Topic 1: Errors in Analytical Measurements

***Key Notes*

Measurement errors	• *All measurement processes are subject to measurement errors that affect numerical data and which arise from a variety of sources.*
Absolute and relative errors	• *An absolute error is the numerical difference between a measured value and a true or accepted value. A relative error is the absolute error divided by the true or accepted value.*
Determinate errors	• Also known as systematic errors, or bias, these generally arise from determinate or identifiable sources causing measured values to differ from a true or accepted value.
Indeterminate errors	• Also known as random errors, these arise from a variety of uncontrolled sources and cause small random variations in a measured quantity when the measurement is repeated a number of times.
Accumulated errors	• *Where several different measurements are combined to compute an overall analytical result, the errors associated with each individual measurement contribute to a total or accumulated error.*

Measurement errors

The causes of measurement errors are numerous and their magnitudes are variable. This leads to uncertainties in reported results. However, measurement errors can be minimized and some types eliminated altogether by careful experimental design and control. Their effects can be assessed by the application of statistical methods of data analysis and chemometrics. Gross errors may arise from faulty equipment or bad laboratory practice; proper equipment maintenance and appropriate training and supervision of personnel should eliminate these. Nevertheless, whether it is reading a burette or thermometer, weighing a sample or timing events, or monitoring an electrical signal or liquid flow, there will always be inherent variations in the measured parameter if readings are repeated a number of times under the same conditions. In addition, errors may go undetected if the true or accepted value is not known for comparison purposes. Errors must be controlled and assessed so that valid analytical measurements can be made and reported. The reliability of such data must be demonstrated so that an end-user can have an acceptable degree of confidence in the results of an analysis.

Absolute and relative errors

The absolute error, E_A, in a measurement or result, X_M, is given by the equation

$$E_A = X_M - X_T$$

where X_T is the true or accepted value. Examples are shown in Figure 1 where a 200 mg aspirin standard has been analyzed a number of times. The absolute errors range from -4 mg to +10 mg.

The relative error, E_R , in a measurement or result, X_M , is given by the equation:

$$E_R = (X_M - X_T)/X_T$$

Often, E_R is expressed as a percentage relative error, 100 E_R . Thus, for the aspirin results shown in Figure 1, the relative error ranges from -2% to +5 %. Relative errors are particularly useful for comparing results of differing magnitude.

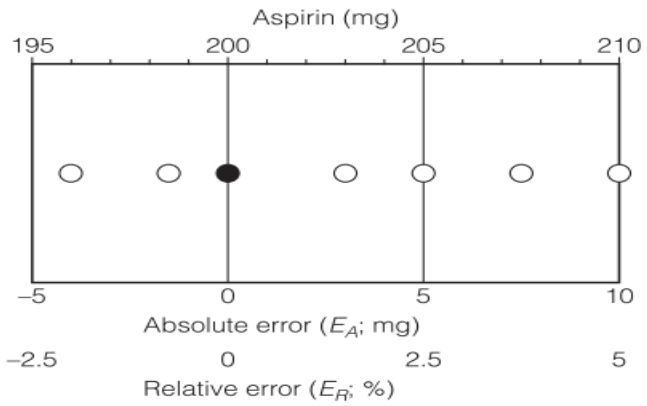


Fig. 1. Absolute and relative errors in the analysis of an aspirin standard.

Determinate Errors

There are three basic sources of determinate or systematic errors that lead to a bias in measured values or results:

- The analyst or operator;
- The equipment (apparatus and instrumentation) and the laboratory environment;
- The method or procedure.

It should be possible to eliminate errors of this type by careful observation and record keeping, equipment maintenance and training of laboratory personnel.

Operator errors can arise through carelessness, insufficient training, illness or disability. Equipment errors include substandard volumetric glassware, faulty or worn mechanical components, incorrect electrical signals and a poor or insufficiently controlled laboratory environment. Method or procedural errors are caused by inadequate method validation, the application of a method to samples or concentration levels for which it is not suitable or unexpected variations in sample characteristics that affect measurements. Determinate errors that lead to a higher value or result than a true or accepted one are said to show a positive bias; those leading to a lower value or result are said to show a negative bias. Particularly large errors are described as gross errors; these should be easily apparent and readily eliminated.

Determinate errors can be proportional to the size of sample taken for analysis. If so, they will have the same effect on the magnitude of a result regardless of the size of the sample, and their presence can thus be difficult to detect. For example, copper(II) can be determined by titration after reaction with potassium iodide to release iodine according to the equation

$$2Cu^{2+} + 4I^{-} \longrightarrow 2CuI + I_2$$

However, the reaction is not specific to copper(II), and any iron(III) present in the sample will react in the same way. Results for the determination of copper in an alloy containing 20%, but which also contained 0.2% of iron are shown in Figure 2 for a range of sample sizes. The same absolute error of +0.2% or relative error of 1% (i.e. a positive bias) occurs regardless of sample size, due to the presence of the iron. This type of error may go undetected unless the constituents of the sample and the chemistry of the method are known.

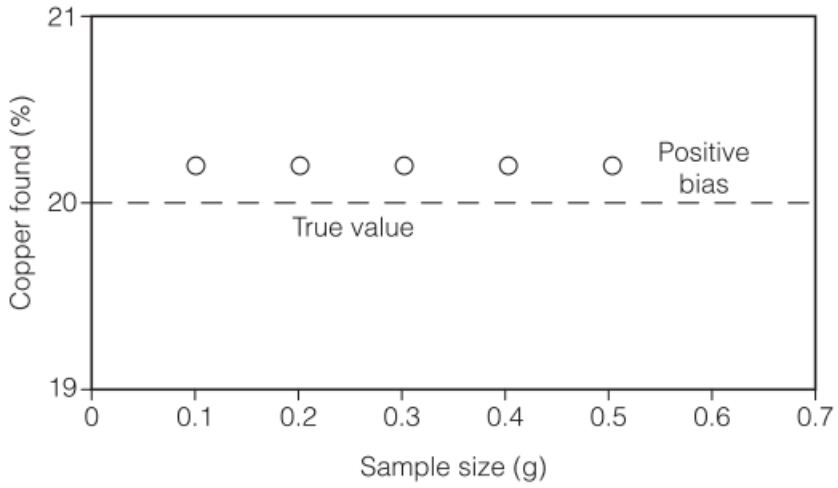


Fig. 2. Effect of a proportional error on the determination of copper by titration in the presence of iron.

Constant determinate errors are independent of sample size, and therefore become less significant as the sample size is increased. For example, where a visual indicator is employed in a volumetric procedure, a small amount of titrant is required to change the color at the end-point, even in a blank solution (i.e. when the solution contains none of the species to be determined). This indicator blank is the same regardless of the size of the titer when the species being determined is present. The relative error, therefore, decreases with the magnitude of the titer, as shown graphically in Figure 3. Thus, for an indicator blank of 0.02 cm^3 , the relative error for a 1 cm 3 titer is 2%, but this falls to only 0.08% for a 25 cm 3 titer.

Indeterminate errors

Known also as random errors, these arise from random fluctuations in measured quantities, which always occur even under closely controlled conditions. It is impossible to eliminate them entirely, but they can be minimized by careful experimental design and control. Environmental factors such as temperature, pressure and humidity, and electrical properties such as current, voltage and resistance are all susceptible to small continuous and random variations described as noise. These contribute to the overall indeterminate error in any physical or physicochemical measurement, but no one specific source can be identified.

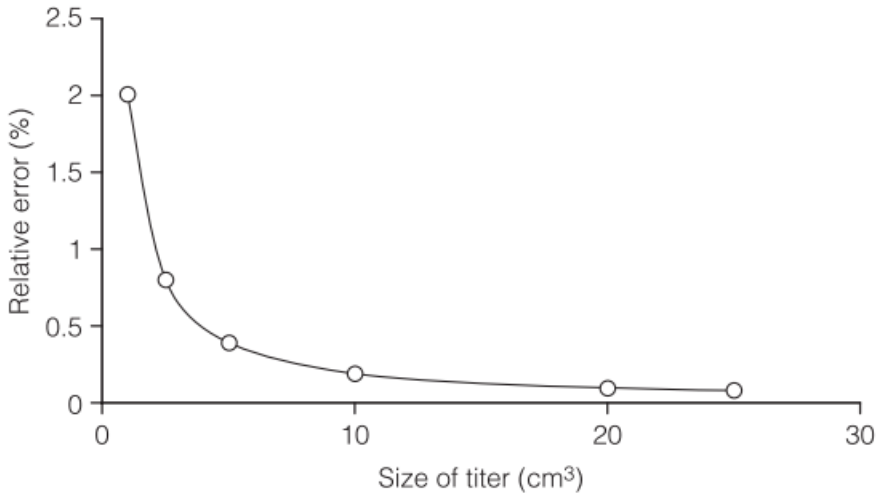


Fig. 3. Effect of a constant error on titers of differing magnitudes.

A series of measurements made under the same prescribed conditions and represented graphically is known as a frequency

distribution. The frequency of occurrence of each experimental value is plotted as a function of the magnitude of the error or deviation from the average or mean value.

For analytical data, the values are often distributed symmetrically about the mean value, the most common being the normal error or Gaussian distribution curve. The curve (Fig. 4) shows that:

- Small errors are more probable than large ones,
- Positive and negative errors are equally probable, and
- The maximum of the curve corresponds to the mean value.

The normal error curve is the basis of a number of statistical tests that can be applied to analytical data to assess the effects of indeterminate errors, to compare values and to establish levels of confidence in results (Topics in the following chapters)

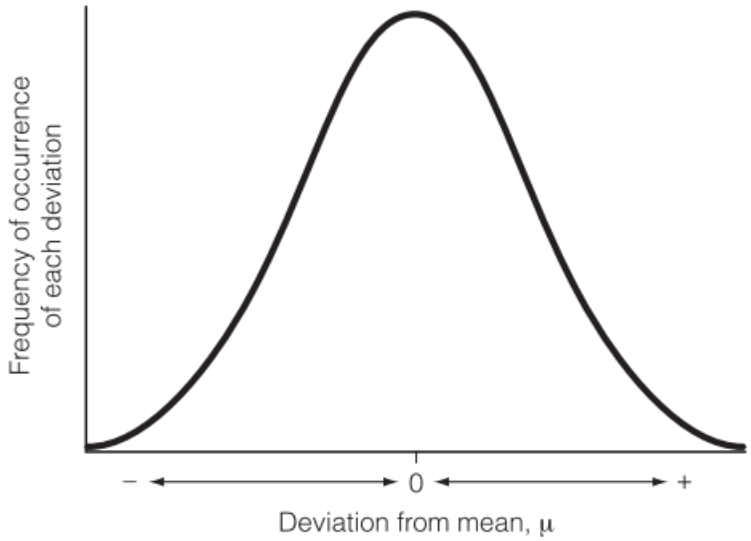


Fig. 4. The normal error or Gaussian distribution curve.

Accumulated errors

Errors are associated with every measurement made in an analytical procedure, and these will be aggregated in the final calculated result. The accumulation or propagation of errors is treated similarly for both determinate (systematic) and indeterminate (random) errors.

Determinate (systematic) errors can be either positive or negative, hence some cancellation of errors is likely in computing an overall determinate error, and in some instances this may be zero. The overall error is calculated using one of two alternative expressions, that is

- Where only a linear combination of individual measurements is required to compute the result, the overall absolute determinate error, E_T , is given by

$E_T = E_1 + E_2 + E_3 + \ldots\ldots$

E_1 , E_2 and E_3 etc., being the absolute determinate errors in the individual measurements taking sign into account

- Where a multiplicative expression is required to compute the result, the overall relative determinate error, E_{TR} , is given by

$E_{TR} = E_{1R} + E_{2R} + E_{3R} + \ldots\ldots$

E_{1R} and E_{2R} etc., being the relative determinate errors in the individual measurements taking sign into account.

The accumulated effect of indeterminate (random) errors is computed by combining statistical parameters for each measurement.

Topic 2: Assessment of Accuracy and Precision

***Key Notes*

Accuracy	• ***Accuracy*** is the closeness of an experimental measurement or result to the true or accepted value.
Precision	• ***Precision*** is the closeness of agreement between replicated measurements or results obtained under the same prescribed conditions
Standard deviation	• ***The standard deviation*** of a set of values is a statistic based on the normal error (Gaussian) curve and used as a measure of precision..
Relative standard deviation	• ***Relative standard deviation (coefficient of variation)*** is the standard deviation expressed as a percentage of the measured value
Pooled standard deviation	• **A standard deviation** can be calculated for two or more sets of data by pooling the values to give a more reliable measure of precision.
Variance	• *This is the **square of the standard deviation**, which is used in some statistical tests.*
Overall precision	• *An estimate of the **overall precision** of an analytical procedure can be made by combining the precisions of individual measurements.*
Confidence interval	• *This is the range of values around an experimental result within which the true or accepted value is expected to lie with a defined level of probability.*

Accuracy and precision

These two characteristics of numerical data are the most important and the most frequently confused. It is vital to understand the difference between them, and this is best illustrated diagrammatically as in Figure 1. Four analysts have each performed a set of five titrations for which the correct titer is known to be 20.00 cm^3 . The titers have been plotted on a linear scale, and inspection reveals the following:

- *The average titers for analysts **B** and **D** are very close to 20.00 cm^3 - these two sets are therefore said to have **good accuracy**;*
- *The average titers for analysts **A** and **C** are well above and below 20.00 cm^3 respectively - these are therefore said to have **poor accuracy**;*
- *The five titers for analyst **A** and the five for analyst **D** are very close to one another within each set – these two sets therefore both show **good precision**;*
- *The five titers for analyst **B** and the five for analyst **C** are spread widely within each set - these two sets therefore both show **poor precision**.*

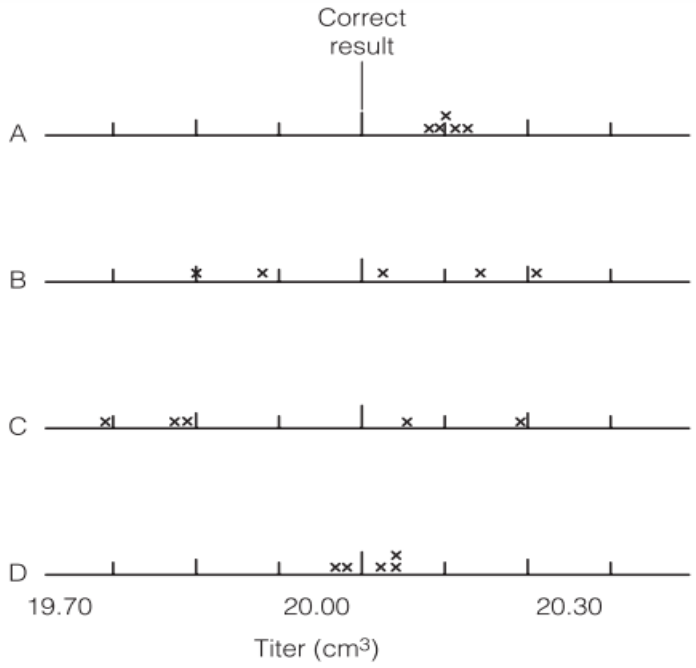


Fig. 1. Plots of titration data to distinguish accuracy and precision.

It should be noted that good precision does not necessarily produce good accuracy (analyst A) and poor precision does not necessarily produce poor accuracy (analyst B). However, confidence in the analytical procedure and the results is greater when good precision can be demonstrated (analyst D).

Accuracy is generally the more important characteristic of quantitative data to be assessed, although consistency, as measured by precision, is of particular concern in some circumstances. Trueness is a term associated with accuracy, which describes the closeness of agreement between the average of a large number of results and a true or accepted reference value. The degree of accuracy required depends on the context of the analytical problem; results must be shown to be fit for the purpose for which they are intended. For example, one result may be satisfactory if it is within 10% of a true or accepted value whilst it may be necessary for another to be within 0.5%.

By repeating an analysis a number of times and computing an average value for the result, the level of accuracy will be improved, provided that no systematic error (bias) has occurred.

Accuracy cannot be established with certainty where a true or accepted value is not known, as is often the case. However, statistical tests indicating the accuracy of a result with a given probability are widely used (vide infra).

Precision, which is a measure of the variability or dispersion within a set of replicated values or results obtained under the same prescribed conditions, can be assessed in several ways. The spread or range (i.e. the difference between the highest and lowest value) is sometimes used, but the most popular method is to estimate the standard deviation of the data (vide infra).

The precision of results obtained within one working session is known as repeatability or within-run precision. The precision of results obtained over a series of working sessions is known as reproducibility or between-runs precision. It is sometimes necessary to separate the contributions made to the overall precision by within-run and between-runs variability. It may also be important to establish the precision of individual steps in an analysis.

Standard deviation (SD)

This is the most widely used measure of precision and is a parameter of the normal error or Gaussian curve (Previous Topic, Fig. 4). Figure 2 shows two curves for the frequency distribution of two theoretical sets of data, each having an infinite number of values and known as a statistical population.

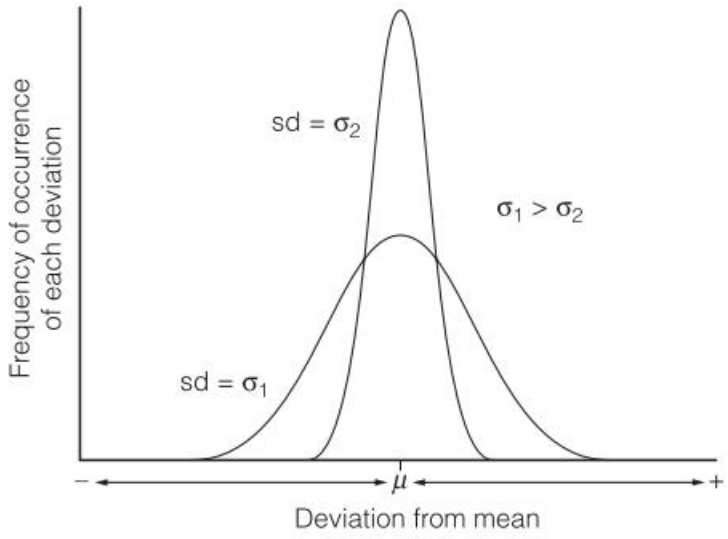


Fig. 2. Normal error or Gaussian curves for the frequency distributions of two statistical populations with differing spreads.

The maximum in each curve corresponds to the population mean, which for these examples has the same value, m. However, the spread of values for the two sets is quite different, and this is reflected in the half-widths of the two curves at the points of inflection, which, by definition, is the population standard deviation, σ. As σ_2 is much less than σ_1 , the precision of the second

set is much better than that of the first. The abscissa scale can be calibrated in absolute units or, more commonly, as positive and negative deviations from the mean, μ.

In general, the smaller the spread of values or deviations, the smaller the value of σ and hence the better the precision. In practice, the true values of μ and σ can never be known because they relate to a population of infinite size. However, an assumption is made that a small number of experimental values or a statistical sample drawn from a statistical population is also distributed normally or approximately so. The experimental mean, $\bar{x}$ *, of a set of values* x_1 , x_2 , x_3 ,…….x_n *is therefore considered to be an estimate of the true or population mean, μ, and the experimental standard deviation, s, is an estimate of the true or population standard deviation, σ.*

A useful property of the normal error curve is that, regardless of the magnitude of m and s, the area under the curve within defined limits on either side of m (usually expressed in multiples of ± σ) is a constant proportion of the total area. Expressed as a percentage of the total area, this indicates that a particular percentage of the population will be found between those limits. Thus, approximately 68% of the area, and therefore of the population, will be found within ±1σ of the mean, approximately 95% will be found within ±2σ and approximately 99.7% within ±3σ . More practically convenient levels, as shown in Figure 3, are those corresponding to 90%, 95% and 99% of the population, which are defined by ±1.64σ, ±1.96σ and ±2.58σ respectively. Many statistical tests are based on these probability levels.

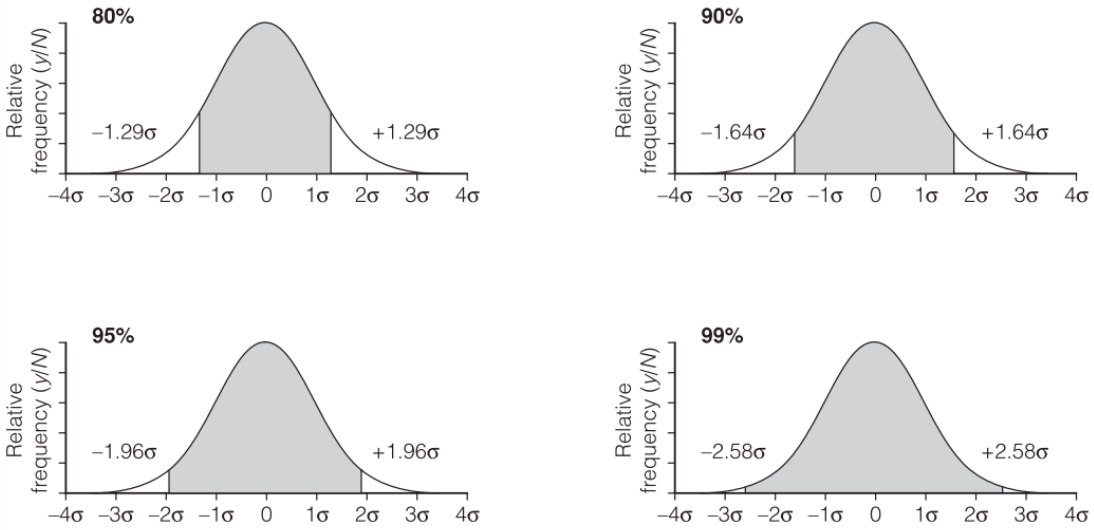


Fig. 3. Proportions of a population within defined limits of the mean.

The value of the population standard deviation, s, is given by the formula:

$$\sigma = \sqrt{\frac{\sum_{i=1}^{i=N}(x_i - \mu)^2}{N}} \quad \text{.....(1)}$$

where xi represents any individual value in the population and N is the total number of values, strictly infinite.

The summation symbol, ∑, is used to show that the numerator of the equation is the sum for i = 1 to i = N of the squares of the deviations of the individual x values from the population mean, μ. For very large sets of data (e.g., when N >50), it may be justifiable to use this formula as the difference between σ and s will then be negligible. However, most analytical data consists of sets of values of less than ten and often as small as three.

Therefore, a modified formula is used to calculate an estimated standard

deviation, s, to replace σ , and using an experimental mean, $\bar{x}$, to replace the population mean, μ:

$$s = \sqrt{\frac{\sum_{i=1}^{i=N}(x_i - \bar{x})^2}{N-1}} \qquad (2)$$

Note that N in the denominator is replaced by N-1, which is known as the number of degrees of freedom and is defined as the number of independent deviations ($x_i - \bar{x}$) used to calculate s. For single sets of data, this is always one less than the number in the set because when N-1 deviations are known the last one can be deduced as, taking sign into account, $\sum_{i=1}^{i=N}(x_i - \bar{x})^2$ must be zero (see Example 1 below).

