Principles of Physical Chemistry by Puri and Sharma
2. VOGEL'S Textbook of Quantitative Chemical Analysis by J Mendham, R.C. Denney, J.D.Baranes, M.J.K.Thomas
3. Quantitative Analysis by R.A.Day, Jr.A.L.Underwood
4. Basic Concepts of Analytical Chemistry by S.M.Khopkar
5.Instrumental Method of Chemical Analysis by B.K.Sharma

Unit - 3 Gravimetric Analysis

Structure

3.0 Introduction

In the previous unit, we studied volumetric analysis. Now in this unit, we will learn about gravimetric analysis.Gravimetric analysis or quantitative estimation by weight, is the process of isolating and weighing an element or a definite compound of the element in as pure a form as possible. A large portion of the determination in the gravimetric analysis is concerned with the transformation of the element or radical into a stable, pure compound that can be readily converted into a form suitable for weighing.

In gravimetric analysis, the material to be analysed is precipitated by some suitable reagent which after drying is weighed. The different steps involved in the gravimetric analysis are:

1. Precipitation:

The substance whose gravimetric estimation is to be done is taken in the solution in the soluble form. To this solution, we add some of the reagent which precipitates the desired components under certain experimental conditions.

2. Filtration:
After the precipitation of the substance completely, the next step is filtration which simply involves the isolations of the precipitate form quantitatively from the solution. For this purpose we can use filter paper, sintered glass crucible, asbestos mats, platinum mats, etc

.**3. Washing:**
The washing is done in order to remove the undesirable ions sticking on the precipitate which may give higher yields. Washing and filtration are done simultaneously.The excess washing is also harmful as it takes away a slight part of the precipitate to the filtrate.

4. Drying:
The drying of the precipitate is done by placing the funnel containing filter paper with precipitate on the metallic chimney which is kept on a tripod stand with wire gauze.

5. Ignition and Weighing:
The dried precipitate as much as possible is collected on glazed paper and covered with an inverted funnel. Some portion of the precipitate remains to stick on filter paper. Ignite this paper to remove carbon completely and then convert back the precipitate with a suitable reagent. The whole process is called ***ash- treatment.***

Based on determining the mass of a pure compound to which the analyte is chemically related, gravimetric analysis is of three types:

1. Precipitation gravimetry: It is the chemical analysis in which the constituent of the substances in solution is determined by the measurement of weight of the corresponding precipitate.

2. **Electrodeposition gravimetry:** In electrodeposition, the desired constituent is deposited or isolated at an electrode by the passage of an electric current. The weight of the desired constituent is then calculated by the difference in weight of the electrode before and after the electrodeposition.

3. Volatilization gravimetry: In volatilization, the sample is decomposed by a known stoichiometric reaction in which one of the products is volatilized. The amount of vaporized constituent is then calculated by the difference in weight of the electrode before and after the volatilization.

Advantages of gravimetric analysis:

- It is accurate and precise when using modern analytical balances.

- Possible sources of error are readily checked, since filtrates can be tested for completeness of precipitation and precipitates may be examined for the presence of impurities.
- It is an absolute method, i.e. it involves direct measurement without any form of calibration being required.
- Determinations can be carried out with relatively inexpensive apparatus.

Limitations of gravimetric analysis:

- It takes a long time to complete.
- Gravimetric analysis is typically limited to analysing a single element or a small group of elements at a time.
- Methods are frequently complicated, and a minor error in a procedure can often spell disaster for the analysis.
- Gravimetric analysis is based on mass measurement.

Precipitation

When the gravimetric procedure involves precipitation through a chemical reaction, the following important chemical steps are involved in gravimetry:

- The sample being analysed is weighed accurately.
- The weighed sample is dissolved in a suitable solvent.
- Species that interfere with measurement are often removed by using suitable separation methods.
- The experimental environment is adjusted like adjustment of pH is carried out by adding buffer solution, change of oxidation state, concentration or dilution of the sample, or adding masking agents.
- A suitable inorganic precipitating reagent is added.
- Precipitation is usually carried out in a hot dilute solution. Precipitate is then separated from the mother liquor by filtration.
- Precipitation is washed with a suitable solution.
- The dried precipitate formed as a result of ignition is finally weighed and then calculate the percent of substance present in the sample.

Formation of Precipitate

The Precipitate of an electrolyte can be represented as,

$$M^+ + X^- \leftrightarrows MX(s)$$

Example

$$AgNO_3 + . HCl \rightarrow AgCl + HNO_3$$

It is formed by many stages:

(i) Saturation: It occurs when the solution contains the maximum amount permitted by its solubility at specified conditions.

(ii) Supersaturation: It occurs when a solution phase contains more of the dissolved salt than the equilibrium condition requires.

(iii) Nucleation: The initial step from Saturation to Supersaturation is called nucleation, Which is a process that leads to the smallest particles that are capable of spontaneous growth.

(iv) Crystal growth: When a nucleus forms, it shows continuous growth of particles by the deposition of the precipitate. Greater supersaturation produces a much faster crystal growth rate. The greater the supersaturation faster is the rate of crystal growth.

Objectives

After studying this unit, you will be able to understand the following:

- isolation of weighable precipitate.
- know optimum conditions for Precipitation.
- know the precipitation from a homogeneous solution.
- know about the purity of precipitation.
- know the washing and ignition of precipitates.
- know how to remove the impurities from the precipitates.
- calculate the percent of substance in the sample.

3.1 Precipitation from Homogeneous Medium

In the gravimetric methods, the precipitate is obtained by adding the precipitating reagent to the given solution. In such cases, excess of the precipitating reagent is either adsorbed on the surface of the precipitate or forms a soluble complex ion with the metal. Therefore close control of the concentration of a precipitated ion becomes necessary where, So this method will yield incorrect results.

The main aim of the precipitation reactions is the separation of a pure solid phase into a compact and dense form that could be filtered easily. In order to get a precipitate in an analytically desirable form and size, a new technique called **precipitation from the homogeneous medium** was developed which in the supersaturation is held to a minimum. In this technique, the precipitating reagent is not added as such but is gradually and uniformly liberated by a homogeneous chemical reaction within the solution.

Advantages of Precipitation from Homogeneous Medium

- If the two reagents were mixed directly then the extent of supersaturation does not reach as high a value in homogeneous precipitation as would exist.
- The undesirable accumulation of the precipitating reagent is eliminated and the precipitate is formed under the conditions in which the coprecipitation is enormously minimized.
- The precipitate may be obtained in a very dense and easily filterable form.
- By varying the rate of the chemical reaction producing the precipitate in the homogeneous solution, the physical appearance of the precipitate may be altered. It has been found that the slower the reaction, the larger the crystals formed.

In this method, the reagent is generated slowly by a homogeneous chemical reaction within the solution. Then it is allowed to react with the ion to form the precipitate. Such a precipitate is dense and filterable; coprecipitation is reduced to a minimum. A few examples of precipitation from homogeneous solution are:

(i) Oxalates: Calcium oxalate has been precipitated by neutralizing an acid solution of calcium containing excess oxalate by the hydrolysis of urea

.

$$Ca^{2+} + H_2C_2O_4 + CO(NH_2)_2 + H_2O \rightarrow CaC_2O_4\,(s) + CO_2 + 2NH_4^+$$

In this process calcium oxalate may be precipitated even in the presence of magnesium ions and phosphate ions. Thus the crystals of $CaC_2O_4\,H_2O$, precipitated, are much larger and more perfect than those produced in conventional anionic precipitation.

(ii) Sulphates: Sulphonic acid has been used to liberate ions within the solution.

$$NH_2SO_3H + H_2O \rightarrow NH_4^+ + H^+ + SO_4^{2--}$$

Dimethyl sulphate is also a good source of sulphate ions. The hydrolytic reaction has been used for the precipitation of barium, strontium, calcium as well as lead.

$$(CH_3)_2SO_4 + 2H_2O \rightarrow 2CH_3OH + 2H^+ + SO_4^{2-}$$

(iii) Phosphates: Insoluble phosphates may be precipitated by generating the phosphate ion from trimethyl or triethyl phosphate by hydrolysis. Zirconium is precipitated as zirconium phosphate by heating with sulphuric acid.

(iv) Hydrous oxides: These are gelatinous whether formed under ordinary analytical conditions or homogeneously.

Willard and his co-workers have obtained dense precipitates of iron and aluminum by precipitation with urea in the presence of certain anions. The succinate ion is best for aluminum, and formate is best for iron. The precipitates are of indefinite composition but contain basic salts of aluminum and succinate or of iron and formate. Coprecipitation of foreign ions is less than when the hydrous oxides are precipitated by the addition of ammonia.

SAQ-1

(i).Which analytical technique is based on the precipitate weight?

a. Gravimetry b. Precipitation titration

c. Acid-base titration d. Conductometric titration

(ii).Which of the following is similar to electroplating?

a. Volatilization b. Precipitation

c. Electrogravimetric d. Thermogravimetric

3.2 Purity of Precipitation

The accuracy of the gravimetric results depends upon proper precipitation and the purity of the precipitate. The precipitated particles should be in the granular form. They should not be

contaminated with other foreign particles. To achieve these requirements, prior knowledge of some analytical phenomena is necessary.

1. Digestion:

- Digestion is the process of allowing the precipitate to stand for a certain duration on the water bath.
- As a result, the fine precipitated particles have a higher solubility than the larger precipitated particles and tend to pass into solution and then reaggregate on the surface of the larger particles.
- Therefore, It helps to grow the precipitated particles into a more compact and perfectly crystalline form. This reduces the surface area and thus eliminates the loosely adsorbed impurities.
- As a result of digestion, the precipitate undergoes a major degree of self-purification.

2. Co-precipitation:

The contamination of the precipitate with foreign substances which are soluble in the mother liquor is called co-precipitation.

- The process of digestion minimizes the effect of co-precipitation.
- Precipitation should be carried out in dilute solutions.
- The precipitating agent should be added slowly and with constant stirring.
- Precipitation should be carried out in hot conditions depending on the nature of the precipitate.
- The precipitation should be carried out in the presence of an electrolyte. i.e. in the presence of mineral acids.

3. Post-precipitation:

The phenomenon of precipitation of a foreign substance by delayed action after the primary precipitate is formed is known as post-precipitation e.g.

- In Agl an acetate of silver and $BaSO_4$ precipitate in alkali nitrates. Appreciable errors may also be introduced by post-precipitation. This is the precipitation that occurs on the surface of the first precipitate after its formation. It occurs with sparingly soluble substances which form supersaturated solutions.

- They usually have an ion in common with the primary precipitate. e.g.CaC_2O_4 precipitation in the presence of magnesium. The latter separates out as MgC_2O_4 gradually upon CaC_2O_4. The longer the time of contact the greater the precipitation which generally occurs on the surface of the first precipitate.

Difference between Gravimetric and Volumetric analysis:

S.No.	Gravimetric analysis	Volumetric analysis
1.	A type of quantitative analysis, in which we can determine the weight of an unknown compound in a sample.	A type of quantitative analysis, in which we can measure the amount of an unknown compound using its volume.
2.	A precipitate, which can be separated from the sample solution, is formed during the gravimetric analysis process.	Titration is used for volumetric analysis, which involves adding small amounts of a reagent (of known concentration) that can react with the analyte to measure the volume of the analyte.
3.	The final result of a gravimetric analysis is given in grams.	The final result of volumetric analysis is presented in milliliters.
4.	It involves chemical reactions which can precipitate the desired compound.	It involves chemical reactions which can change the color of the chemicals.

3.3. Co-precipitation

The contamination of undesirable constituents with the desired precipitate is called **co-precipitation.** Under normal conditions these impurities are soluble.
eg. nitrates, chlorides, and permanganates of various metals co-precipitate with $BaSO_4$ precipitate. and Copper, iron, phosphoric acid, etc co-precipitate from alloys together with stannic acid precipitate.

On the basis of conditions under which precipitation occurs, these are the following types: Adsorption co- precipitation, occlusion, inclusion, isomorphous coprecipitation, chemical Co-precipitation, and post-precipitation.

1. Adsorption Co-precipitation or surface Co-precipitation

- It is the adsorption of ions, polar molecules, and other contaminants by the surface of the precipitate. eg.

  (i) when ferric hydroxide is precipitated in ammonia solution, the $Fe(OH)_3$ particles adsorb OH^- ions, become negatively charged, and therefore take various cations such as Mg^2, Ca^{2+} , Co^{2+}, Mn^{2+}, Ni^{2+} etc, with them into the precipitate when they coagulate.

  (ii) If ferric hydroxide is precipitated in a slightly acidic solution, the $Fe(OH)_3$, particles adsorb Fe^+ ions and become positively charged. Therefore, the precipitate adsorbs various anions such as CI^-, SO_4^{2-} etc from the solution.
- This effect, when extraneous substances which are not themselves precipitated by a given precipitate under particular conditions are included in the precipitate, is known as co-precipitation.
- Adsorption largely depends on the specific surface of the adsorbent.
- The amount of adsorption depends upon the concentration of the adsorbed ions, temperature, and nature of the adsorbate.
- The amount of adsorption decreases with increasing temperature, an increase in the concentration of a substance, and a decrease in absorbing surface.

2. Occlusion

- It is the carrying down of an impurity in the interior of a particle of precipitate.

- It occurs through the adsorption of the impurity on the surface of the growing crystals.
- Occlusion can also occur from the formation of chemical compounds between the main precipitate and the co-precipitated impurity.
- Crystalline precipitates such as $BaSO_4$, and CaC_2O_4, undergo occlusion most readily.
- Co-precipitation occurring in imperfections like cracks and micro capillaries present in crystals is also known as **internal adsorption.**
- The amount of occlusion varies with the concentration of the impurities in the solution and is largely determined by the order of mixing of the solutions.
- Occlusion is much decreased when precipitation is carried out in a medium that contains an excess of common ions of the compound.

3. Inclusion

It refers to the mechanical trapping of a portion of the solution (and solutes in it) surrounding a growing crystal.

4. Isomorphous coprecipitation

Substances capable of forming mixed crystals are said to be isomorphous. This type of co-precipitation is called **isomorphous co-precipitation or bulk coprecipitation,** in which a joint crystal lattice is formed by two or more substances of different compositions.

5. Chemical coprecipitation

It is the precipitation of substances formed during the chemical interaction of precipitate, together with the main precipitate.eg.

(i) when H_2S is passed through the $PbCl_2$ solution, a black precipitate of PbS is formed. An excess of $PbCl_2$ produces $[PbCl]^+$ complex and as a result white $[PbCl]_2S$ coprecipitates simultaneously with PbS precipitate.

(ii) Similarly, the addition of excess alkali to a mixture of Cr^{3+} and Zn^{2+} ions coprecipitates Zn^{2+} and Cr^{3+} ions as hydroxides and causes the formation of $Zn(CrO_2)_2$ and $Cr(ZnO_2)_3$ precipitates, whereas no CrO_2 and ZnO_2 ions appear in the solution.

SAQ-2

(i). Impurities absorbed in the surface of the precipitate are called

a. Inclusion b. Mechanical Entrapment
c. Surface adsorption d. Post precipitation

(ii). when foreign ions get trapped in a growing crystal is called

a. Inclusion b. Mechanical Entrapment
c. Surface adsorption d. Occlusion

3.4 Post-Precipitation

Post-precipitation is the precipitation of impurities after the main precipitate has been formed. Post-precipitation is due to the adsorption of the reagent on the precipitate formed, chemical interaction, the formation of a solid solution, the fact that the main precipitate starts to serve as nuclei of crystallisation, and so on. It depends upon the following factors:

- It increases with time.
- It increases at higher temperatures.
- It occurs whether the contaminant causing post-precipitation is added to the liquid after or before.

For Example when a mixture of Ca^{2+} and Mg^{2+} ions is treated with $C_2O_4^{2-}$ ions, the less soluble calcium oxalate starts to precipitate. On standing, $C_2O_4^{2-}$ ions adsorb on the surface of the CaC_2O_4 precipitate. As a result, the solubility product of magnesium oxalate is increased, and MgC_2O_4 is post-precipitated on the calcium oxalate precipitate.

Cause of post-precipitation- The post-precipitating substance is supersaturated in the solution from which it separates. The precipitate on which it forms acts as a nucleus on which the precipitation may occur afterward.

Differences between post-precipitation and co-precipitation-

S.No.	Post-precipitation	Co-precipitation
1.	The contamination increases with time as the precipitation is left in contact with the mother liquor.	The contamination decreases somewhat upon aging.
2.	Some degree of contamination is obtained when the impurity is added after the precipitate is formed.	impurity is present during the precipitation.
3.	The contamination may be much greater than in co-precipitation.	It may amount to as much as 100% of the weight of pure precipitate.

3.5 Washing and ignition of precipitates

Washing of precipitates

Washing of precipitate is to remove the contamination on the surface. The composition of the wash solution will depend upon the tendency of the precipitate to undergo peptization. In such cases, water is avoided. A solution of strong electrolyte is employed. This solution should have a common ion with the precipitate in order to reduce solubility errors and should be easily volatilised in the preparation for weighing. Hence ammonium salts are used to wash liquids. Hot solutions are preferred. Wash solutions are divided into three categories as follows:
(i) Solutions that prevent precipitation from becoming colloidal and passing through filter papers e.g. use of ammonium nitrate for washing precipitate of ferric hydroxide.
(ii) Solutions that reduce the solubility of precipitate should be avoided.

(iii) Solutions that prevent the hydrolysis of salts of weak acids and weaker bases.

Washing with many small portions is much more effective than a single

Ignition of Precipitates

The precipitate may contain adsorbed water, occluded water, sorbed water, and essential water as water of hydration. Ignition temperatures are based on the chemical properties of the substance. Heating should be continued till constant weight and should be uniform. The weight of filter paper ash should also be accounted for. The optimum temperature can be ascertained.

Ignition at high temperatures is required for the complete removal of water that is occluded or very strongly adsorbed and for the complete conversion of some precipitates to the desired compound. Gelatinous precipitates such as hydrous oxides adsorb water quite strongly and must be heated to very high temperatures to remove water completely. The ignition of CaC_2O_4 to CaO requires a high temperature for a complete reaction.

SAQ-3

(i)Washing is done to

a.Remove impurities b.Remove Primary absorbed layer

c.Remove Secondary absorbed layer d.All of the above

(ii)Next step after washing of precipitate is

a.Ignition b. Dryness

c.Weighing d.Calculation

3.6 Contamination and their removal

Prevention of co-precipitation

Coprecipitation causes great errors in gravimetry. Following are some important measures that can be used to prevent or decrease coprecipitation.

- Expedient scheme of analysis.
- Reasonable choice of precipitating reagent
- Optimum conditions for precipitation like pH, temperature, rate of addition of precipitant, order of mixing the solutions, etc.
- Retardation of the crystallisation process.
- Removal of coprecipitated ions as stable complexes.

- Rapid separation of the precipitate from the solution in the case of post-precipitation.
- Dilution of the solution in order to reduce the concentration of co-crystallized impurities.
- To wash away the adsorbed impurities and mother liquor from the precipitate.

Reprecipitation

Impurities that have been trapped by the precipitate particles due to occlusion, coprecipitation, and post-precipitation processes are removed by reprecipitation, in which the precipitate, after filtering off and washing, is dissolved in a suitable solvent and the solution is neutralised. Hence the desired ion is reprecipitated with the same or another precipitant.

For example, if the Ca^{2+} ion is precipitated as CaC_2O_4 in the presence of Mg^{2+} a small amount of MgC_2O_4 is coprecipitated. Which is removed by filtering off the precipitate, washing it slightly, and dissolving it in HCl. The resulting solution contains all the Ca^{2+}ions present in the original solution and a small amount of Mg^{2+} ions present initially in it. If acid is neutralised then CaC_2O_4 is reprecipitated, and the Mg^{2+} concentration during the second precipitation is much lower than the first. The resulting CaC_2O_4 precipitate is almost free from MgC_2O_4. If the attempts to reduce co-precipitation or to remove impurities fail, the amount of the impurity co-precipitated with the desired precipitate is determined and the correction is introduced into the result.

Prevention of post-precipitation

In order to prevent post-precipitation, acidities must be regulated very carefully, and ions that are likely to be post-precipitated should be protected by complex ion formation.

