Priti Malhotra

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Basic Techniques and Methods

Ane Books Pvt. Ltd.

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Priti Malhotra
Department of Chemistry
Daulat Ram College
University of Delhi
New Delhi, India

Fellow of School of Climate Change
and Sustainability
Institute of Eminence
University of Delhi
New Delhi, India

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My beloved father whose heavenly blessings constantly encourage and cheer me in my academic endeavour.

Preface

Analytical Chemistry is pivotal to every research and investigative study. It is an integral part of every synthetic procedure. Students and teachers of all branches of science, such as Chemistry, Biochemistry, Physics, Geology, Life Sciences, Forensic Sciences, etc., rely upon this particular branch of Chemistry. In their journey to achieve their goals they heavily bank upon analytical methods.

For validation of instrumental methods and their pertinent selection, knowledge of the basic principles involved in the design of the analytical techniques is highly significant. The aim of this book is to address the basic principles of a few commonly used analytical techniques, which empowers the user with holistic skills while using the instruments. The knowledge of the strengths and limitations of a particular analytical technique further enlightens the users' approach towards handling the analyte sample and its investigations on the instrument. All chapters give a sufficiently deep insight into the basic principles of the technique and the schematic design of the instrument. The basic knowledge of the fundamental principles behind the working of an instrument not only helps in making an apt choice of the method for analysis, but also infuses confidence and command on its usage. From sampling, recording to interpreting the data, all fall in the right sequence and enable a comfortable and spontaneous run of the analytical procedure.

With growing environmental concerns, the instrumental methods of qualitative and quantitative analysis are more eco-friendly and safe for the surroundings as they do not involve the use of any chemicals, polluting gases, nor do they generate any hazardous chemical reactions.

Therefore, the importance of *Spectroscopic*, *Electroanalytical*, and *Thermal Methods* of analysis is further emphasized and advancements in the analytical techniques are also encouraged.

How an instrument can be automated and interfaced with computers for fast and accurate data handling has also been explained in the last chapter which lays emphasis on the need for employing computerized instruments. An attempt has been made to mention pertinent problems along with their solutions so that the application of the basic concepts is thoroughly understood by the student, Thus, an endeavour has been made to render this book comprehensive and useful for the reader.

Hoping to succeed in my mission.

New Delhi, India Priti Malhotra

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About the Author

Prof. (Dr.) Priti Malhotra is a Professor at Daulat Ram College, University of Delhi, India. She has been involved in undergraduate teaching for 30 years and has made significant contribution to the field of inorganic chemistry and analytical chemistry. She has published more than 40 research papers in international journals and has also guided and mentored several Ph.D. students and interns. She has actively carried out research projects at the University of Delhi and organised and attended many national and international conferences in the field of chemistry. She is also a Fellow at School of Climate Change and Sustainability, Institute of Eminence, University of Delhi.

Chapter 1
Statistical Methods of Analysis

1.1 Introduction

Every chemical analysis involves data analysis in such a way that the results have minimum errors and uncertainties. All measurements whether it is weight, volume, or pH may not necessarily coincide with the actual values. Although reliability of the data obtained can be ensured by repetitive measurements yet the total possibility of error cannot be ruled out. Therefore, the degree of uncertainty associated with every measurement requires to be decreased such that it reaches an acceptable value. This can be achieved by discussing the typical types of errors and problems faced during data interpretation of chemical analysis.

1.2 Significant Figures

Representation of the analytical data obtained from various measurements necessarily requires certain fixed number of digits. The figures used to estimate the uncertainty involved in the measurements are called significant figures. The number of significant figures is the number of digits which are necessary to express results which are consistent with the precision of measurement. Significant figure in a number is taken as all the certain digits plus one doubtful or uncertain digit. For instance, if the volume of liquid is measured by a burette, which is graduated, to 0.1 ml, any volume reported should reflect this. Thus, if the least count of burette is 0.1 then a measurement of 11.5 ml is more significant than 11.50 ml although they are numerically same. Reading 11.5 ml implies that the actual reading could be between 11.4 and 11.6, whereas 11.50 implies that the actual reading lies between 11.40 and 11.60 but is only possible when the burette would have the least count of 0.01 ml. Therefore, there are only 3 significant figures in 11.5. All non-zero integers are significant. As mentioned, in case of burette reading, zero is not a significant figure since the burette is graduated to 0.1 ml and not 0.01 ml. Zeroes between two non-zero digits

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are always counted as significant figures. For example, 30.9 and 3.09 both have 3 significant figures.

Calculations of Significant Figures

When adding figures having different number of digits, the result should contain the minimum number of decimals. For example,

$$12.2 + 6.413 + 2.36 + 9.4 = 30.393$$

Since the fewest decimal is 9.4 so the result should also contain only one decimal and should be rounded off to **30.4**.

Similarly while finding the result of multiplication or division, the minimum number of digits should be retained in the final answer whereas while calculating retain in each factor one more significant figure than is contained in the factor having the largest uncertainty. For example,

$$2.34 \times 2.342 \times 0.4312 \times 41.2862$$

Should be carried out using the values.

$$2.34 \times 2.342 \times 0.431 \times 41.29$$

And the result with three significant figures will be 2.36.

1.3 Types of Errors and Their Causes

Error in chemical analysis is commonly encountered in almost every observation. The measured value of a property is never the accurate value of the property. The difference between the two is called error.

Error in any observation is actually difference between the observed value and true value.

$$E = (O - T)$$

where

E error in the experiment
O observed value
T True value

percentage error $(E\%) = \frac{E}{T} \times 100$.
per thousand error $E_{O/100} = \frac{E}{T} \times 1000$.

Classification of Errors

Accuracy and precision of a measured quantity are invariably affected by mainly two types of errors:

A. **Determinate errors,**
B. **Indeterminate errors.**

A. **Determinate Errors**: Those errors which can be either avoided or corrected are known as determinate errors. Determinate errors have definite values and are quite systematic so they can presumably be rectified or overcome. Depending upon its origin, determinant errors can be generally classified as

(a) Instrumental errors.
(b) Operative errors.
(c) Methodic errors.
(d) Personal error or human errors.

(a) ***Instrumental errors***: Instrumental errors are those which are usually an integral part of the instrument arising either due to manufacturing defect, environmental factors, or zero error in reading any instrument. The reliability of the instrument generally has range which is provided in the form of a table equating various ranges. Glasswares such as pipettes and burettes measuring flasks used in volumetric analysis, instruments using electronic circuits, such as pH meter, are all calibrated at a certain temperature. When used at different temperature, the measurement differs and so error creeps in. Uncalibrated weights, ungraduated glassware, and pH meters also invite errors. Sometimes voltage fluctuation and long power cuts may cause standarization defects and major errors. Such errors may be minimized in spectrometric analysis by using blanks.

(b) ***Operative errors***: These errors generally arise due to defective operations of various kinds. Erroneous results are expected when hygroscopic materials are weighed, excess amount of indicators are taken, incomplete drying of samples, uncontrolled bumping during heating, weighing crucibles when still hot, and ignition of precipitate at an inaccurate temperature.

(c) ***Methodic errors***: If unsuitable experimental methods or defective experimental techniques are followed, then methodic errors are evolved. These are most dangerous errors and cannot be minimized or corrected unless the condition of the determination is changed. Such an error is well exemplified when a wrongly standardized pH meter is used or when faulty detector responses occur in spectroscopic and chromatographic methods. Some sources of methodic error also include coprecipitation, post-precipitation, side reactions, low solubility of precipitate, and various impurities in reagents. Kjeldahl's method used for the estimation for nitrogen does not give consistent results with nitrogen-containing rings like pyridine compounds. Another common methodic error arises due to large difference between the percentage compositions of analyte sample and the standard solutions used for calibration. Sometimes methodic calibration using blank sample also helps to minimize the errors to a great extent.

(d) ***Personal errors or human errors***: Such errors lie in the handling technique by the individual analyst and are not related with the method or procedure. Visual faults in taking exact readings or judgement of colour, carelessness, ignorance, and physical limitations of the experiment majorly lead to such errors. Sometimes mechanical loss of material in various steps of analysis, improper washing, insufficient cooling, and mathematical errors in calculation also lead to such systematic errors.

Running a blank determination or running a parallel and controlled determination also helps to check these errors. In certain analytical techniques like chromatography and spectrophotometry, some amplification methods are also used to minimize these errors. Therefore, to minimize such errors a suitable choice of equipment, an appropriate method of analysis, and proper calibration of apparatus as well as the methodology become an essential feature of any chemical analysis.

B. **Indeterminate Errors**: Another type of error an analyst generally comes across is accidental or random error which arises from uncertainties in a measurement that are unpredictable and cannot be controlled by the analyst. Such errors are called indeterminate errors and they follow a random distribution. These errors creep in due to slight variation that occurs in successive measurements made by the analyst and over which the analyst has no control. Using a mathematical law of probability, making a large number of observations drawing a normal distribution curve (Gaussian curve) and its interpretation can help greatly to minimize these errors (Fig. 1.1).

It can be interpreted from the curve that small errors occur more frequently than large ones and there are equal probabilities for positive and negative errors of same magnitude to occur. Curves with narrow peaks and steep slopes indicate high precision whereas broad curves indicate low precision. Such random errors are quite unique and cannot always be overcome.

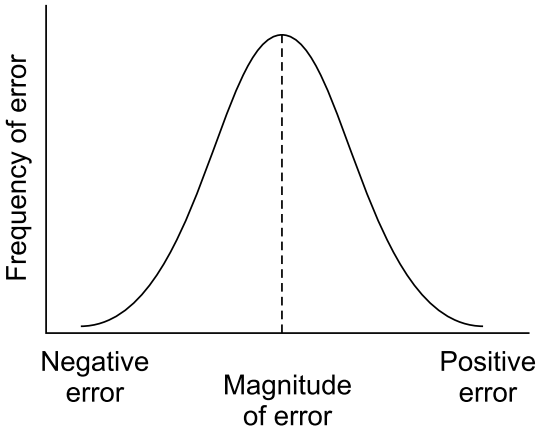


Fig. 1.1 Gaussian curve for indeterminate errors

1.4 Precision and Accuracy

Precision is defined as the variation in repeated measurements and the closeness of a series of results to each other. Precision expresses the reproducibility of the results and can often be achieved readily. A series of measurements may replicate each other and yet not be accurate. There are various ways of expressing precision which are mainly expressed in terms of mean and median, range, average deviation, standard deviation, relative standard direction variance, standard deviation of the mean, relative standard deviation of the mean, and confidence limit. It is important that every analytical exercise should indicate the precision in these ways of expression.

The Mean and Median

When results of successive determinations differ with each other to a small or great extent, then their average value is expected to be closer to the real value. Two common ways of determining average are mean and median.

$$\text{The Mean :} \qquad \overline{x} = \frac{x_1 + x_2 + x_3 + \cdots x_n}{n} = \frac{\sum_{i=1} n_{x_i}}{n}$$

If x_1, x_2, x_3, … x_n are values of various successive measurements x_i represents the ith measurement and n is the number of independent measurements.

The Median

When odd number measurement values are arranged in increasing order then the middle term is chosen or for an odd number of measurement n, the selection of median may be done directly, take the value of $\frac{n+1}{2}$th measurement and if even measurements are there, then the average of $\frac{n}{2}$th and $\frac{n+1}{2}$th measurement is taken as the median.

Example Calculate the mean and median for the following result:

12.08, 12.10, 12.05, 12.20, 12.16, 12.34

$$\text{Mean} \quad \overline{x} = \frac{12.08 + 12.10 + 12.05 + 12.20 + 12.16 + 12.34}{6} = \frac{72.93}{6} = 12.155$$

To find median, the data is arranged in increasing order.
12.05, 12.08, 12.10, 12.16, 12.20 12.34

$$\text{Median} = \frac{12.10 + 12.16}{2} = 12.13$$

Determining precision in terms of mean and median precision can be determined by finding the deviation of the value (x_i) from the mean or the median

$$\text{precision} = |x_i - \overline{x}| \text{ or } |x_i - x_{med}|$$

$$\text{also that,} \qquad |x_i - \overline{x}| = d$$

Precision by range

The difference between the maximum and minimum values in the set of date is called the range. Range $R = x_{\max} - x_{\min}$.

Precision by average deviation and relative average deviation $\overline{d}$

Average deviation is found by taking the means of the difference of individual measured value and the mean of the measurement average deviation

$$\overline{d} = \frac{[(x_1 - \overline{x}) + (x_2 - \overline{x}) + \cdots (x_n - \overline{x})]}{n}$$

where $x_1, x_2, \ldots x_n$ are subsequent measurements and $\overline{x}$ is the mean value

$$\overline{d} = \frac{\sum_{i=1}^{i=n} [(x_i - \overline{x})]}{n}$$

x_i individual measured values
$\overline{x}$ mean of the measurement

Example: Find the average deviation $\overline{d}$ from the following date: 32.35,32.41, 32.12 and 32.40.

$$\overline{x} = 32.35 + 32.41 + 32.12 + 32.40$$
$$= \frac{129.28}{4} = 32.32$$
$$\sum_{i=1}^{i=4} |x_i - \overline{x}| = |32.35 - 32.32| + |32.41 - 32.32| + |32.12 - 32.32| + |32.40 - 32.32|$$
$$= 0.03 + 0.09 + 0.20 + 0.08$$
$$= \frac{0.4}{4} = 0.1$$
$$\overline{d} = 0.1$$

$$\text{Relative average deviation } \% = \frac{\overline{d}}{x} \times 100$$
$$= \frac{0.1}{32.32} \times 100 = 0.309$$

$$\text{Relative average deviation } \% = \frac{\overline{d}}{x} \times 1000$$
$$= \frac{0.1}{32.32} \times 1000$$
$$= 3.09$$

Standard Deviation

Standard deviation is calculated by taking square root of the mean of square of the difference between the individual measured value x_i and the mean

$$S = \sqrt{\frac{\sum (x_i - \overline{x})^2}{n-1}}$$

for small values of n.

n number of measurements,
$n-1$ degree number of freedom.

Relative standard deviation (RSD) $\% = \frac{S}{\overline{x}} \times 100$.
Relative standard deviation 0/00 $= \frac{S}{\overline{x}} \times 1000$.

Variance S^2: The square of the standard deviation is called the variance
Variance, $S^2 = \frac{\sum (x_i - \overline{x})^2}{n-1}$

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

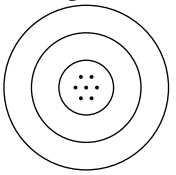
This measure is often expressed as a coefficient of variation (CV)

$$CV = \frac{S \times 100}{x}$$

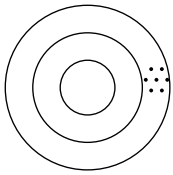
Comparison of Accuracy and Precision

Accuracy is the difference between true value and the value obtained whereas precision is defined as the variability among replicate measurements. It is seen that precision always accompanies accuracy but a good precision does not mean good accuracy. Very often the same errors may be made repeatedly so in spite of precision, accuracy may not be obtained. Sometimes experimental procedures may be precise but due to some constant and unknown errors the results may be inaccurate.

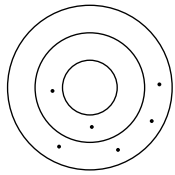
For example,



(a) Precise and accurate



(b) Precise but not accurate



(c) Imprecise and inaccurate

Example Suppose at a target shooting practise a series of bullet have to hit a bull's eye (target). Figure (*a*) shows both precision and accuracy as all the bullets are repeatedly hitting the same target (bull's eye). But in figure (*b*) all the bullets are hitting at a different target, which show precision in the replicate exercises but not the accuracy. Figure (*c*) shows that neither the right target is hit nor all the bullets are hitting the same target, so this is neither precise nor accurate.

Accuracy

Accuracy is defined as the closeness of the observed value to the true value and it expresses the correctness of a measurement. The concordance between the observed value and the most probable value is accuracy. The accuracy of measurement can be expressed in two possible ways:

Absolute Method and Comparative Method

Absolute Method: This is a method of replication. If experiments are repeated for several times then the consistency of the results in the repeated experiments offers a measure of accuracy. But sometimes due to unavoidable or unidentifiable reasons the errors may be repeated and accuracy may yet not be obtained. In such a case, the difference between the mean of an adequate number of results and the true value may be taken as the measure of the accuracy of the method. The accuracy of the method can be established by also varying the amounts involved during the measurements or standardizing the method by making variations in some other parameters.

Comparative Method: Accuracy can be established by using more than one type of analytical techniques independently for determining the measurements. For example, gravimetric, titrimetric, spectrophotometric, etc. techniques may be used to solve one problem. Thus, accuracy is achieved if two independent techniques give the same result. Using standard samplers and comparing the values with those of reference sample using the same technique (e.g. running blank samples spectroscopic measurement) is also a very commonly used comparative method to ensure accuracy. Therefore, accuracy can be judged only when exact values or reported values are compared with the measured values and then using mathematical and statistical methods to check the reliability of the result. The expression of accuracy can be done in the following ways or units.

Methods of Expressing Accuracy: The accuracy of a measurement can be expressed in terms of

(a) Absolute error,
(b) Relative error, and
(c) Relative accuracy.

(a) ***Absolute error***: The difference between the true value and the measured value with regard to the sign is known as absolute error.

$$E = |x_m - x_t|$$

The unit is the same as the units of the measurement.

(b) ***Relative error***: It is determined as the ratio of the difference between true values and measured value to the true value.

$$E_R = \frac{(x_m - x_t)}{x_t}$$

It gives the error relative to the size of the measured value.

(c) ***Relative accuracy***: It is the measured value expressed as the percentage of the true value.

$$\text{Relative accuracy} = \frac{\text{Measured value}}{\text{True value}} \times 100$$
$$= \frac{x_m}{x_t} \times 100$$

1.5 The Confidence Limit

Statistical theory allows us to estimate the range within which the true value might fall within a given probability defined by the experimental mean and standard deviation. This range is called confidence interval and the limits of the range are called the confidence limit. It is highly probable that the true value falls within range. The confidence limit is generally,

$$\text{Confidence limit } = \overline{x} + \frac{t_s}{\sqrt{n}}.$$

where t depends upon the number of degrees of freedom v and the confidence level required. The values of t at different confidence levels and degrees of freedom are given in Table 1.1.

It can be seen that on increasing the numbers of replicate measurement both the values t and $\frac{S}{\sqrt{n}}$ decrease, so that the confidence interval is small.

Table 1.1 Values of t at different confidence levels and degrees of freedom

No. of observation	No. of degree of freedom	Probability levels		
n	n − 1	90%	95%	99%
2	1	6.314	12.706	63.66
3	2	2.920	4.303	9.925
4	3	2.353	3.182	5.841
5	4	2.132	2.776	4.604
6	5	2.015	2.571	4.032
7	6	1.943	2.447	3.707
8	7	1.895	2.365	3.500

The limitations of estimation of confidence interval lie in the number of repeated analysis. Although to avoid unnecessary time and money, only a suitable number of determinations may be performed to obtain reliable results and not always does a large number ensures any great accuracy and precision.

Example The following results are obtained for a Na_2CO_3 and HCl titration: 93.50, 93.58, and 93.43% of Na_2CO_3. The range within which true values lie with 95% confidence.

Solution:

Since $n = 3$, degree of freedom v $= 3 - 1 = 2$

$$\text{Mean} \quad \overline{x} = \frac{93.50 + 93.58 + 93.43}{3}$$
$$= 93.50\%$$

$$\text{Standard deviation} \quad S = \sqrt{\frac{(x_1 - \overline{x})^2 + (x_2 - \overline{x})^2 + (x_3 - \overline{x})^2}{n - 1}}$$
$$= \sqrt{\frac{(93.5 - 93.5)^2 + (93.58 - 9.5)^2}{3 - 1}}$$
$$= 0.75\%$$

At 95% confidence level and 2 degrees of freedom $t = 4.303$

$$\text{confidence limit} = \overline{x} \pm \frac{t_s}{\sqrt{n}}$$

$$= 93.5 \pm \frac{4.303 \times 0.075}{\sqrt{3}}$$
$$= 93.5 \pm 0.196$$

1.6 Test of Significance

In developing a new analytical technique or methodology it is very essential to parallelly run an accepted and established standard method and then make a comparison of the results.

The Students' *t*-Test

The aim of this test is to compare a mean value with a true value so that a new methodology can be accepted. W.S. Gosset, an English Chemist (using pen name of student), proposed a test to know the significance of a new method as compared to a standard method. Described as students' *t*-test has following steps:

(i) Two sets of replicate measurements are made by two different methods, one the new method and the other the standard method. The two ways in which *t*-test can be used are

   (a) A series of repeated measurements are made for a single sample with same concentrations and by two methods.
   (b) Repeated measurements are done with a set of different samples having different concentrations using the same two methods.

(ii) The students' *t*-value is calculated by applying the following equation. For the first method (*a*)

$$t = |\overline{x} - \mu| \frac{\sqrt{n}}{S}$$

where μ is the true mean value, S is the standard deviation, and is the average value for n number of observation.

For the second method (*b*), the difference (*Di*) between each of the paired measurement on each sample is calculated retaining its sign and the average difference is calculated to find standard deviation S_d.

$$S_d = D_i - \overline{D}$$

Then, *t*-value is given as

$$t = \frac{\overline{D}}{S_d}\sqrt{n}S_d$$

$$S_d = \frac{\sqrt{\sum (D_i - D)^2}}{n-1}$$

(iii) The calculated t-value is compared with the tabulated t-value for a given number of measurements at the desired confidence level (Table 1.1). If the calculated t-value exceeds the tabulated value then there is a significant difference between the results of the two methods at that confidence level. If it is does not exceed the tabulated value then it ensures the viability of the new method.

Sometimes it is also beneficial to compare two experimental means. If a sample has been analysed by two different methods, yielding means and and standard deviation S_1 and S_2, then first step is to calculate a t-value

$$t = \frac{|\overline{x}_1 - \overline{x}_2|}{S}\sqrt{\frac{n_1 n_2}{n_1 + n_2}}$$

where n_1 and n_2 are number of individual results obtained by the two different methods. Here, it is assumed that standard deviations in both cases S_1 and S_2 are nearly the same. In the next step, one refers to the t-table, with degree of freedom given by $(n_1 + n_2 - 2)$ and desired confidence level. If the value in the table is greater than the t calculated from the data, then it is not a significant difference between the mean values and it indicates that the experiment is reliable.

F-Test

This is another test which is used to compare the precision of two sets of data, whether obtained from two different analytical methods or two different laboratories. This test is designed to differentiate the two methods on the basis of their standard deviation.

$$F = \frac{S_1^2}{S_2^2}$$

where ${S_1}^2 > {S_2}^2$ so, $F > 1$.

If the calculated value of F exceeds as tabulated at a desired confidence level, then there is a significant difference between the values of the two methods (Table 1.2).

1.7 Rejection of Result

When a number of readings are taken using the same methodology or a series of experiments and measurements are carried out to ensure accuracy, then sometimes few of the results may differ remarkably from others. Then there is a dilemma whether

Table 1.2 F-values at the 95% confidence level

n − 1 for smaller S^2	n − 1 for larger S^2				
	3	4	5	6	10
3	9.28	9.12	9.01	8.94	8.79
4	6.59	6.39	6.26	6.16	5.96
5	5.41	5.19	5.05	4.95	4.74
6	4.76	4.53	4.39	4.28	4.06
10	3.71	3.48	3.33	3.22	2.98
20	3.10	2.87	2.71	2.60	2.35

to still retain or reject a particular result. The following rules may assist to decide whether to retain or reject a particular result.

Rules Based on Average Deviation

Initially, the mean values and average deviation () of good results are determined, then the deviation of the suspected result from the mean of good result is determined. If the suspected result, i.e. if the deviation of the suspected value from the mean is at least four times the average deviation, then the rejection of the suspected result is allowed.

Rule Based on the Range Q-Test

Another test applied before deciding on the rejection or retention of a particular result is the *Q*-test.

The following steps are followed for determining *Q*-test:

(i) The data is arranged in a decreasing order.
(ii) The range (ω) of the result is calculated.
(iii) The difference (a) between the suspected result and its nearest neighbour is found.
(iv) The difference (a) obtained in step (iii) is divided by range (ω) in step (ii) to obtain the rejection quotient Q.

$$Q = \frac{a}{\omega} = \frac{\text{Suspected Value} - \text{Nearest Value}}{\text{Largest Value} - \text{Lowest Value}}$$

(v) The above calculated values of Q are compared with the values given in Q-table. If Q is greater than value in Q-table then the suspected result is rejected (Table 1.3).

Example Amount of Cu present in a sample 1 g of gold gave the results: 0.218, 0.219, 0.218, 0.230, 0.220, 0.215. Apply Q-test to justify the rejection of suspected value of 0.230.

Solution: Arrange in decreasing order

Table 1.3 Value of rejection quotient, Q at 90% confidence level

No. of observations	$Q_{0.90}$
3	0.94
4	0.76
5	0.64
6	0.56
7	0.51
8	0.47

0.230, 0.220, 0.219, 0.218, 0.215

$$a = 0.230 - 0.220 = 0.01$$
$$\omega = 0.230 - 0.215 = 0.015$$
$$Q = \frac{a}{\omega} = \frac{0.01}{0.015} = 0.67$$

The value of Q at $n = 5$ is 0.64, since.
$0.67 > 0.64$.
the Q-test allows the rejection of suspected value of 0.230.

Solved Problems

1. The values obtained for the determination of cadmium in a sample are 4.3, 4.1, 4.0, 3.2 μg/g. Can the value 3.2 be rejected.($Q_{\text{critical}} = 0.831$ for sample size of 4).

Solution:

Applying Q-test

$$Q = \frac{|3.2 - 4.0|}{4.3 - 3.2} = \frac{0.8}{1.1} = 0.727$$

$Q_{\text{calculated}} < Q_{\text{critical}}$ hence the result 3.2 μg/g must be retained.

2. An alloy of tin gave mean of four determinations as 8.27% with a standard deviation $s = 0.17\%$. Calculate 95% confidence limit for the true value.

Solution:

For 4 determinations $(n - 1)$ degrees of freedom $= 3$.
For 95% confidence level (CL), t-value $= 3.18$.
For 95% CL true value $= 8.27 \pm \frac{3.18 \times 0.17}{\sqrt{4}}$.
$= 8.27\% \pm 0.27\%$.
Thus, the true value at 95% confidence limit lies in the range 8% to 8.54% for tin in the alloy.

Unsolved Problems

1. In replicate analysis, the carbohydrate content of a glycoprotein is found to be 12.6, 11.9, 13.0, 12.7, 12.5 g of carbohydrate per 100 g of protein. Find the 50% and 90% confidence intervals for the carbohydrate content.
2. For the numbers 116.0, 97.9, 114.2, 106.8, and 108.3, find the mean, standard deviation, and 90% confidence interval for the mean.
3. The following sets of data are found for chloride analysis: 103, 106, 107, and 114 m eg/L. One value 114 is doubtful. Can it be retained or rejected at 95% confidence level?
4. An analyst reported the following percentage of FeO in a sample: 16.65, 16.70, 16.68, 16.60, 16.58, and 16.63. For this set of results, calculate mean, median, range, average deviation, standard deviation, and coefficient of variation.
5. Calculate the standard deviation and variance of the following set of analytical results 12.67g, 12.69g, and 13.03g.
6. Differentiate between:
   (a) Accuracy and precision.
   (b) Random and systematic error.
   (c) Mean and median.
   (d) Absolute and relative error.
   (e) Variance and standard deviation.
   (f) Determinate and indeterminate errors.
7. Define detection limit.
8. For what purpose *Q*- and *T*-test are used.
9. Define errors. Mention their causes.
10. What are the different ways of expressing precision? Justify that good precision does not assume good accuracy.

Answers

1. $50\% = 12.5 \pm 0.1$, $90\% = 12.5 \pm 0.3$
2. 108.6, 7.1, 108.6 ± 6.8
3. No
4. 16.64, 0.12, 0.04, 0.046, 0.28
5. 0.20, 0.04

Chapter 2
Sampling

2.1 Introduction

Sampling is an integral part of every chemical analysis. The reliability of a chemical analysis cannot be ensured unless appropriation is exercised in sampling. Proper knowledge of the history of the sample, its transmission and storage along with the statistical background of the sample is highly important. The sample used for analysis is also known as analyte and should be a true representative of the bulk material. Among the various factors that govern the accuracy of a test method, parameters associated with sampling play a major role. Few blunders in sampling which are very obvious or natural may lead to pit fall in sampling and erroneous results. For example, a random choice of sample without the knowledge of its history should be avoided. During the course of handling the sample, its composition should not change. The separation of particles on the basis of size and using any adhesive tapes can cause serious errors and mislead the results. Transmission and storage of samples are further great concerns for sampling techniques. Depending upon whether the samples are solid, liquid, or gaseous in nature, the sampling scheme involves a certain protocol. A detailed consideration of statistics of sampling gives following relationship:

$$n \propto \frac{1}{R^2}$$

where R is the relative standard deviation and n is the number of particle. It is the number of analyte particles which is more important than the amount (mass) of the sample. Statistical criteria for ideal sampling also focuses on level of confidence, standard deviation, and unbiased estimate of population variance which means that every particle should get equal exposure to the methodology of analysis, so that every portion of the sample gets equal chance of going through the analysis. Sample stability due to environmental factors, its variability with position and with time

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are further major concerns for designing sampling procedures. Certain legislation guidelines associated with various solid, liquid, or gaseous samples also influence the sampling protocol.

2.2 Gases

Sampling of gases and vapours does not require any considerations of size because here the particle size is the molecule. Handling of gaseous samples largely depends upon its source. Whether the gas is obtained from the environment industrial emissions or indoor collection. Gases found in open systems pose larger difficulty in sampling than those in closed systems such as pipes or cylinders. Gaseous pollutants found in industrial emissions have to follow certain legislation and guidelines which also vary for different places and countries. The type of effluents, their level or concentration in the air, and the duration of exposure are the key factors to be seriously considered before beginning with the sampling technique.

Industrial emissions may be monitored at the spot where the gas leaves a shack or exhaust and where the concentrations are much higher than when dispersed. The volume of air sampled decides its concentration, followed by rate of sampling, duration of sampling, and sample storage where minimum time of storage free from heat and light is required.

Sample techniques adopted for gases obtained from industrial environment can be categorized as follows.

1. **Gas Sampling Vessels**

Vessels made of glass or metals whose capacity ranges from 0.01 to 10 L can be used for simply trapping a gaseous sample from industrial laboratory or a workshop emissions where there are high concentration of gases. Gas sampling syringes fitted with a simple valve can also be used (Fig. 2.1).

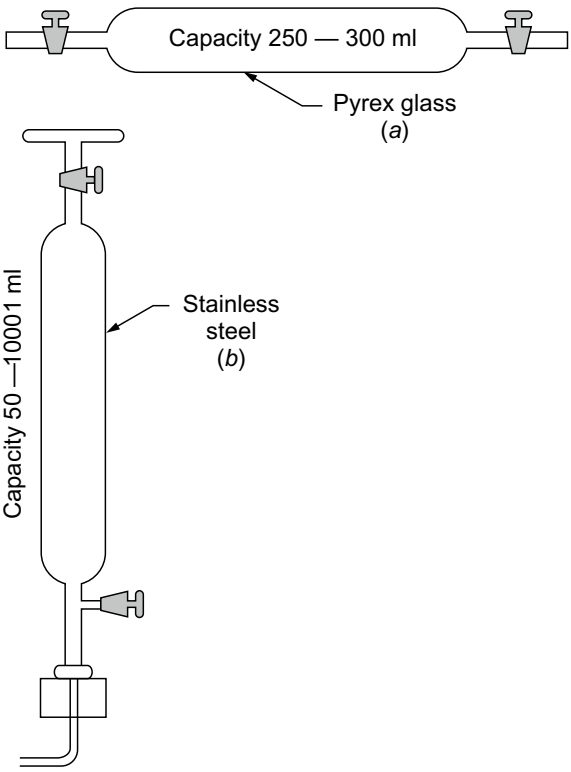

Fig. 2.1 Sample vessels **a** glass, **b** metal

2. **Static Sensors**

They are adsorbent sensors containing an adsorbent layer which are sealed in foiled containers and for its use, they are removed from the foil and clipped to the clothing worn by the worker. The analyte reaches the adsorbent via diffusion and takes long for a required concentration to be adsorbed and is also not suitable, where gas concentrations in the surrounding are already low. If too many gases are adsorbed and due to some chemical reaction at the surface of adsorbent takes place which produces colour then different colours should be produced for different gases, or even variation in concentration should produce variation in colours. Special electro-chemical sensors can be used for a particular gas or vapour, for example, 'Breath analysers' employed for measuring ethanol concentration are equipped accordingly.

3. **Entrapment**

It is a modified method of absorption supported by active collection devices, where the atmospheric air is pumped mechanically through a trap. By adjusting the pumping speed and time the concentration levels of gas sample can be varied and gases having low concentration can be increased in concentration for sampling. Liquid traps have also been used in which after pumping the gas, it is allowed to react and produce a

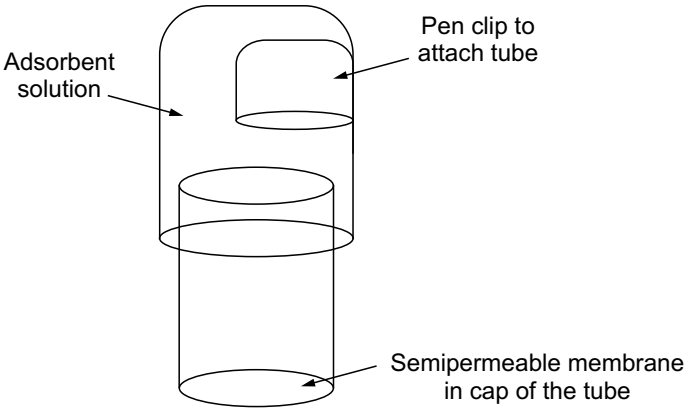

Fig. 2.2 Liquid sensor system for gases and vapour sampling

liquid sample which can be stored for long. For example, even low concentrations of SO_2 can be trapped by allowing it to react with H_2O_2.

$$SO_2 + H_2O_2 \rightarrow H_2SO_4$$

Sulphuric acid is produced which can be easily titrated volumetrically or potentiometrically. When the level of gases in the industrial environment suddenly drops or rise signalling a risk or hazard, then such devices can be programmed to indicate hazard warnings (Fig. 2.2).

In case of solid adsorbents where high concentration of analyte is expected, an adsorbent bed of granular silica gel coated with such a reagent is used so that it produces a characteristic colour with the sample. Breath analyser tubes were originally designed on this pattern, acidic dichromate adsorbed on silica was used which gave green colour when reduced by alcohol.

2.3 Diffusive Samplers

In this method of sampling instead of using active pump to collect vapours, passive diffusion is done. For example, NO_2 is measured using diffusing sampling, an acrylic tube containing absorbent triethanolamine is used which forms nitrite which can be calorimetrically determined. Although the absolute sensitivity is lower than for active pumping but the results are quite reliable.

In general, all entrapment techniques can provide good accuracy for a wide range of analytes.

2.4 Real-Time Analysis

For an instant flash analysis, an extremely sensitive device is required so that it can be used for real-time monitoring of environmental systems.

Since such devices are based on various principles, they are accordingly categorized as follows:

(a) ***Electrochemical sensors***: They use of a gas-phase electrolytic cell to reduce or oxidize and as a particular gas reaches the cell, a redox reaction occurs at the electrodes which generates a current proportional to gas concentration in the sensor zone. If the concentration indicates a risky level, it triggers an alarm warning everybody. For example, levels of Cl_2 in water industry, HCl or NH_3 in chemical process plants, other gas leaks in various industries, oxygen levels, etc. These alarms can be of great advantage.

(b) ***Specialist sensors***: Specialist sensors are designed to sample and detect the presence of gas simultaneously. Gas-sensing abilities of silicon nano-wire-based devices, Pt-based carbon nanotubes, TiO_2 nanotubes, and ZnO-based liquid petroleum gas sensors have been well recognized in the recent past.

(c) ***Infrared spectroscopy***: Gaseous molecule like SO_2, NO_2, CO, NO O_3, and CO_2 absorb IR radiation at characteristic wavelength even at low concentration. Generally, a portable IR spectrometer is engaged along with the sampling methods. In these instruments, a mirror system inside the sample cell causes the radiation travel to and fro many times and thus increase the sensitivity.

(d) ***Chromatography***: Gas chromatography also promotes the analysis of most gases and vapours as the sample is not suitable for pumping into the system. Although the method only gives an indication of total organic vapour but with an instant and fast response.

Liquid

Liquid sampling majorly focuses on drinking water and its purity checks. There are several types of water matrices that are sampled and analysed, including surface water (rivers, lakes, and runoff water), ground water and spring water, saline water, or water from the atmosphere (rain, snow, fog, dew). A homogenous liquid sample is of prime consideration at the beginning of any sampling protocol. Depending upon whether a number of discrete samples are obtained to be analysed separately or individual samples are taken to make a bulk and analyse a composite sample.

Discrete sampling can be performed with very simple apparatus like glass or polythene bottle of 50–1000 ml, Knudson bottle, or a bomb sampler (Fig. 2.3).

Liquid sampler

The problem with taking a large number of samples lies in their storage, transport, handling time, and cost involved in collection of samples. Therefore, instead of taking average of all the results, *composite sampling* is preferred because in this an average sample is taken from the bulk prior to final analysis. Sometimes whichever form

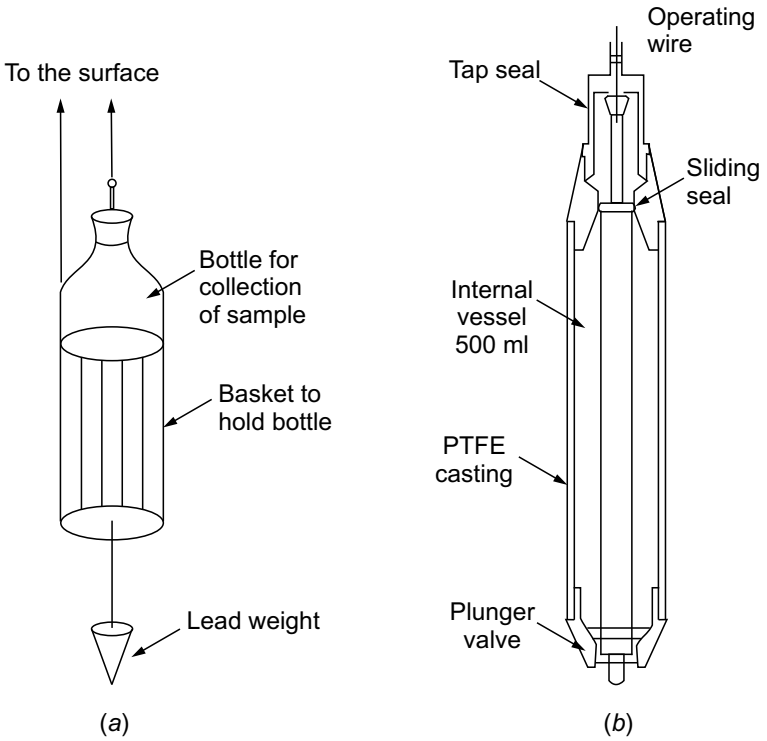

Fig. 2.3 Liquid sampler

of sample collection is employed it is generally important to carry out some form of pretreatment to stabilize the sample and prevent its composition change till the analysis is complete. Not all samples have a big *hold times* and some may require a physical or chemical *preservation* methodology to increase its *hold time.* Sample preconcentration is also required when water samples are analysed for important anions, cations, or some organic species. Direct injection into GC or HPLC columns, solvent extractions, and various other techniques can assist in ensuring required concentrations of samples.

Solids

Sampling of solids is the most daunting task because most gases and liquids are homogenous and contain a large number of particles whereas in solids particle size is of great significance. In solid the area and source of sample collection can also lead to great variation in results. Size of a solid sample generally refers to the mass or volume but statistically it means the number of samples taken. In order to obtain a representative sample from a bulk solid, special care must be taken to minimize sampling errors. The probability p of extracting a single particle, A from a mixture of A and B is

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

n_a = number of type A particles in the total population $(n_a + n_b)$.

Thus, for n particles removed, probability = np.

For solid B there will be nq particles where $q = \frac{n_b}{n_a + h_b}$ and if $q = 1 - p$ then relative standard deviation $R = \frac{\text{standard deviation}}{\text{number of particles withdrawn}}$

where standard deviation $s = \sqrt{npq}$

i.e.

$$R = \frac{s}{np} = \frac{\sqrt{npq}}{np} = \frac{\sqrt{q}}{np}$$
$$n = \frac{1 - p}{pR^2}$$

This indicates the number of particles required to give sampling variability R. Since mass of n particles

$$m = n \times \frac{4}{3}\pi r^3 P$$
$$\therefore \quad m = \frac{(1 - p)}{pR^2} \times \frac{4}{3}\pi r^3 p$$

since $m \infty r^3$, particle size will depend on mass of the material required for a given sampling variance, after calculating the minimum mass for each of the bulk samples, the minimum number of samples necessary for a certain sampling variation at a particular confidence level are determined.

The true result.

$$\mu = \overline{x} \pm ts\sqrt{n}$$
$$t = \text{ students } t \text{ test}$$

where $\overline{x}$= mean value

so the error being the difference between the true result and the mean value

$$\mu - \overline{x} = E = tR/\sqrt{n}$$
$$\text{or} \qquad n = \frac{t^2 R^2}{E^2}$$

E here will be sampling error. For 95% confidence levels $t = 1.96(\sim 2)$

$$n = \frac{4R^2}{E^2}$$

$$\text{then} \quad n = \frac{4V_S}{A^2}$$

if $E =$ accuracy A

and $R =$ sampling variability

so $V_S =$ sampling variability.

Example How many samples are needed for 95% confidence level, relative standard deviation of 9.9% so that measured value of analyte does not vary by more than 5% amount the measured mean?

Solution:

$$n = \frac{t^2 \times R^2}{E^2}$$

$$= \frac{4 \times (9.9)^2}{(5)^2} \quad t = 2 \text{ for 95\% confidence level}$$

$$= 16 \text{ samples}$$

$$\text{For value} \quad t = 2.13$$

$$n = 17.78$$

for $t = 2.11$, $n = 17.45$ can go on till a constant value of n is found.

After calculating the number of samples required for analysis, the next step is to obtain the samples from the bulk with uniformity. In a homogeneous bulk material which consists of identically sized sections, known as matrix, the desired number of samples are taken randomly and then either analysed separately or combined to make to a new bulk to be used for analysis. However, random sampling does not mean the indiscriminate collection of samples rather, it refers to a strictly following sampling protocol designed to minimize the errors.

Solved Problem

1. In a random sampling of 10,000 Units from an industry, 2% error in sampling is expected. What is the expected number of faulty units in the sample and standard deviation.

Solution:

The expected number is

$$0.02 \times 10000 = 200$$

$$\text{Standard deviation} = \sqrt{10,000 \times 0.2 \times 0.98}$$

$$= 14$$

Unsolved Problems

1. How big a sample of an equimolar mixture of oxygen and nitrogen measured at 273°C absolute temperature and 10^{-6} mm pressure must be taken if the sampling error is not to exceed 0.1%.
2. What role does history of a sample play in sampling?
3. On what aspects are statistical criteria for sampling based?
4. How is sampling of gases done. Illustrate by an example of gas sample vessel.
5. Describe real-time analysis with reference to sensors.
6. What are the salient features of liquid sampling?

Chapter 3
Spectroanalytical Techniques

Among the various analytical techniques used, spectroanalytical techniques have a widely acclaimed application either in qualitative or quantitative determinations (identification). It greatly encompasses spectrophotometry which involves electromagnetic radiations that interact with matter and cause either electronic transitions, molecular rotation, or vibrational excitations. To bring about these changes, a given molecular species absorbs radiation only in specific regions of the spectrum where the radiation has the energy required to raise the molecules to some excited state. A display of absorption versus wavelength is called an absorption spectrum of that molecular species and serves as a 'fingerprint' for identification. Emission spectroscopy method where emission spectra analyses a molecule has also developed in a great way and is a significant tool for qualitative and quantitative estimations.

3.1 Origin of Spectra

Spectroscopy involves interaction of electromagnetic radiation with matter. Electromagnetic radiation comprises energy packets called photons and the radiant power of a beam of radiation is proportional to the number of photons per unit time, when a photon collides with a molecule, it transfers its energy to the molecule which in turn rises to some excited state. The energy of photons is given by

$$E = n\,h\,\nu$$

where

n number of photons,
ν frequency of electromagnetic radiation,
h Planck's constant,

P. Malhotra, *Analytical Chemistry*,
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$$\text{or} \qquad \Delta E = nh\frac{c}{\lambda}$$

where

c speed of light,
λ wavelength, and
ΔE is the energy absorbed during transition.

In spectrochemical measurements, both amplitude and frequency are significant but since the detectors are not so well equipped to give fast responses to frequencies, and amplitude cannot be measured, devices are designed to measure radiant power. Radiant power P is proportional to the square of wave amplitude and is a measure of the amount of energy transmitted in the form of electromagnetic radiation per unit time.

$$P = E\phi$$
$$P = h\nu\phi$$

where

E energy of photon,
P radiant power,
ϕ photon flux (number of photons per unit time).

Electronic transition in a molecule or ion from a lower energy state E_1 to a higher energy state E_2. The spectrochemical analysis is based on two factors:

(a) **The interaction of radiation with a chemical species**.
(b) **The range of frequencies used in spectroscopy (Fig. 3.1)**.

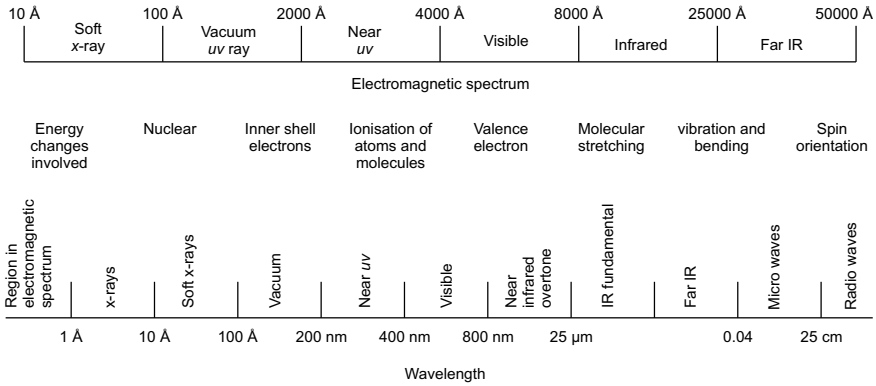


Fig. 3.1 Schematic diagram of electromagnetic spectrum covering *uv* and visible region

In case of ultraviolet and visible spectroscopy the transitions that result in the absorption of electromagnetic radiation in this region of the spectrum are transitions between electronic energy levels. This part of a molecule responsible for light absorption or reflected from it is called a **chromophore**. Any substance that absorbs visible light appears coloured when white light is transmitted through it. A very essential condition for spectrochemical **analysis** is that a source of monochromatic light is desired. A beam that carries radiation with a very small wavelength spread approximating one discrete wavelength is said to be **monochromatic**.

3.2 Interaction of Radiation with Matter

When continuous radiation passes through a transparent material, the interaction of radiation with matter takes place throughout the entire electromagnetic spectrum. As a result of absorption of radiation, various types of provocations are produced within the molecules simultaneously. Radiations may interact with the chemical species to initiate reflection, refraction, and diffraction or scattering which may further produce emission of radiations, phosphorescence, fluorescence, etc. Depending upon the energy of the photons absorbed various types of excitations and transitions are caused by absorption (Table 3.1).

In lowest energy, radio-frequency range and energy transitions are associated with reorientation of nuclear spin states of substances in a magnetic field. In the slightly higher energy microwave region, there are changes in electron spin states for substances with unpaired electron when in a magnetic field. In the infrared region

Table 3.1 Wavelength range for various transitions

Type of spectroscopy	Wavelength range	Type of transition
Gamma ray emission	0.005–1.4 Å	Nuclear
X ray absorption, emission, fluorescence and diffraction	0.1–100 Å	Inner electron
Vacuum ultraviolet absorption	10–180 nm	Bonding electrons (valence electrons)
Ultraviolet visible absorption emission and fluorescence	180–780 nm	Bounding electron
Infrared absorption and Raman scattering	0.78–300 μm	Rotation/vibration of molecules
Microwave absorption	0.75–375 mm	Rotation of molecules
Electron spin resonance	3 cm	Spin of electron in a magnetic field
Nuclear magnetic resonance	0.6–10 m	Spin of nuclei in a magnetic field

absorption causes changes in rotational and rotational-vibrational energy states, whereas absorption of radiation in the visible and ultraviolet regions cause changes in the energy of the valence electrons along with rotational-vibrational changes. X-rays cause the ejection of inner electrons from matter, and the highest energy gamma rays can cause changes in the nucleus.

Therefore, using the interactions of radiation with matter, spectroscopy, and its various techniques were developed and several samples or analytes were analysed. The analytes are initially at its lowest energy state or ground state, then the stimulus causes some of the analyte species to undergo a transition to a higher energy or excited state. By measuring the electromagnetic radiation emitted as it returns to the ground state or by measuring the amount of electromagnetic radiation absorbed or scattered as a result of excitation, a detailed information can be obtained about the analyte (Fig. 3.2).

3.3 Fundamental Laws of Spectroscopy

Absorption of light by a molecule which causes excitations is governed by fundamental laws of spectroscopy. There are two laws:

(i) Lambert's law and (ii) Beer's law.

(i) **Lambert's Law**:

When a beam of monochromatic radiation passes through homogenous absorbing medium, the rate of decrease of intensity of radiation with thickness of absorbing medium is proportional to the intensity of the incident radiation

$$\frac{-dI}{dx} \propto I \tag{3.1}$$

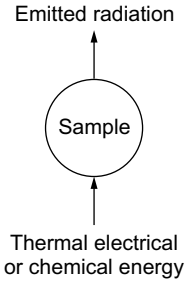
where

$\frac{dI}{dx}$ rate of decrease in intensity with thickness x,
I incident radiation.

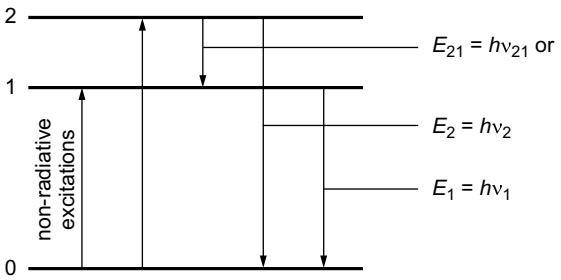
(ii) **Beer's Law**:

When a beam of monochromatic radiation is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with thickness of the absorbing solution is proportional to the concentration of the solution

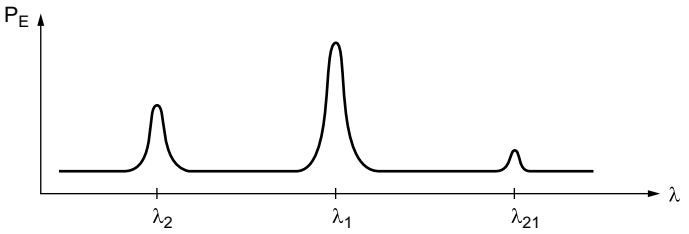
$$\frac{-dt}{dx} \propto C \tag{3.2}$$



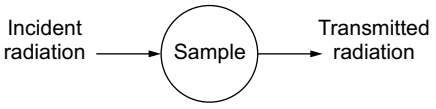
(*a*)



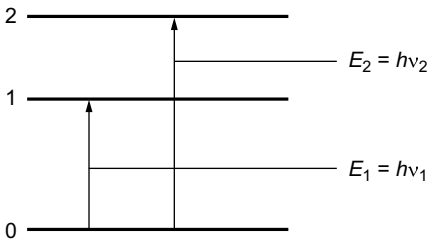
(*b*) Energy level diagram



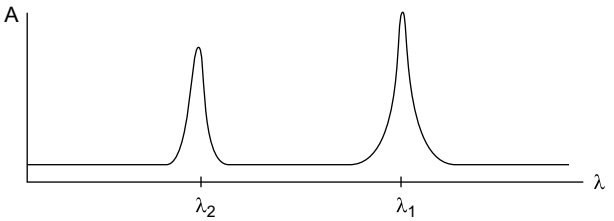
(*c*) The resulting spectrum showing radiant power emitted P_E as a function of wavelength λ.



(*d*)



(*e*)



(*f*) Absorption spectrum as a function of wavelength

Fig. 3.2 **a** Pattern of energy flow. **b** Energy level diagram. **c** The resulting spectrum showing radiant power emitted PE as a function of wavelength

where

C concentration of the solution in moles/litre.

Lambert–Beer's Law:

This law is a combination of both the laws which can be stated as 'when a beam of monochromatic radiation is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with thickness of the absorbing solution is proportional to the intensity of incident radiation as well as to the concentration of the solution

$$\frac{-dI}{dx} \propto CI \qquad \text{(combining Eqs. 3.1 and 3.2)}$$
$$\frac{-dI}{dx} = KCI$$

where K = proportionality constant or absorption coefficient whose value depends upon the nature of absorbing medium.

If I_0 = intensity of incident radiation

I = intensity of radiation after passing through any thickness x of the medium

$$\int_{I_0}^{I} \frac{dI}{I} = -\int_{x=0}^{x=x} KCdx$$

$$\ln I = -KCx \tag{3.3}$$

$$\frac{I}{I_0} = e^{-KCx}$$

$$\text{from Eq. (3)} \quad \log \frac{I}{I_0} = -\frac{K}{2.303}Cx$$

$$\therefore \quad \log \frac{I_0}{I} = \in Cx$$

where $\in = \frac{-K}{2.303}$

$$A = \log \frac{I_0}{I}$$

where A = absorbance or optical density of the solution.

Lambert–Beer's law can be represented as

$$A = \log \frac{I_0}{I} = \in Cx$$

A absorbance or optical density,
C molar concentration of the absorbing solution,
$\in$ molar absorptivity or molar extinction coefficient,
x thickness of the absorbing medium (or path length).

The path length $x = l$.
The ratio $\frac{I}{I_0}$ is called transmittance which is inverse log of absorbance

$$\begin{aligned} A &= -\log T \\ \frac{I}{I_0} \times 100 &= \text{ percentage transmittance} \\ &= \%T \\ \log \%T &= \log \frac{T}{I_0} + \log 100 \\ &= -\log \frac{I_0}{I} + 2 \\ &= -A + 2 \qquad \text{where } A = \in Cl \\ A &= 2 - \log \%\mathrm{T} \end{aligned}$$

From $A = \in Cl$.

It follows that molar absorptivity is the absorbance of 1 mol solution having a path length of 1 cm.

Comparison of Molar Absorptivity and Absorbance

Absorbance is an extensive property of a substance whereas absorptivity is an intensive property. If there is a change of concentration and in the thickness of the container, the value of molar absorptivity will remain constant within Beer's law range but absorbance will change significantly. Although both molar absorptivity and absorbance will vary with wavelength.

Example A solution containing 1.00 mg iron (as thiocyanate complex) in 100 ml was observed to transmit 70.0% of the incident light compared to an appropriate blank, (a) what is the absorbance of the solution at this wavelength? (b) what fraction of light would be transmitted by a solution of iron four times as concentrated?

Solution:

$$\begin{aligned} T &= 0.700 \\ A &= \log \frac{1}{0.700} \\ &= \log 1.43 = 0.155 \\ 0.155 &= ab\,(0.0100 \text{ g/L}) \end{aligned}$$

$$ab = 15.5 \text{ L/g}$$

$$\text{Therefore} \quad A = 15.5 \text{ L/g}(4 \times 0.0100 \text{ g/L})$$
$$= 0.620$$

$$\text{or} \quad \frac{A_1}{A_2} = \frac{abc_1}{abc_2} = \frac{C_2}{C_1}$$
$$A_2 = A_1 \times \frac{C_2}{C_1}$$
$$= 0.155 \times 4$$
$$= 0.620$$
$$\log \frac{1}{T} = 0.620$$
$$T = 0.240$$

Mixtures of Absorbing Species

When two absorbing species in solution have overlapping spectra, then the total absorbance, A at a given wavelength will be equal to the sum of the absorbances of all absorbing species.

$$A = a_1 bc_1 + a_2 bc_2$$
$$A = \in_1 bc_1 + \in_2 bc_2$$

where c_1 and c_2 are the molarities of two species and $\in_1$ and $\in_2$ are their molar absorptivities at that wavelength.

3.4 Validity of Beer-Lambert's Law and Deviation from Beer-Lambert's Law

In accordance with Beer's law a plot of absorbance against concentration should be a straight line passing through the origin but if bending of the straight line is observed, it indicates the deviation from Beer–Lambert's law.

Validity of this law is ensured by the following conditions:

(a) The light used must be monochromatic in nature. Deviations may occur if monochromatic light is not used because at different wavelengths, different values of absorbance will be obtained (Fig. 3.3).

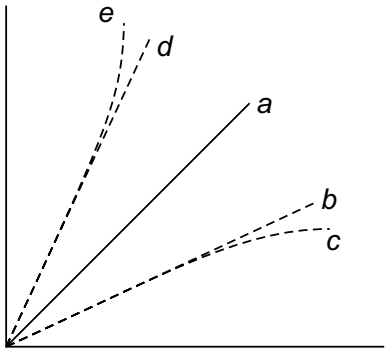


Fig. 3.3 Relative deviation from Beer-Lambert's law

a = Adherence to Beer-Lambert's law
b and c = negative deviation
e and d = positive deviation

(b) The law is applicable only to dilute solutions. It may hold good over a wide range of concentrations only if the colour does not change with concentration. Even impurities affect the absorption of light at higher concentrations. Denser solutions also loose transparency to light and sometimes become optically heterogeneous.

At concentrations of 10^{-3}M or less, the refractive index is constant but at higher concentration the refractive index varies along with absorptivity.

(c) Adverse effects are expected if the coloured solute ionizes, dissociates or associates in solution. For example, Benzyl alcohol tetramerises in chloroform

$$4C_6H_5 - CH_2OH \rightleftarrows C_6H_5CH_2(OH)_4$$

The monomer absorbs at 2.750 μ whereas polymer absorbs at 3.000 μ thus showing positive deviation.

Dilution enhances dissociation. For example, dichromate ion changes colour on dilution

$$\underset{\substack{\text{(orange)}\\ \lambda_{max}375\text{nm},450\text{nm}}}{Cr_2O_7^{-2}} + H_2O \rightleftharpoons 2HCrO_4^- \rightleftharpoons 2H^+ + \underset{\substack{\text{yellow}\\ \lambda_{max}350\text{nm}}}{CrO_4^{-2}}$$

Shows positive deviation on ionization.

(d) Presence of certain impurities in the analyte which causes stray fluorescence. Turbidity caused by suspended particles makes radiations scattered and absorbed rather than transmitted in straight lines through the sample.

(e) Deviations are also expected due to instrumental errors. If the width of slits is wider than required, then undesired radiations would change the absorbance of the sample. The ratio between the spectral slit width and the bandwidth of the absorption band is also important. Narrow absorption bands also cause serious departures from Beer–Lambert's law.

3.5 Types of Spectra

Radiation from an excited source is characterized by emission spectra in which the power of the emitted radiation is plotted as a function of wavelength or frequency. Depending upon the figure generally, three types of spectra appear.

(a) Line spectra,
(b) Band spectra, and
(c) Continuum spectra.

(a) ***Line spectra***: The line spectra is made of series of sharp, well-defined peaks caused by excitation of individual atoms. Line spectra are produced in the visible and ultraviolet regions when the radiating species are individual atomic particles that are well separated in the gas phase. Every particle in the gas behaves independent of one another and the spectrum consists of a series of sharp lines with widths of about 10^{-5} nm.