In summary, the calculation of an estimated standard deviation, s, for a small number of values involves the following steps:

- *calculation of an experimental mean;*
- *calculation of the deviations of individual x i values from the mean;*
- *squaring the deviations and summing them;*
- *dividing by the number of degrees of freedom, N - 1, and*
- *taking the square root of the result.*

Note that if N were used in the denominator, the calculated value of s would be an underestimate of s. Estimated standard deviations are easily obtained using a calculator that incorporates statistical function keys or with one of the many computer software packages. It is, however, useful to be able to perform a

stepwise arithmetic calculation, and an example using the set of five replicate titers by analyst A (Fig. 1) is shown below.

Example 1

	x_i/cm^3	$(x_i - x)$	$(x_i - x)^2$
	20.16	−0.04	1.6×10^{-3}
	20.22	+0.02	4×10^{-4}
	20.18	−0.02	4×10^{-4}
	20.20	0.00	0
	20.24	+0.04	1.6×10^{-3}
Σ	101.00		$\mathbf{4 \times 10^{-3}}$
$\bar{x}$	**20.20**		

$$s = \sqrt{\frac{4 \times 10^{-3}}{4}} = 0.032 \text{ cm}^3$$

Relative standard deviation (RSD)

The relative standard deviation, RSD or s r, is also known as the coefficient of variation, CV. It is a measure of relative precision and is normally expressed as

a percentage of the mean value or result:

$$s_r = \left(s/\bar{x}\right) * 100 \text{.....(3)}$$

It is an example of a relative error (previous Topic) and is particularly useful for comparisons between sets of data of differing magnitude or units, and in calculating accumulated (propagated) errors. The RSD for the data in Example 1 is given below.

s_r= (0.032/20.20) *100=0.16%

Pooled standard deviation

Where replicate samples are analyzed on a number of occasions under the same prescribed conditions, an improved estimate of the standard deviation can be obtained by pooling the data from the individual sets. A general formula for

$$s_{pooled} = \sqrt{\frac{\sum_{i=1}^{i=N_1}(x_i - \bar{x}_1)^2 + \sum_{i=1}^{i=N_2}(x_i - \bar{x}_2)^2 + \sum_{i=1}^{i=N_3}(x_i - \bar{x}_3)^2 + \ldots + \sum_{i=1}^{i=N_k}(x_i - \bar{x}_k)^2}{\sum_{i=1}^{i=k} N_i = k}} \qquad (4)$$

where N_1, N_2, $N_3 \ldots N_k$ are the numbers of results in each of the k sets, and $\bar{x}_1$, $\bar{x}_2$, $\bar{x}_3$, . . . $\bar{x}_k$, are the means for each of the k sets.

the pooled standard deviation, s pooled, is given by the expression:

Variance

The square of the standard deviation, σ^2 , or estimated standard deviation, s^2 , is used in a number of statistical computations and tests, such as for calculating accumulated (propagated) errors (previous Topic and below) or when comparing the precisions of two sets of data (following chapter)

Overall precision

Random errors accumulated within an analytical procedure contribute to the overall precision. Where the calculated result is derived by the addition or subtraction of the individual values, the overall precision can be found by summing the variances of all the measurements so as to provide an estimate of the overall standard deviation, i.e.

$$s_{overall} = \sqrt{(s_1^2 + s_2^2 + s_2^2 + \cdots \ldots)}$$

Example

In a titrimetric procedure, the buret must be read twice, and the error associated with each reading must be taken into account in estimating the overall precision. If the reading error has an estimated standard deviation of 0.02 cm^3 , then the overall estimated standard deviation of the titration is given by:

$$s_{overall} = \sqrt{(0.02^2 + 0.02^2)} = 0.028\, cm^3$$

Note that this is less than twice the estimated standard deviation of a single reading. The overall standard deviation of weighing by difference is estimated in the same way.

If the calculated result is derived from a multiplicative expression, the overall relative precision is found by summing the squares of the relative standard deviations of all the measurements, i.e.

$$s_{r\,(overall)} = \sqrt{(s_{r1}^2 + s_{r2}^2 + s_{r2}^2 + \cdots \ldots .)}$$

Confidence interval

The true or accepted mean of a set of experimental results is generally unknown except where a certified reference material is being checked or analyzed for calibration purposes.

In all other cases, an estimate of the accuracy of the experimental mean, $\bar{x}$, must be made. This can be done by defining a range of values on either side of $\bar{x}$ within which the true mean, μ, is expected to lie with a defined level of probability. This range, which ideally should be as narrow as possible, is based on the standard deviation and is known as the confidence interval, CI, and the upper and lower limits of the range as confidence limits, CL. Confidence limits can be calculated using the standard deviation, σ, if it is known, or the estimated standard deviation, s, for the data. In either case, a probability level must be defined, otherwise the test is of no value.

When the standard deviation is already known from past history, the confidence limits are given by the equation

$$CL(\mu) = \bar{x} \pm \frac{z\sigma}{\sqrt{N}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(5)$$

where z is a statistical factor related to the probability level required, usually

90%, 95% or 99%. The values of z for these levels are 1.64, 1.96 and 2.58, respectively, and correspond to the multiples of the standard deviation shown in Figure 3.

Where an estimated standard deviation is to be used, σ is replaced by s, which must first be calculated from the current data. The confidence limits are then given by the equation:

$$CL(\mu) = \bar{x} \pm \frac{ts}{\sqrt{N}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

where z is replaced by an alternative statistical factor, t, also related to the probability level but in addition determined by the number of degrees of freedom for the set of data, i.e. one less than the number of results. It should be noted that (i) the confidence interval is inversely proportional to $\sqrt{N}$, and (ii) the higher the selected probability level, the greater the confidence interval becomes as both z and t increase.

A probability level of 100 percent is meaningless, as the confidence limits would then have to be ± ∞.

The following examples demonstrate the calculation of confidence limits using each of the two formulae.

Example 3

The chloride content of water samples has been determined a very large number of times using a particular method, and the standard deviation found to be 7 ppm. Further analysis of a particular sample gave experimental values of 350 ppm for a single determination, for the mean of two replicates and for the mean of four replicates. Using equation (5), and at the 95% probability level, z = 1.96 and the confidence limits are:

1 determinations CL(m) = 350 ± = 350 ± 14 ppm

2 determinations CL(m) = 350 ± = 350 ± 10 ppm

4 determinations CL(m) = 350 ± = 350 ± 7 ppm

Example 4

The same chloride analysis as in Example 3, but using a new method for which the standard deviation was not known, gave the following replicate results, mean and estimated standard deviation:

Chloride/ppm	Mean	Estimated standard deviation
346 359 350	351.67 ppm	6.66 ppm

Using equation (6), and at the 95% probability level, $t = 4.3$ for **two degrees of freedom**, and the confidence limits are:

$$3 \text{ determinations} \quad CL(\mu) = 352 \pm \frac{4.3 \times 6.66}{\sqrt{3}} = \mathbf{352 \pm 17 \text{ ppm}}$$

The wider limits given by equation (6) when the standard deviation is estimated with only three results reflects the much greater uncertainty associated with this value, which in turn affects the confidence in the degree of accuracy. To demonstrate good accuracy, the confidence interval, CI, should be as small as possible and increasing the number of replicates will clearly achieve this. However, due to the $\sqrt{N}$ *term in the denominator, to reduce the interval by, say, a factor of two requires an increase in the number of replicates by a factor of four as shown by Example 3.*

Unfortunately, the law of diminishing returns applies here, so if the CI is to be halved again, the number of replicates must be increased from four to sixteen. Similarly, in Example 4, the number of replicates would have to be increased from three to twelve to halve the CI, which would represent an unacceptable amount of time and money for most analytical laboratories.

Topic 3: Significance Testing

***Key Notes*

Significance tests	• These are statistical tests used to compare individual values or sets of values for significant differences
Outliers	• *A measurement or result that appears to differ significantly from others in the same set of replicates is described as an outlier.*
Q-test	• The Q-test is used to determine whether to reject or retain a suspected outlier.
F-test	• The F-test enables the precisions of two sets of data to be compared using their variances.
t-test	• The t-test is used to compare two experimental means, an experimental mean with a known value or sets of pairs of experimental values.
Analysis of variance	• F-tests can be applied to several sets of data to assess and compare different sources of variability.

Significance tests

Significance tests involve a comparison between a calculated experimental factor and a tabulated factor determined by the number of values in the set(s) of experimental data and a selected probability level that the conclusion is correct. They are used for several purposes, such as:

- *To check individual values in a set of data for the presence of determinate errors (bias);*
- *To compare the precision of two or more sets of data using their variances;*
- *To compare the means of two or more sets of data with one another or with known values to establish levels of accuracy.*

Tests are based on a null hypothesis - an assumption that there is no significant difference between the values being compared. The hypothesis is accepted if the calculated experimental factor is less than the corresponding tabulated factor, otherwise it is rejected and there is said to be a significant difference between the values at the selected probability level. The conclusion should always be stated clearly and unambiguously.

Probability levels of 90%, 95% and 99% are generally considered appropriate for most purposes, but it should be remembered that there are also corresponding 10%, 5% or 1% probabilities, respectively, of the opposite conclusion being valid. For example, if a test indicates that the null hypothesis is correct and that there is no significant difference between two

values at the 95% probability level, it also allows the possibility that there is a significant difference at the 5% level.

Outliers

Inspection of a set of replicate measurements or results may reveal that one or more is considerably higher or lower than the remainder and appears to be outside the range expected from the inherent effects of indeterminate (random) errors alone. Such values are termed outliers, or suspect values, because it is possible that they may have a bias due to a determinate error. On occasions, the source of error may already be known or it is discovered on investigation, and the outlier(s) can be rejected without recourse to a statistical test. Frequently, however, this is not the case, and a test of significance such as the Q-test should be applied to a suspect value to determine whether it should be rejected and therefore not included in any further computations and statistical assessments of the data.

Q-test

Also known as Dixon's Q-test, this is one of several that have been devised to test suspected outliers in a set of replicates. It involves the calculation of a ratio, Q_{exptl}*, defined as the absolute difference between a suspect value and the value closest to it divided by the spread of all the values in the set:*

Q_{exptl}=|*suspect value* − *nearest value*|/(*largest value*–*smallest value*)

Q_{exptl} *is then compared with a tabulated value,* Q_{tab} *at a selected level of probability, usually 90% or 95%, for a set of n values (Table 1). If* Q_{exptl} *is less than* Q_{tab} *, then the null hypothesis that there is no significant difference between the suspect value and the other values in the set is accepted, and the suspect value is retained for further data processing. However, if* Q_{exptl} *is greater than* Q_{tab}*, then the suspect value is regarded as an outlier and is rejected. A rejected value should NOT be used in the remaining calculations.*

Table 1. Critical values of Q at the 95% (P = 0.05) level for a two-tailed test

Sample size	Critical value
4	0.831
5	0.717
6	0.621
7	0.570
8	0.524

Example 1

Four replicate values were obtained for the determination of a pesticide in river water

0.403, 0.410, 0.401, 0.380 mg dm^{-3}

Inspection of the data suggests that 0.380 μg dm^{-3} is a possible outlier.

$$Q_{exptl} = |0.380 - 0.401|/(0.410 - 0.380) = 0.021/0.03 = 0.70$$

$$Q_{tab} = 0.83 \text{ for four values at the 95\% probability level}$$

As Q_{exptl} is **less** than Q_{tab}, 0.380 μg dm^{-3} is not an outlier at the 95% level and should be retained.

Example 2

If, in Example 1, three additional values of 0.400, 0.413 and 0.411 mg dm^{-3} were included, 0.380 mg dm^{-3} is still a possible outlier.

Q_{exptl}= |0.380 − 0.400 |/*(0.413 - 0.380) = 0.020/0.033 = 0.61*

Q tab = 0.57 for seven values at the 95% probability level

Now, as Q_{exptl} is greater than Q tab, 0.380 mg dm^{-3} is an outlier at the 95% level and should be rejected. Note that because the three additional values are all around 0.4 mg dm^{-3}, the suspect value of 0.380 mg dm^{-3} appears even more anomalous.

F-test

This test is used to compare the precisions of two sets of data which may originate from two analysts in the same laboratory, two different methods of analysis for the same analyte or results from two different laboratories. A statistic, F, is defined as the ratio of the population

variances, σ_1^2/σ_2^2, or the sample variances, s_1^2/s_2^2, of the two sets of data where the larger variance is always placed in the numerator so that F ≥1.

If the null hypothesis is true, the variances are equal and the value of F will be one or very close to it. As for the Q-test, an experimental value, F_{exptl}, is calculated and compared with a tabulated value, F_{tab}, at a defined probability level, usually 90% or 95%, and for the number of degrees of freedom, N - 1, for each set of data. If F_{exptl} is less than F_{tab}, then the null hypothesis that there is no significant difference between the two variances and hence between the precision of the two sets of data, is accepted. However, if F_{exptl} is greater than F_{tab}, there is a significant difference between the two variances and hence between the precisions of the two sets of data.

Some values of F_{tab} at the 95% probability level are given in Table 2. The columns in the table correspond to the numbers of degrees of freedom for the numerator set of data, while the rows correspond to the number of degrees of freedom for the denominator set. Two versions of the table are available, depending on the exact purpose of the comparison to be made: a one-tailed F-test will show whether the

precision of one set of data is significantly better than the other, while a two-tailed F-test will show whether the two precisions are significantly different.

Table 2. Critical values of F at the 95% (P = 0.05) level for a two-tailed test

ν_1	5	7	9
ν_2			
5	7.146	6.853	6.681
7	5.285	4.995	4.823
9	4.484	4.197	4.026

ν_1 = number of degrees of freedom of the numerator. ν_2 = number of degrees of freedom of the denominator

The application of a two-tailed F-test is demonstrated by the following example.

The two-tailed tabular value for F with 7 degrees of freedom for both the numerator and the denominator is

$F_{7,7}$ = 5.00 at the 95% probability level

Example 3

A proposed new method for the determination of sulfate in an industrial waste effluent is compared with an existing method, giving the following results:

Method	Mean/g dm^{-3}	No. of replicates	No. of degrees of freedom	s/mg dm^{-3}
Existing	72	8	7	3.38
New	72	8	7	1.50

Is there a significant difference between the precisions of the two methods?

$$F_{exptl} = s^2_{existing} \Big/ s^2_{new} = \frac{(3.38)^2}{(1.50)^2} = 5.08$$

As F_{exptl} is greater than F_{tab} , the null hypothesis is rejected; the two methods are giving significantly different precisions.

This test is used to compare the experimental means of two sets of data or to compare the experimental mean of one set of data with a known or reference value. A statistic, t, is defined, depending on the circumstances, by one of three alternative equations.

Comparison of two experimental means, $\bar{x}_A$ and $\bar{x}_B$

$$t = \frac{(\bar{x}_A - \bar{x}_B)}{s_{pooled}} \times \left(\frac{NM}{N+M}\right)^{1/2} \qquad (1)$$

where s pooled is the pooled estimated standard deviation for sets A and B, and N and M are the numbers of values in sets A and B respectively. If N = M, then the second term reduces to $\left(\frac{N}{2}\right)^{1/2}$. *A simplified version of equation (4), can be used to calculate* s_{pooled} *as there are only two sets of data.*

$$s_{pooled} = \left\{ \left[(N-1)s_A^2 + (M-1)s_B^2 \right] / \left[N + M - 2 \right] \right\}^{1/2} \qquad (2)$$

In some circumstances, the use of equation (1) may not be appropriate for the comparison of two experimental means. Examples of when this may be the case are if

- *The amount of sample is so restricted as to allow only one determination by each of the two methods;*

- *The methods are to be compared for a series of samples containing different levels of analyte rather than replicating the analysis at one level only;*

- *Samples are to be analyzed over a long period of time when the same experimental conditions cannot be guaranteed.*

It may therefore be essential or convenient to pair the results (one from each method) and use a paired t-test where t is defined by

$$t = \frac{\bar{x}_d}{s_d} \times N^{1/2} \qquad (3)$$

$\bar{x}_d$ being the mean difference between paired values and s_d the estimated standard deviation of the differences.

Comparison of one experimental mean with a known value, μ

$$t = \frac{(\bar{x} - \mu)}{s} \times N^{1/2} \qquad (4)$$

Using the appropriate equation, an experimental value, t_{exptl}, is calculated and compared with a tabulated value, t_{tab}, at a defined probability level, usually between 90 and 99%, and for $N-1$ degrees of freedom (equations (3) and (4)) or $(N+M-2)$ degrees of freedom (equation (1)). If t_{exptl} is less than t_{tab}, then the null hypothesis that there is no significant difference between the two experimental means or between the experimental mean and a known value is accepted, i.e. there is no evidence of a bias. However, if t_{exptl} is greater than t_{tab}, there is a significant difference indicating a bias.

Both one-tailed and two-tailed t-tests can be used, depending on circumstances, but two-tailed are often preferred (Table 3). The application of all three t-test equations is demonstrated by the following examples.

Table 3. Critical values of t at the 95% and 99% (P = 0.05 and 0.01) levels for a two-tailed test

Number of degrees of freedom	95 percent level	99 percent level
2	4.30	9.92
5	2.57	4.03
10	2.23	3.10
18	2.10	2.88

Example 1

Two methods for the determination of polyaromatic hydrocarbons in soils were compared by analyzing a standard with the following results:

No. of determinations by each method:	10	
No. of degrees of freedom:	18	
UV spectrophotometry:	$\bar{x} = 28.00$ mg kg^{-1}	$s = 0.30$ mg kg^{-1}
Fluorimetry:	$\bar{x} = 26.25$ mg kg^{-1}	$s = 0.23$ mg kg^{-1}

Do the mean results for the two methods differ significantly?

Equation (2) is first used to calculate a pooled standard deviation:

$$s_{pooled} = \left\{ \left[(N-1)s_A^2 + (M-1)s_B^2 \right] / \left[N + M - 2 \right] \right\}^{1/2} = \{(9 \times 0.3^2 + 9 \times 0.23^2)/18\}^{1/2}$$

$$s_{pooled} = 0.267 \text{ mg kg}^{-1}$$

Then equation (1) is used to evaluate t_{exptl}

$$t_{exptl} = \frac{(\bar{x}_A - \bar{x}_B)}{s_{pooled}} \times \left(\frac{NM}{N+M} \right)^{1/2} = \{(28.0 - 26.25)/0.267\} \times 5^{1/2} = \mathbf{14.7}$$

For 18 degrees of freedom, the two-tailed value of t_{tab} at the 95% probability level is 2.10, and at the 99% level it is 2.88.

As t_{exptl} is greater than t_{tab} at both the 95 and 99% probability levels, there is a significant difference between the means of the two methods.

Example 2

A new high performance liquid chromatographic method for the determination of pseudoephedrine in a pharmaceutical product at two different levels was compared with an established method with the following results:

Pseudoephedrine per dose (mg)	
Method 1	Method 2
59.9	58.6
59.3	58.3
60.4	60.5
30.7	29.4
30.2	30.4
30.1	28.9

Do the means of the two methods differ significantly?

Because the two levels of pseudoephedrine differ considerably, equation (3) for a paired t-test is used to calculate t_{exptl}. The differences between the pairs of values are 1.3, 1.0, −0.1, 1.3, −0.2 and 1.2 mg per dose, and the estimated standard deviation of the differences from their mean of 0.750 mg per dose is 0.706 mg per dose. Substitution of these values into the equation gives

$$t_{exptl} = \frac{\bar{x}_d}{s_d} \times N^{1/2} = (0.750/0.706) \times 6^{1/2} = \mathbf{2.60}$$

For 5 degrees of freedom, the two-tailed value of t_{tab} at the 95% probability level is 2.57. As t_{exptl} is **greater** than t_{tab}, there is a significant difference between the means of the two methods. (Note: using equation (1) would give a t_{exptl} value of 0.08 and an incorrect conclusion.)

Example 3

A method for the determination of mercury by atomic absorption spectrometry gave values of 400, 385 and 382 ppm for a standard known to contain 400 ppm. Does the mean value differ significantly from the true value, or is there any evidence of systematic error (bias)?

$\bar{x}$=389 ppm s = 9.64 ppm μ = 400 ppm

Using equation (4) to evaluate t_{exptl}

$$t_{exptl} = \frac{(\bar{x}-\mu)}{s}\ x\ N^{1/2} = \frac{(389-400)}{9.64}\ x\ 3^{1/2} = 1.89$$

For 2 degrees of freedom, the two-tailed t_{tab} value is 4.30 at the 95% probability level. As t_{exptl} is less than the two-tailed value of t_{tab}, the mean is not significantly different from the true value. There is, therefore, no evidence of a systematic error, or bias.

Analysis of variance

Analysis of variance, also known as ANOVA, is a statistical technique for investigating different sources of variability associated with a series of results. It enables the effect of each source to be assessed separately and compared with the other(s) using F-tests. Indeterminate or random errors affect all measurements, but additional sources of variability may also arise. The additional sources can be divided into two types:

- Additional random effects, described as random-effect factors;
- Specific effects from determinate sources, described as controlled or fixed effect factors.

Where one additional effect may be present, a one-way **ANOVA** is used, whilst for two additional effects, two-way **ANOVA** is appropriate. Both involve much lengthier calculations than the simpler tests of significance, but facilities for these are available with computer packages such as Microsoft Excel and Minitab.

Typical examples of the use of **ANOVA** are:

- Analysis of a heterogeneous material where variation in composition is an additional random factor;
- Analysis of samples by several laboratories, methods or analysts where the laboratories, methods or analysts are additional fixed-effect factors;
- Analysis of a material stored under different conditions to investigate stability where the storage conditions provide an additional fixed-effect factor.

Topic 4: Calibration and Linear Regression

**Key Notes

Calibration	• Calibration is the process of establishing a relation between a detection or measurement system and known amounts or concentrations of an analyte under specified conditions.
Correlation coefficient	• The coefficient is used to assess the degree of linearity between two variables, e.g. an instrument response and an analyte mass or concentration.
Linear regression	• Calculations to define the best straight line through a series of calibration points represented graphically are described as linear regression.
Limit of detection	• The smallest mass or concentration of an analyte that can be measured quantitatively at a defined level of probability defines a limit of detection.
Standard addition	• This is a calibration procedure that avoids matrix interference by measuring instrument response for an analyte in both the sample and a sample to which known amounts of an analyte standard have been added.
Internal standardization	• This is a calibration procedure where the ratio of the instrument response for an analyte to that of an added standard is measured for a series of analyte standards and samples. .
Internal normalization	• Internally normalized results give the relative composition of a mixture by expressing the instrument response for each analyte as a fraction or percentage of the sum of the responses for all of the analytes.

Calibration

Many quantitative analytical procedures rely on instrumental measurements where a property of the analyte(s) is monitored by a suitable detection system. The detector generates an electrical signal, the magnitude of which is determined by the mass or concentration of the analyte. Before using a particular analytical procedure to analyze samples, it is first necessary to establish the detector responses to known amounts of the analyte (calibration standards) over a selected mass or concentration range, a process known as calibration.

The relation between the two variables is often linear (directly proportional), but there is generally an upper limit to the range of values beyond which a curved or curvilinear relation is observed. In some instances, there may be no direct linear relation at all, or a logarithmic or more complex mathematical correlation may be found.

Calibration data are generally used to construct a calibration graph, where detector response is plotted on the ordinate axis (y-values) and mass or concentration of the analyte on the abscissa axis (x-values) as shown in Figure 1.

The graphs are often linear, being defined by the equation

$$y = bx + a \quad \text{.....(1)}$$

where b is the slope and a the intercept on the y-axis. In some cases, it is prefer able to plot a logarithmic function of the detector response or analyte concentration to obtain a linear calibration curve.