The post-precipitation can also be reduced by adding finely divided solids such as broken glass, silica gel, and barium sulphate. The addition of aluminium ions or by adding a sulphur compound such as cysteine the post-precipitation of zinc sulphide on copper sulphide can be slowed down.

SAQ-4

(i). Crystal graphic defect is called

a. Inclusion b. Mechanical Entrapment
c. Surface adsorption d. Post precipitation

(ii). Which error always gives a positive error?

a. Inclusion b. Mechanical Entrapment
c. Surface adsorption d. Post precipitation

3.7 Summary

- In gravimetric analysis, the material to be analysed is precipitated by some suitable reagent which after drying is weighed. The different steps involved in the gravimetric analysis are:
  1. Precipitation 2. Filtration 3. Washing 4. Drying 5. Ignition and Weighing

- Based on determining the mass of a pure compound to which the analyte is chemically related, gravimetric analysis is of three types:
  1.Precipitation gravimetry 2. Electrodeposition gravimetry 3. Volatilization gravimetry

- The main aim of the precipitation reactions is the separation of a pure solid phase into a compact and dense form that could be filtered easily. In order to get a precipitate in an analytically desirable form and size, a new technique called **precipitation from the homogeneous medium** was developed which in the supersaturation is held to a minimum. In this technique, the precipitating reagent is not added as such but is gradually and uniformly liberated by a homogeneous chemical reaction within the solution.

- The accuracy of the gravimetric results depends upon proper precipitation and the purity of the precipitate. The precipitated particles should be in the granular form. They should not be contaminated with other foreign particles.
- The contamination of undesirable constituents with the desired precipitate is called co-precipitation. On the basis of conditions under which precipitation occurs, these are the following types: Adsorption co- precipitation, occlusion, inclusion, isomorphous coprecipitation, chemical coprecipitation, and post-precipitation.

- Post-precipitation is the precipitation of impurities after the main precipitate has been formed. Post-precipitation is due to the adsorption of the reagent on the precipitate formed, chemical interaction, and the formation of a solid solution.

- Washing of precipitate is to remove the contamination on the surface. The composition of the wash solution will depend upon the tendency of the precipitate to undergo peptisation. In such cases, water is avoided. A solution of strong electrolyte is employed.

- Ignition at high temperatures is required for the complete removal of water that is occluded or very strongly adsorbed and for the complete conversion of some precipitates to the desired compound.

- Impurities that have been trapped by the precipitate particles due to occlusion, coprecipitation, and post-precipitation processes are removed by reprecipitation, in which the precipitate, after filtering off and washing, is dissolved in a suitable solvent and the solution is neutralised. Hence the desired ion is reprecipitated with the same or another precipitant.

3.8 Terminal Questions

Q-1: What is the principle of gravimetric analysis?
Answer:_____
_____
_____

Q-2: What is the distinction between gravimetric and volumetric analysis?
Answer:_____
_____
_____

Q-3: What do you mean by precipitation gravimetry?
Answer:_____
_____
_____

Q-4: Name the process that contaminates the precipitates and also carries the precipitate solution containing soluble impurities.
Answer:_____
_____
_____

Q-5: What is the use of the sintered crucible in gravimetric analysis?
Answer:_____
_____
_____

Q-6: What are the limitations of gravimetric analysis?

Answer:_____
_____
_____

Q-7: What conditions must be met in order for the analysis to be accurate?
Answer:_____
_____
_____

Q-8: What is meant by volatilisation gravimetric analysis?
Answer:_____
_____
_____

Q-9: What is meant by the electrodeposition type of gravimetric analysis?
Answer:_____
_____
_____

Q-10: What are the various applications of gravimetric analysis?
Answer:_____
_____
_____

Q-11: What is the phenomenon of Co-precipitation?
Answer:_____
_____
_____

Q-12: What precautions should be taken for proper precipitation?
Answer:_____
_____
_____

Q- 13: What is the process of digestion? How does it reduce the effect of Co-precipitation?
Answer:_____
_____
_____

Q-14: What is the purpose of washing liquid during gravimetric estimation?
Answer:_____
_____
_____

Q-15: How do the precipitation in dilute solution and the addition of a precipitating agent slowly and with constant stirring reduce the effect of Co-precipitation?
Answer:_____
_____
_____

3.9 Answers

SAQ-1 (i) a. (ii). c. SAQ-2 (i). c (ii). D
SAQ-3 (i) d. (ii). a SAQ-4 (i). a. (ii). d

Suggested readings

1. Principles of Physical Chemistry by Puri and Sharma
2. VOGEL'S Textbook of Quantitative Chemical Analysis by J Mendham, R.C. Denney, J.D.Baranes, M.J.K.Thomas
3. Quantitative Analysis by R.A.Day, Jr.A.L.Underwood
4. Basic Concepts of Analytical Chemistry by S.M.Khopkar
5.Instrumental Method of Chemical Analysis by B.K.Sharma
6. Statistical Methods in Analytical Chemistry by Peter C. Meier and Richard E. Zund
7. Modern Analytical Chemistry by David T Harvey

Unit-4: Separation techniques

Contents

4.1. Introduction

Separation techniques are a set of methods used to isolate and purify individual components from a mixture. In various fields such as chemistry, biochemistry, pharmaceuticals, and environmental sciences, separation techniques play a crucial role in analyzing and manipulating substances. The primary objective of separation techniques is to separate the desired component(s) from a complex mixture while minimizing contamination and ensuring high purity. These techniques rely on the differences in physical or chemical properties of the components in order to achieve separation. There are several commonly used separation techniques, each with its own principles and applications. Filtration involves passing a mixture through a porous material or filter, allowing the liquid or gas to pass while retaining the solid particles. This method is used to separate solids from liquids or gases. Distillation is a technique based on differences in boiling points. It involves heating a liquid mixture to vaporize the components and then condensing the vapor back into a liquid, thereby separating substances based on their volatility. Chromatography is a versatile technique that separates components based on their differential affinity for a stationary phase and a mobile phase. There are various

types of chromatography, including gas chromatography (GC), liquid chromatography (LC), and thin-layer chromatography (TLC), each suited for different types of mixtures. Extraction involves separating a desired substance from a mixture using a solvent that selectively dissolves the target component. This technique is particularly useful for separating organic compounds from complex mixtures. Electrophoresis exploits the movement of charged particles in an electric field to separate components based on their charge and size. It is commonly used in biochemistry to separate proteins, DNA fragments, and other biomolecules. Centrifugation employs high-speed spinning to separate components based on their density and sedimentation rate. The denser particles settle at the bottom of the centrifuge tube, allowing for their isolation from the rest of the mixture. These are just a few examples of separation techniques, and there are many other specialized methods available depending on the specific requirements of a given separation process.

Objectives

- To discuss the principle, techniques and application of solvent extraction
- To discuss types of chromatography and its application
- To discuss the tine layer, column, and ion exchange chromatography
- To discuss instrumentation of HPLC and its applications

4.2. Solvents Extraction

Solvent extraction, also known as liquid-liquid extraction or simply extraction, is a separation technique that has been widely used for centuries. Its history can be traced back to ancient times when it was employed for various purposes such as perfume extraction, medicinal plant processing, and metal extraction. The solvent extraction process typically involves several stages, including contact, mixing, settling, and separation. The mixture is brought into contact with the solvent phase, allowing the solute to transfer preferentially to the solvent phase. The two phases are then mixed to facilitate mass transfer. After mixing, they are allowed to settle, and the phases separate based on their densities. The solvent phase, now containing the extracted solute, is further processed to recover the solute, while the aqueous phase may undergo additional treatments or be discarded. The selection of the appropriate solvent, extraction conditions (such

as temperature, pH, and agitation), and equipment depends on the nature of the solute and the desired separation efficiency.

Principle

Solvent extraction is based on the differential solubility of components in two immiscible phases, typically a solvent and an aqueous solution. The process involves the transfer of a solute (the desired component) from one phase to another, resulting in the separation of the solute from the original mixture. The key components involved in a solvent extraction process are:

- **Feed Solution:** This is the initial mixture containing the solute to be extracted.
- **Extractant:** It is a solvent with a high affinity for the solute, enabling its transfer from the feed solution to the solvent phase.
- **Solvent Phase:** It is a non-aqueous phase (often an organic solvent) that acts as the extracting medium.
- **Aqueous Phase:** This phase contains the residual components that are not extracted and remains after the separation.

The principle of solvent extraction can be understood through the following key steps:

- **Contact between Two Immiscible Phases:** The two liquid phases, typically an aqueous phase and an organic solvent phase, are brought into contact to allow for mass transfer. These two phases do not mix or dissolve in each other, forming distinct layers.
- **Distribution of Solute:** When the two phases come into contact, the solute of interest in the original mixture distributes between the two phases based on its solubility in each. The solute can either dissolve more readily in the aqueous phase or the organic solvent phase, depending on its chemical nature and the properties of the phases.
- **Partitioning and Equilibrium:** The solute partitions or distributes itself between the two phases based on the solubility or affinity of the solute for each phase. Equilibrium is established between the solute concentrations in the two phases, depending on the solute's solubility in each phase and the conditions of the extraction process.
- **Extraction of Solute:** The solute is selectively extracted from the original mixture into the solvent phase. This occurs because the solute's solubility or affinity for the solvent phase is higher than its solubility in the aqueous phase.

- **Separation and Recovery:** After the extraction, the two liquid phases are allowed to separate based on their density or by using separation techniques such as settling, decantation, or centrifugation. This enables the isolation and recovery of the solvent phase containing the extracted solute.

The efficiency and selectivity of solvent extraction depend on various factors, including the choice of solvents, the chemical nature of the solute and the original mixture, the pH and temperature conditions, and the presence of other compounds that may interfere with the extraction process.

Techniques:

Solvent extraction is the most widely used method. The extraction of natural products progresses through the following stages:

i. The solvent penetrates into the solid matrix;
ii. The solute dissolves in the solvents;
iii. The solute is diffused out of the solid matrix;
iv. The extracted solutes are collected.

Any factor enhancing the diffusivity and solubility in the above steps will facilitate the extraction. The properties of the extraction solvent, the particle size of the raw materials, the solvent-to-solid ration, the extraction temperature and the extraction duration will affect the extraction efficiency. Solvent extraction is involves the transfer of a solute (substance to be extracted) from one solvent phase into another, where the solute is more soluble. The choice of technique depends on factors such as the nature of the solute, the solvents involved, the required degree of separation, and the desired efficiency.

A system of two immiscible liquid is required for the separation of material by solvent extraction. The active constituent should be unevenly soluble in the system thereby enabling extraction of the constituent from one phase to the other. The efficiency of extraction is determined by Distribution Co-Efficient (D).

$$D = \frac{\text{Total weight (gms.) of solute in the Organic Phase}}{\text{Total weight (gms.) of solute in the Aqueous Phase}}$$

If one of the two liquids contains a solute, this method is found to be more appropriate. The system, in this case is first shaken and then allowed to settle. Some of the solute is transferred to the other liquid. Each of the liquid in a mixture of two immiscible liquids of this kind is mentioned as a phase. Thus, some of the solutes is transferred from one phase to another phase. The amount transferred depends on the relative affinity of the solute for each of the two solvents (Relative Solubility).

The immiscible system may involve two organic solvents. The extraction for this system may be impaired due to formation of emulsion. Solvent extraction is a common technique in forensic toxicology related to biological matrices. Solvent extraction method has now been upgraded and made automated viz. Accelerated Solvent Extraction (ASE). In case of solid non-biological matrices, continuous extraction by a soxhlet may be employed i.e. continuous extraction.

Each technique has its advantages and limitations, and the selection should be based on the specific requirements of the extraction process. The few commonly used solvent extraction techniques are:

- **Simple solvent extraction**:

This is the basic technique where the solute is transferred from one solvent phase to another. It involves mixing the two immiscible solvents (usually an organic solvent and an aqueous phase containing the solute) and then separating the two phases. The solute preferentially dissolves in one of the solvents and can be separated by physical separation techniques such as decantation or centrifugation.

- **Counter-current extraction**:

This technique improves the efficiency of solvent extraction by allowing continuous contact between the two solvent phases. The extraction and stripping (separation of solute from the solvent) stages are performed in a counter-current flow system, allowing for a higher degree of extraction and reduced solvent usage. The simplified explanation of counter-current extraction is that:

Two immiscible liquid phases are introduced into a separation apparatus, such as a liquid-liquid extraction column. The solute-containing liquid mixture is initially added to one of the phases, often referred to as the feed phase.

The feed phase is then brought into contact with the second liquid phase, known as the extracting phase or solvent phase. The two phases are allowed to interact, facilitating the transfer of solutes between them.

As the solutes distribute themselves between the two phases, their concentrations in each phase reach an equilibrium state based on their solubilities and partition coefficients. The solutes preferentially partition into the phase that provides more favorable interactions with the solute molecules. The two liquid phases are then separated, and a portion of the extracting phase, now enriched with the desired solutes, is transferred to the next stage of the extraction apparatus. This transfer is done countercurrently, meaning that the extracting phase flows in the opposite direction to the feed phase.

The process is repeated for multiple stages, with each stage allowing for further partitioning and separation of the solutes. The countercurrent flow of the liquid phases enhances the efficiency of the extraction process by maximizing the concentration gradient and facilitating the transfer of solutes between the phases.

Counter-current extraction is commonly used in various industries, including pharmaceutical, chemical, and biochemical processes.

- **Solid-phase extraction (SPE):**

In this technique, a solid material is used as a stationary phase to extract the solute from a liquid phase. The solid phase, commonly packed in a column or a cartridge, selectively adsorbs the solute, which can later be eluted using an appropriate solvent. Solid-phase extraction (SPE) is a sample preparation technique that involves the selective retention and subsequent elution of target analytes using a solid sorbent.

Retention Step:

Analyte (A) + Solid Sorbent (S) ⟶ [Analyte-Sorbent Complex]

In this step, the target analyte interacts with the solid sorbent, forming a complex or adsorbing onto the sorbent surface. The interaction can be based on various mechanisms such as

hydrophobic interactions, electrostatic interactions, or specific chemical interactions (e.g., affinity-based interactions).

Washing Step:

[Analyte-Sorbent Complex] + Washing Solvent (W) ⟶ [Removal of Interfering Substances]

After the retention step, the sorbent containing the analyte is typically washed with a suitable solvent to remove unwanted matrix components or interfering substances. The washing step helps in improving the purity of the retained analyte.

Elution Step:

[Analyte-Sorbent Complex] + Elution Solvent (E) ⟶ Analyte (A)]

Finally, the retained analyte is eluted from the solid sorbent using an elution solvent. The elution solvent disrupts the interaction between the analyte and the sorbent, allowing the analyte to be released in a purified form.

- **Supercritical fluid extraction**

Supercritical fluids, such as supercritical carbon dioxide, can be used as solvents for extraction. Under specific conditions of temperature and pressure, a supercritical fluid exhibits properties of both liquids and gases, making it an efficient solvent for certain applications. Supercritical fluid extraction is often used for extracting delicate or temperature-sensitive compounds.

- **Microwave-assisted extraction (MAE):**

This technique utilizes microwave irradiation to accelerate the extraction process. The mixture of solute and solvent is exposed to microwave energy, which promotes the solute's release into the solvent. MAE is known for its rapid extraction and high efficiency.

- **Multiple-stage solvent extraction:**

In cases where a high degree of separation is required, multiple stages of solvent extraction can be performed. This involves a series of extraction steps, with each step using fresh solvent and increasing the overall separation efficiency.

Applications

i. Solvent extraction is employed for the purification and separation of organic and inorganic compounds. It is used in the production of fine chemicals, pharmaceuticals, petrochemicals, and dyes.

ii. It is plays a crucial role in the extraction and purification of metals from ores. It is widely used in the mining industry for the recovery of valuable metals such as copper, uranium, gold, and rare earth elements.

iii. It is used for the removal of pollutants and contaminants from soil and water. It aids in the cleanup of contaminated sites and helps in the treatment of wastewater.

iv. It is an integral part of the nuclear fuel cycle. It is used for the separation and purification of uranium and plutonium from nuclear materials.

v. Solvent extraction can be used the analysis of trace metal ions in water samples, solvent extraction can be employed to preconcentrate the metals for subsequent determination by techniques such as atomic absorption spectroscopy (AAS) or inductively coupled plasma mass spectrometry (ICP-MS).

vi. Solvent extraction plays a crucial role in speciation analysis, where the determination of different chemical species of an element is required.

vii. Solvent extraction is commonly used in the analysis of food and beverage samples. It allows for the extraction and concentration of various analytes, such as flavor compounds, additives, pesticides, or contaminants, from complex matrices. This enables their subsequent analysis and determination.

Advantages of Extraction:

- Selective separation of target compounds from complex mixtures.
- Versatility in using different solvents and extraction methods.
- Can be used for large-scale extractions.
- Can concentrate and purify target compounds for further analysis.

4.3. Chromatography

Chromatography is a widely used separation technique in analytical chemistry that is used to separate, identify, and quantify individual components in a mixture. It is based on the

differential distribution of components between a mobile phase (a fluid or gas) and a stationary phase (a solid or liquid). The basic principle of chromatography involves the movement of a sample, called the analyte, through a chromatographic column or surface, where it interacts with the stationary phase. The analyte is separated into its individual components based on their varying affinities for the stationary and mobile phases. There are several types of chromatography, including:

i. Liquid Chromatography (LC):
   a) High-Performance Liquid Chromatography (HPLC)
   b) Gas Chromatography (GC)
ii. Thin-Layer Chromatography (TLC):
iii. Ion-Exchange Chromatography
iv. Affinity Chromatography:
v. Size-Exclusion Chromatography (SEC)

Principle of chromatography

The principle of chromatography is based on the differential partitioning or distribution of components between two phases: a stationary phase and a mobile phase. The separation is achieved by exploiting the varying affinities of the components for these two phases. Chromatography is derived from the Greek word *chroma*, which means “color” and *graphein*, which means "to write". So the word chromatography means "color writing". A Russian botanist M.S. Tswett invented chromatography in 1903, while studying the coloring materials in plant life. The first analytical use of chromatography was described by James and Martin in 1952, for the use of gas chromatography for the analysis of fatty acid mixtures The principle can be explained through the following key steps:

Stationary phase: The stationary means doesn't move. It either a solid or a liquid supported on a thin film on the surface of inert solid, through which the sample contained in the mobile phase percolates.

Mobile phase: This phase flowing over the surface of the stationary phase. The mobile phase may be either a liquid or a gas.

The mobile phase flows through the stationary phase and carries the components of the mixture with it. The various components of the mixture travel at different rates, causing them to separate.

Equilibrium and Partitioning: When the sample is introduced into the chromatographic system, the components interact with both the mobile phase and the stationary phase. Equilibrium is established between the components' distribution or partitioning between the two phases.

Differential Affinities: Each component in the sample has a different affinity or interaction strength with the stationary phase compared to the mobile phase. Some components may have a stronger affinity for the stationary phase and therefore spend more time interacting with it, leading to slower movement through the system. Others may have a stronger affinity for the mobile phase and elute more quickly

Separation: As the sample migrates through the chromatographic system, the components separate based on their differential affinities. The components with stronger interactions with the stationary phase will have a longer retention time, while those with stronger interactions with the mobile phase will have a shorter retention time.

Detection and Analysis: Once the components have passed through the chromatographic system, they are detected and analyzed.

In the chromatography substance are separated due to their relative affinities for the stationary and mobile phase. The distribution coefficient ‘K’ governs the distribution. The fraction with a greater affinity to stationary phase travels slower and at a shorter distance, while that with a lesser affinity travels faster and longer.

$$K = \frac{\text{concentration of a component in moving phase}}{\text{concentration of that component in the stationary phase}}$$

Advantages of Chromatography:

- High separation efficiency and resolution
- Wide range of applications and techniques available.

- Ability to analyze and separate a wide variety of compounds, including volatile and non-volatile substances.
- Possibility for automation and high-throughput analysis in modern chromatographic systems.