(b) ***Band spectra***: The band spectra consist of several groups of lines so closely spaced that they are not completely resolved. Small molecules or gaseous radicals are the source of the bands. It is formed when the instrument cannot resolve a series of closely spaced lines.

(c) ***Continuum spectra***: The line and band spectra superimpose to give rise to continuum spectra. When solids are heated to in candescence then due to the phenomenon of black-body radiation, a number of atomic and molecular oscillations are produced. The thermal energy causes this excitation in the condensed solid and with increasing temperature the energy peaks shift to shorter wavelength. Heated solids make good sources for infrared, visible, and ultraviolet radiation for analytical instruments (Fig. 3.4).

Solved Problems

1. A solution containing 1.00 mg of coloured complex ion in 100 ml was observed to transmit 70% of the incident light. (a) what is the absorbance of this solution? (b) what fraction of light would be transmitted for a four times more concentrated solution.

Solution:

(a)

$$T = 0.700$$

$$A = \log \frac{1}{0.700} = \log 1.43 = 0.155$$

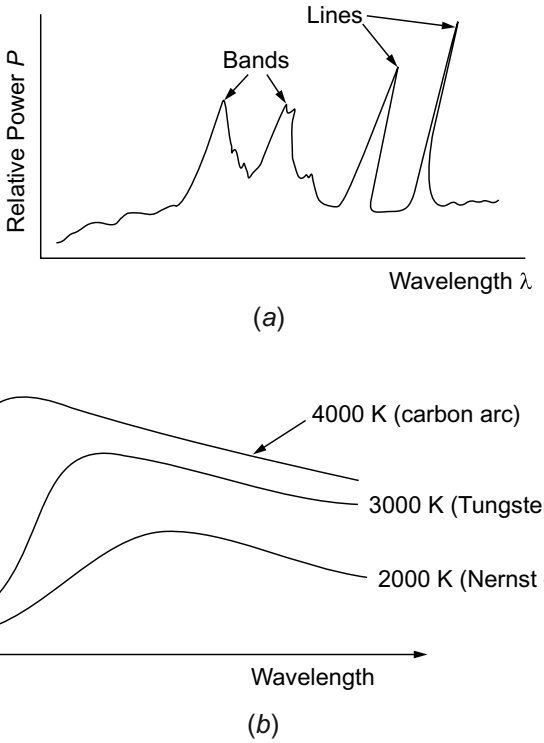


Fig. 3.4 **a** Line and band spectra, **b** continuous spectra

(b)

$$0.155 = ab(0.0100 \text{ g/L})$$
$$ab = 15.5 \text{ L/g}$$

Therefore,
$$A = 15.5 \text{ L/g}(4 \times 0.0100 \text{ g/L}) = 0.620$$
$$\log \frac{1}{T} = 0.620$$
$$T = 0.240$$

2. $K_2Cr_2O_7$ solution of molarity 1×10^{-3} M shows an absorbance of 0.200 at 450 nm and an absorbance of 0.050 at 530 nm. $KMnO_4$ solution of 1.1×10^{-4} M shows no absorbance at 450 nm and an absorbance of 0.420 at 530 nm. Calculate the concentration of $K_2Cr_2O_7$ and $KMnO_4$ present in a solution which exhibits an absorbance of 0.370 and 0.710 at 450 and 530 nm, respectively, in a cell at 10 mm width.

Solution:

$$\text{For} \quad K_2Cr_2O_7 \quad \text{at} \quad 450\ \text{nm} \in = \frac{0.200}{1 \times 10^{-3}} = 200$$

$$\text{For} \quad K_2Cr_2O_7 \quad \text{at} \quad 530\ \text{nm} \in = \frac{0.050}{1 \times 10^{-3}} = 50$$

$$\text{For} \quad KMnO_4 \quad \text{at} \quad 450\ \text{nm}, \in = 0$$

$$KMnO_4 \text{ at } 530\ \text{nm}, \in = \frac{0.420}{1 \times 10^{-4}} = 4.2 \times 10^3$$

$$A\lambda_1 = (a_1c_1)\lambda_1 + (a_2c_2)\lambda_1 \text{ when } \lambda_1 = 450\ \text{nm}$$

$$A\lambda_2 = (a_1c_1)\lambda_2 + (a_2c_2)\lambda_2 \text{ when } \lambda_2 = 530\ \text{nm}$$

$$0.370 = 200c_1 + 0 \times c_2 \text{ at } 45\ \text{nm}$$

$$\therefore \quad 0.710 = 50c_1 + 4200c_2 \text{ at } 530\ \text{nm}$$

$$\text{and} \quad c_1 = 1.90 \times 10^{-3} MK_2Cr_2O_7$$

$$c_2 = 1.46 \times 10^{-4} MKMnO_4$$

Unsolved Problems

1. A 20 ppm solution of a complex was found to give an absorbance of 0.80 in a 2 cm cell. Calculate the absorptivity of the molecule.
2. A compound (molecular mass = 180) has an absorptivity of 286 $cm^{-1}g^{-1}L$. What is its molar absorptivity?
3. A compound having molecular weight 280 absorbed 65% of the radiation in a 2 cm cell at a concentration of 15.0 μg/ml. Calculate its molar absorptivity.
4. Compound A shows an absorption maximum at 267 nm (a = 157) and a trailing shoulder at 312 nm (a = 12.6). Compound B has an absorption maximum at 312 nm (a = 186) and does not absorb at 267 m. A solution containing the two compounds exhibits of 0.726 and 0.544 in a 1 cm cell at 267 and 312 nm , respectively. What are the concentrations of A and B in mg/L?
5. Discuss the origin of UV–visible spectroscopy.
6. Define: absorbance, molar extinction coefficient, transmittance percentage transmission. How are they related?
7. Write the mechanism involved in electronic transitions.
8. Describe the fundamental laws of spectroscopy.
9. Write a short note on the validity of Beer–Lambert's law and its deviation.
10. How does Beer–Lambert's law apply to mixture solutions.

Answers

1. 200 $cm^{-1}g^{-1}L$.
2. 5.5×10^4 cm^{-1} mol^{-1} L.
3. 4.25×10^3 cm^{-1} mol^{-1} L.
4. 2.61 mg/L.

Chapter 4
Ultraviolet and Visible Spectral Methods

Absorbance or transmittance of a radiation falling in the ultraviolet and visible region of the spectrum is considered to be one of the oldest physical methods used for quantitative analysis and structural elucidation. Ultraviolet and visible spectrophotometry are mainly used for quantitative analysis and needs to be aided by IR and NMR spectroscopy for structural determinations. Absorption in the *uv* region (4000–2000 Å) leads to *uv* spectrophotometry and in the visible region (8000–40,000 Å) leads to colorimetry. Both *uv* light and visible light spectroscopically behave similarly.

The absorptions which respond to ultraviolet and visible regions engage electronic transition of various types. Those involving, π, σ, and *n* electrons, absorption involving *d* and *f* electrons and charge transfer spectral absorptions.

P. Malhotra, *Analytical Chemistry*,
https://doi.org/10.1007/978-3-031-26757-4_4

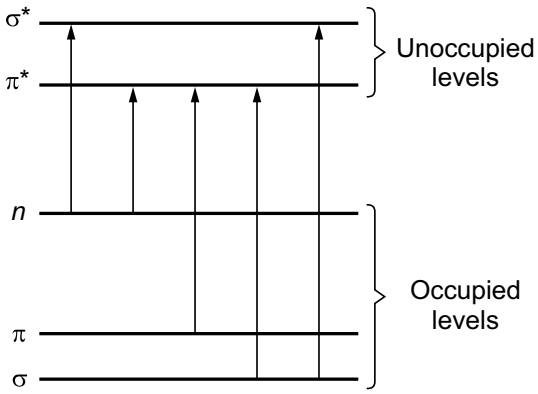


Fig. 4.1 Electronic transitions

4.1 Nature of Electronic Transitions

Electronic transitions in a molecule which take place due to absorption of radiation follow **Franck Condon** principle. According to this principle, electronic transition is very rapid compared to nuclear motion and generally occur without changes in internuclear distance. As a molecule absorbs energy, an electron is promoted from an occupied orbital to an unoccupied orbital of greater potential energy. In general, the most probable transition is from the highest occupied molecular orbital (HOMO) to lowest unoccupied molecular orbital (LUMO). For most molecules, the lowest energy occupied molecular orbitals are the σ orbitals, the π orbitals lie at somewhat higher energy levels and orbitals that hold unshared pairs, the nonbonding *n* orbitals lie at even higher energies, above which lies unoccupied π* antibonding and σ* antibonding orbitals. Some important transitions are.

σ → σ* in alkanes;
σ → π* in carbonyl compounds;
π → π* in alkanes, carbonyl compounds, alkanes, and azo compound;
n → σ* in nitrogen, oxygen, sulphur, and halogens;
n → π* in carbonyl compounds (Fig. 4.1).

The transition elements absorb in the *uv*–visible region due to *d*–*d* transitions and charge transfer spectral absorption giving rise to colorimetry, i.e. in the visible range.

4.2 Instrumentation

Block diagram for measuring absorption of radiation is given below (Fig. 4.2).

Radiation Sources

The incident radiation to which the analyte is exposed needs to be received from a radiation source having some basic requirements. The source used in absorption

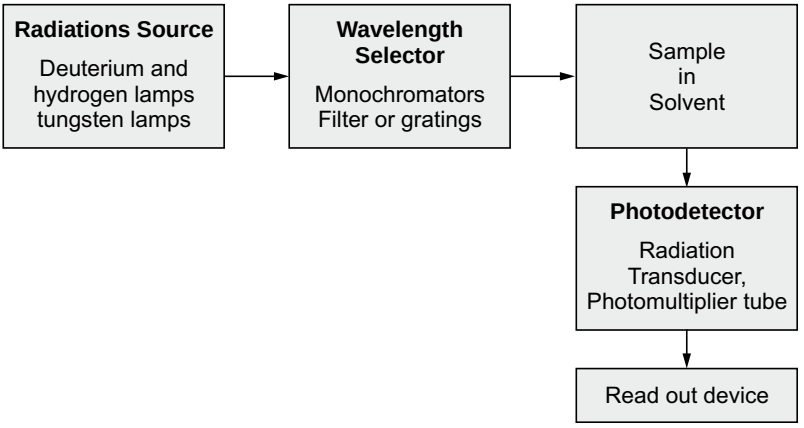


Fig. 4.2 Block diagram for uv–visible spectrophotometry

spectrophotometry should provide sufficient radiant energy over the desired wavelength region, and should be able to maintain a constant intensity over the time phase of measurement. To provide a continuous source of energy in the particular wavelength region, various lamps have been designed.

1. **Deuterium and Hydrogen Lamps**: A deuterium lamp is a low-pressure gas-discharge light source often used in spectroscopy when a continuous spectrum in the ultraviolet region is required. The electrical excitation of deuterium or hydrogen at low pressure is capable of producing a continuous spectrum in the ultraviolet region. Deuterium follows the reaction:

$$\mathrm{D_2} + \mathrm{Ee} \rightarrow \mathrm{D_2^*} \rightarrow \mathrm{D'} + \mathrm{D''} + h\mathrm{v}$$

$$\text{energywise} \qquad \mathrm{Ee} \rightleftharpoons \mathrm{E_{D_2^*}} \rightleftharpoons \mathrm{E_{D'}} + \mathrm{E_{D''}} + h\mathrm{v}$$

where $D^*{}_2$ is the excited deuterium atom and E'_{D} and $E^{''}{}_D$ are two kinetic energies.

The *uv* range of 190–400 nm matches with the output range produced by deuterium and hydrogen lamps (160 nm–800 nm). Although at wavelengths > 400 nm, it is not a continuous spectrum but emission lines and superimposed bands are present. In deuterium and hydrogen discharge lamps, the shape of the aperture between two electrodes is such that the discharge exits in a narrow path with a constant power supply maintaining constant intensity.

2. **Tungsten Filament Lamp**: It is the most common source of radiation and is bared on black-body radiation as its temperature is 2870 K. There is a drawback in the tungsten lamp that it emits the major part of radiant energy in the near-infrared region and only a small part falls in visible region. Another improved lamp, tungsten-halogen lamps also called quartz halogen lamps are also used and

(a) (b)

Fig. 4.3 **a** Deuterium lamp **b** Hydrogen lamp used in uv-visible spectrophotometers

have a longer usage time. They contain small quantity of iodine inside the quartz chamber which has tungsten filament. The sublimed tungsten reacts with iodine to form volatile WI_3 which increases its life and also focusses on the radiations in the ultraviolet range and so is widely used (Fig. 4.3).

3. **Light Emitting Diodes (LED)**: Light emitting diodes which are a *pn*-junction device and produce radiant energy when forward biased. It produces continuous radiation of a narrow range. Diodes made from gallium aluminium arsenide (λ_m = 900 nm), gallium arsenic phosphide (λ_m = 650 nm) and gallium nitride and (λ_m = 450) *nm* are generally used and are safe, long shelf life and are ecofriendly.

Wavelength Selectors: Since radiation of single wavelength, i.e. monochromatic radiation is essentially required for spectrophotometry, it is important to isolate a narrow band of wavelength from the radiation source. For this purpose, generally filters or monochromators are used.

Filters are of two types: *interference filters* and *absorption filters*.

Interference filters: Using the phenomenon of optical interference, these types of filters provide narrow bands of radiation; A layer of dielectric between two metallic films is sandwiched between two glass plates or transparent material (Fig. 4.4).

This assembly reinforces a particular wavelength and other wavelengths phase out due to destructive interference.

Absorption filters: Presence of dye suspended in gelatin or coloured glass between transparent glass plates behaves like an absorption filter. This effective bandwidth is between 30 and 250 nm but the light transmitted is low in intensity and although the desired radiation is obtained but with reduced transmittance. They are of two types, cut-off and band-pass filters.

Monochromators: As one of the essential conditions of Beer–Lambert's law, light meant for absorption should consist of only one wavelength. Monochromators are such devices which are designed for spectral scanning, and provide single wavelength radiation. Generally, all monochromators consist of similar components.

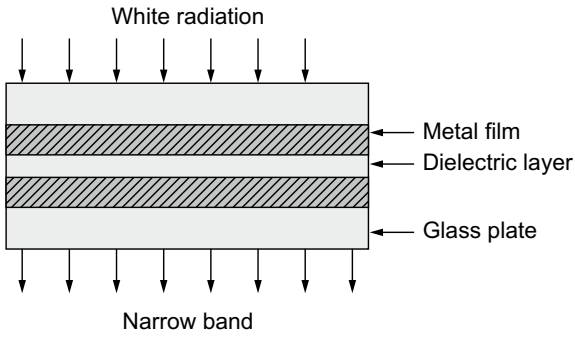


Fig. 4.4 Interference filter

An entrance slit, a dispersing unit with a prism and grating, and an exit slit. Some lens or mirrors are also used in coordination so that parallel beams may also be produced and focussed on a focal plane. Depending upon the required range, the materials used for construction of component are normal glass for visible range, quartz for *UV*, and alkali halides for infrared. The prism monochromators use prisms as dispersing unit whereas the grating monochromators use grating as the dispersing unit. A 60° prism often made up of quartz is commonly used to disperse visible, ultraviolet, and infrared radiation. In gratings type usually 300 to 2000 groves/mm are constructed for ultraviolet and visible region, and the gratings need to be identical in size, equally spaced and parallel to each other (Fig. 4.5a, b).

Prisms are good at dispersing wavelength which do not overlap but they give non-linear dispersion, whereas gratings give linear dispersion but overlaps of wavelength are possible.

Resolving Power of Monochromators: It is described as the ability to separate adjacent images which have different wavelengths.

$$R = \frac{\lambda}{\Delta\lambda}$$

where

R Resolving power
λ average wavelength of two images
$\Delta\lambda$ their difference

The *uv*–visible monochromators generally give 10^3 to 10^4 range of resolving power. All types of gratings also give a good resolving power if they are longer gratings and have smaller spacings.

$$R = \frac{\lambda}{\Delta\lambda} = nN = \frac{2Nb\sin r}{\lambda}$$

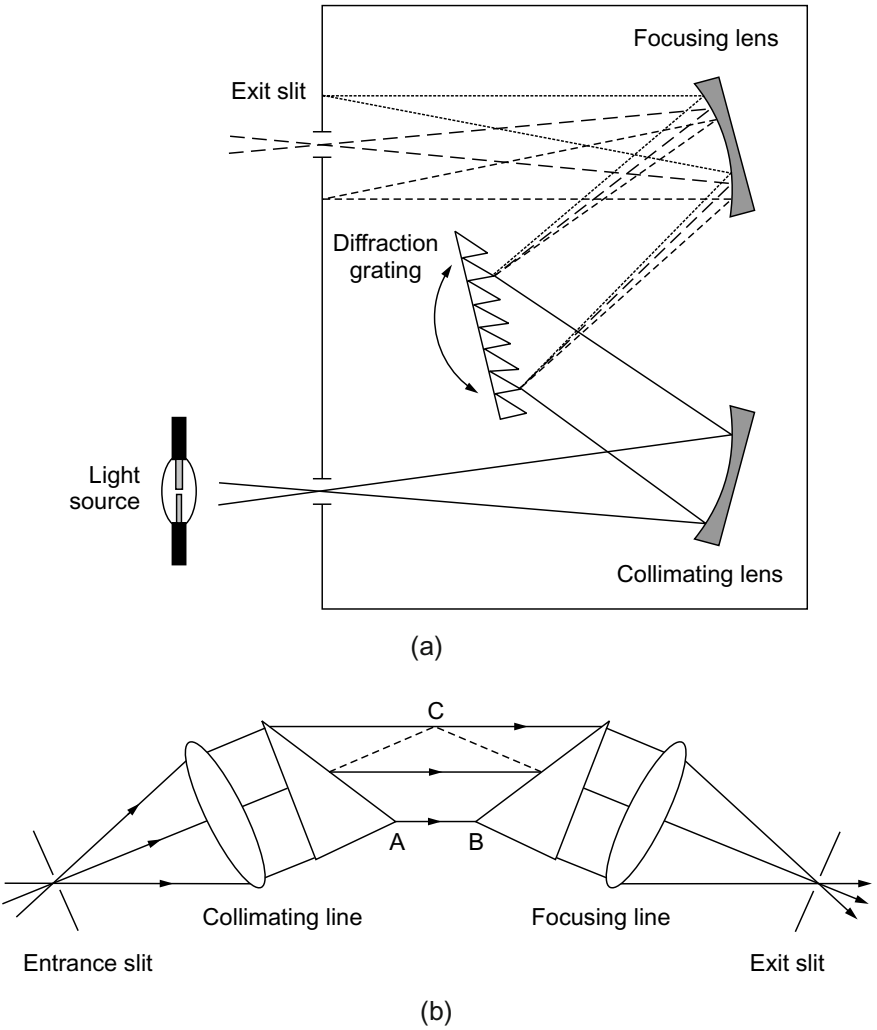


Fig. 4.5 **a** Grating monochromator **b** Prism monochromator

where

n diffraction order and
N no. of grating illuminated.
b width of individual groove
r angle between the diffracted ray and the grating normal.

Sample Containers: The absorbance data obtained in uv–visible spectroscopy majorly depends on sample handling and the cells in which the samples are kept. The cells or cuvettes in which the samples and solvents are held should be transparent throughout the visible and near-infrared regions for which quartz or fused

silica or plastic containers are provided. It is also essential to use clean cells or cuvettes for every spectroscopic run. *Sample cells* should be of constant thickness, the surface facing the incident light must be optically flat. The material should be inert to the solvent and temperature resistant and should transmit light of the particular wavelength.

Detectors: The role of a detector is to convert the electromagnetic radiation emitted from the sample into a current flow which can be detected on the read-out device. Sometimes the signals received ought to be amplified before being conveyed to the reader. In order to detect radiations, three types of photosensitive devices are used:

(a) Photovoltaic cells,
(b) Photoemissive cells, and
(c) Photomultiplier tubes,

where detection is based on photoconduction and use photoelectric effect.

(a) ***Photovoltaic cells***: Also known as barrier-layer photovoltaic cell and is used to measure radiation signal in the visible region, similar to human eye. These cells although do not need any external source of electrical energy, but respond only at high output levels and for a small period of time when used continuously.

If consists of a flat copper or iron electrode on which lies a semiconductor (e.g. selenium) layer coated with metallic silver or gold (Fig. 4.6).

When the radiation falls on selenium surface, electrons are ejected from selenium–silver junction and they create a potential difference between silver and the base resulting in a photocurrent. The amount of current will vary with the intensity of incident radiation. Because of low internal resistance the current produced by photovoltaic cell cannot be amplified easily and they show fatigue effects which are reduced by using restricted radiations.

(b) ***Photoemissive cells***: These type of cells are available for ultraviolet and visible wavelength regions. Photoemissive cells are used in commercial phototubes. It consists of a wired anode and semi-cylindrical cathode supporting photoemissive surface. When radiation is incident upon the cathode, photoelectrons are emitted, collected on anode, and then returned from external circuit. The number of electrons ejected from a photoemissive surface is directly proportional to radiant power of the beam that strikes that surface and are independent of voltage difference (Fig. 4.7).

Photoemissive cells or phototubes are more sensitive than photovoltaic cells and can also be used at low intensities and their output current can be amplified to provide sufficiently readable signals.

(c) ***Photomultiplier tubes***: Photomultiplier tube is mostly used in the spectrophotometer and is more sensitive than phototube for the visible and ultraviolet regions. It consists of a photoemissive cathode, which the photon strikes and a series of electrodes (dynodes), each one charged at a successively higher

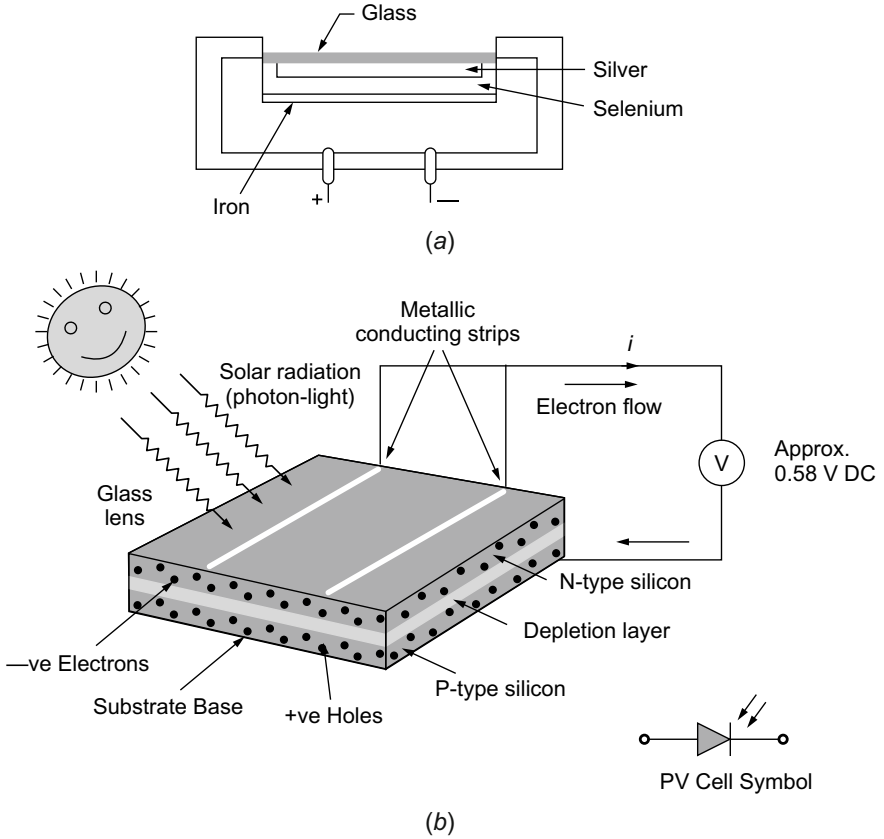
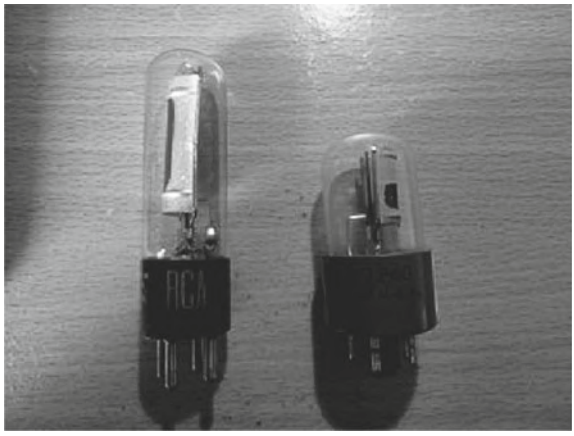


Fig. 4.6 **a** Barrier-layer photovoltaic cell, **b** Working of photovoltaic cell

Fig. 4.7 Photoemissive cell



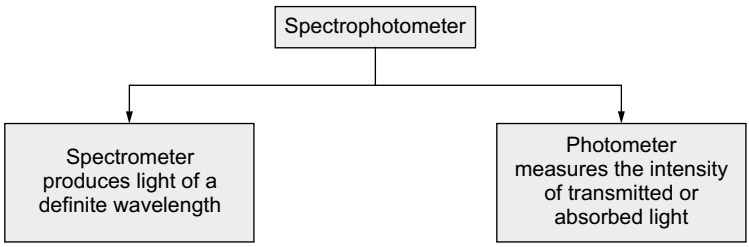
potential. The cathode surface emits electrons which are accelerated towards dynodes. The first dyanode D_1 has potential difference of 90 V then the second dyanode has risen in potential further 90 V above the cathode. This way by subsequent collections to Dyanode 'the number of electrons' get enhanced by 10^6–10^4 times after repetitions, and the resulting current converted to voltage can be easily measured. For each electrons emitted, several photons are created at the end, so that a powerful signal is created even for a small radiation, thus making it a highly sensitive and fast responding detector.

The only limitation of photomultiplier tubes is that they can measure low power radiation because light of high intensity can damage the photoemissive surface and thus they are housed in such cases which prevent them to be exposed to even broad day light, further, to eliminate thermal dark currents, cooling the detector enhances the performance of photomultiplier tubes (Fig. 4.8).

Readout device: The measure of performance from ultraviolet–visible photometry and its accuracy lies in what is achieved from the readout device. Generally, the signals produced by detectors require either analog meters, recorders, digital voltmeters, or displays of computer systems, sometimes low-frequency responses, amplification, and frequency modulations are desired which are later transformed into an alternating current (ac) frequency. This transformation reduces drifts and noise problems. After amplification by an ac amplifier the signals are converted back to dc (direct current) by a rectifier as they are required by readout devices. These days all reader devices are digitalized converting the analog signals to digital signals using a dc convertor.

4.3 Types of *UV*–Visible Spectrophotometers

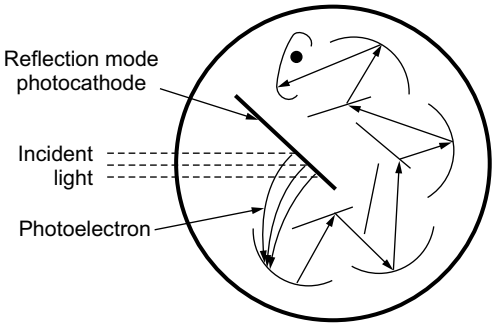
A spectrophotometer is a combination of two units:



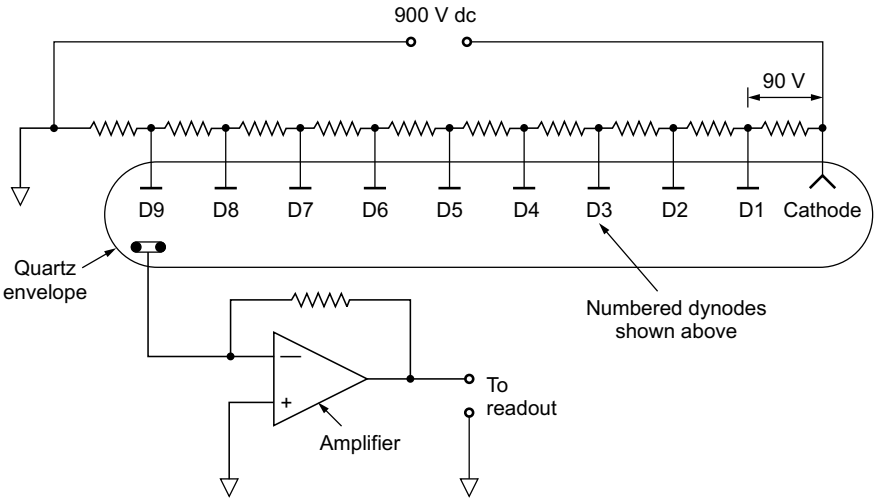
Several spectrophotometers designed for *uv* and visible regions are commercially available (Fig. 4.9).

Types of spectrophotometers: Depending upon the number of beam operations, three types of spectroscopic instruments are generally used.

(*a*) Photomultiplier tube (PMT)
(head-on type)



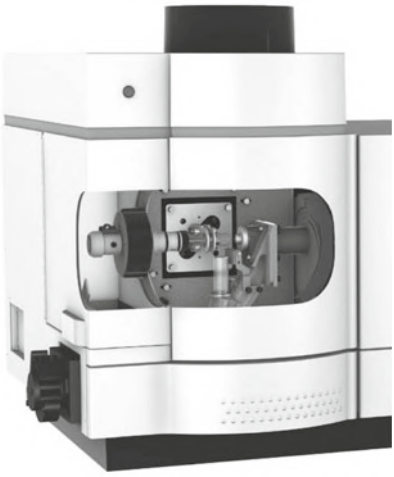
(*b*) Cross section of
photomultiplier tube (PMT)



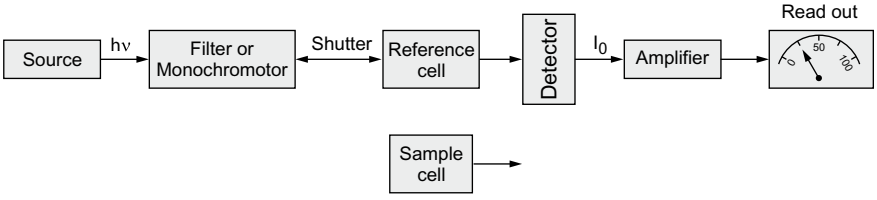
(*c*) Electrical circuit diagram of photomultiplier tube

Fig. 4.8 **a** Photomultiplier tube **b** Cross section of photomultiplier tube **c** Electrical circuit diagram of photomultiplier tube

Single-beam instruments, double-beam instruments, and multichannel instruments.



(a)



(b)

Fig. 4.9 **a** A commercial uv–visible spectrophotometer **b** Design of single-beam spectrophotometer

Single-beam spectrophotometer

The single-beam spectrophotometer as the name suggests is meant to measure a single sample at one time. As shown in Fig. 4.9, radiation from the filter or monochromator passes through either the reference cell or the sample cell before striking the photodetector.

Double-beam spectrophotometer

In double-beam spectrophotometers, the monochromatic beam is split by a beam splitter into two parallel beams one passing through the sample and other passing through the reference and both finally hitting either two different matching detectors or a single detector. The two outputs are amplified and their ratio is determined electronically or by a computer and displayed by the readout device (Fig. 4.10).

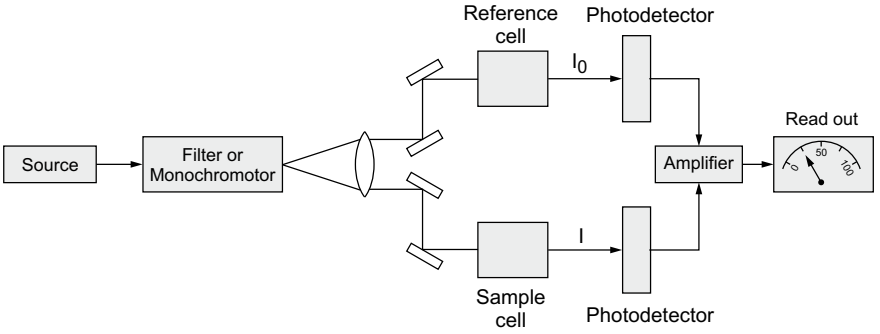


Fig. 4.10 A double-beam in-space instrument

In another type of double-beam monochromator, the beams are separated with time by a rotating sector mirror that alternates in such a way so that the beam is directed through reference cell at one time and at other time through sample cell.

The pulses of radiation are created which one by one pass through sample and reference cells and are reflected to the photodetector. This is a preferred arrangement because of lack of choosing similar detectors as in double beam in space (Fig. 4.11).

Advantages of double-beam instruments

(i) In a single run, both the reference and sample can be measured and an instant comparison can be made.

(ii) The ratio of the powers of the sample and reference beams is constantly obtained and errors due to difference in fluctuations and intensity of source are minimized.

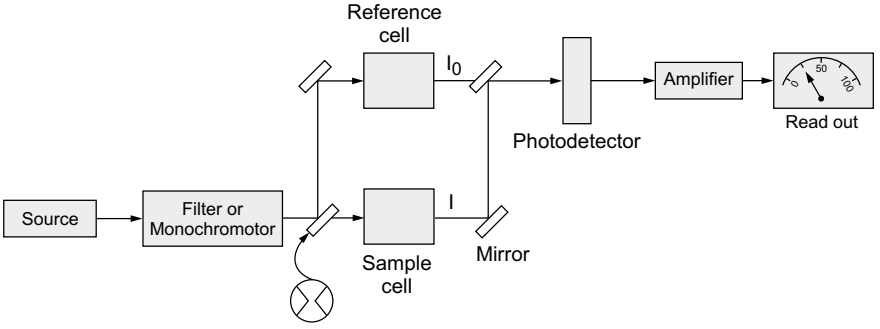


Fig. 4.11 A double beam in time instrument

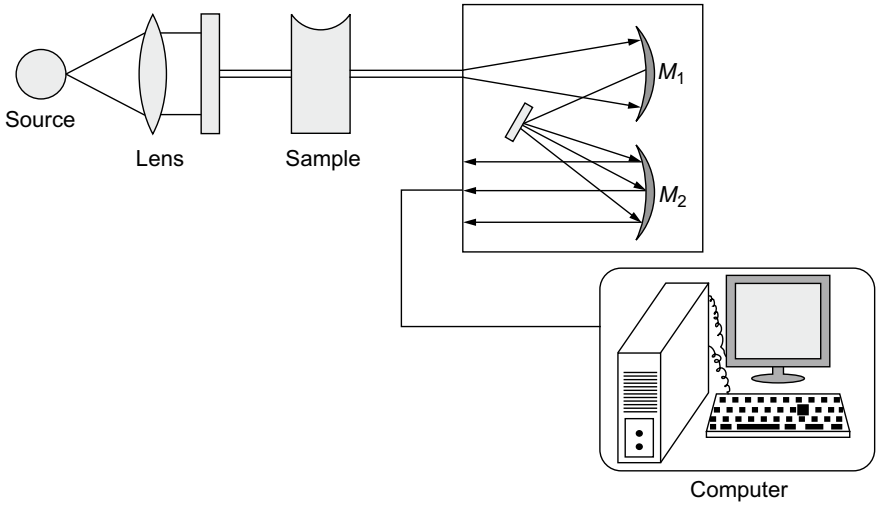


Fig. 4.12 Design of a multichannel instrument

(iii) It gives a fast scanning of both sample and reference together on the readout device.

Multichannel Instrument

They are generally similar to double beam in time spectrophotometers. In this assembly, the dispersive system is a grating spectrograph placed after the sample or reference cell. The dark current measurements are first taken and stored in computer memory, then the spectrum of source is obtained and saved after dark current subtraction, later the spectrum of the sample is obtained from which the dark current subtraction is done and the ratio of both the source and example gives absorbance. Charged coupled device, which behaves like photomultiplier tube is the basis of multichannel instrument and is capable of obtaining spectrum in a few milliseconds and is also capable of scanning intermediates of fast reactions (Fig. 4.12).

4.4 Choice of Solvents

Solvent selection is a very important part of ultraviolet spectroscopy. The essential characteristics desired in suitable solvents are, firstly, it should not absorb ultraviolet radiation in the same region as the sample, and secondly should not affect the fine structure of the absorption band in any way. Polarity of the solvent is also a great concern because hydrogen bonding or any other interaction forming solute–solvent complex formation can deviate the absorption band. A wide range of solvents are available for use in ultraviolet region. The table links some commonly used solvents and their transparency range.

Acetonitrile 200 nm	*n*-hexane 201 nm
Chloroform 240 nm	Methanol 205 nm
Cyclohexane 195 nm	Isooctane 195 nm
1,4-Dioxane 215 nm	Water 190 nm
95% Ethanol 205 nm	Trimethyl phosphate 210 nm

The most common ones are cyclohexane, 95% ethanol and 1,4 dioxane and when polar solvent is also desired, 95% ethanol is suitable. It is important to ensure that the solvents used are 'spectral grade' and free from contamination of benzene.

4.5 Concept of Chromophore

The factor leading to the absorption of radiation is governed by the arrangement of electrons in the absorbing molecule or ions. The absorbing groups in a molecule are called chromophores. A molecule containing a chromophore is named chromogen. Two types of chromophores are known in organic molecules.

(i) Chromophores which contain the π -electrons only and undergo $\pi \rightarrow \pi^*$ transition due to unsaturation of C = CandC ≡ C bonds.
(ii) Chromophores which contain π electrons and *n* (non-bonding) electrons which undergo $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transition attributed to the presence of > C ≡ O, −N ≡ N−, C ≡ N, $-NO_2$, −COOH, $-CONH_2$ groups.

Auxochrome: An auxochrome is that atom or group of atoms which do not give rise to its own absorption bands but when in conjugation with a chromophore, it changes both the wavelength and intensity of the absorption band characteristic of that chromophore. They also enhance the colour obtained by the chromophoric group in visible spectrometry. The auxochrome is able to influence the chromophore by extending its non-bonding or unshared electrons towards conjugation. For example, absorption maxima of benzene C = C chromophore $\lambda_{max} = 255$ nm.

Auxochrome benzene	$\in_{max} = 203$
Amino –NH_2	$\lambda_{max} = 280$ nm
Phenol,	$\in_{max} = 200$
– OH	$\lambda_{max} = 275$ nm
Chlorobenzene	$\in_{o\,max} = 200$
– Cl	$\lambda_{max} = 265$ nm
Bromobenzene	$\in_{o\,max} = 360$
– Br	$\in_{max} = 245$ nm
	$\lambda_{max} = 295$

(where λ_{max} = wavelength corresponding to maximum absorbance $\in_{max}$).

4.6 Shifting of Absorption Bands and Change in Intensity

The characteristics of absorption bands, for any chromophoric group, their wavelength and intensities are sensitive to solvent, pH and inductive effects, other than auxochrome influences. These charges affect the shifting of λ_{max} and absorbance.

Bathochromic shift: (Red Shift) is defined as increase in value of wavelength (λ_{max}) caused by either an auxochrome present or by change of solvent polarity.

Hypsochromic shift: (Blue Shift) is referred to the shift of wavelength to lower value due to removal of auxochrome or polarity change of the solvent.

Hyperchromic shift: indicates increase in intensity of absorption band thus increasing the $\in_{max}$ value generally caused by inclusion of an auxochrome (Fig. 4.13).

Hypochromic Shift: Groups which alter the geometry of a chromophore cause decrease in intensity of the absorption band $\in_{max}$ value. The various factors which influence the shifting in absorption bands and alterations in intensity can be summarized as follows:

(1) When π electrons get delocalized due to conjugation, the energy of $\pi \rightarrow \pi^*$ transition decreases and absorption maxima shifts to higher wavelength, For example, divinyl elthylene absorbs $\lambda_{max} = 256$ nm (conjugated double bonds) whereas diallyl (unconjugated) absorbs $\lambda_{max} = 180$ nm.
(2) When two similar chromophores are present in a molecule but they are far from conjugation then they absorb at same wavelength but double the intensity. For example, di allyl CH_2=CH–CH_2CH_2CH=CH_2.

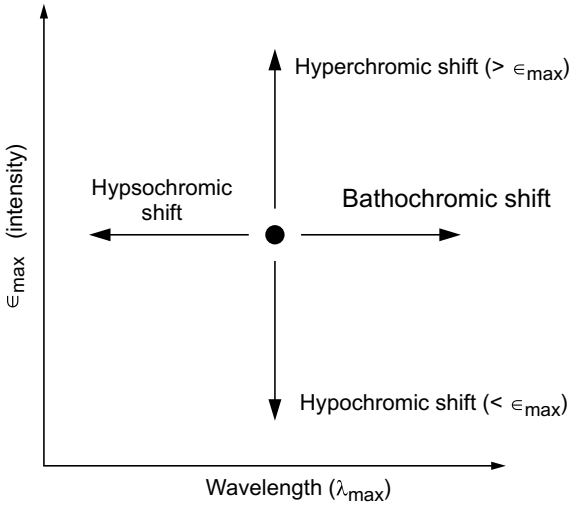


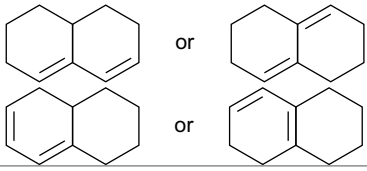
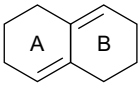
Fig. 4.13 Types of shifts of absorption bands

(3) In polar solvent red shift, *i.e.* increase in wavelength is observed in $\pi \rightarrow \pi^*$ transitions.
(4) Stearic factors which deviate the planarity of the π bonded molecules also influence the shifting of wavelength and change of the intensity, the *cis* isomer of a conjugated double bonded molecule absorbs at lower wavelength, for *example, cis* isomer of cinnamic acid absorbs at $\lambda_{max} = 168$ nm and trans at $\lambda_{max} = 272$ nm, as the *cis* isomer is more hindered than *trans.*
(5) Effect of solvent polarity is quite a pronounced effect and leads to substantial shifts in wavelength and intensity. Increase in polarity of the solvent stabilizes the non-bonding electron and destablizes the π^* which leads to a blue shift in $n \rightarrow \pi^*$ transition and a red shift in $\pi \rightarrow \pi^*$ transition, For example, for mesityl oxide (4 methyl–3 ene pent–2 one):

Solvent	λ_{max}	Transition
Hexane	230	$\pi \rightarrow \pi^*$
	329	$n \rightarrow \pi^*$
Water	243	$\pi \rightarrow \pi^*$
	303	$n \rightarrow \pi^*$

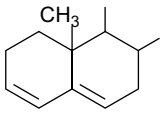
4.7 Application of *UV*–Visible Spectral Methods

UV–visible spectral methods are among the most versatile and frequently used methods for qualitative and quantitative determination of compounds containing absorbing groups. Since absorption of ultraviolet or visible radiation generally results from excitation of bonding electrons, the absorption of bands can be correlated to the bonds formed by the atoms and thus it could prove to be valuable for identifying functional group in a molecule. Structural analysis of organic molecules depends heavily on ultraviolet spectroscopy. For calculating λ_{max} values certain empirical rules known as Woodward–Fieser rules are as follows:

alicylicdienes C = = C—C = = C Heteroannular diene (2 double bonds in different rings) Homo diene (2 double bonds in same rings)	1,3 butadiene  	λ_{max} 217 nm 214 nm 253 nm
Exocyclic double bonds cause red shift of 5 nm	 An exocyclic conjugated double bond	

Empirical Rules for dienes

Parent	Homoannular	Heteroannular
Increments for	(*Cisoid*)	(*Transoid*)
Parent	λ = 253 nm	λ = 214 nm
Increments for double bond extending conjugation	30	30
Alkyl substituent or ring residue	5	5
Exocyclic double bond	5	5
Polar groupings	0	0
—$OCOCH_3$	6	6
—OR		
—Cl, –Br	5	5
—NR_2	60	60

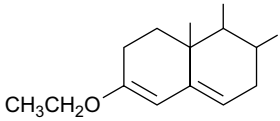


Transoid 214 nm

CH_3 3 × 5 = 15 nm

Exocyclic double bond = 5

Observed = 236 nm.



Transoid 214 nm

CH_3 3 × 5 = 15

Exocyclic = 5

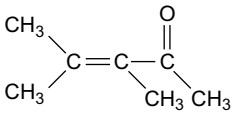
OR = 6

240 nm

Observed = 241 nm.

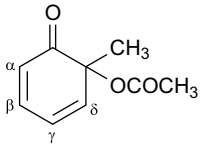
Rules for Enones: The conjugation of a double bond with a carbonyl group leads to intense absorption (ϵ = 8000 to 20,000) due to $\pi \rightarrow \pi^*$ transition of the carbonyl group.

The absorption is found between 220 and 250 nm in simple enones. The $\pi \rightarrow \pi^*$ transition is much less intense (ϵ = 50 to 100) and appeal at 310 to 330 nm and does not get much affected by structural modifications of the chromophore whereas $\pi \rightarrow \pi^*$ transitions get affected in a predictable way.



Acyclic enone =215 nm.

αCH_3	10
$\beta\ CH_3$	$2 \times 12 = 24$
	249 nm.
Observed	249 nm.

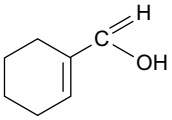


Cyclic enone = 215 nm.

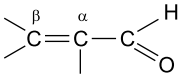
Conjugated double bond	= 39
Homocyclic diene	= 30
δ Ring residue	18
	302 nm.
Observed	300 nm.

α, β **Unsaturated aldehyde, acid, and esters**

Generally same rules as for enones are followed with absorption around 217 nm.



αβ dialkyl = 217 nm.
Double bond is in
6 memebered ring 217 nm.



Parent = 208nm.

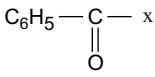
With ⟶ α or β alkyl groups 220 nm.
⟶ α, β or β, β alkyl groups 230 nm.
⟶ α, β alkyl groups 242 nm.

Aromatic Compounds: The absorptions that result from transitions within the benzene chromophore are quite complex as they are less intense at 230 nm and more intense at 202 nm. Substitution on the benzene ring can cause bathochromic and hyperchromic shifts.

Table 4.1 Table exhibiting red shift values for different auxochromes

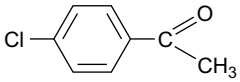
Increment in nm for substitution			
Auxochrome	Ortho	Meta	Para
alkyl	+ 3	+ 3	+ 10
OH,OR	+7	+7	+25
Cl	0	0	+10
Br	+2	+2	+15
NH_2	+13	+13	+58
$NHCOCH_3$	+20	+20	+45
NR_2	+20	+20	+85
– O–	+11	+20	+75

Rules for calculating λ_{max} of acylbenzene and its derivatives.
Scott derived a set of rules for calculating λ_{max} for the derivatives of acyl benzene.



(i) The basic value = 246 nm, if *x* is an alkyl group or alicyclic residue.
(ii) If *X* is halogen atom, the basic value becomes 250 nm.
(iii) The basic value is 230 nm, if *X* = OH or *X* = OR, further substitutions on ortho, para, and meta positions give red shifts (Table 4.1).

Example



Basic value = 246 nm
1 parachloro = +10 nm
λ_{max} = 256 nm

Basic value = 246 nm
1 parachloro = +10 nm
$\lambda_{max} = 256nm$

Absorption by Inorganic Species

Due to non-bonding electrons and their excitation, inorganic anions also exhibit ultraviolet absorption bonds.

Example:	NO_3^-	313 nm
	CO_3^{-2}	217 nm
	NO_2^-	360 nm and 280 nm
	N_3^-	230 nm

Complexes of transition metals absorb in the visible range and appear coloured. Their intensities depend on the nature of ligand and oxidation state of the metal. Ions of lanthanoids and actinoids also show absorption bands which are sharper than those of transition metals.

4.8 Molar Compositions

Applications of ultraviolet and visible spectroscopy to determine molar compositions are among the most widely used ones. The molar compositions of complexes or simple compounds can be determined by finding the molar ratio of two components of a complex, for example,

$$nM + pL \longrightarrow M_nLp$$

Where M = metal; L = Ligand

Spectrophotometric methods to determine molar compositions are of the following types:

(a) **Yoe's mole ratio**; (*b*) **Job's method of continuous variation**
(b) **Slope ratio method.**

(a) ***Yoe's mole ratio*:** This is a very convenient and popular method for measuring coloured complexes in solutions. For this method, a series of solutions are prepared containing a constant amount of the metal ion and varying the amounts of ligand or the vice versa, a plot of absorbance as a function of concentration ratios at a particular wavelength is drawn. The absorbance *versus* concentration ratio curve rises linearly from the origin when both reactants are colourless and breaks sharply to a horizontal straight line at molar ratio of the compounds in the complex. The intersection of the extrapolated linear segments determines the ratio of mole of ligand to mole of metal (Fig. 4.14)

However, a complex that undergoes appreciable dissociation in solution gives a continuous curve, which only becomes parallel to the mole ratio axis when a large excess of the variable component is added. It is often seen that such a curve breaks sharply at the correct molar ratio if the ionic strength of the solution is adjusted to a suitable value be the addition of an indifferent electrolyte. Thus, in such cases, it is possible to get the true composition of the complex (Fig. 4.15).

(b) **Job's method of continuous variation:** This is a widely used technique to elucidate the composition of a complex. Suppose, between species M and L the

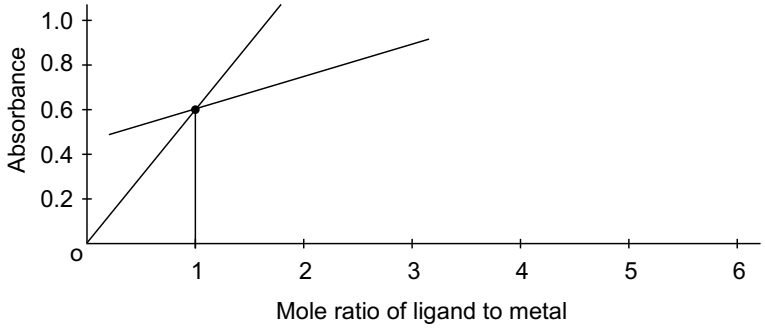


Fig. 4.14 Showing 1:1 complex (ML)

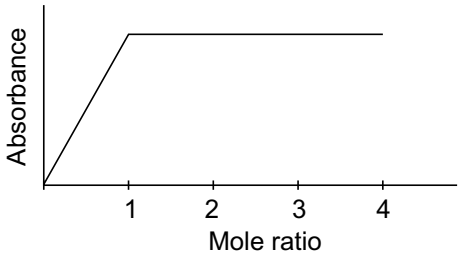


Fig. 4.15 Shows the result of the molar ratio method applied to the complex between biphenyl carbazone and mercuric ions

possible ratios are ML_1, ML_2, ML_3 and if one complex ML_2 predominates, Job's method of continuous variation enables us to identify the stoichiometry of the predominant complex.

The conventional procedure involves mixing of *M* and *L* and dilute to constant volume so that the total concentration $[M] + [L]$ is constant. As shown in the following table 10 mM solution of *M* and *L* could be mixed to give various *L*:*M* ratios, keeping the total concentration constant. The absorbance of each solution is measured for λ_{max} for the complex and plotted as absorbance *versus* mole fraction of *L*, graphically. The corrected absorbance values were calculated as corrected absorbance = measured absorbance:

$$A = \in_M bM_T + \in_L bL_T$$

where $\in_M$ and $\in_L$ are molar absorptivities of pure *M* and pure *L*,

b = path length, and M_T and L_T are the total concentrations of *M* and *L* in the solution (Table 4.2).

Maximum absorbance is reached at the composition of the predominant complex indicating by the mole fraction.

Maximum absorbance occurs when mole fraction of $L = \frac{n}{n+1}$.

Table 4.2 Mole ratio table for jobs method

mL of 10 mM of M	ml of 10 m M of L	Mole ratio L:M	Mole fraction of L
1.00	9.00	9:1	0.9
2.00	8.00	4:1	0.8
3.00	7.00	7:3	0.7
4.00	6.00	3:2	0.6
5.00	5.00	5:5	0.5
6.00	4.00	2:3	0.4
7.00	3.00	3:7	0.3
8.00	2.00	1:4	0.2
9.00	1.00	1:9	0.1

If the predominant complex is ML_3 then maximum absorbance will occur at mole fraction ~ 0.75. (Fig. 4.16).

(c) ***Slope ratio method*:** This method is applicable only when a single complex is formed by the reaction of metal *M* and *l*igand *L*.

$$nM + pL \rightleftharpoons M_nLp$$

If two sets of solutions are prepared; first set with fixed concentration of *L* but much greater than the variable concentration of the metal ion. The concentration of the complex formed will be proportional to C_M.

$C_M/n = M_LL_P$

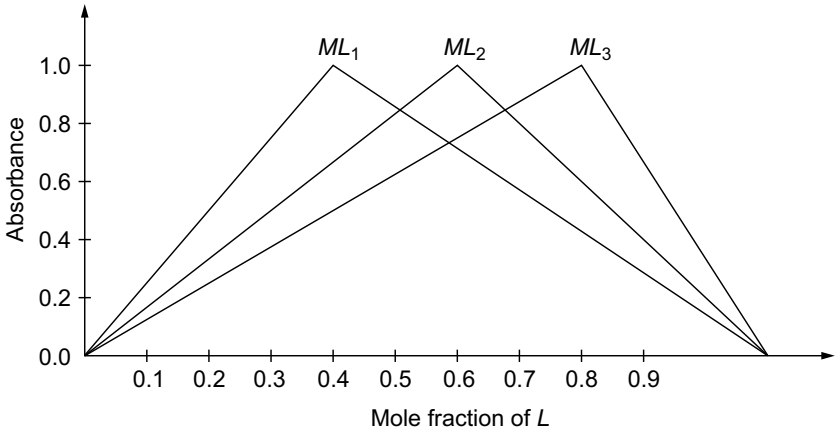


Fig. 4.16 Showing absorbance versus mole fraction

If absorptions due to all other species are ignored then absorbance of M_nLp

$$\mathrm{A} = \in \cdot \frac{C_m}{n}$$

where $\in$ = molar extinction coefficient and cell length = 1 cm.

The plot of absorbance against C_M will be a straight line equal to $\frac{\in}{n}$.

In another set, solution of fixed concentration of metal ion with its large concentration and variable concentration of ligand is prepared.

Assuming that all the ligand is complexed and the concentration of the complex formed is taken as

$$M_L L_P = \frac{C_L}{P}$$

and absorbance $A = \in \frac{C_L}{P}$

Absorbance *versus* C_L will give a straight line, and the slope $\frac{\in}{P}$.

Taking ratio of the slopes, one can obtain P: n which is expected to correspond to M: L ratio of the complex.

However, this method is only useful if formation of only one type of complex between M and L throughout all concentrations is ensured.

4.9 Quantitative Analysis

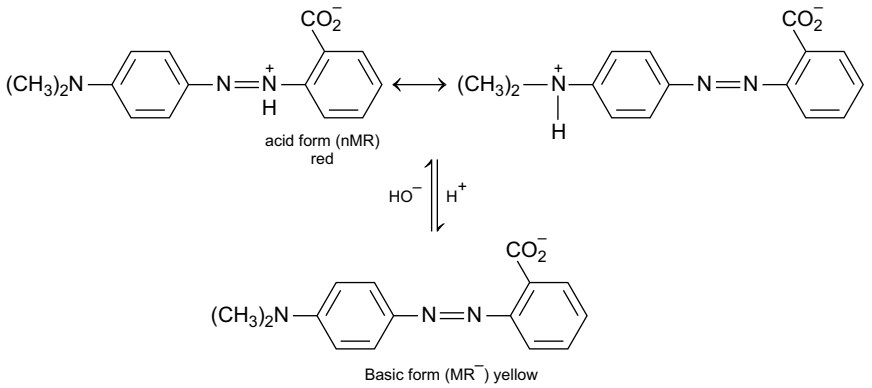
Various types of quantitative measurements are also possible using spectroscopic methods. These include the determination of concentration of a substances, pK values of an indicator, instability constants of complexes, and molecular weight determinations.

Determinations of concentration of a solution: For determination of the concentration of a substance, wavelength of its maximum absorption is selected. The absorbance of many standard solutions is measured and then compared with the absorbance of unknown solution. The absorbance recorded for different known concentrations of solutions is plotted on an absorbance versus concentration plot and referred to as a calibration curve for unknown sample, this way it becomes easy to identify the concentration of the unknown solution.

Determination of pK_{In} value of an Indicator: Determinations of the dissociation of acid–base indicator are very well performed using spectrophotometric studies. For example, while acid dissociation constant of methyl red is found, the equilibrium is considered as

$$HMR \overset{K_a}{\rightleftharpoons} H^+ + MR^-$$

$$pK_a = -\log K_a$$



$$HMR \rightleftharpoons H^+ + MR^-$$

$$pK_a = -\log K_a$$

$$K_a = \frac{[H^+][MR^-]}{[HMR]}$$

$$pK_a = pH - \log \frac{[MR^-]}{[HMR]}$$

Using the above equation pK_a is determined spectrophotometrically because both *HMR* and MR^- exhibit strong absorption peaks in the visible portion of the spectrum. The maximum absorptions are shown at different wavelengths $\lambda_{A(HMR)}$ and $\lambda_{B(M\overline{R})}$ when a graph is plotted (Fig. 4.17).

In accordance with Beer's law for both *HMR* and MR^- at wavelengths λ_A and λ_B, the relative amounts of *HMR* and MR^- present in a solution are determined as a function of *pH* using the following equations:

$$A_A = \epsilon_{A(HMR)}\,[HMR] + \epsilon_{A(M\overline{R})}\,\left[MR^-\right]$$

$$A_B = \epsilon_{B(HMR)}\,[HMR] + \epsilon_{B(M\overline{R})}\,\left[MR^-\right]$$

where ϵ_{AHMR}, $\epsilon_{A(MR^-)}$, $\epsilon_{\mathrm{B(HMR)}}$ and $\epsilon_{B(MR^-)}$ are molar absorptivities of HMR and MR^- at wavelengths λ_A and $\lambda_{B,}$ respectively, in a cell of 1 cm path lengths. A_A and A_B are absorbance at wavelengths λ_A and $\lambda_{B,}$ respectively. Solving the above equation one can calculate pK_a values if *pH* values are also known.

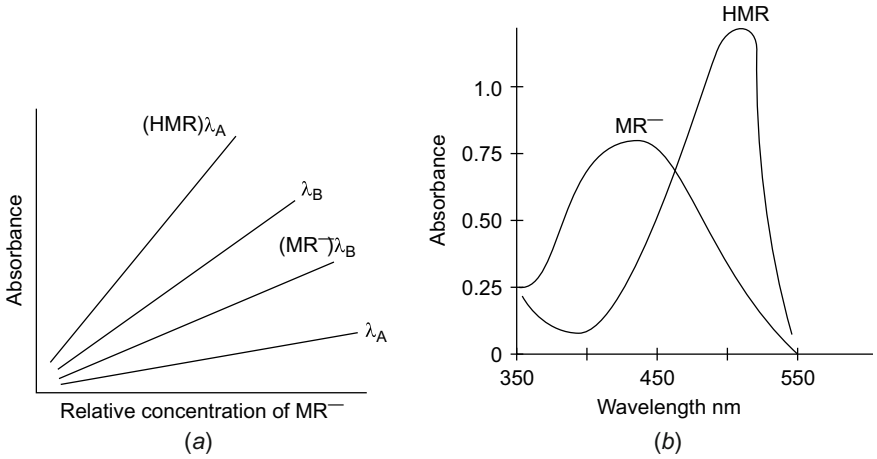


Fig. 4.17 **a** Maximum absorption at different wavelengths with respect to concentration **b** Maximum absorbance with respect to wavelength

Therefore $pK_{In} = pH - \log \frac{[MR^-]}{[HMR]}$.
where In = Indicator.