Unknown levels of the analyte are determined from the graph by interpolation. Where a linear relation has been established, a calibration factor can be used to convert detector response to mass or concentration of analyte when analyzing samples. Theoretically, the graph should pass through the origin, but frequently in practice there is a small positive intercept due to traces of analyte in the reagent blank or contributions to the detector signal by other components in the standards. Calibration points also show a degree of scatter due to the effects of experimental errors in preparing the standards, or noise in the measuring circuitry. A line of best fit through the points, known as a regression line, is therefore drawn or computed.

Calibration graphs may show curvature, particularly at higher mass or concentration levels, but this does not invalidate their use if the data are reproducible. However, it is advisable to prepare additional standards to define the curve more closely, and the use of a factor to compute analyte levels in samples is precluded. Statistical methods are used to assess calibration data

- For linearity or otherwise;
- To calculate the parameters defining a calibration curve;
- To assess the effects of determinate and indeterminate errors on standards and samples.

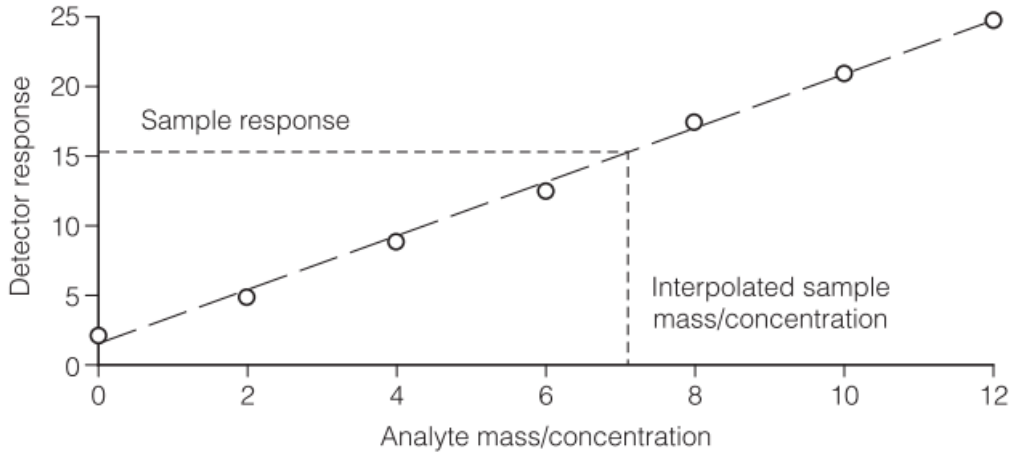


Fig. 1. A typical calibration graph.

Correlation coefficient

The correlation coefficient, r, indicates the degree of linearity between x and y and is given by the expression

$$r = \frac{\sum_{i=1}^{i=N}\{(x_i - \bar{x})(y_i - \bar{y})\}}{\left\{\left[\sum_{i=1}^{i=N}(x_i - \bar{x})^2\right]\left[\sum_{i=1}^{i=N}(y_i - \bar{y})^2\right]\right\}^{1/2}} \qquad (2)$$

where x_1y_1; x_2y_2; x_3y_3;....x_n,y_n are the co-ordinates of the plotted points, $\bar{x}$ and $\bar{y}$ are the means of the x and y values respectively, and Σ indicates sums of terms (see standard deviation equations (1), (2) and (4), Topic B2).

The range of possible values for r is $-1 \leq r \leq +1$. A value of unity indicates a perfect linear correlation between x and y, all the points lying exactly on a straight line, whilst a value of zero indicates no linear correlation. Values may be positive or negative depending on the slope of the calibration graph. These alternatives are illustrated in Figure 2 (a) to (c).

Most calibration graphs have a positive slope, and correlation coefficients frequently exceed 0.99. They are normally quoted to four decimal places. (Note that graphs with a slight curvature may still have correlation coefficients exceeding about 0.98 (Fig. 2(d)), hence great care must be taken before concluding that the data shows a linear relation. Visual inspection of the plotted points is the only way of avoiding mistakes.)

Linear regression

When inspection of the calibration data and the value of the correlation coefficient show that there is a linear relation between the detector response and the mass or concentration of the analyte, it is necessary to draw a line of best fit through the plotted points before they can be used as a working curve. Although this can be done by eye, a more accurate method is to employ linear regression.

It is invariably the case that, due to the effects of indeterminate errors on the data, most of the points do not lie exactly on the line, as shown in Figure 1.

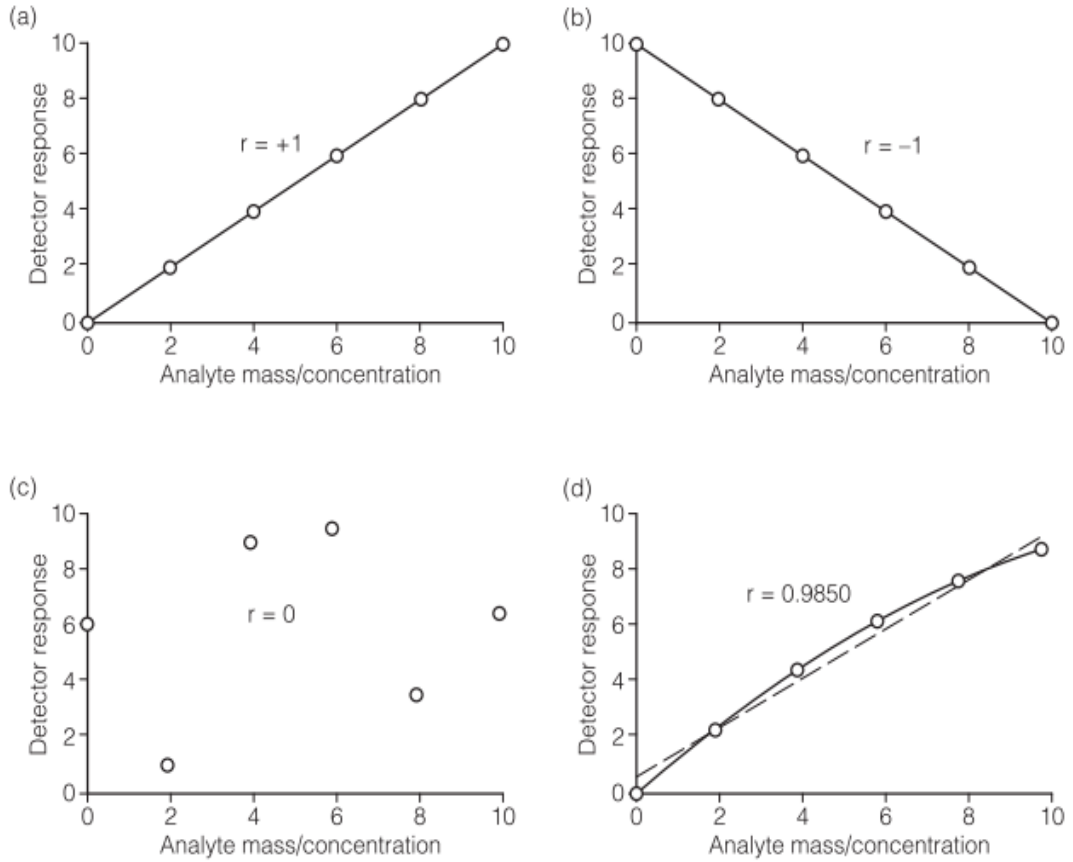


Fig. 2. Examples of correlation coefficients. (a) Perfect positive correlation; (b) perfect negative correlation; (c) no correlation, and (d) curved correlation.

Linear regression enables a line of best fit through the points to be defined by calculating values for the slope and y-axis intercept (b and a respectively in equation (1)), and the method of least squares is commonly used for this purpose. An assumption is made that only errors in the detector responses (y-values) are significant, any errors in the values for the mass or concentration of the analyte being neglected.

The deviations in the y-direction of the individual plotted points from the calculated regression line are known as y-residuals (Fig. 3) and the line represents the regression of y upon x. The method of least squares minimizes the sum of the squares of the y-residuals by equating them to zero in defining equations for the slope and intercept of the regression line. For the slope, b

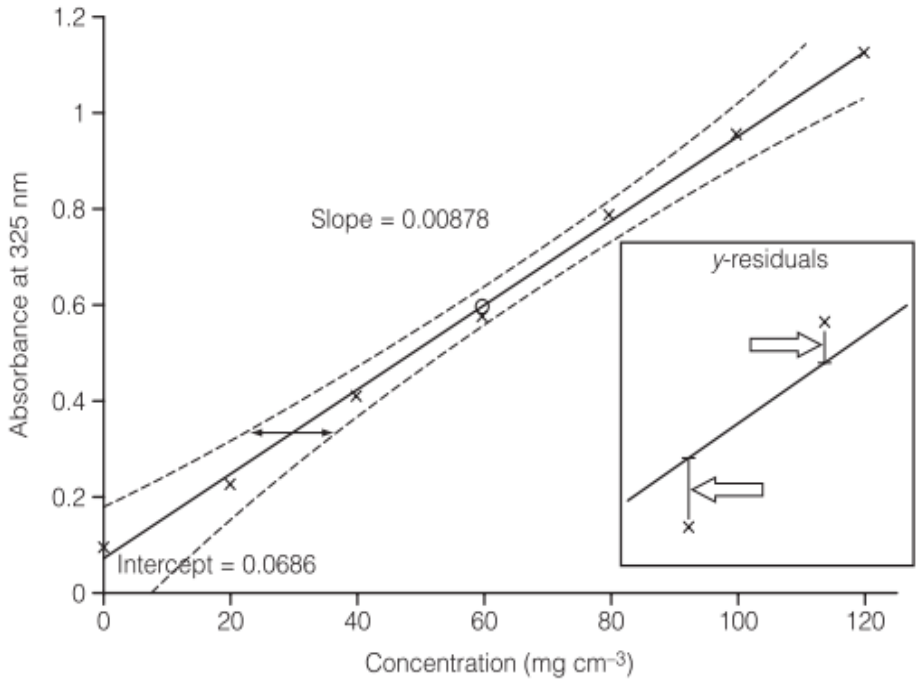


Fig. 3. Calibration graph, regression line, slope and intercept values for the UV spectrophotometric determination of an active ingredient in a sun cream. ——— = regression line; ○ = centroid, $\bar{x}$, $\bar{y}$; ---------- = confidence limits lines at the 99 percent level; ◄——► = confidence limits for sample concentration of 30 mg cm^{-3}. Inset: Illustration of y-residuals.

Example

A calibration graph was prepared as part of a validation procedure for a new method to determine an active constituent of a sun cream by UV spectrophotometry. The following data were obtained:

Analyte conc. (mg cm^{-3})	0	20	40	60	80	100	120
Absorbance at 325 nm	0.095	0.227	0.409	0.573	0.786	0.955	1.123

The data is first checked for linearity by calculation of the correlation coefficient, r, and visual inspection of a plotted curve. Some calculators and computer software can perform the computation from the raw data, but it is instructive to show the full working, for which tabulation is preferable.

x_i	y_i	$(x_i - \bar{x})$	$(x_i - \bar{x})^2$	$(y_i - \bar{y})$	$(y_i - \bar{y})^2$	$(x_i - \bar{x})(y_i - \bar{y})$
0	0.095	–60	3600	–0.5004	0.2504	30.024
20	0.227	–40	1600	–0.3684	0.1357	14.736
40	0.409	–20	400	–0.1864	0.0347	3.728
60	0.573	0	0	–0.0224	0.0005	0
80	0.786	20	400	0.1906	0.0363	3.812
100	0.955	40	1600	0.3596	0.1293	14.384
120	1.123	60	3600	0.5276	0.2784	31.656
Σ 420	**4.168**	**0**	**11200**	**0**	**0.8653**	**98.340**
$\bar{x} = 60$	$\bar{y} = 0.59543$					

Substitution of the totals in columns 4, 6 and 7 in equation (2) gives

$$r = 98.340/(11200 \times 0.8653)^{1/2} = 98.340/98.445 = 0.9989$$

Figure 3 and the correlation coefficient of 0.9989 show that there is a good linear relation between the measured UV absorbance and the analyte concentration.

The slope and *y*-axis intercept of the regression line, given by equations (3) and (4) respectively are

$$b = 98.340/11200 = 0.00878 \quad a = 0.59543 - (0.00878 \times 60) = 0.0686$$

The y-axis intercept, slope and analyte masses or concentrations calculated by interpolation from the regression line are all affected by errors. Additional equations can be used to obtain the following statistics:

- Estimated standard deviations for the slope and intercept;
- Estimated standard deviations for analyte masses or concentrations determined from the calibration graph;
- Confidence limits for analyte masses and concentrations at selected probability levels;
- Limit of detection of the analyte (vide infra).

Confidence limits over the entire range of the calibration graph at selected probability levels, e.g. 95 or 99 percent, can be displayed (dashed curves, Fig. 3). A horizontal line drawn through a given experimental point on the regression line and intersecting the confidence limits lines on either side gives the upper and lower limits for that particular mass or concentration. Figure 3 shows the 99% limits, the narrowest interval being at the centroid, of the graph, and widening steadily towards each end.

Some calculators and computer packages have the ability to perform the regression calculations described. Where there is a nonlinear relation between the detector response and the mass or concentration of the analyte more complex curvilinear or logarithmic regression calculations are required.

Limit of detection

For any analytical procedure, it is important to establish the smallest amount of an analyte that can be detected and/or measured quantitatively. In statisticalterms, and for instrumental data, this is defined as the smallest amount of an analyte giving a detector response significantly different from a blank or background response (i.e. the response from standards containing the same reagents and having the same overall composition (matrix) as the samples, where this is known, but containing no analyte). Detection limits are usually based on estimates of the standard deviation of replicate measurements of prepared blanks.

A detection limit of two or three times the estimated standard deviation of the blanks above their mean, $\bar{x}_B$, is often quoted, where as many blanks as possible (at least 5 to 10) have been prepared and measured.

This is somewhat arbitrary, and it is perfectly acceptable to define alternatives provided that the basis is clear and comparisons are made at the same probability level.

Standard addition

Where components of a sample other than the analyte(s) (the matrix) interfere with the instrument response for the analyte, the use of a calibration curve based on standards of pure analyte may lead to erroneous results. Such matrix interference effects can be largely if not entirely avoided by

preparing calibration standards where known amounts of pure analyte are added to a series of equal sized portions of the sample, a procedure known as spiking. In addition, one portion of sample is not spiked with analyte. (Note: if spiking sample solutions with analyte changes the volume significantly, volume corrections must be applied.)

The effects of the matrix on measurements of the analyte in both the spiked and unspiked samples should be identical. The instrument responses are then used to construct a calibration graph where the x-axis values are the added amounts of analyte and the response for the unspiked sample is at x = 0 (i.e., the curve does NOT pass through the origin). The regression line is calculated and extrapolated back to give a negative intercept on the x-axis at y = 0, which corresponds to the amount of analyte in the sample (Fig. 4).

Example

The calcium level in a clinical sample was determined by flame emission spectrometry using a standard addition method, which gave the following data:

Spiked calcium (ppm)	0	10	20	30	40	50
Emission intensity at 423 nm	0.257	0.314	0.364	0.413	0.468	0.528

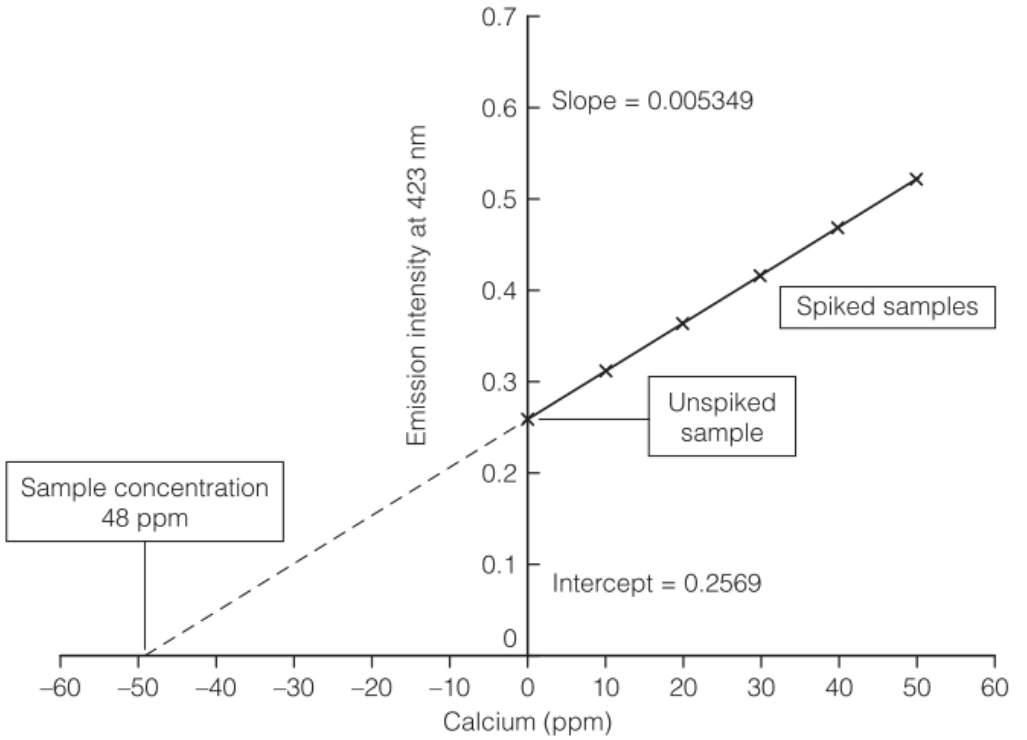


Fig. 4. Standard addition calibration graph, regression line, slope and intercept values for the flame emission determination of calcium in a clinical sample.

Detailed calculations of the correlation coefficient, r, and the slope and intercept values have not been given, but should be set out as in the previous example if a suitable calculator or computer program is not available.

The amount of calcium in the sample can be read from the extrapolated graph or calculated from the slope, b, and the intercept, a

Calcium concentration in sample = a/b = 0.2569/0.005349 = 48 ppm

Internal standardization

For some analytical techniques, particularly chromatography, variations in experimental conditions can adversely affect the precision of the data. A calibration procedure whereby a constant amount of a selected substance, the internal standard is added to all samples and analyte standards alike compensates for variations in sample size and other parameters. The ratio of the detector response for the analyte in each standard to the corresponding response for the added internal standard is plotted on the y-axis of a calibration graph against the mass or concentration of the analyte on the x-axis. The correlation coefficient, slope and intercept values can be computed as shown previously, and response ratios for the analyte and added internal standard in the samples can then be used to determine the amount of analyte in the samples by interpolation on the graph. If only one or two analyte standards are prepared, the amount of analyte in a sample can be calculated by simple proportion, i.e.

$$\frac{analyte\ in\ sample}{analuyte\ in\ standard} = \frac{response\ ratio\ for\ sample}{response\ for\ standard}$$

Internal normalization

For some purposes, only the relative amounts of the analytes in a multicomponent mixture are required. These are normalized to 100 or 1 by expressing each as a percentage or fraction of the total. Internal normalization is of particular value in quantitative chromatography where several components of a sample can be determined simultaneously, and absolute levels are not of interest. The relative composition is calculated from the instrument response, peak area in the case of a chromatographic analysis, for each component in the mixture using the formula

$$\%x_i = \frac{A_x}{\sum_{i=1}^{i=n} A_i}\ x\ 100$$

where x i is one of n components and A is the measured area or response.

Example

Figure 5 is a chromatographic record (chromatogram) of the separation of a 5- component mixture. The measured peak areas (using electronic integration with a computing-integrator, computer and chromatography data processing software or geometric construction such as triangulation, $(^1/_2\ x\ base\ x\ hight)$ and percentages by internal normalization, which must total 100 percent, are given in Table 1

(e.g., for component 1, relative percent = (167.8/466.94) x 100 = 35.9 percent).

Table 1. Peak areas and percentage composition by internal normalization for a 5-component mixture

Component	Measured peak area (arbitrary units)	Relative percent
1	167.8	35.9
2	31.63	6.8
3	108.5	23.2
4	80.63	17.3
5	78.38	16.8
Totals	466.94	100.0

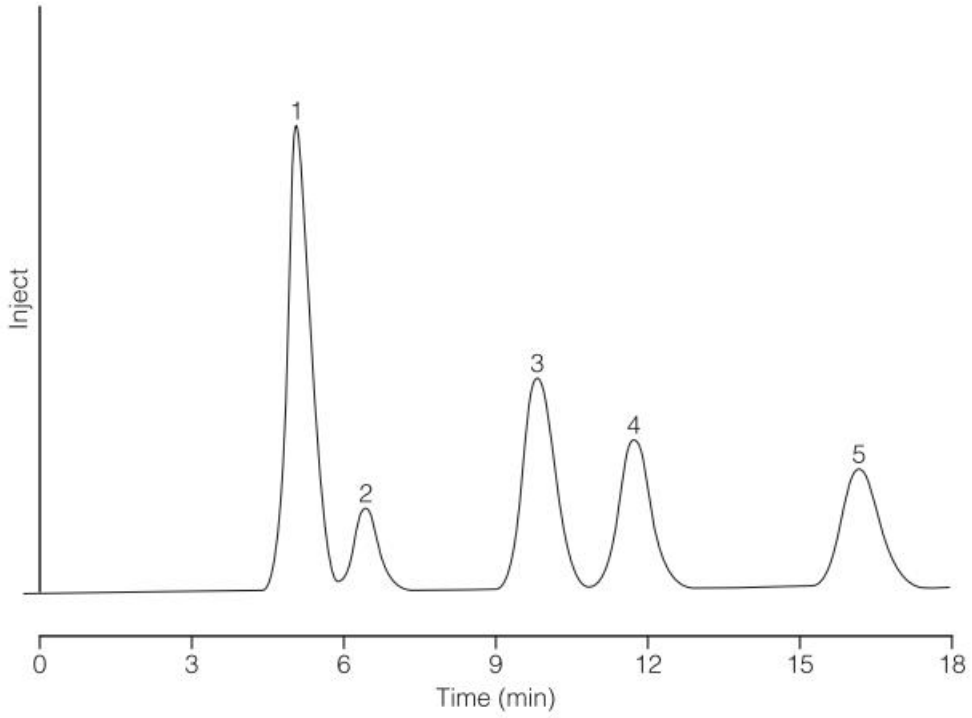
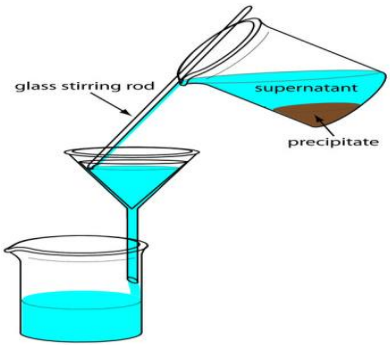


Fig. 5. Chromatogram of a 5-component mixture.

Gravimetric methods are quantitative methods that *are based on determining the mass of a pure compound to which the analyte is chemically related.* **Gravimetric methods of analysis are based on mass measurements with an analytical balance (an instrument that yields highly accurate and precise data)** and can be classify into:

1. **Precipitation gravimetry**, *the analyte is separated from a solution of the sample as a precipitate and is converted to a compound of known composition that can be weighed*.