4.4. Paper chromatography

Paper chromatography is a type of chromatographic technique that utilizes a specialized paper as the stationary phase. It is a simple and cost-effective method used to separate and analyze components in a mixture based on their differential affinity for the paper and a solvent (mobile phase). The paper chromatography is of two types;

i. Paper adsorption chromatography
ii. Paper partition chromatography

In paper adsorption chromatography, paper immobilized with silica or alumina acts as adsorbent (stationary phase) and solvent as mobile phase. However, in paper partition chromatography, the liquid (mainly moisture) present in the pores of the cellulose fibers of the filter paper act as stationary phase and solvent as mobile phase.

Principle:

Paper chromatography works on the principle of differential migration of components in a mixture between a stationary phase (the paper) and a mobile phase (the solvent). The stationary liquid is called as stationary phase and moving liquid is named as mobile phase or developing solvent. The components in the mixture have varying affinities for the paper and the solvent, causing them to move at different rates along the paper. The components of the mixture migrate with different rates, which is the basis for their separation. The separation can be observed as spots at different positions on the TLC paper.

Components of paper chromatography

The various components of paper chromatography are;

- Stationary phase or paper,
- Mobile phase,
- Chromatographic chamber

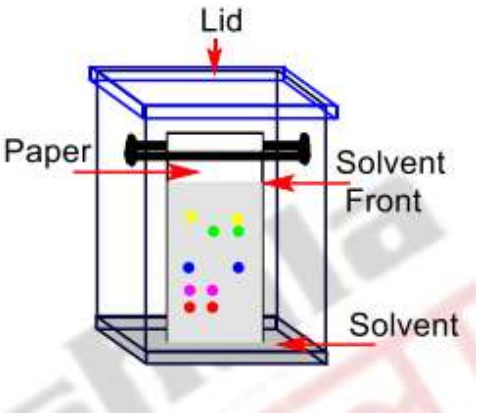
i. **Stationary phase or paper used:** the paper is used as stationary phase. The choice of the filter paper depends upon the nature of β-cellulose, 0.3-1% α98-99% solvent and sample to be separated, type of the analysis i.e quantitative and qualitative, and thickness of the paper.

ii. **Mobile phase:** The polar solvents and buffer solutions are used as mobile phase in paper chromatography. Sometimes, to improve the separation of the components, binary mixtures of the solvents are used. For example; to use hydrophilic mobile phase, mixtures of propanol: ammonia: water, methanol: water and methanol: glacial acetic acid: water can be used as developing solvents.

iii. **Chromatographic chamber:** The chromatographic chamber is commonly made up of glass, and less commonly of plastic or stainless steel. The size of the chamber is variable and can be selected on the basis of length of filter paper used.

Process of paper chromatography

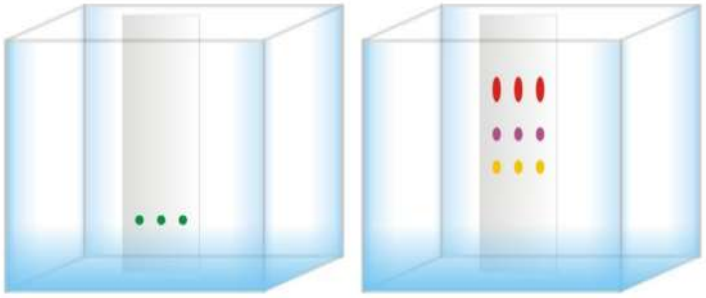
- **Preparation:** A piece of filter paper or chromatography paper is taken and a small spot of the mixture to be analyzed is applied near the bottom of the paper. The spot is generally made using a capillary tube or a fine dropper.
- **Development:** The bottom of the paper is immersed in a solvent, which acts as the mobile phase. As the solvent ascends the paper by capillary action, it carries the components of the mixture with it.



- **Separation:** As the solvent moves up the paper, the different components of the mixture separate into distinct bands or spots. This occurs because each component interacts differently with the stationary phase (paper) and the mobile phase (solvent).
- **Visualization:** Once the solvent front nears the top of the paper, it is removed from the developing chamber. The separated components on the paper can be visualized using various techniques depending on the nature of the components. For example, the paper can be treated with reagents, exposed to UV light, or subjected to thermal activation to make the spots visible.
- **Analysis:** The distance traveled by each component (from the spot to the solvent front) is measured and compared to reference standards or known components to identify and quantify the components present in the mixture.

4.5. Thin layer chromatography

Thin layer chromatography is type of planar chromatography. The important difference between TLC and other chromatography is of particular technique, in place of physical phenomenon like adsorption, partition etc. in TLC the stationary phase consists of a thin layer of sorbent (e.g. silica gel, cellulose powder, alumina) coated on a inert, rigid backing material such as glass plate, aluminum foil or silver foil. As a result, the separation process takes place on a flat 2D surface. TLC is an analytical tool widely used because of its simplicity, relative low cost, high sensitivity, and speed of separation. This analytical technique can be performed as a means of monitoring the progress of a reaction, identify compounds present in a given mixture, and determine the purity of a substance. Characteristics of stationary and mobile phase in TLC are as follows. The stationary phase present in TLC is finally divide powder. Stationary plate spread over supporting material which is made up of glass or aluminium. The thickness of stationary phase should be 250 micrometer and the particle size in stationary phase will be 1-40 micrometer. While the mobile phase is always liquid that is used in TLC. Both polar and non polar liquid can also be used in TLC.

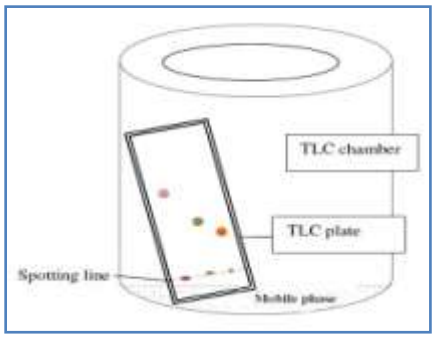


Principle

It is based on the principle of adsorption chromatography or partition chromatography or combination of both, depending on adsorbent, its treatment and nature of solvent employed. In this technique finally divide stationary phase is used and it is spread over a supporting plate and a mobile phase is also used. This mobile phase migrate over these supporting material or stationary phase against the gravitational force. The mechanism behind this migration of mobile phase is the capillary action. During this movement, the compounds with higher affinity to stationary phase travel slowly while the others travel faster. Thus, separation of components in the mixture is achieved. Once separation occurs, the individual components are visualized as spots at a respective level of travel on the plate. Their nature or characters are identified by means of suitable detection techniques.

Experimental

TLC system consists of following components.



- **TLC plate:** TLC plates can be prepared in lab but usually commercially available, with standard particle size ranges to improve reproducibility. These plates are prepared by mixing the adsorbent, such as silica gel, with a small amount of inert binder like gypsum

and water. This mixture spreads as thick slurry on a non-reactive carrier sheet, generally made up of glass, thick aluminum foil or plastic.

- **TLC chamber:** TLC chamber helps to maintain a uniform environment inside for proper development of spots. It also prevents the evaporation of solvents, and keeps the process dust free.
- **Mobile phase:** This comprises of a solvent or solvent mixture which is suitable to separate the chemical mixture. The solvents used should be chemically inert with the sample, a stationary phase.

Methodology

To run a thin layer chromatography plate, three steps - spotting, development and visualization are carried out.

Firstly draw a line using a ruler and pencil approximately 1 cm from the edge of the TLC plate. Put a small spot of solution containing sample with the help of TLC pipettes. Make sure enough sample is spotted on the plate. After the first spot is applied, leave it to dry. The sample should be applied to the same spot three to four time depending on how dilute the sample is never touch the TLC plate on the surface, but carefully held only by the edges. This will avoid possible contamination due to perspiration. Plate is developed in TLC chamber which contain solvent or mobile phase. A small amount of suitable solvent is poured into the chamber to a depth less than 1cm. A moistened filter paper in the mobile phase is placed on the inner wall of the chamber. Close the chamber with a glass cover for a few minute to let the solvent vapors ascend the filter paper and maintain equal humidity. Now placed the TLC plate in the chamber, in such a way that the sample does not touch the surface of eluent in the camber and closed it with a lid. The solvent travel up the stationary phase by capillary action meets the sample mixture and carries it up the plate. Once the mobile phase has reached approximately two-third up the plate, remove it from the chamber and let it dry. Mark the solvent front immediately. In Visualization process the simple is visualized colored compounds, as the spots can be directly observed after development. The silica gel on the TLC plate is impregnated with a fluorescent material that glows under ultraviolet (UV) light and dark spots will appear on a glowing background. These dark spots should be circled with a pencil to mark their locations. Sometimes it is not possible to see the spots on the plate as these are UV inactive compounds. For these

types of compounds, a number of chemical stains can be used. Iodine is among the most common stains. Most organic compounds will form a dark-colored complex with iodine and dark brown spots will appear on the plate.

Any individual solute (steroids, drugs, dyes etc) will move by a constant ratio with respect to the solvent front, under constant condition of temperature, solvent system and adsorbent. This is known as retardation factor or R_f value.

$$\mathbf{R_f} = \frac{\textit{distance travel by solute molecule from origin}}{\textit{distance travel by solvent front from origin}}$$

Applications

i. TLC can be used to check the purity of the sample by comparing it with standard or authentic sample. If any impurity is present, then it shows extra spots and this can be detected easily.

ii. Thin layer chromatography can be used in purification, isolation and identification of natural products like volatile oil or essential oil, fixed oil, waxes, alkaloids, glycosides, steroids etc. In spite of these TLC is employed to identify the compound of interest in mixture, extracted from plant, animal or microbial sources by running the TLC of both standard and extracted sample together and matching their Rf value.

iii. It is extremely useful in isolation, separation and characterization of biochemical metabolites or constituent from its body fluids, blood, plasma, serum, urine etc.

iv. It is used for separation and identification and characterization of colors, various cosmetic products, preservatives and sweetening agent. It will perform easily as it does not require any sophisticated equipment and is also time-efficient.

v. TLC can perform to monitor the progress and rate of reaction at particular intervals. To determine this it is observed that at the beginning of a reaction the entire spot is occupied by the starting chemicals or materials on the plate. As the reaction starts taking place the spot formed by the initial chemicals starts reducing and eventually replaces the whole spot of starting chemicals with a new product present on the plate. The formation of an entirely new spot determines the completion of a reaction.

vi. Quantitatively, TLC is used to monitor the purity of several drugs, including sedatives, antihistamines, analgesics, tranquilizers, and steroids and separate different metabolites present in a drug. TLC can be used to identify the presence of drug residues and antibiotics in food, such as poultry, beef, pork, milk, and fish among others.

vii. TLC can be effectively used in forensic studies where body fluids, such as urine and blood can be tested for the presence of drugs and it nature. For example acidic and neutral drugs are identified by using octadecyl silica in the stationary phase, while plain silica and octadecyl silica are used to identify basic drugs.

A special application of TLC is in the characterization of radio labeled compounds, where it is used to determine radiochemical purity.

4.6. Column Chromatography

As discussed earlier, column chromatography was developed by American Chemist D.T. Day in 1900 and M.S. Tswett the Polish botanist in 1906. It is the most useful and common method for the separation and purification of solids as well as liquids. It involves use of solid stationary phase and liquid mobile phase. The separation is based upon the distribution of the components of a mixture between stationary phase and mobile phase. The separation process occurs as the sample is carried down the column by a mobile phase, typically a solvent or a mixture of solvents. Different compounds in the sample interact differently with the stationary phase due to variations in their polarity, size, and other properties. This leads to differences in the rate of movement of the compounds through the column, resulting in their separation.

Principle

The principle of column chromatography is purely adsorption based; the column chromatography involves the passage of a mixture of compounds, called the sample or analyte, through a column packed with a stationary phase material. The stationary phase can be a solid adsorbent or a liquid immobilized on a solid support. Silica gel and alumina are commonly used solid stationary phases, while liquid stationary phases include hydrophilic or hydrophobic polymers.in which components are separated on differential adsorption basis. When sample with various components is introduced in the column, the rate at the components move downward is different. The components with lower affinity for the adsorbent move faster and eluted rapidly while the components with higher affinity for the adsorbent move slowly and eluted in the end. This results in the separation of the components.

Components of column chromatography:

- **Stationary phase:**

The stationary phase in the column chromatography is also termed as adsorbent because it works of the principle of adsorption. A stationary phase must fulfill the following requirements. Particles should have uniform size with spherical shape. 2) The adsorbent should be stable chemically as well as mechanically. 3) It should be inexpensive and easily available. 4) It should be used with wide range of compounds. The stationary phase is selected on the basis of various factors like; nature of the components, length of the column, adsorption affinity of the various components, and quantity of adsorbent required for separation.

- **Mobile phase**

The mobile phase in column chromatography acts performs various functions i.e to introduce sample into the column, to develop the bands in the column (as developing solvent) and to elute the components from the column.

The main points to be remembered during the selection of mobile phase are; 1) The components of a sample must be soluble in the solvent system chosen. 2) It should be of low boiling point so that components can be recovered at the end. 3) The polarity should be appropriate. Some of polar solvents used in column chromatography are petroleum ether, cyclohexane, acetone, carbon tetrachloride, toluene, esters, benzene, water etc.

- **Column**

A column is a cylindrical tube made up of glass. It is required for filling the stationary phase. The material used for the fabrication of column should be inert towards various solvents and the components to be separated. The columns may be attached with reservoir at the top and collector at the bottom to make the process easier. A column can have dimensions of 10:1, 30:1 and 100:1, length: diameter.

Steps involved in column chromatography

- **Packing the Column:** Choose an appropriate column size (glass or plastic) and add a layer of glass wool or cotton at the bottom to act as a support. Fill the column with a stationary phase material, such as silica gel or alumina, which is usually pre-washed and dried. The stationary phase should be packed uniformly without any air pockets.

- **Loading the Sample:** Dissolve the mixture you want to separate in a suitable solvent and add it to the top of the column. This is typically done using a pipette or a syringe. The sample is adsorbed onto the stationary phase.
- **Elution:** Begin the elution process by adding an eluting solvent to the top of the column. The eluting solvent should be chosen based on the polarity of the compounds in the mixture. The most polar compound will elute slower, while less polar compounds will move faster through the column.
- **Collecting Fractions:** As the solvent passes through the column, different compounds in the mixture will move at different rates. Collect the eluted fractions in separate containers, usually test tubes or vials, in a systematic manner.
- **Analyzing Fractions**: Once all the fractions are collected, analyze them individually to determine the purity and identify the desired compound. Common analytical techniques include TLC, spectroscopy (UV-Vis, IR, NMR), and mass spectrometry.
- **Combining Fractions:** If desired, fractions containing the desired compound can be combined based on the analysis results. This can increase the concentration of the desired compound and reduce the volume for further processing.

Application

Column chromatography has a wide range of applications in various fields, including organic chemistry, biochemistry, pharmaceuticals, and natural product isolation

i. Column chromatography is extensively used to purify natural products such as alkaloids, flavonoids, and terpenoids.

ii. Column chromatography is used to separate complex mixtures of organic compounds into individual components. For example, it can be used to isolate specific isomers or enantiomers of a compound.

iii. This chromatography is used extensively in the development and production of pharmaceuticals. It can be used to purify drug candidates, separate impurities, and ensure the purity and potency of final products.

iv. This chromatography is used in the purification of proteins and other biomolecules.

v. This chromatography is used to analyze environmental samples such as soil, water, and air for the presence of pollutants and other contaminants.

vi. This chromatography is used in forensic laboratories to separate and identify drugs and other substances found at crime scenes.

vii. This chromatography is used in quality control of various products such as food, cosmetics, and industrial chemicals.

Advantages

i. Column chromatography can be performed using various stationary and mobile phases, making it a versatile technique. Different column types, such as normal phase, reversed phase, or ion exchange, can be employed depending on the specific requirements of the bullet analysis.

ii. Column chromatography can be easily scaled up or down to accommodate different sample sizes. This flexibility makes it suitable for handling both small-scale laboratory analyses and larger-scale forensic investigations.

iii. Column chromatography can be a relatively inexpensive technique compared to other separation methods, such as high-performance liquid chromatography (HPLC) or gas chromatography (GC). It requires simple equipment and consumables, which can reduce the overall cost of analysis.

Disadvantages

i. Column chromatography is generally a time-consuming process. It requires the loading of the sample onto the column, elution with the mobile phase, and subsequent collection of fractions.

ii. Due to the time-consuming nature of column chromatography, the throughput is relatively low. The technique may not be suitable for analyzing large numbers of bullet samples quickly, especially in high-volume forensic laboratories where rapid analysis is crucial.

iii. The success of column chromatography heavily relies on the expertise and skill of the operator. Factors such as column packing, sample loading, and elution conditions need to be carefully controlled to achieve optimal separation. Inexperienced operators may encounter difficulties and obtain inconsistent results.

iv. During the chromatographic process, there is a possibility of sample loss or degradation. Inadequate handling or improper elution conditions can lead to the loss of valuable analytes

or the degradation of compounds of interest, affecting the accuracy and reliability of the analysis.

4.7. High Performance Liquid Chromatography

HPLC stands for high-performance liquid chromatography. As its name suggest, high performance means that this analytical technique used for proper separation, identification and for quantification of components in a mixture**.** HPLC is an instrumental form of liquid chromatography and gives high performance due to the small particle size of the stationary phase. The particle size of stationary phase is 3.5 to 10 micrometer. Due to smaller size, surface area of the particle is high and ultimately HETP (height equivalent to theoretical pressure) increased and thereby help in achieving more efficient separation of the components of the mixture than those used in conventional liquid chromatography.

In this chromatography particle size of stationary phase is small and due to small size, packing of stationary phase will be high. Due to tight packing flow rate of mobile phase is reduced. So that to increase the flow rate or to increase efficiency or to increase the separation of the mixture high pressure is applied. The applied pressure is about 1000-4000 psi. Because of the use of high pressure in this technique, it is sometimes also known as high pressure liquid chromatography. So we can say HPLC is modern application of liquid chromatography.

HPLC has the ability to separate, and identify compounds that are present in any sample that can be dissolved in a liquid in trace concentrations as low as parts per trillion. Because of this versatility, HPLC is used in a variety of industrial and scientific applications, such as pharmaceutical, environmental, forensics, and chemicals.

Principle

HPLC is highly automated and extremely sensitive technique as compared to column chromatography because the components of a mixture are separated from each other due to their different degrees of interaction with the absorbent particles. This causes different elution rates for the different components and leads to the separation of the components as they flow out the column. Solvents are used as mobile phase. In general organic compounds are analyzed using HPLC and these organic compounds are soluble in polar solvents. Some commonly used solvents as mobile phase are methanol, acetonitrile and water. Acidifiers or basifies or buffer

solution are added to the solvent to achieve better separation. As these neutralize the ionized analytes or compounds present in the column. If analyte or compounds present in column will be ionized so affinity with stationary phase will be lost and proper separation does not take place. Means every analyte immediately come with the mobile phase. Particle size of stationary phase ranges 3.5 to 10 micrometer. There are two types of stationary phase. i) Normal stationary phase for example silica gel is used and ii) Reversed stationary phase for example octa decyl silane silica gel etc. There are generally two types of columns are used. Normal phase column and reversed phase column.