Determination of instability constants: Determination of degree of dissociation by determining equilibrium concentrations can faster measure instability constants for a complex.

$$K = \frac{c\alpha^2}{(c - \alpha)}$$

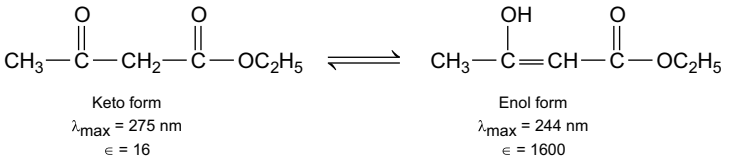
Molecular weight determinations: Since Beer's law is useful in finding concentrations of solutions, it can also lead to molecular weight determinations. Molecular weights of compounds can be measured spectrophotometrically by preparing suitable derivatives of the compounds which is transparent towards *uv*–visible spectra. For example, molecular weight of an amine can be found by converting it into amine picrate whose optical density can be measured at $\lambda_{max} = 380$ nm and then C is calculated as

$$C = \frac{\log \frac{I_o}{I_t}}{\in_{\max} \times l}$$

measured C can lead to molecular weight determination.

4.10 In Tautomeric Equilibria

The percentage of tautomeric isomers in a tautomeric equilibrium can also be measured by *UV* spectroscopy. For example, ethylacetoacetate has both keto and enol forms responding to *uv* range.



Since keto form has weak $n \rightarrow \pi^*$ band whereas enol form has intense band λ_{max} = 244 nm,. whose intensity can give the measure of the proportion of enol form present in the equilibria.

Similar to tautomeric determinations, the octahedral-square planar equilibria, square-planar-tetrahedral equilibria, and *cis–trans* equilibria can also be determined by *UV* spectroscopy.

Solved Problems

1. *The* molar absorptivity of a phenolic compound at a wavelength of 356 nm in 0.1 M HCl is 400, in 0.2 M NaOH is 17, 100 and pH 9.50 (buffer solution) is 9800. Calculate the pK of phenolic compound.

   Solution: The phenolic equilibrium can be represented as

$$RH \rightleftharpoons R^- + H^+$$

   If x is concentration of RH the 1 – x is the concentration of R^- at equilibrium.
   molar absorptivity $\in_{RH} = 400$ and $\in_{R^-} = 17{,}100$.
   At pH 9.5 the molar absorptivity is 9800

$$\therefore \quad 9800 = 400(RH) + 17100\left(R^-\right)$$
$$9800 = 400x + 17100(1 - x)$$
$$x = \frac{7300}{16700} \text{and} 1 - x = 1 - \frac{7300}{16700}$$

   Applying,

$$pK = pH - \log \frac{R^-}{RH}$$
$$= 9.5 - \log \frac{1 - x}{x}$$
$$= 9.5 - \log \frac{9400}{7300}$$

$$= 9.5 - 0.11 = 9.39$$

2. Find the formula for the complex between Fe^{+2} and O-phenanthroline from the following data. The total concentration of the metal and ligand was kept constant at 3.15×10^{-4} M and 510 nm wavelength.

x_L	Absorbance
0.0	0.000
0.1	0.116
0.2	0.231
0.3	0.347
0.4	0.462
0.5	0.578
0.6	0.693
0.7	0.809
0.9	0.347
1.0	0.000

Solution: The plot of absorbance versus mole fraction of ligand was extrapolated to get maximum absorbance.

The intersection of extrapolated lines corresponds to a mole fraction of 0.75 for the ligand.

$$Y = \frac{x_L}{1 - x_I} = \frac{0.75}{1 - 0.75} = 3 \text{ i.e. } ML_3$$

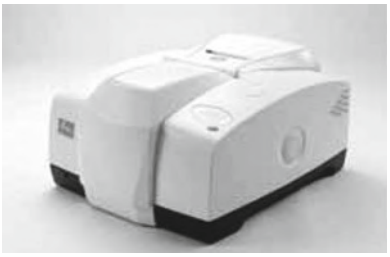
So, the formula of the complex = [Fe(O-phenanthroline)$_3$].

Unsolved Problems

1. State the differences between single- and double-beam spectrophotometers and explain how each measures the transmittance of a sample.
2. What are the advantages and disadvantages of decreasing monochromator slit width?
3. Describe a photomultiplier tube and state why it is a sensitive photodetector?
4. Write the mechanism involved in electronic transitions and state how spectral bands are displayed.
5. Distinguish between hyperchromic and hypochromic shift.
6. Explain the nature of absorption bands shown by the following compounds:(i) benzene (ii) acetophenone (iii) alkenes
7. How are samples quantitatively analysed by spectrophotometric method?

8. Write a short note on the solvents used in UV–visible spectroscopy and the basis of their choice.
9. Comment upon the resolving power of a monochromator.
10. How many types of chromophoric groups are known in organic molecules?

Chapter 5
Infrared Spectroscopy



5.1 Introduction

Infrared (IR) absorption, emission, and reflection for molecular species originate from transitions of molecules from one vibrational or rotational energy state to another. The atoms in a molecule do not remain in fixed relative positions but vibrate about some mean positions, along with rotations thus giving various energy levels. When a molecule absorbs infrared radiations, transitions between vibrational and rotational energy levels take place leading to vibrational and rotational bands. The vibrational and rotational energy levels arise due to change in dipole moment. *Thus, for absorption in the IR region, a molecule must undergo change in dipole moment during vibrations or rotations. The alternating electric field of the radiation interacts with the molecule and causes changes in the amplitude of one of its motion.* Vibrations cause regular fluctuation in dipole moment and its electric field interacts with the electric field associated with the radiation. Similarly, rotation of asymmetric molecules around their centre of mass results in periodic dipole moment fluctuations which further interact with external radiation field.

P. Malhotra, *Analytical Chemistry*,
https://doi.org/10.1007/978-3-031-26757-4_5

Table 5.1 Range of wave number

Region	Wave number $\left(v^{-1}cm^{-1}\right)$	Wavelength (λ) μm
Near IR	12,800−4000	0.78−2.5
Middle IR	4000−200	2.5−50
Far IR	200−10	50−1000
Most used	4000−670	2.5−1.5

Homonuclear species like H_2, N_2, O_2 or Cl_2 do not absorb IR radiations because no net change in their dipole moment occurs during any vibration or rotation.

IR region of electromagnetic radiation lies in between visible and microwave regions covering a wide range of 12,500−100 cm^{-1} of wave number. This range has been further divided as given in Table 5.1.

The near-IR region is used for quantitative determination of species, whereas the middle IR region applies to qualitative measurements. Far-IR region is particularly useful for inorganic solids.

5.2 Molecular Vibrations, Vibrational Modes, and Vibrational Frequency

Molecular Vibrations: IR radiations are energetic enough only to cause molecular movements and to absorb IR radiation a molecule must undergo a net change in dipole moment as it vibrates or rotates. Molecular vibrations which mean fluctuation of atoms from its relative positions in a molecule and rotations, in general, can be classified as follows Fig 5.1.

More energy is required for stretching than for bending, therefore stretching frequencies are higher than bending frequencies. Also symmetric stretchings are

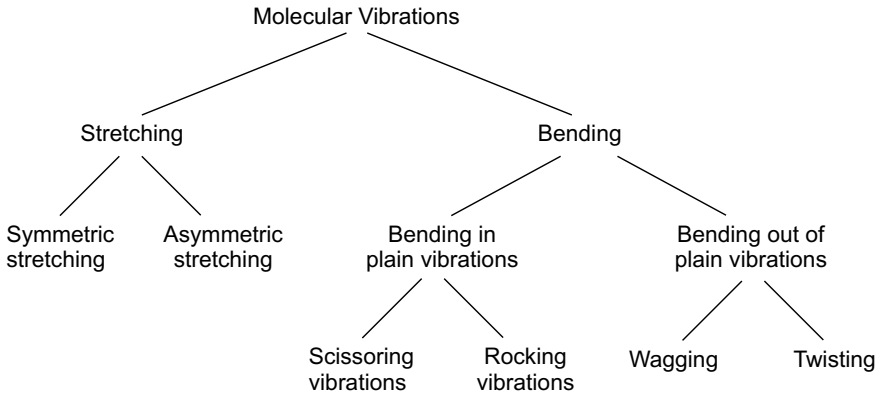


Fig. 5.1 Types of molecular vibrations

easier than asymmetric stretching and so it has lower frequency than for asymmetric stretching. Bending vibrations have lower force constants and are highly sensitive to the surroundings.

Vibrational energies are all quantized and the energy differences between quantum states correspond to the mid-IR region. The transitions in vibrational energy levels can be brought about by absorption of corresponding energy of radiations which matches the ΔE, difference in energy of vibrational quantum states, accompanied by changes in dipole moment.

Vibrational Modes: The number of possible vibrations in a poly-atomic molecule can be determined only by knowing its degree of freedom. Each atom in a molecule has three degrees of freedom in *x*-, *y*-, and *z*-directions and if there are *N* atoms in a molecule there are total of 3*N* degrees of freedom. For non-linear molecules, there are six degrees which are rotational and translational whereas there are five such degrees for linear molecules. Therefore, for non-linear molecules 3N-6 numbers of possible vibrations and for linear molecules 3N–5 possible vibrations are present Fig. 5.2.

Vibrational Frequency: The model of a diatomic molecule *A B* showing vibrations may be considered as simple harmonic motion whose frequency is measured by Hooke's law.

$$\text{Frequency} \qquad \nu_m = \frac{1}{2\pi}\sqrt{\frac{K}{\mu}}\text{Hz}$$

where K = force constant of the bond,
μ = reduced mass,
$\mu = \frac{m_A m_B}{m_A + m_B}$

$$\text{wave number} \qquad \bar{\nu} = \frac{1}{2\pi C}\sqrt{\frac{K}{\mu}}$$

$$\text{C} = \text{Velocity of light}$$

Vibrational energies E_v like all other molecular energies are quantized and the possible vibrational energy levels for any particular system may be calculated from the Schrodinger equation. For the simple hormonic oscillator this is given as.

$$E_\nu = \left(\nu + \frac{1}{2}\right)h\nu_m$$

where ν = vibrational quantum number,

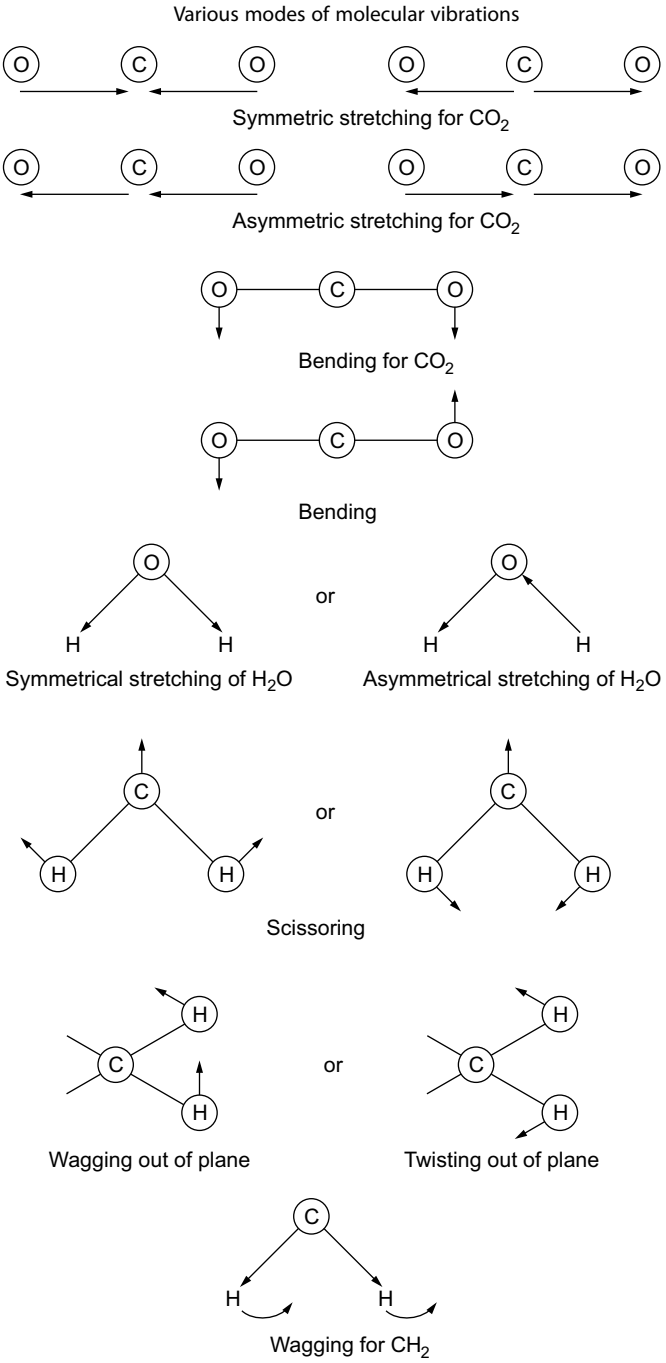


Fig. 5.2 Various modes of molecular vibrations

$v = 0, 1, 2, \ldots..$

Absorptions occur when the energy difference corresponds to the wave number of spectral lines absorbed which lies in IR region of electromagnetic radiation. Vibrational frequency according to.

$$\nu_m = \frac{1}{2\pi}\sqrt{\frac{K}{\mu}}$$

depends on force constant K which is a measure of bond strength, and is inversely proportional to the reduceSd mass, so increase in reduced mass reduces vibrational frequency. Lighter atoms like C, N, and O vibrate at higher frequency due to their low reduced mass. The force constants for triple bond, double bond, and single bond are in order $C \equiv C > C = C > C - C$ and so are the frequencies $2100 > 1600 > 1200\ \text{cm}^{-1}$ respectively.

Example: Calculate the stretching frequency of C–H bond ($K = 5 \times 10^5$ dyne cm^{-1}).

reduced mass

$$\mu_{C-H} = \frac{m_C \times m_H}{m_C + m_H}$$

$$= \frac{12 \times 1}{13} \times \frac{1}{6.023 \times 10^{23}}$$

$$= 1.532 \times 10^{-24}$$

$$\overline{\nu} = \frac{1}{2\pi C}\sqrt{\frac{K}{\mu}} = 1.532 \times 10^{-24}$$

$$= \frac{1}{2\pi \times 3 \times 10^{10}}\sqrt{\frac{5 \times 10^5}{1.532 \times 10^{-24}}}$$

$$= 3030\ \text{cm}^{-1}$$

5.3 Theory of IR Absorption Spectroscopy

For each normal mode of vibration, there is a potential energy relationship, and the differences between the energy levels of a given vibration are equal, thus leading to a single absorption peak for each vibration accompanied by a change in dipole moment Fig 5.3.

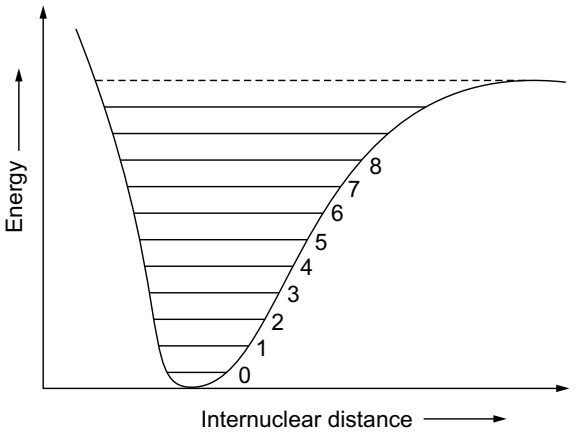


Fig. 5.3 Vibrational energy levels

Vibrational energy levels are equally spaced as expected unless these levels converge due to deviation from harmonic oscillation which is normally expected at higher vibrational quantum numbers, then the equation.

$$E_{\mathrm{v}} = \left(\mathrm{v} + \frac{1}{2}\right) h\nu_m \qquad (\text{where } \mathrm{v} = 0, 1, 2, \ldots)$$

also gets deviated.

The selection rules for IR absorption are mainly dependent on the oscillating dipole moment which should be non-zero for infrared activity and more polar a bond, more intense will be its IR spectral band. During absorption-only transitions for which $\Delta v = \pm 1$ can occur, where Δv implies the change in vibrational quantum number between two vibrational energy levels. The transitions from $v0$ to $v = 1$ which means at room temperature most molecules are at $v = 0$ and such a transition gives rise to fundamental frequency. The transition $0 \rightarrow 2$ has poor intensity and is known as first overtone whereas $0 \rightarrow 3$ is second overtone of negligible intensity and transition from $v1$ to higher energy levels are observed only at high sample temperature.

Absorption in the IR regions is often influenced by vibrational couplings which occur due to common bonds between vibrating groups or common atoms to the two vibrations. Sometimes coupling between stretching and bending may also take place when the angle change is also involved in stretching. As a result of vibrational coupling, the position of an absorption band corresponding to a given organic functional group cannot be specified exactly. For example, the C–O stretching frequencies in methanol and 2 butanol are:

	C—O Stretching
CH_3OH	1034 cm^{-1}
C_2H_5OH	1053 cm^{-1}
$CH_3—\underset{\displaystyle OH}{\underset{\vert}{CH}}—CH_2—CH_3$	1105 cm^{-1}

These are due to coupling of adjacent C–C and C–H vibrations with C–O.

5.4 Instrumentation

The IR absorption spectra are recorded by IR spectrometers which are made up of the same basic components as those in the *uv*–visible spectroscopy. The components of IR spectrometers are as follows:

(a) Radiation Source	(b) Sampling Area
(c) Monochromator	(d) Detector
(e) Amplifier	(f) Recorder

These components are arranged according to the following scheme Fig. 5.4.

(a) **The IR Radiation Sources**: IR sources consist of an inert solid that is heated electrically to a temperature between 1500 to 2200 K producing continuum black-body radiation with maximum intensity ranging between 5000 cm^{-1} and 5900 cm^{-1}. The commonly used light sources for the absorption of infrared radiation in IR spectrometers are

(i) **Incandescent Wire Source**: Although normal incandescent lamps with ordinary tungsten filament are convenient source for the near-IR region of 4000 to 12,800 cm^{-1}, but glass or quartz strongly absorbs in the far-infrared region. So to overcome this difficulty an incandescent wire source in which a rhodium wire heater is sealed in a ceramic cylinder serves as a good source. A lightly wound spiral of nichrome wire heated to 1100 K by electric current also acts

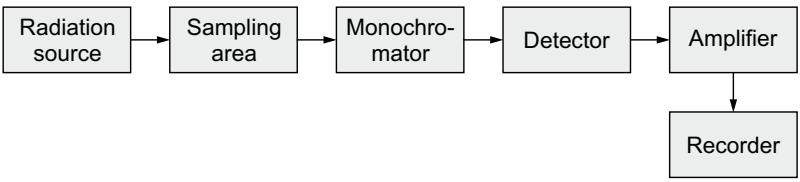


Fig. 5.4 Scheme of IR instruments

as an incandescent source which is easy to maintain and does not require any cooling.

(ii) **The Nernst Glower**: Nernst glower lamp which is most widely used IR source consists of a hollow rod which is approx. 2 mm in diameter and 30 mm in length made of rare earth oxides like yttrium, thorium, and zirconium. To make this rod conducting it is heated to 1500–2000 °C as it gives radiation up to 7000 cm^{-1}which is steady and intense for long duration of spectral output, however the only disadvantage of Nernst Glower is its frequent mechanical breakdown.

(iii) **Globar Source**: A Globar consists of a cylindrical rod of sintered silicon carbide being 5 cm long and 5 mm in diameter which emits in IR region of ~ 5000 cm^{-1}after heating between 1300 and 1700 °C. Although it produces less intensity than Nernst glower but gives more satisfactory spectral output and its temperature control is quite conveniently handled with a self-starting advantage.

(iv) **Mercury Arc**: It is a device in which high-pressure mercury arc is used, created by mercury vapours in a quartz tube. Flow of electricity within mercury vapours produces internal plasma sources from which continuous infrared radiation is obtained without any thermal treatment.

(v) **The Carbon Dioxide Laser Source**: Laser (light amplification by stimulated emission of radiation) has wide applications as a source of radiation in spectroscopic techniques because of their ability to produce narrow, extremely intense beams of radiation and their light amplifying characteristics. A carbon dioxide laser is used as an IR source which produces a band of radiation in the range of 900 to 1100 cm^{-1} consisting ~ 100 closely spaced discrete lines.

(b) **Sample Cells and Sample Handling**: In IR spectroscopy, sample handling is the most difficult part because no good solvents are transparent throughout the region of interest and in all three types of samples solid, liquid, and gases and may be used successfully for characterization by this method. Although solid, liquid, and gaseous samples need to be handled differently but the sample containers should be transparent to IR radiation which implies to NaCl or KBr like salts only. The common techniques for sampling in IR may be discussed as follows.

Gases: Samples which are in the gaseous state or are volatile are made to expand into a cylindrical cell having NaCl walls under vacuum. The cell should contain reflecting internal surfaces so that number of reflections increase path length.

Liquid samples or Solutions: Which contain water or alcohol are difficult to use as they attack alkali metal halides used as cell windows hence water-insoluble window materials, such as barium fluoride or calcium fluoride, are used. Even silver nitrate can be used but it is expensive. However in absence of a suitable solvent, pure liquid drop is squeezed between two plates of NaCl and then placed in the path of the beam. Increase in sensitivity can be obtained by increasing path length. Provisions for variable path lengths can produce solvent compensation and differential analysis.

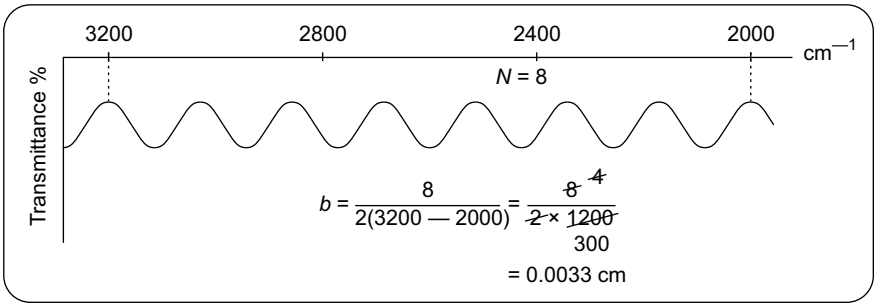


Fig. 5.5 Transmittance versus. wavelength graph

Infrared solution cells are constructed with windows sealed and separated by thin gaskets of teflon, copper, or lead which are surfaced by mercury. The complete set is mounted in a stainless steel holder. Flow-through cells are useful for continuous analysis of liquids and accuracy of measurement is obtained by repetitive sampling applications.

The thickness b of very narrow IR cells can be determined by interference fringe method. The sample thickness is selected to obtain a transmittance range of 15–70%. For most liquids a thin layer of 0.01–0.05 mm in thickness serves the purpose Fig 5.5.

$$\lambda = \frac{2b}{N}$$

$$\Delta N = \frac{2d}{\lambda_1} - \frac{2d}{\lambda_2} = \frac{2b}{\overline{\nu}_{m1}} - \frac{2b}{\overline{\nu}_{m2}}$$

$$b = \frac{\Delta N}{2(\overline{\nu}_{m1} - \overline{\nu}_{m2})}$$

where N is the number of fringes between wavelengths λ_1 and λ_2 and v_{m1} and v_{m2} are respective wave numbers.

As in Fig. 5.5 transmittance is plotted against wavenumber. The reflected radiation interferes with the transmitted beam to produce the interference maxima and minima which are found in this case at N = 8.

Solids: Solid sampling is an important task while making IR measurements because to find a solvent whose peaks do not overlap is very difficult. Most of the organic compounds exhibit numerous absorption bonds throughout the mid-IR region. To overcome various hurdles following methods of sampling of solids for infrared spectroscopy are prevalent.

Solid Films: For amorphous solids, the sample is deposited on the surface of a KBr or NaCl cell by evaporation of the solid solution. Polymers and resins like amorphous substances are dissolved in reasonable volatile solvent and dropped on an NaCl plate

whereas the solvent is evaporated by soft heating, a thin homogenous film is deposited on the plate which can be mounted and measured through IR spectrometers.

Mulls: Mull technique is followed when a finely ground sample is mixed with Nujol (mulling agent) resulting in a paste. This paste is then transferred to the mull plates and the plates are pressed together to adjust the thickness of the sample so that 60% to 80% adsorption takes place to give strongest bands. Because the particles have been powdered multiple reflections and refractions are reduced. Mull technique of sampling is quite useful for qualitative analysis by IR spectroscopy.

Pellets: The pellet technique involves the grinding and mixing of the particles in a vibrating ball mill with potassium bromide. This is then pressed against a die at high pressure to produce a transparent disc which appears like KBr wafers. An advantage of using KBr as a mulling agent in place of Nujol is that it does not produce bands in IR region like Nujol. Other benefits are that KBr pellets can be stored for longer duration and sample concentrations can be suitably adjusted to give fine spectrum. Quantitative analysis can be accurately done while sampling the solids by pellet technique.

(c) **Monochromators**: The monochromators, which are placed next to the sample in IR spectrometry are similar to the ones used in *uv*–visible spectrometry. Their main objective is to produce light of single wavelength or desired frequency either using the prism monochromators or grating monochromators which have been discussed in Chap. 4 (Sect. 4.2).
(d) **Detectors**: Detection of signals obtained by IR spectroscopy are best done by using (a) thermal detectors and (b) photon detectors.

Thermal detectors, which can be used over a wide range of wavelengths, possess temperature-sensitive elements whose heating rate and cooling rate should be very rapid. **Photon detectors** use the quantum effects of the infrared radiation to change the electrical properties of a semiconductor.

The various types of detectors used in IR spectroscopy are.

(i) **Bolometers** : Also known as thermistors, change their resistance when heated. Steps of metals like platinum or nickel or any semiconductor (germanium) are used as they change their resistance as a function of temperature. A bolometer works on the principle of Wheatstone bridge. On one of the balancing arm of the bridge, when IR radiation falls on the bolometer, the change in resistance causes change in current flowing for galvanometer G and the amount of current flowing through G means the intensity of radiation Fig 5.6.

(ii) **Thermocouples**: It consists of a couple of two metal pieces which are fused and a voltage develops between two junctions, which changes as the temperature changes. Very often thin wires of bismuth and antimony are either welded together or evaporated onto a nonconducting support whose absorption of heat is enhanced by blacking the surface and reduce loss of energy by casing it in steel. In IR spectroscopy, one such junction (cold junction) is kept at constant

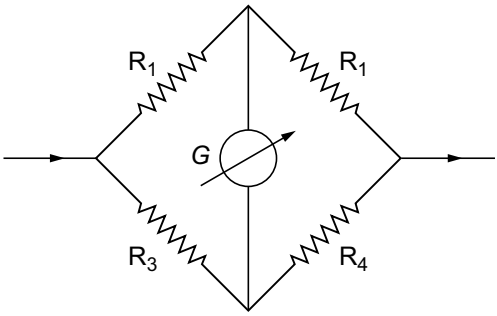


Fig. 5.6 Wheatstone bridge: Arrangement found in bolometers

temperature and the other welded junction increases the temperature of the junction by absorbing IR radiation. It is the temperature difference between the two junctions which generate potential difference and thus measures the IR radiation on the hot junction Fig 5.7.

Golay cells

The Golay pneumatic detector works on the principle of expansion of gases and is generally used in commercial spectrophotometer. It consists of a small metal cylinder closed by a blackened metal plate at one end and thin silver plate at the other end. It is filled by xenon gas through which the IR radiation absorbed by black plate is transmitted to the thin silver plate (diaphragm) leading to its expansion. Light following from a lamp on this diaphragm is reflected to a photocell. Movement of the diaphragm due to expansion changes the output of the cell. Golay detector is as sensitive as thermocouple detector in the mid-infrared region.

Pyroelectric Detectors

Pyroelectric detectors are an important class of infrared transducers constructed from single crystalline wafers of pyroelectric materials having special thermal and electrical properties. Triglycine sulphate $(NH_2CH_2COOH)_3 \cdot H_2SO_4$ is an important pyroelectric material used in the construction of infrared transducers.

Signal Processors and Readouts

The detection of signals from IR detection need to be first, amplified and then displayed on the readout device. This function is successfully performed by several types of readout devices like digital meters, recorders, LCD panels, cathode ray tubes, and computer displays.

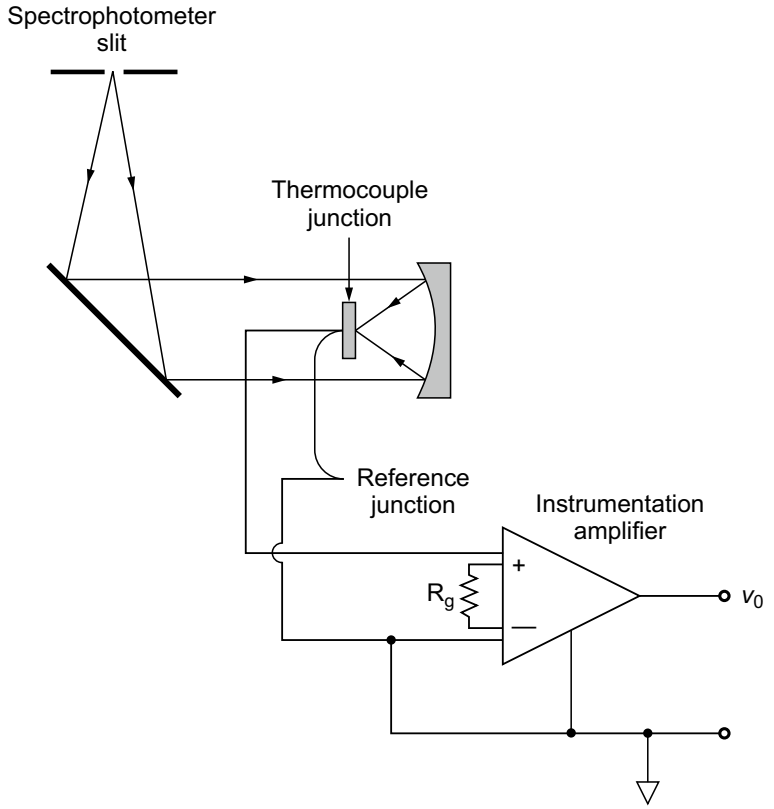


Fig. 5.7 Design of a thermocouple

5.5 Comparison of IR and *UV*–Visible Spectrophotometers

IR Spectrophotometer	*UV*–Visible Spectrophotometer
Source In IR instruments a Nernst or Glowers lamp is used as light source	In *UV*–visible models a deuterium tungsten lamp is used as a radiation source
Monochromator An IR monochromator gratings or a prism is made of alkali halides	In *uv*–visible monochromator gratings or prisms are made of corex glass or quartz
The monochromator is placed after the sample and before the detector to avoid stray light	The monochromator is placed before the sample
Detector The detector for IR radiation is a thermocouple or a bolometer	Photomultiplier tubes are used as detectors in *uv*–visible spectrophotometers

(continued)

(continued)

IR Spectrophotometer	*UV*–Visible Spectrophotometer
Recording Percentage transmittance is measured against wave number	Absorbance is measured against wavelength
Sample handling is highly specific in IR	Sample handling is simple

5.6 IR Fingerprint Regions

To make full use of IR spectroscopy for qualitative analysis of various organic, inorganic, and biological species is carried out on the basis of spectral comparisons. The characteristic spectral zones are compared with the responses obtained and a suitable conclusion is drawn. For this a fingerprint region is required so that characteristic group frequencies and significant changes with changes in atoms or their structures are used as a measuring scale. Therefore to identify a compound by IR technique, a systematic examination of certain IR regions and their comparison with the fingerprint regions are important. Some important regions are as follows:

- Hydrogen stretching regions 3700–2700 $cm^{-1}$are due to O–H and N–H stretching vibrations. Aliphatic C–H = 3000–2800 cm^{-1}in C_2H_2, C–H appears at 3300 cm^{-1}, aldehydic C–H at 2745 – 2710 cm.
- Triple bonds –C≡N and –C≡C show peaks at 2180 to 2120 cm^{-1} and 2260 to 2190 cm^{-1}, respectively.
- Carbonyl stretching vibrations range from 1770 to 1700 cm^{-1}. Those carbonyl which are from ketones, aldehydes, acids, amides, and carbonates have absorption peaks at 1700 cm^{-1} whereas those from esters, acid chlorides, and acid anhydride show higher range ~1770 to 1725 cm^{-1}.
- Esters have supportive peaks of C–O–R at 1200 $cm^{-1}$and aldehydes have C–H peak above 2700 cm^{-1}.
- Aromatic compounds show C = C peaks between 1650 and 1450 $cm^{-1}$with variations due to substitutions –C = C– and –C = N absorb between 1690 and 1600 cm^{-1}.
- C–Cl stretching vibration occurs at 700 to 800 cm^{-1} and other inorganic groups such as sulphate, phosphate nitrate, and carbonate absorb IR radiation in the fingerprint region of < 1200 cm^{-1} Table 5.2.

Table 5.2 Group frequencies of organic functional groups

Bond	Type of compound	Frequency range cm^{-1}	Intensity
C—H	Alkanes	2850–2970 1340–1470	Strong Strong
C—H	Alkenes	3010–3095 675–995	Medium Strong
C—H	Alkynes $-C \equiv C - N$	3300	Strong
C—H	Aromatic rings	3010–3100 690–900	Medium Strong
O—H	1. Monomeric alcohols, phenols 2. Hydrogen bonded alcohols, phenols 3. Monomeric carboxylic acids Hydrogen bonded carboxylic acid	1. 3590–3650 2. 3200–3600 3. 3500–3650 2500–2700	Variable Variable Variable Variable
N—H	Amines, amides	3300–3500	Variable
C=C	Alkenes	1610–1680	Variable
C=C	Aromatic rings	3010–3100	Variable
$C \equiv C$	Alkynes	2100–2260	Strong
C=N	Amines, amides	1180–136z	Strong
C—O	Alcohols, ethers, carboxylic acids and esters	1050–1300	Strong
C=O	Aldehydes, Ketones acids & esters	1690–1760	Strong

5.7 Application of IR Spectroscopy to Organic Compounds

Identification of organic compounds is highly dependent on IR spectroscopy because of the common presence of functional groups in these compounds. Alkanes, alkenes, alkynes, cyclics, or aromatics commonly absorb due to C–H vibrations. An organic compound is identified by first examining the group frequency region of $3600-1250\ cm^{-1}$ which indicates the functional group present. Secondly a comparison is done of the sample IR spectra and that of a pure compound sample having same functional group. The comparison with the fingerprint region ($1200–600\ cm^{-1}$) majorly helps to identify the compound accurately whereas group frequency region is used to find the functional group. Diverse organic group frequency region $3600–1250\ cm^{-1}$ is used to find the functional group. Compounds like carbohydrates, lipids, amino acids, proteins, nucleic acids, enzymes, and other biochemical compounds have been extensively studied by IR spectroscopy. Steroids, hormones and pharmaceuticals are also identified by IR Fig. 5.8.

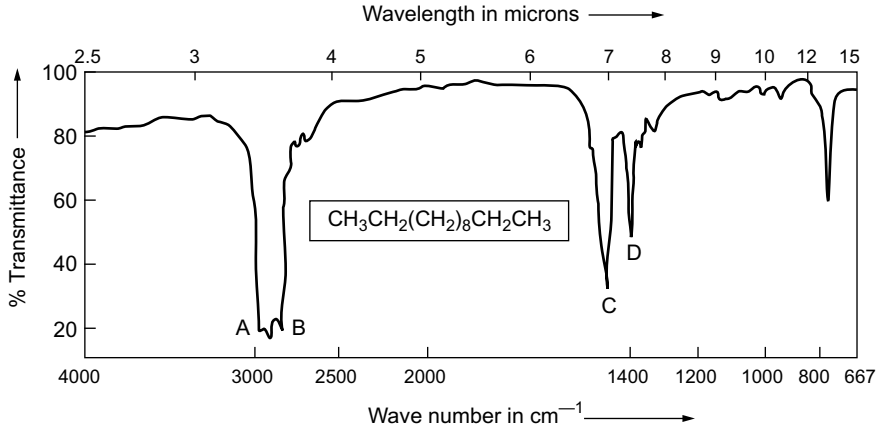


Fig. 5.8 IR spectrum of dodecane

Example: IR spectrum of dodecane.

A = 2960 cm^{-1}	O—H $_{str}$
B = 2870 cm^{-1}	C—H_{str} in methyl\methylene
C = 1465 cm^{-1}	C—H is CH_2
D = 1372 cm^{-1}	C—H bond is CH_2
E = 770 cm^{-1}	CH_2 (rocking)

IR Spectra of ethyl alcohol.

A = 3330 cm^{-1}	O—H group
B = 2960 cm^{-1}	C—H str (asymmetric)
C = 2924 cm^{-1}	C—H str (symmetric)
D = 1050 cm^{-1}	C—H str (for primary alcohols)

Example: The IR spectra of carbonyl compound of formula C_4H_8O are given. Identify the compound.

IR spectra of carbonyl compound (Fig. 5.9).

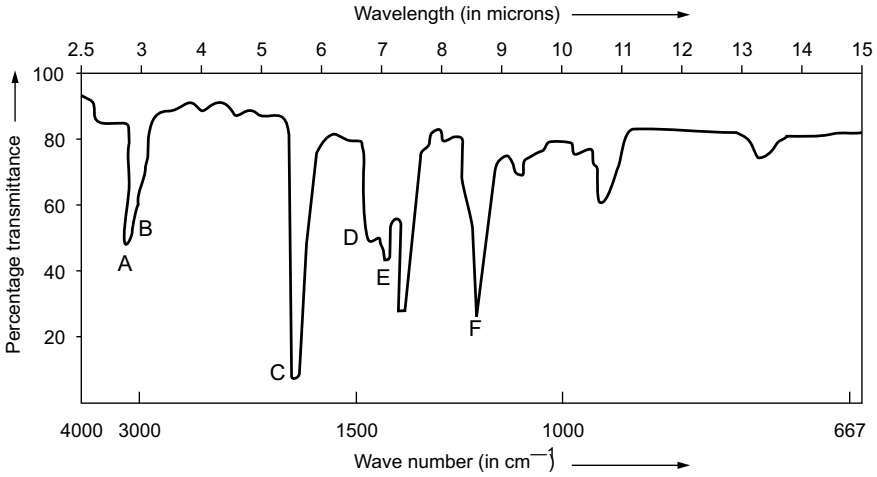


Fig. 5.9 IR spectra of carbonyl compound

A = 3002 cm^{-1}	C—Hstr. olefines/aromatics,
B = 2940 cm^{-1}	C—H str.,
C = 1715 cm^{-1}	C = O str.,
D = 1450 cm^{-1}	C—H methyl methylene,
E = 1466 cm^{-1}	CH_2 bend in CH_2
F = 1360 cm^{-1}	CN—H bend in CH_3

The absence of band at 2720 cm^{-1} indicates the absence of aldehyde group. So the possible structure is $H_3C—CH_2—\overset{\overset{O}{\|}}{C}—CH_3$.

5.8 Application of IR Spectroscopy to Inorganic Compounds

Mainly coordination compounds respond to IR spectroscopy because the coordination of the ligand atom with the metal ion results in the movement of electron density from the ligand atom causing polarization of the bond and thus making it IR active. The ligand–metal (L—M) bond also changes the symmetry of the molecule to make the bond IR active. For example, water H_2O in inorganic salts is either lattice water or coordinated as ligand to metal. The lattice water absorbs at 3550–3200 cm^{-1} and 1630–1600 due to antisymmetric, symmetric stretching and bending of HOH. When it is coordinated to the metal, it exhibits other modes, rocking and

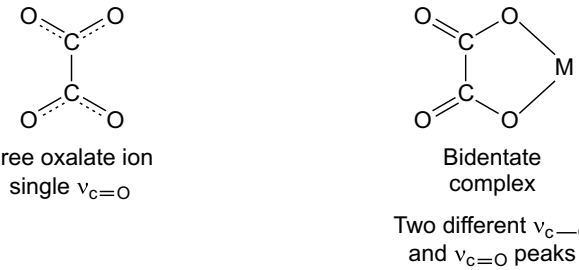


Fig. 5.10 O slate ion and its nature of binding to metal

wagging in the region 900–800 and 600–500 cm^{-1} respectively. An extra band at 500–400 cm^{-1}which occurs due to M—O stretching can also be detected.

IR spectra of N_2Ogroups can be detected when an anionic N_2Ogroup is present, it shows three IR bands at 1335, 1250, and 850 cm^{-1} whereas coordination through nitrogen as in $\left[Co(NO_2)_6\right]^{-3}$, all the IR-active bands change position and an additional band at 625 $cm^{-1}$is observed. When $N_2O$is coordinated through oxygen atom bands occur at 1468 cm^{-1} and 1065 cm^{-1} respectively. Many chelating ligands like oxalate and carbonate can be analysed using IR. The bridged and terminal ligands can also be detected by IR spectroscopy and a suitable structure of the complex is drawn on the basis of IR results Fig 5.10.

Bridging v_{c-O} absorbs at lower frequency than terminal v_{c-O}.

5.9 Quantitative Applications of FT-IR Spectroscopy

The application of infrared spectroscopy as a quantitative tool varies widely as it requires high-resolution instruments. Quantitative work acquired precision only after the advent of Fourier transform infrared spectrometers. The mathematical operations known as Fourier transformation (FT) provide a powerful method to increase signal-to-noise ratio (S/N) of an instrument and thus increase its resolution power Fig 5.11.

Advantages of FT-IR Spectroscopy

(i) FT-IR models have increased sensitivity as compared to dispersive IR models. They are fast and so multiple spectra can be recorded to give accuracy.
(ii) The FT spectroscopy is designed to give more radiation energy by means of using a circular optical slit.
(iii) Stray radiations are totally eliminated and all frequencies are modulated.

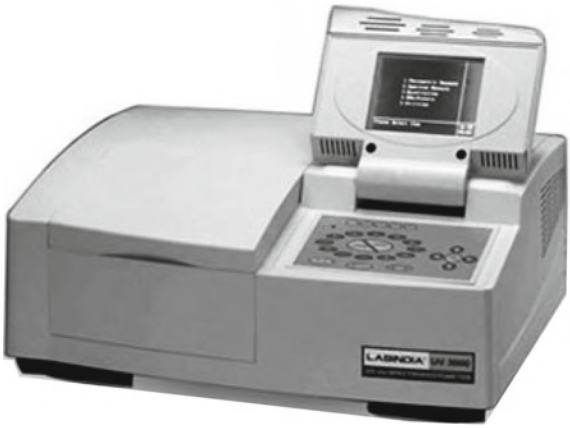


Fig. 5.11 A FT-IR spectrometer

(iv) Provision for internal calibration and data processing lead to convenient rapid and precise measurements.

Therefore quantitative analysis gains higher precision due to the FT-IR instruments.

Quantitative infrared analysis is based on Beer's law. The molar absorptivities ($\in$) can be calculated at particular wavelengths and the spectrometer is scanned in a narrow region near the wavelength of maximum absorption. The baseline method involves the selection of an absorption band of an analyte which does major absorption, and the subsidiary absorptions are either too weak or have distantly placed bands. The value of the incident energy P_o is obtained by drawing a straight line tangent to the spectral absorption curve at the position of the sample absorption band and then measuring the distance from this tangent to the % T line. The transmitted power P_t is measured at the point of maximum absorption of the analyte. The value of $\frac{P_o}{P_t}$ against concentration for a series of standard solutions, and the unknown concentration is determined from the calibration curve.

The difference method is applied to quantitative infrared determinations when greater accuracy is desired. In the vicinal range of concentrations of the unknown sample, a series of standard solutions are prepared and are placed in the reference beam along with the unknown sample in the sample beam and the differences are plotted against concentration. The concentration of the unknown is that concentrations on the curve where the difference is zero.

For quantitative measurements, the single-beam systems are more beneficial than the double-beam systems as they have greater signal–noise (S/N) ratio and result in greater sensitivity and better accuracy.

Solved Problems

1. Calculate the vibrational frequency of C–D bond if the vibrational frequency of C–H bond is 3060 cm^{-1} and the force constants for C–H bond and C–D bond are nearly the same.

   Solution

$$\nu_{C-H} = \frac{1}{2\pi C}\sqrt{\frac{K_{C-H}}{\mu_{C-H}}}cm^{-1}$$

similarly

$$\nu_{C-D} = \frac{1}{2\pi C}\sqrt{\frac{K_{C-D}}{\mu_{C-D}}}$$

$$\frac{\nu_{C-H}}{\nu_{C-D}} = \sqrt{\frac{\mu_{C-D}}{\mu_{C-H}}}$$

if

$$K_{C-H} = K_{C-D}$$

$$\mu_{C-H} = 1.532 \times 10^{-24}g$$

$$\mu_{C-D} = 2.847 \times 10^{-24}g$$

$$\frac{\nu_{C-H}}{\nu_{C-H}} = 1.363$$

$$\nu_{C-D} = 3060\ cm^{-1}$$

$$\nu_{C-D} = 2245\ cm^{-1}$$

Unsolved Problems

1. The infrared spectrum of a carbonyl compound shows a vibrational absorption peak of CO at 2170 cm^{-1}. Calculate the corresponding peak of ^{14}CO.
2. Calculate the vibrational absorption peak for HF if its force constant is 9×10^6dynes/cm.
3. How many vibrational modes are expected in ethane, benzene, HCl, and water molecules?

4. Explain, why C = C stretching in salicylic acid is observed at 1665 cm^{-1} while in p-hydroxybenzoic acid it is observed at 1680 cm^{-1}*?*
5. What is necessary for a molecule to absorb infrared radiation?
6. Explain why O–H stretching frequency is observed at higher range than C–H stretching.
7. How many fundamental vibrational frequencies are expected to be observed in the IR spectrum of CO_2*?*
8. How are lattice water and coordinated water distinguished by IR spectra?
9. Why water cannot be used as a solvent in IR spectroscopy?
10. What are the factors which influence the positions of IR absorption bonds from their normal values?
11. Describe the effect of intermolecular and intramolecular hydrogen bonding on the positions of IR absorption frequency of a compound by giving examples.
12. What are the main components of instruments used in IR spectrometers?
13. Which are the commonly used sources of radiation in IR spectroscopy? Explain.
14. Differentiate between the mull and pellet techniques of sampling used in IR spectroscopy.
15. Describe the various types of detectors used in IR spectroscopy.

Answers

1. $2.08 \times 10^{-3} cm^{-1}$
2. $3.99 \times 10^{-3} cm^{-1}$
3. 19, 30, 1, 3

Chapter 6
Atomic Absorption Spectroscopy

6.1 Basic Principles of Atomic Absorption Spectroscopy

Atoms which cannot rotate or vibrate show only electronic transitions. Since these transitions are discrete or quantized, *i.e.* a fixed amount of energy is associated with every transition, line spectra are observed. To generate spectra for an element, the sample has to be atomized, *i.e.* the constituents of the sample are decomposed and converted into gaseous elementary particles which are atoms or ions. Depending upon the measurements made for electronic transitions of atoms or ions, atomic spectroscopy can be classified into three categories:

(i) **Atomic absorption spectroscopy.**
(ii) **Atomic emission spectroscopy.**
(iii) **Atomic fluorescence spectroscopy.**

Based on absorption, emission, and fluorescence phenomena, respectively, all the three categories of spectra are observed in the visible and ultraviolet regions.

Large number of gaseous metal atoms in their ground state are capable of absorbing wavelength of their specific resonance wavelength from a radiation source. Hence, if light of the resonance wavelength is passed through a flame containing the atoms of the sample, then part of light will be absorbed and the extent of absorption will be proportional to the number of ground-state atoms present in the flame. This is the basic principle of atomic absorption spectroscopy (AAS) whereas atomic fluorescence spectroscopy (AFS) is based on the re-emission of absorbed energy by free atoms in the form of fluorescence. In AAS determinations, the absorbance is measured and the concentration of the analyte is related to the signal by the Lambert–Beer law, usually called Beer's law.

Atomic spectroscopy is based on the fact that atoms absorb definite amount of radiation of energy to get excited to another electronic state. Atoms absorb light of particular wavelength and undergo electronic transitions which are characteristic of a particular element. Both absorption and emission of radiant energy by atoms are

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the basic atomic spectroscopy which turn out to be powerful analytical tools. The various methods for atomic spectroscopy are summarized as follows Table 6.1.

Although the absorption and emission of radiant energy furnish great deal of information which are efficiently used for both qualitative and quantitative analyses, there are certain limitations which apply to this field of atomic spectroscopic methods. They have limited ability to distinguish among oxidation states and chemical surroundings of the elemental sample and cannot detect the presence of nonmetallic elements. These limitations have been overcome by various modifications in optical components.

The extent of analysis and its precision depends upon the distribution of number of atoms in the excited state and the ground state given by Boltzmann distribution equation:

$$\frac{N_j}{N_o} = \frac{P_j}{P_o} \exp\left(-\frac{E_j}{KT}\right)$$

where N_j and N_o are the number of atoms in the excited state and the ground state, respectively, K = Boltzmann constant (1.38×10^{-16} ergs/deg),

T = Temperature (Kelvin scale),

E_j = energy difference in ergs between the excited state and the ground state,

$\frac{P_j}{P_o}$ = ratio of statistical weights for ground and excited states.

$$\frac{N_j}{N_o} = \frac{P_j}{P_o} e^{-\frac{E_f}{KT}}$$

Table 6.1 Various methods of atomic spectroscopy

Method	Energy source	Measured unit
Absorption	*Source of atomization*	
Flame atomic absorption spectroscopy (FAAS)	Flame (1700−3200 °C)	Absorption of radiation
Electrothermal absorption spectroscopy	Electric furnace (1200−3000 °C)	Absorption of radiation
Emission Flame emission spectroscopy (FES)	Flame (1700−3200 °C)	Intensity of radiation
Atomic fluorescence spectroscopy (AFS)	Flame (1700−3200 °C)	Intensity of scattered radiation
Electric arc	Plasma from dc arc (4000−6500 °C)	Intensity of radiation
Electric spark	Plasma from ac spark 4500	Intensity of radiation
Inductively coupled plasma (ICP)	Argon plasma produced by high frequency magnetic field	Intensity of radiation

Therefore, the ratio depends largely on the temperature and the energy difference between the excited state and the ground state. It is also important to note that fraction of excited+state atom is temperature dependent whereas the fraction in the ground state is virtually constant.

Atomic absorption spectra of most elements are simple in character and are more sensitive to inter-element interferences than emission spectroscopy. Although elements whose resonance lines (lines arising from ground state) are from low energy values are more sensitive than those whose resonance lines are from high energy values, in case of flame emission spectroscopy.

Emission line wavelength

For example,

Na	589 nm	More sensitive
Zn	213.9 nm	Less sensitive

In atomic absorption spectroscopy (*AAS*), the absorbance A is given by

$$A = \log \frac{I_o}{I_t}$$
$$= KLN_0$$

I_o = intensity of incident light,

I_t = intensity of transmitted light,

whereN_o = concentration of atoms in the flame (no. of atoms /*me*),

L = pathlength through the flame,

K = a constant related to the absorption coefficient.

6.2 Instrumentation

Just as is required in any other absorption spectrophotometry, atomic absorption spectrophotometry also follows similar sequence of a light source, a cell (the flame), a monochromator, and then a detector Fig 6.1.

Before the steps of instrumentation begin, pretreatment of sample and sample delivery are of great importance in all types of atomic spectrometry. The samples require to be dissolved in a solution of substance that either do not interfere with absorption measurements or if they interfere, they should be removed or masked by complexing reagents. The accuracy of analysis also depends largely on sample induction into the flame or plasma. The most widely acclaimed sampling method is *Nebulization.*

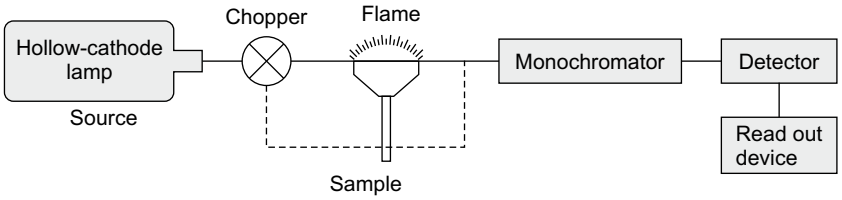


Fig. 6.1 Schematic diagram of AAS

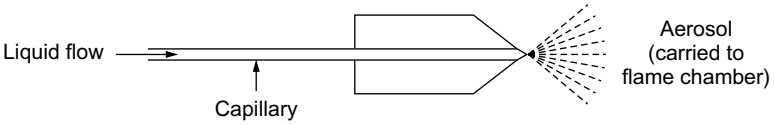


Fig. 6.2 Design of a nebulizer

To provide a continuous flow of aerosol into a flame, nebulization of liquid sample is a popular technique used in most atomic spectroscopy. Before the liquid sample enters the flame chamber it is first converted into small droplets or mist of solution. This process in called *nebulization.* Pneumatic nebulization is a commonly known technique of this process. The sample stream produced through a small hole into a high velocity gas jet intersects the gas stream parallelly or perpendicularly. The high velocity gas jet withdraws the liquid from the sample which oscillates to produce a laminar flow finally forming a cloud of droplets in the spray chamber Fig 6.2.

The Role of Flames in AAS and FES

There are two ways that free atoms can be transformed into excited electronic states:

Either by absorption of additional thermal energy from the flame or by absorbing radiant energy from an external source of radiation.

In Flame Emission Spectroscopy (FES), the energy from the flame is sufficient to vapourize the atom as well as remove the electrons from the free atoms from the ground state to the excited state. The intensity of the radiation then emitted by these excited atoms is determined by FES. In AAS, the free electrons generated by flame only are the sample cell and the incident radiation absorbed by free atoms is used for excitation and is recorded for analysis. For better detections, electrothermal furnaces have also been used in AAS. In both FES and flame AAS, the sample solutions is introduced as an aerosol into the flame so that the ions are converted to free atoms which are qualitatively and quantitatively analysed. If the source of exciting radiation is placed at right angles to both the flame and the optical axis of the spectrometer, another method known as atomic fluorescence spectroscopy has originated, as can be seen from Fig. 6.3.

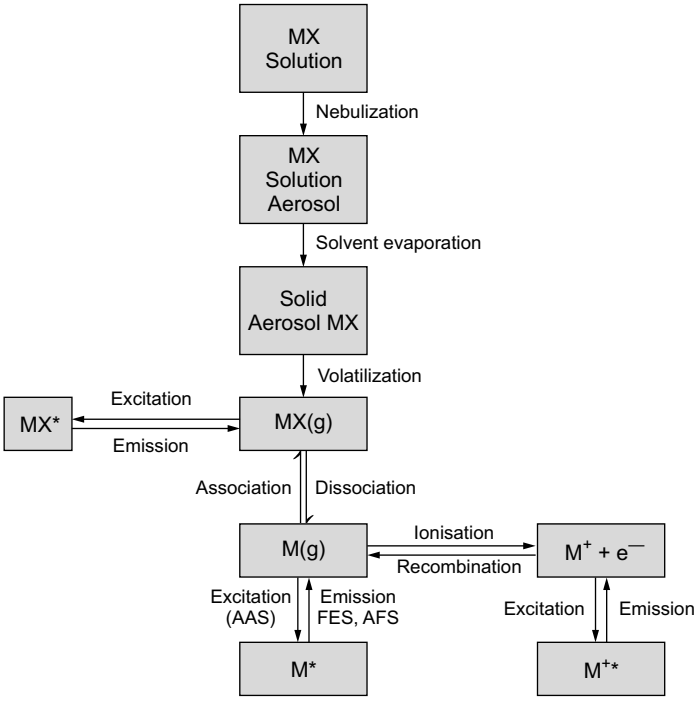


Fig. 6.3 Flame atomization of a salt MX generating excited M*

Essential requirements of flame

Flame temperature, flame composition, flow rates of both fuel gas and oxidant gas, and position of the flame all make important criteria for various forms of atomic spectroscopy. Flame temperatures are generally greater than 2000 K which can also be achieved by burning the fuel gas in an oxidant gas like air, nitrous oxide, or oxygen with nitrogen or argon. Flow rates of both the fuel gas and oxidant gas are decided according to the spectroscopy method involved and the nature of sample. Among different flame compositions acetylene–air mixture is suitable for many metal determinations.

Commonly used flames, their burning temperatures, and their velocities are given in Table 6.2.

Few metals which can be easily vapourized to atomic vapours require propane–air flame whereas *Al* and *Ti* require acetylene nitrous oxide flame to achieve high temperature. Determination of certain metals requires specific position of flame as maximum absorption takes place at a certain region of the flame Fig 6.4.

The fuel/oxidant ratio not only influences the temperature of the flame but also determines the concentration of unexcited and excited atoms in the flame which in turn influences the intensity of the spectra obtained.

Table. 6.2 Flames, their burning temperatures, and their velocities

Flame mixture	Maximum temperature °C	Maximum flame velocity cm/s
Hydrogen–oxygen	2677	–
Hydrogen-air	2045	–
Propane-air	1725	–
Propane-oxygen	2900	–
Acetylene-air	2250	160
Acetylene-oxygen	3060	1130
Acetylene-nitrous oxide	2955	180
Hydrogen–argon–entrained air	1577	–

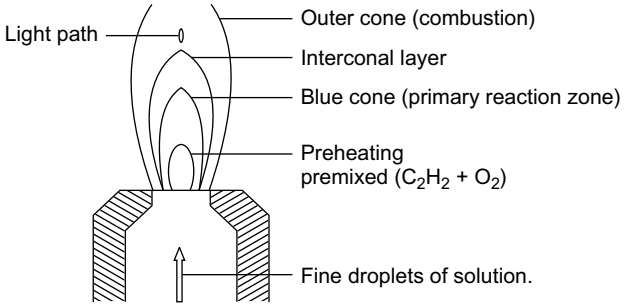


Fig. 6.4 Various regions of a flame

Graphite Furnace

For atomization non-flame methods are preferred as they are safer. A graphite furnace is one of them, which is heated electrically and so provides electrothermal atomization. The graphite tube furnace consists of a hollow graphite cylinder of 50 mm long and 9 mm width and surrounded by a metal jacket maintaining a gas space in between the two. An inert gas like argon is circulated in the gas space, water in the metal container, and electrical current is passed through graphite tube for heating it at a temperature sufficient to evaporate the solvent from the sample solution.

The sample is converted to ash and then vapourized to produce metal atoms by enhancing the current. Each graphite tube can be used for 100–200 analysis and provides absorption signals with enhanced sensitivity. Other advantages are that it requires very small sample size and solid sample can be used directly. However, there are background absorption effects and some loss of sample during ashing in graphite furnace Fig 6.5.

Various other atomization techniques include glow discharge atomization, hydride atomization, and cold vapour atomization which involve a glow discharge cell, hydride generation in a quartz tube, and cold vapour atomization for mercury, respectively.

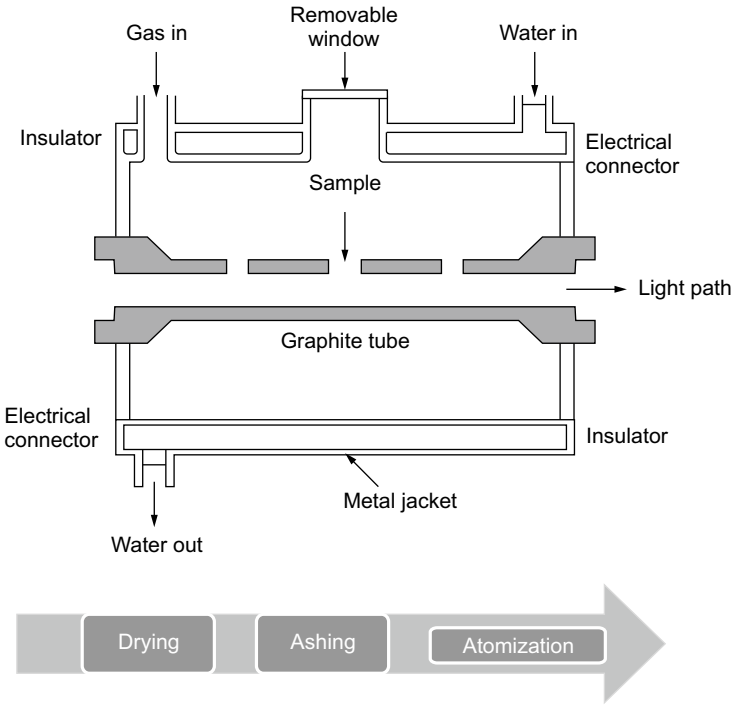


Fig. 6.5 A graphite furnace with a cross-sectional view

Radiation Sources

For Beer's law to be obeyed during atomic absorption spectroscopy measurements, radiation source having narrow bandwidth relative to the width of an absorption line or band is essentially required. The limited width of atomic absorption lines has to be matched with the source having even narrower bandwidth than the absorption line width. Such requirements are easily catered by hollow cathode lamps and electrode discharge lamps.