In gravimetric titrimetry, the analyte is converted to a sparingly soluble precipitate. This precipitate is then filtered, washed free of impurities, converted to a product of known composition by suitable heat treatment, and weighed. **For example**, a precipitation method for determining calcium in water, a certain volume of water is taken and an excess of oxalic acid, $H_2C_2O_4$, is added to an aqueous solution of the sample. After that, ammonia is added (to

neutralizes the acid) and **causes essentially all of the calcium in the sample to precipitate as calcium oxalate. The reactions are:**

$$2NH_3 + H_2C_2O_4 \rightarrow 2NH_4^+ + C_2O_4^{2-}$$
$$Ca^{2+}(aq) + C_2O_4^{2-}(aq) \rightarrow CaC_2O_4(s)$$

The **CaC_2O_4** precipitate is filtered using a weighed filtering crucible, then dried and ignited. This process converts the precipitate entirely to **calcium oxide**. The reaction is:

$$CaC_2O_4(s) \xrightarrow{\Delta} CaO(s) + CO(g) + CO_2(g)$$

After cooling, the crucible and precipitate are weighed, and the mass of calcium oxide is determined by subtracting the known mass of the crucible. The calcium content of the sample is then computed.

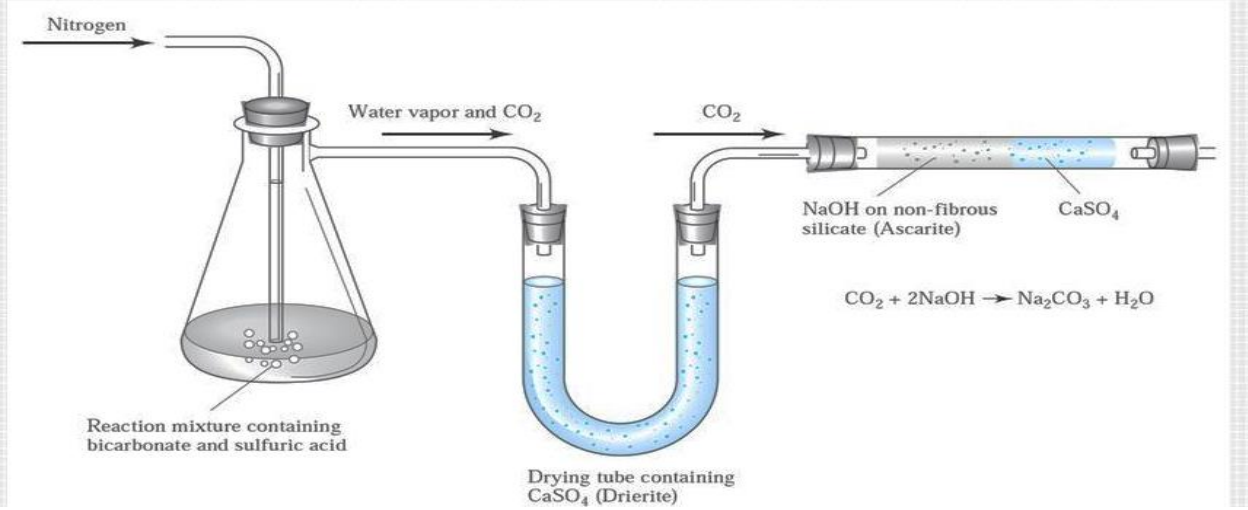
2. **Volatilization gravimetry**, in this technique, *the analyte is separated from other constituents of a sample by converting it to a gas of known chemical composition and the mass of the gas then serves as a measure of the analyte concentration.*

The two most common gravimetric methods based on volatilization are those for determining water and carbon dioxide. Water is quantitatively distilled from many materials by heating. In direct determination, water vapor is collected on any of several solid desiccants, and its mass is determined from the mass gain of the desiccant. The indirect method in which the amount of water is determined by the loss of mass of the sample during heating is less satisfactory because it must be

assumed that water is the only component that is volatilized. This assumption can present problems, however, if any component of the precipitate is volatile. Nevertheless, the indirect method is widely used to determine water in items of commerce. **For example:** Determination of sodium hydrogen carbonate ($NaHCO_3$) in antacid tablets. In this **Exp**. a weighed sample of the finely ground a tablet is treated with dilute H_2SO_4 to convert the sodium hydrogen carbonate to carbon dioxide:

$$NaHCO_3 + H_2SO_4 \longrightarrow CO_{2(g)} + H_2O_{(l)} + NaHSO_4$$

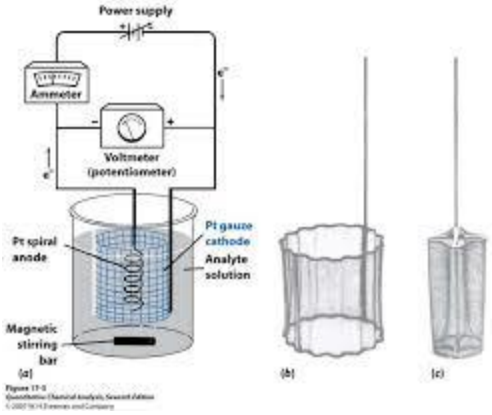
This reaction is carried out in a flask connect to a weighed absorption tube that contains an absorbent that retains the carbon dioxide selectively as it removed from solution by heating as shown in the Figure below:



The difference in mass of the tube before and after absorption is used to calculate the amount of sodium hydrogen carbonate.

In fact, the precipitation methods are considered to be the most commonly used methods compared to volatilization methods and have many applications in the gravimetric analysis.

3. **In Electrogravimetry,** the analyte is separated by deposition on an electrode by an electrical current and the mass of this product then provides a measure of the analyte concentration.



Properties of *Precipitates* and *Precipitating Reagents*:

A gravimetric precipitating agent should **react specifically or at least selectively** with the analyte. **Specific reagents**, *which are rare*, react only with a single chemical species. **Selective reagents**, *which are more common*, react with a limited number of species. In addition to specificity and selectivity, the ideal precipitating reagent would react with the analyte to give a product that is:

i. The precipitate form should have **low solubility {***so that no significant loss of the solid occurs during the filtration and washing***}...it is known that the solubility product (K_{SP}).** *The precipitation is practically complete when the*

value of K_{SP} **does not exceed** 10^{-8}**. Therefore, compounds with** K_{SP} ***value >*** 10^{-8} **are not used as precipitated forms in gravimetric analysis.**

ii. The precipitate **easily filtered and washed** *(free of contaminants). Generally, ppts. consisting of relatively large crystals are very convenient. This is because, they hardly clog the filter pore, and their specific surface is not extensive they do not readily adsorb impurities from solution and easily washed free from letter.*

Amorphous precipitates (colloidal, gelatinous) such as $Al(OH)_3$ have extensive specific surfaces and therefore adsorb considerable amount of impurities which are difficult to wash off. Moreover, the filtration is very slow in such cases.

iii. The precipitate should be of **known composition**. [for example; the precipitation of iron by ammonia solution as follows;

$$Fe^{3+} + 3OH^- \longrightarrow Fe(OH)_3$$

The precipitate contains variable amounts of water which depend on the condition of precipitation. This formula $Fe(OH)_3$ is not known exactly, so it should be written correctly as $Fe_2O_3.XH_2O$. since it is difficult to determine its molecular formula as X is unknown amount. Therefore, the precipitate $Fe(OH)_3$ must ignited at certain temperature to remove all water molecules and a compound of quit definite composition is formed, exactly correspond to the formula Fe_2O_3.

iv. The precipitate **unreactive with constituents of the atmosphere**;

(i.e., must have adequate chemical stability) during the final weighing measurement because it is difficult to weight the ppt. if its compositions are changed). F**or example**: *CaO precipitates readily absorb H_2O and CO_2 from air (which makes weighing difficult); therefore, they are sometime converted into $CaSO_4$ by treatment with H_2SO_4 in the crucible, and excess acid is removed by evaporation).*

v. The precipitate is convenient ***if the content of the element being determined in the precipitate should be as low as possible (why?)*** *Because, errors in the determination (e.g. weighed errors, losses due to solubility of the precipitate or incomplete transfer to the filter, etc) have less effect on the final result of the analysis.*

For example, in case that we want determine Cr in such sample. If the Cr has been precipitated as $BaCrO_4$ or Cr_2O_3. It was found that, the absolute error in weighing 3.5 times more in case of Cr_2O_3. Suppose the loss of 1 mg of precipitate in analysis correspond to **the following errors in determination of the weight of Cr:**

Weighed form Cr_2O_3	**Weighed form $BaCrO_4$**
152 mg **Cr_2O_3** contains 104 mg Cr	253.3 mg **$BaCrO_4$** contains 52 mg Cr
1 mg **Cr_2O_3** contains X mg Cr	1 mg **$BaCrO_4$** contains X mg X=104/
104*1/152 = 0.7 mg Cr	X=52*1/253.3=0.2 mg Cr

The precision of the analytical results will depend (to the certain extent) on the following:

- Choice the type of suitable precipitating agent
- The amount of precipitating added
- The conditions of precipitation such as Temp., pH of solution and the concentration of reactants.

The gravimetric methods are very accurate because it is possible to weigh the substance with highly precision by using the analytical balance up to five digits level. Consequently, the accuracy and precision of gravimetric methods depend on:

- The precipitation technique, and
- The properties of the formed precipitate.

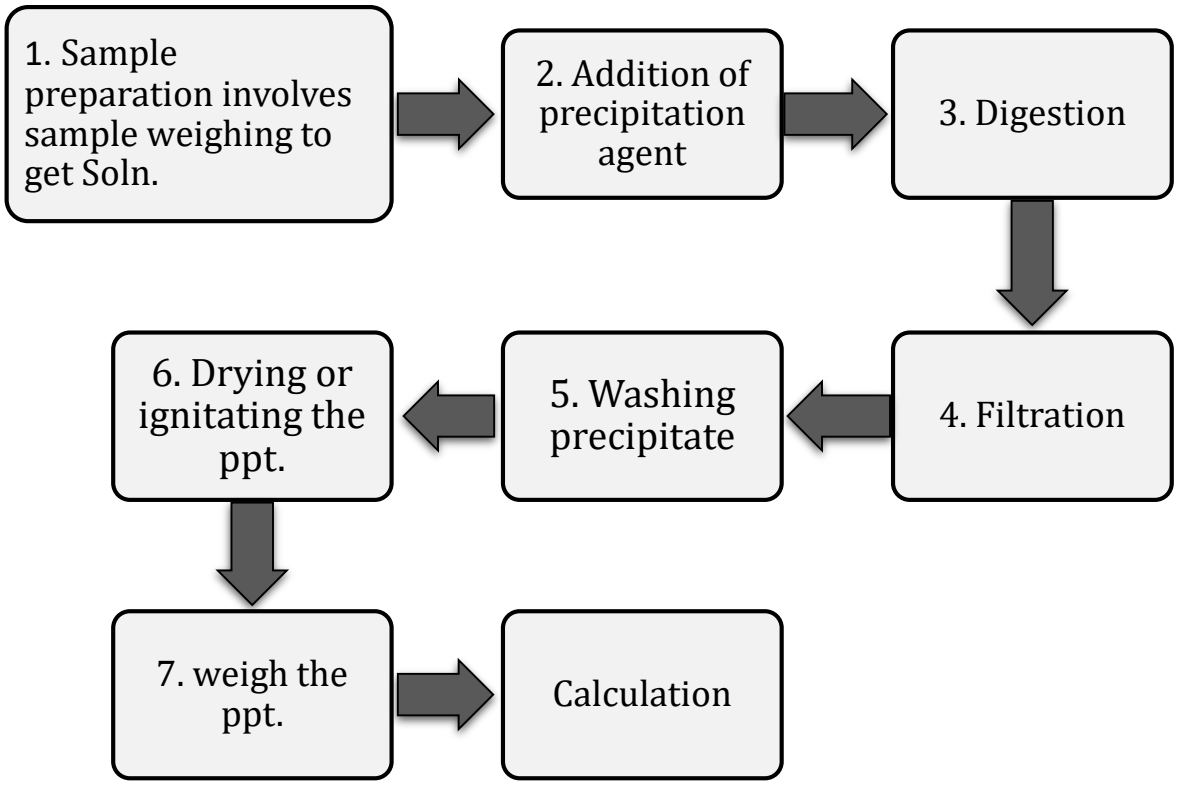
However, to get high precision, the following requirements should be satisfied and can be summarized as follows:

1. The ppt. should be stoichiometric and reproducible
2. Low soluble in washing solution
3. Minimum interferences of elements or other compound in the precipitation system
4. The ppt. must have low surface area, provided that the adsorption of impurities should be minimal.
5. The ppt. a high thermal stability, provided that it is dried appropriately without change in its structure.
6. The dried ppt. should be high stable.

Steps of Gravimetric Analysis

The steps required in a gravimetric analysis, after the sample has been dissolved, can be summarized as follows:

1. Preparation of solution 2. Precipitation 3. Digestion
4. Filtration 5. Washing 6. Drying or igniting
7. Weighing 8. Calculations



The first step of gravimetric analysis usually involves preparation of sample and solution and some form of preliminary separation maybe necessary to eliminate interfering materials. Also, we must adjust the solution conditions to maintain low solubility of the precipitate and to obtain it in a suitable form for filtration. Adjustment of the solution conditions prior to precipitation may also mask potential interferences. Factors that must be considered include the volume of the solution during precipitation, the concentration range of the test substance, the presence and concentration of other constituents, the temperature, and the pH. **Note:** *The pH is important because it often influences*

both the solubility of the analytical precipitate and the possibility of interferences from other substances

Particle size and filterability of precipitates

Precipitates consisting of large particles are generally desirable for gravimetric work because these particles are easy to filter and wash free of impurities. In addition, precipitates of this type are usually purer than are precipitates made up of fine particles.

Factors that Determine the Particle Size of Precipitates

The particle size of solids formed by precipitation varies enormously. Therefore, two types of ppt. occur as follows:

Colloidal suspensions whose tiny particles are invisible to the naked eye (10^{-7}-10^{-4} cm in diameter). Moreover, it shows no tendency to settle from solution and are not easily filtered.

Note. *It is very difficult to filter the particles of a colloidal suspension. To trap these particles, the pore size of the filtering medium must be so small that filtrations take a very long time. With suitable treatment, however, the individual colloidal particles can be made to stick together, or coagulate, to produce large particles that are easy to filter.*

Crystal suspensions is temporary dispersion of such particles in the liquid phase. The particles of a crystalline suspension tend to settle spontaneously and are easily filtered.

The experimental variables that effect on the particle size of ppt. are summarized as:

Precipitate solubility, temperature, concentration of reactant, and the rate at which reactant are mixed.

……

Precipitate formation has been studied for many years, but the mechanism of the process is still not fully understood. The net effect of these variables can be accounted for, at least qualitatively, by assuming that the particle size is related to a single property of the system called **relative supersaturation**, where

relative supersaturation **(RSS)** $= \frac{Q-S}{S}$ ……(*Von Weimarn equation*)

where, Q is the concentration of the solute at any instant, and S is its equilibrium solubility. Generally, precipitation reactions are slow so that, even when a precipitating reagent is added drop by drop to a solution of an analyte, some supersaturation is likely. Experimental evidence indicates that the particle size of a precipitate varies inversely with the average relative supersaturation during the time when the reagent is being introduced.

Thus, when $(Q-S)/S$ **is large**, the precipitate tends to be colloidal, and when $(Q-S)/S$ is **small,** a crystalline solid is more likely.

Therefore, usually keeping ***Q*** is low and ***S*** is high during precipitation. Several steps are commonly taken to maintain favorable conditions for the precipitation process.

Therefore, **we can keep RSS small**, by

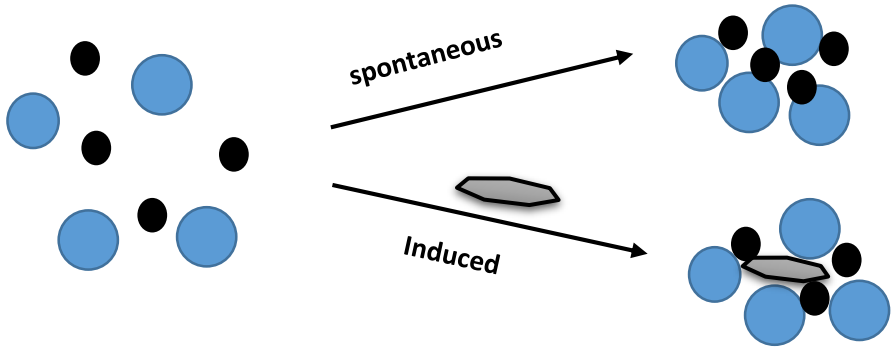
1. Using dilute solutions and reagent {This keep ***Q*** low}
2. Slowly adding the precipitating reagents with effective string {this keeps ***Q*** low & stirring prevents local excess of reagent}
3. Precipitate from hot solution (increase ***S***). The solubility should not be too great or the precipitation will not be quantitative (with less than 1 ppt remaining). The bulk of the precipitation may be performed in the hot solution, and then the solution may be cooled to make the precipitation quantitative.
4. Precipitation at low pH as is possible to maintain quantitative precipitation.

During the above steps; decreasing in degree of contamination is possible. *The concentration of impurities is kept lower and their solubility is increased, and the slower rate of precipitation decreases their chance of being trapped*)

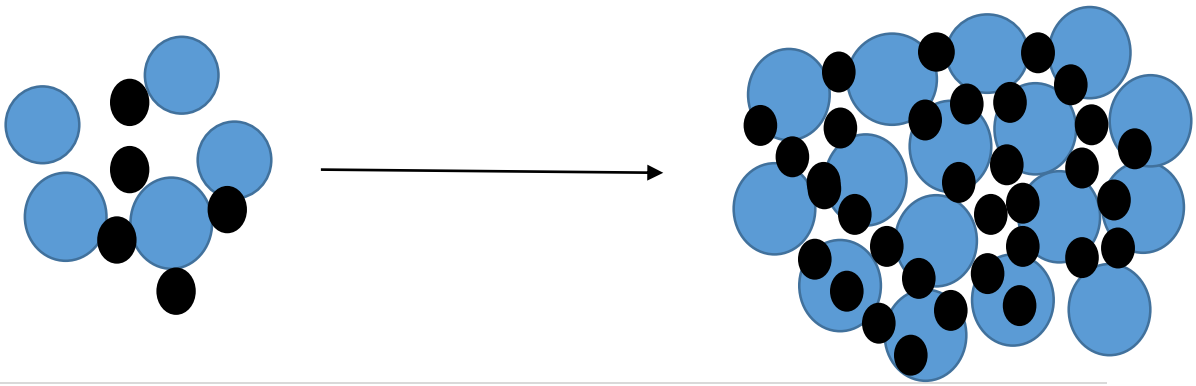
Mechanism of Precipitate Formation

The effect of relative supersaturation (RSS) on particle size can be explained if we assume that precipitates form in two ways: by **nucleation** and by **particle growth.** The particle size of a freshly formed precipitate is determined by the mechanism that predominates.

1. ***In Nucleation a minimum number of atoms, ions, or molecules join together to give a stable solid by spontaneous and induced process. In spontaneous nucleation will occur by its own while induced nucleation requires a seed particle to get thing started (dust, another crystal, glass fragment…).***



2. ***In particle growth* the three dimensional growth of a particle nucleus into larger crystal**

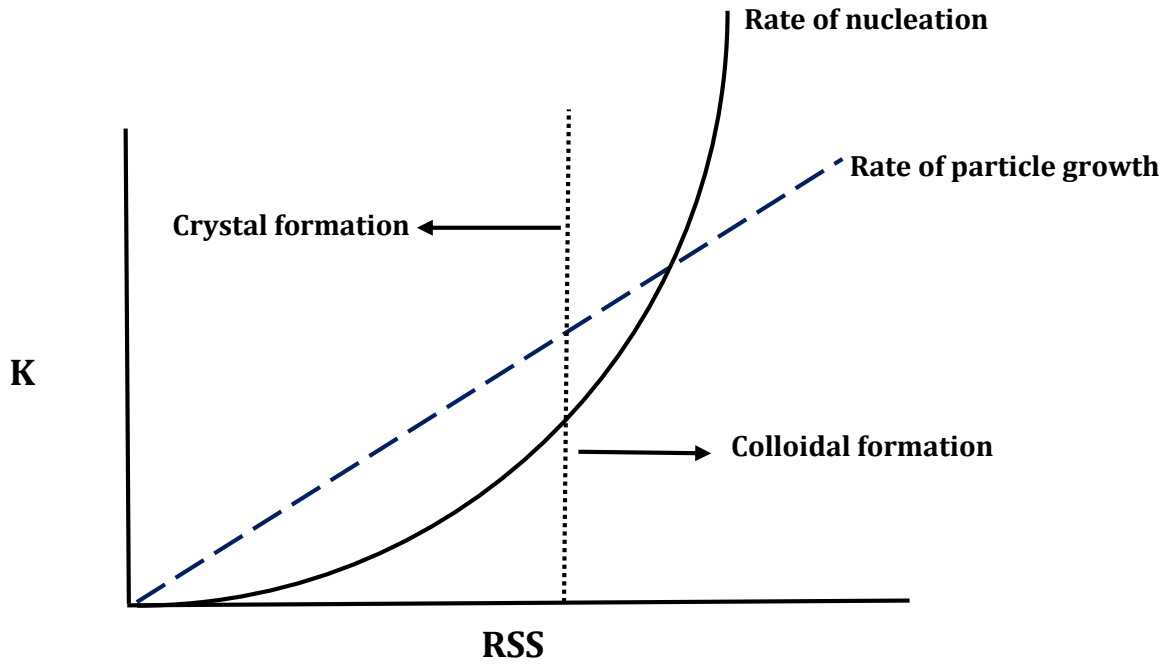


Once a nucleation has formed, other ions are attracted to the site. This will result in the formation of large, filterable particles. If done properly, it also reduces contamination since they do not "fit in" to the crystal structure.