Instrumentation:

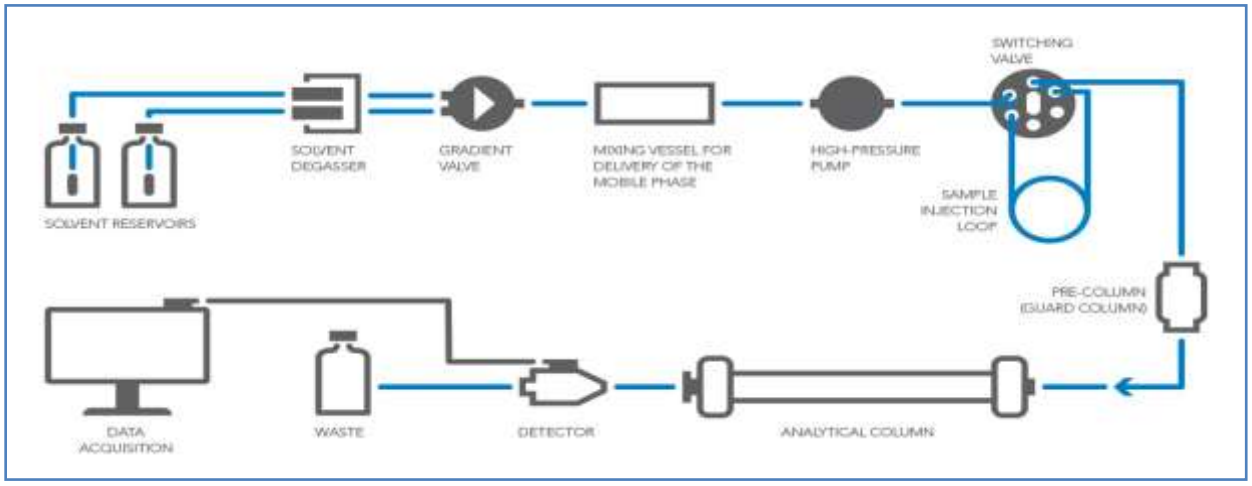


Fig.2.4: Diagrammatical representation of HPLC

The main components of HPLC are as follows:

1. **Solvent Reservoir:** The solvent reservoir holds the solvent, which is referred to as the mobile phase. Two types of reservoir are used.
    a. **Binary system:** In this type two reservoir are present.
    b. **Quaternary system:** In this type four reservoir are present.

In HPLC we use specific type of solvent that are filtered known as HPLC grade solvent. This solvent is highly pure.

2. **Degasser:** It is used to remove the gases which are dissolved in mobile phase or in solvent. Degassing is done by using vacuum pump. So vacuum pump remove the any type of gas or air present in solvent and make it suitable for better separation. If these gases interact with

mobile phase and go to the column, proper functioning of the column does not take place i.e. proper separation may hindered.

3. **Solvent Mixing Valve:** This valve used to mix the solvent. If we use binary system two solvents will get mix together and if we use quaternary system, four solvents mix together.
4. **HPLC Pump:** Two types of pumps are used.
    a. **Constant pressure pump:** Pressure of the pump is constant so that flow rate may vary and this will not do proper separation.
    b. **Constant flow rate pump:** Flow rate is constant and pressure may be changed. So according to our column resistance pressure will be changed and due to that changed pressure flow rate of the column does not affected. Generally 1 ml/min flow rate is used in HPLC.
5. **Precolumn or Guard Column:** HPLC used precolumn or guard column to remove impurity of the solvent. As the name suggest precolumn, means it is used before the analytical column. As stationary phase is same in guard column. Solvent first go through the guard column after that it will go to the analytical column. So any type of contamination present in the solvent is removed by this column, before it go to analytical column. Size of the guard column is low as compared the analytical column. Internal diameter is 4-5 mm and length is 2-10 cm.
6. **Sample Injector: Three types of sample injectors are used.**
    a. **Septum Injector**: In this type one auto selling in present and solvent is injected using micro siring.
    b. **Stop flow septum less injector:** In this type of injector firstly we have to stop the mobile phase then we open the upper part of the column and after that we put the sample here and again start the flow of mobile phase.
    c. **Microvolume** sampling injector: This is the modern and sophisticated injector. By using this Injector highest accuracy will be achieved and auto sampling technique is applied by using this method
7. **Analytical Column:** Length of analytical column is 10-30 cm and internal diameter is 4-5 mm. this column is made up of stainless steel which can resist the high pressure used in HPLC. During packing of analytical column 14000-15000 psi pressure are applied.
8. **Detector:** It is used to separate compound bands as they elute from the high pressure column. There are several types of detector used in HPLC. Some of them are as follows.

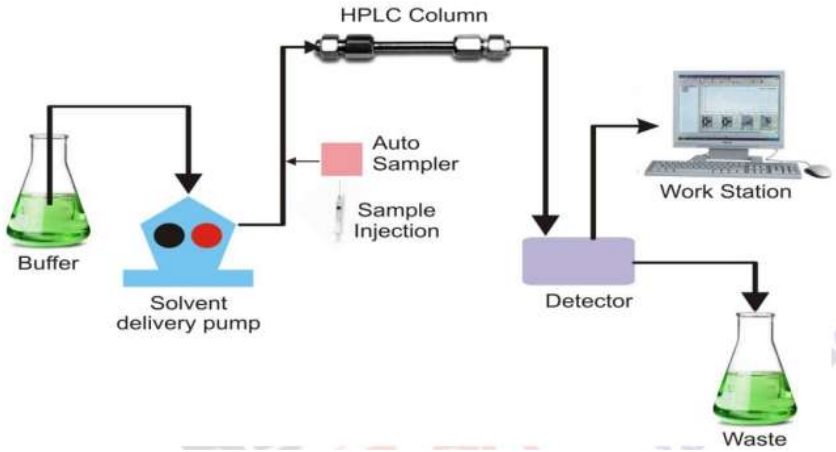
i. **Bulk property detector:** Like refractive index detector.
ii. **Solute property detector:** Like florescence detector, UV detector.
iii. **Multipurpose detector:** It is combination of 2 or 3 types of detector.
iv. **Electrochemical detector:** Like colometric, emperometric detector.
v. **Mass detector:** It is also known as LCMS. It is highly sensitive and most commonly used detector in HPLC.

The information is sent from the detector to a computer which generates the chromatogram. The mobile phase exits the detector and is either sent to a waste, or collected, as desired.

Methodology

To run HPLC following process goes on.

- The sampler brings the sample mixture to be evaluated into a stream of mobile phase which is flowing at a defined pressure.
- Now the injected mixture does flow over the stationary phase inside the column under the influence of pressure along with the mobile phase.
- During this flows based on the affinity of individual compounds in the mixture towards stationary and mobile phase, some compounds get eluted out of the column first before others.
- Outside the column they are sent into a detector where individual compounds are detected and recorded in computer installed chromatography software.
- The recordings (preferably in the form of quantitative peaks) are compared with those of standard compound's HPLC values, and the individual compounds are identified. So the overall theory of HPLC is relative separation and detection of compounds.



Application

i. HPLC can be use in detection of phenolic compounds and other contaminants present in drinking water. It also behaves as bio-monitor of pollutants.

ii. It is used to determine quantify of steroids, cocaine and other drugs in blood, urine etc. Forensic analysis of textile dyes can also be performed by using this technique.

iii. It helps in measurement of quality of soft drinks and water, sugar analysis in fruit juices, polycyclic compounds analysis in vegetables. Preservative analysis can also be done by this technique.

iv. It is used in Urine analysis, antibiotics analysis in blood. Many disorders related to body metabolism, those related to endocrine and exocrine gland secretion, alteration in body fluids are diagnosed by HPLC analysis of concerned fluids. For example, detection of bilirubin, biliverdin in hepatic disorders and endogenous Neuropeptides in extracellular fluid of brain can be performed by using HPLC.

v. It is a mandatory tool in most of the scientific research such as medical, biological, chemical, biochemical, and photochemical research. This technique finds its major application to analyze and quantify the molecules and can easily distinguished the components with similar chemistry and properties.

vi. It can be used to control drug stability and pharmaceutical quality. HPLC also analyzed any new molecule under development or in a preclinical trial to see their concentration in the blood after certain intervals of administration. This helps to evaluate the metabolic profile, plasma concentration, bioavailability, etc. of the formulation or chemical moieties under development.

4.8. Ion exchange chromatography

As it name suggest ion exchange chromatography, means ion exchanger resin will be there and due to presence of that resin ion exchange will take place i.e. sample is ionized and that sample will exchange with counter ion present in stationary phase and ultimately separation will take place. This technique separates charged or polar molecule in a mixture. Only hydrophilic molecules can be separated out from this technology.

In this type of chromatography separation occurs as a result of formation of ionic or electrostatic bond between the charged group of biomolecules and an ion exchange resin bearing opposite charge. Ions exist in a state of equilibrium between the mobile phase and stationary phases which give rise two possible formats, anion and cation exchange are referred to as counter ion (Fig. 2.1). These exchangeable matrix counter ions may include protons (H^+), hydroxide groups (OH^-), single charged mono atomic ions (Na^+, K^+, Cl^-), double charged mono atomic ions (Ca^{2+}, Mg^{2+}), and polyatomic inorganic ions (SO_4^{2-}, PO_4^{3-}) as well as organic bases (NR_2H^+) and acids (COO^-). Cations are separated on cation-exchange resin column and anions on an anion exchange resin column.

It is one of the most important adsorption chromatography, performed for separation of peptides, proteins, nucleic acids and related biopolymers which have different molecular sizes and molecular nature with electronic charge. Advantage of using ion chromatography is that only one interaction involved during the separation as compared to other separation techniques; therefore, ion chromatography may have higher matrix tolerance.

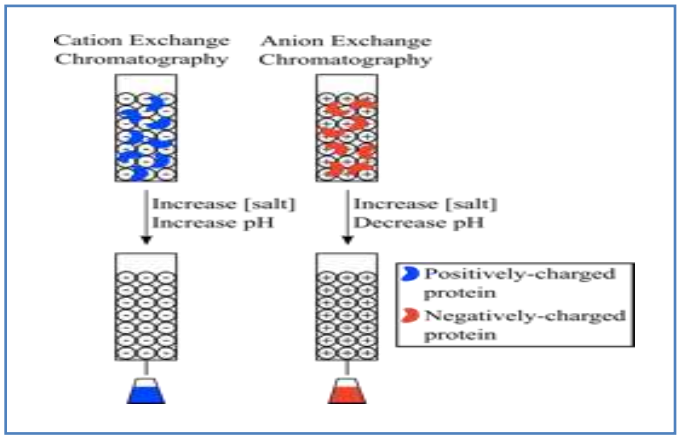


Fig. 2.1: Diagrammatical representation of ion exchange chromatography.

Stationary phase is solid and have resin polymers forming network. Most commonly used cellulose, agarose and polymethacrylate. These stationary phases consist of an immobile matrix which covalently bound to charged molecules. Mobile phase is liquid containing sample that is to be separated.

Principle

Ion exchange chromatography is based on the reversible electrostatic interaction of charged species with the ion exchange matrix and ultimately separation takes place. On the basis of ions separated, the ion exchange chromatography can be divided into two categories.

a. Anion Exchange Chromatography:

$$\text{Regin-OH}^- + \text{A} \longrightarrow \text{Regin-A} + \text{OH}$$

(in solution) (in eluting solution)

Anion exchanges regin

When molecule of interest is negatively charged then anion exchange chromatography is used. In this process anion in a mobile (liquid) phase exchanges with another anion that is previously bound to a positively charged solid support or matrix. Commonly used anion exchange resins are Q-resin, a Quaternary amine; and DEAE resin, Diethylaminoethane. Anion exchange chromatography is used both for preparative and analytical purposes and may also be usedchromatographically, to separate anions and medicinally
to remove an anion from gastric contents or bile acids in the intestine.

b. Cation Exchange Chromatography:

$$\text{Regin-H}^+ + \text{M}^+ \longrightarrow \text{Regin-M}^+ + \text{H}^+$$

(in solution) (in eluting solution)

Using cation exchange regin

Cation exchange chromatography is used when the desired molecules to separate are cations in mobile phase. Positively charged molecules are attracted to a negatively charged solid support. S-resin, sulfate derivatives; and CM resins, carboxylate derived ions are commonly used as cation exchange resins.This type of chromatography is used both for preparative and

analytical purposes and can separate a large range of molecules from amino acids and nucleotides to large proteins.

Instrumentation

Typical Ion exchange chromatography instrumentation includes the following components.

1. **Pump:** It is one of the most important components in the ion exchange chromatography system which has to provide a continuous constant flow of the eluent through the IC injector, column, and detector. The constant-flow pump is the most widely used.
2. **Injector:** Sample is injected in column by using injection valve. Liquid samples may be injected directly and solid samples need only to be dissolved in an appropriate solvent. Injectors should provide the possibility of injecting the liquid sample within the range of 0.1 to 100 ml of volume with high reproducibility and under high pressure (up to the 4000 psi).
3. **Columns:** Column is made up of stainless steel, titanium, glass or an inert plastic according to its ultimate use. The column can vary in diameter from about 2mm to 5 cm and in length from 3 cm to 50 cm depending on whether it is to be used for normal analytical purposes, microanalysis, high speed analyze or preparative work.
4. **Guard Column:** It is placed anterior to the separating column. This serves as a protective factor that prolongs the life and usefulness of the separation column. They are dependable columns designed to filter or remove particles that clog the separation column
5. **Suppressor:** This device reduces the background conductivity of the chemicals used to elute samples from the ion-exchange column which improves the conductivity measurement of the ions being tested. It is membrane-based devices which are designed to convert the ionic eluent to water as a means of enhancing the sensitivity.
6. **Detectors:** Detector used in ion exchange chromatography has wide range to run instrument both on analytical and preparative scale. Generally electrical conductivity detector is used.
7. **Data system:** In routine analysis a pre-programmed computing integrator may be sufficient but for higher control levels, a more intelligent device is necessary, such as a data station or minicomputer.

Methodology

Separation through ion exchange chromatography carried out in a column packed with ion exchange. Generally these ion exchangers are commercially available, made up of styrene and divinyl benzene. Selection of ion exchanger depends upon charge of particle to be separated. To separate anion "Anionic exchanger" for example DEAD-cellulose is used, whereas to separate cations "Cationic exchanger" such as CM-cellulose is used. The following four steps are involved in the separation process.

1. **Equilibration:** The first step is the equilibration of the stationary phase to the desired start condition. When equilibrium is reached all stationary phase charged group associated with exchangeable counter ions such as chloride or sodium.
2. **Sample application and wash:** The second step is sample application and wash. The column is filled with ion exchanger then the sample is applied followed by the buffer. Generally tris-buffer, pyridine buffer, acetate buffer, citrate and phosphate buffers are used. The sample buffer should have the same pH and ionic strength as the starting buffer in order to bind all appropriated charged molecule. The goal in this step is to bind the target molecule and wash out all the unbounded materials.
3. **Elution:** In the third step elution, biomolecules are released from the bioexchanger by a change in buffer composition. A common way is to increase the ionic strength with sodium chloride, or another simple salt, in order to desorb the bound molecule. The particles which have high affinity for ion exchanger will come down the column along with buffers.
4. **Regeneration:** The final step is regeneration, removes all molecules still bound. This ensures that the full capacity of the stationary phase is available for next run.

Application

i. Ion exchange chromatography is most effective method for water purification. As hard water is one of the common problems in most parts of the world and it is necessary to make hard water soft for drinking purpose. The calcium and other salts present in water are removed by this method.

ii. Complete deionization of water or a non-electrolyte solution is performed by exchanging solute cations for hydrogen ions and solute anions for hydroxyl ion.

iii. An important use of ion-exchange chromatography is the routine analysis of amino acid mixtures. The 20 principal amino acids from blood serum or from the hydrolysis of proteins are separated in a few hours, and their concentrations are determined automatically as the amino acid "Autoanalyzer" is based on in exchange principle.

iv. Separation of many vitamins, other biological amines, carbohydrates, nucleotides, Proteins and organic acids and bases etc are performed with the help of ion exchange chromatography.

v. In biochemistry ion exchange chromatography used for separation of drugs and metabolites from blood, urine, etc which find application in clinical diagnosis. This separation technique effectively used in purification of enzymes after extracting from the tissues.

4.9.Summary

Extraction is a process used to separate a desired compound or analyte from a complex mixture. It involves selectively transferring the target compound from one phase (usually a liquid) to another phase. The separation is based on differences in solubility, polarity, or other properties between the target compound and the surrounding mixture. Common extraction methods include liquid-liquid extraction and solid-phase extraction. Extraction is widely used in various fields, including pharmaceutical analysis, environmental monitoring, and forensic investigations. Chromatography is a broad term that encompasses several techniques used for separation and analysis of mixtures. It involves the distribution of components between a stationary phase and a mobile phase. The different components of the mixture interact differently with the stationary and mobile phases, leading to their separation based on various physical and chemical properties. Common types of chromatography include gas chromatography (GC), liquid chromatography (LC), and thin-layer chromatography (TLC). Chromatography is widely used in analytical chemistry, biochemistry, pharmacology, and other scientific disciplines for qualitative and quantitative analysis, identification of compounds, and purification purposes. All types of chromatography work on the same principle and used mobile phase and stationary phase separation of biological samples. In thin layer chromatography the stationary phase is used and it is spread over a supporting plate and a mobile phase is also used. This mobile phase migrate over these supporting material or stationary phase against the gravitational force. In ion exchange chromatography, separation occurs as a result of formation of ionic or electrostatic bond between

the charged group of biomolecules and an ion exchange resin bearing opposite charge. HPLC has the ability to separate, and identify compounds that are present in any sample that can be dissolved in a liquid in trace concentrations as low as parts per trillion HPLC used for identification and for quantification of components in a biological mixture.

4.10. Terminal Question

Q.1. Write the about solvent extraction methods.

Answer: ----------

What is chromatography, write the principle and application of chromatography

Answer: ----------

Q.2. Write the principle and instrumentation of HPLC.

Answer: ----------

Q.3. Discuss the working principle of paper chromatography

Answer: ----------

Q.4. Discuss the working principle of ion exchange chromatography.

Answer: ----------

Q.5. Write the principle of thin layer chromatography.

Answer: ----------

4.11. Further reading

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UNIT 5: NANO CHEMISTRY

Structure

5.1 INRODUCTION

Nanotechnology is the science and engineering of making materials, functional structures and devices on the order of a nanometer scale. In scientific terms, "Nano" means 10^{-9} m, where 1 nanometer (nm) is equivalent to one thousandth of a micrometer (μm), one millionth of a millimeter, and one billionth of a meter. In Greek, "nanotechnology" derives from the *nanos* which means dwarf and *technologia* means systematic treatment of an art or craft. In this unit you should come to know about the nano-materials properties, synthetic methods, characterization methods and applications.

Objectives

- Why the nanoscale is a magical point on the dimensional scale
- The reasons why nanoscale has become so important
- Synthetic methods
- Characterizations tools (XRD, TEM, SEM, AFM, etc.) for Nanomaterials
- Applications

5.2 NANOMATERIALS- AN INRODUCTION

Nanoscale is a magical point on the dimensional scale: Structures in nanoscale (called nanostructures) are considered at the borderline of the smallest of human-made devices and the largest molecules of living systems. Nanotechnology has become a very active and vital area of research which is rapidly developing in industrial sectors and spreading to almost every field of science and engineering. There are several major research and development government programs on nanostructured materials and nanotechnology in the whole word. This field of research has become of great scientific and commercial interest because of its rapid expansion to academic institutes, governmental laboratories and industries. By the turn of this century, nanotechnology is expected to grow to a multibillion-dollar industry and will become the most dominant technology of the twenty-first century.

Nanostructures are loosely defined particles, grains, functional structures and devices with dimensions in the 1–100 nanometer (nm) range. Nanostructures include quantum dots, quantum wires, grains, particles, nanotubes, nanorods, nanofibers, nanofoams, nanocrystals, nanoprecision self-assemblies and thin films, metals, intermetallics, semiconductors, minerals, ferroelectrics, dielectrics, composites, alloys, blends, organics, organominerals, biomaterials, biomolecules, oligomers, polymers, functional structures, and devices. The fundamental physical and biological properties of materials are remarkably altered as the size of their constituent grains decreases to a nanometer scale.

The reasons why nanoscale has become so important:

1. The quantum mechanical properties of electrons inside matter are influenced by variations on the nanoscale. By nanoscale design of materials it is possible to vary their micro and macroscopic properties, such as charge capacity, magnetization and melting temperature without changing their chemical composition.

2. A key feature of biological entities is the systematic organization of matter on the nanoscale. Developments in nanoscience and nanotechnology would allow us to place man-made nanoscale things inside living cells. It would also make it possible to make new materials using the self-assembly features of nature. This certainly will be a powerful combination of biology with materials science.

3. Nanoscale components have very high surface to volume ratio, making them ideal for use in composite materials, reacting systems, drug delivery, and chemical energy storage (such as hydrogen and natural gas).

4. Macroscopic systems made up of nanostructures can have much higher density than those made up of microstructures. They can also be better conductors of electricity. This can result in new electronic device concepts, smaller and faster circuits, more sophisticated functions and greatly reduced power consumption simultaneously by controlling nanostructure interactions and complexity.