Hollow Cathode Lamps

Hollow cathode lamps are capable of producing narrow lines of required frequency whereas monochromators generally cannot produce lines narrower than 10^{-3} to 10^{-2} nm. They contain vapour of the same element which is being analysed. They are the most commonly used radiation source for atomic absorption measurements.

The hollow cathode lamp (Fig. 6.6) is filled with Ne or Ar at 130–700 pa (1–5 torr) pressure. The cathode is made of the element whose analysis is desired and is sealed in a glass tube. It consists of tungsten anode and when a voltage of ~500 V is applied between anode and cathode, the gas gets ionized and the positive ions are accelerated towards the cathode. The electrons generate a current of 5−15 mA which further accelerates the cations to ionize the metal atoms from the surface of cathode.

Their energy is high enough to produce a sputter of metal atoms from the cathode into the gas phase. These gaseous atoms get excited by giving energy while colliding with the electrons and emit photons. This atomic radiation has the same emit bandwidth which the sample absorbs while being present in the flame or furnace. The lamp is at lower temperature than the flame or furnace so lamp emissions consist of narrower bandwidth and are monochromatic than the absorption bandwidth of atoms in the flame. The purpose of a monochromator in AAS then is to select one line from hollow cathode lamp and eject emissions from flame or furnace which are not required Fig 6.7.

Every hollow cathode lamp emits the spectra of the metal of which its cathode is made from. Sometimes cathode tube of a mixture of several metals is also commercially available which are alloyed for cathode purposes and enable the analysis of more than one element.

Electrode Discharge Lamps

Another well-known radiation source for AAS and AFS is electrode discharge lamps which give radiations of much higher intensities than hollow cathode lamp. The lamp consists of a sealed quartz tube filled with inert gas like argon at high pressure and the metal salt (iodide) of metal whose spectra is desired. Intense radio frequency or microwave radiations are generated without using any electrodes. The excited inert gas atoms gain energy from these radiations and collide with the metal atoms which in turn absorb energy and give the absorption spectrum. Electrode discharge lamps are commercially available and are equipped for analysis of 15 or more elements, although they are not as efficient as hollow cathode lamps.

Chopper: A chopper which is placed between the radiation source and the flame is meant to break the continuous light coming from the lamp. In the form of a rotating wheel, it is able to produce intermittent light which further produces an alternating current. This helps in measuring the absorption of light without interference from the constant light coming from the flame as only the pulsating or alternating current produced is amplified and recorded. This gives better resolution of the absorption spectrum.

Monochromator: In AAS the role of monochromator is highly confined to the isolation and selection of resonance line from other unabsorbed lines emitted from the

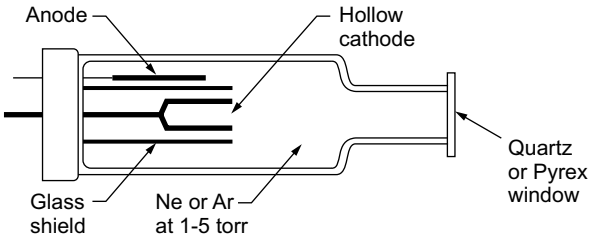


Fig. 6.6 Hollow cathode lamp

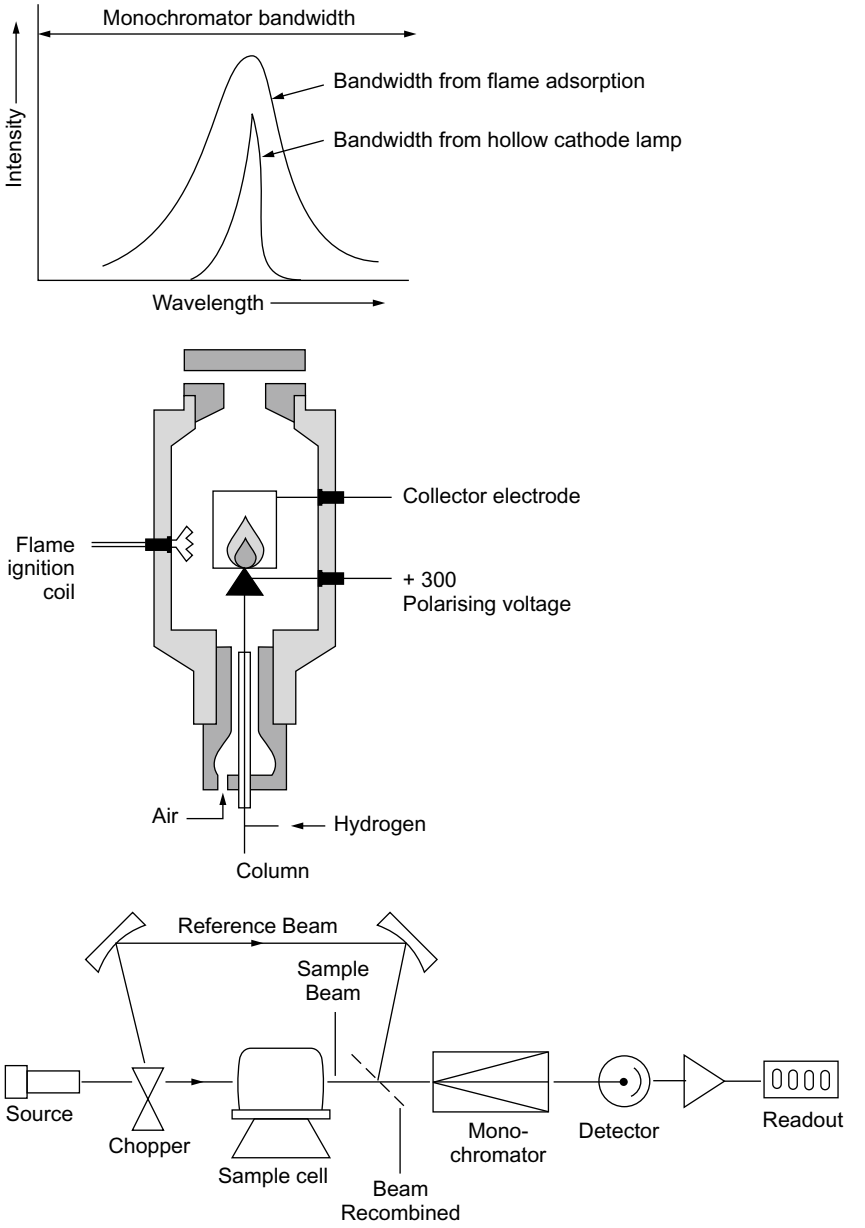


Fig. 6.7 **a** Relative bandwidths of hollow cathode lamp, flame, and monochromator **b** An electrode discharge lamp **c** A chopper in use

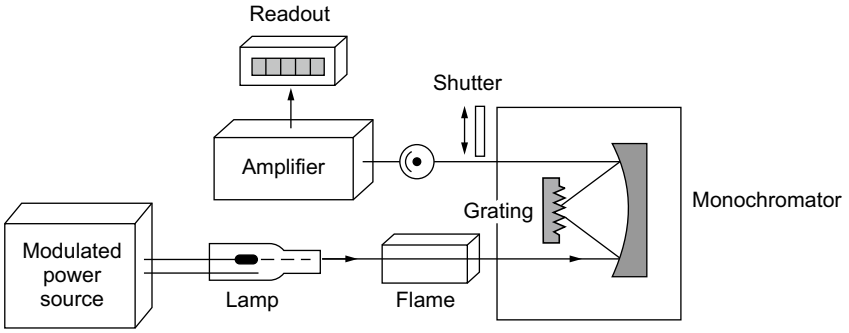


Fig. 6.8 A monochromator used in AAS

lamp. For this purpose most of the commercial instruments preferably use diffraction gratings as it produces more uniform dispersion and gives higher resolution.

Detectors: The most commonly used detector in almost every AAS instrument is photomultiplier tube which responds to the selected resonance line as well as a few emissions from the flame which are distinguished by the chopper. The amplifier also is designed such that flame emissions are removed and absorptions of resonance lines only are detected by the photomultiplier tube Fig 6.8.

6.3 Single-and Double-Beam Atomic Absorption Spectrophotometers

Single-Beam Instruments: In single-beam atomic absorption spectrophotometer, the hollow cathode lamp is placed ahead of the flame and a mechanical chopper is placed between the lamp and the flame. Without the sample being placed in the flame the instrument is operated and a blank of the metal from hallow cathode lamp is obtained on the recorder. Then a sample is nebulized in the flame and the output is recorded. The two output signals are compared to detect the analyte Fig 6.9.

Double-Beam Instrument: In this type of instrument both the sample and reference can be analysed together. The beam from the hollow cathode source is split by a mirrored chopper which then passes through the flame containing the sample and the reference. Later they enter the monochromator and detector and produce the signal. The ratio of the signals is amplified and collected by the readout device.

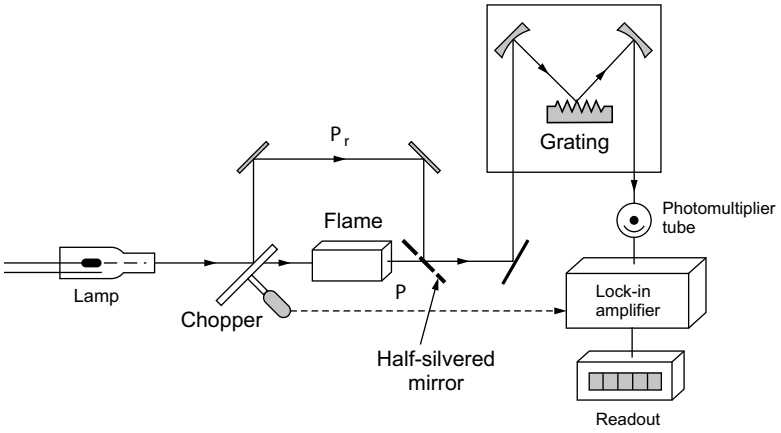


Fig. 6.9 A double-beam instrument

6.4 Interferences in Atomic Absorption Spectroscopy

Interference is defined as those effects which change the signals keeping fixed the concentration, temperature, pressure, and environment of the sample.

In atomic absorption methods, mainly three types of interferences occur which influence the analysis and detection.

Spectral Interferences

The spectral interferences arise when overlapping of signal lines take place. Separation between the narrow lines obtained from hollow cathode lamp when reduced than 0.1 Å, they cause spectral interferences. The various reasons of spectral interferences and their preventions are discussed as follows:

- When two or more metals absorb at wavelengths very close to each other, for example, vanadium and aluminium absorb at 3082.11 Å and 3082.15 Å, respectively. To prevent this interference a change in wavelength for Al can be exercised by observing its absorption at 3092 Å.
- Presence of other elements or molecules in the sample or signals produced by flame or furnace can be avoided by using high-resolution spectrometers which may also be equipped for making Zeeman background correction.
- Spectral interferences are also produced when elements upon oxidation form stable diatomic oxides and are not completely atomized. For when Y and Ba are present *Y forms YO* which gives a broad molecular emission (Fig. 6.10) similarly probability of formation of WO_3, when solid tungsten W sample is taken are there, which can be overcome by heating tungsten powder with hydrogen at 1000−1200 °C so that reduction takes place and interference is avoided.

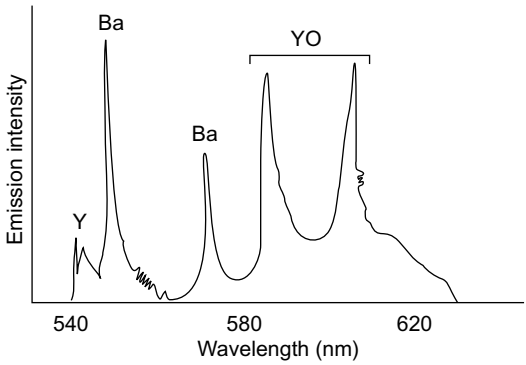


Fig. 6.10 Spectral interference due to oxide formation

- Chances of spectral interferences are even there when concentrated solutions of elements such as Ti, Zr, and W are aspirated in the flame as it leads to the formation of by products which cause scattering of the beam.
- Spectral interference caused by scattering of light due to the presence of organic compounds in the matrix whose incomplete combustion has remaining carbon. This kind of scattering is not produced if flame atomization is treated with variable flame temperature and by varying the fuel–oxidant ratio.

Chemical Interferences

The result from various chemical processes occurring during atomization which alter the absorption spectra of the analyte gives way to chemical interferences. Some component of the sample may alter the rate of vapourization of salt particles that contain the analyte or some chemical reaction may take place which change the vapourization behaviour of the solid are few other causes of chemical interferences. Sometimes as a result of a physical process also some atoms are released which lead to chemical interference during vapourization, for example, very often analysis of Ca^{2+} is accompanied by the release of SO_4^{2-} and PO_4^{3-} ions which release non-volatile salts and slow down the process of atomization. The more stable calcium sulphates and phosphate salts are formed which do not readily break into atoms. Sometimes cations also interfere in atomic absorption, although rarely. For example, Al and B interfere with alkaline earth metals and Mg interferes with Ca determinations.

Chemical interferences can be minimized by adding some chemicals called releasing agents EDTA and 8-hydroxyquinoline may be added to protect Ca^{2+} from SO_4^{2-} and PO_4^{2-}. Another releasing agent can be La^{3+} or Sr^{2+} which may preferentially react with phosphate to prevent calcium to form its phosphate salt and create interference. 8-hydroxy quinoline also masks aluminium and prevents its interference in calcium and magnesium measurements.

Many other precautions like using higher flame temperatures and using fuel rich flames can also help in eliminating many chemical interferences.

Self-absorption

Various parameters such as variation in rate of sample uptake and atomization due to changes in gas flow rates, alteration in sample viscosity, and density lead to interferences of self-adsorptions. They may also be caused when samples dissociate or ionize due to variation in physical parameters. For example, *ionization interference* poses a hinderance in the analysis of alkali metals at low temperature and some other metals at high temperature. Alkali metals have low ionization potentials so are easily ionized. As the order of ionization potential predicts, potassium is much more ionized than sodium.

For any element, a gas-phase ionization reaction is

$$\mathrm{M(g)} \nu \mathrm{M^{+}(g)} + \mathrm{e^{-}(g)}$$

$$\kappa = \frac{\left[\mathrm{M^{+}}\right]\left[\mathrm{e^{-}}\right]}{[\mathrm{M}]}$$

In case of potassium as an analyte, this ionization can be prevented by adding a solution of CsCl of 1 ppm as cesium has even lower ionization potential than *K*. Cesium chloride solution can play the role of an ionization suppressor. *Dissociation* of metal compounds in case of metals like La, Al, and Ti when aspirated into the flame form their metal oxide or hydroxide first, which further dissociate as follows:

$$\mathrm{MO} \nu \mathrm{M^{+2}} + \mathrm{O^{-2}}$$

$$\mathrm{M(OH)_2} \nu \mathrm{M^{+2}} + 2\mathrm{OH^{-}}$$

The heat of the gases in the furnace can cause different types of dissociations and association reactions of which metal oxide and hydroxide play important role in the final output of absorption spectra of atomic absorption. Anions are different from oxygen, like chloride Cl ion produced on dissociation of NaCl

$$\mathrm{NaCl} \nu \mathrm{Na^{+}} + \mathrm{Cl^{-}}$$

also decrease the intensity of the spectra obtained.

Therefore, almost all interferences can be removed or minimized by adopting certain measures but sometimes adaptation of background correction facilities also can be undertaken.

6.5 Atomic Line Broadening

One of the most important parameters in AAS is the shape of the spectral line emitted by the source as narrow lines reduce interferences. Line width, devoid of any overlapping of spectral lines, is quite significant. Ideally, line width should be zero

but the atomic spectral line broadens which is highly dependent on temperature. For a given emission line, the line width is proportional to square root of the temperature. Line width = $\Delta\lambda_{1/2}$ is defined as the width of line in λ when measured at half of the maximum signal. The various reasons for line broadening are briefly either due to uncertainty effect, Doppler effect, pressure effect, or Zeeman effect.

The uncertainty effect arises due to the inherent uncertainty in the number of atoms in the ground state and the excited state. The number of electrons in the ground state stays longer than in the excited state and so it generally causes broadening by 10^{-4} Å units.

Doppler broadening is caused due to the frequently changing density of those atoms which move towards the recorder, and move away from it.

The Doppler shift $\Delta\lambda$ is also related to the velocity of the movement of atoms.

$$\frac{\Delta\lambda}{\lambda_0} = \frac{V}{C}$$

where C = velocity of light,

λ_0 = wavelength of the original line,

V = velocity of moving to and fro signals from the recorder.

In AAS the random motion of excited atoms causes **Doppler broadening** of the wavelength. Spectral lines then even tend to overlap due to random motion.

The pressure effect arises due to the collisions among the ions or atoms which increase with rise in temperature. Such an effect of natural collisions can be seen during the broadening in hollow cathode lamp due to the collisions of absorbing atoms.

6.6 Background Correction Methods

The absorbance obtained for an analyte may be diverted from the true absorbance, especially higher absorbance can be corrected using background correction methods. Atomic spectroscopy should offer background corrections to distinguish analyte signal from absorption, emission, and optical scattering of the sample matrix, the flame, plasma, or red-hot graphite furnace. Anything other than the sample if causes any signal disturbs the original signal, it is termed as background signal. The total output should be dealt with using background correction techniques.

Figure 6.11 shows absorption spectrum of bronze containing Fe and Cu. Sharp atomic signals with a maximum absorbance near 1.0 are superimposed on a broad background with an absorbance of 0.3. For good result, background absorbance is subtracted. Techniques generally employed for background correction may be summed up as follows: **deuterium arc background corrections, Zeeman background corrections, and the Smith–Hieftje system**.

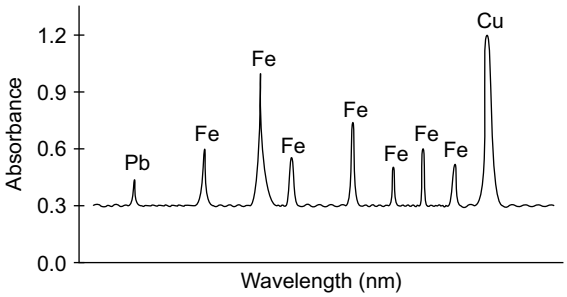


Fig. 6.11 Graphite furnace absorption spectrum of bronze

The method of **Deuterium arc** uses two lamps, a high-intensity deuterium arc lamp and the hollow cathode lamp of the element to be measured. In a double-beam analysis both the sample and the reference are exposed to both the sources, deuterium and hollow cathode. Both the sample and the reference are equally affected by the background absorptions which are eliminated by taking the ratio of the intensities of two beams.

The Zeeman background correction is based on the Zeeman effect of splitting of magnetic field. When a magnetic field is applied parallel to the light path through a furnace, absorption or emission line of analyte atoms is split into three components, out of these three components two are shifted to higher and lower wavelengths but one remains fixed. A string magnetic field is first applied and the spectra of the sample is taken then the spectra of the sample is observed after taking off the magnetic field. The difference between the two gives the correction signals (Fig. 6.12). When there is no magnetic field, signals for sample and background both are observed whereas only background is observed in the presence of the field. The Zeeman effect method for background correction is advantageous because the resonance line wavelength of the analyte only is used in contrast to the deuterium arc method in which broadband is used which is a wider range than the analyte wavelength.

The Smith–Hieftje system of background correction is applied on the basis of self-absorption. Hollow cathode lamp is operated alternatively under both low current and high current which, respectively, give a normal and a broad emission line. This indicates the background absorbance during high current and its absence during

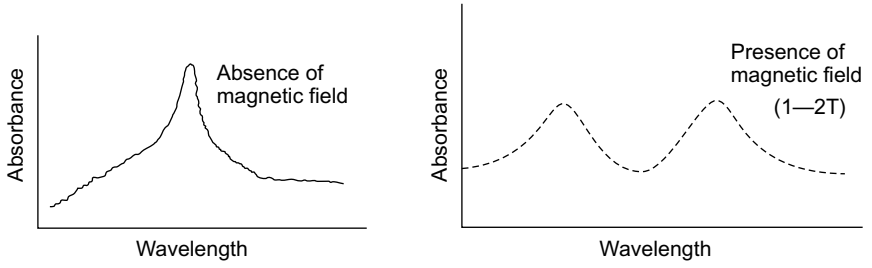


Fig. 6.12 Zeeman effect on Co in a graphite furnace

low current. The difference of absorbance between high current and low current measurements gives the corrected analysis.

6.7 Application of Atomic Absorption Spectroscopy

In the recent past, AAS has become a very popular tool for analysis because of its being swift and accurate method of analysis. It is capable of determining many elements together and that too it can detect smallest amount of the element. Atomic absorption finds valid applications in every branch by chemical analysis, ranging from analytical chemistry, ceramics, mineralogy, biochemistry, water supplies, metallurgy, and soil analysis. Atomic absorption shows superior detectability for those elements that emit below 300 nm because of the high thermal energy required to excite the atoms for emission at these wavelengths.

Qualitative analysis: AAS can be utilized for the analysis of as many as 67 metals. Typical non-metals which can be analysed are phosphorous and boron. Alkali and alkaline earth metals although can be determined by flame photometry, *i.e.* emission methods, it is also used for isotopic analysis of lithium.

Quantitative analysis is based upon the amount of radiation absorbed by the sample and the calibration curves drawn. Apart from the sensitivity of the instrument, every element also has its sensitivity and detection limit while responding to AAS. Quantitative analysis can be very well seen in clinical analysis while analysing metals in accurate amounts in biological fluids and tissues such as whole blood, plasma, urine, saliva, brain tissue, liver, muscle tissue, semen, etc. Analysing water for its metal content also emphasizes the quantitative application of AAS.

Sensitivity and *detection limits* are important criteria in AAS. Sensitivity implies at that range of concentration of the element (μ g/ml) which shows 1% absorbance (0.0044) and 99% transmittance. Sensitivity is generally expressed by the slope of the calibration graph.

Detection limit is that minimum concentration of the analyte whose response can be well demarcated or distinguished from the detection of the blank response. Generally, it is such that concentration of an element in solution which gives a signal equal to twice the standard deviation of the series of measurements is near blank level. Both sensitivity and detection limits are largely influenced by flame temperature flame type and pressure. The detection limit also varies with the variation in the spectral bandwidth of the element (Table 6.3).

The atomic absorption method is very sensitive and is generally more accurate than many analytical procedures used for similar determinations.

Few more areas where atomic absorption spectroscopy finds its wide applications are in the determination of trace metals in biological materials and food stuff. Presence of toxins like Cu, Zn, and Ni in food can be early detected. Ca, Mg, Na, and K can be determined in blood samples and serum for diagnosis of various deficiency

Table. 6.3 Sensitivity limits (ppm)

Element	Å	Sensitivity (ppm)	Element	Å	Sensitivity (ppm)
Al	3092	1.0	Mg	2852	0.001
Ba	5535	0.2	Pb	2476	0.56
Ca	4427	0.05	Si	2516	0.80
Cu	3247	0.1	Sr	4607	0.1
Pb	2170	0.01	U	3515	100.0
Ni	2320	0.1	As	1937	2.0
K	7665	0.01	Bi	2231	0.1
Na	5890	0.01	Co	2407	0.1
Sn	2354	0.5	Fe	2483	0.1
Zn	3601	50.0	Hg	2537	1.0
Sb	2176	0.1	Pt	2659	0.01
Be	2349	0.1	Ag	3281	0.01
Cr	3579	0.1	Ti	3681	1.0
Au	2428	1.0	Zn	2139	0.01

and diseases. Presence of Pb in petrol is another important area where AAS can be used for analysis.

Unsolved Problems

1. Discuss the basic principle involved in atomic absorption spectroscopy.
2. Give the schematic diagram of the instrumentation used in atomic absorption spectroscopy.
3. Describe a hollow cathode lamp.
4. Explain briefly the types of interferences commonly found in atomic absorption spectroscopy.
5. Describe the applications of atomic absorption spectroscopy.
6. Illustrate the methods used in background correction in atomic absorption spectroscopy.
7. Define nebulization and explain how is it carried out.
8. What are the factors which cause atomic line broadening? Explain Doppler broadening.
9. Define detection limit and sensitivity of an atomic absorption spectroscopy.
10. How are spectral and chemical interferences prevented or corrected in atomic absorption spectroscopy?

Chapter 7
Atomic Emission Spectroscopy

7.1 Introduction

Atomic emission spectroscopy (AES) deals with the excitation of atoms or elementary ions to their higher excitation state and the study of the radiation emitted by the excited sample. When the excited species rapidly relax back to lower states, they give rise to ultraviolet and visible line spectra. The electronic transitions in atoms use excitation sources like flames, arcs, or sparks and argon plasma and are quite sensitive to these sources. However, their low susceptibility to chemical interferences because of their high temperature makes them an efficient method for measuring the analyte. The amount of sampling is very low and is more convenient than in atomic absorption methods, even solids can be directly analysed. Emission spectroscopy is considered as the most reliable method for quantitative elemental analysis. Above all, it is an analysis in which there is no loss of sample-throughout the procedure.

P. Malhotra, *Analytical Chemistry*,
https://doi.org/10.1007/978-3-031-26757-4_7

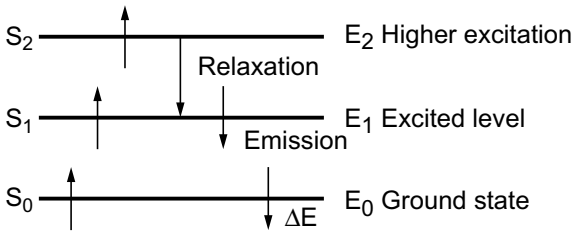


Fig. 7.1 Types of transitions

7.2 Principles of Emission Spectroscopy

Emission spectra are based on the age-old principle of flame test performed for alkali and alkaline earth metals. When metal salts are introduced in the flame, they emit their characteristic colours. Similarly, when sufficient energy is absorbed by the elementary particle, electronic excitations are produced leading to emissions which can be seen in a spectroscope (Fig. 7.1).

A definite wavelength is associated with every emission and has a fixed position in the spectra when passed through a spectroscope. Truly, emission spectroscopy is complimentary of absorption spectroscopy. The atomic spectrum emitted by a sample is used to determine its composition as every element is identified by the wavelength at which it is measured and its concentration is measured by the intensity of the emitted radiation. Elements in an unknown spectrum can be identified by comparing it with the spectrum of the known elements.

The emission lines are usually determined photographically specially in case of electric spark or electric arc excitations.

Depending upon the sources of radiation, atomic emission spectroscopy can be classified as.

Radiation source

(a) Flame emission spectroscopy (Flame photometer).
(b) Plasma emission spectroscopy (Inductively coupled plasma).

7.3 Flame Emission Spectroscopy (FES)

Flame emission method is obtained by recording the complimentary emission lines (transmittance *vs.* wavelength) of absorption spectrometry. Flame emission spectroscopy is also known as flame photometry in which the sample is sprayed into the flame for excitation. The flame is capable of transforming the solid or liquid sample first into the vapour state and then decomposing it to molecules or atoms. The thermal energy from the flame excites the electrons to higher energy state and then when they return to the ground state, radiations are emitted. Every element emits at a particular wavelength, and a characteristic spectra which may be either line spectra, continuous

spectra, or band spectra is obtained. Line spectra are obtained from atoms, continuous spectra are obtained from bulk material or from incandescent solids like iron or carbon by heating them till they glowed. Band spectrum originates from excited molecules.

The excited electrons may either return straight to the ground state or some stage in between, thus giving rise to many spectral lines. The most prominent line is due to the transition between the lowest excited state to the ground state and is a preferred transition for atoms and ions. Characteristic sharp lines are emitted by neutral atom. At high flame temperature, the alkali and alkaline earth metals get ionized and the spectra changes resemble that of an element lying in a group lower than it. The intensities of emission are also lowered for ions than for neutral atoms as the concentration of the neutral atoms in the flame decreases. The intensity of lines depends upon the following factors:

(1) Time of exposure.
(2) Concentration of the element in the matrix.
(3) Sensitivity of the photographic emulsion.
(4) Conditions of developing film.

Earlier for flame photometry, only cool flame was used and only a small fraction of atoms of most elements is excited by flame which increases with increase in temperature. Therefore, in routine only few elements have been determined by flame photometry. The easily excited metals like Na, K Ca, and Li are determined by flame photometry. However, with flames such as oxyacetylene and nitrous oxide acetylene over 60 elements can now be determined by FES. Good sensitivity is observed because the difference between ground zero and first excited state is small, but finite signal is measured, as restricted response also restricts the background noise and interference.

7.4 Distribution Between Ground and Excited States

Since most atoms are in ground state initially and then when by absorbing energy they get excited to higher energy states (or the excited state) the ratio of their populations at a given temperature is calculated from Maxwell–Boltzmann expression:

$$\frac{N_e}{H_o} = \frac{g_e}{g_o} e^{-(E_e - E_o)/KT}$$

where

N_e = population in excited state,

N_o = population in ground state,

g_e and g_o are statistical weights of excited and ground state,

E_e and E_o are the energies of two states,

K = Boltzmann constant (1.3805×10^{-16} erg K^{-1}).

The statistical weights represent the probability of electrons residing in the given energy levels.

Example: The lowest excited state for Na lies at 3.371×10^{-19} J/atom above the ground state. The degeneracy of excited state is 2 and ground state is 1. (*i*) Calculate the fraction of Na atoms in excited state in air–acetylene flame at 2600 K. (*ii*) Would the fraction of atoms in the excited state increase if the temperature increases to 2610 K.

Solution:

(i) $\frac{N_e}{N_o} = \frac{g_e}{g_o} e^{-\frac{\Delta E}{KT}}$

$$= \frac{2}{1} e^{-\frac{\left(3.371 \times 10^{-19} J\right)}{1.381 \times 10^{-23} J/K\,(2600 K)}}$$
$$= 1.67 \times 10^{-4} = 0.016\%$$

(ii) when temperature is 2610 K so it increases

$$\frac{N_e}{N_o} = \frac{2}{1} e^{-\frac{\left(3.371 \times 10^{-19}\right)}{1.381 \times 10^{-23} \times 2610}}$$
$$= 1.74 \times 10^{-4} = 0.017\%$$

It can be seen that the relative population ratios for elements like sodium are low at lower temperature and are higher at higher temperature (Table 7.1).

Normally, the excited state population is measured in flame emission methods (FES) and the ground-state population is measured in atomic absorption methods although both methods are equally affected by temperature variations and the chemical reactions taking place in the flame.

Table 7.1 N/N_o values at different temperatures

Resonance line (nm)	N/No		
	2000 K	3000 K	10,000 K
Na 589.0	9.9×10^{-6}	5.9×10^{-4}	2.6×10^{-1}
Ca 422.7	1.2×10^{-7}	3.7×10^{-3}	1.0×10^{-1}
Zn 213.8	7.3×10^{-15}	5.4×10^{-10}	3.6×10^{-3}

7.5 Instrumentation of FES

Flame photometers use flames as its radiation source which are much lower energy source compared to the electrical sources. The flame which produces a simple emission spectrum with few lines is the most suitable for flame photometer. The flame used in the flame photometer should possess the following characteristics (Fig. 7.2):

(a) the flame should be capable of evaporating the liquid droplets from the sample solution and from a solid residue;
(b) should be able to decompose the solid to atoms; and finally.
(c) should excite the atom.

The above characteristics are governed by the flame temperature, fuel–oxidant ratio, and also amount of solvent entering the flame other than the nature of fuel and solvent. Relatively cool flames, e.g. air–propane mixtures are commonly used in flame photometer and the emitted radiation is isolated by an optical filter, generally an interference filter is used. These signals are then converted to electrical signals by the photodetector, a photomultiplier. Layout of a simple flame photometer is as follows.

When air is passed through a pressure regulator and flowmeter into an atomizer which introduces the liquid sample into the flame at stable and reproducible rates, the fuel gas which is usually propane is supplied to the burner, gets mixed with air, and the mixture gets burned. The radiation from the flame where the sample is sprayed first passes through lens and then an optical filter. The optical filter is usually as interference filter which acts as a monochromator which only allows the radiation characteristic of elements to pass through to reach the photodetector. The photodetector is the photomultiplier tube which first amplifies the signals received and then sends these signals to readout device. Only those detectors are used in which the temperature is uniformly controlled (Fig. 7.3a and b).

Using a double-beam flame photometer is more advantages as it observes greater precision. A standard solution of lithium is continuously run and is used as a reference. The intensities of the lines produced by Na or K containing samples are compared to Li and are quantitatively estimated by their ratios (Fig. 7.4).

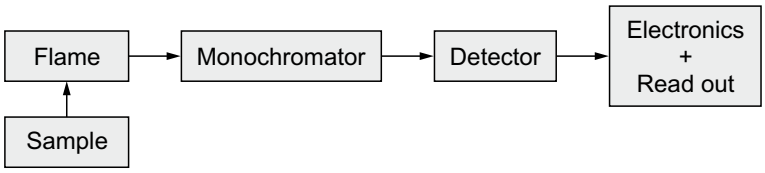
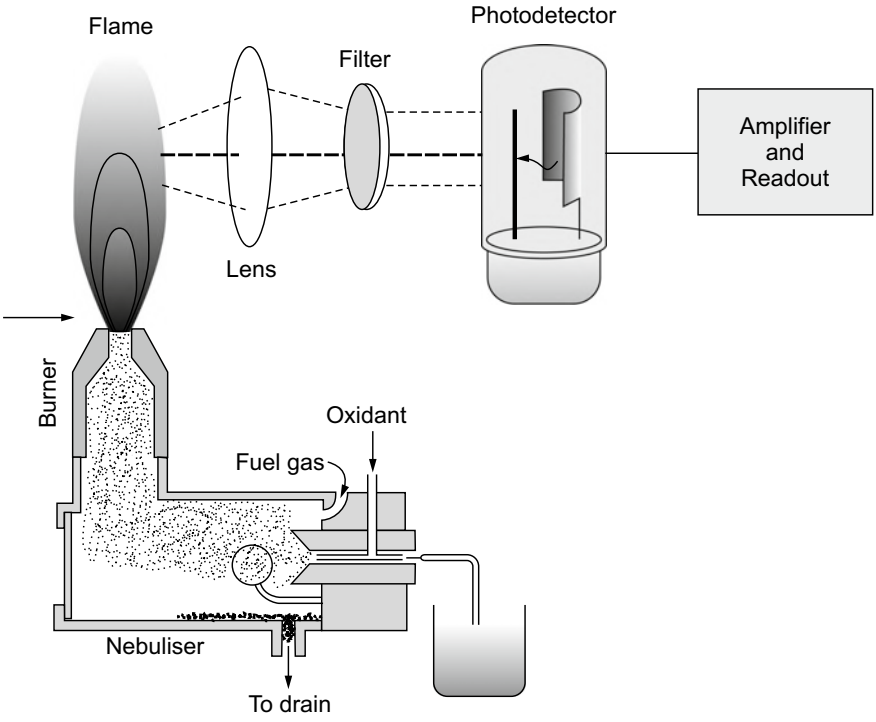


Fig. 7.2 Schematic diagram of flame emission instrument

(*a*) A flame photometer



(*b*) Layout of a flame photometer

Fig. 7.3 **a** A flame photometer. **b** Layout of a flame photometer

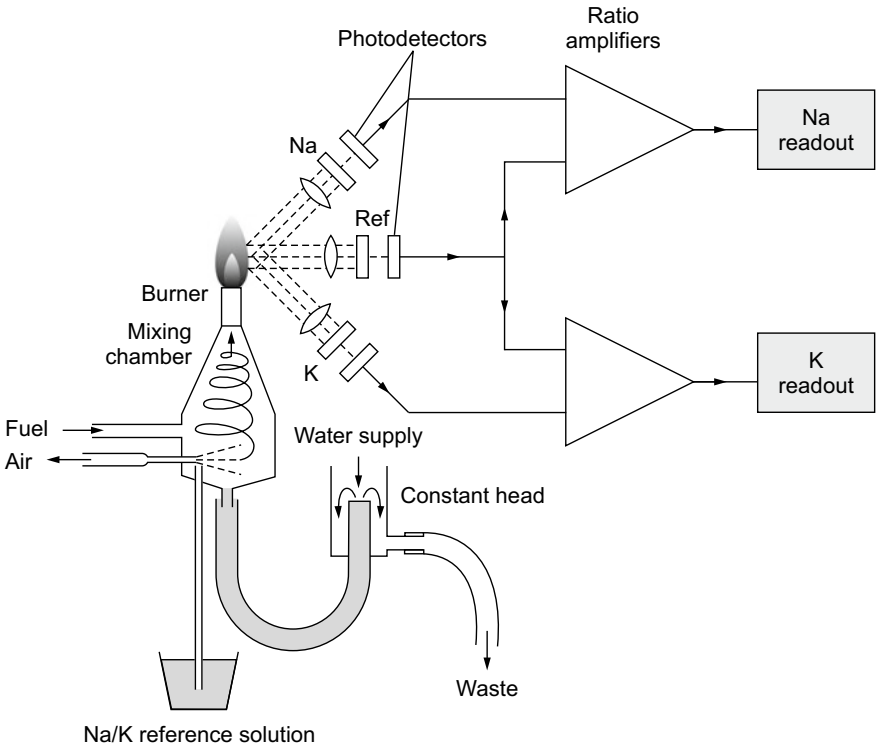


Fig. 7.4 Double-beam flame photometer

7.6 Interferences in Flame Photometry

Similar to AAS, mainly three types of interferences are found in flame photometry. **Spectral interference**, **chemical interference,** and **self-absorption** due to ionization and dissociation into ions are the common categories of interferences. Spectral interferences are overcome by using monochromators and calibrating such that suitable spectral lines are chosen. Taking blank solutions also help in eliminating background interference. Self-absorption weakens the intensity of spectral lines and affects the resonance line more strongly. Such interferences are prevented by controlling the concentrations of the solution and avoiding the presence of various salts and acids. Also addition of reductant can eliminate the formation of certain anions and some chelating agents so as to block the cations produced due to dissociation are prevented from causing interference.

7.7 Evaluation of Results

The results obtained from flame emission spectroscopy are evaluated in three different ways.

Calibration curve: They help to find the concentration of the analyte by comparing the measured emission values with the standard curves obtained for standard solutions. At least four standard solutions should be measured in the optimum absorbance range 0.1–0.4. Even the test solution may be diluted to give absorbance reading in the range 0.1–0.4. The spectra of the unknown sample and various standards are photographed alternately on the same plate under the same set of conditions. Comparison of the spectral lines can give the concentration of the sample.

Internal standard method: This involves the addition of fixed amount of a reference material, which is also known as internal standard, to both the sample solution and the standard solution. After the excitation the emission spectra of the analyte and the internal standard are measured simultaneously either by two photodetectors or measuring together with a double-beam spectrometer. The two measurements made were the intensity of the sample emission line and the intensity of the internal standard emission line. The concentration of these two can be determined by the ratio of the intensities of the internal standard and the sample. The internal standard method is capable of avoiding the variations arising due to chemical interferences and self-absorption.

For example, in the course of analysing Mn in steel, iron may be used as the internal standard and the intensities of manganese and iron are determined. By finding the ratios of the intensities of their spectral lines and plotting on a graph the percentage composition of Mn in steel can be determined.

Standard addition method: This is another method to evaluate the results of emission spectroscopy. In this method, two solutions are taken, the unknown sample solution and a second solution which contains the same amount of the measured amount of the unknown solution, in addition to a measured amount of standard solution of the element. A standard calibration curve is plotted with the spectral lines obtained, and this is how an unknown sample is determined.

7.8 Advantages of Flame Photometry

Flame photometry has always been recognized as one of the advantageous methods found among the various emission spectroscopic techniques. Use of AAS in the emission mode has enhanced sensitivity and fewer background interferences than in the flame atomic absorption spectroscopy. Flame emission spectroscopy or flame photometry has following benefits:

(i) Its cost of running and maintenance is low.

(ii) An excellent method for elemental trace analysis. A few milligrams or even low amount is enough for analysis.
(iii) Around 60 elements can possibly be analysed in FES.
(iv) Can be successfully used to analyse metals, metalloids, and some other elements.
(v) It can be used for routine quality control work and forensic studies.
(vi) This method is ideally used to analyse alloys, soils, environmental, and biological samples. The only drawback being that after the analysis the sample gets destroyed.

7.9 Plasma Emission Spectroscopy

In all types of previously discussed atomic absorption (AAS) spectroscopy methods and in atomic emission spectroscopy methods, thermal or electrical energy is widely used for excitation of atoms. For atomization, followed by excitation generally the radiation sources used are flames, furnaces, and electric discharge. Both flames and furnaces produce high temperatures which sometimes are either not sufficient to cause ionizations and excitations or cause self-absorption at high temperatures. Electrical discharges also produce very high temperatures (~7500 K) but do not give reproducible results. To overcome all these shortcomings, the use of a plasma as an atomization source for emission spectroscopy has been developed in the past.

A plasma may be defined as a gas which contains majorly electrons, positive ions, and neutral molecules. Such an ionized gas mixture has high energy content and shows high electrical conductivity. The gaseous ions are capable of dissociating as well as ionizing the atoms and they also excite, then to achieve higher energy levels from which emissions are produced on returning to the ground states.

Mainly three types of plasma sources are known: **inductively coupled plasma (ICP)**, **direct current plasma (DCP),** and **microwave-induced plasma (MIP)**. Out of these, ICP is the most preferred one because it has higher sensitivity and least background interferences.

Generally when a strong electric field is created in an inert gas like argon, it causes ionization of the gas which produces a plasma cloud. Such plasma sources operate at very high temperatures (between 7000 and 15,000 K), thus large number of excited atoms are obtained and the source can reproduce atomization with greater precision. A plasma source can also be handled simply and can be used as a source of radiation for liquid samples where an arc or spark source does not work. The greatest advantage of plasma source lies in the fact that it can produce the spectra for a large number of elements simultaneously.

7.9.1 Inductively Coupled Plasma Source (ICP)

Since argon gas is frequently used for emission analysis, its ions along with electrons and the cations from the sample mainly constitute the plasma and can absorb sufficient power from an external source. It can maintain the high temperature for long duration and further ionizations can help to prolong it. Therefore, use of argon gas is the key feature of inductively coupled plasma source. The design of a typical ICP source, known as torch, consists of three concentric quartz tubes, through which streams of argon gas flow over the opening on the top. The stream of argon carries the sample in the form of an aerosol which passes through the central tube (Fig. 7.5).

A radio-frequency generating induction coil is fixed around the top opening of the tube and it radiates 0.5 to 2 KW of power at 27–40 megahertz frequency. Through induction from the concentric tubes this radiation reaches the aerosol in the centre and causes its excitation. The ionizations are initiated by a spark from a Tesla coil and the resulting ions and electrons flow in circular path due to their interaction with the fluctuating magnetic field produced by the induction coil. This causes heating of the plasma and the helical pattern provides thermal stability and thermal isolation of the outermost quartz tube. This isolation is possible due to the tangential flow of argon around the walls of the tube so that the sample is introduced into the cool central hole of the tube. The radiation emitted from the centre of the plasma is then used for analysis.

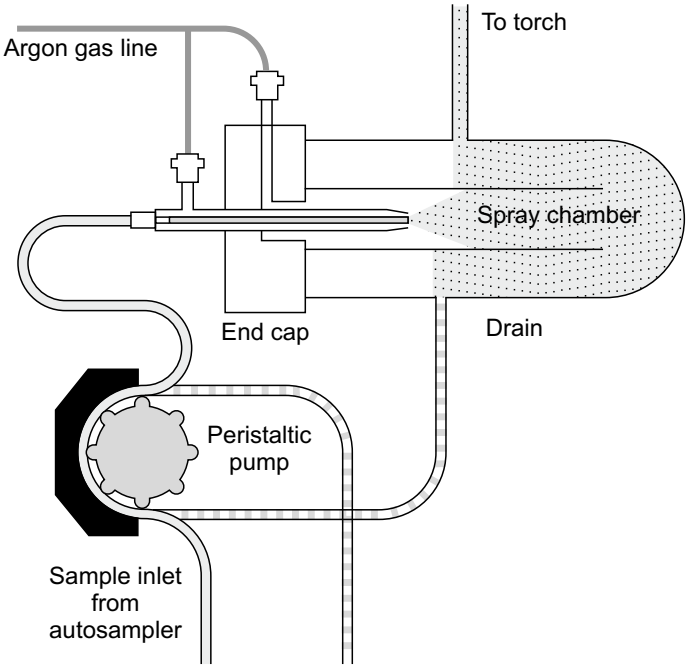


Fig. 7.5 Design of an ICP torch

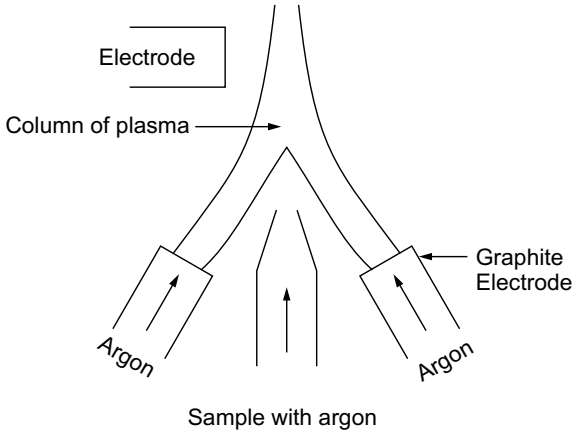


Fig. 7.6 DCP source

The gas temperature in ICP is ~ 7000 K which is suitable to eliminate chemical interference as the sample inside the plasma stays for a very short while (~2 milli seconds). Monochromators are used to eliminate spectral interferences and obtain single wavelength as plasma emissions are polychromatic.

7.9.1.1 Direct Current Plasma (DCP)

A wide range of elements can be analysed for their emission spectra using direct current plasma source. The spectra produced by DCP source have fewer lines than those produced by ICP source because they are mainly produced by atoms and not ions. The DCP plasma source consists of a high voltage discharge between two graphite electrodes and a third electrode is placed in an inverted Y-shape because such an arrangement improves the stability of the discharge. Similar to ICP, the sample is nebulized at flow rate of 1 ml min^{-1} along with argon gas which gets ionized by high voltage discharge. A current of ~20 A is generated by the ionization of argon gas which further gives more ions and so the current is sustained. The temperature between the arc is ~ 8000 K and it is this area where the sample is aspirated to get atomized and excited.

Although the sensitivity of DCP source in emission spectroscopy is lower than ICP but less argon is required and power supply is simple. Organic solutions having high solid content can be handled better with DCP than with ICP but the electrodes in DCP require higher maintenance though at a lower cost than ICP (Fig. 7.6).

7.9.1.2 Sample Introduction

In ICP the sample used is in the form of a solution which is introduced into the central quartz tube where argon gas is flowing at the rate of 1 L/min. For this purpose, a

concentric glass nebulizer is used. The sample is made to erupt from a nozzle in such a way that the high velocity gas breaks up the liquid into fine droplets of various sizes. Among the various types of nebulizers used, the crossed flow nebulizer is most commonly used. The sample is thrusted into the mixing chamber at a flow rate of 1 ml/ min by using a rhythmic pump and is nebulized by the streamlined flow of argon gas at the rate of ~ 1L min^{-1}.

Samples containing higher percentage of solid require a V-shaped channel in which the sample solution is pumped. Also addition of a spray chamber before the plasma is placed greatly reduces the particle size of the aerosol. Ablation techniques are used to produce particulate aerosol. Two types of ablation methods, laser oblation and spark ablation, are available, both of them are meant to transfer particulate and mobilized aerosol into the plasma.

7.9.2 ICP Instrumentation

ICP instrumentation pattern is almost similar to the one used for AAS or flame emission spectroscopy. Just that the atomization unit in AAS is graphite furnace, in flame photometer, there are various types of flames whereas in ICP it is fulfilled by ICP torch. The instrumentation assembly of ICP consists of the following components with its schematic diagram given in Fig. 7.7.

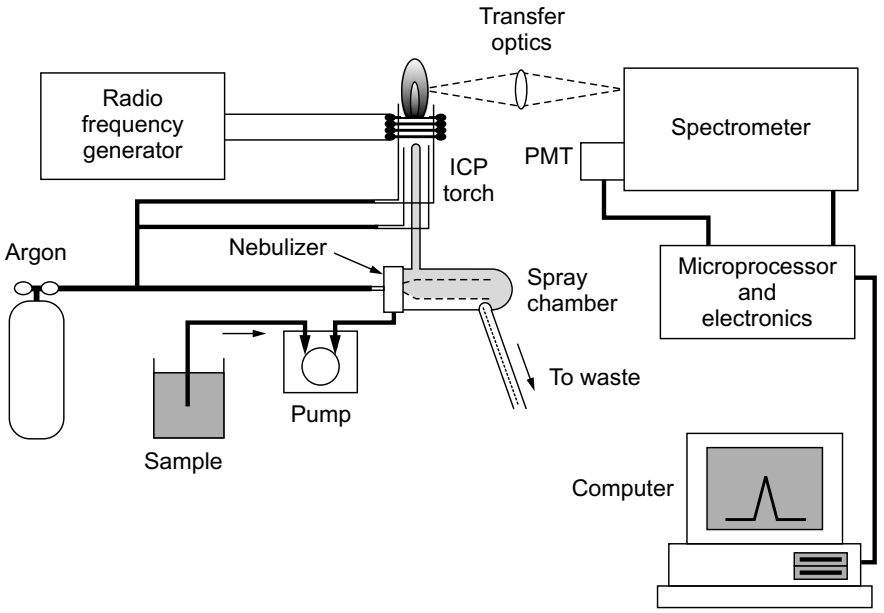


Fig. 7.7 Schematic diagram of an ICP instrument assembly

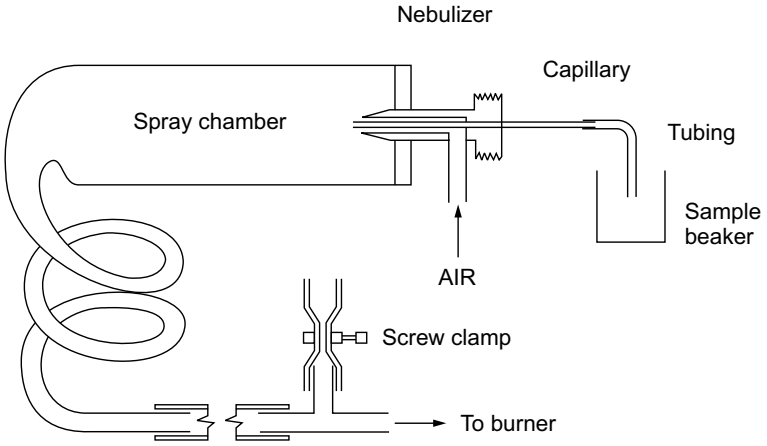


Fig. 7.8 Design of a crossed flow nebulizer

(*a*) Nebulizer, (*b*) Pumps, (*c*) spray chamber, (*d*) sample introducer, (*e*) ICP torch, (*f*) radio-frequency generator, and (*g*) detector (Fig. 7.8).

Nebulization is a process in which the liquid breaks into fine mist of small droplets and nebulizers are used for this purpose, nebulizers function as devices which transform the liquid samples to aerosols so that the broken droplets of the sample can be easily transferred into the plasma. To enable the liquid sample to forcefully enter the nebulizer, the high-pressure pumps are used. Spray chambers play a vital role as they are used to transfer the liquid droplets into the plasma. While in process they also withdraw some remaining liquid from the sample. Sample injectors are basically sample holders and they hold the sample till the pressure created by the pump pushes it into the nebulizer. ICP torch functions as the main plasma source, as described earlier. Radio-frequency generator provides power for the generation of plasma discharge. The detector used in ICP instrumentation is photomultiplier tubes which first amplify the emitted radiations and then measure their intensities.

7.9.3 Application of ICP-AES

Inductively coupled plasma source in atomic emission spectroscopy has a wide range of applications because of certain acclaimed advantages mentioned below:

1. ICP can perform for a wide range of sample concentrations 0.1 to 1000 $\mu g\ ml^{-1.}$.
2. Simultaneously many elements can be analysed from a sample which may also help to attain precision.
3. Higher solid content in solutions can be handled with greater accuracy by ICP-AES.

4. More simplified analysis is carried out with ICP-AES techniques, and therefore are conveniently used for analysis of agricultural products and foods. Metal traces in commercial solvents, oils, alcohols, beverages, and blood samples can be analysed for metals like Ca, Cu, Fe, Mg, Mn, K, and Zn. Al in blood, Cu in brain tissue, Se in liver, and Na is breast milk and urine samples can also be easily analysed.

While comparing ICP-AES with AAS, the AAS becomes an obvious choice for an analyst because lower skills are required for its operation than used for ICP-AES. The detection limits are better in ICP-AES than in AAS although interferences are fewer in AAS than ICP-AES. The ability of ICP-AES technique to measure many elements simultaneously is the highlight of this method; however, ICP-AES is more expensive than AAS and has higher operating cost than AAS.

Unsolved Problems

1. What are the differences between atomic absorption spectroscopy and flame emission spectroscopy?
2. Discuss how atomic absorption spectroscopy has advantages above flame emission spectroscopy.
3. How does the spectra obtained from excited molecules differ from those obtained from excited atoms?
4. Give the schematic diagram of instrument used in flame emission spectroscopy.
5. What is Plasma emission spectroscopy?
6. How many types of plasma sources are known and which one is preferred?
7. What are the advantages of ICP?
8. How does ICP differ from DCP?
9. State the different components of ICP instrumentation.
10. State various applications of ICP-AES.

Chapter 8
Thermal Methods

8.1 Introduction to Thermal Methods of Analysis and Its Instrumentation

Chemical analysis is very often based on temperature changes because many physical and chemical properties of chemicals vary as a function of temperature. Therefore, those methods of analysis in which physical and chemical changes of a compound are measured as a function of temperature are called thermal methods (Fig. 8.1).

- **Thermogravimetry (TG):** It is a technique in which the variation in weight is recorded with change in temperature or time. The instrument commonly used is thermobalance which measures mass and a curve of mass *versus* temperature with time is recorded.
- **Differential thermal analysis (DTA):** When the sample and reference are both subjected to a controlled temperature exercise then the temperature difference of the sample and reference is measured as function of temperature. In this method also, measurements are made using DTA apparatus and the dm/dt or ΔT versus temperature curve is drawn.
- **Differential scanning calorimetry (DSC):** This is a technique in which a controlled temperature programme is carried out with the sample and the reference and the difference in energy absorbed or released by the sample and reference is measured as a function of temperature. Use of calorimeter to measure $\Delta H/dt$ is important and a graph of $\Delta H/dt$ *versus* temperature is drawn.
- **Evolved gas analysis (EGA):** In this method, volatile products evolved during thermal analysis are quantitatively and qualitatively analysed. For detection of the gases evolved thermal conductivity cells are used to measure thermal conductivity which is plotted against temperature to obtain a characteristic curve.

P. Malhotra, *Analytical Chemistry*,
https://doi.org/10.1007/978-3-031-26757-4_8

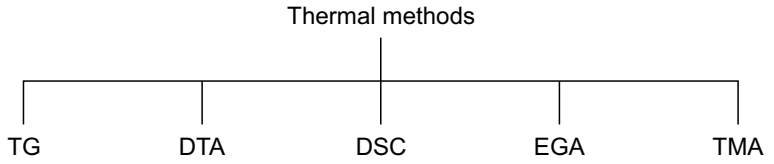


Fig. 8.1 Classification of thermal methods

- **Thermomechanical analysis (TMA):** Following this technique, the dimensional changes occurring in the samples due to heat treatment are measured. Since it measures change in volume or length, the change in volume is plotted against temperature and a curve is obtained.

Among the most widely used techniques of thermal analysis are TG, DTA, and DSC whereas EGA is used along with anyone of them.

8.2 Instrumentation of TG

Thermogravimetry is a valuable technique for determining the purity of a sample and measure a sample with great precision. An efficient thermal balance is an essential part of this technique.

Main Components of a Thermobalance

The major components of a thermobalance also referred as null point balance are categorized as (*a*) thermal unit, (*b*) sample unit, and (*c*) balance unit (Fig. 8.2).

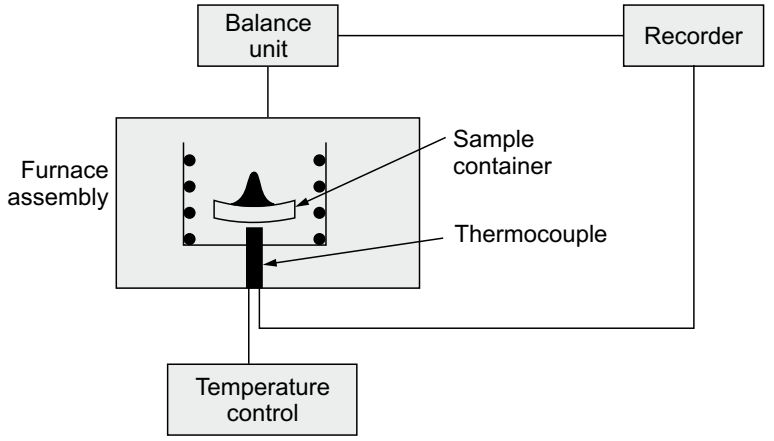


Fig. 8.2 Main components of a thermobalance

(a) Thermal unit

- Should be able to reach the maximum desired temperature (~150 to ~2400 °C)
- The rate of heating should be constant and should allow rerun of the exercise.
- Rapid heating and cooling provisions should exist.
- Good coordination between heating rates and the recorder so that accuracy in recording of heating temperatures is ensured.

(b) Sample unit

- The sample holder should occupy the hottest zone of the heating unit or furnace
- The shape and geometry of the sample container can alter the slope of thermogravimetric curve so a flat plate-shaped crucible is preferred.
- The material of the sample should itself be heat resistant in that particular temperature range and should also not get affected by the gases evolved from the furnace.

(c) Balance unit

- It should record the continuous weight change of the sample with respect to temperature and time, quite accurately
- The balance mechanism needs to be well shielded from the furnace and from the effects of corrosive effluents.
- The sensitivity of the balance should be very high and be capable of carrying out accurate isothermal studies.
- Sample size and sample history being major concerns should be optimum (Fig. 8.3).