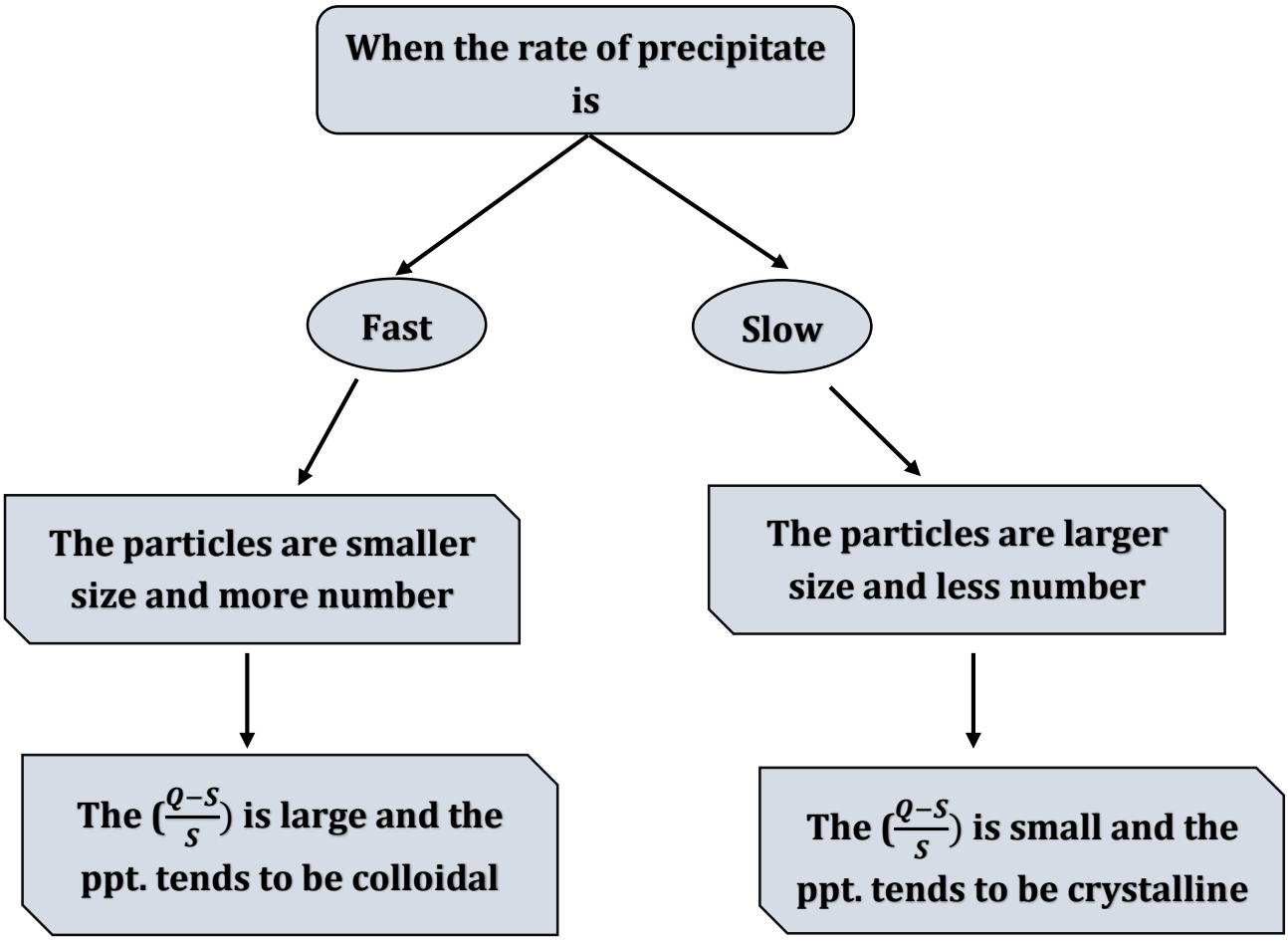
If **nucleation predominates**, a precipitate containing a large number of small particles results, and if **growth predominates**, a smaller number of larger particles is produced. The **rate of nucleation** is believed to **increase enormously with increasing RSS** while the **rate of particle growth** is only moderately (i.e., linearly) with by **RSS.**

Therefore, when a precipitate is formed at **high RSS**, nucleation is the major precipitation mechanism, and a large number of small particles is formed (colloidal suspensions tend to be formed)

While, at **low RSS**, the rate of particle growth tends to predominate, and deposition of solid on existing particles occurs rather than further nucleation. (crystalline suspensions tend to be formed).



Therefore, according to Von Wiemarm, super-saturation *(A supersaturated solution* ***is an unstable solution that contains a higher solute concentration than a saturated solution)*** plays an important part during the determination of the particle size of precipitate. Thus, due to the particle size is affected by rate of precipitation, we can conclude that:



Colloidal Precipitates

Individual colloidal particles are so small that they are not retained by ordinary filters. Moreover, Brownian motion prevents their settling out of solution under the influence of gravity. Fortunately, however, we can coagulate, or agglomerate, the individual particles of most colloids to give a filterable, amorphous mass that will settle out of solution.

Coagulation of Colloids

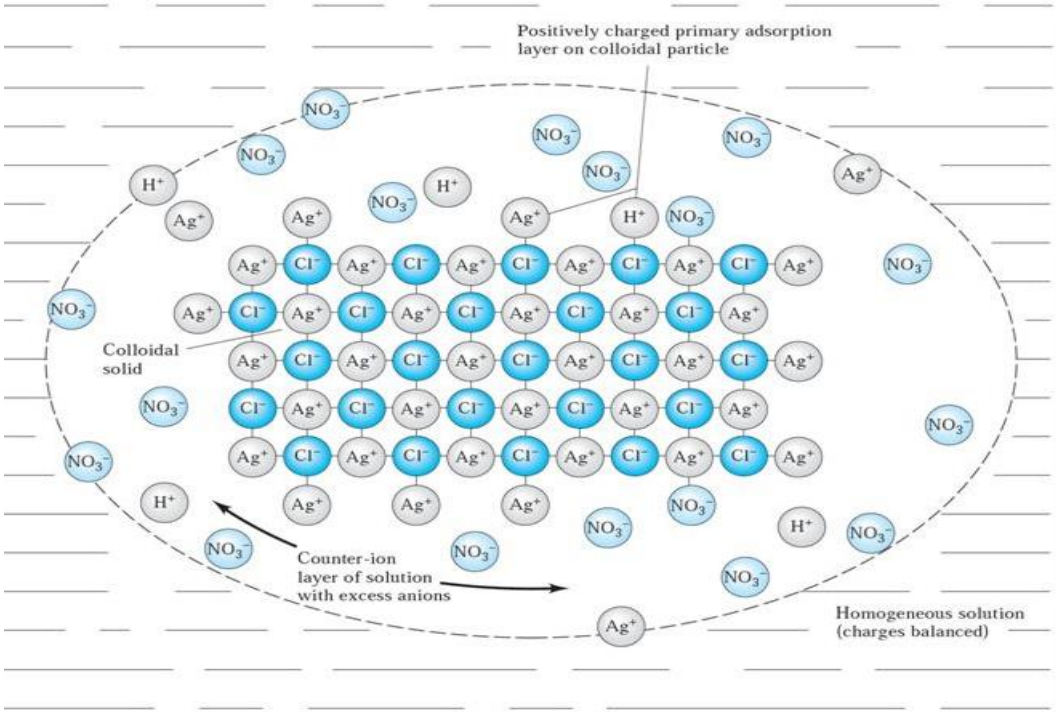
Coagulation can be hastened by heating, by stirring, and by adding an electrolyte to the medium. To understand the effectiveness of these measures,

we need to look into ***why colloidal suspensions are stable and do not coagulate spontaneously***. Colloidal suspensions are stable because all of the particles of the colloid are either positively or negatively charged and thus repel one another. The charge results from cations or anions that are bound to the surface of the particles. We can show that colloidal particles are charged by placing them between charged plates where some of the particles migrate toward one electrode while others move toward the electrode of the opposite charge. The process by which ions are retained on the surface of a solid is known as Adsorption (***Adsorption is a process in which a substance (gas, liquid, or solid) is held on the surface of a solid. In contrast, absorption is retention of a substance within the pores of a solid.***). The adsorption of ions on an ionic solid originates from the normal bonding forces that are responsible for crystal growth. For example, a silver ion at the surface of a silver chloride particle has a partially unsatisfied bonding capacity for anions because of its surface location. Negative ions are attracted to this site by the same forces that hold chloride ions in the silver chloride lattice. Chloride ions at the surface of the solid exert an analogous attraction for cations dissolved in the solvent.

The kind of ions retained on the surface of a colloidal particle and their number depend in a complex way on several variables. For a suspension produced in a gravimetric analysis, however, the species adsorbed, and hence the charge on

the particles, can be easily predicted because lattice ions are generally more strongly held than others. (***The charge on a colloidal particle formed in a gravimetric analysis is determined by the charge of the lattice ion that is in excess when the precipitation is complete)***. For example, when silver nitrate is first added to a solution containing chloride ion, the colloidal particles of the precipitate are negatively charged as a result of adsorption of some of the excess chloride ions. This charge, though, becomes positive when enough silver nitrate has been added to provide an excess of silver ions. The surface charge is at a minimum when the supernatant liquid does not contain an excess of either ion.

Figure 1 shows a colloidal silver chloride particle in a solution that contains an excess of silver nitrate. Attached directly to the solid surface is the primary



adsorption layer, which consists mainly of adsorbed silver ions. Surrounding the charged particle is a layer of solution, called the counter-ion layer, which contains sufficient excess of negative ions (principally nitrate) to just balance the charge on the surface of the particle. The primarily adsorbed silver ions and the negative counter-ion layer constitute an electric double layer that imparts stability to the colloidal suspension. As colloidal particles approach one another, this double layer exerts an electrostatic repulsive force that prevents particles from colliding and adhering.

Figure 1: A colloidal silver chloride particle suspended in a solution of silver nitrate

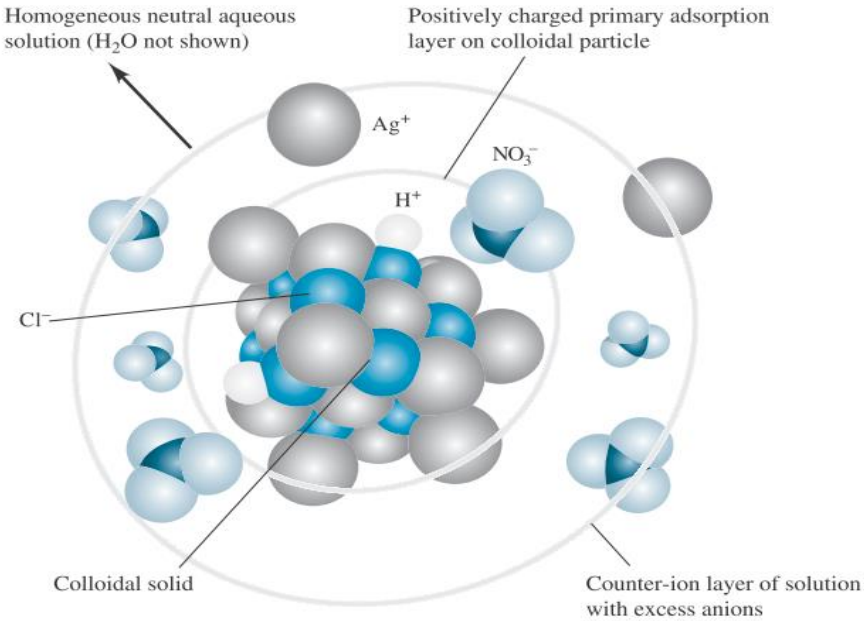


Figure 1: *A colloidal silver chloride particle suspended in a solution of silver nitrate*

- *In the beginning, there are very little free chloride ions in the solution due to the excess of Ag^+.*
- *The outer layer of the precipitate contains both Ag^+ and Cl^- which tend to attract additional Ag^+ to surface $^-$. This layer is called Primary adsorbed layer. This layer is simply an extension of the precipitate but we have run out of Cl^-.*
- *The counter ion in excess (nitrate) is attracted to this layer by electrostatic forces-maintain electrical neutrality (balance the charged on the surface of the particle). The overall structure tends to appear negative to the solution, it attracts water molecules that move with the particle.*
- *At higher concentrations of Ag^+, there are a higher charge on the precipitate. This results in a larger particle.*
- *The primary adsorbed silver ions layer and the negative counter-ion layer constitute an electrical double layer imparts stability to the colloidal suspension. As colloidal particles approach one another, this double layer exerts an electrostatic repulsive forces that prevents particles from colliding and adhering.*
- *Net result: At higher Ag^+ concentrations and with large counter ions we tend to form stable colloids. The charged structures have difficulty approaching each other since they are similarly charged.*
- *Note: the same type of problem will occur when chloride is in excess towards end of precipitation.*

- *Below a brief comparison between the primary adsorbed layer and counter ions layer:*

Primary adsorbed layer	***Counter ions layer***
It is an integral part of the crystal and present in solution at high concentration.	*It is an ion of opposite charge to the prim adsorbed ion which preferred in higher concentration than another*
The ions are bounded by chemical bonds with crystal	*The ion is bounded with adsorbed ion by Electrostatic forces.*

The factors which determine the nature of the adsorbed counter ion include:

- *Concentration ion*
- *The charge of ion*
- *The nature of the compounds which contain this ion.*

The more concentration and charge of the ion, the more capability of adsorption. In the case of equality between the concentration and charge for ions, the most adsorbed ion it has a capability to form a sparingly soluble compound with the primary adsorbed.

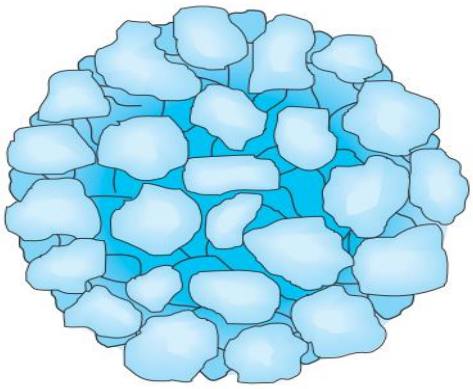
For example, in precipitating of SO_4^- ion by adding an excess of barium chloride $BaCl_2$, the Ba^{2+} ion is the primary adsorbed ion, and in the case of the presence of nitrate, perchlorate or chloride ions in solution, the nitrate ion is the counter ion

which forms with forms with Ba^{2+} ion an compound of less soluble than the remainder ions.

$BaSO_4$: Ba^{2+}: NO_3^-

Coagulation: is the process where the colloidal particles' lump together into larger particles. Hence the coagulation occurs by decreasing the repulsive forces among its particles where individual particles approach one another closely enough to permit agglomeration.

The following diagram shows the coagulated colloidal particles consist of a huge number of particles arranged irregularly.



Experimentally, how we can reduce the effect of this repulsion forces?

Two common approaches for causing a colloidal to coagulate are heating and adding an electrolyte to the solution.

1. *Heating the colloidal while stirring: this significantly decreases the number of absorbed ion per particle. This reduces the counter ion layer making it easier for the particles to approach each other.*
2. *Edition of electrolyte solution: unrelated, non-interfering ionic compound can be added to the solution. This reduces the volume of solution that contains sufficient ions of opposite charge to neutralize the particles (i.e. reduces of charge and thickness of electrical double layer). If the concentration of electrolytic solution added is high, its activity increases allowing the particles to approach each other closely. The result is that a smaller structure is formed.*

Hence, the process of removing or reducing the charge from the colloid precipitate particles and growth to larger size is called coagulation. "The precipitated formed in this way is of two types:

- *Precipitate contains of a high content of water is called hydrophilic (i.e. water loving) and this resulting from the colloidal solutions named lyophilic. If the water present as a solvent, the solution is known as hydrophilic and the precipitate is called "gel" which mean viscous. There are unlimited examples of this type, some of these $Fe(OH)_3$ and $Ai\ (OH)_3$*

Coagulation of a hydrophilic colloid is more difficult, and it produces a gelatinous precipitate that is difficult to filter because it tends to clog the pore of the filter. In addition, gelatinous precipitates adsorb impurities readily, because of their very large surface area.

- *Precipitate contains less amount of solvent named as hydrophobic (i.e. has little attraction of water) and the suspension solutions is called lyphobic. A solution of this type is called a 'sol'« An example such as: AgCl, CuS etc… Coagulation of a hydrophobic colloid is fairly easy and result in crudy precipitate, it loses water easy by 110°C.*

Peptization of Colloids

Peptization is the process by which a coagulated colloid reverts to its original dispersed state (i.e. reverse of coagulation). When a **coagulated colloid** is washed with water (to remove excess counter ion or trapped impurities) can result in peptization **(why)**? This is because of water removes the action of an electrolyte solution that is responsible for coagulation from the inside liquid ich is in contact with the solid. When the electrolyte is removed, what is happened?

1- The counter ions layer will increase in size., and
2- The repulsive forces responsible for colloid stat will recover thereby the bonding among the coagulated mass particles break down.

There are several common approaches that can be used to govern this problem:

A-*Use a volatile electrolyte:* a relative volatile salt can be used to wash the precipitate This displaces the less volatile, excess counter ion. Heating the precipitate (during drying) will remove the volatile electrolyte. Examples, for AgCI, wash with dilute HNO_3. Dry the precipitate at 110°C will remove HNQ_3.

B- Digestion and aging

Digestion –heating the solution for about an hour after precipitate formation. This helps to remove weakly bound water.

Aging–storing the solution, unheated, overnight. This allows rapped contamination time to' work their way out.

Both can result in a denser precipitate that is easier to filter.

Crystalline Precipitates

Crystalline precipitates are generally more easily filtered and purified than are coagulated colloids. In addition, the size of individual crystalline particles, and thus their filterability, can be controlled to some extent.

Methods of Improving Particle Size and Filterability

The particle size of crystalline solids can often be improved significantly by maintaining the **RSS** to be low during the interval **(**minimizing **Q** or maximizing **S)**, or both, in the Equation of Von Weimran. The value of Q is can often be minimized by **using dilute solutions** and **adding the precipitating reagent slowly**, with **good mixing**. Often, S is increased by precipitating from **hot solution** or by **adjusting the pH of the precipitation medium**. (Digestion improves the purity and filterability of both colloidal and crystalline precipitates).

Digestion of crystalline precipitates (without stirring) for some time after formation frequently yields a purer, more filterable product. The improvement in filterability undoubtedly results from the dissolution and recrystallization that occur continuously and at an enhanced rate at elevated temperatures. Re-crystallization apparently results in bridging between adjacent particles, a process that yields larger and more easily filtered crystalline aggregates.

Some important terms that effect on purity of crystalline Precipitates

Post-precipitation

When the precipitation is allowed to stand in contact with the mother liquor, a second substance will slowly form a precipitate with the precipitating reagent. Moreover, Post-precipitation is a slow equilibrium process.

Examples: include precipitation of copper as the sulfide in presence of zinc.

Copper sulfide is formed first but if not directly filtered, zinc sulfide starts to precipitate on the top of it. The same is observed in the precipitation of as the oxalate in presence of magnesium. At is, when you desire to precipitate Ca^{2+} with oxalate ion ($C_2O_4^{2-}$) in the presence of Mg^{2+} the $Mg_2C_20_4$ does not form immediately because it tends to form supersaturation solutions. But if the mother liquor left the precipitate for a long time before filtration, the $Mg_2C_20_4$ will precipitated along with $Ca_2C_20_4$

Re-precipitation

A drastic but effective way to minimize the effects of adsorption is re-precipitation. In this process, the filtered solid is re-dissolved and re-precipitated.

The first precipitate usually carries down only a fraction of the contaminant present in the original solvent. Thus, the solution containing the re-dissolved precipitate has a significantly lower contaminant concentration than the original, and even less adsorption occurs during the second precipitation. Re-precipitation adds substantially to the time required for an analysis. However, it is often necessary for

such precipitates as the hydrous oxides of iron(III) and aluminum, which have extraordinary tendencies to adsorb the hydroxides of heavy-metal cations such as zinc, cadmium, and manganese.

Occlusion is a type of co-precipitation in which a compound is trapped within a pocket formed during rapid crystal growth.

Co-precipitation: *is a process in which normally soluble compounds are carried out of solution by a precipitate or tendency of the precipitate to carry down of otherwise soluble species or compounds either within solid or on the surface of a solid as it precipitates.* ***(or when otherwise soluble compound is precipitated along with your analyte)****. This does not include material that would be normally be insoluble.*

Contamination of a precipitate by a second substance whose solubility product has been exceeded is not co-precipitation. There are four types of co-precipitation: **surface adsorption, mixed-crystal formation**, **occlusion, and mechanical entrapment**. Surface adsorption and mixed-crystal formation are equilibrium processes, while occlusion and mechanical entrapment arise from the kinetics of crystal growth.

- **Surface Adsorption:** the surface of our precipitate will contain some primary adsorbed ions. As a result, the counter ion will also be present. Example; in our AgCI example the precipitate will commonly contain some nitrate. This occurs with colloidal and crystalline precipitates. Worse with colloidal because the surface is significantly larger. If nitrate ion is co-precipitate, our results will be

to high –nitrate weights more than chloride Further, the results will be variable since the surface area may differ from sample to sample lower weight counter ion will result in our results being too low.

Dealing with surface adsorption

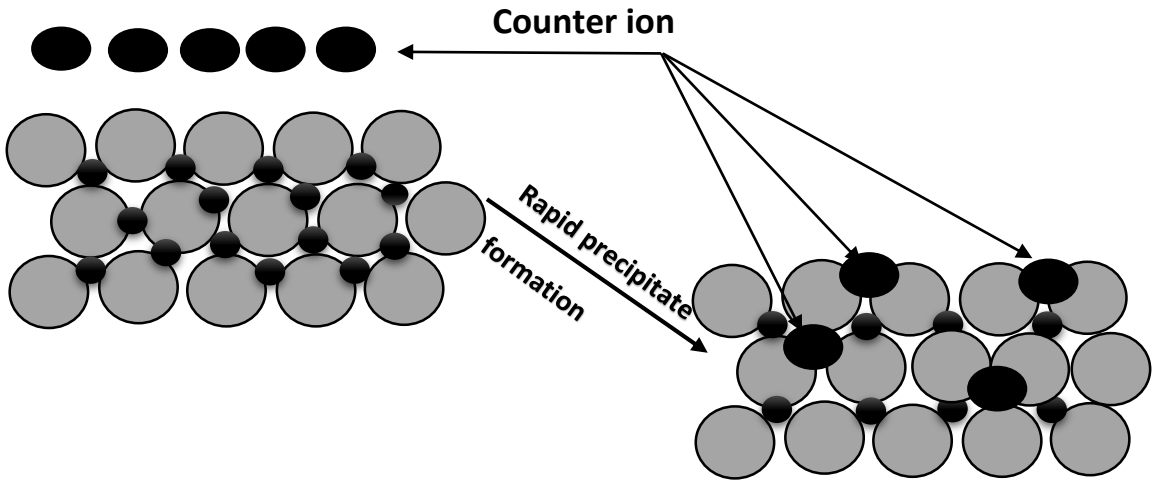
1- Washing: this may help but not much. The attraction of counter ion is usually too strong so you only get a limited exchange.

2- Washing with a volatile electrolyte: this a better approach since the excess can be removed during drying- For AgCI, washing with HCI is reasonable approach.

3- Re-precipitation: the filtered precipitate is dissolved and then precipitated a second time. Only a small portion of the contaminate makes it into the second solution. The second precipitates will occur. This approach is one of last resort since it significantly adds to analysis time.

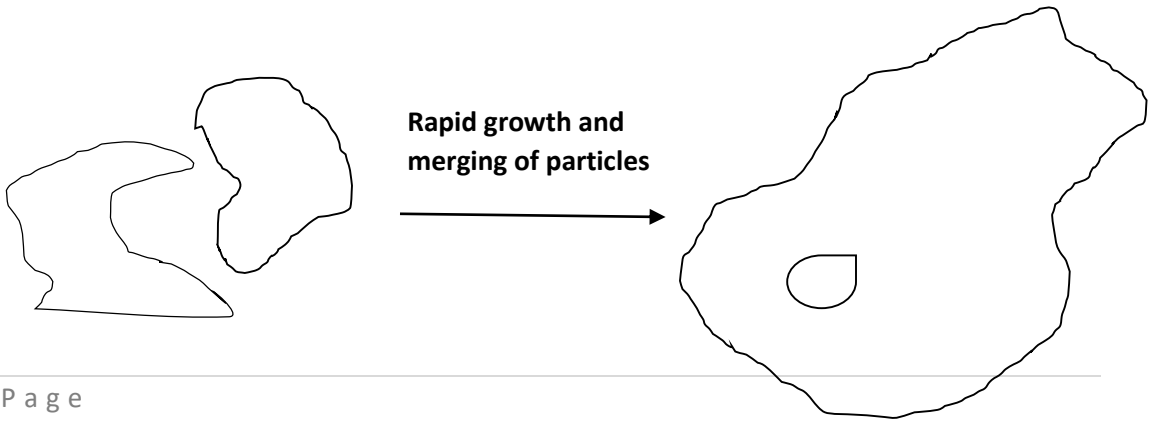
- **Mixed crystal formation**: if similar ions are present, they can replace our analyte ion in the crystal lattice during precipitation. Similar ions have the same charge and have sizes within 5% of our analyte. Example: determination of SO_4^{-2} as $BaS0_4$. The presence of Pb or Sr will cause a mixed crystal containing either $PbS0_4$ or $SrS0_4$. This is a problem with both colloidal and crystalline precipitates. There is no easy solution to minimize the problem. So, when encountered, your only choices are to remove the interferences prior to precipitation or to select a different reagent.