An abundance of scientific data is now available to make useful comparisons between nanosize materials and their counterpart microscale or bulk materials. For example, the hardness of nanocrystalline copper increases with decreasing grain size and 6 nm copper grains show five times hardness than the conventional copper.

Atomic and Molecular Basis of Nanotechnology

According to the quantum mechanics Heisenberg Uncertainty Principle the position and momentum of an object cannot simultaneously and precisely be determined. The Heisenberg Uncertainty Principle helps determine the size of electron clouds and hence the size of atoms. It applies only to the subatomic particles like electron, positron, photon, etc. It does not forbid the possibility of nanotechnology, which has to do with the position and momentum of such large particles like atoms and molecules. This is because the mass of the atoms and molecules are quite large and the quantum mechanical calculation by the Heisenberg Uncertainty Principle places no limit on how well atoms and molecules can be held in place. The historic birth of the nanotechnology is commonly credited to Feynman. Historically nanotechnology was for the first

time formally recognized as a viable field of research with the landmark lecture delivered by **Richard P. Feynman**, the famous Noble Laureate physicist on December 29th 1959 at the annual meeting of the American Physical Society. His lecture was entitled "There's Plenty of Room at the Bottom - An invitation to enter a new field of physics". Feynman stated in his lecture that the entire encyclopedia of Britannica could be put on the tip of a needle and in principle there is no law preventing such an undertaking. "Rearranging the atoms" to build various nanostructures and nanodevices and behavior of "Atoms in a small world" which included atomic scale fabrication as a bottom-up approach as opposed to the top-down approach that we are accustomed to. Bottom-up approach is self-assembly of machines from basic chemical building blocks which is considered to be an ideal through which nanotechnology will ultimately be implemented. Top-down approach is assembly by manipulating components with much larger devices, which is more readily achievable using the current technology. It is only appropriate to name the nanometer scale "the Feynman (φ nman) scale"after Feynman's great contribution and we suggest the notation "φ" for it like Å as used for Angstrom scale and μ as used for micron scale.

One Feynman (φ) ≡1 Nanometer (nm) = 10 Angstroms(Å)= 10^{-3}Micron(μ) = 10^{-9}Meter(m)

5.3 Uses of Nanodimensional Materials

Nanomaterials:

Size (approx.) diam. in nm	Materials	Example
1–10	Metals, semiconductors, magnetic materials	Nano-crystals and clusters (quantum dots)
1–100	Ceramic oxides	Other Nanoparticles
1–100	Metals, semiconductors, oxides, sulfides, nitrides	Nano-wires
1–100	Carbon, layered metal chalcogenides	Nano-tubes
pore diam. 0.5–10	Zeolites, phosphates etc.	Nanoporous solids
Several nm^2–μm^2	Metals, semiconductors, magnetic materials	2-Dimensional arrays (of nano particles)

thickness 1–1000	A variety of materials	Surfaces and thin films
Several nm in the 3D	Metals, semiconductors, magnetic materials	3-Dimensional structures (super lattices)

Diamondoids

The smallest diamondoid molecule was first discovered and isolated from a Czechoslovakian petroleum in 1933. The isolated substance was named adamantane, from the Greek for diamond. The bio-nanotechnology point of view diamondoids are in the category of organic nanostructures with over 20,000 variants. Diamondoids are recently named as the molecular building blocks (MBBs) for nanotechnology. Diamondoids have diamond-like fused ring structures which can have applications in nanotechnology. They have the same structure as the diamond lattice, i.e., highly symmetrical and strain free. Diamondoids offer the possibility of producing variety of nanostructural shapes. We expect them to have the potential to produce possibilities for application as molding and cavity formation characteristics due to their organic nature and their sublimation potential. They have quite high strength, toughness, and stiffness compared to other known molecule. Some derivatives of diamondoids have been used as antiviral drugs for many years. Due to their flexible chemistry, their exploitations as Molecular Building Blocks (MBBs), to design drug delivery and drug targeting are being examined. In Figure below adamantane ($C_{10}H_{16}$), diamantane ($C_{14}H_{20}$), and triamantane ($C_{18}H_{24}$), the smaller diamondoid molecules, with the general chemical formula $C_{4n+6}H_{4n+12}$ are reported.

Figure: Molecular structures of adamantane, diamantane and trimantane, the smaller diamondoids, with chemical formulas $C_{10}H_{16}$, $C_{14}H_{20}$and $C_{18}H_{24}$, respectively.

The possibility of introducing six linking groups to adamantane and thus being able to form three-dimensional structures out of connecting several adamantanes is its major potential feature which has attracted a great deal of attention. Some of these MBBs are electrical conductors, some are semiconductors, some are photonic and the characteristic dimension of each is a few nanometers. For example, carbon nanotubes are about five times lighter and five times stronger than steel. Adamantane (a diamondoid) is a tetrahedrally symmetric stiff hydrocarbon that provides an excellent building block for positional (or robotic) assembly as well as for self assembly. In fact, over 20,000 variants of adamantane have been identified and synthesized and even more are possible, providing a rich and well-studied set of MBBs. It should be pointed out that MBBs with three linking groups, like graphite, could only produce planar or tubular structures. MBBs with four linking groups can form three dimensional diamond lattices. MBBs with five linking groups can create 3-dimensional solids and hexagonal planes. The ultimate present possibility is MBBs with six linking groups.

Application: In pharmacology the two adamantane derivatives, Amantadine (1-adamantaneamine hydrochloride) and Rimantadine (α-methyl-1-adamatane methylamine hydrochloride) have been well-known because of their antiviral activity. The main indication of these drugs is prophylaxis and treatment of influenza A viral infections.

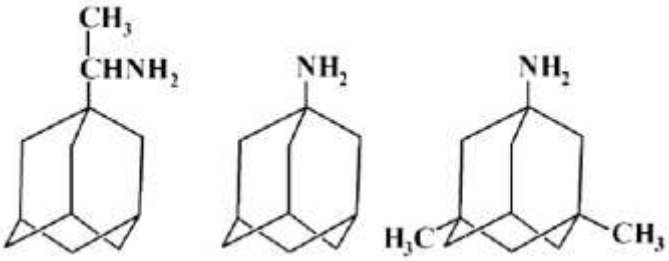


Figure: Amantadine (left) and Rimantadine (center) and Memantine (right), the three adamantane derivatives used as antiviral drugs

Improvement of thermal stability

Diamondoids are specially adamantane's derivatives and diamantane, can be used for improvement of thermal stability and other physicochemical properties of polymers and preparation of thermosetting resins which are stable at high temperatures. For example diethynyl diamantane has been utilized for such an application.

For Drug Delivery and Drug Targeting

Adamantane derivatives can be employed as carriers for drug delivery and targeting systems. Due to their high lipophilicity, attachment of such groups to drugs with low hydrophobicity would lead to increment of drug solubility in lipidic membranes and thus uptake increases. Short peptidic sequences (like proteins and nucleic acids), lipids and polysaccharides can be bound to adamantine and provide a binding site for connection of macromolecular drugs as well as small molecules. Higher affinity of adamantane-bearing ligand to DNA, instead of RNA, arises from the presence of adamantane and leads to DNA stabilization. This fact can be exploited for using such ligands as stabilizing carrier in gene delivery. Adamantine causes lipophilicity increase as well as DNA stabilization. On the other hand, a targeting sequence can be utilized in order to achieve intracellular targeting.

For Host-Guest Chemistry

The paramount aim in host-guest chemistry is to construct molecular receptors by self-assembly process so that such receptors could to some extent to gain molecular recognition capability. Calixarenes, which are macrocyclic compounds, are some of the best building blocks to design molecular hosts in supramolecular chemistry. The outstanding feature of these adamantane-bearing crown ethers (which are called “Diamond Crowns”) is that α-amino acids can be incorporated to the adamantano-crown backbone. This family of compounds provides valuable models to study selective host-guest chemistry, ion-transports and ion-complexation.

Carbon Nanotubes

Carbon nanotubes were discovered by Iijima in 1991 using an electron microscope while studying cathodic material deposition through vaporizing carbon graphite in an electric arc evaporation reactor under an inert atmosphere during the synthesis of Fullerenes. The nanotubes produced by Iijima appeared to be made up of a perfect network of hexagonal graphite rolled up to form a hollow tube. The nanotube diameter range is from one to several nanometers which is much smaller than its length range which is from one to a few micrometers. A variety of manufacturing techniques has since been developed to synthesize and purify carbon nanotubes with tailored characteristics and functionalities. Controlled production of single walled carbon nanotubes (SWCNT) is one of the favorite forms of carbon nanotube which has many present

and future applications in nanoscience and nanotechnology. Laser ablation chemical vapor depositions joined with metal-catalyzed disproportionation of suitable carbonaceous feedstock are often used to produce carbon nanotubes. SWCNTs could behave metallic, semi-metallic, or semi-conductive one-dimensional. The carbon nanotubes and fullerenes are shown to exhibit unusual photochemical, electronic, thermal and mechanical properties.

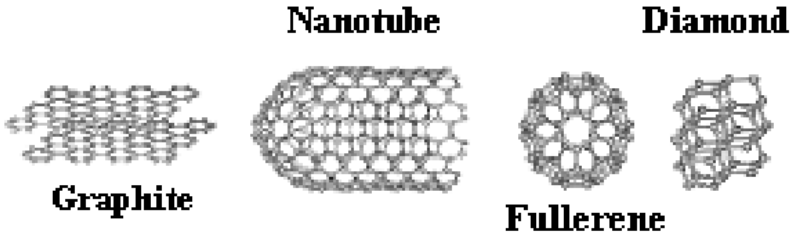


Figure: The four allotropes of carbon

Cyclodextrins and Liposome

Like carbon nanotubes, buckyballs and diamondoids the biologists have been making their own advances with other nanoscale structures like cyclodextrins, liposomes and monoclonal antibodies. These biological nanostructures have many applications including drug delivery and drug targeting.

Cyclodextrins are cyclic oligosaccharides, their shape is like a truncated cone and they have a relatively hydrophobic interior. They have the ability to form inclusion complexes with a wide range of substrates in aqueous solutions. This property has led to their application for encapsulation of drugs in drug delivery.

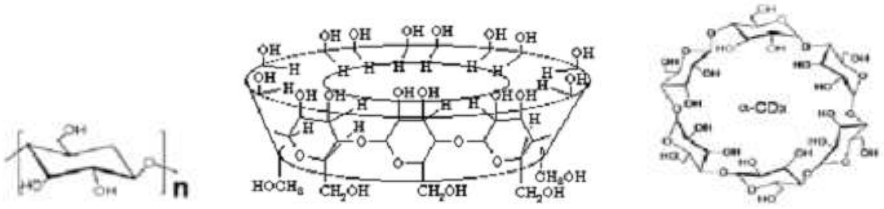


Figure : Chemical formula and structure of Cyclodextrins

For n=6 it is called α-CDx, n=7 is called β-CDx, n=8 is called γ-CDx. Cyclodextrins are cyclic oligosaccharides. Their shape is like a truncated cone and they have a relatively hydrophobic interiors.

Liposome is a spherical synthetic lipid bilayer vesicle, created in the laboratory by dispersion of a phospholipid in aqueous salt solutions. Liposome is quite similar to a micelle with an internal aqueous compartment, which are in nanoscale size range. The self-assemble based on hydrophilic and hydrophobic properties and they encapsulate materials inside. For example, a recent commercially available anticancer drug is a liposome, loaded with doxorubicin, and is approximately 100-nanometer in diameter.

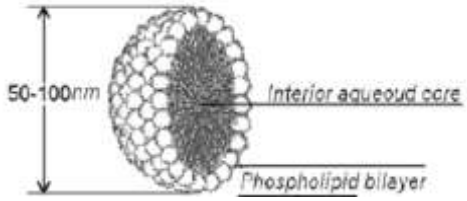


Figure: Cross section of a liposome

5.4 Synthesis of Nanodimensional Materials

The synthesis of nanomaterials and assembling the nanostructures into ordered arrays to turn into them functional and operational are vital aspects of nanoscience. The materials or structures include nanoparticles, nanowires, nanotubes, nanocapsules, nanostructured alloys and polymers, nanoporous solids and DNA chips.

Synthesis of Metal-Oxide Nanoparticles:

Liquid–Solid Transformations

Metal oxides can be found in the form of a single crystal (pure/defective), powder, polycrystalline (crystals with various orientations), or thin film. Various methods are available in the literature for the synthesis of metal oxides, the selection of which is often dependent on the desired properties and application. Early methods to synthesize solid-state materials, especially mixed-metal oxides, demanded high temperatures (1000–3000 °C) and long reaction times

(1 hour to 1 month). Solid-state materials like halides, hydrides and chalcogenides (oxides, sulphides, etc.), oxides and sulphides have found wide application particularly because of their nonstoichiometry, thermal and mechanical properties, as well as catalytic activity.

The syntheses of nanoscale materials are generally grouped into two broad categories: “bottom-up” and “top-down.” “Bottom-up” preparation methods are of primary interest to chemists and materials scientists because the fundamental building blocks are atoms or molecules such as size, shape, stoichiometry, surface area, pore size, and surface decoration of the end product. The most common and widely used “bottom up” wet chemical method for the preparation of nanoscale oxides has been the sol-gel process. Other wet chemistry methods, including novel microemulsion techniques, oxidation of metal colloids, and precipitation from solutions, have also been used.

Coprecipitation methods:

The method to prepare nanoparticles of metal oxide ceramics is the precipitation method. This process involves dissolving a salt precursor,usually a chloride, oxychloride, or nitrate. The corresponding metal hydroxides usually form and precipitate in water by adding a basic solution such as sodium hydroxide or ammonium hydroxide to the solution. The resulting salts then washed away and the hydroxide is calcined after filtration and washing to obtain the final oxide powder. This method is useful in preparing composites of different oxides by coprecipitation of the corresponding hydroxides in the same solution. One disadvantage of this method is the difficulty to control the particle size and size distribution. Very often, fast (uncontrolled) precipitation takes place resulting in large particles.

SOL-GEL PROCESSING

Sol-gel techniques have been known for the preparations of metal oxides. The process is typically used to prepare metal oxides via the hydrolysis of metal reactive precursors, usually alkoxides in an alcoholic solution, resulting in the corresponding hydroxide. Condensation of the hydroxide molecules by giving off water leads to the formation of a network of metal hydroxide. When hydroxide species undergo polymerization by condensation of the hydroxy network,

gelation is achieved and a dense porous gel is obtained. The gel is a polymer of a three-dimensional skeleton surrounding interconnected pores. Removal of the solvents and appropriate drying of the gel is an important step that results in an ultra-fine powder of the metal hydroxide. Heat treatment of the hydroxide is a final step that leads to the corresponding ultra-fine powder of the metal oxide. Depending on the heat treatment procedure, the final product may end up in the form of a nanometer scale powder, bulk material or metal oxides.

$$\mathrm{M(OR)}_y + x\mathrm{H_2O} \longleftrightarrow \mathrm{M(OR)}_{y-x}\mathrm{(OH)} + x\mathrm{ROH}$$

Solvothermal technique

Solvothermal techniques have also been used to synthesize metal oxide and as well as semiconductor chalcogenides nanoparticles. The metal complexes are decomposed thermally either by boiling the contents in an inert atmosphere or by using an autoclave. A suitable capping agent or stabilizer such as a long-chain amine, thiol, and trioctylphospine oxide [TOPO] is added to the reaction contents at a suitable point to hinder the growth of the particles and hence, provide stabilization against agglomeration. The stabilizers also help in dissolution of the particles in different solvents. Synthesis of nanoparticles using an organometallic precursor in TOPO as solvent, and toluene as solvent in nujol are some important innovations in recent years.

Gas–Solid Transformations-

Chemical vapor deposition

CVD is a general term applied to the deposition of solid materials from chemical precursors in the vapor phase. The process for compound formation involves reaction between a volatile precursor of the material to be deposited with other reactive gases. CVD processes have been used in various Industrial applications and technologies such as the fabrication of electronic devices, the manufacture of cutting tools and the formation of nanoparticles. The following CVD processes are commonly used for the formation of Nanoparticles-

1. CVD—chemical vapor deposition (thermally activated or pyrolytic)
2. MOCVD—Metalorganic CVD
3. PACVD—Plasma-assisted CVD
4. PCVD—Photo CVD

MOCVD is also a thermal CVD processes, but the sources differ in that they are metal-organic gases or liquids. Metal-organic compounds are organic compounds that contain a metal atom, particularly compounds in which the metal atom has a direct bond with a carbon atom, their use offers significant advantages

5.5 Synthesis of Semiconductor Nanoparticles

5.6 Study of different characterization tools

Characterization of Nanomaterials and nanostructures has been largely based on certain vital enhancement of conventional characterization methods developed for bulk materials. For example, X-ray diffraction (XRD) has been widely used for the determination of crystalline character, crystallite size, crystal structures and lattice constants of nanoparticles, nanowires and thin films. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are together with electron diffraction have been commonly used in the characterization of nanoparticles to get an idea of the size, shape and defects present in these materials. The characterization and manipulation of individual nanostructures require not only extreme sensitivity and accuracy, but also atomic-level resolution.

Optical spectroscopy is used to determine the size of semiconductor quantum dots. Scanning probe microscopy (SPM) is a relatively new characterization technique and has found widespread application in nanotechnology. The two branches of SPM are scanning tunneling microscopy (STM) and atomic force microscopy (AFM) both STM and AFM are true surface imaging techniques that can produce topographic images of a surface with atomic resolution in all three dimensions in combination with appropriately designed attachments, STM and AFM have found a much broader range of applications, such as nano-indentation, nano-lithography and patterned self-assembly. Almost all solid surfaces whether they are hard or soft, electrically conductive or not, can be studied with STM/AFM. Surfaces can be studied in a gaseous medium such as air or vacuum or in liquid.

X-ray diffraction (XRD)

XRD is extensively used to study the crystal structure of solids, defects and stresses. In XRD, a beam of X-rays, with wavelength ranging from 0.07 to 0.2 nm, is diffracted by the crystalline specimen according to Bragg's law:

$$\lambda = 2d\,\mathrm{Sin}\theta$$

where d is the interplanar distance and λ is the wavelength of the X-rays.

The intensity of the diffracted beam is measured as a function of the diffraction angle (2θ) and the specimen's orientation. The diffraction pattern can be used to identify the crystalline phases and their structural characteristics. XRD is non-destructive and does not require detailed sample preparation.

$$\mathrm{D} = \frac{K\lambda}{\beta\, Cos\theta}$$

where λ is the X-ray wavelength, β is the full width at half maximum (FWHM) height of a diffraction peak, θ is the diffraction angle, and K is Scherrer's constant, which is of the order of unity for a spherical crystal.

However, nanoparticles often form twinned structures; hence Scherrer's formula may not always give true particle sizes. The X-ray diffraction provides only an average crystallite size.