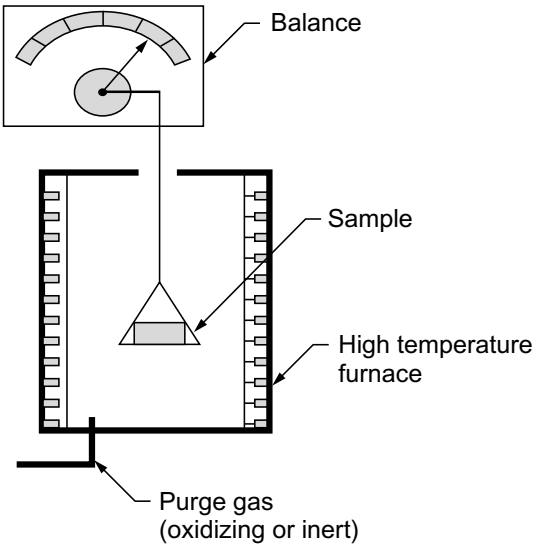


Fig. 8.3 Thermobalance with furnace heating

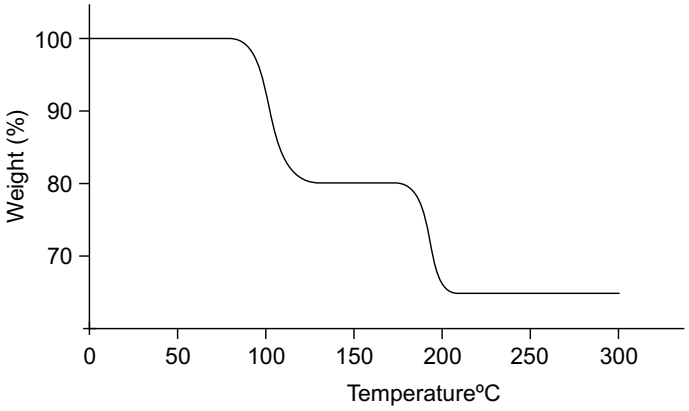


Fig. 8.4 TGA of hydrogen carbonate

In modern days, the thermobalances used are all computers controlled which facilitates a wide range of heating, cooling, and isothermal modes and also allows changes in sample atmosphere conditions automatically. The resolution and analysis time as well as the gases evolved which accompany thermal decomposition can also be monitored and finally detected by making it computer controlled (Fig. 8.4).

Factors affecting Thermogravimetric Curves: Two factors broadly affect TG curves: Instrumental effects and sample characteristics.

Instrumental effects: Heating rates influence the decomposition, fast heating rates have higher decomposition temperature than slow heating rates. For example, for 10% decomposition of polystyrene in nitrogen at 1 °C per minute the temperature is 375 °C whereas for 5 °C per minute heating rate the 10% decomposition takes place at 394 °C. For a reversible reaction, heating rates do not have any influence (Fig. 8.5).

Heating rates also influence the detection of intermediate compounds formed.

Furnace atmosphere: The surrounding gases present in the TG furnace also greatly affect the TG curves. For example, decomposition of $CaCO_3$ in CO_2 atmosphere will take place at higher temperature than in N_2 atmosphere. Thermobalances where heating in vacuum is possible is normally preferred.

Sample holder: Shape and material of sample holder have varied effects on TG curves. Shallow dishes enable rapid gaseous exchange so are preferred over cone-shaped crucibles.

Sample Characteristics: The various sample characteristics which affect TG curves are weight of sample, particle size, compactness of the sample, and prehistory of the sample majorly affect the TG curves. Smaller sized particles decompose faster and at lower temperature than larger sized particles. TG curves also get altered by the enthalpy of the reaction. Exothermic and endothermic reactions differently affect the TG curves.

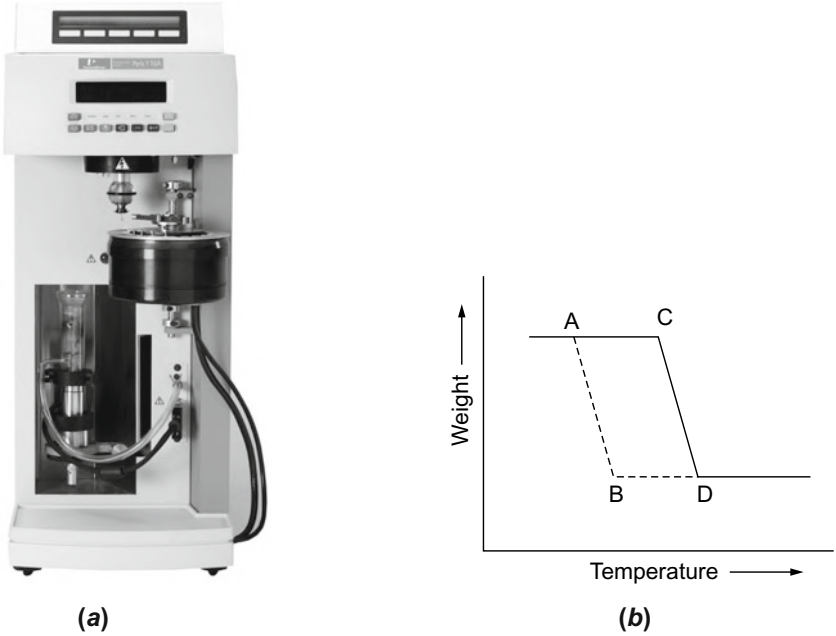


Fig. 8.5 **a** TG instrument. **b** Effect of heating rate on TG curve

8.3 Applications of Thermogravimetry

Among the various applications, the following four applications are of major importance:

1. Determination of purity and thermal stability of primary and secondary standards.
2. Direct application to various analytical methods.
3. Suitable weighing method and drying temperatures for gravimetric analysis.
4. Determination of percentage composition of mixtures and alloys.

Purity determination using TG methods is a commonly used tool. Analytical reagents used as primary standards can be efficiently checked for its moisture content as their purity affects the calculations to be applied for analytical exercises. Reagents like $Na_2\ CO_3$, $Na_2S_2\ O_3$, and EDTA which are commonly used in titrations require purity checks before being used for titrations. Thermal stability of reagents like EDTA has also been determined by TG method. Drying temperatures of gravimetric precipitates and stability limits at various temperatures are determined by TG curves.

Thermogravimetry is efficiently used to determine the composition of binary mixtures using pyrolysis curve. Without being aware of the initial weight of the sample automatic determination of a mixture is made possible by TG method. For example, as reported by Duval, automatic determination of a mixture of calcium

and magnesium as their oxalates has been successfully performed. There are three ranges of temperature in which calcium oxalate monohydrate decomposes, whereas magnesium oxalate has only two ranges.

$$\begin{aligned}
CaC_2O_4.H_2O &\rightarrow CaC_2O_4 + H_2O && 100 - 250\,^{\circ}C\\
CaC_2O_4 &\rightarrow CaCO_3 + CO && 400 - 500\,^{\circ}C\\
CaCO_3\ Ca\ O &+ CO_2 && 650 - 850\,^{\circ}C\\
Mg\ C_2O_4.2H_2O\ Mg\ C_2O_4 &+ 2H_2O && 100 - 250\,^{\circ}C\\
Mg\ C_2O_4\ Mg\ O &+ CO + CO_2 && 400 - 500\,^{\circ}C
\end{aligned}$$

The various steps of decomposition can be detected by their pyrolysis curves. Thermal stabilities of various polymers and their density have also been determined by TG methods.

TGA curve of polymers:

(a) PVC polyvinyl chloride.
(b) PMMA (polymethylmethacylate).
(c) LDPE (low-density polyethylene
(d) PTFE (poly-tetrafluoro ethylene) (Fig. 8.6).

It is evident from the TG curve that PVC has a lower decomposition temperature and has a two-stage decomposition process.

Relative efficiencies of different fuels can also be judged by TG methods in addition to their moisture content, volatile matter content, and carbon content determinations.

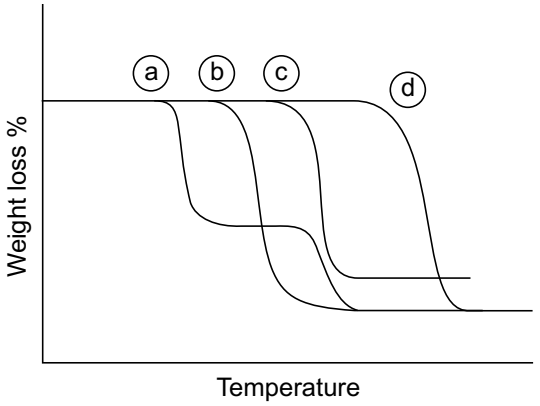


Fig. 8.6 TG curves of few polymers

8.4 Differential Thermal Analysis (DTA)

As indicated by its name differential, both the test sample and an inert reference material are run through a controlled heating or cooling programme and then recording of the temperature difference between the two with respect to time of temperature is done (Fig. 8.7).

The DTA curve remains parallel to the temperature or time axis unless the sample undergoes any physical or chemical changes. In most of the compounds, the exothermic changes are due to chemical reactions like oxidation whereas the physical changes give rise to endothermic curves. The shapes and size of the peak give wide range of information about the sample as sharp endothermic peaks indicate crystallinity change or fusion changes. Furthermore, broad peaks are due to dehydration reactions. Without indicating any weight change DTA curves are capable of detecting physical and chemical changes.

Instrumentation for DTA

The various components of DTA apparatus are as follows:

1. Furnace.
2. Sample holder.
3. DC amplifier.
4. Differential temperature detector.
5. Furnace temperature programmer.
6. Recorder.

Metallic or non-metallic sample holders may be used keeping in mind that sharp exotherms and flat endotherms are obtained from metallic holders and non-metallic holders give sharp endotherms and flat exotherms. Cylindrical-shaped sample holders

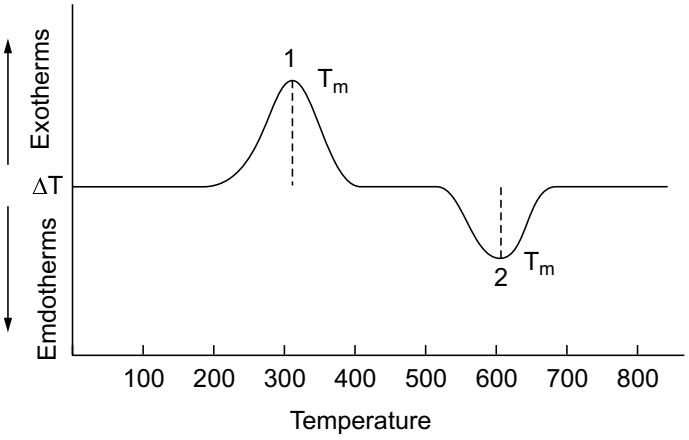


Fig. 8.7 An ideal DTA curve

having grooves in which thermocouple junctions are fitted and preferred. Depending on the required enveloping environment closed lid or open sample holders or crucibles are used.

In DTA instrumentation generally furnaces with a wide range of temperature control having uniform temperature area are suited which may also be capable of controlling rate of heating or cooling.

The recorders used in thermal instruments are tuned with sensors such that the signals are either recorded in a deflection type or in a null type of mode of recording. In deflection type, there is a direct deflection on receiving signals whereas in null type the reference and sample signals are compared.

8.5 Applications of DTA

The scope of DTA methods is wider as compared to TG methods as it includes the identification of both physical transformations and chemical changes. Other than its applications in Analytical Chemistry it is also applied for the determination of physical parameters like heat of reactions, specific heat, melting point, boiling point, and decomposition temperature. DTA helps to study phase transitions and draws phase diagrams of substances. Identification of reaction intermediates and distinction between reversible phase changes and irreversible decompositions can be made possible by DTA because of its higher resolution than TG curves. Characterization of polymers and their thermal stabilities are successfully determined by these methods. Ceramics and metal industry also make good use of DTA techniques.

8.6 Differential Scanning Calorimetry (DSC)

DSC is a thermal technique of analysis in which the instrument measures the difference in the heat flow between the sample and the reference. The energy difference is recorded as a function of time or temperature and the plot of heat flow *versus* temperature is called a thermogram. Suitably made instruments working on the principle of a calorimeter are used which measure the total energy transfer to or from the sample. Mainly three types of DSC instruments are used: power-compensated instruments, DSC instruments, heat flux instruments, and modulated DSC instrument. The power-compensated DSC has the ability to record the energy flow for the sample and reference while heating both at a constant temperature programme whereas in heat flux instrument the heat flow to the sample and reference is measured while changing temperature at a constant rate. Modulated DSC instrument is designed such that it can also record the reverse flow heating and cooling signals which are associated with the heat capacity component of the thermogram.

8.7 Applications of DSC

DSC technique is successfully used for both quantitative and qualitative analyses of various substances like polymers, by identifying their respective glass-transition temperature. Glass-transition temperature is that temperature at which the polymer undergoes changes in volume and expansion, heat flow, and heat capacity. Measurement of enthalpy of fusion made possible by DSC is used to determine the level of crystallinity. Rates of crystallization and rates of polymerization can also be measured by DSC technique. In general, DSC can be used for all applications of conventional DTA and since comparatively smaller sized sample is used it is preferred over DTA in many fields.

Solved Problems

1. Thermogram of a compound which is either MgO, $MgCO_3$, MgC_2O_4 shows mass loss of 45.5 mg from total of 87.5 mg. What compound is it?

   Solution:

   $$MgCO_3 \xrightarrow{\Delta} MgO + CO_2$$
   $$MgC_2O_4 \xrightarrow{\Delta} MgO + 2CO_2$$
   $$Mg\ O \xrightarrow{\Delta} \text{no product}$$
   $$\text{Initial mass} = 87.5\text{mg}$$
   $$\text{mass lost} = 45.5\text{mg}$$
   $$Mg\ CO_3 = 24 + 12 + 48 = 84$$
   $$CO_2 = 12 + 32 = 44$$

   Therefore, from a total of 84 mg (≈87.5 mg) loss of 44 mg (≈45.5 mg) indicates the compound to be Mg CO_3.
2. A mixture of CaO, $CaCO_3$ is analysed. Thermogram shows a reaction between 500° and 900 °C. Where the mass of sample decreases from 125.3 mg to 95.4 mg. What is % composition of the mixture?

   Solution:

   $$\underset{125.3}{CaO + CaCO_3} \xrightarrow{\Delta} \underset{95.4}{CaO + CaO + CO_2}$$

   $$\text{Mass of } CO_2 = 125.3 - 95.4$$
   $$= 29.9$$

   If 44 g of CO_2 is obtained from 100 g $CaCO_3$.

Then $29.9 \frac{100}{4} \times 29.9 = 67.95$

Therefore, in a mixture of CaO + $CaCO_3$ (125.3 mg), 67.95 gm is $CaCO_3$. The percentage composition of

$$\text{CaCO}_3 = \frac{67.95}{125.3} \times 100$$
$$= 54.14\%$$
$$\text{amount of CaO} = 45.85\%$$

Unsolved Problems

1. In analysing 250 mg sample of $Ca(OH)_2$ containing $CaCO_3$ and SiO_2 by TG method, the losses in mass were detected at various temperatures.

   (i) loss of H_2O (100–150 °C) = 18 mg.
   (ii) Dehydration of $Ca(OH)_2$ (510°–560 °C) = 38 mg.
   (iii) Dissociation of $CaCO_3$ (900–950 °C).

   Determine the composition of technical $Ca(OH)_2$.
2. TG of mixture of CaC_2O_4, MgC_2O_4, and silica. is given. Find composition of mixture.
3. What are the different methods of thermal analysis and what are they based on?
4. What are the main components of a thermobalance and what are their functions?
5. Illustrate a thermogravimetric curve and discuss the factors affecting a thermogravimetric curve.
6. Explain differential thermal analysis (DTA) and give an ideal DTA curve.
7. Discuss various components of instrumentation of a DTA apparatus.
8. Explain differential scanning calorimetry (DSC) and its applications.

Answers

1. 62.48%,

2. $CaC_2O_4 = 46.54\%$ $MgC_2O_4 = 33.46\%$

Chapter 9
Electroanalytical Methods

9.1 Introduction

A large number of qualitative and quantitative analyses are based on electrochemical principles and their measurements are based on electrical properties like current, resistance, conductance, and voltage. Evaluation of substances deposited on the electrodes as a result of chemical reactions due to the flow of current is the main focus. All these measurements are directly related to the concentration of the analyte. Electroanalytical methods have vast capabilities and are able to determine the concentrations and activity of elements in their particular oxidation states. All laws of electrochemistry are efficiently applied in various electroanalytical methods and successful analyses are carried out. These techniques are very popular and instruments are available at low cost.

9.2 Classification of Electroanalytical Methods

Electroanalytical methods are conveniently classified on the basis of the electrical quantities measured and variables controlled. There are mainly two branches of electroanalytical methods: the **interfacial methods** and the **bulk methods**. Interfacial methods are those methods in which the activities taking place on the interface of the electrode and thin layer solution are considered, whereas bulk methods involve the activities taking place in the whole bulk of solutions.

Interfacial methods have two categories, one which has no flowing current is the **static method** and the one in which the current flows in the electrochemical cell is the **dynamic method**. The static methods involve potentiometric measurements in which the potentials are measured without drawing any approachable current. These measurements are important due to their selectivity and fast results. The dynamic methods where a substantial amount of current is drawn are either measured under

P. Malhotra, *Analytical Chemistry*,
https://doi.org/10.1007/978-3-031-26757-4_9

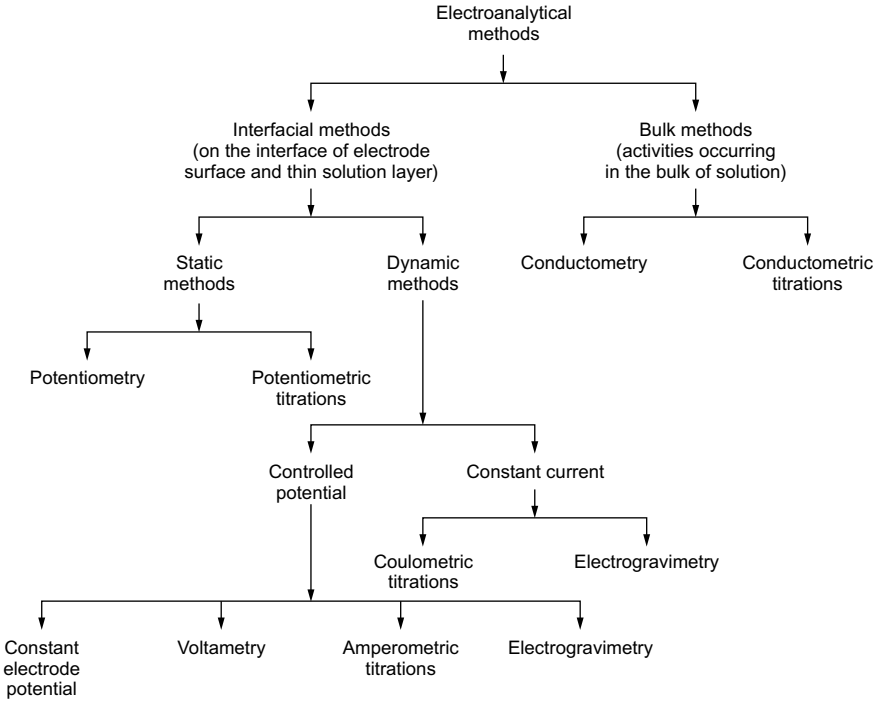


Fig. 9.1 Various categories of electroanalytical methods

controlled potential or under constant current. The currents flowing in electrochemical cells play an important role and a range of variables are measured during electroanalysis. Dynamic methods in which current drawn is maintained at a constant value have various types of measurements done. Coulometric titrations measure the charge and electrogravimetry which measures the mass of substances deported at electrodes. Briefly, electrochemical methods have the following categories (Fig. 9.1).

Potentiometry: This measures the potentials of two non-polarized electrodes under zero current and uses electrochemical laws: Nernst equation.

Voltammetry: Composition of dilute electrolyte solutions is studied under this method by plotting current–voltage graph. Concentrations of oxidized or reduced species are determined while voltage is applied to a small polarizable electrode. When a dropping mercury electrode is used as cathode and mercury pool is used as anode the technique is called polarography.

Conductometry: Using two identical inert electrodes, the conductance (1/R) between them is measured based on the principle of Wheatstone Bridge. Conductometric titrations are performed measuring conductance with a change in volume.

Coulometry: By applying Faraday's laws of electrolysis the relationship between the quantity of electricity passed and the amount of substance deposited on the electrodes are determined.

Oscillometry: In this method, changes in conductance and dielectric constant are observed while using high frequency alternating current and keeping the electrodes barred from touching the solution.

Amperometry: A constant voltage is applied and the concentration of analyte determines the current.

Controlled potential electrolysis: By controlling the potential and monitoring the substances deposited on electrodes by means of oxidation or reduction are measured coulometrically or electrogravimetrically. This measures the separated species although the potential needs to be carefully controlled.

Electrogravimetry: The element in the analyte is determined as it is deposited on a suitable electrode by electrolysis.

9.3 Electrochemical Cells

All operations of electroanalytical methods are based on electrochemical cells. An electrochemical cell consists of two conductor plates called electrodes where the two half reactions of a redox reaction take place and two electrolyte solutions in which these electrodes are dipped. The two electrolyte solutions are made to stay in contact so that electron transfer occurs during the redox reaction. For example, when Zn electrode is dipped in an electrolyte solution of $ZnSO_4$ and Cu electrode is dipped in $CuSO_4$. According to the following reaction, zinc electrode acts as anode and copper electrode acts as a cathode. Between the two solutions, there exists a salt bridge containing ions of another electrolyte (KCl).

$$Zn + CuSO_4 \rightarrow ZnSO_4 + Cu$$

Two half reactions

$$Zn \xrightarrow{-2e^-} Zn^{+2} \qquad \text{(at anode)}$$

$$Cu^{+2} \xrightarrow{-2e^-} Cu \quad \text{(at cathode)}$$

Oxidation takes place at anode and reduction at cathode. The cell is represented as

$$Zn|Zn^{+2}(1m)||Cu^{+2}(1m)|Cu$$

There are two types of electrochemical cells, voltaic (galvanic) cells, and electrolytic cells. In voltaic cells, a redox reaction occurs spontaneously and the flow of electron produces current. Thus, chemical energy gives rise to electrical energy, whereas, in electrolytic cell, electrical energy is applied which forces a non-spontaneous reaction to take place and so converts electrical energy to chemical energy (Fig. 9.2).

Voltaic cell is important for potentiometry and electrolytic cells are important in electrochemical methods such as voltammetry. In voltammetry electroactive substances like metal ions are reduced at an electrode and flow of electrons are responsible for the current generated. By applying appropriate potential a non-spontaneous reaction is made to occur and the resulting current from forced electrolysis is proportional to the concentration of the electroactive substance.

9.4 Electrode Potential

As mentioned earlier, every redox reaction can be divided into two half reactions because every redox reaction has an electron donor (a reducing agent) and an electron acceptor (oxidizing agent). Every half reaction takes place at an electrode which is inert itself and is dipped in solution. Each half reaction generates a definite potential which is the electrode potential and is the interfacial potential difference at the corresponding electrode.

The electrode potential or the standard electrode potential, which is defined as the electrode potential at standard temperature and when the solution in which the electrode is dipped has M concentration ($\mathcal{A} = 1$) and can be measured by joining it with standard hydrogen electrode (SHE). The standard hydrogen electrode consists of acatalytic Pt surface in contact with an acidic solution in which $A_H^+ = 1$ (activity $\mathcal{A}$). A stream of H_2(g) bubbled through the electrode saturates the solution with H_2(aq). The activity of H_2(g) is unity if the pressure of H_2(g) is one bar.

The reaction occurring at the Pt electrode, comes to an equilibrium, which is SHE half reaction:

$$H^+(\text{aq}, \mathcal{A} = 1) + e^- \rightleftharpoons \frac{1}{2}H_2(g\ \mathcal{A} = 1)$$

We arbitrarily assign a potential of zero to the standard hydrogen electrode at 25 °C. The electrode whose potential is to be measured is joined with SHE, such that SHE makes the anode and the electrode as the cathode. The reading on the voltmeter measures the reduction potential of the half reaction taking place at the electrode and thus also measuring the electrode potential. For example, to measure the electrode potential of silver electrode by giving the following half reaction,

$$Ag^+ + e^- \rightarrow Ag(s)$$

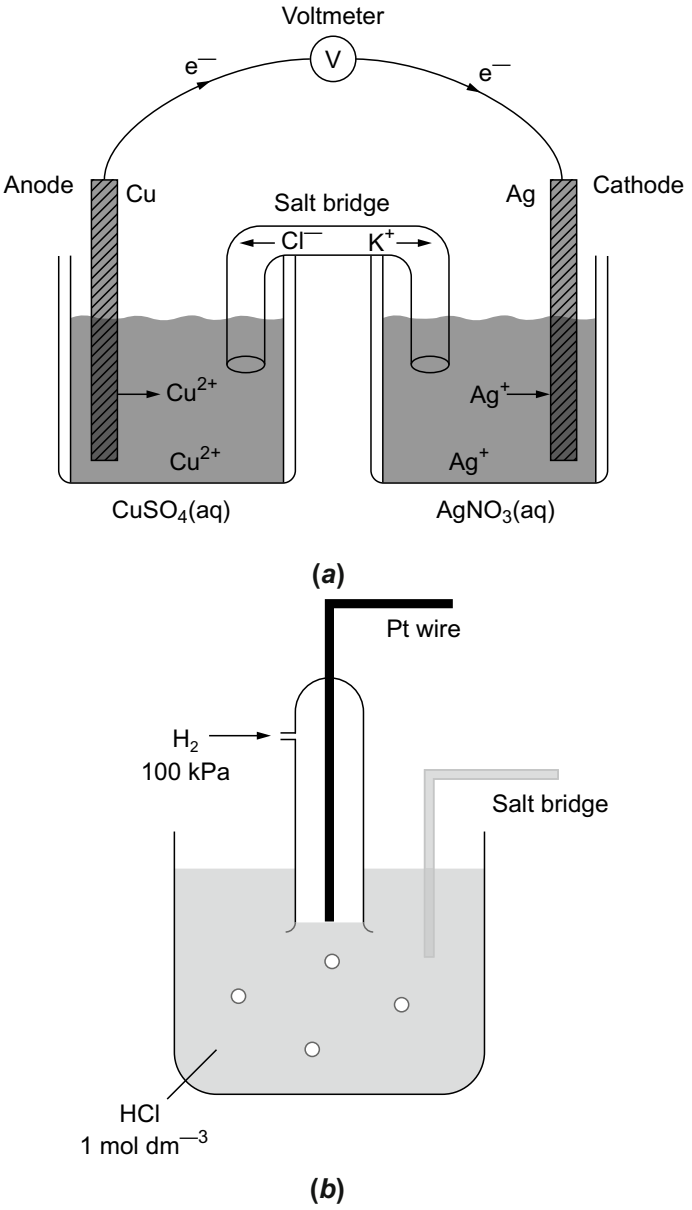


Fig. 9.2 **a** An electrochemical cell. **b** A standard hydrogen electrode (SHE)

The measured $E^\circ = 0.799$ V is the standard reduction potential for the above reaction. The positive sign indicates that electrons flow from left to right through the meter.

Measurement of electrode potential by this method and formation of a cell with SHE can be represented as

$$\mathrm{Pt(S)|H_2(g, \mathcal{A} = 1)|H^+(aq\mathcal{A} = 1)||Ag^+(aq, \mathcal{A} = 1)Ag(S)}$$

By convention, the left electrode (Pt) is attached to the negative (reference) terminal of the potentiometer and the right-hand electrode is attached to the positive terminal. A standard reduction potential is actually the potential difference between the potential of the reaction of interest and the potential of SHE, which has been taken to be zero. Some general conclusions drawn from the values of electrode potential are:

(1) The more positive the electrode potential, the greater the tendency of the oxidized form to be reduced. The more positive the electrode potential, the stronger an oxidizing agent the oxidized form is and the weaker a reducing agent the reduced form is.
(2) The more negative the electrode potential, the greater the tendency of the reduced form to be oxidized. The more negative the reduction potential, the weaker an oxidizing agent the oxidized form is and the stronger a reducing agent and the reduced form is.
(3) The oxidized form of a species in a half reaction has the tendency of oxidizing the reduced form of a species in a half reaction whose reduction potential is more negative than its own, and vice versa. The reduced form in a half-reaction has a tendency of reducing the oxidized form in a half reaction with a more positive potential.

For example, consider the half reactions

$$\mathrm{Fe^{+3} + e^- \rightarrow Fe^{+2}}\ E^\circ = 0.771\ \mathrm{V}$$

$$\mathrm{Sn^{+4} + 2e \rightarrow Sn^{+2}} E^\circ = 0.154\ \mathrm{V}$$

Obviously, Fe^{+3} is capable of oxidizing Sn^{+2} and not the opposite is possible

$$\mathrm{2Fe^{+3} + Sn^{+2} \rightarrow 2Fe^{+2} + Sn^{+4}}\left(E^\circ = 2 \times 0.771 - 0.154\right);\ \left(E^\circ = 1.388\right)$$

Standard electrode potentials of metals are useful in predicting the feasibility of the combination cells formed with other metals. The negative E° values w.r.t. SHE for metals makes them more powerful reducing agents than H, and positive E° values makes them less powerful reducing agent than H. Some typical standard electrode potentials are listed in Table 9.1.

Table 9.1 Some standard electrode potentials of some metals

Metal	Chemical reaction	Potential E at 25 °C (in Volts)
Ag	$Ag^{+} + e^{-} = Ag$	0.799
Al	$Al^{+3} + 3e = Al$	–1.662
Ba^{+2}	$Ba^{+2} + 2e- = Ba$	–2.906
BiO^{+}	$BiO^{+} + 2H^{+} + 3e^{-} = Bi + H_2O$	+0.320
Ca	$Ca^{+2} + 2e^{-} = Ca$	+1.44
Cd	$Cd^{+2} + 2e^{-} = Cd$	–0.403
Co	$Co^{+2} + 2e^{-} = Co$	–0.277
Cu	$Cu^{+2} + e^{-} \rightarrow Cu^{+}$, $Cu^{+} + e^{-} = Cu$	+0.153 V, +0.5221
Fe	$Fe^{+2} + 2e \rightarrow Fe$, $Fe^{+3} + e^{-} = Fe^{+2}$	–0.440 V, +0.771
Hg	$2Hg^{+2} + 2e^{-} \rightarrow Hg_2^{+2}$	+0.920
K	$K^{+} + e^{-} = K$	–2.925
Mg	$Mg^{+2} + 2e^{-} = Mg$	–2.363
Mn	$Mn^{+2} + 2e^{-} = Mn$	–1.180
Na	$Na^{+} + e^{-} = Na$	–2.714
Ni	$Ni^{+2} + 2e^{-} = Ni$	–0.250
Pb	$Pb^{+2} + 2e^{-} = Pb$	–0.126
Pd	$Pd^{+2} + 2e^{-} = Pd$	+0.987
Sn	$Sn^{+2} + 2e = Sn$, $Sn^{+4} + 2e^{-} = Sn^{+2}$	–0.136
Ti	$Ti^{+3} + e^{-} = Te^{+2}$	–0.369
Tl	$Tl^{+} + e^{-} = Tl$	–0.336
Zn	$Zn^{+2} + 2e^{-} = Zn$	–0.763

A relative driving force for a half reaction with respect to Hydrogen electrode can be determined from the values given in the Table 9.1. Even the direction of the reaction between two metals can be concluded by finding E_{cell}, taking one of them at cathode and the other anode. Positive value for the E_{cell} value indicates the spontaneity of the reaction.

9.5 The Nernst Equation

The standard cell potentials are calculated by using two standard electrode potentials of an electrochemical cell using the formula

$$E^{\circ}_{\text{cell}} = E^{\circ}_{\text{cathode}} - E^{\circ}_{\text{anode}}$$

where $E^{\circ}_{\text{cathode}}$ and E°_{anode} are the reduction potentials of the two electrodes which form the cell.

All these are standard potentials and are calculated by unit activity or unit concentrations of the electrolyte. The cell potential is dependent on the concentrations of the species and is different from the standard cell potential. Nernst equation shows the dependence of cell potential on reagent concentrations.

For the half reaction

$$aA + ne^{-} \rightleftharpoons bB \tag{9.1}$$

the Nernst equation for half cell potential

$$E = E^{\circ} - \frac{RT}{nF} \ln \frac{\mathcal{A}_B^b}{\mathcal{A}_A^a} \tag{9.2}$$

where

E° = standard reduction potential
R = gas constant = 8.314 J/K mol.
T = Temperature (K).
n = no. of electrons in the half reaction.
F = Faraday constant 9.649×10^4 C/mol.
$\mathcal{A}_i$ = activity of species i.

Nernst equation at 25 °C

$$E = E^{\circ} - \frac{0.0591}{n} \log \frac{\mathcal{A}_B^b}{\mathcal{A}_A^a} \tag{9.3}$$

Just as multiplying a half reaction by any number does not change the standard reduction potential E°, the half cell potential, E also does not change by multiplying a half reaction by any number.

$$aA + bB \rightleftharpoons cC + dD$$

For a redox reaction, Nernst equation is

$$E_{\text{cell}} = E^{\circ}_{\text{cell}} - \frac{0.0591}{n} \log \frac{\mathcal{A}_C^c \mathcal{A}_D^d}{\mathcal{A}_A^a \mathcal{A}_B^b} \tag{9.4}$$

$$E_{\text{cell}} = E^{\circ}_{\text{cell}} - \frac{0.0591}{n} \log Q \tag{9.5}$$

where Q is the reaction Quotient for the whole cell and

$$Q = \frac{\mathcal{A}_C^c \mathcal{A}_D^d}{\mathcal{A}_A^a \mathcal{A}_B^b}$$

Therefore

$$E = E_0 - \frac{0.0591}{n} \log Q \tag{9.6}$$

Equation (9.6) is valid at any point of the reaction but in the special case when the cell is at equilibrium $E = 0$ and $Q = K$, the equilibrium constant. Therefore, Eq. 9.6 gets transformed into

$$E^\circ = \frac{0.05916}{n} \log K \,(\text{at } 25\ ^\circ\text{C}) \tag{9.7}$$

$$\log K = \frac{nE^\circ}{0.05916}$$

$$10^{\log K} = 10^{nE^\circ/0.05916}$$

$$K = 10^{nE^\circ/0.05916} (\text{at } 25\ ^\circ\text{C}) \tag{9.8}$$

Example 1: Write the Nernst equation for the following half reaction

$$\frac{1}{4} P_4(S,\ \text{white}) + 3\text{H}^+ + 3e^- \rightleftharpoons \text{PH}_3(g)\ E^\circ = -0.046\ \text{V}$$

Solution: Solids are omitted and the concentration of gas is expressed as the pressure of the gas. Thus, the Nernst equation is

$$E = -0.046 - \frac{0.0591}{3} \log \frac{\text{P}_{\text{PH}_3}}{\left[\text{H}^+\right]^3}$$

Example 2: Find the voltage of the cell if the right half cell contains 0.50 M Ag NO_3(aq) and the left half cell contains 0.010 M $Cd(NO_3)_2$(aq) and their standard reduction potentials are 0.799 V and –0.402 V respectively. Write the net cell reaction and state whether it is spontaneous in the forward or reverse direction.

$$\text{Right electrode } 2\text{Ag}^+ + 2\text{e}^- \rightleftharpoons 2\text{Ag(s)}$$
$$E_+^\circ = 0.799\text{V}$$
$$\text{Left electrode } \text{Cd}^{+2} + 2\text{e}^- \rightarrow \text{Cd(S)}$$
$$E^\circ = -0.402\ \text{V}$$

Nernst equation for the right electrode

$$E_+ = E_+^\circ - \frac{0.0591}{2}\log\frac{1}{\left[Ag^+\right]^2} = 0.799 - \frac{0.059}{2}\log\frac{1}{(0.50)^2} = 0.781\ \text{V}$$

left electrode

$$\begin{aligned} E- &= E^\circ - \frac{0.0591}{2}\log\frac{1}{\left[Cd^{+2}\right]} \\ &= -0.402 - \frac{0.0591}{2}\log\frac{1}{[0.010]} \end{aligned}$$

Cell Potential (Voltage)

$$\begin{aligned} E &= E+ - E- \\ &= 0.781 - (-0.461) \\ &= +1.242\ \text{V} \end{aligned}$$

Since it is a +ve value the reaction

$$Cd + 2Ag^+ \rightleftharpoons Cd^{+2} + 2Ag(S)$$

will take place in the forward direction, Cd(S) will get oxidized and Ag^+ will get reduced.

Example 3: Find the equilibrium constant for the reaction

$$Cu(S) + 2Fe^{+3} \rightleftharpoons 2Fe^{+2} + Cu^{+2}$$

Solution: Dividing the reaction into two half reaction,

$$\begin{aligned} 2Fe^{+3} + 2e^- &\rightleftharpoons 2Fe^{+2} \quad E_+^\circ = 0.771\ \text{V} \\ Cu^{+2} + 2e^- &\rightleftharpoons Cu(S) \quad E^\circ - = 0.339\ \text{V} \\ E_{\text{cell}}^\circ &= E_+^\circ - E_-^\circ = 0.771 - 0.339 = 0.432\ \text{V} \end{aligned}$$

Equilibrium constant

$$\begin{aligned} K &= 10^{(2)(0.432)/0.0591} \\ &= 4 \times 10^{14} \end{aligned}$$

Example 4: A solution of 10^{-3} M in $Cr_2O_7^{-2}$ and 10^{-2} M in Cr^{+3}. If the pH is 2.0 what is the potential of half reaction?

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

Solution:

$$\begin{aligned} E &= E^{\circ}_{Cr_2O_7^{-2}/Cr^{+3}} - \frac{0.0591}{6} \log \frac{\left[Cr^{+3}\right]^2}{\left[Cr_2O_7^{-2}\right]\left[H^+\right]^{14}} \\ &= 1.33 - \frac{0.0591}{6} \log \frac{\left(10^{-2}\right)^2}{\left(10^{-3}\right)\left(10^{-2}\right)^{14}} \\ &= 1.33 - \frac{0.0591}{6} \log 10^{27} = 1.33 - \frac{27 \times 0.0591}{6} \\ &= 1.06 \text{ V} \end{aligned}$$

Formal Potential: Dependence of Potential on pH

The potential of a half reaction also depends on the reaction conditions, apart from the standard conditions requiring unity activity and 25 °C at 1 bar. A change in potential takes place if acid concentrations are changed keeping all other species at unit activity. The term Formal potential becomes important as it is defined as the standard potential of a redox couple with the oxidized and reduced forms at 1 M concentrations and at a specified solution conditions. For example, the formal potential of the Ce^{+4}/Ce^{+3} couple in 1 M HCl is 1.28 V. The formal potential $E^{\circ\prime}$ at 0.1 M HCl (pH = 1) can be given as

$$E^{\circ\prime} = E^{\circ} - 0.059$$

in neutral conditions it is

$$E^{\circ\prime} = E^{\circ} - 0.059(7)$$

Sometimes changing the pH also reverses the reaction.

9.6 Potentiometry for Quantitative Analysis

Potentiometry is a method used in electroanalytical chemistry, usually meant to measure the concentration of a solute in solution. In potentiometric measurements, the potential between two electrodes is measured using a high-impedance voltmeter. Using a voltmeter of high impedance ensures a zero net current, there are no net electrochemical reactions, hence the system is in equilibrium. A basic potentiometer

consists of two electrodes whose reduction potentials differ when inserted in a test solution. One of the electrodes is a reference electrode, whose electrode potential is known, the other electrode is the test electrode. The Nernst equation is used which gives a simple relationship between the relative potential of an electrode and the concentration of the corresponding ionic species in the solution. Thus, by measuring the potential of a reversible electrode, the activity or concentration of a component of a solution can be measured. Therefore, potentiometry passively measures the concentration of a solution between two electrodes, affecting the solution very little in the process.

A unique feature of potentiometric measurements is that it measures activity rather than concentration.

When in a single measurement procedure, the electrode potential is measured and the activity of corresponding ionic species in solution is determined the process is called direct potentiometry. This electrode whose potential determines the activity of the ion in the solution is known as indicator electrode.

Direct potentiometry involves two types of measurements. When the ion to be determined is directly involved in the electrode reaction, the electrode is known as electrode of the first kind and when the ion is not directly involved in the electrode reaction the electrode is known as the electrode of second kind. The sensitivity of the electrode plays a major role in direct potentiometry. This can be enhanced by taking the electrode of the same metal as the cation in the test solution, dipped in a solution of known concentration and joined with another electrode with same metal dipped in a solution of unknown concentration. Then the activity of the metal ion in a test solution is given by

$$E_{\text{cell}} = \frac{RT}{nF} \ln \frac{\mathcal{A}_{\text{known}}}{\mathcal{A}_{\text{unknown}}}$$

The use of a pH meter or an ion activity meter to measure the concentration of hydrogen ions or some other ionic species in a solution is a main application of direct potentiometry.

9.7 Types of Electrodes

Potentiometric methods engage a simple assembly and a typical cell for potentiometric analysis is represented as (Fig. 9.3).

The overall assembly includes an indicator electrode, a reference electrode and a potential measuring device. The reference electrode has a fixed potential, and is not affected by the analyte concentration and is made as the anode of the cell i.e. the left electrode. The indicator electrode is the right electrode, is immersed in the analyte solution and its potential is governed by the activity of the analyte. The salt bridge prevents the mixing of the two solutions in which the two electrodes are dipped and due to the mobilities of its ions it helps to maintain a constant potential.

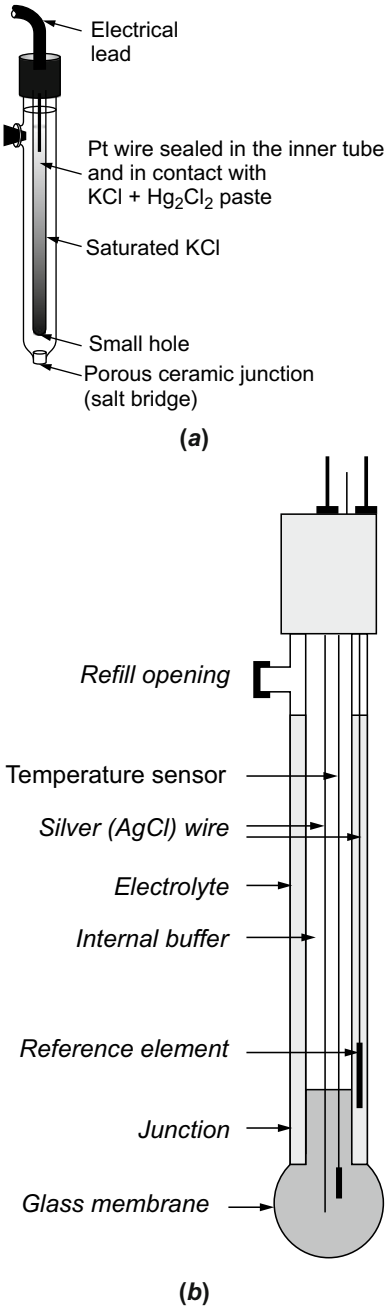


Fig. 9.3 **a** Reference electrode/salt bridge/analyte solution/indicator electrode. **b** The saturated calomel electrode (SCE)

Reference Electrodes

An essential condition for a reference electrode is that its potential should be fixed and stable and not get affected by the passage of small amounts of current during potentiometric measurements. Although in an ideal potentiometric measurement the net current is zero but sometimes a small current passes through the liquid junction.

A commonly used reference electrode is the saturated calomel electrode (SCE). An SCE consists of a mix of mercury and calomel (Solid Hg_2Cl_2) and is covered with a 0.1 M, 1 M, or saturated (4.2 M) solution of potassium chloride having fixed potentials of 0.335, 0.282, and 0.244 V, respectively (with respect to SHE). A platinum electrode is immersed in the above mix (paste) to make a contact with the small mercury pool. A salt bridge serves as the contact between the KCl solution and the test solution and is usually a fibre wetted with saturated KCl solution. The saturated calomel electrode is often used because it is easy to prepare and maintain however it is highly affected by changes in temperature.

The calomel half cell follows the reaction

$$Hg_2Cl_2(S) + 2e^- \rightarrow 2Hg(l) + 2Cl^- \quad E^\circ_{SCE} = 0.244\ V$$

and is affected by the Cl– ion concentration. The half cell is represented as (Fig. 9.4)

$$Hg|Hg_2Cl_2(Satd), KCl\ (xM)||$$

The Silver–silver chloride electrode: The silver/silver chloride is made from a silver wire coated with silver chloride by electrolysis or by dipping into the molten salt. The silver-plated wire is dipped into a KCl solution of known concentration, usually 1.0 M, and saturated with silver chloride. Half cell:

$$Ag|AgCl(satd), KCl(xM)||$$

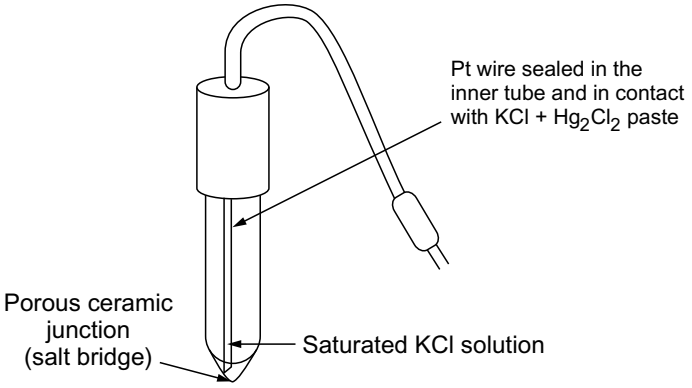


Fig. 9.4 A commercial saturated calomel electrode

and the half reaction is

$$AgCl(S) + e^- \rightleftharpoons Ag(S) + Cl^-$$

The silver-silver chloride electrodes are as important as calomel electrodes and have the advantage that they can be used at temperatures above 60 °C, whereas calomel electrodes cannot. The silver–silver chloride electrode exhibits greater electrical and chemical stability; however, they cannot be used for test solutions containing proteins, sulphide, bromide, and iodide as they react with Ag but are inert towards Hg. Such reactions can cause deposits between the electrode and analyte solution.

The potentials of SCE and silver-silver chloride type reference electrodes at different concentrations and temperatures have been given in Table 9.2.

In reference electrodes, it is essential to keep the level of the internal liquid separated from the level of the liquid in the sample solution so that the electrode solution does not plug the junction by the reaction of analyte with either Ag(1) or Hg(1) ions.

Indicator Electrodes

The indicator electrodes are those which respond to the activity of the ion in the analyte solution. Its potential highly depends on the activity or concentration of the ion which is being measured. Such indicator electrodes are of two types: metallic electrode and membrane electrode. Indicator electrodes meant to quantify metal ions usually consist of a metal rod or wire of the same metal dipped in a solution either containing its cation or a different cation. Preferably a platinum wire on which the metal is electrodeposited so that a thin metal film is coated on it, is used (Fig. 9.5).

The indicator electrode of the membrane type is those in which the potential is developed on a surface between a membrane (usually glass) and a solution. The potential developed on the glass membrane depends linearly on the pH of the solution and can therefore be used to measure the hydrogen ion concentration of the solution. A wide range of membrane electrodes are commercially available which can determine various cations and anions by direct potentiometric measurements. Because of their

Table 9.2 Potentials of different references electrodes

Temperature °C	0.1 M Calomel	3.5 M Calomel	Saturated Calomel	3.5 M Ag. AgCl	Saturated Ag.AgCl
10	–	0.256	–	0.215	0.214
12	0.3362	–	0.252	–	–
15	0.3362	0.254	0.251	0.212	0.209
20	0.3359	0.252	0.247	0.208	0.204
25	0.3356	0.250	0.244	0.205	0.199
30	0.335	0.248	0.241	0.201	0.194
35	0.334	0.246	0.237	0.197	0.189
40	–	0.244	–	0.193	–

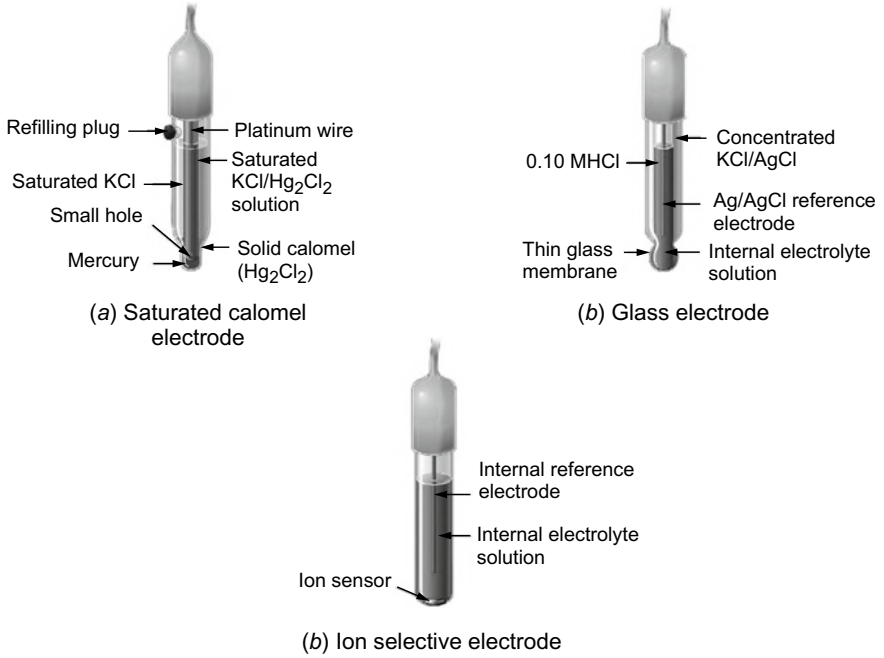


Fig. 9.5 Various Indicator electrodes

high sensitivity and selectivity, they are also categorized **as ion-selective electrodes**. Thus, the selection of electrode is governed by not only the type of ion to be measured but also the type of reaction taking place at the electrode. These reactions vary from acid–base, precipitation and redox reaction, and the corresponding choice of electrodes are glass electrodes, silver electrodes and platinum wires respectively. They also differ in the general mechanism by which ion-selective potential develops on the electrode (Fig. 9.6).

The Glass Electrode

The most common electrode for making pH measurements is the glass electrode. It is a membrane-type electrode and is highly sensitive to hydrogen ion H^+ concentration. The basic arrangement in a glass electrode is that it consists of a bulb filled with 0.1 M HCl and inside this lies the silver-silver chloride electrode. Since the concentration of HCl remains fixed, the potential of silver–silver chloride electrode which is dipped into it will also remain fixed and only the potential of the glass bulb in contact with the test solution will vary.

Dual nature electrodes in which a combination of reference and indicator electrodes are fixed as one single unit are also available. Instead of holding two separate components to be used as reference and indicator electrode, they are held in a combined assembly and with a single unit getting immersed in the test solution the measurement can be carried out, conveniently. It consists of a thin glass bulb

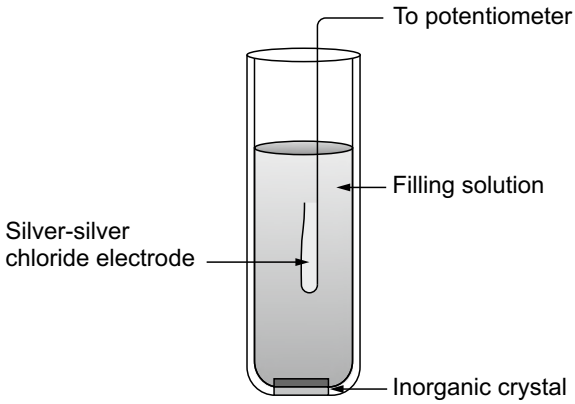


Fig. 9.6 An ion-selective electrode employing an inorganic salt crystal as the ion-selective membrane

containing a thin tube filled with HCl, encasing a silver-silver chloride electrode. This assembly is held in a wide tube containing saturated potassium chloride and saturated silver chloride carrying another silver-silver chloride electrode, all of which are sealed in an insulated case. Soda glass has been used for long in making such glass electrodes but in the recent times lithium glass has been used to prevent alkaline errors. Such combination electrode is a complete cell and a single immersion measures the pH of a test solution.

Although, the internal reference electrode is part of the glass electrode but is not the pH-sensing component, rather the thin glass membrane at the tip is pH-sensitive. This membrane responds to pH 1–9 range. The voltage of ideal pH electrode changes by 59.16 m V for every unit pH change at 25 °C of a solution. For an accurate measurement of H^+ concentration of a solution, the glass electrode must be combined with a reference, usually, SCE (standard calomel electrode) and cell is represented as:

$$\text{Ag.AgCl(s)|HCl(0.1M)|glass|test solution||KCl(sat), } Hg_2Cl_2(s)\big|Hg$$

Advantages of Glass Electrode

(i) Glass electrodes are capable of responding to small volumes of test solution.
(ii) The glass electrode can be used in the presence of strong oxidizing and reducing agents and also in viscous solutions. Substances like proteins and other complexing agents can also be measured.
(iii) It is immune to poisoning and is simple to operate and also gives a quick response.

However, a glass electrode needs special care for handling because of its fragile nature and its surface must be hydrated before it functions as a pH electrode. They also need to be kept immersed in distilled water for long duration before use as ideally, the solution should be similar to that inside the reference section of the electrode. Before using they should be calibrated with standard buffers of pH7 and slight above pH7, and at the same temperatures at which actual measurements are performed (Fig. 9.7).

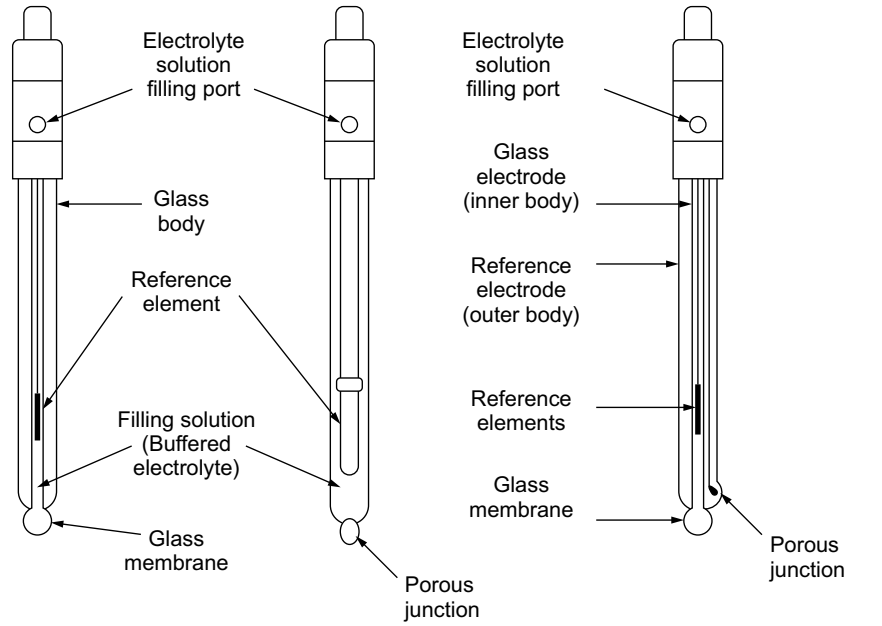


Fig. 9.7 Designs of types of glass electrodes

Ion-Selective Glass Electrode

The response of glass membrane electrode towards cations other than H^+, have also acquired great recognition and application. Many types of membrane electrodes have been developed in which the membrane potential is selective towards given ions in the same way as the potential of glass electrode is selective towards H^+ ions. The cation-sensitive glass electrodes which respond to sodium and potassium ions consist of aluminium oxide in the glass. The overall construction of these electrodes is similar to the pH-sensitive glass electrodes but it is necessary to use them jointly with a reference electrode which is preferably a silver–silver chloride electrode. The different types of ion-selective electrodes are as follows:

(a) **Glass membrane** electrodes for H^+ and certain monovalent cations
(b) **Solid-state electrodes** based on inorganic crystals or conductive polymers
(c) **Liquid base electrodes** using a hydrophobic polymeric membrane saturated with liquid ion exchanger.
(d) **Compound electrodes** with an analyte-selective electrode inside a membrane that separates the analyte from any other species or chemical reaction.

The response of an electrode to the activity of the cation follows the **Nernst equation**

$$E = K + \frac{RT}{nF} \log M^{+n}$$

For singly charged cation ($-\log_a M^+ = \text{pM}$)

$$E = K - 0.0591 \text{ pM}$$

Ion-selective electrode work on the basis of the electric potential difference across the interface between the membrane and the analyte solution, changes in analyte ion concentration in the solution change the potential difference across the outer boundary of the ion-selective membrane. Analyte ion establishes an ion-exchange equilibrium at the surface of the ion-selective membrane.

An example of ion selectivity can be seen while measuring Pb^{+2} ions in tap water. It is obvious that an ion-selective electrode responds to the activity of free analyte and not complexed analyte. Pb^{+2} in tap water at pH = 8 was found $Pb^{+2} = 2 \times 10^{-10}$ M but same water gave a concentration of Pb^{+2} as 3×10^{-9} M by ICP measurement. The reason being that inductively coupled plasma measures the total lead present, whereas the ion-selective electrode measures only the free Pb^{+2} ions. At pH = 4, all the lead which had complexed with OH^- and CO_3^{-2} ions in water was set free and given same concentration of 3×10^{-9} M by ion-selective electrode as found in ICP measurement. Glass electrodes for pH measurements are highly selective, however, sodium ion has its interference known and causes significant interference when $[H^+] \leq 10^{-12}$ M and $[Na^+ \geq 10^{-2}$ M. The relative response to measure an ion A with respect to another (interfering ion) ion x can be evaluated by selectivity coefficient

$$K_{A,x}^{pot} = \frac{\text{response to } x}{\text{response to } A} \qquad (\text{pot} = \text{potentiometric})$$

The smaller the selectivity coefficient, the less the interference by x. It has been found that in the measurement of K^+, Na^+ hardly interferes but Cs^+ and K_b^+ interfere strongly.

Use of a solid-state ion-selective electrode is well known and consists of a fluoride electrode which lies in contact with an inorganic salt crystal of LaF_3 containing Eu^{+2} as an impurity. The electrode is dipped in a solution of 0.1 M NaF and 0.1 M NaCl. These fluoride electrodes are used to measure fluoride ion concentrations in various sources of water supply.

Another type of commonly used inorganic crystal electrode employs Ag_2S for membrane and the electrode responds to Ag^+ and S^{-2} ions. Doping the electrode with CuS, CdS or PbS can lead to the preparation of electrodes sensitive to Cu^{+2}, Cd^{+2} or Pb^{+2} respectively.

Liquid-based ion-selective electrodes are prepared using organic liquid ion exchanger and the electrode has a hydrophobic membrane impregnated with a hydrophobic ion exchanger called an ionosphere, which is selective towards the ion present in the sample.

Compound electrodes contain a conventional electrode surrounded by a membrane that separates from the analyte to which the electrode responds. The usual Ag/AgCl reference electrode is dipped in electrolyte solution and is kept in a 0.1 M HCl solution with a glass electrode, all of them enclosed in a semipermeable membrane of polyethylene, Teflon, or rubber, when CO_2 diffuses through the semipermeable membrane, the pH of the electrolyte is lowered as it makes it acidic. The response of the glass electrode to the change in pH is a measure of CO_2 concentration outside the electrode. All other gases which are either generated in a chemical reaction or are present in an analyte and can alter the pH can be efficiently detected by these electrodes.

Ion-selective compound electrodes are also designed as biochemical electrodes and can be used together with immobilized enzymes to selectively measure specific substrates for ion probe in biological samples. They are referred to as Biosensors and are capable of measuring ions from even complex organic molecules.

Advantages of Ion-Selective Electrodes

(i) Ion-selective electrodes respond linearly to the logarithm of analyte activity over four to six orders of magnitude.
(ii) The Electrodes are non-destructive and non-contaminating.
(iii) Electrodes do not consume the analyte and require a very small concentration of analyte for precise response.
(iv) Response time is short, in seconds or minutes and so they can be used to monitor flow streams in industrial applications.
(v) Colour and turbidity of the analyte solutions do not affect the electrode.