- **Occlusion**: if crystal growth is to rapid, some counter ions do not have to escape from the surface.



This neutralized by a secondary layer of counter ions from the solution. For example, Na^+ may be the counter ion (two per SO_4^{-2}). As more $BaCl_2$ solution is added, the loosely bound Na^+ is replaced by Ba^{2+} and the crystal continues to grow. However, if the growth take place rapidly, all the solution ions may not replace by Ba^{2+} ions.

- **Mechanical entrapment**: when a rapid growth traps a pocket of solution. While the solvent can be removed, the trapped ions will remain after drying.



The best way to deal with problems (occlusion and mechanical entrapment) is to follow things down:

- Using dilute, warm solution during precipitation gives counter ions leave and helps break up pockets.

- Digestion and aging the precipitate provides additions' time for this occur as wen.

Coagulation of a colloid does not significantly decrease the amount of adsorption because the coagulated solid still contains large internal surface areas that remain exposed to the solvent.

In adsorption, a normally soluble compound is carried out of solution on the surface of a coagulated colloid. This compound consists of the primarily adsorbed ion and an ion of opposite charge from the counter-ion layer. The **co-precipitated** contaminant on the coagulated colloid consists of the lattice ion originally adsorbed on the surface before coagulation plus the counter-ion of opposite charge held in the film of solution immediately adjacent to the particle. The net effect of surface adsorption is, therefore, the carrying down of an otherwise soluble compound as a surface contaminant. For example, the coagulated silver chloride formed in the gravimetric determination of chloride ion is contaminated with primarily adsorbed silver ions with nitrate or other anions in the counter-ion layer. The result is that silver nitrate, a normally soluble compound, is **co-precipitated** with the silver chloride.

The difference between post-precipitation and co-precipitation

Post-precipitation	Co-precipitation
The contamination increase with the time that the precipitation is left in contact with the mother liquor	Decrease
Contamination increases the faster the solution is agitated by either mechanical or thermal means	Decrease
Magnitude of contamination much greater	**Less**
The post-precipitation was occurs either the pollutants are found through the precipitation operation or adding after the operation	
The quantity of post-precipitate maybe some time equal to 50% of the true precipitation	
The post-precipitation increase with temperature increasing	

Precipitation from Homogeneous Solution

Homogeneous precipitation is a process in which a precipitate is formed by slow generation of a precipitating reagent homogeneously throughout a solution. Local reagent excesses do not occur because the precipitating agent appears gradually and homogeneously throughout the solution and reacts immediately with the analyte. As a result, the relative supersaturation is kept low during the entire precipitation (solids formed by homogeneous precipitation are generally purer and more easily filtered than precipitates generated by direct addition of a reagent to the analyte solution).

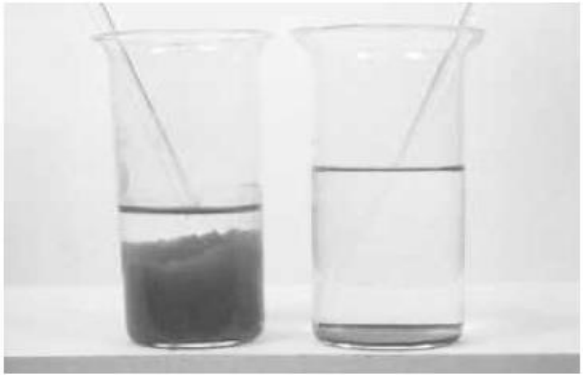
In general, homogeneously formed precipitates, both colloidal and crystalline, are better suited for analysis than a solid formed by direct addition of a precipitating reagent.

1. Urea is often used as example for the homogeneous generation of hydroxide ion. The reaction can be expressed by the equation:

$$(H_2N)_2CO + 3H_2O \longrightarrow CO_2 + 2NH_4^+ + 2OH^-$$

This hydrolysis proceeds slowly at temperatures just below 100°C, with 1 to 2 hours needed to complete a typical precipitation.

Urea is particularly valuable for the precipitation of hydrous oxides or basic salts. For example, hydrous oxides of iron(III) and aluminum [$Fe_2O_3.xH_2O$ and $Al_2O_3.xH_2O$], formed by direct addition of base, are bulky and gelatinous masses that are heavily contaminated and difficult to filter. In contrast, when these same products are produced by homogeneous generation of hydroxide ion, they are dense, are easily filtered, and have considerably higher purity. The Figure below shows hydrous oxide precipitates of aluminum formed by direct addition of base and by homogeneous precipitation with urea. Homogeneous precipitation of crystalline precipitates also results in marked increases in crystal size as well as improvements in purity.



Aluminum hydroxide formed by the direct addition of ammonia (left) and the homogeneous production of hydroxide (right)

The above reaction can also be used the precipitation of Ga, Th, Bi and Sn as hydroxides.

2. Sulfate ion ($S0_4^{2-}$) can be generated by heating a solution containing sulfamic acid. The sulfamic acid hydrolyzes as follows:

$$HSO_3NH_2 + H_20 \longrightarrow H^+ + S0_4^{2-} + NH_4^-$$

Thus, barium sulfate or lead sulfate may be homogeneously precipitate. In the presence of nitric acid, sulfate ion is also formed homogeneously by the following reaction:

$$\mathbf{HSO_3NH_2 + HN0_3 \longrightarrow 2H^+ + SO_4^{2-} + N_2O + H_2O}$$

3. The generation of sulfate ions or phosphate ions from the hydrolysis of their compounds:

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

4. The preparation of oxalate ions from the hydrolysis of dimethyl or diethyl oxalate as a precipitant for Ca.

$$(C_2H_5)_2C_2O_4 + 2H_2O \longrightarrow 2C_2H_5OH + 2H+ + C_2O_4$$

5. The chromate ion as a precipitant for Pb can be generated in simple oxidation of trivalent chromium by using potassium bromate as an oxidizing agent:

$$2Cr^{3+} + BrO_3^- + 5H_2O \longrightarrow 2CrO_4 + Br- + 10\ H^+$$

Advantages of the homogeneous precipitation•

1. The precipitates are slowly formed giving a large size crystal and regular shape.
2. Large sizes of crystals allow ease filtration and washing.
3. The precipitate formed is easily dried to constant weight.
4. The precipitate is a free from the surface impurities (i.e. the degree of co-precipitation is less).
5. The purity of the precipitate and its large crystals allow foe ignition at lower temperature compared to the precipitate that is formed by external addition of precipitant.

Disadvantages:

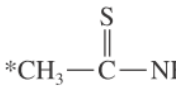
1. Homogeneous precipitation id required longer time than by the ordinary precipitation.
2. It is costly due to the use of organic materials such as, solvents or precipitants.
3. The precipitation is started directly on the wall of the precipitation container making difficult to remove it.

Despite these disadvantages, we cannot give up in using this method largely because it gives us good results compared with the traditions; precipitation method.

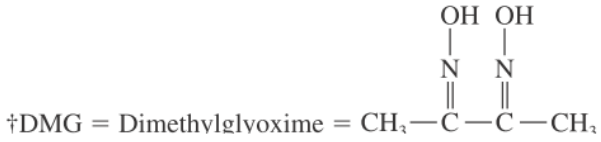
Representative methods based on precipitation by homogeneously generated reagents are given in the Table below.

Methods for Homogeneous Generation of Precipitating Agents

Precipitating Agent	Reagent	Generation Reaction	Elements Precipitated
OH^-	Urea	$(NH_2)_2CO + 3H_2O \rightarrow CO_2 + 2NH_4^+ + 2OH^-$	Al, Ga, Th, Bi, Fe, Sn
PO_4^{3-}	Trimethyl phosphate	$(CH_3O)_3PO + 3H_2O \rightarrow 3CH_3OH + H_3PO_4$	Zr, Hf
$C_2O_4^{2-}$	Ethyl oxalate	$(C_2H_5)_2C_2O_4 + 2H_2O \rightarrow 2C_2H_5OH + H_2C_2O_4$	Mg, Zn, Ca
SO_4^{2-}	Dimethyl sulfate	$(CH_3O)_2SO_2 + 4H_2O \rightarrow 2CH_3OH + SO_4^{2-} + 2H_3O^+$	Ba, Ca, Sr, Pb
CO_3^{2-}	Trichloroacetic acid	$Cl_3CCOOH + 2OH^- \rightarrow CHCl_3 + CO_3^{2-} + H_2O$	La, Ba, Ra
H_2S	Thioacetamide*	$CH_3CSNH_2 + H_2O \rightarrow CH_3CONH_2 + H_2S$	Sb, Mo, Cu, Cd
DMG†	Biacetyl + hydroxylamine	$CH_3COCOCH_3 + 2H_2NOH \rightarrow DMG + 2H_2O$	Ni
HOQ‡	8-Acetoxyquinoline§	$CH_3COOQ + H_2O \rightarrow CH_3COOH + HOQ$	Al, U, Mg, Zn

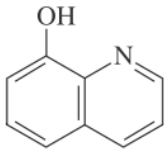


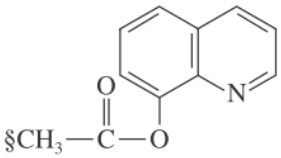
*$CH_3-C(=S)-NH_2$



†DMG = Dimethylglyoxime = $CH_3-C(=N-OH)-C(=N-OH)-CH_3$

‡HOQ = 8-Hydroxyquinoline =





§$CH_3-C(=O)-O$ (quinoline)

How can we avoid the contamination precipitate and what are the methods for the treatment?

First: some types of contamination are difficult to avoid and need pretreatment of contaminated ion as in the case of contamination resulting from the formation of the mixed crystals or due to the spontaneous crystals or due to the spontaneous precipitation. For example, if trace amount of iodine ion present in solution containing chloride ion, iodine ion shall positively precipitate with chloride ion when the precipitation of the later on the form of AgCl.

Second: there are the other cases of contaminations which can be controlled thus decreasing the errors at minimum.

- ❖ Contamination resulting from the formation a local excess of pH as in the precipitation of hydroxides. This can be avoided via using the buffer solutions or homogeneous precipitation.
- ❖ We can avoid the contamination resulting from the internal adsorption by carefully controlling the experimental conditions. For example, the contamination of $BaS0_4$ by adsorption of CI^- can be reduced to minimum if the $BaCI_2$ is added first to H_2SO_4 and not conversely.

❖ In some cases, it is difficult to control the experimental conditions to reduce the contamination by cations, for example, in the precipitation of Ag^+ by adding excess of CI^- to form AqCI , the primary adsorbed layer on the AgCI particles is the negatively CI^- ions and the counter layer are the contaminated positively ions gut if the precipitation has carried out in the presence of dilute $HN0_3$, the H^+ will be predominated as the counter ions. That is, HCI will co-precipitate with AgCI which can be removed at drying the precipitate.

The most important processes that the Analytical Chemist to follow is to avoid the contaminations are:

1 – Digestion 2- Washing 3- Re-precipitation

Digestion of the Precipitate

The precipitate is left hot (below boiling) for 30 min to 1 hour in order for the particles to be digested. Digestion involves dissolution of small particles and re-precipitation on larger ones resulting in particle growth and better precipitate characteristics. This process is called **Ostwald ripening**.

An important advantage of digestion is observed for colloidal precipitates where large amounts of adsorbed ions cover the huge area of the precipitate. Digestion forces the small colloidal particles to agglomerate which decreases their surface area and thus adsorption.

You should know that adsorption is a major problem in gravimetry in case of colloidal precipitate since a precipitate tends to adsorb its own ions present in excess; therefore forming what is called a primary ion layer which attracts ions from solution forming a secondary or counter ion layer. Individual particles repel each other keeping the colloidal properties of the precipitate.

Particle coagulation can be forced by either digestion or addition of a high concentration of a diverse ions strong electrolytic solution in order to shield the

charges on colloidal particles and force agglomeration. Usually, coagulated particles return to the colloidal state if washed with water, a process called peptization. The digestion process is only improving the purity of precipitate, but the filtering process is also improved. So, the digestion is the process of reducing the contamination of the precipitates due the co-precipitation. However, the digestion has limit usefulness for the gelatinous precipitate like hydrous ferric oxide. Also, it is a dangerous and should be avoided if the post-precipitation may be occurred.

Washing the Precipitate

It is crucial to wash the precipitate very well in order to remove all adsorbed species which will add to weight of precipitate. One should be careful not to use too much water since part of the precipitate may be lost. Also, in case of colloidal precipitates we should not use water as a washing solution since **peptization** would occur.

In such situations dilute nitric acid, ammonium nitrate, or dilute acetic acid may be used. Usually, ii is a good practice to check for the presence of precipitating agent in the filtrate of the final washing solution. The presence of precipitating agent means that extra washing is required.

The choice of washing solution is important and depends on the following rules:

1. It has low solubility to the precipitate, but high soluble for the impurities.
2. It should be mixed with an electrolyte to prevent peptization especially for colloidal ppt.
3. An electrolyte solution must add to the precipitate to assist ion exchanging when the adsorbed ions are nonvolatile which is exchanged with volatile ions during drying or ignition.

Solubility losses are reduced by employing the minimum quantity of wash solution constituent with the removal of impurities. That is, the large number of small volume of wash solution in removing impurities is more effective than the small number of large volume. The following expression may be shown to hold:

$$X_n = X_o \left(\frac{\mu}{\mu - v}\right)^n$$

where

$\boldsymbol{X_n} = is\ the\ concentration\ of\ impurity\ before\ washing,$

$\boldsymbol{X_o}\ is\ the\ concentration\ of\ impurity\ after\ n\ washing$

μ=i$s\ the\ volume\ in\ mL\ of\ the\ liquid\ remaining\ with\ the\ precipitate\ after\ decantation.$

V=is the volume of wash solution added to the precipitate in each time, **n** is the number of washing times. It follows from this expression that it is best:

1. **To use a drain (decantation) as far as possible in order to maintain μ at a minimum and,**
2. **To a relatively small volume of liquid and to increase the number of washings.**

Drying and Ignition

The purpose of drying (heating at about 120-150 °c in an oven) or ignition in a muffle furnace at temperatures ranging from 600-1200 °C is to get a material exactly known chemical structure so that the amount of analyte can be accurately determined.

After filtration and washing, the precipitate must be dried to a constant weight. By: **removes excess of solvent & drive off any volatile species**

In some cases, the precipitate is heated to a point where it decomposes to a stable form for weighing. Ignition (strong heating) is used to change the chemical form of some precipitates. For example, ignition $Fe(HC0_2)_3.nH20$ at 850 °C for 1 h gives

Fe_20_3, and ignition $Mg(NH_4)P0_4.6H_20$ at 1100 °C gives $Mg_2P_20_7$. Thermobalance can be used to determine optimum drying times and temperatures. Here, a substance is heated, and its mass is measured as a function of temperature

Advantages of the gravimetriric methods

1. These methods do not require the standardization processes which use the standard solution as in the other method. The gravimetric factor is simply calculated from atomic weights of analyte or compounds at any time.
2. More efficient especially one or two samples are needed for the analysis.
3. it can be used for the determination of an analyte at concentration higher than 1% getting a good sensitivity.

It can be applied for the determination of inorganic ions, neutral species (ike, water, sulfur dioxide, carbon dioxide, iodine etc… it is possible to determine such organic compounds gravimetrically such as, Iactose in milk products, nicotine in milk products, nicotine in insecticides, cholesterol in corn etc.

Disadvantages

1. Less usefulness when a large number of samples needed for analysis.
2. Less sensitivity in the determination of analyte at concentration below 1%.
3. Less precise than other techniques.
4. Non-specific.

Applications of Gravimetric methods

Gravimetric methods have been developed for most *inorganic anions and cations, as well as for such neutral species as water, sulfur dioxide, carbon dioxide, and iodine.* A variety of organic substances can also be determined gravimetrically. Examples include *lactose in milk products, salicylates in drug preparations, phenolphthalein in laxatives, nicotine in pesticides, cholesterol in cereals, and benzaldehyde in almond extracts.* Indeed, gravimetric methods are among the most widely applicable of all analytical procedures.

1. Inorganic Precipitating Agents

Lists of common inorganic precipitating agents are illustrated below. These reagents typically form slightly soluble salts or hydrous oxides with the analyte. As you can see from the many entries for each reagent, few inorganic reagents are selective.

Some Inorganic Precipitating Agents

Precipitating Agent	Element Precipitated*
$NH_3(aq)$	**Be** (BeO), **Al** (Al_2O_3), **Sc** (Sc_2O_3), Cr (Cr_2O_3)†, **Fe** (Fe_2O_3), Ga (Ga_2O_3), Zr (ZrO_2), **In** (In_2O_3), Sn (SnO_2), U (U_3O_8)
H_2S	Cu (CuO)†, **Zn** (ZnO or $ZnSO_4$), **Ge** (GeO_2), As (As_2O_3 or As_2O_5), Mo (MoO_3), Sn (SnO_2)†, Sb (Sb_2O_3), or Sb_2O_5), Bi (Bi_2S_3)
$(NH_4)_2S$	Hg (HgS), Co (Co_3O_4)
$(NH_4)_2HPO_4$	**Mg** ($Mg_2P_2O_7$), Al ($AlPO_4$), Mn ($Mn_2P_2O_7$), Zn ($Zn_2P_2O_7$), Zr ($Zr_2P_2O_7$), Cd ($Cd_2P_2O_7$), Bi ($BiPO_4$)
H_2SO_4	Li, Mn, **Sr**, **Cd**, **Pb**, **Ba** (all as sulfates)
H_2PtCl_6	K (K_2PtCl_6 or Pt), Rb (Rb_2PtCl_6), Cs (Cs_2PtCl_6)
$H_2C_2O_4$	Ca (CaO), Sr (SrO), **Th** (ThO_2)
$(NH_4)_2MoO_4$	Cd ($CdMoO_4$)†, Pb ($PbMoO_4$)
HCl	**Ag** (AgCl), Hg (Hg_2Cl_2), Na (as NaCl from butyl alcohol), Si (SiO_2)
$AgNO_3$	**Cl** (AgCl), Br (AgBr), I(AgI)
$(NH_4)_2CO_3$	**Bi** (Bi_2O_3)
NH_4SCN	Cu [$Cu_2(SCN)_2$]
$NaHCO_3$	Ru, Os, Ir (precipitated as hydrous oxides, reduced with H_2 to metallic state)
HNO_3	Sn (SnO_2)
H_5IO_6	Hg [$Hg_5(IO_6)_2$]
NaCl, $Pb(NO_3)_2$	F (PbClF)
$BaCl_2$	$\mathbf{SO_4^{2-}}$ ($BaSO_4$)
$MgCl_2$, NH_4Cl	PO_4^{3-} ($Mg_2P_2O_7$)

2. Reducing Agents

Lists several reagents that convert an analyte to its elemental form for weighing.

Some Reducing Agents Used in Gravimetric Methods

Reducing Agent	Analyte
SO_2	Se, Au
$SO_2 + H_2NOH$	Te
H_2NOH	Se
$H_2C_2O_4$	Au
H_2	Re, Ir
HCOOH	Pt
$NaNO_2$	Au
$SnCl_2$	Hg
Electrolytic reduction	Co, Ni, Cu, Zn Ag, In, Sn, Sb, Cd, Re, Bi

3. Organic Precipitating Agents

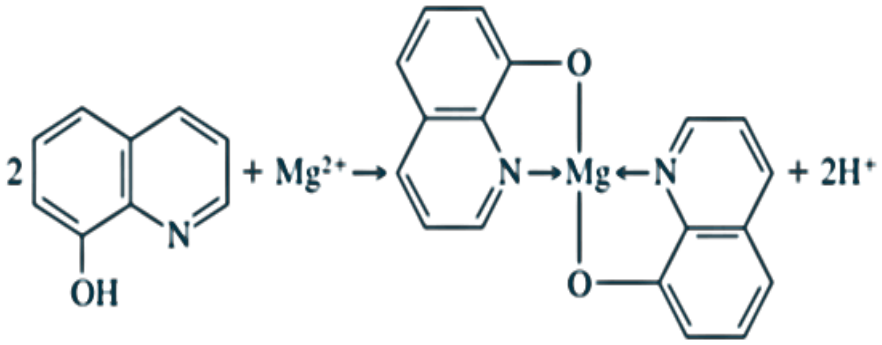
Numerous organic reagents have been developed for the gravimetric determination of inorganic species. Some of these reagents are significantly more selective in their reactions than are most of the inorganic reagents listed in the **Table below**. There are two types of organic reagents: **one forms slightly soluble nonionic products called coordination compounds**, and **the other forms products in which the bonding between the inorganic species and the reagent is largely ionic.** Organic reagents that yield sparingly soluble coordination compounds typically contain at least two functional groups. Each of these groups is capable of bonding with a cation by donating a pair of electrons. The functional groups are located in the molecule such that a five- or six-membered ring results from the reaction. Reagents that form compounds of this type are called **chelating agents**, and their products are

called **chelates.** Metal chelates are relatively nonpolar and, as a consequence, have solubilities that are low in water but high in organic liquids. Usually, these compounds possess low densities and are often intensely colored. Because they are not wetted by water, coordination compounds are easily freed of moisture at low temperatures.

Two widely used chelating reagents are described below

❖ 8-Hydroxyquinoline (oxine)

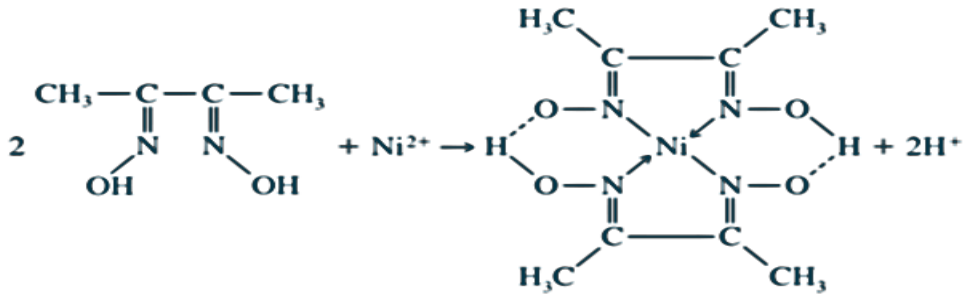
Approximately two dozen cations form sparingly soluble chelates with 8-hydroxyquinoline. The structure of magnesium 8-hydroxyquinolate is typical of these chelates.