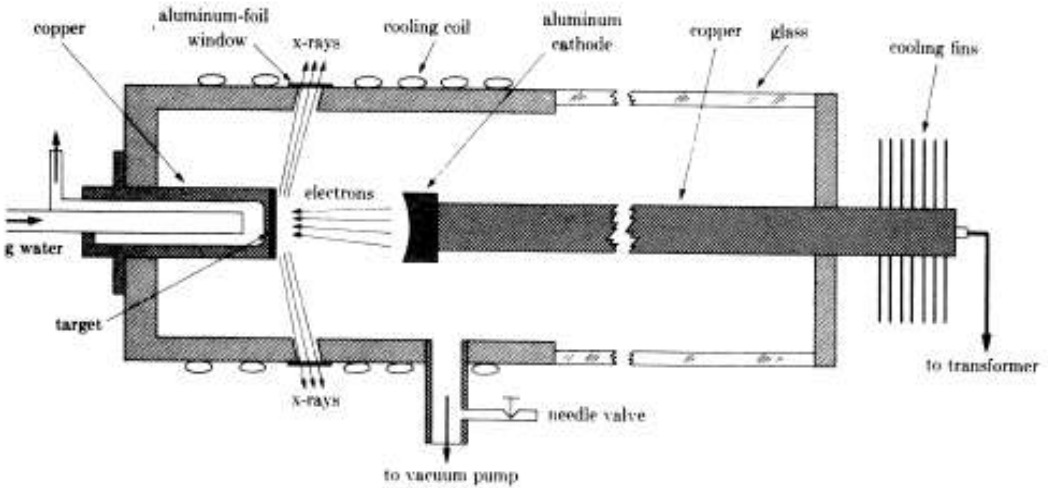


Fig.: Cross section of gas x-ray tube

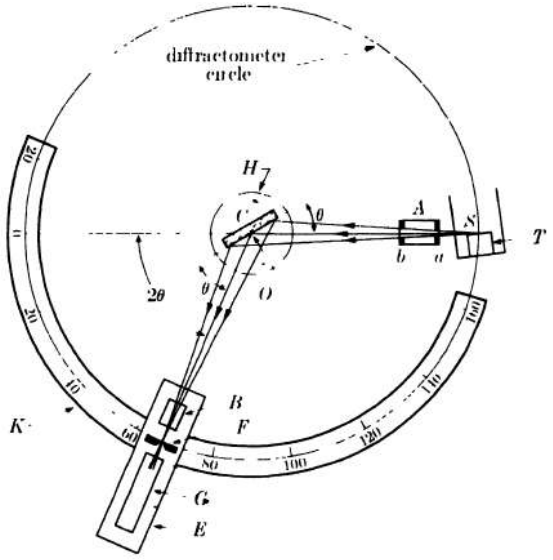


Fig.: X-ray diffractometer (Schematic)

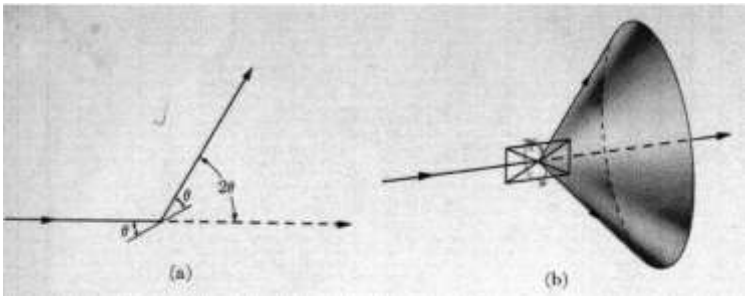


Fig.: Formation of diffracted core of radiation in the powder method

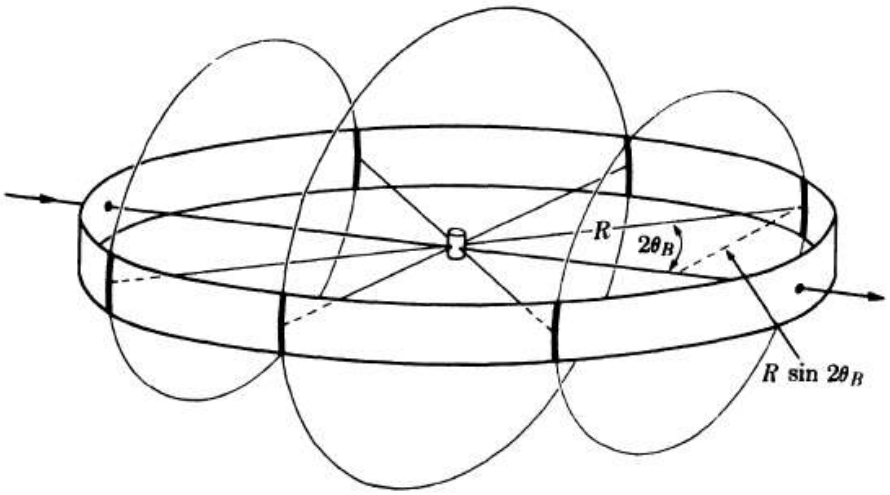


Fig.: Intersection of cones of diffracted rays with Debye Scherrer film

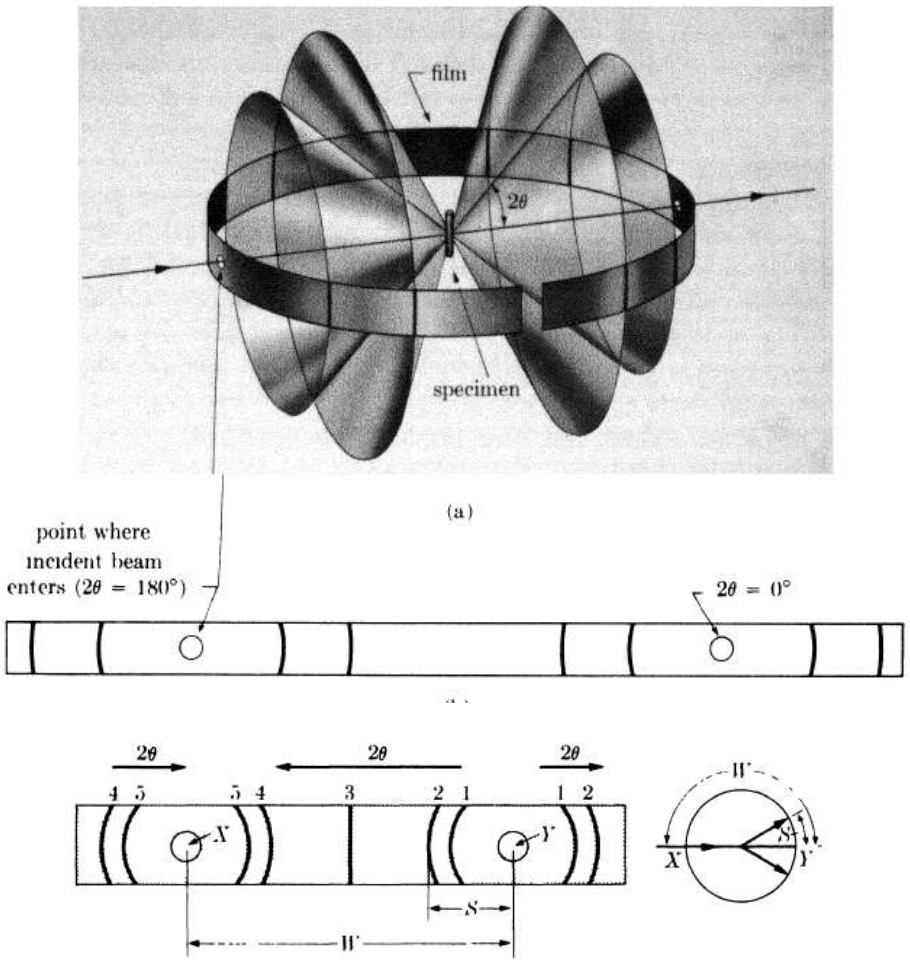


Fig.: Debye Scherrer powder method (a) relation of film to specimen and incident beam amd (b) appearance of film when laid out flat

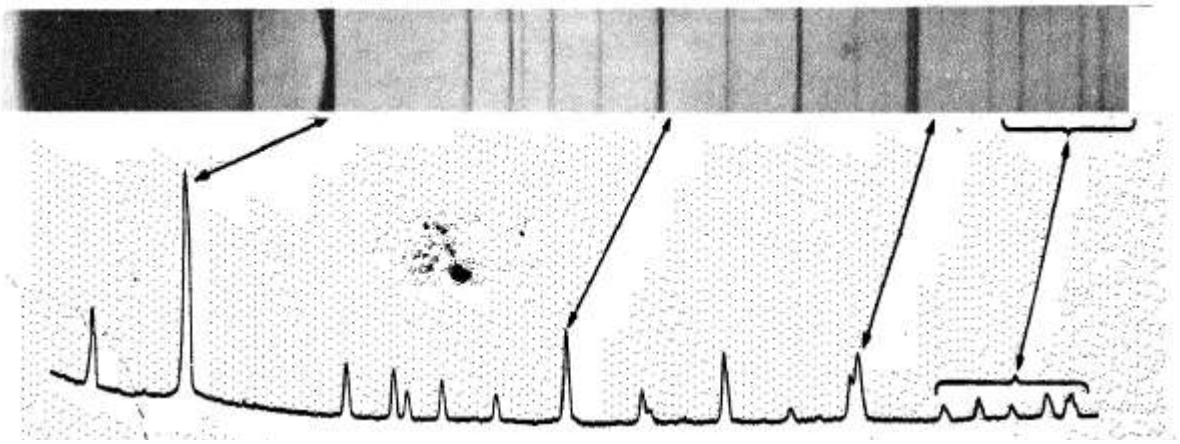


Fig.: Powder pattern of quartz and corresponding microphotometer

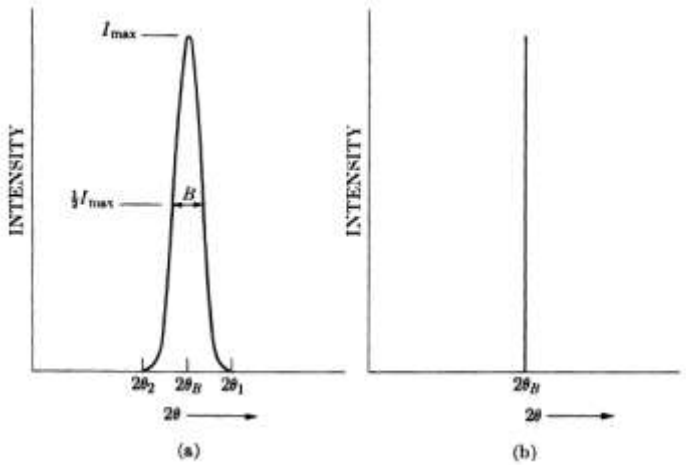


Fig.: Effect of fine particle size on diffraction curves

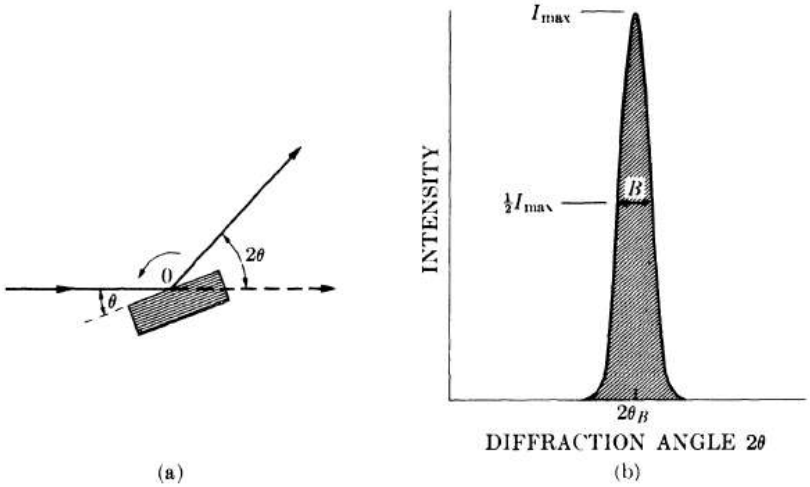
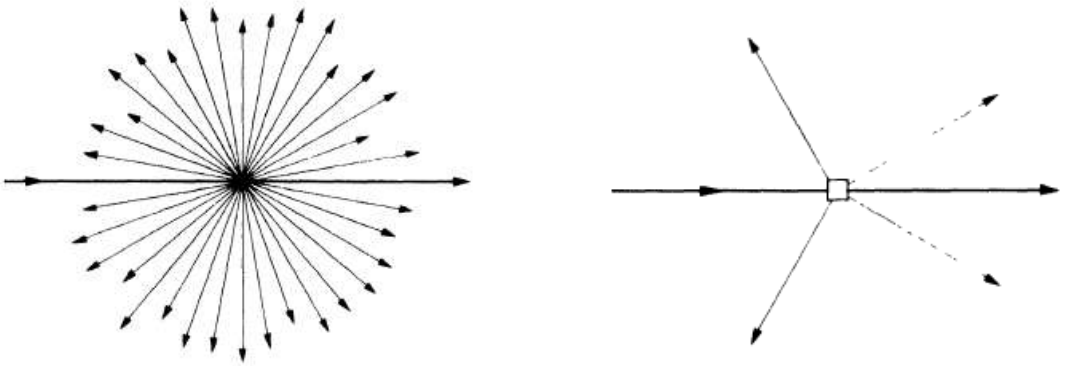


Fig.: Diffraction by crystal rotated through the Bragg angle

$$B = \tfrac{1}{2}(2\theta_1 - 2\theta_2) = \theta_1 - \theta_2.$$

$$t = \frac{0.9\lambda}{B \cos \theta_B},$$



(a) (b)

Fig.:(a) Scattering by an atom (b) diffraction by a crystal

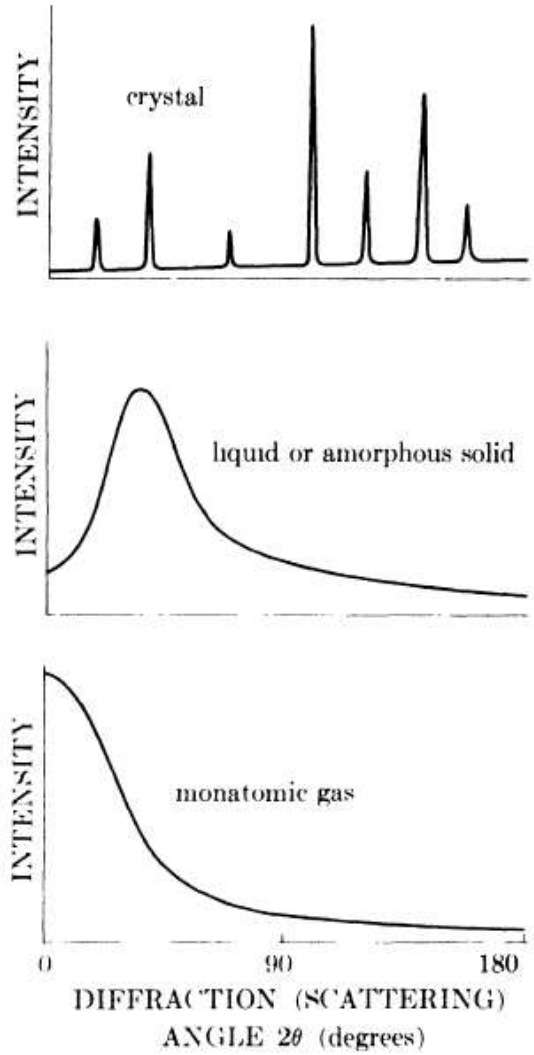


Fig.: Comparative x-ray scattering by crystalline solids, amorphous solids, liquids and monoatomic gases.

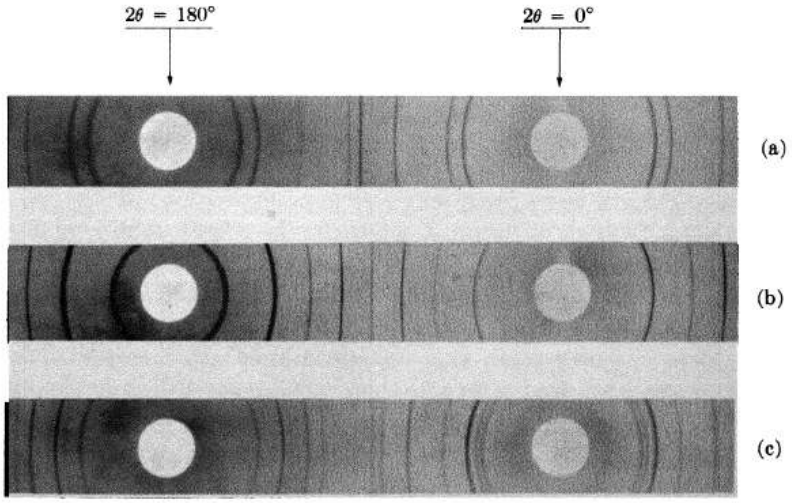


Fig.: Debye Scherrer powder pattern of (a) copper (FCC), (b) tungsten (BCC) and (c) zinc (HCP)

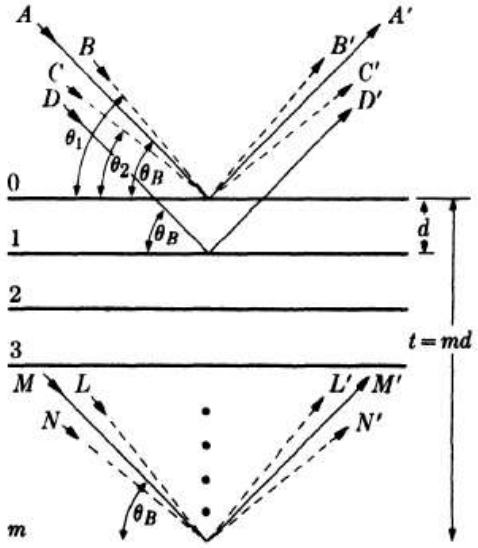


Fig.: Effect of crystal size on diffraction

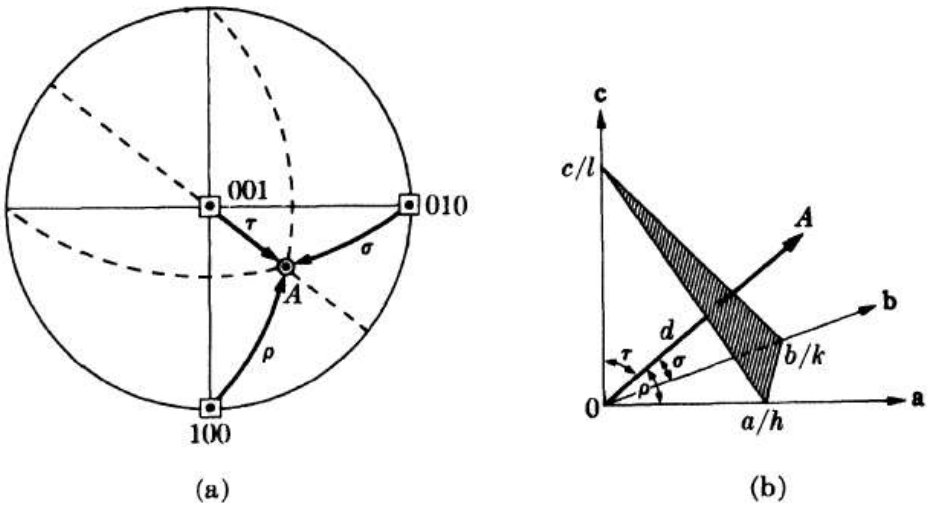


Fig.: Determination of miller indices of a pole

$$h:k:l = pa:qb:rc.$$

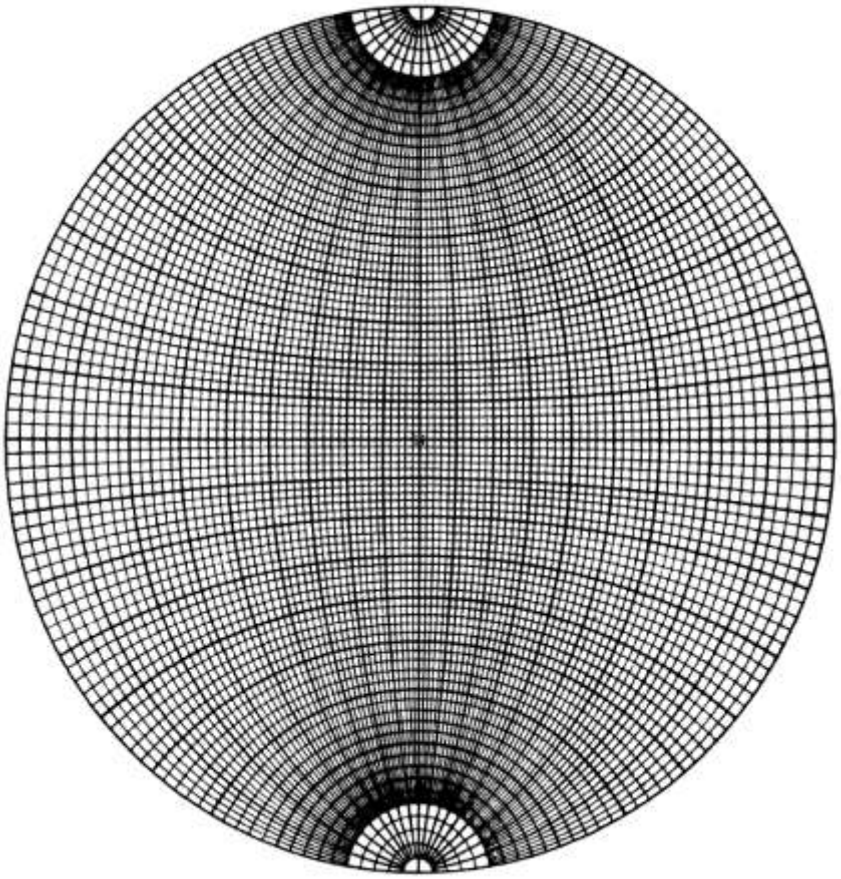


Fig.: Wolf net drown to 2^{o} intervals

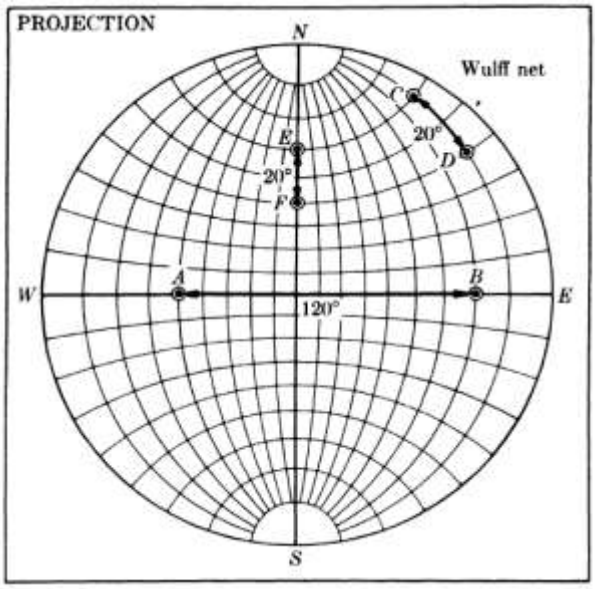


Fig.: Stereographic projection superimposed on wolf net for measurement of angles between Poles