Voltage Conversions between Different Reference Scales

While using different reference electrodes, different electrode potentials are observed for the same set of oxidation and reduction reactions. Conversion of potentials from one reference scale to another is an important aspect. From Fig. 9.8, it can be seen that an electrode at point A which measures –0.461 V from SCE, is +0.033 V with respect to silver-silver chloride. It lies –0.011 V from SCE and +0.230 V from SHE. Using these scales it is convenient to convert potentials from one scale to another.

9.8 Potentiometric Titrations

Potentiometric titrations are those titrations in which the change in electrode potential caused by the addition of titrant is recorded against the volume of the titrant added.

The equivalence point (end point) in potentiometric, titrations are detected by rapid changes in activity causing a sudden change in the emf values. The abrupt change in the potential can usually be identified by a graph of e.m.f values plotted against the volume of the titrating solution. It is the change in emf versus titration

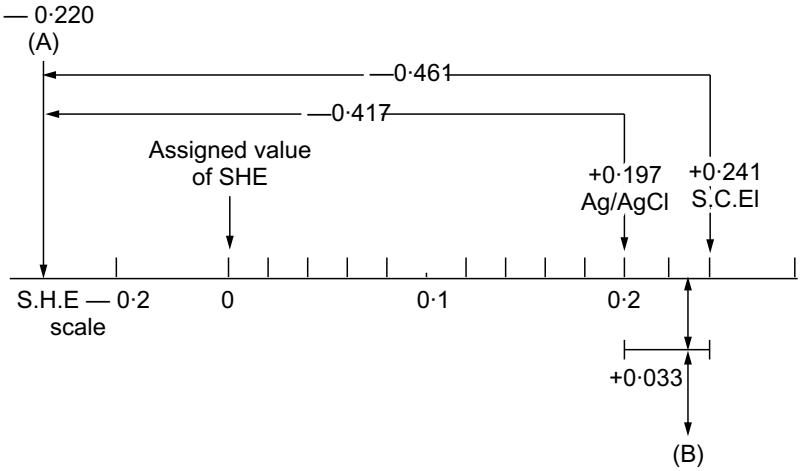


Fig. 9.8 Conversions shown from various reference electrodes

volume rather than the absolute value of the emf that is of interest. Therefore it ensures minimized influence of liquid-junction potentials and activity coefficients (Fig. 9.9).

The emf measurement is done by connecting the electrode system to a pH meter that can function as a millivoltmeter to record the emf values. Potentiometric titrations thus use pH meters as millivoltmeters and consist of an assembly of reference electrode-indicator electrode to record the results. Some instruments are designed in such a way that the recorder continuously records the results of a potentiometric titration and draws a curve instantly (Fig. 9.10).

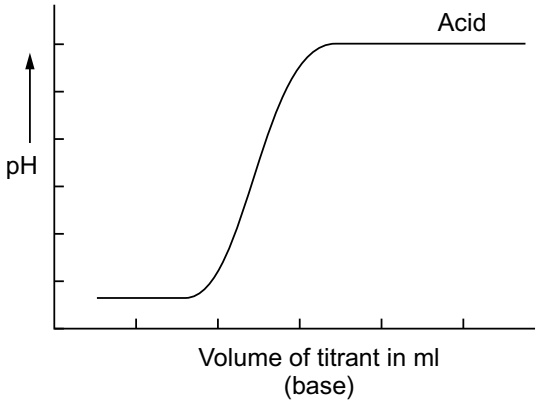


Fig. 9.9 An acid–base potentiometric titration

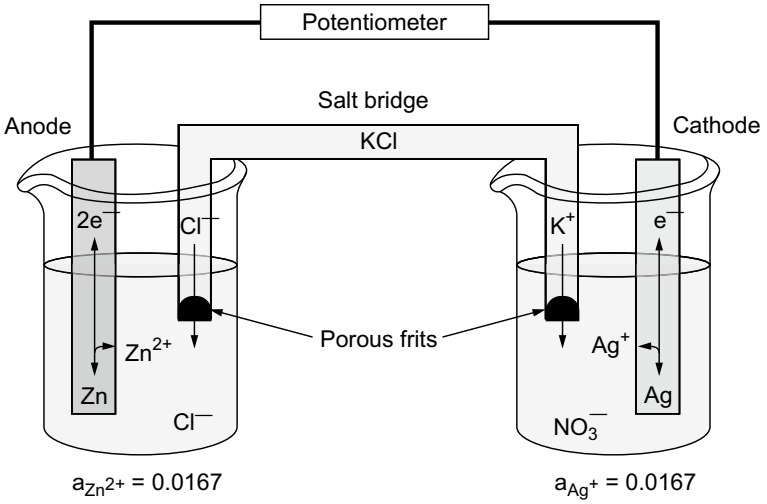


Fig. 9.10 Simple apparatus used for potentiometric titration

Potentiometric titrations (Indirect potentiometry) are always more accurate than direct potentiometry because of the uncertainties involved in potential measurements. The reasons why they are more accurate can be summarized as follows:

(a) The potential reading moves slowly in dilute solutions and especially near the end point the abrupt change becomes more significant.
(b) Small increments of titrant volume are added near the end point, for example, 0.1 or 0.05 ml and even if the exact end point volume is not added, it is determined by extrapolating the emf *versus* titrant volume plot.
(c) Plotting or recording the first or second derivative of a titration curve can more accurately indicate the end point. Since the rate of change of potential is maximum at end point, in the first derivative curve where $\frac{\Delta E}{\Delta V}$ is plotted against volume of the titrant added, the maxima gives end point (Fig. 9.11).

In a second derivative curve of a potentiometric metric titration either $\frac{\Delta^2 E}{\Delta V^2}$ or $\frac{\Delta(\Delta E/\Delta V_1)}{\Delta V_2}$ is plotted against the volume of titrant and the curve should pass through zero at the equivalence point (Fig. 9.12).

A Gran's plot for end point detection further gives accuracy because a linear plot is obtained which can easily identify the end point even at small inflection. If a series of additions of reagent is made in a potentiometric titration, and the cell emf E is read after each addition and antilog of $nFE/2.303\ kT$ is plotted against the volume of reagent added, a straight line is obtained. When extrapolated at the volume axis, it gives the value of volume at the end point. By Gran's plot method only a few observations are needed, the actual volume required at the equivalence point need not be observed. A Gran plot can also be obtained for the titrant beyond the end point.

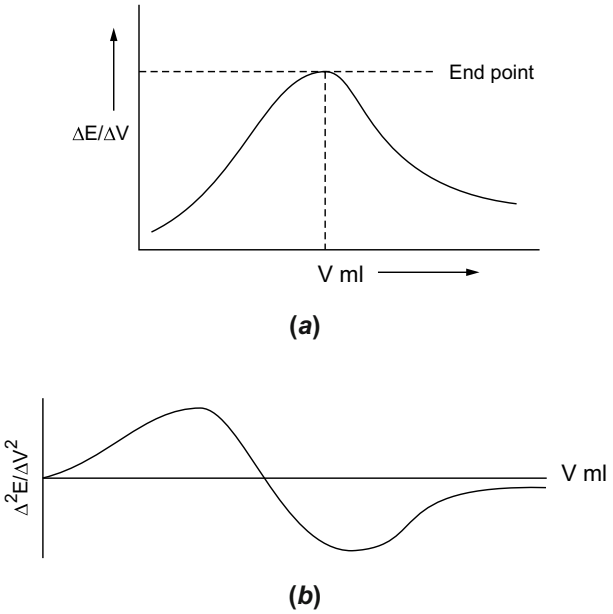


Fig. 9.11 **a** First derivative curve. **b** Second derivative curve

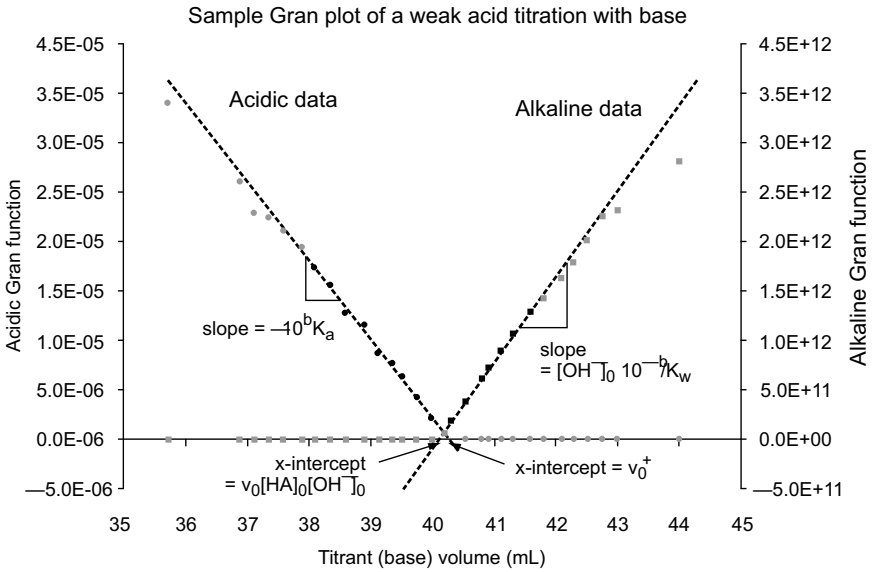


Fig. 9.12 Gran's plot

Potentiometric titrations can not only be performed manually but automatic titrators can also be employed which automatically record the first or second derivative of the titration curve and read out the end-point volume. Detection of end point photometrically where colour change is observed is successfully carried out by automatic titrators. Automatic titrators make volumetric analysis rapid, reproducible, and convenient and a full range of titrations can be carried out automatically.

9.9 Types of Potentiometric Titrations

The potentiometric equivalence point can be applied to generally all types of chemical reactions. On the basis of the types of chemical reaction taking place during the titration, potentiometric titrations are classified as (1) neutralization reaction, (2) complexation reaction, (3) precipitation reaction, and (4) oxidation–reduction reaction.

Types of Titrations

(a) **Acid–base Titrations**

These are based on neutralization reactions

$$H^+ + OH^- \rightarrow H_2O$$

which surely brings changes in H^+ ion concentration. Therefore, the indicator electrode for determining H^+ ion concentration is used along with the standard calomel electrode (SCE). The two electrodes also consist of a salt bridge between them and the assembly is connected to voltmeter to record the changes in EMF, a known volume of acid is kept in the beaker containing the indicator electrode and an automatic stirrer. After each addition of base from burette into the beaker, the change in EMF is measured and is plotted against the volume added.

The change in electrode potential or EMF of the cell is proportional to the change in pH during titration. The point where the EMF shows an abrupt change refers to the end point (Fig. 9.13).

Plotting $\Delta E/\Delta V$ against V ml gives a more convenient way of identifying the end point. The maxima in such a curve gives accurate end point (Fig. 9.14).

Results of acid–base titrations carried out by potentiometric measurements are quite satisfactory and an accuracy is observed if the acids or bases differ in strength by at least $10^5 - 1$.

(b) **Complexometric Titrations**

For a reaction in which a complex is formed as the product of the reaction then a metal electrode is employed whose ion is involved in complex formations. For example, a silver electrode is used for the following reaction:

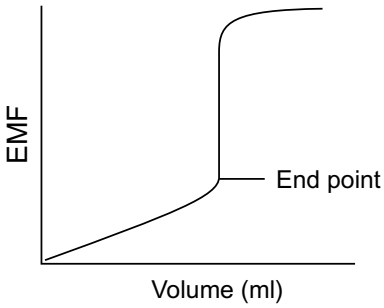


Fig. 9.13 End point determination

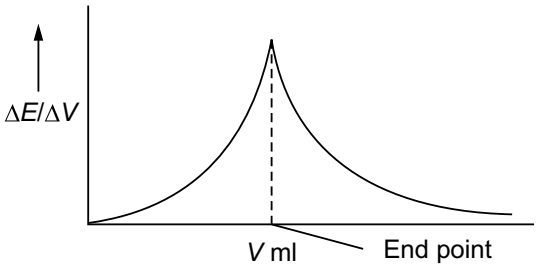


Fig. 9.14 End point determination

$$Ag^{+} + 2CN^{-} \rightleftharpoons \left[Ag(CN)^{2}\right]^{-}$$

$$K_C = \frac{\left[Ag^{+}\right]\left[CN^{-}\right]^{2}}{\left[Ag(CN)_2\right]^{-}}$$

At the equivalence point,

$$2\left[Ag^{+}\right] = \left[CN^{-}\right]$$

Solid silver cyanide gets precipitated after the end point, and further addition of silver does not change the concentration of the complex. The titration curve almost becomes horizontal at the end point (Fig. 9.15).

Metals which cannot be titrated directly, use a back-titration procedure so that the analyte concentration is determined from the difference between the total concentration of the remaining unreacted chelating or complexing agent. For example, by adding known excess of EDTA for the determination of iron(III) so that the remaining unreacted EDTA is back titrated using copper as an indicator electrode in the presence of buffer solution of pH 4.7.

(c) **Precipitation Titrations**

When a precipitation titration is carried out the reaction which is followed is one which has an insoluble product. Insoluble salts formed from metals like mercury,

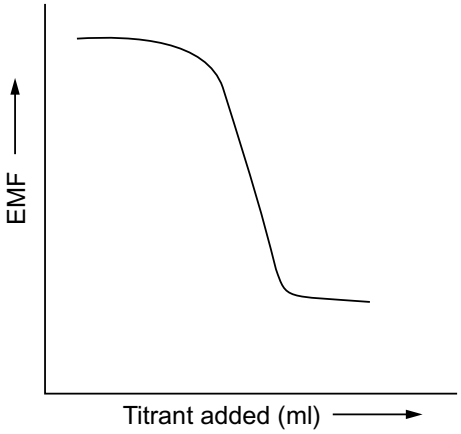


Fig. 9.15 Titration of 0.1N cyanide with 0.1N silver solution

silver, lead, or copper are followed potentiometrically. The ionic concentration at the equivalence point is determined by the solubility product of the sparingly soluble material formed during the titration. The indicator electrode is generally made of the metal involved in the reaction or the ion whose potential is influenced by the concentration of the anion being precipitated. The reference electrode is either saturated calomel or silver–silver chloride electrode. At the end-point, the potential change finally depends on the solubility of the ion being precipitated, indirectly the solubility product. Many examples using silver electrode and standard solution of silver nitrate for the estimation of halides chloride, bromide, and iodide can be cited. The precipitation of silver salt of these halides influences the end point determination. For example,

$$K_{SP}(AgCl) = \left[Ag^{+}\right]\left[Cl^{-}\right]$$

for equilibrium

$$Ag^{+} + Cl^{-} \rightarrow AgCl$$

which is established after sufficient silver nitrate is added so that AgCl is precipitated.

The cell is formed by silver electrode, which consists of silver wire or a platinum wire plated with silver and cased in a glass tube, joined with a reference electrode (SCE or Ag–AgCl) through salt bridge containing saturated solution of potassium nitrate. Nernst equation for determining electrode potential can be given as (Fig. 9.16)

$$E_{Ag^{+}/Ag} = E^{\circ}_{Ag^{+}/Ag} + \frac{0.0591}{1}\log\left[Ag^{+}\right]$$

At the equivalence point

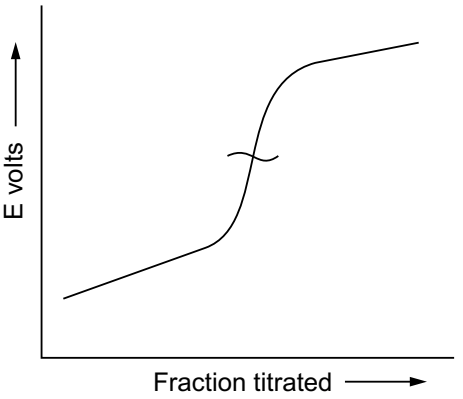


Fig. 9.16 Precipitation titration of silver salt

$$\left[Ag^{+}\right] = \left[Cl^{-}\right]$$

(d) **Oxidation Reduction Titration**

All redox reactions can be followed potentiometrically because the two half reactions, oxidation, and reduction complete to make a cell. The standard potentials of the two half cells may be used to calculate the standard potential of the reactions For example,

$$\begin{aligned} Fe^{+2} &\rightarrow Fe^{+3} + e & E^{\circ} &= -0.76\ V \\ Ce^{+4} + e &\rightarrow Ce^{+3} & E^{\circ} &= +1.61\ V \\ Ce^{+4} + Fe^{+2} &\rightarrow Fe^{+3} + Ce^{+3} & E^{\circ} &= +0.85\ V \end{aligned}$$

A platinum electrode can be used for titration of iron (ii) with cerium (iv). When a ferrous solution is titrated with a standard solution of ferric ions in acidic solution, their electrode potentials with respect to standard hydrogen electrode

$$E = E^{0}_{Ce^{+4}/Ce^{+3}} - \frac{0.0591}{1} \log \frac{Ce^{+3}}{Ce^{+4}}$$

$$E = E^{\circ}_{Fe^{+3}/Fe^{+2}} - \frac{0.0591}{1} \log \frac{Fe^{+2}}{Fe^{+3}}$$

at end point

$$\left[\frac{Fe^{+2}}{Fe^{+3}}\right] = \left[\frac{Ce^{+4}}{Ce^{+3}}\right]$$

equilibrium constant

$$K = \frac{[Fe^{+3}][Ce^{+3}]}{[Fe^{+2}][Ce^{+4}]}$$

at equivalence point $\left[\frac{Fe^{+3}}{Fe^{+2}}\right] = \left[\frac{Ce^{+3}}{Ce^{+4}}\right] = \sqrt{K}$

Thus, on calculating we get at the equivalence point

$$E_{eq} = E^{\circ}_{Ce^{+4}/Ce^{+3}} - \frac{0.0591}{2} \log K$$

$$E_{eq} = E^{\circ}_{Fe^{+3}/Fe^{+2}} + \frac{0.0591}{2} \log K$$

on combining

$$E_{eq} = \frac{E^{\circ}_{Ce^{+4}/Ce^{+3}} + E^{\circ}_{Fe^{+3}/Fe^{+2}}}{2}$$

Thus, the end point potential can measure the titration.

If titration of 100 ml of 0.1 M Fe^{+2} with 0.1 M Ce^{+4} in 1 M HNO_3 is carried out, Each millimole of Ce^{+4} will oxidize one milli mole Fe^{+2} and so the end point will occur at 100 ml.

Titration curve for 100 ml Ce^{+4} 0.1 M Fe^{+2} *versus* 0.1 M Ce^{+4} relative to standard hydrogen electrode shows the change in potential of the titration solution with respect to the addition of volume of Ce^{+4} solution (Fig. 9.17).

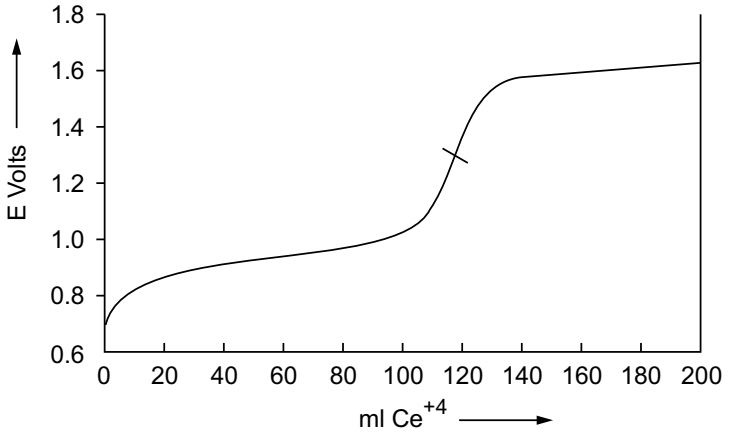


Fig. 9.17 Titration curve of Ce(IV) with Fe(II)

9.10 Advantages of Potentiometric Titrations

Potentiometric titrations can be proved to be more beneficial than many other titrations on the basis of the following advantages:

(i) These titrations can be successfully used for brightly coloured solutions where normal titrations cannot indicate any colour at the end point.
(ii) In non-aqueous conditions also potentiometric titrations can be performed.
(iii) Even in the absence of any internal indicator it can work efficiently.
(iv) For dilute solutions potentiometric titrations give better accuracy
(v) Weak acids and weak bases can be titrated by potentiometric methods.
(vi) Many ions can be titrated in the same solution without the interference of indicators. For example, bromide and iodide can be titrated potentiometrically in the same solution.
(vii) The apparatus required is generally inexpensive, reliable, and easily available.

Solved Problems

1. State whether or not the following reactions will proceed in the forward or backward direction at 25 ºC when all concentrations and pressures are unity.

(a) $Zn + Mg^{+2} \rightarrow Zn^{+2} + Mg$
(b) $Fe + Cl_2(g) \rightarrow Fe^{+2} + 2Cl^-$
(c) $2Ag + Cu^{+2} + 2Cl^- \rightarrow 2AgCl(s) + Cu$

Solution:

(a) $Zn + Mg^{+2} \rightarrow Zn^{+2} + Mg$

$$Zn|Zn|\big|Mg^{+2}\big|Mg$$

$$\begin{aligned} E^{o}_{cell} &= -2.37 - (-0.763) \\ &= -1.607 \qquad \text{(Backward)} \end{aligned}$$

(b) $Fe + Cl_2 \rightarrow Fe^{+2} + 2Cl^-$

$Fe\big|Fe^{+2}\|Cl^-\big|Cl_2|pt$

$$\begin{aligned} E^{o}_{cell} &= 1.3595 - (-0.440) \\ &= 1.7995 \qquad \text{(Forward)} \end{aligned}$$

(c) $2Ag + Cu^{+2} \rightarrow 2Ag^+ + Cu$

$Ag\big|Ag^+\|Cu^{+2}\big|Cu$

$$\begin{aligned} E^{o}_{cell} &= 0.337 - 0.7991 \\ &= -0.4621 \qquad \text{(Forward)} \end{aligned}$$

2. What is the potential of a platinum electrode immersed in a solution of 0.0150 M of KBr in Bromine?

Solution:

$$\mathrm{Br_2}(l) + 2e^- \rightleftharpoons 2\mathrm{Br}^- \quad E^\circ = 1.065$$

$$E = E^\circ - \frac{0.0592}{2} \log \frac{\left[\mathrm{Br}^-\right]^2}{\mathrm{Br_2}(l)}$$

$$E = 1.065 - \frac{0.0592}{2} \log \frac{(0.0150)^2}{1.00}$$

$$= 1.173 \text{ V}$$

Unsolved Problems

1. Find the electrode potential for the half cell when cadmium electrode is dipped in 0.0150 M in Cd^{+2}?
2. Calculate the cell potential for the following reaction where $C_{ZnSO_4} = 5 \times 10^{-4}$

$$\mathrm{PbSO_4}(s) + 2e^- \rightleftharpoons \mathrm{Pb}(s) + \mathrm{SO_4^{-2}} \quad E^\circ = -0.350 \text{ V}$$
$$\mathrm{Zn^{+2}} + 2e^- \rightleftharpoons \mathrm{Zn} \quad E^\circ = -0.763 \text{ V}$$

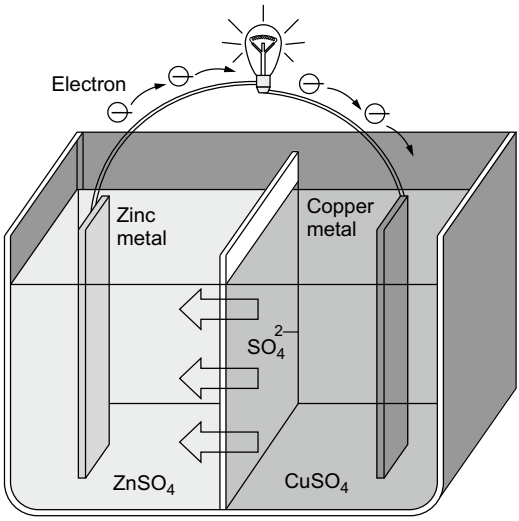
3. How do you classify electrochemical methods and describe electrochemical cells?
4. Define electrode potential and indicate its method of evaluation.
5. Which are the various types of electrodes used in potentiometric methods of analysis.
6. Describe potentiometric titrations.
7. What are the advantages of potentiometric titrations over other methods of titrations?
8. How many types of potentiometric titrations are there? Illustrate.
9. Discuss the pH limitations of ion-selective electrodes.
10. What are the advantages and disadvantages of glass electrodes?

Answers

1. –0.457 V,

2. 0.608 V

Chapter 10
Coulometry



10.1 Basic Principle of Coulometric Method of Chemical Analysis

Those methods which measure an amount of electricity flowing to measure the analyte are categorized under coulometric methods of analysis. These methods are based on the exact measurement of the quantity of electricity that passes through a solution during an electrochemical reaction. All types of reactions in which flow

P. Malhotra, *Analytical Chemistry*,
https://doi.org/10.1007/978-3-031-26757-4_10

of electrons are taking place can be followed by coulometric methods of analysis because it is a chemical analysis based on counting electrons.

Coulometric analysis conceptually follows Faraday's first law of electrolysis according to which for each mole of chemical change at an electrode, n Faraday ($n \times 96{,}487$ coulombs) are required where n is the number of electrons involved in the electrode reaction. This implies that the extent of chemical reaction at an electrode highly depends upon the amount of charge flowing. In other words, it depends on the electricity passing through the electrode.

Since $Q = It.$

where

Q = Charge in coulombs.
I = Current in amperes.
t = Time in seconds.

For a variable current i, the charge is given by the integral

$$Q = \int_0^t i dt$$

Faraday's Law states that n Faraday of electricity forms 1 mol of chemical species on the electrode where n is the number of electrons flowing in the reaction

$$n_A = \frac{Q}{nF}$$

where

n_A = no. of moles of analyte
n = no. of moles of electrons in the analyte half reaction.

Generally, it can be stated that

n Faraday of electricity will deposit 1 mol of a substance during electrolysis where n = charge on the ion.

For example:

2 Faraday of electricity will deposit 63 g (1 mol) of Cu on the electrode.

Similarly, 3 Faraday of electricity will be required to deposit 27 g (1 mol) of Al.

An essential condition for coulometric analysis is that the electrode reaction should proceed with 100% efficiency so that the quantity of electricity measured gives the actual determination, This implies that each faraday of charge must bring about a chemical charge in $1/n$ moles of analyte, where n is the number of electrons flown in the reaction. When the substance being determined is directly involved in the reaction, it is termed as *primary coulometric analysis* and when it reacts in solution with another substance generated by an electrode reaction, it is termed as *secondary coulometric analysis.* Coulometric methods are often as accurate as gravimetric or

volumetric procedures and are usually faster and more conveniently automated than gravimetric methods.

10.2 Types of Coulometric Methods

Generally, two techniques are prevalent for use among coulometric methods:

(a) Controlled potential coulometry and
(b) Controlled current coulometry.

(a) **Controlled Potential Coulometry**

In controlled potential coulometry, the potential of the working electrode is maintained at a constant level and the current decreases exponentially as analyte concentration decreases (Fig. 10.1) with time.

$$q = \int_0^t I\,dt$$

$$I_t = I_o e^{-K't}$$

or $$I_t = I_o 10^{-Kt}$$

where

I_o = Initial current.
I_t = Current at time t

K and K' are the constants.

One can approach the equivalence point when the current decays to a set value. In this method, the current is initially high but decreases rapidly and approaches zero as the analyte is removed from the solution.

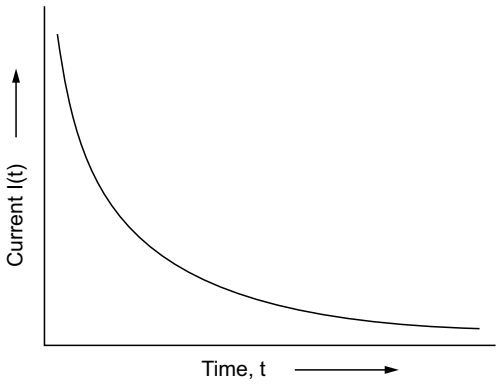


Fig. 10.1 Current vs time with decrease in concentration

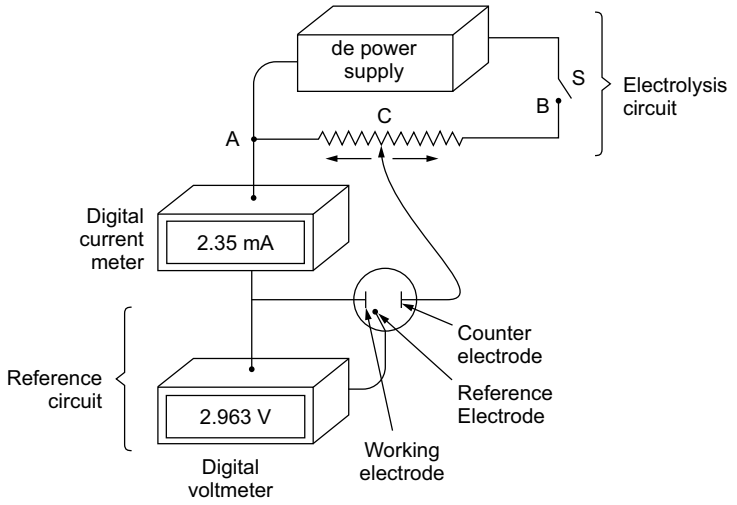


Fig. 10.2 Circuit for controlled potential electrolysis

Controlled potential coulometry is performed when a third auxiliary reference electrode is placed within the electrolytic cell and is connected through a voltage-measuring device and then the potential of one of the electrodes is monitored. The potential of the working electrode is maintained at a constant level so that all the current flowing is used in the reaction taking place at the analyte electrode interface.

The instrumentation for controlled potential coulometry also known as potentiostatic coulometry consists of an electrolysis cell, a potentiostat, and an electronic integrator for determining the charge consumed. The source of current is a potentiostat, which is used in conjunction with a reference electrode (SCE) to control the potential of a working electrode at a constant level relative to the reference electrode (Figs. 10.2 and 10.3).

Generally, two types of cells are used for potentiostatic coulometry. The first type consists of a platinum-gauge working electrode and a platinum-wired second electrode, the two being separated from the analyte by a porous tube containing the same ions in electrolyte as the test solution. An SCE or an Ag–AgCl reference electrode is connected to the analyte solution with a salt bridge containing the same electrolyte. The second type of cell has a mercury cathode which easily reduces elements like copper, nickel, cobalt, silver, and cadmium. Such a mercury pool-type cell dissolves the precipitated elements also and coulometric determination of metal ions becomes easy. Even organic compounds like trichloroacetic acid and 2, 4, 6 trinitrophenol can also be determined as they get reduced at the mercury cathode. Controlled potential coulometric methods have been applied to more than 50 elements

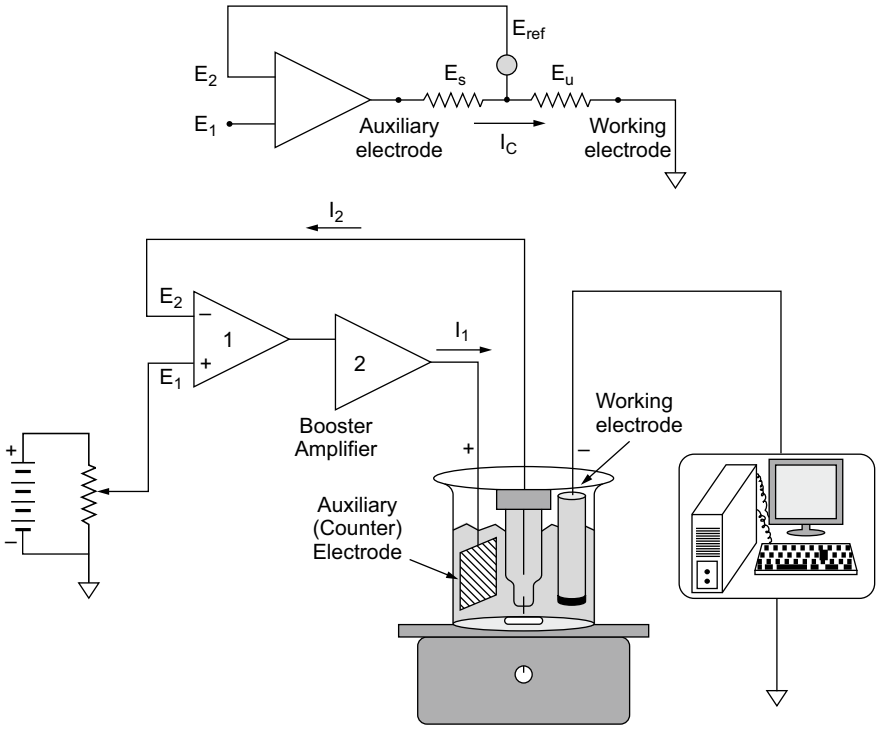


Fig. 10.3 Electrolysis cell for potentiostatic coulometry

in inorganic compounds and, in the majority of the cases, mercury electrodes have found wider applications.

(b) **Controlled Current Coulometry**

In this technique, constant current is passed through electrolytic cell until an indicator indicates the completion of a reaction (end point). The magnitude of current and the time for which it has been passed are major concerns to determine the analyte concentration. Constant current electrolysis is employed to generate a reagent that reacts stoichiometrically with the substance to be determined. Using Faraday's law the quantity of electricity passed is evaluated by timing the electrolysis at constant current. Both of these, the current and time can be measured with high accuracy using a simple technique. Its instrumentation consists of an amperometer which is used as a constant current source, an electrolytic cell, and current as well as time measuring device. The electrolytic cell contains the working (or generator) electrode at which the reagent is electrogenerated and the auxiliary (counter) electrode. The generator electrode is made of platinum, silver, or mercury, whereas the counter electrode is made of platinum. This electrode is placed in a separate glass tube closed at its lower end by a porous glass disc to prevent products from the electrode from reacting with components of the sample. For measuring the current, a carefully calibrated

milliammeter is used. A digital timer is used for measuring the electrolysis time with a precision of 0.01 s (Figs. 10.4 and 10.5).

Current controlled coulometry has been used for a wide range of substances including those which do not react quantitatively at an electrode. Due to their similarity, constant current coulometry is formally same as coulometric titrations are performed. In titrimetric analysis, or conventional volumetric titration, the reagent is added from a burette, in coulometric titrations the reagent is generated electrically

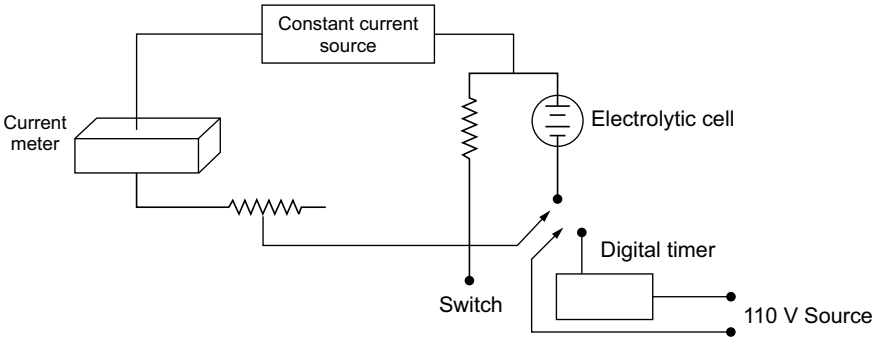


Fig. 10.4 Circuit diagram for current controlled coulometry

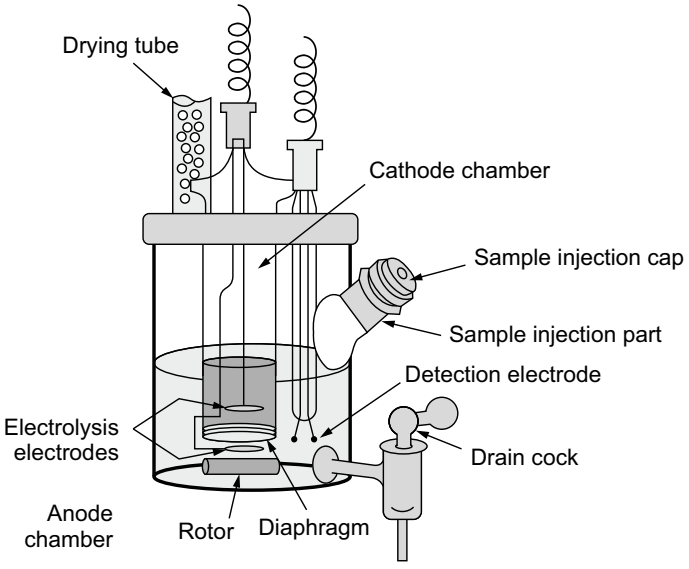


Fig. 10.5 Electrolysis cell for amperostatic coulometry

and its amount is evaluated from the values of current and time. A simple comparison of constant current coulometry and the conventional volumetric titrations can be made as follows:

(i) The standard solution (titrant) in volumetric method can be compared to constant current of known magnitude which is analogous to the molarity of the titrant and the electron being its reagent.
(ii) The time needed to complete the electrolysis, i.e. to reach the end point can be compared to the volume of titrant required for reaching the end point.
(iii) The electronic timer serves as burette and the switch can be compared to stopcock of the burette which helps to stop or start the flow of titrant.

10.3 Coulometric Titrations

The basic requirements of a coulometric titration are: that the reagent generating electrode reaction should proceed with 100% efficiency and the generated reagent should instantly react with the substance which is being determined. Depending upon whether the substance to be estimated is generated directly at one of the electrodes or the substance being determined reacts with another substance generated by the electrode reaction, there are two types of coulometric titrations: **Primary Coulometric Titration and Secondary Coulometric Titration**.

Primary Coulometric Titrations

They involve those titrations in which the analyte undergoes direct reaction at one of the electrodes with 100% efficiency. The titrant is directly generated at the working electrode either by oxidation or reduction of the ion.

An important example of primary coulometric titration is the estimation of halide or sulphide ions from its silver halides or sulphides using silver anode. In the titration of halide X^-, AgX initially gets deposited directly on the electrode or Ag^+ is generated by

$$\begin{aligned} &Ag \rightarrow Ag^+ + e \\ &Ag^+ + X^- \rightarrow AgX \\ \hline &Ag + X^- \rightarrow AgX + e \end{aligned}$$

As X^- ions are consumed, there reaches a stage when the rate at which halide ions are supplied to the electrode is slower than the rate at which Ag^+ ions are formed, thus pushing the Ag^+ into the bulk of the solution. Its diffusion into the bulk solution causes precipitation of AgX in the bulk and not on the electrode. The end point is determined amperometrically, i.e. by measuring the time taken for reaching the end point. Electrically inactive chemical indicators for determining the end point for halide measurements include starch for iodine, dichlorofluorescein for chloride, and eosin for bromide and iodide.

Secondary Coulometric Titrations

The secondary coulometric titration technique is used in the majority of applications. In this method, the substance being determined is allowed to react in solution with another substance generated by an electrode reaction. Generally, an oxidation–reduction buffer serves as the titrant precursor. An active intermediate is generated from the electrode process of the titrant precursor with complete efficiency. This intermediate then reacts completely with the substance being determined at a very fast rate. The end point is that point where generation of the active intermediate ceases. It is easy to generate an intermediate titrant by selecting an appropriate titrant precursor which is always added in excess to the supporting electrolyte. Selection of an appropriate titrant precursor depends upon its standard potential which should lie between that of the unknown analyte and that potential at which the supporting electrolyte reacts. For example, the coulometric titration of iron (II) at a platinum electrode (anode) is a secondary coulometric titration.

A large excess of Ce(III) is added to the solution containing the Fe(II) ions in the presence of 1 M sulphuric acid.

The anodic reaction

$$Fe^{+2} \rightarrow Fe^{+3} + e^{-}$$

The current drops with a decrease in Fe(II) concentration and to maintain a constant current, the applied cell potential is made to increase. This increased potential can cause decomposition of water

$$2H_2O \rightarrow O_2(g) + 4H^{+} + 4e^{-}$$

which is prevented by the oxidation of secondary titrating agent cerium(III), the titrant which is oxidized at a lower potential than water, i.e. potential between H_2O and Fe(II)

$$Ce^{+3} \rightarrow Ce^{+4} + e^{-}$$

The Ce^{+4} ion generated electrolytically oxidizes the remaining Fe(II) ion

$$Ce^{+4} + Fe^{+2} \rightarrow Fe^{+3} + Ce^{+3}$$

Although only a small fraction of Fe^{+2} is oxidized at the anode and greater fraction is oxidized by Ce^{+4} only, 100% current efficiency is maintained by Ce^{+3}. A slight excess of Ce^{+4} indicates the end point which can be detected potentiometrically with a platinum reference electrode pair or spectrophotometrically at a wavelength (400 nm) at which cerium (IV) absorbs strongly.

The instrument used for a constant current source is usually an amperostat which provides a constant current and sometimes also provides constant potential along with constant current. The instrument amperostat can provide a constant current and

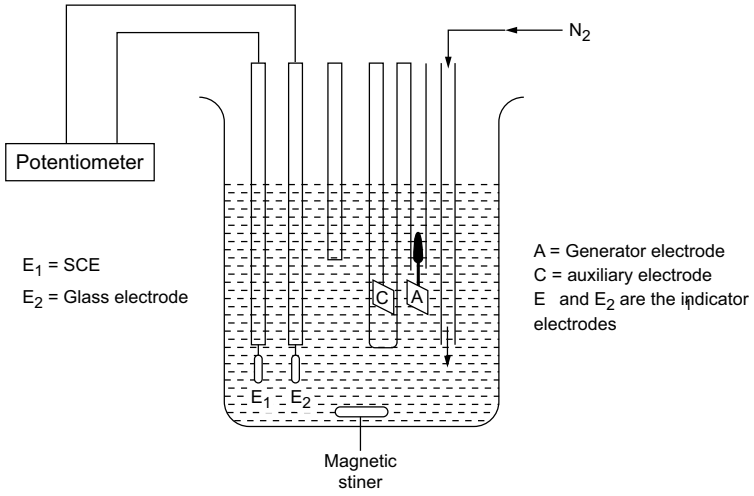


Fig. 10.6 A Coulometric titration cell

very often a constant potential. The titration cell has a beaker with magnetic stirrer and a stream of inert gas (N_2) is allowed to pass through it. The generator electrode of platinum foil (A) and the auxiliary electrode C (also of platinum) are kept in such a way that electrode C is isolated from the bulk of the solution (Fig. 10.6).

The auxiliary electrode is immersed in a suitable electrolyte, whereas the titration cell is charged with the solution from which the titrant will be generated electrolytically. The indicator electrodes E_1 and E_2 are connected to pH meter or spectrophotometer, as the need be, to detect end point.

10.4 Applications of Coulometric Titrations

A wide range of coulometric titrations can be easily performed for analytical purposes. They are applicable to acid–base, redox, precipitation, and complexometric titrations just as these reactions are carried out in other volumetric titrations.

Neutralization (Acid–Base) Titration

Acid–Base titration can be carried out with a high degree of accuracy using electrogenerated H^+ and OH^-. Anodic electrogeneration of hydrogen ion for the titration of bases and cathodic electrogeneration of hydroxide ion for the titration of acids can be achieved through coulometric method.

$$2H_2O + 2e^- \rightarrow H_2(g) + 2OH^- \quad \text{(at cathode)}$$

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^- \quad \text{(at anode)}$$

To make these internal generation of ions, it is essential to keep them separated for which the auxiliary electrode must be placed in a separate chamber otherwise the determination process will get hindered. Coulometric titrations of both weak and strong acids can be performed by using electrogenerated hydroxide ions at the cathode. Similarly, both weak and both strong bases can be titrated with electrogenerated H^+ at the anode. In both the cases, the auxiliary platinum anodes/cathodes must be isolated by a diaphragm from the main solution of acid/base, respectively. The end point of such titrations is determined by potentiometric methods. Examples of coulometric titrations are given as follows (Table 10.1).

Precipitation Titration

For halide ion estimations either mercury (I) ions or silver (I) ions are generated at different types of anodes and precipitation titrations are carried out coulometrically. To generate mercury (I) ions either mercury-plated or mercury pool anodes are used and methanol is added to reduce the solubility of the mercury (I) chloride. Silver (I) is generated from silver anode and is employed for the precipitation of chloride, bromide, and iodide and while detecting the end point, potentiometric method is used.

Examples of coulometric precipitations may be given as follows (Table 10.2).

Table 10.1 Examples of coulometric titrations

Ion generated	Electrode	Electrode reaction	Analytical reaction	Substance titrated
H^+	Na_2SO_4(0.2 M)	$2H_2O \rightarrow 4H^+ + O_2 + 4e^-$	$H^+ + OH^- \rightarrow H_2O$	Bases(weak and strong
OH–	Na_2SO_4(0.2 M)	$2H_2O + 2e^- \rightarrow H_2 + 2OH^-$	$OH^- + H^+ \rightarrow H_2O$	Acid(both weak and strong

Table 10.2 Examples of coulometric precipitation

Ion generated	Reaction	Substance to be determined	Secondary analytical reaction
Ag+	$Ag \rightarrow Ag^+ + e^-$ (at silver anode)	Cl^-, Br^-, I^- Mercaptan (RSH)	$Ag^+ + Cl^- \rightarrow AgCl(s)$ $Ag^+ + Br^- \rightarrow AgBr(s)$ $Ag^+ + I^- \rightarrow AgI(s)$
Hg^{+2}	$2Hg \rightarrow Hg_2^{+2} + 2e$ (at mercury anode)	Cl^-, Br^-, I^-	$Hg_2^{+2} + 2Cl^- \rightarrow Hg_2Cl_2(s)$ $Hg_2^{+2} + 2Br^- \rightarrow Hg_2Br_2(s)$ $Hg_2^{+2} + 2I^- \; Hg_2I_2(s)$
$[Fe(CN)_6]^{-4}$	$[Fe(CN)_6]^{-3} + e \rightarrow [Fe(CN)_6]^{-4}$ (at cathode)	Zn^{+2}	$3Zn^{+2} + 2\,K^+ + 2[Fe(CN)_6]^{-4} \rightarrow K_2Zn_3[Fe(CN)_6]_2(s)$

Redox Titrations

Just as redox reactions form the basis of volumetric redox titrations, similarly, a wide range of redox reactions can be used for coulometric titrations. The redox reagents can be generated coulometrically and the end points are detected either potentiometrically or amperometrically. Those reagents which are unstable towards volumetric redox titrations can be used coulometrically. For example, Mn^{+3}, Ag^{+2}, Ti^{+3}, Cl_2, Br_2, and $(CuCl_3)^{-2}$ (Table 10.3).

Complexometric Titration

Well-known complexing agent, EDTA is generated coulometrically and by using an ammonium nitrate buffer, reduction of ammine at mercury cathode is carried out. The electrogenerated EDTA forms complexes with Ca(II), Cu(II), Zn(II), and Pb(II), so these ions can be titrated and determined by complexometric titration performed coulometrically, complexation reaction:

$$\left[Hg(NH_3)Y\right]^{-2} + NH_4^+ + 2e^- \rightarrow Hg(I) + 2NH_3 + HY^{-3}$$

where Y is EDTA ion and HY is ethylene diamine tetraacetic acid

$$HY^{-3} + Ca^{+2} \rightarrow CaY^{-2} + H^+$$

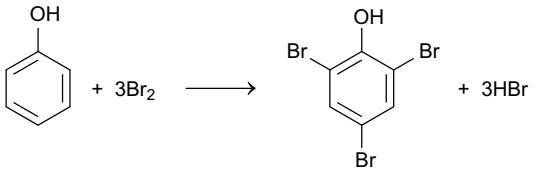
The end point of the titration is detected potentiometrically using the mercury indicator cathode and using a buffer solution of ammonia–ammonium nitrate at pH 8.3.

Organic Titrations

Organic compounds or those compounds which are not electroactive can also be titrated coulometrically.

Table 10.3 Examples of Redox titrations

Ions generated	Electrode reaction	ions estimated
Br_2	$2Br^- \rightarrow Br_2 + 2e^-$ (at anode)	Sb(III), Cl(IV), Tl(I)
Cl_2	$2Cl^- \rightarrow Cl_2 + 2e^-$ (at anode) $2I^- \rightarrow 2I_2 + 2e^-$ (at anode)	I^-, SCN^-, NH_2OH, organic compounds like phenol, mustard gas, 8-hydroxy quinoline
Ce^{+4}	$Ce^{+3} \rightarrow Ce^{+4} + e^-$ (at anode)	Fe(II), $[Fe(CN)_6]^{-4}$ Tl(III), U(IV), As(III)
Mn^{+3}	$Mn^{+2} \rightarrow Mn^{+3} + e^-$ (at anode)	Fe(II), As(III), $H_2C_2O_4$
$CuCl_3^{-2}$	$Cu^{+2} + 3Cl^- + e^- \rightarrow [CuCl_3]^{-2}$ (at cathode)	Cr(VI), IO_3^-, Br_2, V(V)



For example, bromine generated by anodic oxidation of bromide ion may be used to titrate phenol which does not react at any electrode. A visual end point which gets decolourized by an excess of Br_2 is indicated by the dye indigo carmine. Trichloroacetic acid and picric acid also quantitatively reduce at mercury cathode and so they too can be followed by controlled potential coulometric methods.

10.5 Advantages of Coulometric Titrations

The following advantages of coulometric titrations can be highlighted:

(i) Coulometric titrations can be suitable for all types of reactions.
(ii) These methods have high sensitivity and are able to give high precision and accuracy.
(iii) No standard solutions are required, as the electrons or the coulometric charge becomes the primary working standard solution.
(iv) There is no need for storing unstable reagents as they are generated electrolytically and are instantly used.
(v) A very small amount of titrate may be generated when required thus eliminating the difficulty involved in standardization and storage of dilute solutions. The whole analysis can be performed at micro or semi-micro levels.
(vi) Automation in the technique can be easily introduced to enhance precision and accuracy.

Solved Problems

1. Calculate time needed for a constant current of 0.96 A to deposit 0.5 g of Co(II) as elemental cobalt on the surface and deposit Co_3O_4 on the anode. (molar mass of Co = 58.93)

   Solution:

   $$Co^{+2} + 2e \rightarrow Co \text{ (at Cathode)}$$

   $\therefore$ 2 F of electricity required to deposit one mole of Co

   $$\therefore \qquad w = \frac{58.93 \times 0.96 \times t}{2 \times 96500}.$$

where w = mass deposited, i.e. 0.5 g

$$0.5 = \frac{58.93 \times 0.96 \times t}{2 \times 96500}$$

$$t = 1705.76 \text{ s} = 28.4 \text{ min}$$

$$3Co^{+2} + 4H_2O \rightarrow Co_3O_4 + 8H^+ + 2e^- \text{at anode}$$

Since these are 3 mol of cobalt in one mole of Co_3O_4, then amount of Co_3O_4 obtained from

$$0.5 \text{ g of } Co^{+2} = \frac{240.79 \times 0.5}{3 \times 58.93} \times 0.5 \qquad \text{(Molar mass of } Co_3O_4 = 240.79)$$
$$= 0.68 \text{ g of } Co_3O_4$$

$$\therefore \qquad 0.68 = \frac{240.79 \times 0.96 \times t}{2 \times 96500}$$

$$t = 567.75 = 9.46 \text{ min}$$

2. In a coulometric titration of 20 ml of $K_2Cr_2O_7$ with iron (III), reduction of iron takes place in 25 min when a current of 200 mA was passed. Find the normality of $K_2Cr_2O_7$.

Solution:

$$\text{Total charge flowing} = i \times t$$
$$= 0.200 \times 25 \times 60 = 300 \text{ coulombs}$$

$$Fe^{+3} + e^- \rightarrow Fe^{+2}$$

reduction of mole of iron require 1F of charge

$$\therefore \quad 300 \text{ coulomb will give} = \frac{300 \times 1}{96500} = 3.01 \times 10^{-3} \text{g equivalent for 20 ml solution}$$

$$\text{Normality of } K_2Cr_2O_7 = \frac{3 \times 10^{-3} \times 1000}{20}$$
$$= 0.1554 \text{ N}$$

Unsolved Problems

1. Determine the amount of cadmium deposited in 4 h by a current of 2 amperes.
2. The iron in 0.1 g sample was converted to Fe^{+3} and coulometrically titrated by Ti^{+3} ions with a current of 15 mA for 123 s. Calculate the % amount of iron in the given sample.
3. Determine the time taken by a current of 0.5 amps to deposit 0.59 g of silver when the current efficiency was 80%.
4. In a mixture of 35.4 ml of $H_2 + O_2$ was evolved when zinc was reduced in a cell. Find the concentration of zinc in the cell.
5. What are the advantages of constant-current coulometry over controlled potential coulometry?
6. Explain why constant-current coulometric method is called coulometric titration.
7. What are the advantages of coulometric methods of analysis?
8. Discuss the suitable methods to determine end point in coulometric titrations.
9. Give an example of redox titration by coulometric method.
10. Discuss how a complexometric titration can be performed by coulometric method.

Answers

1. 16.71 g
2. 1%
3. 18.6 min
4. 0.0342 g of Zn metal

Chapter 11
Conductometric Methods

11.1 Introduction

Conductometric methods are popular techniques and are the simplest of various electroanalytical methods. Conductometric measurements are based on the measurements of electrolytic conductance which is caused by the movement of ions. The ability of any ion to transport charge depends on the mobility of the ion so that it can transport charge or conduct electricity through the bulk of the solution. Molecules and ions of a solution possess thermal energy and keep colliding which causes random motion but when an electrical field is applied to the solution, the ions move towards oppositely charged electrodes and thus conduct electricity. The mobility of ions is affected by their charge, size, mass, and extent of solvation.

Conductometry is applied in various ways to physicochemical analysis directly or indirectly. Usually, it has three types of applications direct analysis, stream monitoring, and conductometric titrations.

11.2 Important Principles of Conductometric Methods

Solutions can be measured for their electrolytic conductivity by their ability to carry current. This ability to flow current is influenced by the potential gradient. According to Ohm's law, the current flowing I is given by

$$I = \frac{E}{R}$$

where I, the current, is in amperes, E, the potential between the electrodes, is in volts, and R, the resistance offered by the electrolyte solution, is in ohms.

The reciprocal of resistance is called conductance (S)

P. Malhotra, *Analytical Chemistry*,
https://doi.org/10.1007/978-3-031-26757-4_11

$$S = \frac{1}{R}$$

S.I. unit of conductance = mhos or ohm or Siemens S

$$= \Omega^{-1}$$

The resistance R of a conducting substance is directly proportional to its length (d) and inversely proportional to its cross-sectional area A.

$$R \propto \frac{d}{A}$$

$$R = \rho \frac{d}{A}$$

where ρ = specific resistance.

Inverse of specific resistance is known as specific conductance or conductivity (κ) (kappa)

$$\frac{1}{\rho} = \kappa$$

$$\frac{1}{R} = S = \kappa \frac{A}{d}$$

$$\kappa = \frac{d}{RA}$$

where $\frac{d}{A} = G$ = Cell constant.

The units of specific conductance are $\Omega^{-1}\text{cm}^{-1}$ or S cm^{-1}.

The specific conductance or conductivity of a solution depends inversely on the area between the electrodes and directly on the length of the electrodes.

Equivalent Conductivity

Equivalent conductivity may be defined as the conductance of a solution containing one gram equivalent of an electrolyte placed between two sufficiently large electrodes which are spaced one cm apart.

$$\Lambda_{\text{eq}} = 1000 \frac{\kappa}{C_S}$$

Λ_{eq} = Equivalent Conductivity.

where

C_S = concentration of the solution in g equivalent per litre.

Molar Conductivity

Molar conductivity or molar conductance is defined as the conductance of a solution containing one gram mole of the electrolyte when placed between two sufficiently large electrodes placed 1 cm apart.

$$\Lambda_m = 1000\frac{\kappa}{C_m}$$

where C_m is the concentration of the solution in moles/litre.

According to Kohlrausch's Law of independent migration of ions, the conductance of solutions is due to the migration of its ions. At infinite dilution the ions theoretically are independent of one another and each ion contributes to the total conductance

$$\Lambda_m^{\circ} = \Sigma\left(\lambda_+^{\circ}\right) + \Sigma\left(\lambda_-^{\circ}\right)$$

where Λ_m° = molar conductivity at infinite dilution, λ_+° and λ_-° are ionic conductivities of cation and anion respectively at infinite dilution. Both temperature and concentrations of the solution influence its molar conductance. Equivalent conductance and molar conductance increase on dilution. An increase in temperature causes an increase in ionic conductance. For every unit-degree rise in temperature, a 2–3% rise in conductance is expected.

11.3 Measurement of Conductance

Measurement of conductance of electrolytic solutions is done by measuring the resistance offered by the part of solution placed between the electrodes. The resistance is measured using Wheatstone Bridge arrangement but, for direct measurement of conductance, specially designed conductance cells are available commercially. For conductometric measurements, the electrodes are kept in direct contact with the solution and an alternating current (AC) of about 1000 Hz frequency is used. All precautions are taken to avoid Faradic current and polarization at the surface of electrodes.

Conductance cells

For the purpose of measuring conductance and performing titrations, simple conductance cells are known. The basic requirement for a conductance cell is a pair of electrodes dipped in a solution (analyte) and a provision for stirring the solution as well as additions to its content could be done easily. The typical dip-type cell has an open

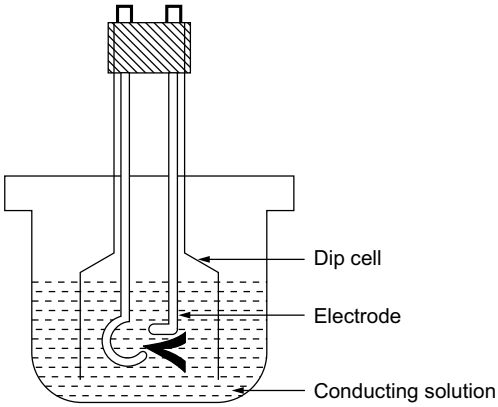


Fig. 11.1 Conductivity cell

container (Fig. 11.1) containing the test solution. The electrodes, generally of platinum, are immersed in the solution to a depth sufficient to cover the electrodes, the length and breadth of the electrodes are selected according to the resistance required. For calibration, a conductance cell with a solution of known conductivity, usually potassium chloride, is employed.

Calibration is rather required over the entire conductance range.

Industrial conductivity probes often employ an inductive method, which has the advantage that the fluid does not wet the electrical parts of the sensor. Here, two inductively coupled coils are used. One is the driving coil producing a magnetic field and it is supplied with accurately known voltage. The other coil is a secondary coil whose one turn is in contact with the liquid and is the sensor. The induced current is the output of the sensor.

Temperature Dependence of Conductivity

The conductivity of a solution is highly temperature dependent, therefore it is important to either use a temperature-compensated instrument, or calibrate the instrument at the same temperature as the solution is being measured. Unlike metals, the conductivity of common electrolytes increases with increasing temperature. Over a limited temperature range, the following relationship is observed between the conductivity of a solution and temperature:

$$\sigma_T \quad = \sigma T_{Cal}[1 + \alpha(T - \sigma T_{Cal})]$$

where

T is the temperature of the sample
T_{Cal} is the calibration temperature
σT is the electrical conductivity at temperature T

Table 11.1 Compensation slope α for some common aqueous solutions

Aqueous solution at 25 °C	Concentrate (mass %)	α %/°C
HCl	10	1.56
KCl	10	1.88
H_2SO_4	50	1.93
NaCl	10	2.14
HF	1.5	7.20
HNO_3	31	31

σT_{Cal} is the electrical conductivity at the calibration temperature T_{Cal} $\propto$ is the temperature compensation slope of the solution.

The temperature compensation slope for most naturally occurring waters is about 2%/°C; however, it can range between 1 and 3%/°C. The compensation slope for some common water solution is listed in the Table 11.1.