The solubilities of metal 8-hydroxyquinolates vary widely from cation to cation and are pH dependent because 8-hydroxyquinoline is always deprotonated during a chelation reaction. Therefore, we can achieve a considerable degree of selectivity in the use of 8-hydroxyquinoline by controlling pH.

❖ Dimethylglyoxime

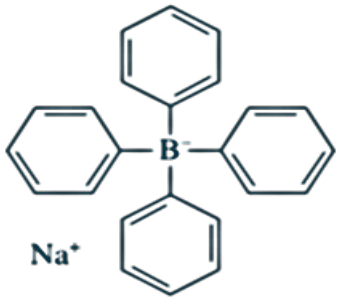
Dimethylglyoxime is an organic precipitating agent of unparalleled specificity. Only nickel(II) is precipitated from a weakly alkaline solution. The reaction is



This precipitate is so bulky that only small amounts of nickel can be handled conveniently. It also has an exasperating tendency to creep up the sides of the container as it is filtered and washed. The solid is conveniently dried at 110°C and has the composition $C_8H_{14}N_4NiO_4$.

Sodium tetraphenylborate, $[(C_6H_5)_4B^-\ Na^+]$

Is an important example of an organic precipitating reagent that forms salt-like precipitates. In cold mineral acid solutions, it is a near-specific precipitating agent for potassium and ammonium ions. The precipitates have stoichiometric composition and contain one mole of potassium or ammonium ion for each mole of tetraphenylborate ion. These ionic compounds are easily filtered and can be brought to constant mass at 105°C to 120°C. Only mercury(II), rubidium, and cesium interfere and must be removed by prior treatment. Organic Functional Group Analysis



Sodium tetraphenylborate.

Several reagents react selectively with certain organic functional groups and thus can be used for the determination of most compounds containing these groups. A list of gravimetric functional group reagents is given in Table above. Many of the reactions shown can also be used for volumetric and spectrophotometric determinations.

Gravimetric Methods for Organic Functional Groups

Functional Group	Basis for Method	Reaction and Product Weighed*
Carbonyl	Mass of precipitate with 2,4-dinitrophenylhydrazine	$RCHO + H_2NNHC_6H_3(NO_2)_2 \rightarrow \underline{R{-}CH{=}NNHC_6H_3(NO_2)_2(s)} + H_2O$ (RCOR′ reacts similarly)
Aromatic carbonyl	Mass of CO_2 formed at 230°C in quinoline; CO_2 distilled, absorbed, and weighed	$ArCHO \xrightarrow[CuCO_3]{230^\circ C} Ar + \underline{CO_2(g)}$
Methoxyl and ethoxyl	Mass of AgI formed after distillation and decomposition of CH_3I or C_2H_5I	$ROCH_3 + HI \rightarrow ROH + CH_3I$ $RCOOH_3 + HI \rightarrow RCOOH + CH_3I$ $ROC_2H_5 + HI \rightarrow ROH + C_2H_5I$ $CH_3I + Ag^+ + H_2O \rightarrow \underline{AgI(s)} + CH_3OH$
Aromatic nitro	Mass loss of Sn	$RNO_2 + \frac{3}{2}\underline{Sn(s)} + 6H^+ \rightarrow RNH_2 + \frac{3}{2}Sn^{4+} + 2H_2O$
Azo	Mass loss of Cu	$RN{=}NR' + 2\underline{Cu}(s) + 4H^+ \rightarrow RNH_2 + R'NH_2 + 2Cu^{2+}$
Phosphate	Mass of Ba salt	$ROP(OH)_2 + Ba^{2+} \rightarrow \underline{ROPO_2Ba(s)} + 2H^+$
Sulfamic acid	Mass of $BaSO_4$ after oxidation with HNO_2	$RNHSO_3H + HNO_2 + Ba^{2+} \rightarrow ROH + \underline{BaSO_4(s)} + N_2 + 2H^+$
Sulfinic acid	Mass of Fe_2O_3 after ignition of Fe(III) sulfinate	$3ROSOH + Fe^{3+} \rightarrow (ROSO)_3Fe(s) + 3H^+$ $(ROSO)_3Fe \xrightarrow[O_2]{} CO_2 + H_2O + SO_2 + \underline{Fe_2O_3(s)}$

*The substance weighed is underlined.

The precipitations conditions

1. Precipitation should be carried out in dilute solution, due regard being paid to the solubility of the precipitate, the time required for filtration and the subsequent operations to be carried out with the filtrate. This will minimize the errors due to co-precipitation.
2. The reagent should be mixed slowly and with constant stirring. This will keep the degree of supersaturation small and will assist the growth of large crystals, a slight excess of the reagent is generally required.
3. Precipitation may be affected under conditions which increase the solubility of the precipitate, thus reducing the degree of supersaturation.
4. Precipitation may be affected in hot solution, provided the solubility and the stability of precipitate permit. At the high temperature.
   - ❖ The solubility is increased with a consequent reduction in the degree of the supersaturation.
   - ❖ Coagulation is assisted and sol formation decreased.
   - ❖ The velocity of crystallization is increased, thus leading to better formed crystals.
5. Crystalline precipitate should be digested for as long as practical, preferably overnight, except in those cases where post-precipitation may occur. Digestion decreases the effect of co-precipitation and gives more readily filterable precipitate.
6. The precipitate should be washed with appropriate dilute solution of an electrolyte.
7. If the precipitate is still appreciably contaminated, the error maybe often reduced by dissolving in a suitable solvent and then re-precipitating it.

Gravimetry is now finished

Thermogravimetry *measures changes in the mass of a sample that occur when it is heated.* These changes relate to the reactions during decomposition, the loss of volatile material and the reactions with the surrounding atmosphere.

Principles

One of the simplest tests that may be applied to an analytical sample is to heat it and observe the changes that occur. These maybe color changes, burning, melting or a variety of other reactions. **The group of techniques that has been developed-to make analytical measurements during heating** is given the general name **"Thermal analysis- TA"** or ***a group of techniques in which a property of the sample is monitored against time or temperature while the temperature of the sample, in a specified atmosphere, is programmed.*** *The program may involve heating or cooling at a fixed rate of temperature change, or holding the temperature constant, or any sequence of these.*

Thermal analysis curve is a plot of the physical property of the sample recorded as a function of the temperature.

Table 1: Lists the more important thermal methods

Technique	**Property**	**Uses**
Thermogravimetry **(TG)** Thermogravimetric analysis **(TGA)**	Mass	Decompositions Oxidation
Derivative Thermogravimetry **(DTG)**	First derivative of mass change	
Differential Thermal Analysis **(DTA)**	Temperature difference	Phase changes reactions
Differential Scanning Calorimetry **(DSC)**	Heat flow	Heat capacity Phase changes reactions
Thermomechanical Analysis **(TMA)**	Deformations	Softening Expansion
Dynamic Mechanical Analysis **(DMA)**	Moduli	Phase changes, Polymer cure
Dielectric Thermal Analysis **(DETA)**	Electrical	Phase changes, Polymer cure
Evolved Gas Analysis **(EGA)**	Gases	Decompositions
Thermophotometry	Optical	Phase changes, Surface reactions

Thermogravimetry analysis (TGA)

It is recording of sample weight changes during controlled temperature programs (dynamical or isothermal). **Δm vs. T**

Differential Thermal Analysis (DTA): temperature difference

Is recording of temperature difference between sample and reference crucible during controlled temperature programs. After calibration the heat flux into/out of the sample (reaction or phase transition enthalpy) can be calculated.

$$\Delta T = T_S - T_R \textit{ vs. } T$$

Differential scanning calorimetry (DSC): heat difference

Heat flux DSC: Measurement of temperature difference between sample and reference crucible during control temperature programs similar to DTA.

Power compensated DSC: Is recording of heating power difference between sample and reference crucible which are kept at the same temperature during controlled temperature programs. Voltage to keep

$\Delta T = T_S - T_R = 0$ vs. T

Generally, TA techniques may be classified into three groups depending upon the way in which the physical property needs to be recorded.

- The absolute value of the property itself can be measured, for example, the sample mass
- The differential method measures the difference between some property of the sample and that of a standard material, for example, their temperature difference.
- The rate at which the property changes with temperature can be measured. These form the basis of derivative measurements and very often may be interpreted on a kinetic basis.

Technique	**Output**
TGA	Δm *vs* T
DTA	ΔT(S.-Ref.) *vs* T
DSC	Power *vs* T

Advantages and Disadvantages of Thermal Analysis

Advantage	Disadvantage
Accurate	Destructive
Low detection limit (up to 10^{-7} g)	Limited range of samples
Reliable data	Time consuming
Easy to use	Usually not qualitative
Rather cheap	
Minimal sample preparation	

Thermogravimetry or thermogravimetnc analysis: it is defined as a technique in which the mass of a substance is measured as a function of temperature while the substance is subjected to a controlled temperature program. The resulting Δm versus T curve provides information concerning the thermal stability and composition of the initial sample, we intermediate products that may form, and the composition of the solid residue, if any. To obtain useful information, the sample should evolve a volatile product as a result of various physical and chemical processes taking place in the sample on heating.

- The resulting plot is known as the **thermogram**. It is a plot of mass percent as a function of time or temperature.
- The mass will change and only if the analyte reacts to produce a gas as a product.
- Otherwise the Physical or chemical changes that go no: involve consumption or liberation of a gas cannot be detected.

The measured weight loss curve (thermogram) gives information on:

- Changes in sample composition
- Thermal stability.
- Kinetic parameters for chemical reactions in the sample**.**

TGA can provide information about:

1. **Physical phenomena**, such as second-order phase transitions, including vaporization, sublimation, absorption, adsorption, and desorption.
2. **Chemical phenomena** including chemisorptions, desolvation (especially dehydration), decomposition, and solid-gas reactions (e.g., oxidation or reduction)
3. **Determine selected characteristics** of materials that exhibit either mass loss or gain due to decomposition, oxidation, or loss of volatiles (such as moisture).

TGA; Phenomena causing mass changes

Physical	Chemical
Gas adsorption	**Decomposition**
Gas desorption	**Break down reactions**
Phase transitions Vaporization & Sublimation	**Gas reactions**
	Chemisorption (adsorption by means of chemical instead of physical forces)

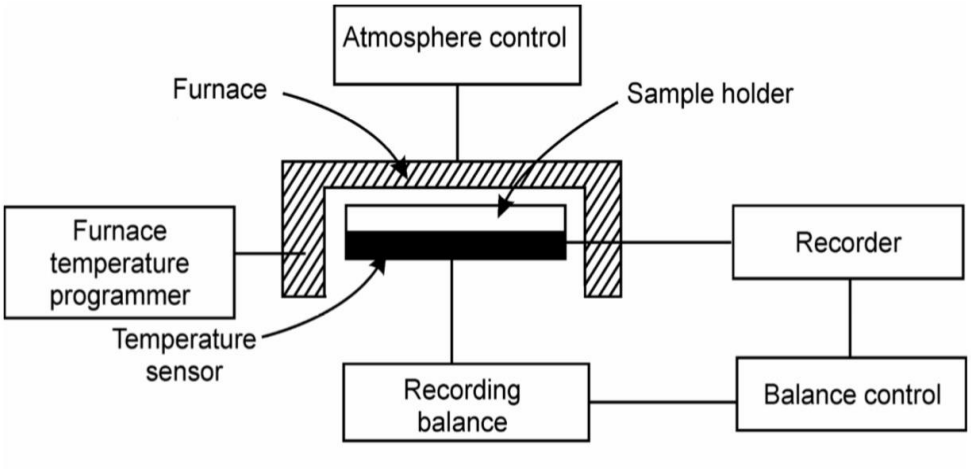
A derivative weight loss curve (DTG curve) can be used to tell about at which weight loss is most apparent.

Derivative thermogravimetrv (DTG) is not a separate technique but involves plotting the first derivative of the TG with respect to either time or temperature.

TGA curve: is plotted with the rate of mass loss on the ordinate, plotted downward, and the temperature or time on the abscissa, increasing from left to right. This can be achieved by an analysis of the TG curve as a separate operation in the dedicated computer part of the equipment.

TGA instrumentation

1. Sample holder (**metallic /ceramic pans**)
2. Microbalance
3. Programmable heater **(furnace)**
4. Gas flow control **(atmosphere control)**
5. Temperature or heating control **(thermostat)**
6. Temperature sensor **(thermocouple)**
7. Read-out **(recorder)**



Schematic diagram of TGA instrumentation

Uses of TGA in Analytical Chemistry

Qualitative analysis	Quantitative analysis
• Determination of the Temp. of decomposition possible and only if the mass • Stability range for the sample. • Identification of the type of the reaction taking place. • Identinfication of the product of the reaction from the decomposition temperature.	• Based on the original mass of the sample, the loss in mass and the temperature at which the loss in mass has occurred • Evaluation of the percentage composition of the sample.

Applications of TGA

1. **Analysis of mixtures** {Each part of mixture behave differently temperature change}
2. **Oxidation studies** {Study of the oxidation of metals at a constant Temp}
3. **Reduction studies** {Study of the reduction of a solution/suspension of metal(s)}
4. **Exact chemical identification** {Different TGA behaviors of identical samples with different history}

Common applications of TGA are:

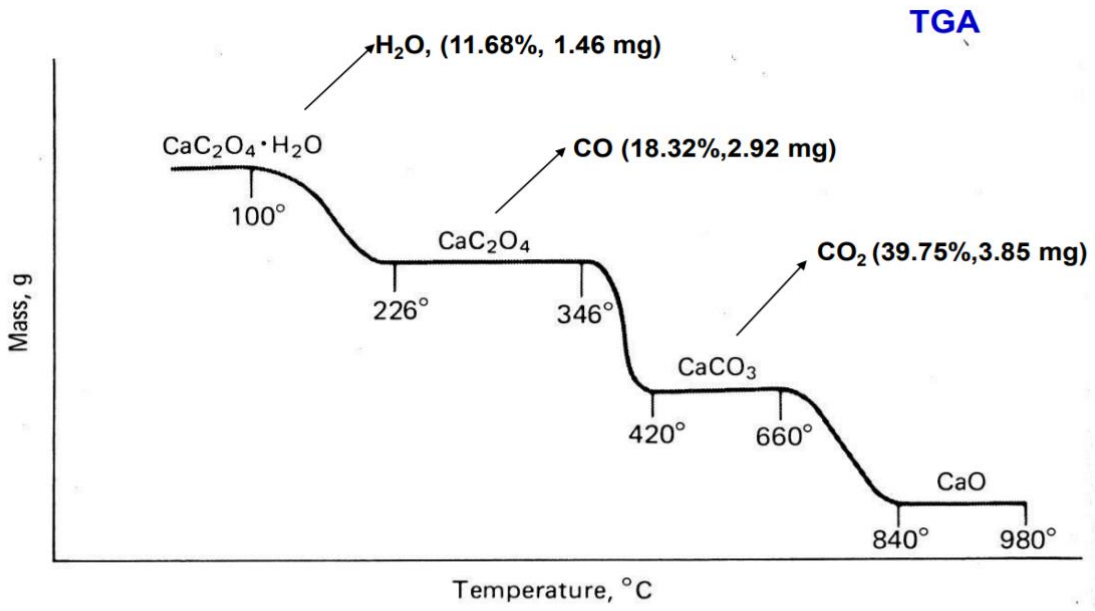
1. Materials characterization through analysis of characteristic decomposition patterns.
2. Studies of degradation mechanisms and reaction kinetics.
3. Determination of organic content in a sample.
4. Determination of inorganic (e.g. ash) content in a sample, which may be useful for corroborating predicted material structures or simply used as a chemical analysis.
5. Decomposition. / oxidation reactions in materials.
6. Physical processes: vaporization, sublimation, desorption, etc., in materials.
7. Decomposition mechanisms / physical processes in polymers.

Application of TGA in Thermal Stability

- **TGA** can be used to evaluate the thermal stability of a material. In a desired temperature range, if a species is thermallv stable, there will be no observed mass
- Negligible mass loss corresponds to little or no slope in the TGA trace.
- Also gives the upper use temperature or a material. Beyond this temperature the material will begin to degrade.

- Performance fibers can be compared using TGA as an evaluation of thermal stability.
- From the TGA, polyoxazole (PBO) has the highest thermal stability of the four fibers as it is stable up to 500 °C.
- Ultra-high-molecular-weight polyethylene has the lowest thermal stability, as it begins to degrade around 200 °C.

Calcium oxalate monohydrate (12.51 mg)

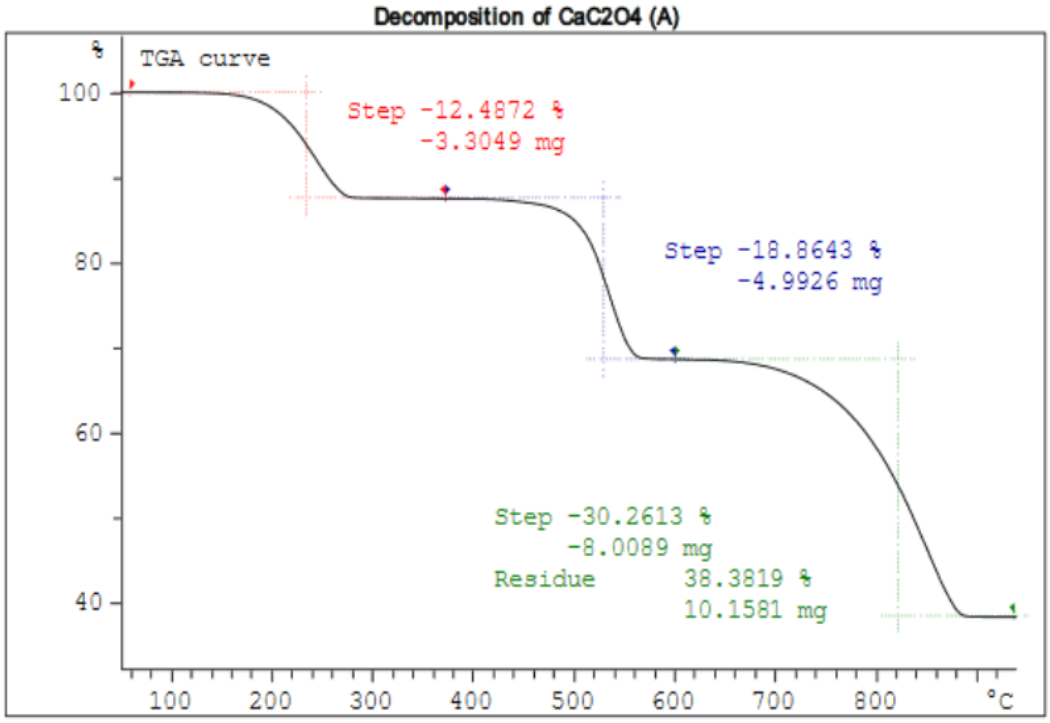


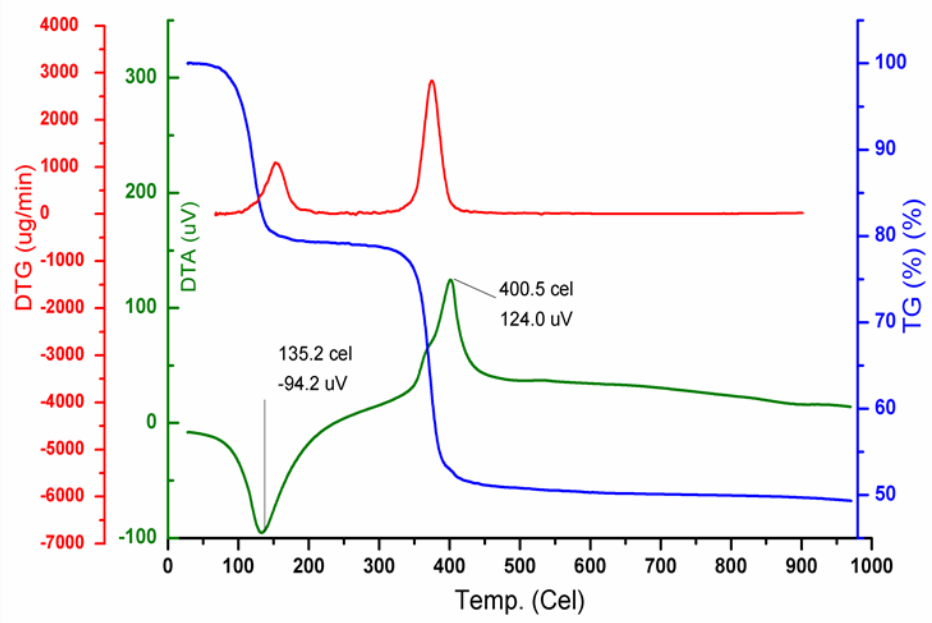
TGA thermogram for $CaC_2O_4.H_2O$ in an inert atmosphere

$CaC_2O_4.H_20 \longrightarrow CaC_2O_4 + H_20$ (T ~100°C)

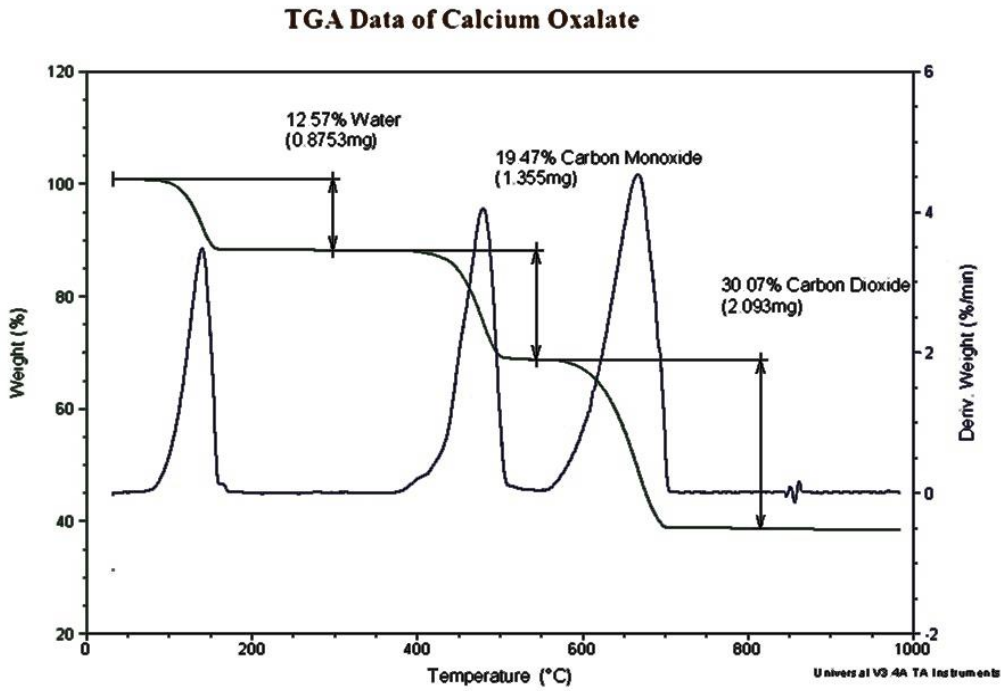
$CaC_2O_4 \longrightarrow CaCO_3 + CO$ (T ~500°C)

$CaCO_3 \longrightarrow CaO + CO_2$ (T ~800°C)


Decomposition of CaC2O4 (A)
%
TGA curve
100
80
60
40
Step -12.4872 %
-3.3049 mg
Step -18.8643 %
-4.9926 mg
Step -30.2613 %
-8.0089 mg
Residue 38.3819 %
10.1581 mg
100 200 300 400 500 600 700 800 °C


DTG (ug/min)
DTA (uV)
TG (%) (%)
400.5 cel
124.0 uV
135.2 cel
-94.2 uV
Temp. (Cel)

TGA – Thermogravimetric Analysis: Example



Factors affecting the shape of thermogravimetric curves

Instrumental factors (thermobalance)	Sample characteristics
❖ Heating rate	❖ Amount of sample
❖ Furnace atmosphere	❖ Particle size
❖ Sample holder geometry	❖ Sample packing
	❖ Thermal conductivity

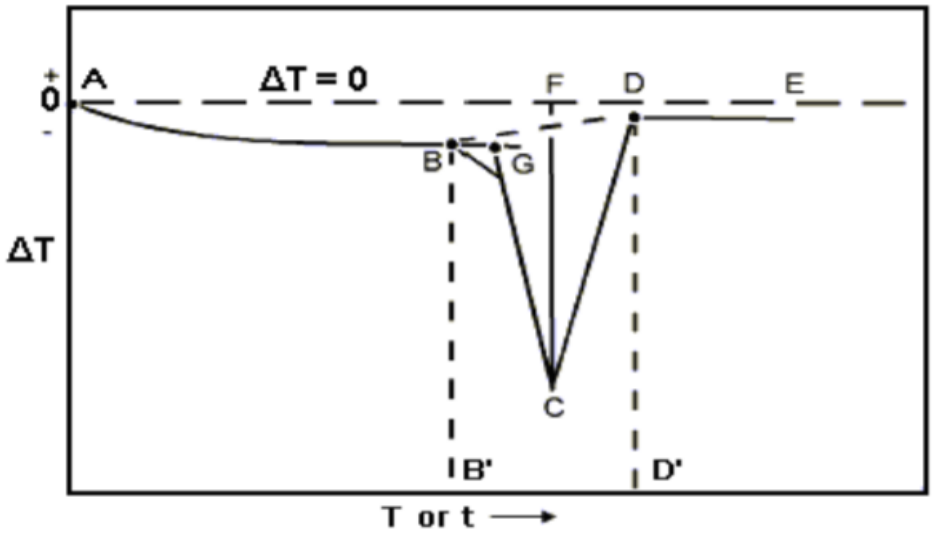
Errors in TGA

1. Drin effect or buoyancy effect (weight gain with temperature).
2. External vibrations.
3. Thermocouple (position and decomposition).
4. Heat of reaction.
5. Thermal conductivity
6. Mass and packing of sample.