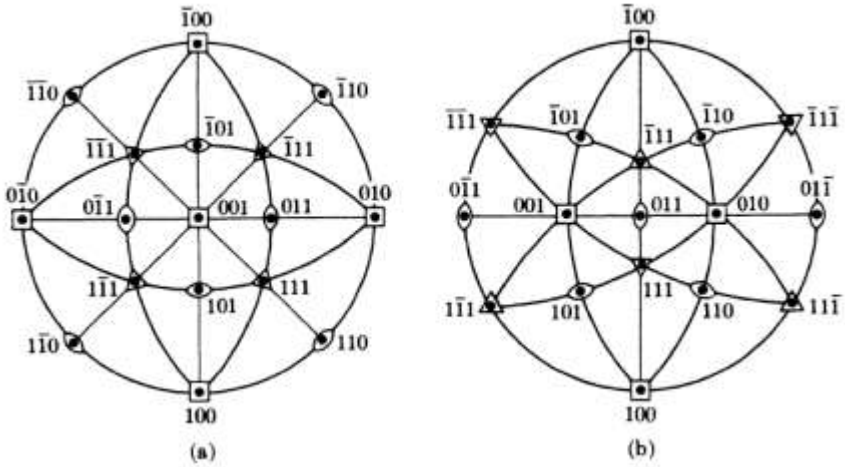


Fig.: Standard projections of cubic crystals (a) on (001) and (b) on (011)

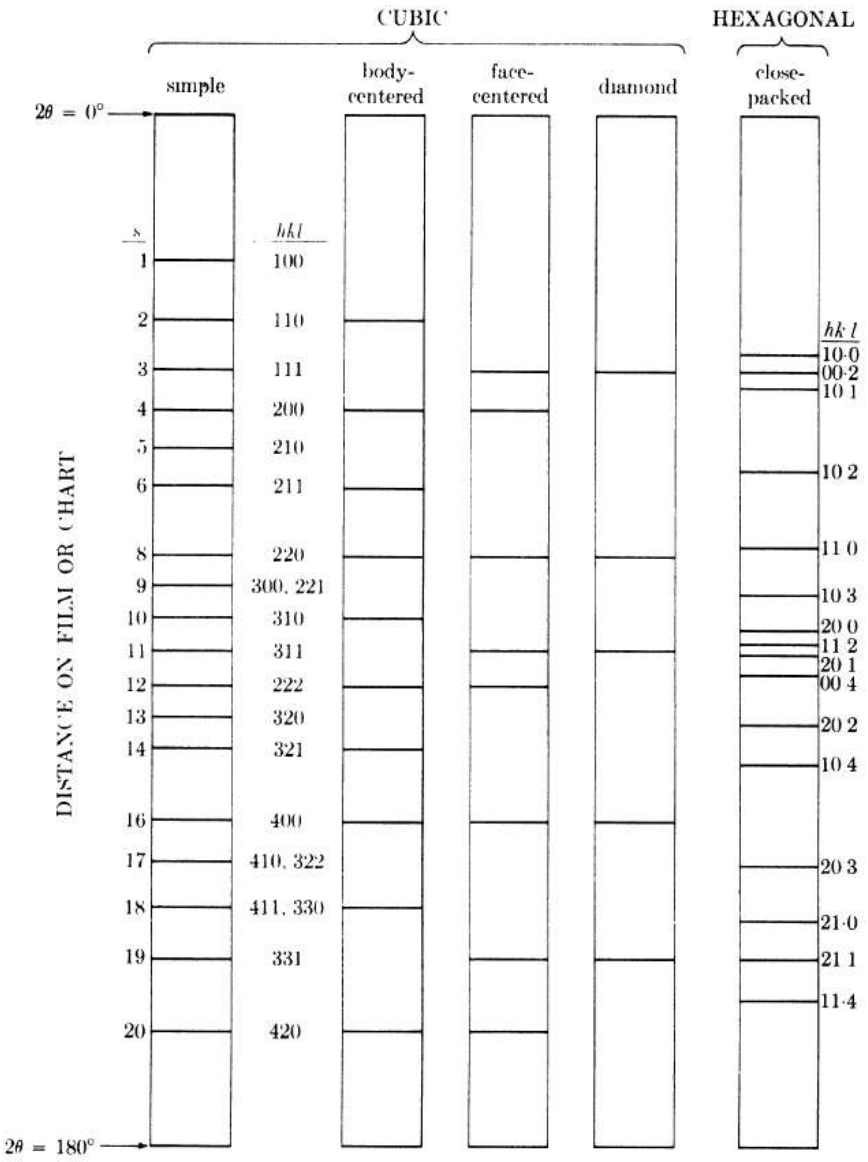


Fig.: Calculated diffraction patterns for various lattices, $s = h^2 + k^2 + l^2$

INTERPLANAR ANGLES (IN DEGREES) IN CUBIC CRYSTALS BETWEEN PLANES OF THE FORM $\{h_1k_1l_1\}$ AND $\{h_2k_2l_2\}$

$\{h_2k_2l_2\}$	$\{h_1k_1l_1\}$						
	100	110	111	210	211	221	310
100	0 90						
110	45 90	0 60 90					
111	54.7	35.3 90	0 70.5 109.5				
210	26.6 63.4 90	18.4 50.8 71.6	39.2 75.0	0 36.9 53.1			
211	35.3 65.9	30 54.7 73.2 90	19.5 61.9 90	24.1 43.1 56.8	0 33.6 48.2		
221	48.2 70.5	19.5 45 76.4 90	15.8 54.7 78.9	26.6 41.8 53.4	17.7 35.3 47.1	0 27.3 39.0	
310	18.4 71.6 90	26.6 47.9 63.4 77.1	43.1 68.6	8.1 58.1 45	25.4 49.8 58.9	32.5 42.5 58.2	0 25.9 36.9
311	25.2 72.5	31.5 64.8 90	29.5 58.5 80.0	19.3 47.6 66.1	10.0 42.4 60.5	25.2 45.3 59.8	17.6 40.3 55.1
320	33.7 56.3 90	11.3 54.0 66.9	61.3 71.3	7.1 29.8 41.9	25.2 37.6 55.6	22.4 42.3 49.7	15.3 37.9 52.1
321	36.7 57.7 74.5	19.1 40.9 55.5	22.2 51.9 72.0 90	17.0 33.2 53.3	10.9 29.2 40.2	11.5 27.0 36.7	21.6 32.3 40.5
331	46.5	13.1	22.0				
510	11.4						
511	15.6						
711	11.3						

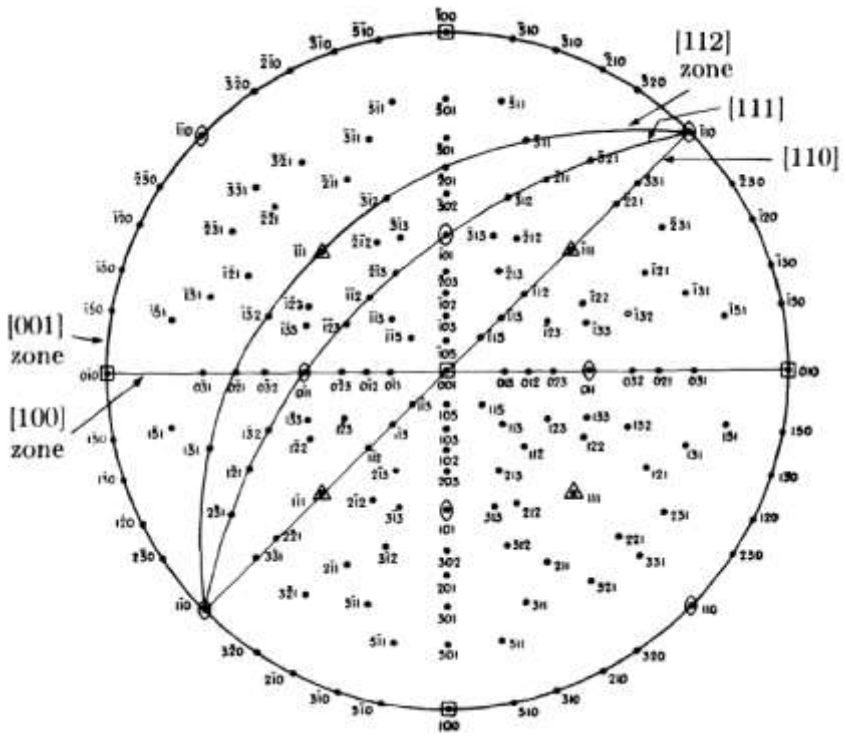


Fig.: Standard (001) projection of a cubic crystal

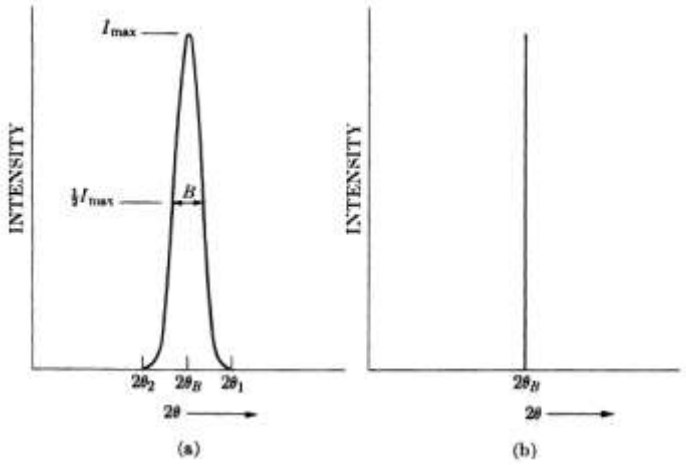


Fig.: Effect of fine particle size on diffraction curves

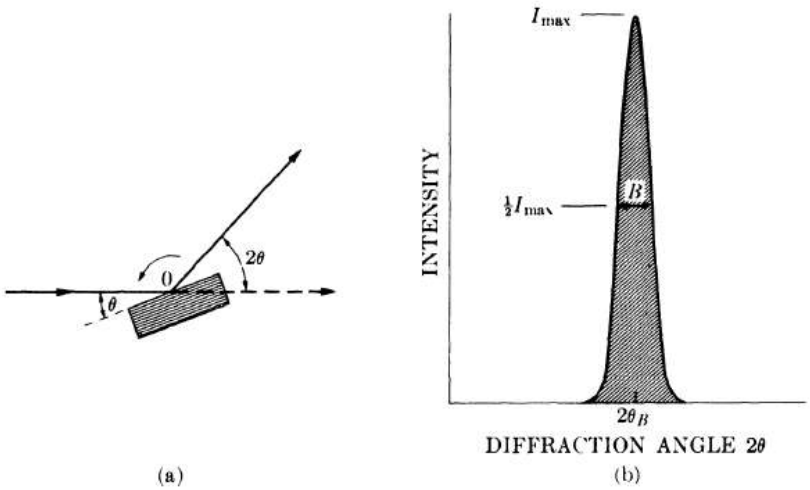
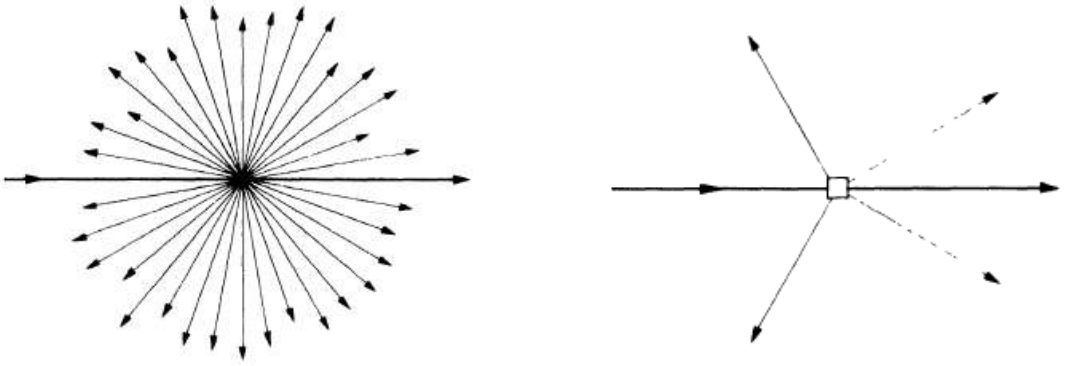


Fig.: Diffraction by crystal rotated through the Bragg angle

$$B = \tfrac{1}{2}(2\theta_1 - 2\theta_2) = \theta_1 - \theta_2.$$

$$t = \frac{0.9\lambda}{B \cos \theta_B},$$



(a) (b)

Fig.:(a) Scattering by an atom (b) diffraction by a crystal

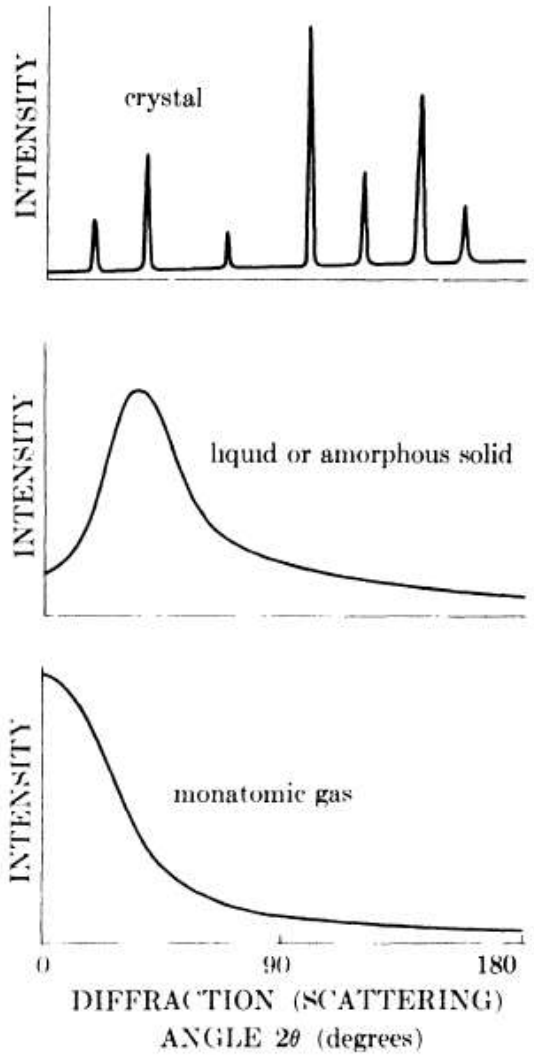


Fig.: Comparative x-ray scattering by crystalline solids, amorphous solids, liquids and monoatomic gases.

SMALL ANGLE X-RAY SCATTERING (SAXS) is another powerful tool for characterizing nanostructured materials. Strong diffraction peaks result from constructive interference of X-rays scattered from ordered arrays of atoms and molecules.

The formation of crystal phases in the synthesized samples was investigated by performing the X-ray diffraction measurements at room temperature. The XRD measurements on powder samples were performed with the XPERT-PRO or other equipped with Cu-Kα radiation (λ = 1.54060 Å) operated at 30 kV and 30 mA. Samples were scanned in the angle range of 10–80^{o} with a scanning rate of 0.1 degree / min. generally. A digital photograph of X-ray diffractometer is shown in figure.

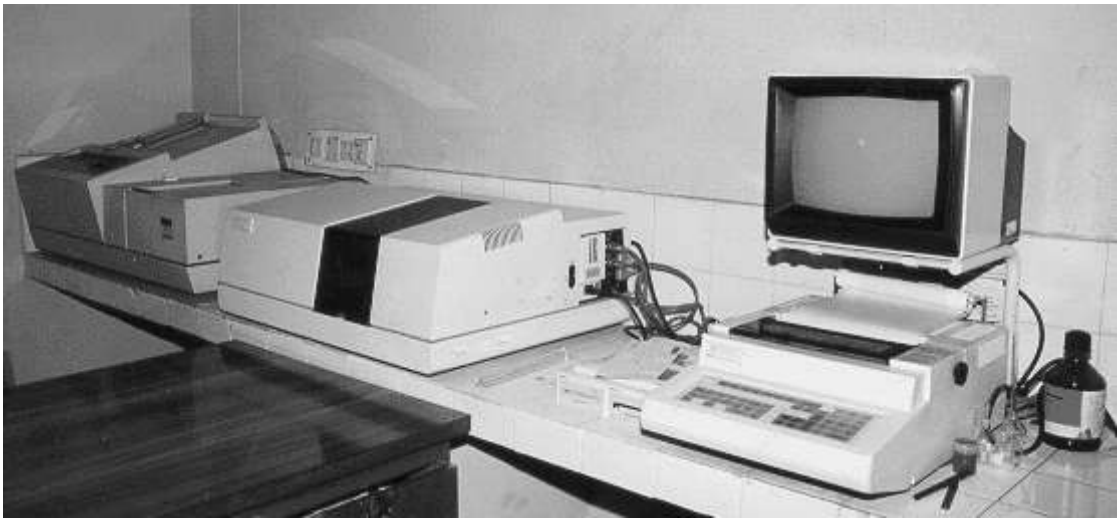


Fig. : Digital photograph of X-ray diffractometer.

SCANNING ELECTRON MICROSCOPY (SEM)

Scanning electron microscopy (SEM) is one of the most popular and widely used techniques for the characterization of Nanomaterials and nanostructures. SEM can be effectively used to characterize specimens down to a resolution of a few nanometers (nm), with image magnifications achievable in the range of ~10 to over 3,00,000. In addition to information on surface topography, SEM can also provide useful information on chemistry, crystal orientation and internal stress distribution.

SEM consists of an electron gun to emit electrons that are focused into a beam, with a very fine spot size of ~5 nm. Electrons are accelerated to energy values in the range of a few hundred eV to 50 KeV and rastered over the surface of the specimen by deflection coils. As the electrons strike and penetrate the surface, a number of interactions that result in the emission of electrons and photons from the sample occur and SEM images are produced by collecting the emitted electrons on a cathode ray tube (CRT).

The various SEM techniques are differentiated on the basis of what is subsequently detected and imaged. The principle images produced in SEM are of three types: secondary electron images, backscattered electron images and elemental X-ray maps.