Wheatstone bridge

Electrolytic conductance measurements are generally based on Wheatstone bridge-like arrangement, modified to operate on alternating current. As shown in (Fig. 11.2), R_3 and R_x are ratio arms kept at a ratio of 0.1, 1, or 10 or may be equal (i.e. $R_1 = R_2$). R_x is the resistance of the conductance cell and R_3 is the resistance of standard arm. Parallel to R_x is the capacitor to which an A.C galvanometer is connected. At zero current on galvanometer,

$$R_x = \frac{R_1}{R_2} \times R_3$$

However, for high resistance like in nonaqueous systems or molten salts, use of Wheatstone bridge is unsatisfactory for conductance measurements.

11.4 Applications of Conductance Measurements

Conductance measurements are usually performed in aqueous solutions because water is a poor conductor ($\kappa_{H_2O} = 5 \times 10^{-8}$ mho per cm at 25 °C). Repeatedly distilled water must be used as it gives a constant value of κ. Conductance measurements can be applied for analysis for a wide range of analytes some of which may be discussed below:

1. **Concentration of strong electrolytes**: It can be determined conveniently because solutions of strong electrolytes show a linear increase in conductance with increasing concentration, especially at high concentrations. It reaches a maximum and then diminishes. It's the interionic interaction which hinders the ionic

$$R_x = \frac{R_1}{R_2} \times R_3$$

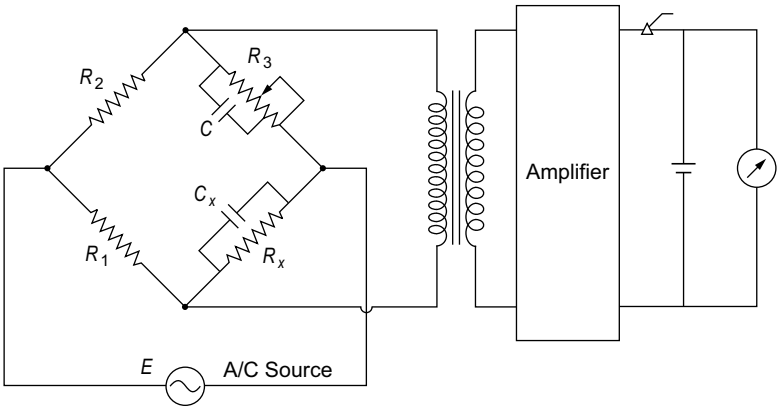


Fig. 11.2 Wheatstone bridge for conductance measurement

mobility and so the conductance decreases with an increase in concentration after showing a maximum.

2. **Analysis of fuming nitric acid**: Fuming nitric acid can be analysed for NO_2: H_2O content by conductivity measurements. Conductance is measured for HNO_3 before and after treatment with KNO_3. It is mainly done on the basis of calibration curves of conductance measurements.
3. **Basicity of organic acids**: Sodium salts of a large number of organic acids were measured for their conductance and Ostwal gave the empirical relation:

$$B = \frac{\lambda_{1024} - \lambda_{32}}{10.8}$$

where

B = basicity of acids

λ_{1024} and λ_{32} are the equivalent conductivities of N/1024 and N/32 solutions.

4. **Solubility of sparingly soluble salts**: The solubility of sparingly soluble salts is expressed as the solubility product. That is the product of the concentration of ions in the solution which are in equilibrium with the solid ion. These concentrations can be determined by conductivity measurements. For example,

$$PbSO_4 \rightleftharpoons Pb^{+2} + SO_4^{-2}$$

assuming a constant concentration of the solid,

Concentration of Pb^{+2} = concentration of SO_4^{-2}

$$= C$$

$$K_{SP} = C^2$$

The measurement of conductance κ of the saturated solution can lead to the determination of the concentration

i.e. $C = \frac{\kappa}{\Lambda_o}$

$$K_{SP} = C^2$$

$\therefore$ $K_{SP} = [\kappa/\Lambda_o]^2$

K_{SP} = Solubility Product

where

Λ_o = molar conductance at infinite dilution
κ = conductance.

5. **Ionic product of water**:

$$H_2O \rightleftharpoons H^+ + OH^-$$

ionization constant $K = \frac{[H^+][OH^-]}{H_2O}$

$$K_\omega = [H^+][OH^-]$$

K_ω = ionic product of water.

and molar conductance at infinte dilution can be given as

$$\Lambda_m^\circ = \lambda_{(H^+)}^\circ + \lambda_{(OH^-)}^\circ$$

$$\Lambda_m^\circ = \frac{\kappa}{C} \times 100$$

and

$$\lambda_{H^+} = 349.8\ \Omega cm^{-1}$$

$$\lambda_{OH^-} = 198.6\ \Omega cm^{-1}$$

$$\lambda_{H_2O} = 349.8 + 198.6$$

$$\Lambda_m^{\circ} = 548.4\ \Omega\text{cm}^{-1}$$

Experimentally pure water at 25 °C gives conductance

$$\kappa = 5.54 \times 10^{-8} \Omega\,\text{cm}^{-1}$$

Thus $$\left[\text{H}^+\right] = \frac{1000 \times 5.54 \times 10^{-8}}{548.4} = 1.01 \times 10^{-7}$$

$$\left[\text{OH}^-\right] = \frac{1000 \times 5.54 \times 10^{-8}}{548.4} = 1.01 \times 10^{-7}$$

$$K_\omega = 1.01 \times 10^{-7} \times 1.01 \times 10^{-7}$$

$$= 1.02 \times 10^{-14}$$

6. **Degree of dissociation of weak electrolytes**: The degree of dissociation (α) of weak electrolytes is given by the ratio

$$\alpha = \frac{\Lambda_m}{\Lambda_m^{\circ}}$$

of molar conductance to the molar conductance at infinite dilution.
Degree of dissociation α can be related to dissociation constant

$$K = \frac{C\alpha^2}{(1-\alpha)}$$

Therefore, we can find the degree of dissociation and dissociation constant by measuring molar conductivities at any point.

7. **Conductometric titrations**: Conductometric titration is the most promising application of conductance measurement and can be applied to all types of ionic reactions. Conductometric titrations provide convenient means for locating the end point in titration. The conductance method can be used to follow the course of titration if a great difference in specific conductance exists in the solution before and after the addition of the titrant. Conductometric titrations and its various types are discussed in detail in Sect. 11.5.

11.5 Types of Conductometric Titrations

Those titrations in which the conductivity of the solution changes sharply at the end point, can be performed conductometrically. In conductometric titrations the variation of the electrical conductivity of a solution during the course of a titration is followed. During conductometric titration, the titrant is added from the burette and

the conductivities are recorded. The values of conductivities are then plotted against the volume of the titrant added. Due to linear dependence of conductivity on the concentration of the ions present, two straight lines are obtained which intersect at a point known as end point or equivalence point.

For example, a 0.001 M HCl solution is titrated with 0.1 M NaOH. Initially, the conductance is high in HCl solution due to H_3O^+ which is highly conducting ($\lambda_+ = 350$) and as it is replaced by a lower conducting Na^+ ion ($\lambda_+ = 50$) due to the addition of NaOH, the conductance drops linearly. After complete neutralization, the end point is indicated after which conductance again increases steeply largely due to OH^- ions which are good conductors ($\lambda_- = 198$). As the concentration of OH^- ion increases, the rising branch of the titration curve is constituted (Fig. 11.3).

A curve may be obtained near the end-point owing to hydrolysis, dissociation of the reaction product, or appreciable solubility in case of precipitation reactions. In this case, the two branches are extrapolated to meet at the end point. More acute is the angle formed at the point of intersection, higher are the ionic conductances of the reactants. In the above curve the H_3O^+ branch is steeper than OH^- branch because of the higher ionic conductivity of H_3O^+ than the ionic conductivity of OH^-.

To restrict the volume change, the titrant should have ten times higher concentration than the solution being titrated. However, conductometric titrations are more valuable at low titrant concentration. In order to get straight line graphs, it is also essential to keep the total volume of the solutions constant during the titration. Whenever required, a correction to the reading can be applied

$$\text{Actual conductivity} = \left(\frac{v + V}{V}\right) \times \text{observed conductivity}$$

v = volume of the titrant or reagent added

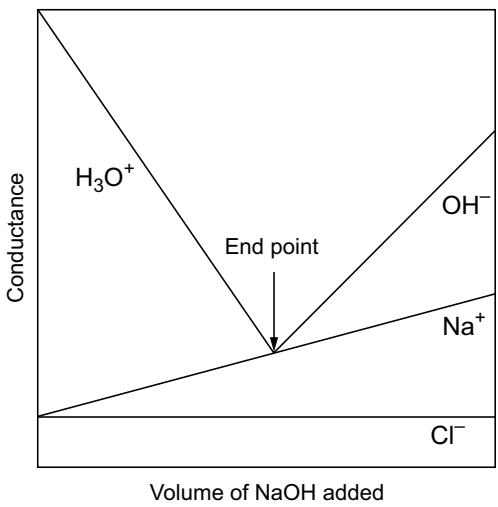


Fig. 11.3 Titration curve for HCl versus NaOH

$$V = \text{initial volume}$$
$$v + V = \text{final volume}$$

The correction factor is on the basis of the fact that conductivity is a linear function of dilution.

Coinciding with a range of volumetric reactions, conductometric titrations also range from acid–base titrations, replacement reactions, precipitation titrations, redox titrations, and complexometric titrations. Types of conductometric titrations are classified totally on the basis of the types of ionic reactions which take place during the titration and reach to completion at the equivalence point. They may be discussed as follows:

(a) **Acid–base titrations** are based on the neutralization reaction and their conductivity measurements rely on the varied mobilities of H^+ and OH^-. Conductivity measurements are plotted against the volume of the base added and end points are determined. The point of intersection of two lines is obtained for two ions involved in the reaction. In actual practice, one gets a curve near the end point due to the heat of neutralization getting evolved raises the temperature, change in volume, and interionic interaction. The branches of the curve can be extended to give the end point.

Strong acid–strong base

NaOH versus HCl

$$\left[H^+ + Cl^-\right] + \left[Na^+ + OH^-\right] \rightarrow Na^+ + Cl^- + H_2O$$

Conductometric titrations of strong acid and strong base have an advantage because the relative change in conductivity is almost independent of the concentration of strong acid being titrated. Equal accuracy is observed for both dilute and concentrated solutions (Fig. 11.4).

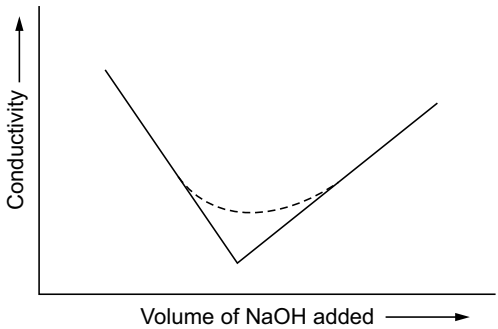


Fig. 11.4 Titration curve for strong acid–strong base

Strong acid–weak base

$$\left[H^+Cl^-\right] + NH_4^+OH^- \rightarrow NH_4^+Cl^- + H_2O$$

Initially, the highly conducting H^+ shows high conductivity but it undergoes a steep drop in conductivity due to the replacement of H^+ by NH_4^+ ions. After neutralization, increase in the volume of NH_4OH does not change the conductance because it is a weakly ionized electrolyte (Fig. 11.5).

Weak acid–strong base

$$\left[CH_3COO^-H^+\right] + \left[Na^+OH^-\right] \rightarrow \left[CH_3COO^-Na^+\right] + H_2O$$

After the end point, excess NaOH shows a rapid rise in conductivity because NaOH being a strong base ionizes to high degree of dissociation and increase in OH^- concentration increases the conductivity (Fig. 11.6).

Weak acid–weak base

$$\left[CH_3COO^-H^+\right] + \left[NH_4^+OH^-\right] \rightarrow CH_3COONH_4 + H_2O$$

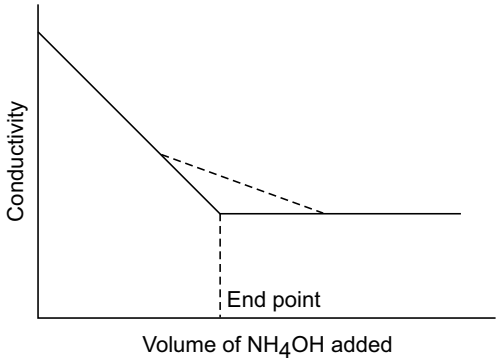


Fig. 11.5 Titration curve for strong acid–weak base

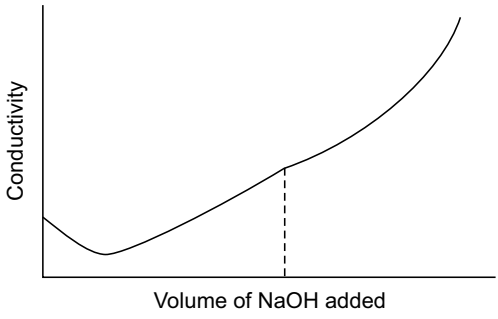


Fig. 11.6 Titration curve of weak acid–strong base

The first decrease (curve 1) is due to the nonconducting weak acid and its decreasing anion concentration. It passes through the minimum and then increases with increasing salt concentration and then an abrupt change near the end point due to weakly dissociated base (Fig. 11.7).

Replacement Reactions

Reaction of a strong acid with salts of weak acid and strong base like sodium acetate and hydrochloric acid can be estimated conductometrically.

$$\left[CH_3COO^-Na^+\right] + \left[H^+Cl^-\right] \rightarrow \left[CH_3COO^-H^+\right] + NaCl$$

A small increase in conductivity near the end point takes place chloride ions have higher conductance than acetate ions and after the end point, there is a rapid increase due to excess of HCl (Fig. 11.8).

Precipitation reactions

Precipitation reactions also involve replacement of ions. To precipitate a cation, such a titrant is selected whose cation has the least mobility and, similarly, it is selected for precipitating an anion.

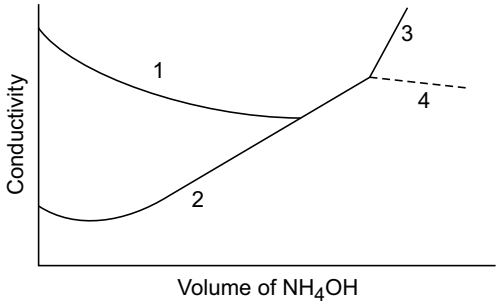


Fig. 11.7 Titration curve of weak acid–weak base

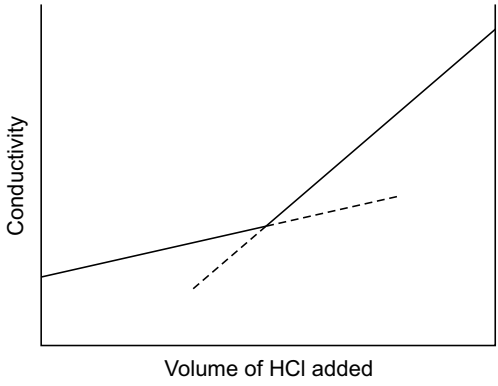


Fig. 11.8 Titration curve of strong acid with salt of weak acid and strong base

For example,

$$\left[K^+ + Cl^-\right] + \left[Ag^+ + NO_3^-\right] \rightarrow \left[K^+ + NO_3^-\right] + AgCl$$

Since both Cl^- and NO_3^- ions have the same conductance, so initially a parallel line is observed which rapidly rises after the end point due to the increase in conductance by excess of silver nitrate (Fig. 11.9).

When both products are insoluble, conductivity decreases at first and then increases. For example, for $MgSO_4$ versus $Ba(OH)_2$ titration, it increases after the end point due to excess of $Ba(OH)_2$.

$$MgSO_4 + Ba(OH)_2 \rightarrow BaSO_4 + Mg(OH)_2$$

Redox reactions

Many redox reactions can be followed conductometrically. For example,

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

The decrease in H^+ ion decreases conductivity sharply and then remains constant. In low initial acid concentration, good results are found (Fig. 11.10).

Complex Formation Reaction

Complexometric titrations can also be followed conductometrically. A common complex formation reaction is the determination of mercury by cyanide ion. A solution of nitrate and perchlorate of mercury (II), is titrated by cyanide ions the conductivity rises only slightly, but, after equivalence point, it increases sharply because of the addition of K^+ and CN^- ions.

$$Hg^{+2} + 2ClO_4^- + 2K^+ + 2CN^- \rightarrow Hg(CN)_2 + 2KClO_4$$

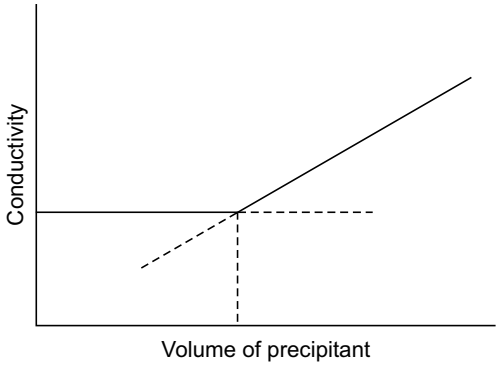


Fig. 11.9 Titration curve of precipitation reaction

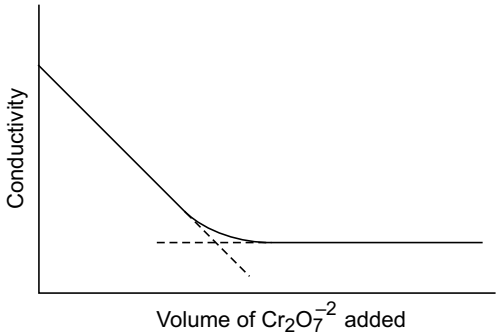


Fig. 11.10 Titration curve for a redox reaction

Halides, cyanides, and thiocyanates can be estimated conductometrically by complex formation from perchlorates.

In the titration of KCl with $Hg(ClO_4)_2$, the graph shows two inflection points, the first one due to the formation of $HgCl_4^{-2}$ and the second one due to the formation of K_2HgCl_4

$$Hg(ClO_4)_2 + 4KCl \rightarrow HgCl_4^{-2} + 2K^+ + 2KClO_4$$

$$HgCl_4^{-2} + 2K^+ \rightarrow K_2HgCl_4$$

Conductometric titrations are applicable in all those reactions where the ionic content is markedly less at the equivalence as compared to its concentration before and after the equivalence point. For very high ionic concentrations, the conductometric methods are not suitable because in this case there is negligible change in conductance near end point. For acid–base titrations, conductometric titrations are quite suitable while for redox titration potentiometric titrations give better results (Fig. 11.11).

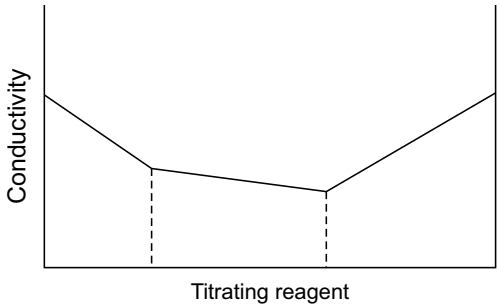


Fig. 11.11 Titration curve for a complexation reaction

11.6 Advantages and Disadvantages of Conductometric Titrations

Like all other titrations, conductometric titrations can be carried out for generally all types of reactions particularly those where ions are an integral part of reactants as well as products and they must differ in their respective conductivities. The conductance method can be used to follow the course of titration if a great difference in specific conductance exists in solution. Based on these, conductometric titrations have a lot of advantages which may be summed up as follows.

(i) Conductometric titrations can be efficiently used in the case of determining coloured liquids where normal titrations do not identify end points by coloured indicators.
(ii) Dilute solutions can also respond to conductometric methods.
(iii) Even weak acids can be measured by conductometric methods of titrations.
(iv) Conductometric titrations can be spared from measuring actual conductance value, instead the value on Wheatstone bridge can be directly plotted against the volume of titrant added to measure the analyte.
(v) Conductometric titrations can be carried out for turbid solutions also.
(vi) The results are quite accurate.

Disadvantages of Conductometric Titrations

(i) When salt levels have increased beyond a point, they hinder the mobility of ions and influence the accurate measurement of conductivity.
(ii) Sometimes, high concentration of ions mask the conductivity and hence accuracy in results is not found.
(iii) Majorly only redox and acid–base titrations give accurate results when performed conductometrically.

11.7 High-Frequency Titration

High-frequency titration is a type of conductometric titration in which two electrodes are mounted on the outside of the beaker or vessel containing the solution to be analysed and alternating current source in the megahertz, range is used to measure the course of titration.

Conductometric methods are all based on the mobility of ions in an electric field. When alternating current is used to generate electric field, it prevents the deposition of ions and maintains the movement of the ions for a short distance between, alternations. With an enhanced frequency of alternating current, the ions will keep vibrating and will not be able to acquire their full speed and such a situation leads to a significant molecular polarization. Due to this polarization, the electrons of the molecule drift towards positive electrode and the nuclei get attracted towards negative electrode when the molecule is exposed to external electric field (Fig. 11.12).

Fig. 11.12 Instrument for high-frequency conductometric titration

This movement of electrons and nuclei in the opposite direction causes distortion of the molecule upon applying electric field and gets removed by the removal of electric field. Under the effect of applied electric field the molecules which possess inherent permanent dipoles, e.g. H_2O, CH_3COCH_3, $CHCl_3$, etc., orient themselves +ve to −ve and vice versa and show oriental polarization in addition to temporary distortion polarization. Both these types of distortion polymerization, orientation, and temporary distortion give rise to brief currents. Such a current is very small and insignificant at low frequencies. At high frequencies, few megahertz (radio frequencies) the conduction current along with polarization current (both becoming of the same order) become quite significant and together add to conductometric measurements. Weakly conducting solutions can also be measured with accuracy when high-frequency titrations are carried out. High-frequency titrations have thus found many applications in a wide range of samples. Non-conducting samples, organic compounds, and binary samples can be measured by placing them between two plates of a capacitor and following their energy of absorption. Direct high-frequency titrations can be used for acid–base titrations and complexometric titrations also. Analysis of mixture of organic compounds and determination of dielectric constant are facilitated by this method. The high value of dielectric constant helps in determining a very small amount of water in binary organic liquids calibration curves prepared from known solutions can help to analyse mixtures of ortho and para xylenes, hexane and benzene, methyl alcohol and ethyl alcohol, and many other binary mixtures. EDTA titrations for analysing Mg^{+2}, Ca^{+2} and Zn^{+2} leading to complexometric titration have been successfully carried out.

Advantages of High-frequency titrations

As compared to conductometric titrations, the high-frequency titrations have certain advantages which may be highlighted as follows:

(i) Any special type of conductivity cell is not required for this method.
(ii) The analyte solutions are not in contact with the metal electrodes, therefore the possibilities of polarization of electrodes and any other interaction of the solution with the metal is prevented.
(iii) Platinum electrodes generally pose the catalytic effect on its surface which is avoided as the electrodes do not contact the sample.
(iv) The titrimetre shows variation in oscillating current which is connected to a potentiometric recorder and its values are recorded and plotted against time.
(v) Organic compounds which do not dissociate into ions showing poor conductivity can also be analysed by high-frequency titrations. Thus, even non-aqueous solutions can be measured.

Disadvantages of High-frequency titrations

(i) The design of the technique is slightly complexed and faces difficulty in tuning the instrument.
(ii) For quantitative estimations, its sensitivity is not greater than that of Wheatstone bridge.

Solved Examples

1. A 0.0109N NH_4OH solution shows electrical conductivity of 1.02×10^{-4} ohms/cm^2. Find the dissociation constant of NH_4OH. (λ° for $NH_4OH = 281$)

   Solution:
   The equivalence conductance

$$\lambda = \frac{K \times 1000}{C}$$
$$= \frac{1.02 \times 10^{-4} \times 1000}{0.0109}$$
$$= 9.38$$

   degree of dissociation $\alpha = \frac{\lambda}{\lambda^\circ}$

$$\alpha = \frac{9.38}{281} = 0.0334$$

   Therefore, dissociation constant

$$K = \frac{\alpha^2 C}{1-\alpha} = \frac{(0.0334)^2(0.0109)}{(1-0.0334)} = 1.26 \times 10^{-5}$$

2. If the specific conductance of 0.02 M HCl is 7.92×10^{-3} ohms, what is the molar conductance of 0.02 MHCl solution:
   Solution:

$$\Lambda_m = \frac{K}{C} \times 1000 = \frac{7.92 \times 10^{-3} \times 1000}{0.02} = 396 \text{ ohm}^{-1}$$

Unsolved Problems

1. A solution of 0.01 NaOH when titrated against HCl conductometrically gives the following readings 0(218), 4(230), 9(247), 10(256), 11(269), 12(285), 14(323), 17(380). Calculate the number of moles of NaCl present in solution.
2. A solution of 0.1N KCl has a conductance of 0.01315 and 0.105 N acetic acid has a conductance of 5.58×10^{-3}. What is the dissociation constant of CH_3COOH acid?
3. Define specific conductance and equivalent conductance.
4. What is the effect of dilution on (i) conductance, (ii) specific conductance, (iii) equivalent conductance and (iv) molecular conductance?
5. What are conductometric titrations and what are its various types?
6. What are the advantages of conductometric titrations?
7. Describe an ideal conductivity cell.
8. State how changes in temperature affect conductivity.
9. Discuss various applications of conductance measurements.
10. Write a short note on High-frequency titrations.

Answers

1. 0.820 g
2. 1.88×10^{-5}

Chapter 12
Solvent Extraction

It is among the most widely followed separation techniques in which a solution is treated with another solvent immiscible with the first so that solute transfer takes place. The extraction of an analyte or group of analytes occurs from one liquid phase to another and is also known as liquid–liquid extraction. The versatility of this method is well recognized and its popularity lies in the fact that it requires a very simple instrument, just a separating funnel and so is very economical. This technique can be used for the preparation, purification, separation, and enrichment of an analyte at even the smallest concentrations. A rapid and clean separation of both organic and inorganic substances can be achieved by solvent extraction. Analytical separations are done because of the distribution of substances between two phases.

12.1 Basic Principle and Classification

Basic principle on which the process of solvent extraction takes place is based on the **Nernst Distribution Law**. The Nernst distribution law states that if a substance which is soluble in both liquid phases is allowed to remain in contact with both, then at equilibrium the ratio of activities of the material in each phase is a constant known as partition coefficient.

$$K = \frac{a_1}{a_2}$$

or

$$K_D = \frac{S_o}{S_{aq}}$$

where

S_o = solubility in organic layer.
S_{aq} = solubility in aqueous layer.

P. Malhotra, *Analytical Chemistry*,
https://doi.org/10.1007/978-3-031-26757-4_12

K_D = distribution coefficient.

The term distribution ratio D may be used to account for the total concentration of species in two phases. Nernst distribution law can be extended to use distribution ratio being equal to the ratio of the total concentration of the species in two phases:

$$D = \frac{C_o}{C_{aq}}$$

D = distribution ratio.
C_o = total concentration of species in the organic phase.
C_{aq} = total concentration of species in the aqueous phase.

When it is assumed that there is no association, dissociation, or polymerization then under idealized conditions

$$K_D = D$$

Limitation of Nernst distribution law

Basically, there are two limitations of Nernst distribution law:

(a) The solute which is to be distributed, should not react with any of the solvents
(b) The solute should not undergo any change in its molecular state in the solvents, i.e. it should neither dissociate nor associate.

Percentage of extraction $E\%$

If V_{aq} and V_{org} represent the volume of aqueous and organic phases, respectively, and E moles of Solute, A, out of 100 mol of A, is present in organic phase and (100-E) moles of A in aqueous phase then,

$$D = \frac{C_o}{C_{aq}} = \frac{\frac{E}{V_{org}}}{\frac{100-E}{V_{aq}}}$$

$$= \frac{E}{V_{org}} \times \frac{V_{aq}}{100 - E}$$

$$E = \frac{100D}{D + \frac{V_{aq}}{V_{org}}}$$

$$E\% = \frac{100D}{D + \frac{V_{aq}}{V_{org}}}$$

where $E\%$ = percentage extraction

Thus, to increase the percentage extraction, volume of the organic phase should be increased.

If $V_{aq} = V_{org}$.

then $E\% = \frac{100D}{D+1}$.

Therefore, a more efficient way of increasing the extracted amount is to use the same volume of organic liquid to perform successive extractions. When a solute is extracted from an aqueous solution by performing successive extraction with smaller portions of the same volume of solvent, the complete separation of the solute takes place which indicates that multiple small-scale extractions are more efficient than single extraction.

Classification of Extractions

Based on the nature of the extracted species formed or the process by which extraction takes place, extractions have been classified into the following categories:

(a) Chelate extraction where the extraction of a metal ion proceeds via chelate formation or a closed ring structure formation.
(b) Extraction by solvation because, in the organic phase, the extracted species gets solvated.
(c) Extraction by ion pair formation as it forms a neutral uncharged species which gets extracted in the organic phase.
(d) Synergistic extraction in which two extractants are used. This enhances the percentage of extraction.

12.2 Sequence and Mechanism of Extraction

The reactions may though vary from system to system but, generally, all extraction processes involve the following mechanism following three basic steps.

(a) **Formation of a distributable species**: In solvent extraction the distributable species is the uncharged or neutral molecule whose formation is an essential step for extraction as a charged species cannot be extracted. Therefore, uncharged species may be formed either by the formation of chelation, solvation, or ion pair formation. For instance, there occurs the reaction of the metal ion in the water phase with the complexing agent to form either a neutral complex or an extractable ion pair

$$M + nL \rightarrow ML_n$$

where

M = metal
L = Ligand
n = Valency of metal.

$$M^{+n} + xL \rightarrow ML_x^{n+}$$

$$ML_x^{+n} + nA^- \rightarrow \left(ML_x^{+n} nA^-\right)$$

The metal may also react with other complexing agents to form other complexes like MA_n and may further undergo hydrolysis to form $M(OH)_n$ in steps. All these intermediate reactions may also occur.

For example, the extraction of copper with 1, 10 phenanthroline in chloroform forms an ion pair

$$\mathrm{Cu}^{+2} + o\text{-phen} \rightleftharpoons \left[\mathrm{Cu(phen)}^{++}\right]$$

$$\mathrm{Cu(phen)}^{++} + 2\mathrm{ClO}_4^- \rightleftharpoons \left[\mathrm{Cu(phen)}^{++} : 2\mathrm{ClO}_4^-\right]$$

and Iron (III) with ether forms anionic complex

$$\mathrm{Fe}^{+3} + 4\mathrm{Cl}^- \rightarrow \mathrm{FeCl}_4^-$$

$$\mathrm{FeCl}_4^- + \mathrm{H}^+ \rightleftharpoons \left[\mathrm{H}^+, \mathrm{FeCl}_4^-\right]$$

both cation and anion get solvated by ether

$$\left[\mathrm{H(ether)}^+, \mathrm{FeCl}_4^- (\mathrm{ether})_4\right]$$

The stability of such a coordinated complex depends upon the acidity of the metal ion, basicity of the ligand, and many other stereochemical considerations. High charge also enhances the speed of extraction of metal ion, for example, Fe(III) is extracted faster than Fe(II) by diethyl ether.

(b) **Distribution of distributable species**: After the formation, the distributable species moves across the boundary until an equilibrium is established and the equilibrium concentrations satisfy the distribution law.

$$(ML_n)_o \rightleftharpoons (ML_n)_w$$

o = organic
w = water.

(c) **Interaction in the organic phase**: After moving across the phase boundary, the extractable complex may undergo polymerization or dissociation or may undergo interaction with other components in the organic phase.

All these possible interactions will affect the distribution leading to a wide gap between D and K_d which is further also governed by pH

$$D = \frac{K_D}{1 + \frac{K_a}{[H^+]_{aq}}}$$

where $$Ka = \frac{[H^+]_{aq}[C_6H_5COO^-]_{aq}}{[C_6H_5COOH]_{aq}}$$

for an acid equilibria like that of benzoic acid.

$$C_6H_5COOH \rightleftharpoons C_6H_5COO^- + H^+$$

$$K_D = \frac{(C_6H_5COOH)_{ether}}{(C_6H_5COOH)_{water}}$$

12.3 Methods of Solvent Extraction

There are three methods used for solvent extraction. These are (i) batch extraction (ii) continuous extraction and (iii) counter-current extraction.

Batch Extraction

The apparatus used for solvent extraction is the separating funnel in which generally a solute is extracted from an aqueous solution into an immiscible organic solvent. The solute in an aqueous solution is well shaken for a few minutes with the organic solvent in which it is not soluble. The phases separate in such a way that the denser bottom layer is aqueous layer and is removed. The process can be repeated several times to ensure good separation or high separating efficiency (Fig. 12.1).

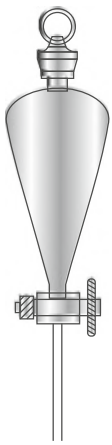


Fig. 12.1 Diagram of separating funnel

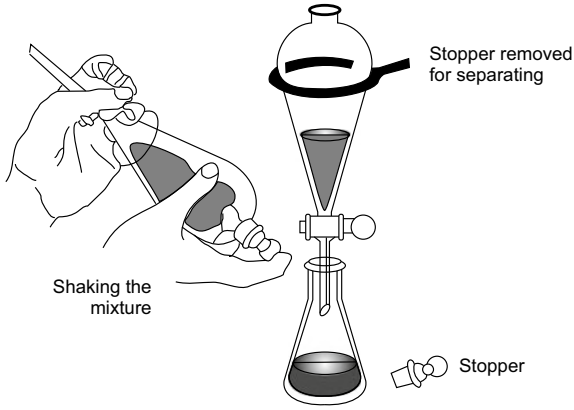


Fig. 12.2 Separating funnel showing continuous extraction

For *example,* a sample of ethyl alcohol having benzene as an impurity can be extracted by this technique. When water is added, it goes with alcohol and benzene being insoluble in water forms a separate layer which can be separated by withdrawing the heavier aqueous layer from the bottom of the separating funnel.

Continuous Extraction

In the case of low distribution coefficient between the organic solvent or aqueous layer, i.e. the solubility in both the solvents is not much different than a large number of multiple batch extractions are carried out. For such a continuous extraction procedure a special extraction equipment known as a continuous extractor has been designed for both the cases, when organic solvents are higher than water or are heavier than water (Fig. 12.2).

The isolation of organic compound from aqueous solution is based on the fact that, due to the presence of inorganic salts, the solubility of many organic compounds in water decreases considerably which reduces the loss of organic solvent in the extraction process.

Counter-current Extraction

A special counter-current extract or designed by Craig is used for this type of extraction method. It consists of 300–4000 separating chambers connected in the four of a train. In a specialize counter current extraction, the two phases flow through one another in opposite directions. This enhances the efficiency because fresh extractant is brought into contact with solute-depleted phase and the solute-enriched extractant is brought into contact with a fresh aqueous solution. Following a binomial expansion, the amount of solute in either of the phases can be calculated.

Extraction by Chelate Formation

Metal extraction procedures majorly involve chelate formation by metals with chelating ligands. Ligands which coordinate with metal at more than one donor atoms and form a cyclic compound with the metal. Various types of chelating agents

are used for metal extraction and have a certain selectivity. Anionic ligands are used as masking agents of cations, neutral chelates are extracted by organic solvents and cationic chelates like Cu (2–9 dimethyl) 1–10 phenanthroline, pair with the perchlorate anion form neutral ion pair which is easily extracted. There are many factors which govern the stability of metal chelates including the basicity of the ligand and the no. of atoms involved in making ring-like metal chelate complexes. For minimum steric hindrance five- or six-membered rings are more stable in metal complexes.

Many of the chelating agents commonly used are organic compounds, are weak acids that ionize in water, and are added to the organic phase. The metal displaces the Hydrogen of the acids and gets coordinated with the organic compound.

At equilibrium, the chelating agent distributes between aqueous and organic phases:

$$(HR)_{aq} \rightleftharpoons (HR)_{org}$$

$$D_{HR} = \frac{[HR]_{org}}{[HR]_{aq}}$$

in aqueous phase

$$(HR)_{aN} \rightleftharpoons H^+ + R^-$$

and $$K_a = \frac{[H^+]_{aq}[R^-]_{aq}}{[nR]_{aq}}$$

$$M^{+n} + nR^- \rightleftharpoons MR_n$$

$$K_f = \frac{[MR_n]_{aq}}{[M^{+n}]_{aq}[R^-]^n_{aq}}$$

where

K_f = formation constant of the chelate

Distribution of metal chelate between organic and aqueous phases gives

$$[MR_n]_{aq} \rightleftharpoons [MR_n]_{org}$$

$$D_{MR} = \frac{[MR_n]_{org}}{[MR_n]_{aq}}$$

D_{MR} = Distribution coefficient of MR_n.

The distribution ratio D when the chelate MR_n is in the organic phase and the metal ions M^{n+} in the aqueous phase

$$D = \frac{[MR_n]_{org}}{[M^{+n}]_{aq}}$$

$$D = \frac{D_{MR_n} K_f K_a [HR]_{org}}{D_{HR}[H^+]_{aq}} = \frac{\mathrm{K}[HR]_{org}}{[H^+]_{aq}}$$

where $K = \frac{D_{MR_n} K_f K_a}{D_{HR}}$.

Interpretation of mathematical expression leads to the conclusion that the extraction not only depends on the chelating reagent concentration, (HR) but also on H^+ concentration, i.e. pH of solution. Therefore, extraction efficiency can be affected only by changing reagent concentration and pH. Since metal concentration M^{+n} term is not in the equation, the overall extraction does not depend upon the initial metal concentration. More stable the chelate (larger K_f value) and higher are the solubility of chelate in organic phase (larger D_{MR}), greater is the extraction efficiency which can also be enhanced by an acidic reagent (larger K_a) and small D_{HR} value.

If

$$D = \frac{K[HR]^n_{org}}{[H^+]^n_{aq}}.$$

Taking log of both sides

$$\begin{aligned} \log D &= \log K + n \log[HR]_{org} + \log\left[H^+\right]^{-n}_{org} \\ &= \log K + n \log[HR]_{org} + npH \end{aligned}$$

For constant reagent concentration

$$\log D = \log K^* + npH$$

of the straight line, the slope gives the n (Fig. 12.3).

As mentioned previously that, for $V_{org} = V_{aq}$,

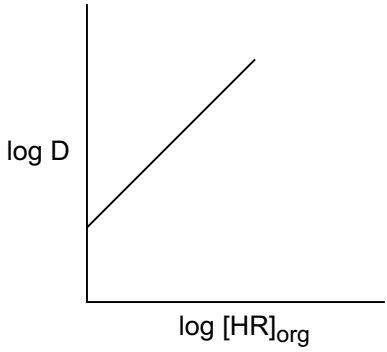


Fig. 12.3 Distribution ratio versus the concentration of chelating reagent

$$D = \frac{E}{100 - E}$$

Taking log
$$\log D = \log \frac{E}{100 - E} = \log K^* + npH$$

for $E = 50\%$

$$\log \frac{50}{100 - 50} = \log K^* + npH$$
$$-\log K^* = n(pH)$$
$$pK^* = npH(1/2)$$
$$pH(1/2) = \frac{1}{n} P^{K^*}$$

Thus, $pH(1/2)$, the pH for 50% extraction, is dependent only on the valence (n) of the ion and the value of const K^* (stability constant. The significance of $pH(1/2)$ lies in determining the stability constants of chelates in aqueous solution as it is inversely proportional to $pH(1/2)$.

12.4 Extraction by Solvation

Another pathway for solvent extraction is by forming associated ion pairs which form neutral species suitable for extraction. The value of the ion pair formation constant (K) is related to the dielectric constant $\in$ and T by the expression

$$K = \frac{4\pi N e^2}{1000 \in K_T} Q(b)$$

where $b = \frac{e^2}{a \in K_T}$

N = Avagadro number
e = charge
K = Boltzmann constant
T = absolute temperature
$Q(b)$ = calculable functions
$\in$ = dielectric constant of the medium
a = empirical parameter dependent upon the distance between charge centres when in contact.

For solvents having low dielectric constants ($\in < 40$), ion associations are of greater degree. Temperature and size of ions also play major roles. It increases with the increase in temperature and decreases with the increase in size of the anion with

donor atoms of ion also influencing the association.

$$\left[Na^{+}NH_2^{-}\right] < \left[Na^{+}NHC_6H_5\right] < \left[Na^{+}N(C_6H_5)_2^{-}\right]$$

Just as dilution enhances degree of dissociation, rise in concentration raises degree of association.

Ions having more electronegative groups decrease association whereas long hydrocarbon chains enhance it. Later, the solubility of associated ion pairs or solvated ions in organic solvent influences the course of extraction. Role of salting out agents further governs the solvent extraction. For example, extraction of gold with tributyl phosphate in HCl increases with the addition of lithium chloride. Increase in salt concentration also lowers the dielectric constant of the aqueous phase and favours the association of ions. Solvent extraction gets improved by solvating salting ions by water and by raising the complex concentration.

There are several extractants which find applications in extraction by solvating solvents likes diethyl ether, ethyl acetate, methyl isobutyl ketone, 4 methyl pentane-2 all of which are oxygenated solvents and organophosphorus solvents like tributyl phosphate (TBP), tributylphosphine oxide (TBPO), and triotylphosphine oxide are used for the extraction of transition metals and *f*-block elements.

12.5 Applications of Solvent Extraction

(i) Determination of Iron as 8-hydroxy quinolates: Ferric ions are extracted from aqueous solutions with a 1% solution of 8-hydroxy quinoline in chloroform by double extraction. Using a spectrophotometer, the optical density is measured and then repeated with oxine solution where again optical density gives the metal analysis. The pH of the aqueous phase ranges from 2 to 10.

(ii) Determination of uranium as 8-hydroxy quinolate: 8 hydroxy quinolate of EDTA at pH 8.8 is used for extracting and analysing where EDTA acts as a masking reagent for Fe, Al, etc., and by measuring optical density, its amount is also determined.

(iii) Extractive separation and direct colorimetric determinations are done by using acetyl acetone, benzoylacetone, dibenzoyl methene, furoyl, thenoyl and thio-thenoyl trifluoro acetone, sulphur-containing ligands like dithizone, cupral, dithol, and xanthates are used for identifying and separating water pollutants.

(iv) Solid-phase extraction is one of the solvent extraction procedure used to isolate proteins from fluids, enrichment of polynumber aromatics, soil gas analysis, and water analysis for the presence of organic material.

(v) Analytical estimation of cations by solvent extraction using organic reagents also finds its applications in (a) determination of nickel as dimethyl-glyoximate complex, (b) determination of molybdenum by thiocyanate, (c) determination of lead by dithizone, and (d) determination of copper as diethyldithiocarbamate, etc.

(vi) Separation of amino acids, qualitative and quantitative analyses of biochemical compounds such as vitamins, peptides, nucleosides, and nucleotides are carried out efficiently by using minimum sample volumes.

(vii) Solvent extraction of not only alkali metals and alkaline earth metals but also transition metals like zinc, uranium, molybdenum, and main group metals is carried out using crown ethers and cryptands which are macrocyclic compounds.

Solved Problems

1. Cerium (IV) was extracted with 2-thenoyltrifluoroacetone in benzene with the distribution ratio of 999.0. The volume of the organic phase was 10 ml and aqueous phase was 25 ml. Find the percentage extraction.

Solution: $D = 999 \quad V_0 = 10\,\text{ml} \quad V_\omega = 25\,\text{ml}$

$$D = \frac{V_\omega / V_0^E}{100 - E}$$

$$= \frac{\frac{25}{10}x}{100 - x}$$

where x is % extraction

$$= \frac{2.5x}{100 - x}$$

$$x = 99.75$$

2. When uranium was extracted with 8-hydroxyquinoline in chloroform the percentage extraction was 99.8% in 25 ml volume of the aqueous and organic phase. Find the distribution ratio.

Solution: $V_0 = 25\,\text{ml}, \quad V_\omega = 25\,\text{ml}, \quad E = 99.8$

$$D = \frac{\frac{V_\omega}{V_0}E}{100 - E} = \frac{\frac{25}{25} \times 9.8}{100 - 99.8} = 499$$

Unsolved Problems

1. Calculate the fraction of a solute *A* extracted from 100 ml of an aqueous phase into 50 ml of an originally pure immiscible organic solvent, if the distribution coefficient is 80 and *A* exists as a monomer in each phase.
2. The distribution ratio for a certain extraction is 10. If 300 mg of solute is dissolved in 100 ml of solvent, *A*, find the amount of solute extracted by one extraction with 100 ml of amount of solute extracted by one extraction with

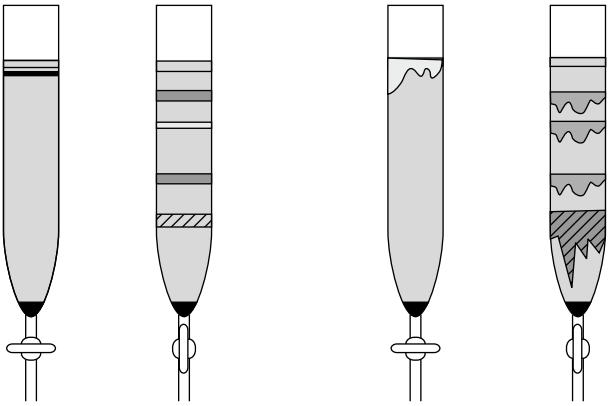
100 ml of solvent *B* and by two extractions with 50 ml of solvent *B* which is immiscible with solvent *A*. (Mol wt. of solute = 71.0)

3. State the law of distribution of a solute between two immiscible solvents. What are its limitations?
4. How many types of solvent extraction methods are available?
5. State the sequence and mechanism of solvent extraction.
6. What do you mean by continuous extraction and how is it more efficient.
7. Describe briefly counter-current extraction method.
8. Explain the process of solvent extraction by chelate formation.
9. Explain the pathway of solvent extraction by forming associated ion pairs.
10. What are the main applications of solvent extraction method of separation?

Answers

1. 0.976
2. 91.0%, 97.2%

Chapter 13
Chromatography



13.1 Introduction to Chromatography

Chromatography is among the most promising methods of separation. Since analytical chemistry primarily involves identification and separation of substances i.e. the resolution of the substances into their components and their subsequent determination, separation is an integral component of analysis. Separation is always essential for purification and isolation of substances, and of all the methods known for separation, chromatographic methods are the most efficient methods.

Chromatography was invented and named by the Russian botanist Mikhail Tsvet in persuit of developing a technique to separate various plant pigments such as chlorophylls and xanthophylls. When solutions of these compounds were passed through a glass column packed with finely divided calcium carbonate, coloured

P. Malhotra, *Analytical Chemistry*,
https://doi.org/10.1007/978-3-031-26757-4_13

bands were observed on the column for the separated components. That is how the name chromatography (chroma means colour and graphy means writing) was given to it. Chromatography seems to have revolutionised chemical analysis and have empowered analytical chemists with a powerful tool to measure distinct analytes in complex samples. The power of chromatography comes from its ability to separate a mixture of compounds, or analytes, and determine their respective identity, chemical structure and concentration.

Definition of Chromatography

According to Keuleman, chromatography may be defined as a physical method of separation of a mixture of solutes brought about by the dynamic partitions or distribution of dissolved materials between two immiscible phases, one of which is called the **mobile phase**, moves past the other called **stationary phase**. Chromatography operates on the same principle as extraction, where one phase is held in place while the other moves past it. The phase that stays in place is known as stationary phase and the phase which moves through the column which is either a liquid or a gas is known as mobile phase. Therefore, the main feature that distinguishes chromatography from most other physical and chemical methods of separation is that two mutually immiscible phases are brought into contact: one phase is stationary and the other mobile. Further it is the nature of mobile phase and stationary phase as well as the type of their interaction which leads to classification of chromatography.

13.2 Classification of Chromatographic Methods

Chromatography can be divided into three basic types that include gas, liquid and supercritical fluid chromatography. Gas chromatography is further divided on the basis of the physical state of the mobile phase and stationary phase. Liquid chromatography is classified on the basis of the type of process involved during the chromatographic separation method which mainly depends on the type of stationary phase. The mobile phase can be a gas or a liquid, whereas the stationary phase can be only a liquid or a solid. The separation of two immiscible liquid phases, one stationary and the other mobile, when undergoes a simple partitioning between the two phases, it is referred to as partition chromatography or liquid–liquid chromatography (LLC). Similarly, when the physical process of adsorptions takes place between the two phases, the stationary phase being solid and the liquid phase as the mobile phase, it is termed as adsorption chromatography or liquid solid chromatography (LSC). Liquid chromatography also extends to ion exchange chromatography, where ionic components of the sample are separated by selective ion exchange ability of the stationary phase. Exclusion chromatography is based on molecular geometry and size of the stationary phase.

Therefore, the categories of chromatography arising from the basic mechanism of interaction of the two phases: stationary phase and the mobile phase may be summarised as follows and illustrated in Figs. 13.1, 13.2, 13.3, and 13.4.

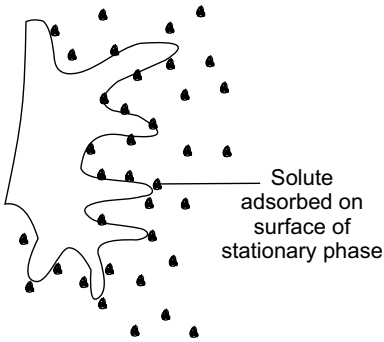


Fig. 13.1 Adsorption chromatography

Adsorption Chromatography

A solid stationary phase and a liquid or gaseous mobile phase are used. Solute is adsorbed on the surface of solid particles and stronger is the adsorption of the solute, slower is its rate by which it travels through the column.

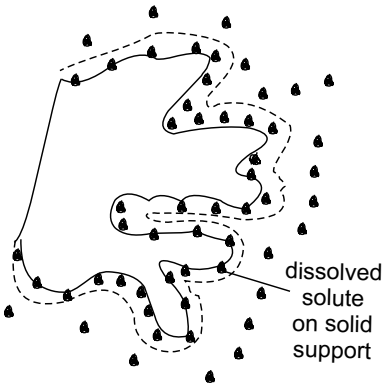


Fig. 13.2 Partition chromatography

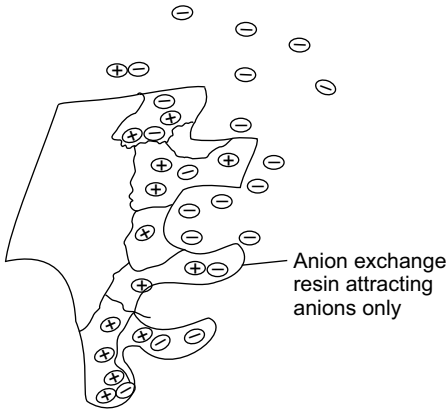


Fig. 13.3 Ion-exchange chromatography

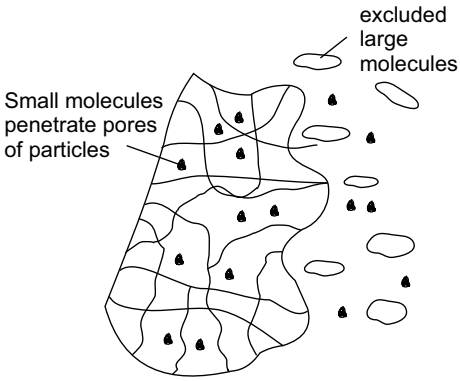


Fig. 13.4 Size exclusion chromatography

Partition Chromatography

A liquid stationary phase is bonded to a solid surface and is moving inside a chromatographic column. The solute equilibrates between the stationary liquid and mobile liquid or gas (Fig. 13.2).

Ion Exchange Chromatography

Usually, a cation exchange or an anion exchange resin is used as a stationary solid phase which attracts the oppositely charged ions from the solute which is the liquid mobile phase.

Molecular Exclusion Chromatography

There are various names given to it: size exclusion, gel filtration or gel permeation chromatography. In this the molecules of mobile phase (gas or liquid) travel on the basis of molecular size. Small molecules take longer to pass through the column whereas larger solute molecules pass through the column faster. The size of pores of the gel used are more permeable for small molecules as they get trapped in pores whereas the large molecules are excluded by the pores.

13.3 Chromatographic Theory

General Theory and Principle of Chromatography: All chromatographic systems have a mobile phase that transports the analytes through the column and a stationary phase coated onto the column or on the resin beads in the column. The stationary phase loosely interacts with each analyte based on its chemical structure, resulting in the separation of each analyte as a function of time spent in the separation column. The analytes which interact less with the stationary phase are transported faster through the system whereas the analytes which strongly interact take long to travel and spend more time with the system. The various components of an analyte are identified by

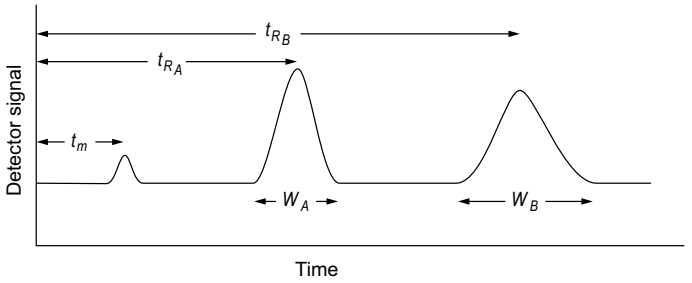


Fig. 13.5 Chromatographic resolution showing retention time

retention time in the system for a given set of conditions. In Gas Chromatography (GC), these conditions include the gas (mobile phase) pressure, flow rate, linear velocity and temperature of the separation column. The pressure of the liquid mobile phase, its flow rate, linear velocity and polarity, they all influence the retention time, as also seen in HPLC. Figure 13.5 shows an illustration of the retention time.

It is clear from the Fig. 13.6 that the minimum time a chemical species will remain in the system is the mobile phase and the time spent is t_m. The peaks B and A represent the retention time of compound B and A as t_{RB} and t_{RA} respectively, indicating higher affinity of compound B for the stationary phase than compound A. A net retention $t_{R'A}$ and $t_{R'B}$ can be calculated by subtracting the retention time of the mobile phase t_m from the peaks t_{RA} and t_{RB}

$$t_{R'A} = t_{RA} - t_m$$
$$t_{R'B} = t_{RB} - t_m$$

Chromatographic Resolution

Since the goal of chromatography is to separate a sample into a series of chromatography peaks, the separation of two chemically similar analytes is characterized mathematically by resolution R_S. Resolution R_S majorly depends on the difference

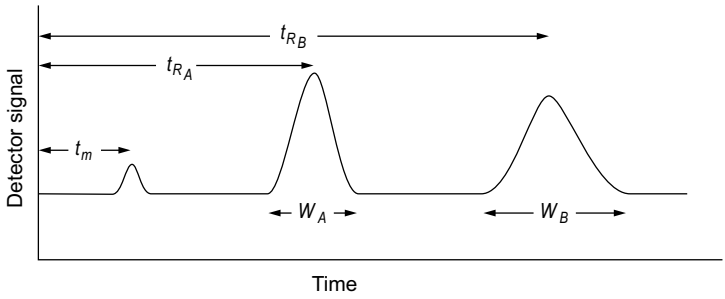


Fig. 13.6 Baseline resolution

in retention times of these analytes

$$R_S = \frac{2(t_{R'B} - t_{R'A})}{W_A - W_B}$$

where $t_{R'A} = t_{RA} - t_m$
$t_{R'B} = t_{RB} - t_m$

W_A and W_B are the peak widths in time units (Fig. 13.6)

$$t_{R'B} - t_{R'A} = \Delta t_R$$

The degree of separation between two chromatographic peaks increases with an increase in their separation Δt_R and the resolution may be improved by either increasing Δt_R, or by decreasing W_A or W_B.

This can be achieved if their distribution coefficient (Partition coefficient K) are different.

Partition Coeffecient

The equilibrium partition coefficient K, describes the spatial distribution of the analyte molecules between the mobile and stationary phases. It is taken as the ratio of the analyte concentration in the solid phase (C_S) and solute concentration in the mobile phase (C_M)

$$K = \frac{C_S}{C_M}$$

Retardation Factor (R_f)

The retardation factor may be described as the ratio of the displacement velocity of solute and the velocity of the standard substance.

It is referred as R_f value

$$R_f = \frac{\text{distance travelled by solute front}}{\text{distance travelled by mobile phase (solvent)}}$$

It plays an important role in paper chromatography and thin layer chromatography.

If R is equilibrium fraction of solute in the mobile phase, then $(1 - R)$ is fraction of solute present in stationary phase.

$$\frac{R}{1-R} = \frac{C_M}{C_S} \times \frac{V_M}{V_S}$$
$$= \frac{\text{Total mass in mobile phase}}{\text{Total mass in stationary phase}}$$

where V_M and V_S are volumes in mobile phase and stationary phase respectively.

$$R = \left[\frac{V_M}{V_M + KV_S}\right] = \left(\frac{1}{1 + K\frac{V_S}{V_M}}\right)$$

where $K = \frac{C_S}{C_M}$.

Similar equations are used in solvent extraction.

Partition Ratio (K')

The partition ratio or capacity ratio K', is related to the equilibrium distribution of the sample within the column to the thermodynamic properties of the column and to the temperature. It is an important quantity in column chromatography and is a measure of the time spent in the stationary phase relative to the time spent in the mobile phase

$$K' = \frac{C_S V_S}{C_M V_M} = K\frac{V_S}{V_M}$$

It also indicates the additional time a solute band takes to elute, as compared with an unretained solute ($K' = 0$), divided by the elution time of unretained band:

$$K' = \frac{t_R - t_M}{t_M} = \frac{V_R - V_M}{V_M}$$

Relative Retention (α)

The relative retentionα (also known as selectivity factor) of two solutes, where solute 1 elutes before solute 2, is given by

$$\alpha = \frac{K_2}{K_1'} = \frac{K_2}{K_1} = \frac{V'_{R\cdot 2}}{V'_{R\cdot 1}} = \frac{t'_{R\cdot 2}}{t'_{R\cdot 1}}$$

The factors influencing relative retention are (1) the nature of stationary and mobile phases and (2) the column operating temperature.

Retention Volume (V_R)

It is the volume of mobile phase that is being eluted by the solvent and is always less than the total volume in the mobile phase and the stationary phase

$$\begin{aligned} V_R C_M &= V_M C_M + V_S C_S \\ V_R &= V_M + K \cdot V_S \end{aligned} \quad \text{where } K = \frac{C_S}{C_M}$$

also $V_R = t_R F_C$.

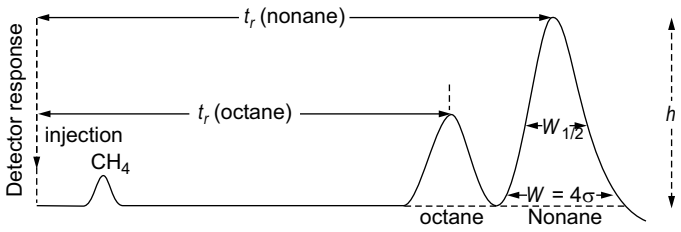


Fig. 13.7 Chromatogram showing retention time t_r and width at half height $W_{1/2}$

where F_C is the rate of flow of the mobile phase

$$V_R = \frac{LF_C}{R_v}$$

$$V_R = LF_C\left(\frac{V_M + KV_S}{vV_M}\right)$$

where L = length of column

v = linear flow rate or average velocity.

Theoretical Plates

An ideal chromatographic peak has a Gaussian shape. If the height of the peak is h, the width at half height, $W_{1/2}$ is measured at 1/2 h. For a Gaussian peak, $W_{1/2}$ is equal to 2.35σ, where σ is the standard deviation of the peak. The width of the peak at the base line, as shown in Fig. 13.7 is 4σ.

A chromatographic column is said to be divided into discrete sections (called theoretical plates) in which a solute molecule equilibrates between the mobile and stationary phases. Retention of a compound on a column can be described by the number of theoretical equilibration steps between injection and elution. More are the equilibration steps, more the theoretical plates and so narrower is the bandwidth when the compound emerges.

For any peak in Fig. 13.7, the number of theoretical plates is computed by measuring the retention time and the width at half height:

$$\text{Number of plates on column } N = \frac{5.55t_r^2}{W_{1/2}^2}$$

Retention and width can be measured in units of time or volume but both t_r and $W_{1/2}$ must be expressed in the same units. If a column is divided into N theoretical plates, then the plate height, H, is the height of one plate.