Differential Thermal Analysis (DTA)

DTA: *is a technique in which the temperature difference between a substance and a reference material is measured as a function of temperature while the substance and reference material are subjected to the same controlled temperature program.*

DTA curve is recording the temperature difference (AT) *(that plotted on the ordinate with endothermic reactions downwards)* and temperature or time on the abscissa increasing from left to right.



Formalized DTA curve

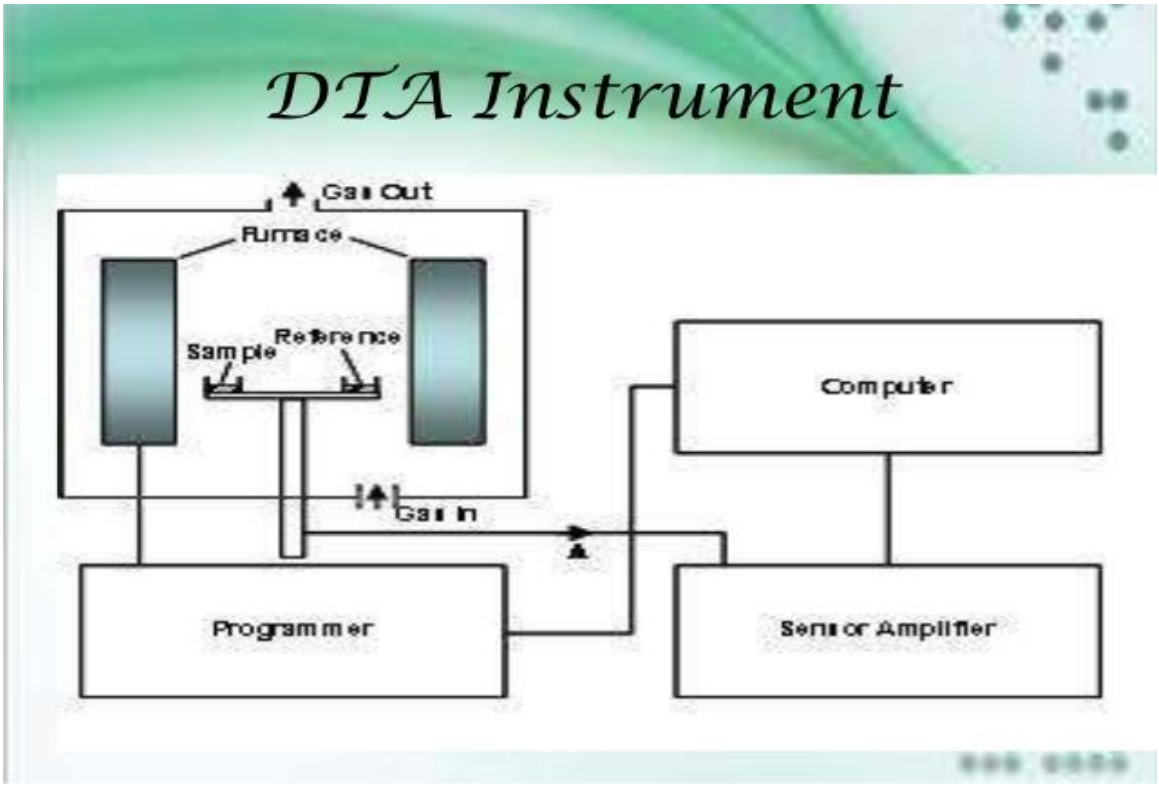
AB and DE	The base line (corresponds to the portion or portions of the DTA curve for which ΔT is approximately zero)
BCD	Peak area: is that portion of the DTA curve, which departs from and subsequently returns to the base line. An endothermic peak or endotherm, is a peak where the temperature of the sample falls below that of the reference material, i.e., **ΔT is negative**. An exothermic peak or exotherm, is a peak where the temperature of the sample rises above that of the reference material, i.e., **ΔT is positive**.
B'D'	Peak width is the time or temperature interval between the points of departure from and return to the base line.
CF	Peak height is the distance, vertical to the time or temperature axis, between the interpolated base line and the peak tip
BCDB	Peak area is the area enclosed between the peak and the interpolated baseline

- Any change physical or chemical will involve energy change i.e. the energy flow in and out of the system.
- The area under the curve is proportions to me amount of energy transferred in or out the sample.
- Integration of the area under the curve will be required for calculation of the energy flow (any energy flow will lead to change in the temperature of the sample).
- The temperature difference of about 0.2°C is the resolution that can be achieved. **Therefore**, the method is more sensitive than all other thermal methods.

DTA; Phenomena causing changes in heat / temperature

Physical	**Chemical**
Adsorption (exothermic)	Oxidation (exothermic]
Desorption (endothermic)	Reduction (endothermic)
A change in crystal structure (endo – or exothermic)	Break down reactions (endo-or exothermic)
Crystallization (exothermic)	Chemisorption (exothermic)
Melting (endothermic)	Solid state reactions (endo-or exothermic)
Vaporization (endothermic)	
Sublimation (endothermic)	

DTA or heat flux DSC instrumentation



Schematic diagram of DTA (or heat flux DSC) instrumentation

- Sensors plus amplifier (Cu for low temperature or Pt-Rh for high temperature).
  Two thermos sensors (thermocouples) are placed in direct contact with the reference and the sample. The third thermocouple records ne temperature of the furnace
  - Furnace and temperature controller Ceramic or Ag (high thermal conductivity)
  - Atmosphere control
  - Reference material; (alpha alumina AI_2O_3 or SiC)
  - It should be inert and should not undergo decomposition in the temperature range in which heating is carried out.
  - Recorder and read-out (fast-response data acquisition)

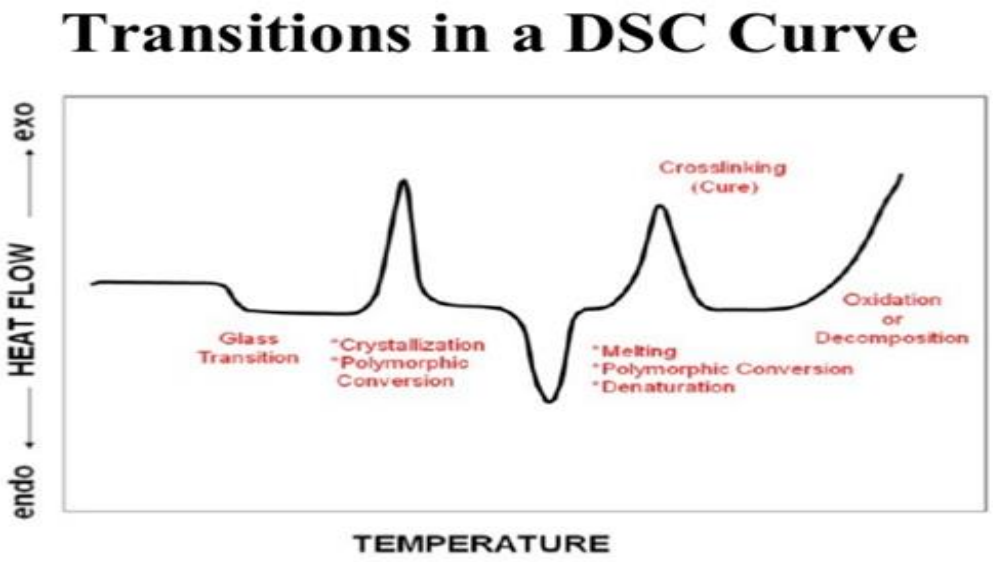
- The instrumentation for DTA and hear flux DSC is identical, but the method of recording the signal is different.

In DTA, the difference in temperature is recorded as a function of time or temperature. While, in DSC, the signal is modified and recorded.

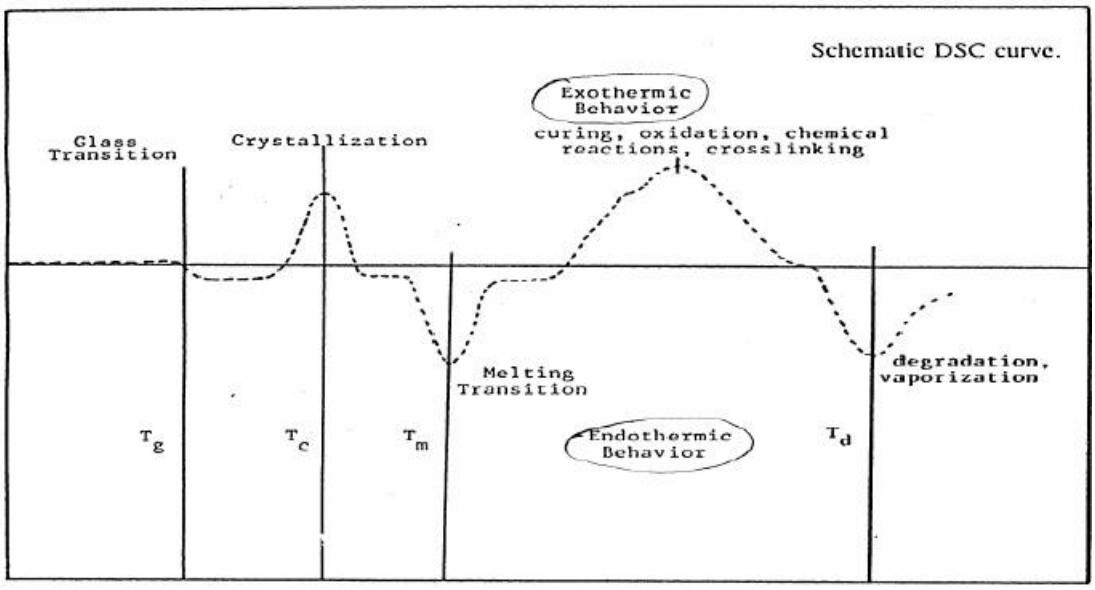
Applications of DTA

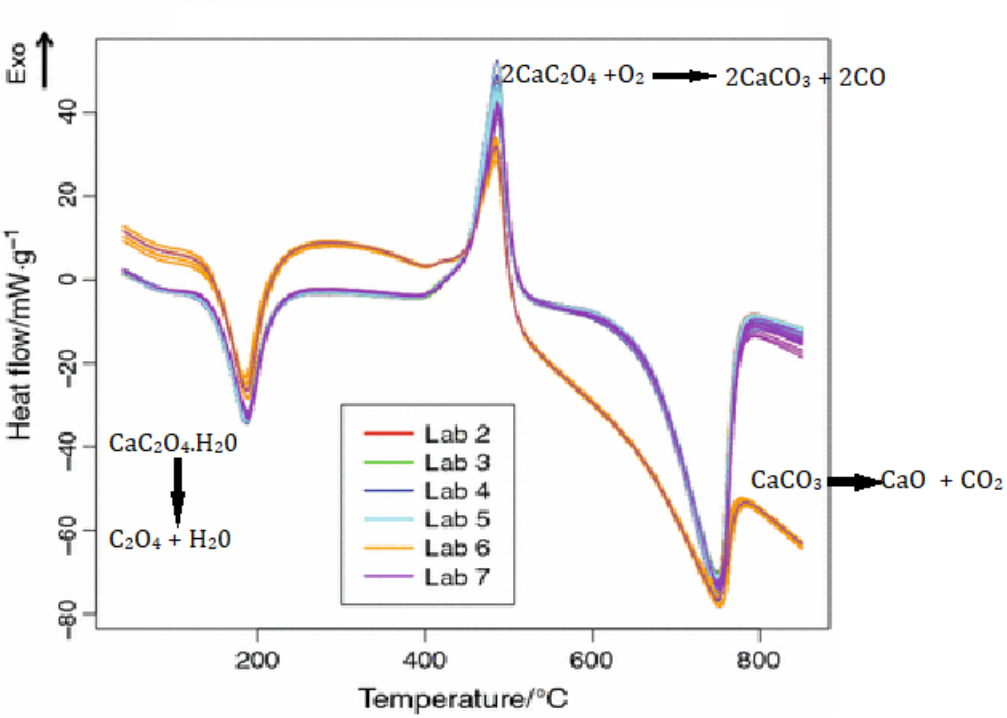
Heat of reaction, ***Corrosion, Identification of polymers***, ***Ceramic and metals industry,*** *and* ***Structure studies*** *involves: Decomposition temperatures, Phase transitions, Melting and crystallization, Thermal stability*

- ✓ *The* ***glass transition temperature*** *(Tg) for a polymer indicates a transformation a rigid to flexible structure, it causes a change in heat capacity and hence –a shift in the baseline. It is an important property, since below the Tg the polymer loses its flexible working behavior.*
- ✓ *Partial* ***crystallization*** *of the glassy form gives rise to an* ***exothermic*** *peak, while* ***Melting*** *of the crystalline polymer results in an* ***endothermic*** *signal.*
- ✓ *In an oxidative atmosphere the polymer undergoes an* ***oxidative decomposition***, *while m an inert one an* ***endothermic degradation*** *leading to carbon formation occurs.*



General transitions through DTA analysis of an organic polymer





DTA curve of calcium oxalate

Factors affecting the shape of DTA curves:

Instrumental factors	Sample characteristics
❖ Heating rate	❖ Amount of sample
❖ Furnace atmosphere	❖ Particle size
❖ Furnace size and shape	❖ Packing density
❖ Sample holder geometry	❖ Thermal conductivity
	❖ Heat capacity
	❖ Degree of crystallinity

The **heating rate** (normally in the 5 -10°C range) and **the amount of sample** are critical in the DTA as well.

In general, using **a small amount of sample:**

1. Yields maximum resolution of peaks.
2. Yields best quantitative results.
3. Yields more regular peak shapes.
4. Permits best thermal contact win the sample holder.
5. Allows quick removal of volatile
6. Minimizes thermal, gradients within the sample.
7. Permits the use of higher heating rates.

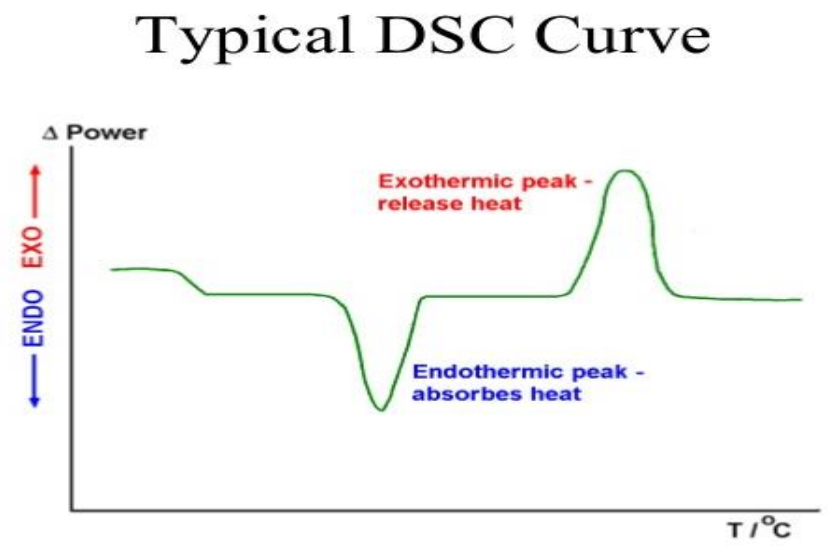
While, using a **larger amount of sample**

1. Allows detection or small thermal effects.
2. Provides more precise Qualitative results.
3. Provides greater quantities of volatiles for evolved gas analysis.

TGA-DTA is combining the two techniques TGA and DTA for comprehensive study of a materials thermal behavior. While TGA only measures changes caused by mass loss, DTA also register changes in material where no mass loss occurs, e.g. crystal structure changes, melting, glass transition, etc.

Differential Scanning Calorimetry (DSC) is a technique in which the difference in energy input into a substance and a thermally inert reference material is measured as a function of temperature, while the substance and reference material are subjected to a controlled temperature program.

The DSC curve records the heat flow (ΔH/ΔT), i.e. the thermal energy transferred to the sample or the reference material in unit time, the signal is recorded in mcal/s, mJ/s or mW units.



This curve can be used to calculate enthalpies of transitions. This is done by integrating the peak corresponding to a given transition. $\Delta H = K.A$

ΔH; is the enthalpy of transition, K; is the calorimetric constant, and A; is the area under the curve.

The calorimetric constant will vary from instrument to instrument, and can be determined by analyzing a well-characterized sample with known enthalpies of transition. Interpretation and evaluation of DSC curves can be made similarly to those obtained by the DTA method. The only difference is that the proportionality constant. K is essentially an electronic conversion factor which is **independent temperature**.

This is very attractive, feature of the method and is primarily responsible for its popularity.

There are two basic types of DSC;

1) Heat flux DSC

- In-concert heating of sample and reference
- Measurement of temperature change (same DTA).

2) Power-compensated DSC

- Separate heating of sample and reference.
- Identical temperature difference.
- Measurement of electrical power.

❖ The two temperatures are equalized by supplying energy to the system.

❖ Amount or energy supplied to the system is measured.

❖ Should correspond to amount of energy utilized by sample.

❖ The area under the curve represents the amount of energy supplied to the system i.e. the amount of energy used by the system.

❖ Heat-flux DSC can be used to higher temperatures, while the power compensation system is more accurate for quantitative work.

Applications of DSC

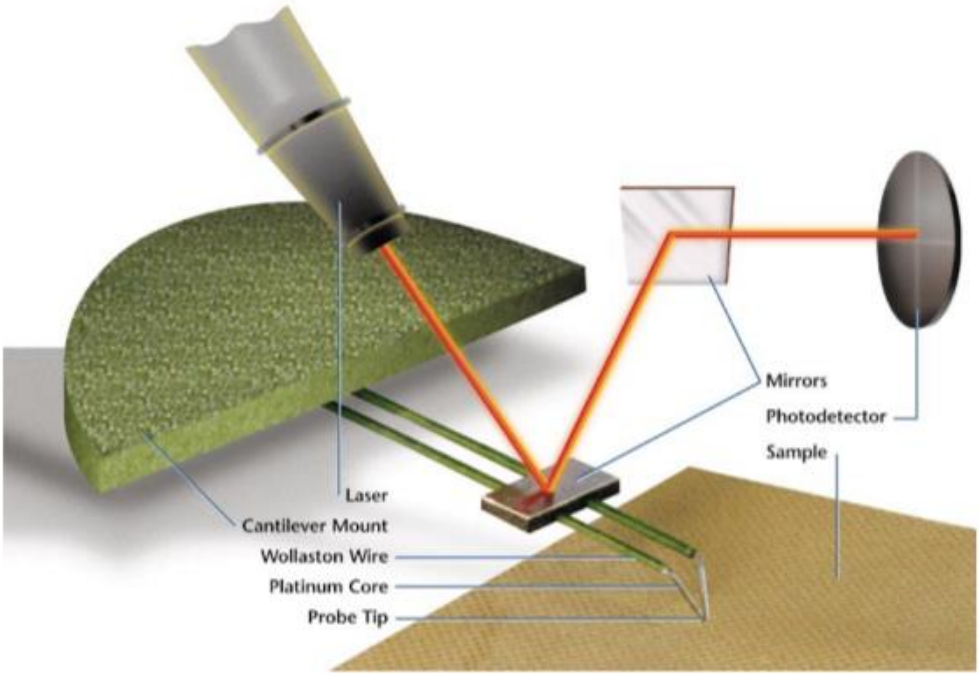
Glass transition temperature, Crystallinity and crystallization rate, Reaction kinetics, Heat of reactions, and Corrosion.

Microthermal analysis combines thermal analysis with atomic force microscopy. It is actually a family of scanning thermal microscopy techniques in which thermal properties of a surface are measured as a function of temperature and used to produce a thermal image. **In microthermal analysis** the tip of an atomic force microscope is replaced by a thermally sensitive probe such as a thermistor or thermocouple. The surface temperature can be changed externally or by the probe acting both as a heater and as a temperature-measuring device.

A micro thermal analysis apparatus (pictured in Figure below), can be operated in either a constant temperature mode or a constant-current mode. The constant-temperature mode is simplest and most often used.

In constant-temperature mode, the electrical power needed to keep the probe temperature constant is obtained as the probe is rastered over the sample surface in contact mode. As the probe encounters parts of the surface that differ in thermal properties, varying amounts of heat flow from the probe to the sample. When the probe touches a region of high thermal conductivity, it cools off, and more power is required to keep it at a constant temperature.

Although microthermal analysis is a very new technique, commercial instruments are available. Applications to pharmaceuticals, polymers, and foods have been reported. The technique also has applications in the ceramics industry and in imaging biomedical samples.



Microthermal analysis apparatus

Micro-thermal analysis is now being used commercially to visualize the spatial distribution of phases, components and contaminants in polymers, pharmaceuticals, foods, biological materials and electronic materials. This review outlines various applications that have been described in the literature to date, the topics ranging from multi-layer packaging materials and interphase regions in composites, to the use of the technique as a means of surface treatment.

Thermal analysis is now finished