Fig.: Digital photograph of SEM

Transmission electron microscope (TEM)

The size and shape of the particles would be analyze by High-resolution transmission electron microscopy HR-TEM (TEM, Tecnai G_2 S-Twin). The HR-TEM instrument was equipped with an ultra-high resolution objective lens and was operated at an accelerating voltage of 80 kV. To perform the measurements, 2 mg of powder sample was dispersed in ethanol by sonicating for 1h and one drop of the well-dispersed sample solution was deposited on to a carbon coated copper grid having 400 mesh/mm. The dried grid was used for HR-TEM measurements. A digital photograph of TEM is shown in Figure.

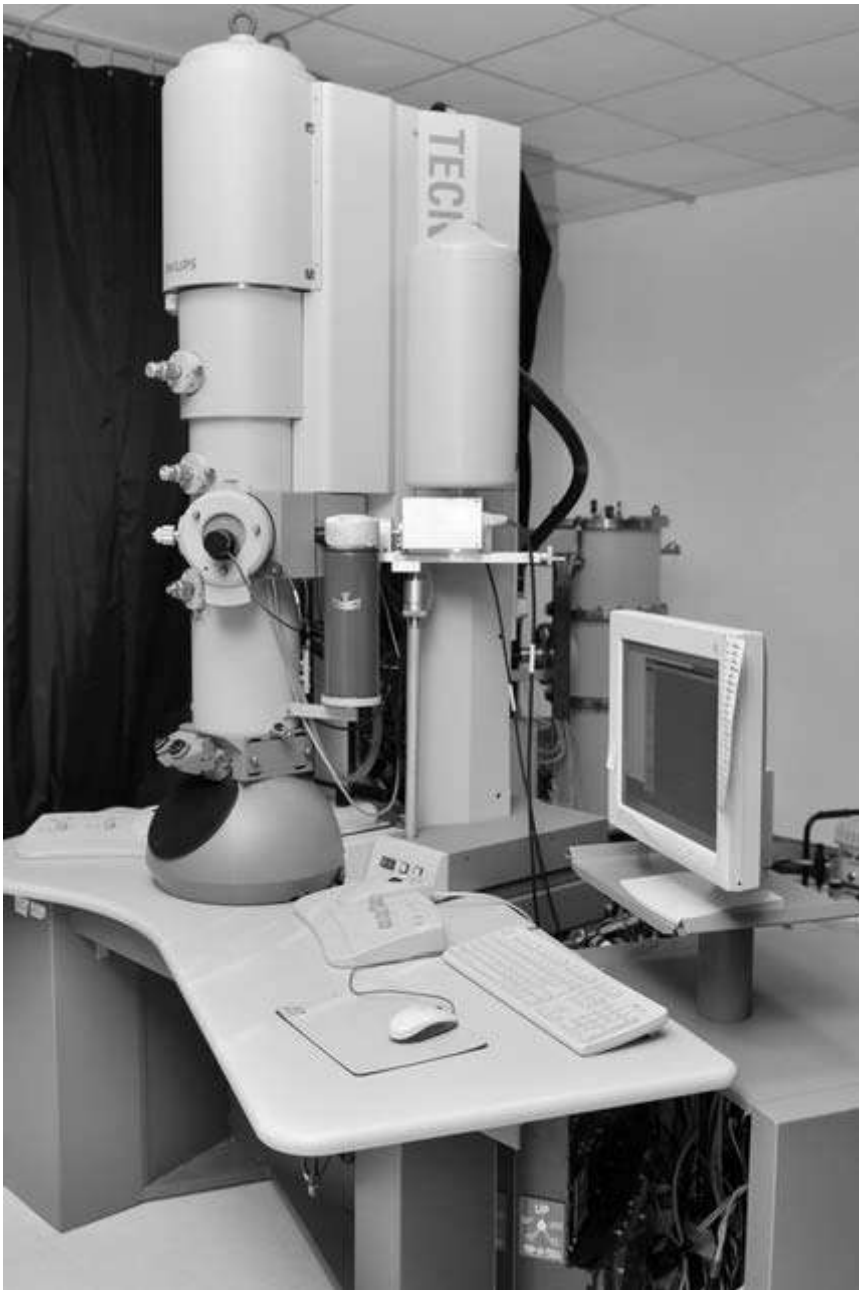


Fig.: Digital photograph of Transmission electron microscope.

Electron–matter interaction

When a high-energy primary electron interacts with an atom, it undergoes either inelastic scattering with atomic electrons or elastic scattering with the atomic nucleus. In an inelastic collision with an electron, the primary electron transfers part of its energy to the other electron.

When the energy transferred is large enough, the other electron will emit from the sample. If the emitted electron has energy of less than 50 eV, it is referred to as a *secondary electron (electron emitted from one of the orbitals of the incident atom)*. Since the secondary electron energy is small, the SE images are highly sensitive to topographic variations. *Backscattered electrons* are the high-energy incident electrons that are elastically scattered and essentially possess nearly the same energy as the incident or primary electrons. The probability of backscattering increases with the atomic number of the sample material. Although backscattering images cannot be used for elemental identification, useful contrasts can develop between regions of the specimen that differ widely in atomic number, *Z*. Hence the BSE image can provide atomic number contrast in addition to topographic contrast.

An additional electron interaction in SEM is that the *primary electron* collides with and ejects a *core electron* from an atom in the sample. The excited atom will decay to its ground state by emitting either a characteristic X-ray photon or an Auger electron, both of which have been used for chemical characterization. The energy of the emitted characteristic X-ray or Auger electrons are specific to the chemistry of the incident atom and hence study of these can provide useful information on the chemistry of the sample investigated. By focusing the electron beam to fine points, it is possible to get localized information on chemistry variations. However, it should be remembered that such information on characteristic X-ray (and hence information on chemistry) is generated from a depth of approximately a micron and hence the information is averaged over this depth although the beam size could be

finer on a lateral scale. Combined with chemical analytical capabilities, SEM not only provides an image of the morphology and microstructures of bulk and nanostructured materials and devices, but can also provide detailed information of chemical composition and distribution.

Imaging

The theoretical limit to an instrument’ s resolving power is determined by the wavelengths of the electron beam used and the numerical aperture about the system. The resolving power, *R*, of an instrument is defined as:

$$R = \frac{\lambda}{2NA}$$

where λ is the wavelength of electrons used and *NA* is the numerical aperture, which is fixed on each objective and condenser lens system and a measure of the electron gathering ability.

Scanning Tunneling Microscope (STM):

Nanotechnology received its greatest momentum with the invention of scanning tunneling microscope (STM) in 1985 by Gerd K. Binnig and Heinrich Rohrer, staff scientists at the IBM's Zürich Research Laboratory. To make headway into a area of molecule sized devices, it would be necessary to survey the landscape at that tiny scale. STM allows imaging solid surfaces with atomic scale resolution. It operates based on tunneling current, which starts to flow when a sharp tip is mounted on a piezoelectric scanner approaches a conducting surface at a distance of about one nm (1 φ). This scanning is recorded and displayed as an image of the surface topography. Actually the individual atoms of a surface can be resolved and displayed using STM. By bringing the tip very close to the surface (a few fractions of nanometer), and by applying an electrical voltage (U 4V) to the tip or sample, a small electric current (0.01nA-50nA) can flow from the sample to the tip or reverse. The STM is based on several principles, the most important of which are as the follows:

(i) The quantum mechanical tunneling effect and related advanced electronics that allows the investigator to see clearly the surface.

(ii) Advanced piezoelectric effect which allows one to precisely scan the tip with nano-level control.

(iii) A feedback loop which monitors the tunneling current and accurately coordinates the current and the positioning of the tip.

Atomic Force Microscopy (AFM)

It originated from scanning tunneling microscopy (STM). Where atomic and molecular forces are monitored and used for the surface characterization at the atomic scale rather than a tunneling current. The forces are detected by a probe tip mounted on a flexible cantilever, as shown in figure given below. Deflection of the cantilever, to a good approximation, is directly proportional to the acting force. It is optically or electronically monitored with high precision. The deflection signal is used to modulate the tip–sample separation in the way it is done in STM with the tunneling current. While scanning, one can obtain a profile of atomic and molecular forces over the sample surface. The sensitivity of AFM to the electronic structure of the sample surface,

inherent in STM, is largely absent. Therefore it allows characterization of nonconducting materials.

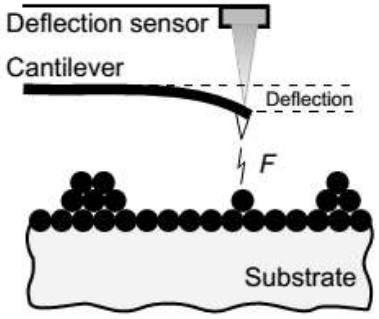


Figure: Atomic Force Microscopy (AFM), tip–sample geometry

There are three principle types of imaging modes of the sample surface in AFM: (i) contact, (ii) tapping, and (iii) non-contact modes.

In the contact mode, the probing tip is always in contact with the sample surface and surface structure is obtained from the deflection of the cantilever. The force on the tip is repulsive with a mean value of about 10^{-9} N. This force is set by pushing the cantilever against the sample surface with a piezoelectric positioning element.

In the tapping mode, the probe tip is periodically in contact with the sample surface and surface structure is obtained from the change in the vibration amplitude or phase of the oscillating cantilever.

In the non-contact mode the probe tip is not in contact with the sample surface and surface structure is obtained from the change in the vibration amplitude or resonant frequency of the oscillating cantilever.

In the contact mode, there is a high possibility that the strong repulsive force acting between the sample surface and the probe tip will destroy the sample surface and/or the tip apex. So, the tapping and non contact modes are widely used because these modes are gentler than the contact mode.

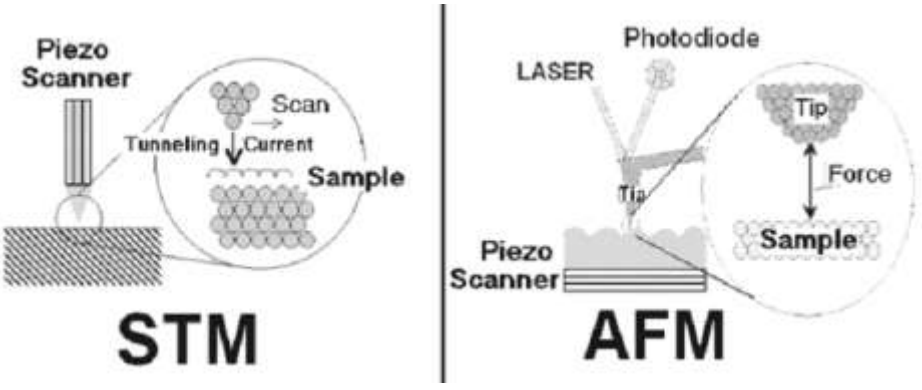
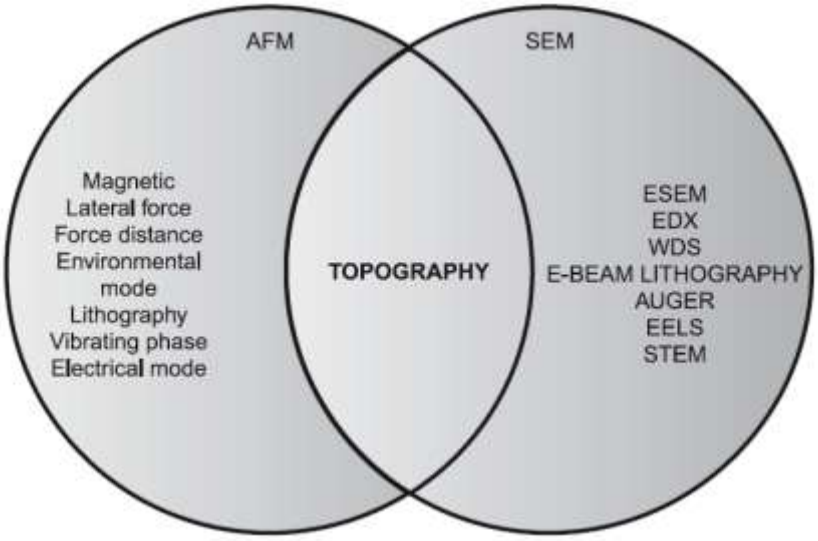


Figure : Comparison of the operations of a scanning tunneling microscope (STM) and an atomic force microscope (AFM).



Raman spectroscopy:

The room temperature Raman spectra of the synthesize products would be recorded in the range of 200–1200 cm^{-1} using a Raman spectrometer equipped with a microscope and a high-performance CCD detector. The 514 nm line of argon-ion laser was used to illuminate the sample. For recording the Raman spectra 180° scattering geometry was used. A photograph of Raman spectrometer is shown in figure.

Figure: A photograph of Raman spectrometer

Field emission scanning electron microscope (FE-SEM)/ scanning electron microscope (SEM)

Surface morphologies of synthesized samples would be study using FE-SEM and SEM. Samples were coated with gold before the measurements. A generally working distance of 10 mm was maintained and a 15 kV acceleration voltage was used to perform the measurements. A digital photograph of SEM is shown in figure.

Fig: Digital photograph of SEM.

Transmission electron microscope (TEM)

The size and shape of the particles would be analyze by High-resolution transmission electron microscopy HR-TEM. The HR-TEM instrument was equipped with an ultra-high resolution objective lens and was operated at an accelerating voltage of 80 kV. To perform the measurements, 2 mg of powder sample was dispersed in ethanol by sonicating for 1h and one drop of the well-dispersed sample solution was deposited on to a carbon coated copper grid having 400 mesh/mm. The dried grid was used for HR-TEM measurements. A digital photograph of TEM is shown in figure.

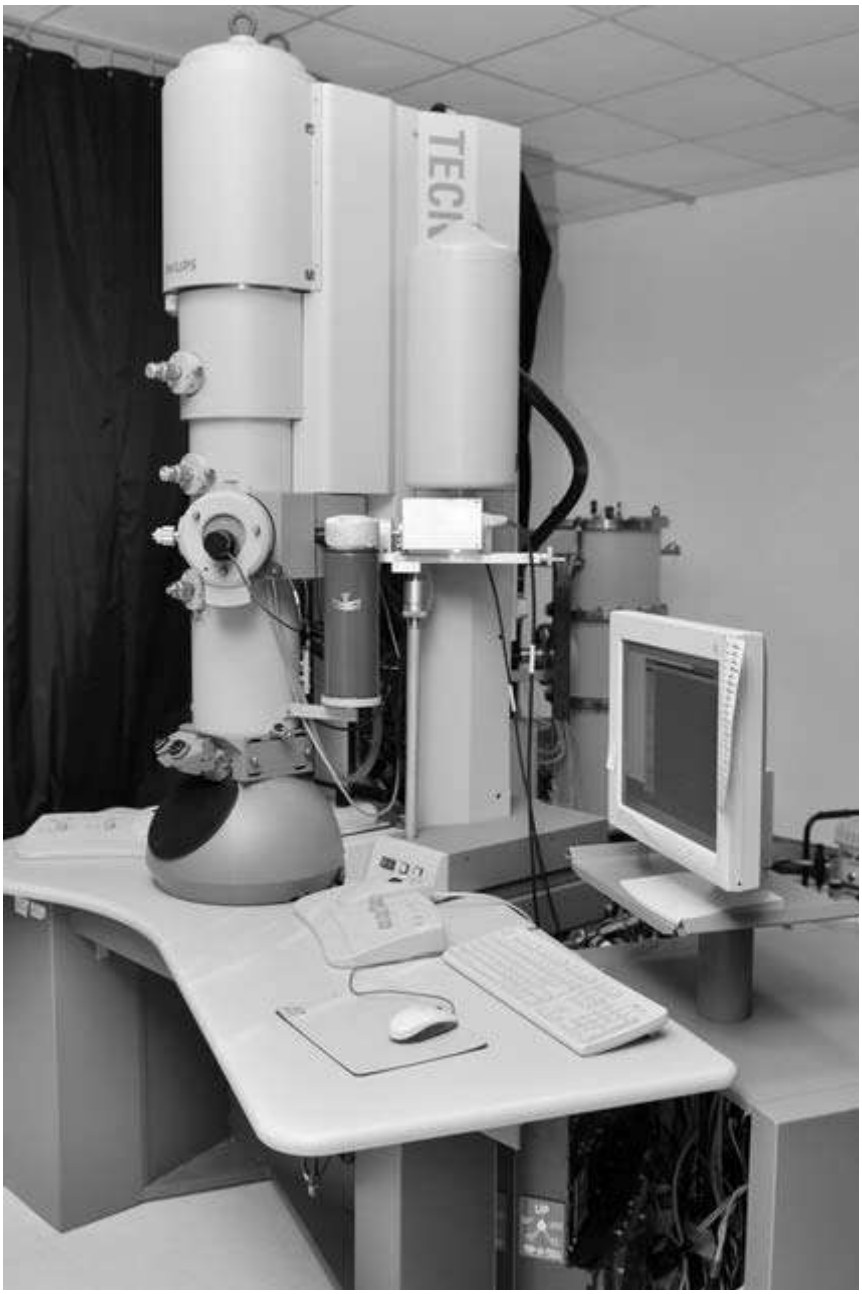


Fig.: Digital photograph of Transmission electron microscope

Vibrating sample magnetometer (VSM)

The magnetic properties of the synthesized samples would be measure at room temperature by the means of VSM at maximum applied field of 17.5 Koe. A small amount of powder ~ 10 mg was put in the butter paper and makes it tight to avoid the movement of sample inside the sample holder. A digital photograph of VSM is shown in figure.

Fig.: Digital photograph of vibrating sample magnetometer

5.7 SUMMARY

Application of Nano science

In the emerging field of nanotechnology, a goal is to make nanostructures or nano-arrays with special properties with respect to those of bulk or single-particle species. Oxide nanoparticles can exhibit unique physical and chemical properties due to their limited size and a high density of corner or edge surface sites. Particle size is expected to influence three important groups of properties in any material. The firstone comprises the structural characteristics, namely, the lattice symmetry and cell parameters. Bulk oxides are usually robust and stable systems with

well-defined crystallographic structures. However, the growing importance of surface-free energy and stress with decreasing particle size must be considered: Changes in thermo-dynamic stability associated with size can induce modification of cell parameters and/or structural transformations, and in extreme cases, the nanoparticle can disappear because of interactions with its surrounding environment and a high surface-free energy. To display mechanical or structural stability, a nanoparticle must have a low surface-free energy. As a consequence of this requirement, phases that have a low stability in bulk materials can become very stable in nanostructures. In technological applications, nano metal oxides are used in the fabrication of microelectronic circuits, sensors, piezoelectric devices, fuel cells, coatings for the passivation of surfaces against corrosion, and as catalysts. For example, almost all catalysts used in industrial applications involve an oxide as active phase, promoter, or "support." In the chemical and petrochemical industries, products worth billions of dollars are generated every year through processes that use oxide and metal/oxide catalysts. For the control of environmental pollution, catalysts or sorbents that contain oxides are employed to remove the CO, NO_x and SO_x species formed during the combustion of fossil-derived fuels. Furthermore, the most active areas of the semiconductor industry involve the use of oxides. Thus, most of the chips used in computers contain an oxide component.

5.8 TERMINAL QUESTIONS

1. How small a number of atoms is necessary until the properties of the original metal are lost?
2. How does an ordered accumulation of atoms behave, when it is no longer under the influence of its ambient bulk matter?
3. What are the future directions for nanotechniques and the applications of the new materials, for example, in microelectronic devices?
4. How X-ray is useful for the characterization of Nanomaterials and why?
5. Give the characterization techniques used for the Nanomaterials analysis and what information they give and use of that techniques too?
6. For the surface study of the materials which kind of techniques are used?
7. Describe 'Quantum Dots' and their application?

8. Give the Debye Scherrer Formula for the calculation of Average Particle Size

5.9 ANSWERS

1. If a metal particle, initially having bulk properties, is reduced in size to a few hundred or dozen of atoms, the density of states in the valence and conductivity bands decreases to such an extent that the electronic properties change dramatically; that is, conductivity, magnetism, and soon begin to disappear.

2. See Section 5.2

3. See Section 5.3

4. See Section 5.2

5. See Section 5.6

6. See Section 5.6

7. See Section 5.6

8. See Section 5.5

SUGGESTED READINGS

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