Plate height is expressed as

$$H = L/N$$

where L = length of column.

The smaller the plate height, the narrower the peaks. The ability of a column to separate components of a mixture is improved by decreasing plate height. An efficient column has more theoretical plates than an inefficient column. Different solutes behave as if the column has somewhat different plate heights, because different compounds equilibrate between the mobile and stationary phases at different rates. Plate heights are ~100 to 1000 μm in gas chromatography, ~10 μm in high performance liquid chromatography, and <1 μm in capillary electrophoresis.

13.4 Factors Affecting Chromatographic Separations

Chromatographic separations are influenced by many factors such as (i) adoptability, (ii) selectivity, (iii) fraction capacity, (iv) speed

(i) **Adoptability**

The suitability of a method so that it allows a cenvenient separation depends upon the physical and chemical properties of the analyte to be separated. For example, depending upon the components of analytes being macromolecular which are volatile, can be separated by exclusion chromatography whereas components which are nonvolatile can be separated by HPLC or liquid chromatography. Optimization of mobile phase and stationary phase also decides the adoptibility of a method for an analyte. Since the quantity of mixture analyte that can be separated by chromatographic methods is quite limited, they cannot be used for synthetic routes. If components of analytes are highly sensitive to temperature, then methods like ion exchange chromatography is more suitable than liquid–liquid or liquid solid chromatography.

(ii) **Selectivity**

For a satisfactory separation of two solutes in a reasonable time chemical selectivity is a priority which highly influences the choice of method as well as the resolution. Selectivity can be manipulated changing the composition of mobile, changing the column temperature, changing the composition of the stationary phase and using special chemical effects. For example, separation of anisole and benzene with a mobile phase of 50% mixture of water and methanol gave $K = 4.5$ for methanol and $K = 4.7$ for anisole with $\alpha = 1.04$ whereas when aqueous 37% tetrahydrofuran was used for mobile phase, K values wer 3.9 and 4.7 and $\alpha = 1.20$ indicating better separation and preventing overlap of peaks.

Values of a separation factor are greatly influenced by molecular properties like weight, fundamental group, shape, isomer, optical properties and polarities of the components. Faster separations are expected for different functional grpups by liquid chromatography or GLC. Highly specific reactions for example those in biochemical separations of biopolymers, GCC is a convenient choice. Isomer selectivity *e.g.* substituted pyridines are separated by gas solid methods similar to the separations of

ortho and para isomers of other compounds. Separations can be greatly influenced by making changes in chemical composition of stationary phases or inducting a complexing agent which may form complex with one of the analyte component. For example, separation of olefins can be improved by impregnating the solvent with a silver salt as a result of complexation reaction.

(iii) **Fraction Capacity**

The maximum number of components that are expected to be separated from an analyte also provides an insight into the choice of methodology to be undertaken. Improvements on peak capacity which means increasing K and α values when attempted, can automatically lead to more number of separations. Thus, number of separation bands will depend on efficiency of separation.

(iv) **Speed**

The rate of solute and migration equally affect the choice of chromatography method as well as the resolution of chromatogram. Speed is experimentally dependent on volumetric flow of mobile phase rather than linear flow. As selectivity factor α is directly related to migration rates, which predominantly affect the retention factor and so the separation of components.

13.5 Applications of Chromatography to Qualitative and Quantitative Analysis

Chromatography not only rules among the separation methods of chemicals but is also proficiently applied to qualitative and quantitative analysis of the separated species. Chromatograms, which are the pattern of separated substances obtained by chromatography can be interpreted in such a manner so that qualitative analysis of the analyte becomes feasible. Very often additional support from spectroscopic methods become important and together with chromatographic survey can lead to a complete analysis. Without chromatographic analysis at the primary level, spectroscopic identification cannot be accurate. For example, presence of ~30 amino acids in a sample of protein hydrosylate can be seen in chromatographic analysis. Chromatograms are capable of providing a certain indication for the absence of a certain component in an analyte.

Quantitative analysis is provided by chromatographic measurements done on the basis of various parameters like peak heights and peak areas of chromatograms. When column temperature, eluent flow rate and rate of sample injection are closely controlled, peak areas give useful informations and especially when compared with the standard chromatogram, quantitative measurements are enabled. A series of external standard solutions are prepared and their chromatograms are obtained and a plot of peak area or height is drawn as a function of concentration. It should give a straight line passing through the origin and is used as a calibration curve. Quantitative chromatography can lead to better results using internal standards as it prevents the

errors caused by sample ejection. A measured quantity of internal standard is added to the sample and to each standard and the ratio of analyte to internal standard peak areas or heights can give a precise quantitative measure.

With the growth of chromatography as one of the premiere method for separating closely related chemicals, many technical advancements have been made in the existing methods. Few of them have been discussed in the following pages.

13.6 Paper Chromatography

All forms of chromatography work on the same principle of having a stationary phase and a mobile phase. In paper chromatography, the stationary phase is a very uniform absorbent paper and the mobile phase is a suitable liquid solvent or mixture of solvents. Paper chromatography is the simplest and most inexpensive form of chromatography. **Paper chromatography** is an analytical technique essentially meant for separating and identifying mixtures that are or can be coloured. The discovery of paper chromatography in 1943 by Martin and Synge provided, for the first time, the means of surveying constituents of plants as well as separating them with there identification. Further credits for developing the present form of paper chromatography was shared by R. Consden and A. H. Gordon. Originally, paper chromatography was used to separate mixtures of dyes and amino acids only but later it was developed for separating cations and anions also from inorganic solutions (see Fig. 13.8).

13.7 Theoretical Aspect of Paper Chromatography

In principle paper chromatography is a type of partition chromatography in which substances are distributed between two liquids. One of the two liquids make the stationary phase, usually water held in the pores of Whatmann paper, while the other is mobile phase, an organic solvent which is either immiscible or partially miscible with the stationary phase. Solutes get partitioned between the water held in the pores of the paper and the organic solvent which comprises the mobile phase. It is the capillary action which is responsible for the rise of the mobile phase pre-adsorbed on the filter paper. Due to the difference in their respective rates of migration of the components of the mixture, their partition coefficients vary enough to make them appear as spots at different points on the filter paper thus representing a chromatogram. Besides the difference in the partition coefficient of the components, their capillary action and preferential adsorption also play a significant role for the spots to appear on the filter paper.

The technique follows a simple start by drawing a base line on the filter paper and applying a drop of the test solution on the filter paper and then the spot is dried. In a closed chamber (a glass jar) the edge of the filter paper is dipped into the solvent (moving phase) known as developing solvent. After a while, when the solvent have

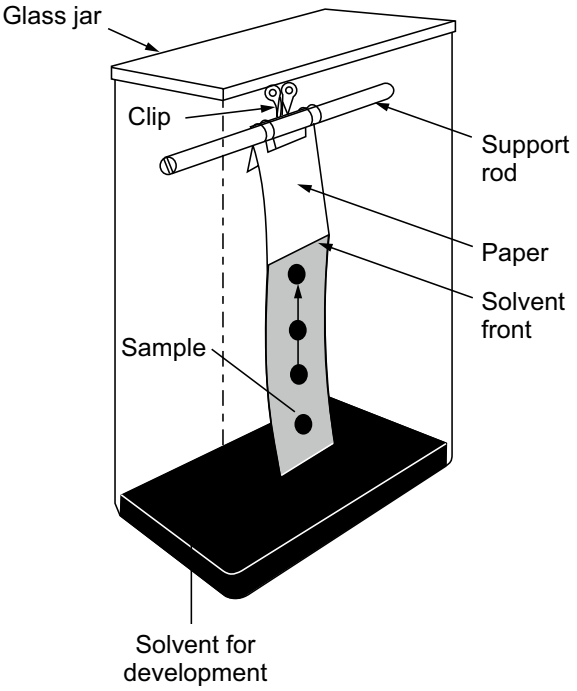


Fig. 13.8 Paper chromatography

moved for a while and has reached a suitable height, the paper is removed and dried and the various spots are visualised by suitable visualising agents.

The dragging forces which make the substances move along the movement of the solvent depend upon various factors. The rate of the flow of solvent and the difference in solubility of the two substances in the same solvent are the main influencing factors. The R_f value which is given as the ratio of distance travelled by the solute from the base line to the distance travelled by the solvent from the baseline, is a function of partition coefficient.

$$R_f = \frac{\text{Distance travelled by the solute from the baseline}}{\text{Distance travelled by the solvent from the baseline}}$$

Each compound has a characteristic, R_f value which is a constant with respect to a solvent in a similar environment like temperature, type of paper, duration and direction of flow. The following factors influence the R_f value:

(i) The nature of the solvent, its polarity and solubility
(ii) The quality of the paper
(iii) The temperature
(iv) The nature of the analyte mixture
(v) The developing solvent (see Fig. 13.9).

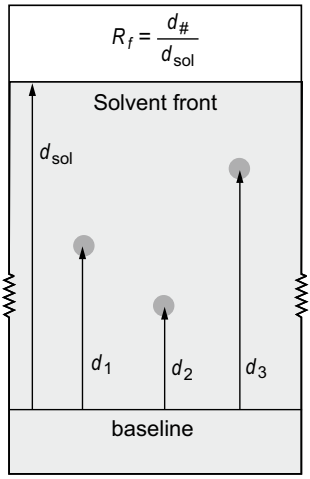


Fig. 13.9 Diagram representing R_f value

Types or Modes of Paper Chromatography

Types of paper chromatography is mainly based on the way the chromatogram is developed on the paper. Broadly five types of paper chromatography can be performed:

1. **Ascending Paper Chromatography**

According to its name, it is a type of chromatography in which the chromatogram ascends. In this type the development of the paper occurs due to the solvent movement or travel in upward direction on the paper. The solvent is placed in a tank at the bottom in which the paper is vertically suspended such that the tip of the paper just dips into the solvent and the spots remain well above the solvent. Since the solvent and solute are both travelling upwards it is termed as ascending paper chromatography. The different compounds present in the mixture analyte travel till different heights. It has been clarified that the capillary action on the pores of the paper are accountable for the solvent rise. The solvent dissolves the components of the mixture and it is the difference in the solubility of the compounds and their degree of adsorption by the paper, that makes them move different heights. That, s how the separation of constituents of a mixture is completed.

2. **Descending Paper Chromatography**

Descending paper chromatography is the preferred method of paper chromatography in most applications. Here the development of paper occurs due to the downward movement of the solvent on the paper. The solvent tank is at the top of a tall glass jar. The movement of the solvent is assisted by gravity besides capillary action. The solutes migrate from their original position under the force of solvent flow and get separated on the basis of their R_f values and generate a paper chromatogram.

The rate of flow of solvent is greater in this technique.

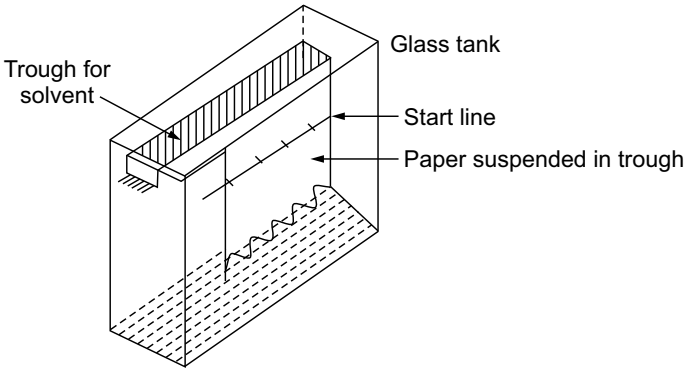


Fig. 13.10 Descending paper chromatography

The main advantage of the descending method over the ascending method is that it holds good for long pieces of paper giving better separation and since it is aided by gravity it develops faster (see Fig. 13.10).

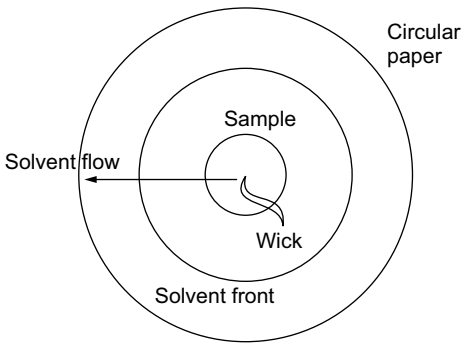
3. Radial or Circular Paper Chromatography

Aslo known as horizontal paper chromatography. In this a circular filter paper is used with a wick in the centre. The sample solution is applied at the upper end of the wick placed in the centre of the filter paper. After drying the spot on the filter paper, it is fixed horizontally on the petridish possessing the solvent so that the tongue or the wick of the paper dips into the solvent. The paper is covered by a petridish cover. The solvent rises thorough the wick and when the solvent front has moved through a sufficient large distance, the components get separated in the form of concentric circular zones (see Fig. 13.11).

4. Ascending Descending Chromatography

It is combination of both ascending and descending types of paper chromatography. The upper part of the ascending chromatography can be folded over a glass rod

Fig. 13.11 Circular chromatography



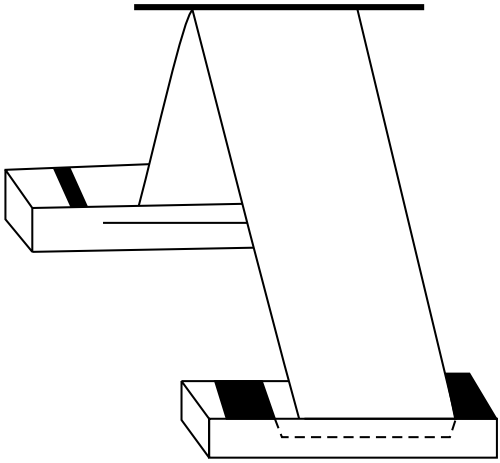


Fig. 13.12 Ascending descending chromatography

and allowing the paper to become descending after crossing the rod. It involves the movement of solvent vertically up and down both to give better separation (see Fig. 13.12).

5. **Two Dimensional Paper Chromatography**

It consists rechromatographing of the separated mixture at right angles to the earlier direction of development using a different solvent for the later direction of development. To implement this methodology a square or rectangular paper is used. The sample is applied to one of the corners and as shown in Fig. 13.13, the whole procedure is followed for two solvents subsequently.

This technique is convenient for the separation of compounds which have similar R_f values.

13.8 Experimental Measures for Qualitative and Quantitative Analysis

1. **Selection of Suitable Chromatographic Method**

The choice of a suitable chromatographic method depends upon the nature of substances to be separated. The difference in the R_f values of the analyte component,

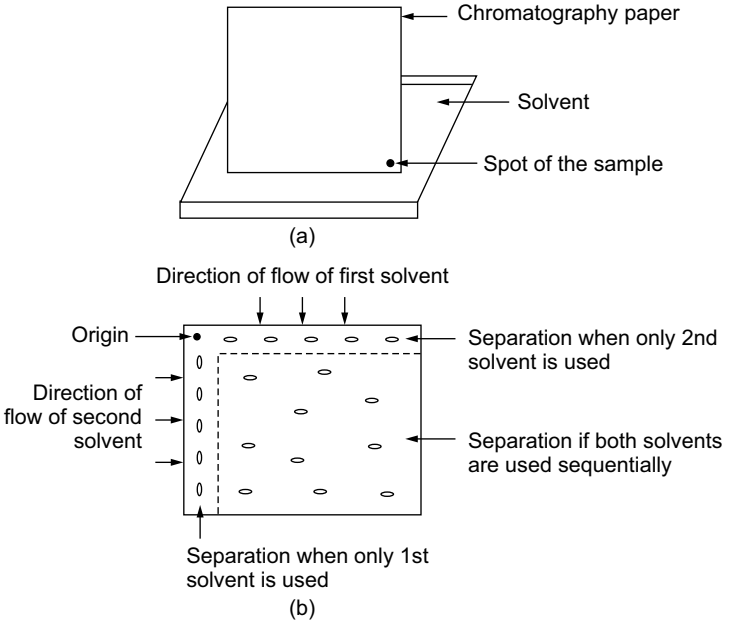


Fig. 13.13 Two dimensional paper chromatography **a** Spot of the sample on paper **b** Chromatogram showing separation

their solubility and the colours produced after developing them are other important parameters.

2. **Selection of Chromatographic Paper**

Generally, Whatmann filter papers are extensively used in paper chromatography, commonly used Whatmann filter papers used for chromatography contain 99% cellulose and balance is mineral. It should fulfill the following aspects:

(i) Should be capable of giving good rate of migration of the solvent.
(ii) The spots of the sample should not get diffused on it.
(iii) Should give clear separation.

3. **Sample Preparation**

Solid samples are dissolved in suitable solvents and concentrated solutions are prepared and to avoid diffusion this becomes important.

4. **Spotting**

For ascending chromatography the filter paper used is generally of a suitable size (25 cm $\times$ 7 cm) on which a base line is drawn a little above the bottom edge. Capillaries or micro pipettes are used to put drops on the points marked beforehand on the base line. Before dipping the paper in the solvent, the spots are fully dried-either by blowing hot air or using hair dryer.

13.9 Visualising the Chromatogram

The wet chromatograms obtained after running the solvent through it are dried completely and are put through various means for visualising the spots. The spots are visualised using chemical and physical methods both. Chemical detection) is done by either pressure spraying the chemicals on the paper or dipping the chromatogram in it. Physical methods involve holding of the chromatogram under a *UV* lamp so that the colourless spots can be visualised.

After the spots have been visualised the calculation of R_f values becomes easier. All the distances, the solvent front and the visualised spots, are measured from the base line.

For quantitative estimation the preliminary separation is similar to the qualitative method and the resultant eluate (spots extracted by semimicro extractor) are analysed by various methods. These methods include gravimetric estimation, *UV* spectrometry, colorimetry, Fluorimetry, Polarography, Coulometry and flame photometry.

13.10 Uses and Applications of Paper Chromatography

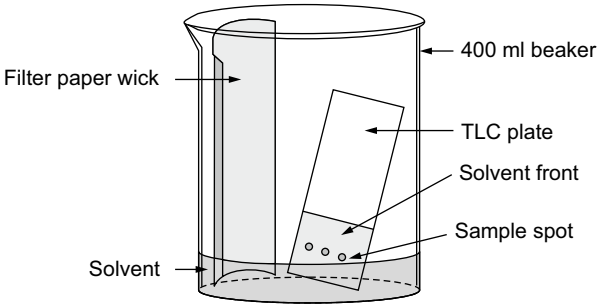
(i) Paper chromatography is specially used for separation of mixture having polar and non polar compounds.
(ii) Paper chromatography is mainly employed for the separation of aminoacids, carbohydrates etc. The spots of aminoacids are visualised by spraying dilute solutions of ninhydrin whereas spots of carbohydrates are seen by spraying hydrogen phthalate.
(iii) Pharmaceutical industry majorly uses this method to ensure drug constituents and also detection of drugs in human and animals are done by this method.
(iv) Food contaminants and adulterants are detected in food samples, syrups, squashes and other drinks.
(v) Apart from organic mixtures, paper chromatography has also been successfully used in separating metals like nickel, manganese, cobalt and zinc from inorganic mixture solutions.
(vi) It is an important analytical method frequently used in biochemical laboratories.

Unsolved Problems

1. Define chromatography and list its classification.
2. State the general principle involved in chromatographic separation.
3. What is chromatographic resolution? Give its expression.
4. Define partition coefficient and partition ratio. How do they differ?
5. Define retardation factor and state its application to chromatography.
6. What are the factors which affect chromatographic separation?

7. State various application of chromatography to qualitative and quantitative analysis.
8. Discuss the theoretical aspect of paper chromatography.
9. Differentiate between ascending and descending paper chromatography.
10. Explain two dimensional paper chromatography.
11. On what basis the type of chromatographic paper is selected?
12. What are the main uses and applications of paper chromatography?

Chapter 14
Thin Layer Chromatography



14.1 Basics of Thin Layer Chromatography

The most common form of chromatography is adsorption chromatography in which the stationary phase is a solid such as alumina or silica gel. The sample substance to be separated is applied at one end of this stationary phase and the mobile phase is allowed to flow through. Thin layer chromatography is based on this fact that the capillary action in the finely divided stationary phase causes the mobile phase to move. Separation occurs when one substance in a mixture is more strongly adsorbed by the stationary phase than the other components in the mixture. Since adsorption is essentially a surface phenomenon, and the degree of separation is dependent on the surface area of the adsorbent available, hence the emphasis is on small particle size of the adsorbent. Based on these concepts thin layer chromatography was innovated by two Russian workers, N.A. Izmailov and M.S. Schraiber. Later it was developed further by Stahl and Demolein.

P. Malhotra, *Analytical Chemistry*,
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Thin layer chromatography (TLC) is one of the most popular and widely used separation techniques. TLC is used for separation of substances in a wide variety of fields. Amino acids from food protein, alkaloid from plants, steroid from the urine of a newborn infant, morphine in the blood of an overdose victim, and pesticides from soil may be separated by TLC with sensitivities of one micrograms or even less. Qualitative analysis by TLC is quite fast and economic although quantitative analysis on the TLC plate may involve additional cost.

In adsorption chromatography distribution coefficient K not only plays a major role but is also temperature dependent.

$$\text{Distribution coeffcient}(K) = \frac{\text{amount of solute per unit of stationary phase}}{\text{amount of solute per unit of mobile phase}}$$

At a given temperature, the relationship between the amount of solute in each phase can be expressed graphically as the adsorption distribution isotherm. The ideal isotherm is obtained as a 45º linear curve when the concentration of the solute in the stationary phase is plotted against its concentration in the mobile phase. A non linear isotherm may also be obtained in a given TLC system which can be seen in the shape of the separated spot on the plate. Spots have either a well defined front half with a tail, appearing as an inverted teardrop or as a normal tear drop, depending on whether distribution isotherm is convex or concave respectively.

TLC concentrations can be regulated so that linear distribution isotherms become linear (see Fig. 14.1).

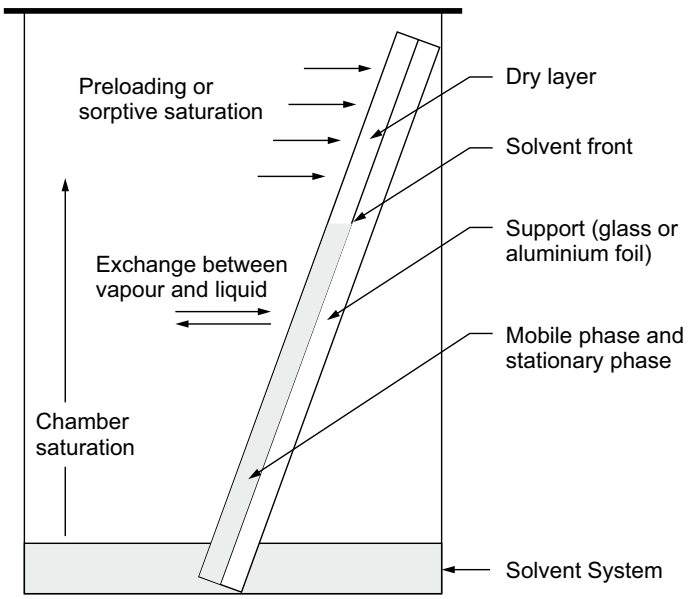

Fig. 14.1 Components of TLC

Thin layer chromatography can be used for various purposes:

(a) to simply check the purity of a substance,
(b) to attempt to separate and identify the components in a mixture and
(c) to obtain a quantitative analysis of one of more or the components present.

14.2 TLC Procedure

Thin layer chromatography is a separation method in which uniform thin layers of sorbent (adsorbent) are applied to a backing as a coating to obtain a stable layer of suitable size. The term sorbent is used in general sense to include layer materials that may be used for either adsorption or partition chromatography. The most common support is a glass plate, but other supports such as plastic sheets and aluminium foil are also used. The four most commonly used sorbent are: silica gel, alumina, Kieselguhr (diatomaceous earth) and cellulose.

Silica gel (silicic acid) is the most popular layer material which is slightly acid and its fine particle adhere well to the support. Binding agent such as plaster of paris is used when binding is weak. Alumina (aluminium oxide) is also widely used as a sorbent which is acidic and chemically more reactive than silicagel. Diatomaceous earth (Kieselguhr) is a chemically neutral sorbent but does not resolve or separate better then silica gel or alumina cellulose is used as a sorbent in TLC when it is convenient to perform a given paper chromatographic separation by TLC so that the time consumed is low and higher sensitivity is required with small amount of sample.

The standard size for TLC plates is 20 × 20 cm. For most separations, the mobile phase is allowed to travel on the layer for ~15 cm on such plates but as the sizes of TLC have decreased with aluminium foil strips, accordingly is the height of mobile phase travel. Micro plates have also found wide applications (see Fig. 14.2).

An aqueous slurry of the finely ground sorbate on the lean surface of a glass or plastic plate or microscopic slide. There are a variety of ways in which adsorbent slurry can be spread on the plates. Either by dipping the slides into the coating material, by spreading manually, by pouring on the plates or by spraying the slurry on the plates. For activating the TLC plates the coating should be dried in oven at 100–105 ºC for 30 min while when coated with silicagel small amount of moisture is required for satisfactory resolution. Air dried silicagel plates sometimes contain iron as impurity for which a preliminary run of methanol: HCl (9:1) is required. The iron moves with the solvent from lower to the upper edge of the plate and further drying of the plate at ~110 ºC leaves it free from iron.

Choice of Solvent

Success in TLC depends largely on the nature of mobile phase and its resolving ability. General rules to be applied for solvent selection may be discussed as follows:

a. Purity of the solvents can be ensured by distillation. For example, presence of ethanol in chloroform or ether can be removed by distillation.

Fig. 14.2 Design of ascending TLC

b. Water content in various solvents are different, so again hygroscopic solvents may be stored on drying agents.
c. The conditions and duration of storage of the solvent can affect reproducibility, since solvents can deteriorate for example by auto oxidation. To prevent this they can be stored in sealed containers.
d. The nature of the substances to be separated and the nature of the adsorbent also play a vital role in the choice of solvent. On cellulose or silica gel, polar substances are treated with polar solvents. Less polar substances should be dissolved in suitable non aqueous solvents and treated on silica gel or alumina as adsorbent mixtures of two or more solvents of different polarity often give better separation, than chemically homogenous solvents. Commonly used solvents are: high boiling paraffins, cyclohexane, benzene petroleum ether, carbon tetrachloride, CS_2, diaethylether acetone, pyridine, chloroform, organic acid, alcohol and water etc.

Sample Application

Sample application is quite a minutely observed aspect of thin layer chromatography. Spots required for quantitative measurements need special care. Usually 0.01–0.1% of solution when applied about 1–2 cm above the edge of the plate requires <1 ml for each spot. For high resolution, the spot should be of minimal diameter and even smaller for quantitative exercise. For applying spots on the TLC plates fine capillary tube or micropipettes are used. Hypodermic syringes and other mechanical dispensers are also available to be used as spot applicator to the TLC plates.

Chromatogram Development

In TLC the development of plates in the form of a chromatogram is the process where the sample is carried through the stationary phase by a mobile phase. The TLC plates are almost developed in the same manner as in paper chromatography. There are various ways in which a chromatogram may be developed but ascending method is the most widely used. As also used in paper chromatography, in the ascending method the mobile phase moves vertically upwards through the plate. The TLC plates are kept in specially designed developing jars which contain developing solvent and the plates after applying the substance are kept vertically tilted in it.

There are a number of factors that influence the performance of the development. It has been observed that the angle at which the plate is supported, affects the rate of development as well as the shape of the spots. An angle of 75° is recommended as optimum for development.

Multiple development when repeated separations are done after drying and reinserting into the developing chamber for redevelopment. **Step wise development** is undertaken when compounds that differ considerably in polarity, and a single development with one mobile phase does not give sufficient separation. Either in increasing order of polarity or in decreasing order of polarity different solvents are used for a sequence of development and depending on the separation a reverse sequence may also be undertaken. In **continuous development** a continuous flow of mobile phase along the length of the plate, with the mobile phase is allowed to evaporate off as it reaches the end of the plate. As the mobile phase evaporates off the end of the plate, a continuous flow of mobile phase is maintained up the layer. **Two dimensional development** technique involves a square plate and two mobile phases to attain better separation.

Visualization Procedures

Two major methods for the location of compounds separated are: physical methods, such as the use of ultraviolet light (360 nm) and chemical methods, reaction with sulfuric acid to produce a brown or black charred area. Use of iodine vapours is an example of another chemical method and a coloured complex is formed which is temporary and reversible. It leaves the substance unchanged chemically after the process, as multi and two dimensional development also can be conveniently visualized.

Iodine

Elemental iodine is a simple, rapid, inexpensive, sensitive and generally non destructive and reversible visualization reagent that will stain substances on a chromatogram more readily than the background. Either a 10% methanolic solution of iodine is sprayed on the chromatogram or crystals of iodine are placed in the tank during development. Iodine has been very useful in identifying a large number of organic compounds like lipids, aminoacids, psychoactive drugs, indoles and steroids. Because of the possibility of undesired reactions, the chromatogram should be

exposed to the iodine vapour as briefly as is necessary to visualize the components: 3. seconds are normally enough. Visualization by iodine gives high sensitivity.

Water as a Visualizing Agent

Water spray has also been used at various places as a non-destructive visualizing agent. The hydrographic compounds are revealed as white areas on a translucent background and gives clear zones. Water visualization has been used for cyclohexanols, hydrocaarbons and bile acids.

pH Indicators

Commonly used pH indicators such as bromocresol green and bromophenol blue are useful for the detection of acidic and basic compounds on TLC plates through a reversible acid–base equilibrium. If desired, the polar nature of the indicator should be such that it can be removed from the sample area. Bromophenol blue (0.5% in 0.2% aqueous citric acid) produces yellow areas on a blue background with aliphatic carboxylic acids. Methyl red may also be used.

Ultraviolet Light

Compounds absorbing ultraviolet light may often be located on thin layer chromatograms if they show differential *UV* absorption relative to the background, or if they reemit the incident light. Such compounds may be visualized by illuminating the plate with a weak source of monochromatic *UV* light. Generally, wavelengths of 254 and 366 nm are used and different substances may respond best to only one wavelength. Many types of compounds are visualized with *UV* light including aromatic amines at 366 nm, alkaloids 254 nm chlorinated pesticides with silver nitrate, 254 nm, and polycyclic aromatic hydrocarbons 366 nm.

14.3 Qualitative and Quantitative Evaluation

The measurement and identification of solutes by TLC are majorly dependent on the extent of the difference between the solute interaction with TLC plate and with the developing solvents. The movement of solute with respect to the solvent maintains a constant ratio known as R_f value (relative front or repartition factor) when under constant conditions of temperature, solvent system and adsorbent.

$$R_f = \frac{\text{Distance moved by compound front from the origin}}{\text{Distance travelled by solvent front from the origin}}$$

R_f values are always less than 1 and help to separate and identify the components of the mixture.

Quantitative measurement of the separated components of the solute needs to be performed in such a way that maximum accuracy can be obtained. Generally, there are two ways this can be performed:

(i) The first one is by using densitometers which operate either in transmission or reflectance mode. It involves the measurement of the photodensity of the spots directly on the thin layer plate. The scanning of the individual spots is usually done along the direction of the development of the plate by either moving the plate under the light source or moving the lamp. The intensity of the reflected light or transmitted light is measured and the difference in the intensities of adsorbent and the solute spots are observed. They are obtained as a series of peaks plotted on a recorder and the areas under the peaks give the measurement of the quantities of the components present in the different spots. To obtain accuracy in the quantitative measurements, known amount of standard mixtures should be spotted and compared with the spots of the sample. Advanced densitometers are equipped with calibration plots and give more reliable and instant measurement of the thin layer chromatogram.

(ii) The second procedure involves separation of components by scrapping off the developed spot after it has been visualized such that it has not been charged or destroyed during the visualization. The scrapped adsorbent containing the component is placed in a centrifuge tube and treated with a suitable solvent to dissolve the solute component. After having spun the dissolved solute in the centrifuge machine, the supernatant liquid is poured out and analysed by using other quantitative methods like

(iii) Gas–liquid chromatography or *uv*-visibile spectrometry. for an alternative measurement, the solute can be extracted by transferring the adsorbent to silica column and then eluted with the solvent. The extract obtained can be analysed by other supportive methods.

Quantitative measurements by TLC are expected to give accurate results only if the R_f values for the components are ranged between 0.3 and 0.7.

14.4 High Performance Thin Layer Chromatography (HPTLC)

High performance thin layer chromatography (HPTLC) is an enhanced form of thin layer chromatography (TLC). Numerous enhancements can be attempted to the basic method of thin layer chromatography by automating different steps involved. Further improvements are undertaken in the methodology so that higher resolution is achieved and more accurate quantitative measurements are performed.

Automation is useful to overcome the uncertainty in droplet size and position when the sample is applied to the TLC plate by hand. Recently automation in sample application has been acquired by the use of piezoelectric devices and inkjet printers.

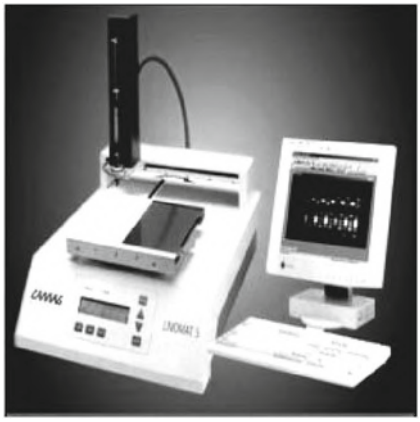

Fig. 14.3 HPTLC instrument

Mechanical application can also be highly improved by using platinum-iridium capillary of fixed volume (100–200 nl) sealed into a glass support capillary of larger bore. The capillary tip is polished to provide a smooth, planar surface of small area (0.05 mm^2) so the surface of the plate does not get damaged (see Fig. 14.3).

The spot capacity can be increased by developing the plate with two different solvents as in two dimensional chromatography mentioned earlier. The procedure involves the sample loaded plate being developed first by one solvent and them after removing it, the plate is rotated by 90º and then developed by another solvent. Instruments for HPTLC which provide automated sample application, plate development, detection and documentation are easily available.

Improvements in the quality of the adsorbent layer also gives rise to high performance TLC. The silica gel used for making TLC plates can be modified in using more pure form with narrower particle size (3–5 μm) to achieve high performance. Also chemically bonded layers are available as reverse phase plates giving enhancement in performance and also greatly reducing the separation time. Quantitative analysis which is based on direct photometric measurements further leads to HPTLC where scanning densitometers are used. As mentioned earlier, scanning of developed spots by densitometry leads to accuracy in the results. The separated compounds are revealed by either using transmitted or reflected radiation and the contrast of dark and light zones on the chromatogram enables precise quantitative measurement thus complimenting high performance TLC.

14.5 Applications of Thin Layer Chromatography (TLC)

Thin layer chromatography has been widely used for qualitative, quantitative and synthetic analysis. TLC gives good results even in trace components. Its high sensitivity has led to various applications.

Purity Check

TLC experiments have been quite successfully used for checking the purity of various products at different stages of their synthetic routes. Both organic and inorganic synthetic pathways can be well assisted by TLC measurements at various points to ensure the product formation and their purity. The main advantage of TLC is that even trace amounts of samples, lead to good resolution. Moisture content and absence or presence of by products during synthesis can be well managed through TLC measurements.

Organic Compounds

Separation, isolation and characterization of organic compounds can be carried out conveniently by TLC method. Characterisation and separation of aliphatic acids like citric acid and tartaric acid have been carried out using butyl-acetate-acetic acid water mixture. Other organic compounds which have been separated have been listed in Table 14.1.

Besides organic compounds, analysis of food stuff have also be performed successfully using TLC (Table 14.2).

Table 14.1 Separation of few organic compounds by TLC

Compounds separated	Adsorbent	Developing solvent
Cis–trans acid	Silica gel–'G'	Benzene-methanol acetic acid (45:8:4) or Benzene-dioxane-acetiocid (90:25:4)
Aromatic acids	Silica gel	Benzene-pyridine (17:3)
Salicylic acid	Aluminium oxide	0.1 m HCl in absolute ethanol
Phenol carboxylic acids	Silica gel 'G'	Benzene, chloroform, acetone or ethlacetate
Bile alcohols	Silica gel	$CHCl_3$–CH_3OH (9: 1)
Glycols	Alumina	hexane–acetone (4: 1) Ether-ethanol (99: 1)
Alkaloids (used in toxicology) (Purine)	Silica gel or aluminium oxide	Spraying agent. alcoholic I_2–KI solution
Alkaloids (Pyrimidine)	Silica gel Acidic alumina	$CHCl_3$: CH_3OH (3: 1) $CHCl_3$: CH_3CH_2OH (19: 1)
O, m and p-nitroanilines	Alumina or silica gel	Dibutyl ether, ethyl acetate-acetic acid (10:10:1)
Aminoacids, Proteins and peptides	Silica gel plates	$CHCl_3$: Methanol: NH_4OH (2: 2: 1) and phenol: water (3: 1)
Cyclic peptides	Silica gel	benzene: ether: methanol (85: 10: 5)
Tetracyclines (Antibiotics)	Silica gel 'G'	n-butanol: methanol: citric acid (4: 1: 2)
Penicillins	Silica gel G	Acetone: methanol (1: 1) and isopropanol: methanol (3: 7)

Table 14.2 Analysis of some food stuff

Component measured	Food stuff	Stationary phase	Mobile phase	Visualising agent
Amino acids	Milk	Cellulose (0.25)	Methanol: chloroform conc. NH_3 (2:2:1)	Ninhydrin
Amino acids	Fruits	Silica gel (0.25)	Chloroform: t-pentanol: acetic acid (30:30:1)	Ninhydrin
Amino acids	Orange juice	Kiesel gel-G	Phenol: water (3:1)	Ninhydrin
Sorbitol	Apples, pears	Kieselguhr-G	Isopropanol, ethylaceto periodate, acetic acid water (54:7:1:2)	Benzidine
Glyceride	Milk from sheep, goat or cow	Silicagel	Light petroleum: diethyl ether, acetic acid (80:20)	Iodine vapours
Glyceride	Butter	Silicagel-G	Chloroform: acetic acid (9:1)	Rhodamine-B clradiation
Vitaminc (Ascorbic acid)	Potatoes	Kiesel gel-G	Oxalic acid: methanol: chloroform (2 g: 20 ml: 60 ml)	Molybdophosporic acid
Vitamin A	Fish, liver	Alumina	Cyclohexane	$SbCl_2$
Vitamin E	Vegetable oils	Kiesel gel (in CO_2 atmosphere)	Dichloromethane, trichloroethylene	$FeCl_3$
Glucose, sorbitol (as adulterant)	Wine	Kieselgel-G	Butanol: acetone: water (4:5:1)	-
Organomercuric compounds	Fish	Alumina, silica gel	Light petroleum, diethyl ether 2:3	Benzophenones
Organomercuric compounds	Eggs	Silica gel	Butanol, acetic acid, water (20:20:1)	Diphenyl carbazone
Aromatic alcohols	Beer	Kiesel gel-G	Chloroform acetone (9:1)	Vanillin/H_2SO_4
Organochlorine	Vegetables	Kiesel gel-G	Heptane, acetone (99:1)	ammoniacal $AgNO_3$
Phenolic acids	Barley	Polyamides	Ethylacetate, acetic acid (19:1)	UV radiation
Artificial colours	Confectionary	Silica gel	Propanol, ammonia (4: 1)	–

Separation of Inorganic Ions

In the recent past TLC has been developed for the separation of metal ions such as Cr(VI), Cr(III), As(III), Cd(II), Tl(III) and Hg(II). They comprise a class of toxic heavy metal ions and from their two, three and four component mixtures, these ions are separated using silica gel 'G'. The separation of metal ions including heavy and toxic metals have been reported by thin layer chromatography by many scientists. The first one was reported by Meynard and Hall in 1949 who separated zinc and iron by radial chromatography on layers of a mixture of aluminium oxide and celite bound with starch. TLC has been used for removal of Cu(II) and Zn(II) ions from contaminated soil. Separation of arsenic (III), anti mony (III) and tin (II) cations by thin layer chromatography on silica gel G have also been achieved successfully.

Quantitative measurements of developed spots find applications in many areas of analysis. Using spot densitometers amino acids are determined where 25% ninhydrin solution in acetone is used as spraying agent. Radioactive methods including isotope dilution technique are employed for quantitative determination of normal as well as radioactive components of mixtures. Sometimes the samples are tagged by either direct reaction with radioactive species or by the addition of unknown quantity of radioactive component to the mixture analyte.

In general, TLC is a very useful and versatile technique for qualitative and quantitative analysis of a wide range of chemicals and natural substances.

Preference of TLC over Paper Chromatography

Though TLC and paper chromatography can be considered as two forms of planar chromatography, TLC is generally preferred over paper chromatography due to the following advantages:

(i) The time taken for the development of the chromatogram is much shorter as compared to paper chromatography.
(ii) TLC plates can be dried at high temperatures without any risk of destruction of the plate whereas chromatographic paper cannot be treated at high temperature as it can easily get destroyed.
(iii) TLC is more sensitive than paper chromatography because spots obtained in TLC are sharp and compact while those obtained in paper chromatography are usually diffused.
(iv) The adsorption capacity of thin layers of the adsorbent is higher than that of paper.

Unsolved Problems

1. For what all purposes can, thin layer chromatography be used.
2. Explain adsorption chromatography. On what principle is it based?
3. Discuss the basic principles underlying TLC.
4. Explain a basic TLC procedure.
5. Comment upon the choice of solvent used in TLC.
6. What are visualizing agents in TLC procedures?
7. How are qualitative and quantitative evaluation done by TLC?

8. How is high performance thin layer chromatography superior to normal TLC?
9. Give various applications of thin layer chromatography.
10. How is TLC useful in separating?
    a. organic compounds
    b. inorganic ions.

Chapter 15
Column Chromatography

15.1 Principle of Column Chromatography

After a series of inventions of chromatography, it was Martin and Singe (1941) who introduced partition column chromatography. It is based on the basic principle of differential rate of adsorption for different substances.

In column chromatography the stationary phase is a solid adsorbent and the mobile phase is a liquid. The sample solution is poured down the column which is packed with the adsorbent. As it flows down, the component which has the highest affinity for the adsorbent is adsorbed in the upper layer of the column. As the affinity of the

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component decreases it gets adsorbed below in the same order. The component which is least adsorbed is found at the bottom of the column. This phenomenon results in partial separation and adsorption of the components in different parts of the column. The segregated zones make marked bands from top to bottom when a suitable solvent is allowed to flow through the column. Such a column which shows distinct bands is called chromatogram and the developing process is known as development of chromatogram.

The extent of adsorption is decided by the mass of solute adsorbed per unit weight of the adsorbent and majorly depends on the concentration of the solute.

It can be derived from Langmuir adsorption isotherm equation.

$$m = \frac{K_1 K_2 C}{1 + K_2 C}$$

where K_1 is a measure of the number of active site per unit weight of the adsorbed solute and depends on the nature of the adsorbent. K_2 is a measure of interaction between solute and solvent and C is the concentration of the solute.

After developing the chromatogram i.e. band, formation in the column, there are two ways to quantify:

a. After development, column of adsorbent is pushed out of the tube and bands are cut by knife and extracted with a suitable solvent. This process of recovery of constituents from the chromatogram is known as *elution.*
b. The washing of column is continued with more solvent (*eluent*) and each component is collected separately as it reaches the end of the column and is eluted out. The least easily absorbed component is carried off first *i.e.*, eluted first. The most strongly adsorbed component is eluted last. The solvent containing the eluted components are called *elute.* Later pure compound is separated from the elute by removing the solvent from it.

15.2 Experimental Methods

Adsorption Column

Adsorption columns are commonly made of narrow glass tubes which are of about 20–30 cm long and 2–3 cm in diameter. Smaller diameters give more distinct bands and so are preferred. The adsorbent is supported on cotton plug or glass wool. At one end a rubber stopper is fixed and the powdered adsorbent is packed inside the column.

Packing of adsorbent needs to be done uniformly such that air channels and air bubbles are excluded. There are generally two methods involved namely wet packing and dry packing, as discussed below (see Fig. 15.1):

(a) **Wet packing**: In a vertically clamped column a slurry of solvent and adsorbent is poured into column through open end and allowed to settle. It is allowed to

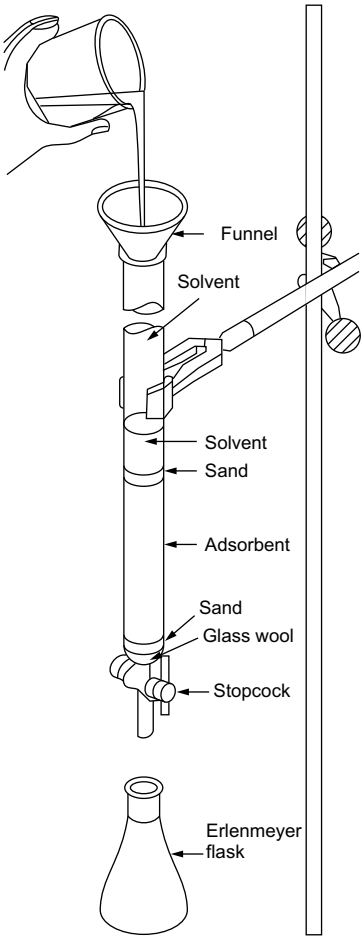

Fig. 15.1 Column used in column chromatography

settle till the column of desired height is finally obtained. The solvent and slurry will flow out slowly through the column thereby increasing the uniformity of the packing process. One of the most commonly used substance to prepare slurries of adsorbent is petroleum ether.

(b) **Dry packing**: In this method dry powdered adsorbent is added to the column in sufficient quantity and sometimes in vacuum the adsorbent is gently tapped for uniform packing.

(c) **Choice of adsorbents**: There are a variety of adsorbents to choose from and in order to obtain columns with efficient separation properties, the adsorbents should have following characteristics:

(i) The particle size ought to be uniform and fine enough to give well defined bands and also maintain uninterrupted flow of solvent.

(ii) The adsorbent should not chemically react with either the eluting solvents or with the sample components nor should they catalyse any dissociation or association of the substances. Although ion exchangers are an exception.
(iii) The adsorbent should possess enough mechanical strength so that its crumbling along with the solvent is prevented. Its physical and chemical properties should not get altered.
(iv) The adsorbent should be insoluble in the solvent used.
(v) The colour of adsorbent should not interfere or hide the colour of the bands in the chromatogram.

A wide range of adsorbents fulfil the above requirements. Aluminium oxide activated by heating till ~200–300 ºC in an air current or carbon dioxide environment, is most commonly used adsorbent. Other adsorbents are silica gels, activated charcoal, calcium sulphate, calcium carbonate, barium and magnesium carbonate, and magnesium oxide. Among organic substances the common adsorbents are various types of polyamides e.g., nylon, perlon etc., which are used for lower fatty acids, synthetic dyes and flavones and quinones. Sucrose, starch and powdered sugar are used for the separation of colouring matter of leaves and various phenols.

Choice of Solvents

The choice of solvents is mainly decided by the type of phenomenon involved. Based on adsorption, partition and exchange phenomenon, a suitable mobile phase (solvent) is chosen. Generally, a single solvent is used for developing the chromatogram and eluting the adsorbed material. The solvents generally employed possess boiling points between 40º and 85 ºC. Commonly used solvent are cyclohexane, carbon disulphide, benzene, carbon tetrachloride, chloroform, methylene chloride, ethyl acetate, ethyl alcohol, acetic acid, ether and acetone and the most widely used medium is petroleum.

Purity of the solvent is an important factor, although for better separations it has been observed that the solution of the mixture should be prepared in a relatively non polar solvent whereas the development of the chromatogram can be done by a polar solvent and elution can be done by even more polar solution. Some of the solvents in increasing order of their polarity can be listed as petroleum ether < carbontetrachloride < cyclohexane < carbon disulphide < ether < acetone < benzene < ester of organic acids < chloroform < alcohols < water < pyridine < organic acids.

In general, the main functions of the solvent are to introduce the mixture to the column, development of the chromatogram and removal of the required content by using the solvent as eluent.

Development of Chromatogram

After the selection of a suitable solvent, the column is wetted by pouring the same solvent and then the sample solution allowed to flow through the column. After the sample solution has traversed through the entire length of the column, the developing solvent is introduced and allowed to flow through the solvent. As the developing solvent moves steadily, the various constituents of the mixture get separated. The different components present in the sample material are adsorbed by the adsorbent to

different extent creating well defined zones of different components. The developed column is known as chromatogram and if the substances separated exhibit colourless bands then ultra violet lamps are used to identify separated bands.

Recovery of the Components by Elution

Once a developed chromatogram is formed in which well defined zones are present, a suitable solvent (or developer liquid) is passed through the column gradually and the components of the mixture are removed from the adsorbent surface. The process of removal of various components from the adsorbent is called elution and the solvent used is called eluent. After collecting the eluted portions by adding the eluent, they are dried by evaporation and the separated components are obtained.

Identification

Sometimes, the eluent is directly flown into a device which measures the adsorbed *uv* light in the eluent and produces peaks on the graph. Chemical identification methods may also be applied by treating the fractions with appropriate reagents. For example vitamin A gives blue colour when treated with antimony chloride in chloroform. In case of ionised substances in aqueous solution, the effluent is passed through a conductivity cell for analysis. Flame ionisation detectors may also be connected to the column base for analysing the eluent.

15.3 Factors Governing Column Chromatography

There are various factors which directly influence the results obtained from column chromatography (see Fig. 15.2).

Migration rate, partition ratio and capacity factor:

The distribution of a solute A between mobile phase and stationary phase at equilibrium is given as

$$A_m \rightleftharpoons A_s$$

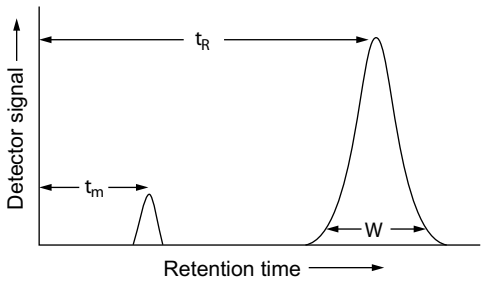

Fig. 15.2 Chromatogram obtained after analysing the separated components with suitable detectors

and equilibrium constant $K = \frac{C_S}{C_m}$ (K is also known as partition ratio)

C_s = molar analytical concentration of the solute in the stationary phase

C_m = is the analytical concentration of the solute in the mobile phase.

If capacity factor = $K^{'}$ (measurement of migration rate)

$$K^{'} = K \times \frac{V_s}{V_m}$$

where V_s and V_m is the volume of stationary and mobile phase respectively.

Migration rate of the solute follows:

$$\overline{v} = \frac{L}{t_R} = \frac{L}{t_m} \times \frac{1}{(1 + k')}$$

where L = length of column

t_R = retention time of the solute

$\overline{v}$ = migration rate

$$K^{'} = \frac{t_R - t_M}{t_M}$$

where T_M = time required to elute non retained components.

Column Efficiency

The efficiency of a column mainly depends on the degree of broadening of band. The diameter of the particles making up the packing and the diameter of the column itself, both affect the column efficiency. The efficiency can be defined in terms of number of theoretical plates N, which can be calculated by simple relationship

$$N = 16\left(\frac{t_R}{W}\right)^2$$

where t_R is the retention time and W is the baseline width of a chromatographic peak.

Column Dimensions + Particle size

Therefore, it is possible to improve the column efficiency by increasing the length/width ratio of the column and keeping the sample/column packing ratios in the range of 1:20 to 1: 100. Column efficiency can be further enhanced by decreasing the particle size of the adsorbent, usual range 100–200 mesh and pore diameter $\leq$20 Å.

Sometimes temperature is also required for good column efficiency as solubilities and rate of adsorptions of the substances vary with temperature, At higher temperature the adsorption is reduced so the rate of elution increases, therefore room temperature is more suitable.

Adsorbent

Not only the particle size but also the nature of adsorbent, its quality and purity greatly affect column efficiency. Equally influential is the way adsorbents are packed in the column. Whether it is dry packing or wet packing, absence of air channels should be ensured because loose packing can lead to diffusion and broadening of the bands.

Solvent

Solvent quality, purity, rate of flow (viscosity) play major roles in column efficiency. Solvents exhibiting too fast or too slow rates of flow should be both avoided and solvents of optimum flow rates need to be chosen for good efficiency.

15.4 Applications of Column Chromatography

Column chromatography finds limited application in the field of analysis, but separation techniques largely rely on it. It is the most commonly used method for separation of mixtures into its pure components which finds tremendous utility in various fields. The following applications are quite important:

(i) Impurities are conveniently removed from the mixture samples and purification is achieved successfully.

(ii) Geometrical isomers of various organic compounds can be separated by column chromatography. Cis–trans isomers of carotenoids, unsaturated carboxylic acids and crocetin dimethyl esters have been separated. It is based on the fact that the isomer which is more strongly adsorbed is the one which can easily bond to the adsorbent.

(iii) Separation of tautomeric mixtures rely on column chromatography because it can be performed at low temperature. For example, Keto and enol forms of *p*-hydroxyl phenyl pyruvic acid and indolyl pyruvic acid could be separated in weakly acidic medium in liquid phase in which the enol form is eluted before the keto form.

(iv) Various plant extracts, natural dyes and chemical dyes can be easily separated into their constituent components.

(v) Another important application of column chromatography lies in the separation of diastereomers. For example, the separation of diastereomeric 7 chloro 7 azobicyclo (4: 1: 0) heptane has been done on silica gel using diethyl ether as solvent.

(vi) Identification and separation of inorganic anions and cations have also been successfully achieved using column chromatography. A number of hydrophobic molecules containing ionic functional groups are deposited on the column packings to separate various ions. A convenient ion chromatography method has been proposed for simple determination of anions SO_4^{-2}, Cl^-, NO_3^- and cations Na^+, NH_4^+, K^+, Mg^{+2} and Ca^{+2} using a single pump a single eluent and a single detector. When 5-sulfosalicylic acid was used as the

eluent, the anions and cations could be conveniently separated on the anion exchange column and cation exchange column, respectively.

Unsolved Problems

1. Explain the terms elution, eluent and development as applied to column chromatography.
2. On what principle is column chromatography based?
3. Explain how separation of a mixture of two components gets affected by column chromatography?
4. Describe briefly the experimental procedure adopted for the separation of a mixture by using column chromatography.
5. Discuss the factors that influence the efficiency of the column.
6. Explain the kind of adsorbents used in column chromatography.
7. Discuss the types of packings used in column chromatography.
8. What is the main function of the solvent used in column chromatography and the various types of solvent used in it?
9. How is a chromatogram developed by column chromatography?
10. Discuss the main applications of column chromatography.
11. Write a short note on column dimensions and column packings used in column chromatography.
12. How can we recover the components of a mixture by column chromatography?

Chapter 16
Gas Chromatography

Gas chromatography is a type of chromatography in which the mobile phase is essentially a gas and the stationary phase is either a solid adsorbent or a non-volatile high boiling liquid held on a solid support. In gas chromatography, the gaseous analyte which is transported through the column is called carrier gas and functions as a mobile phase. In gas–solid adsorption chromatography, analyte is adsorbed directly on solid particles of the stationary phase. Whereas in gas–liquid partition chromatography, the stationary phase is a non-volatile liquid bonded to the inside of the column or to a fine solid support. Immobilized liquid as a stationary phase has a much wider utility than solid. Since 1952, gas chromatography has advanced from using solid spheres to act as the stationary phase (gas–solid chromatography) to using liquid coated resins as the stationary phase, and finally to using covalently bonded stationary phases attached to the wall of a capillary column (gas–liquid chromatography).

Gas chromatography (GC) has further advanced with the simplification of gas–liquid instrumentation. The invention of automation in sample holding and number of samples that can be handled at one time, automated injection and computerized

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result interpretation have all led GC to become the most popular chromatographic method for the separation of non-volatile compounds.

16.1 Principles of Gas Chromatographic Method of Separation

Gas chromatography-specifically gas–liquid chromatography involves a sample being vaporized and injected onto the head of the chromatographic column. The sample is transported through the column by the flow of inert gaseous mobile phase. The schematic diagram of a gas chromatograph is shown in Fig. 16.1b.

In gas chromatography, the components of a vaporized sample are separated when partitioned between a mobile gaseous phase and a liquid or a solid stationary phase held in a column. Elution is done by flowing an inert gaseous mobile phase through the column. The function of the mobile phase is only to transport the analyte through the column and not interact with analyte molecules.

The rate of movement of mobile phase R is given by the expression:

$$R = \frac{V_m}{V_m + K V_s} \tag{16.1}$$

where

V_m volume of the mobile phase
V_s volume of the stationary phase
K partition coefficient

$$K = \frac{C_s}{C_m} = \frac{\text{Concentration in the stationary phase}}{\text{Concentration in the mobile phase}}$$

If a substance has no affinity for the stationary phase then $K = 0$, and it will not be retained by the column, given as t_0 (Fig. 16.2).

A symmetrical Gaussian error function curve is obtained in the gas chromatogram (Fig. 16.2).

The volume of the mobile phase which is required to elute the band V_R can be given as

$$V_R = t_R F_m \tag{16.2}$$

where F_m is the volumetric flow of the mobile phase.

For unreacted component

$$V_0 = t_0 F_m$$

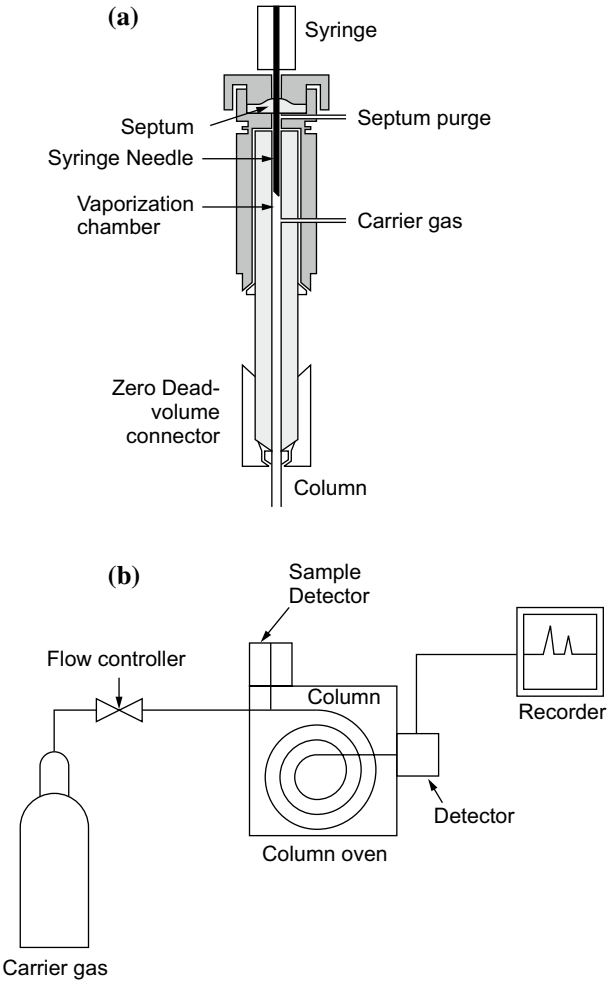


Fig. 16.1 **a** Design of gas chromatographic column. **b** Schematic diagram of a gas chromatograph

and volume of the stationary phase $= V_s$.

Therefore, $V_R = K V_s$ (Fig. 16.3).

A pressure difference is created due to the difference in the rate of flow of gas. The average pressure of gas in the column can be found by considering the compressibility correction (j)

$$j = \frac{3(P_j/P_0)^2 - 1}{2(P_j/P_0)^3 - 1} \tag{16.3}$$

where P_j and P_0 are the inlet and outlet pressures in the column.

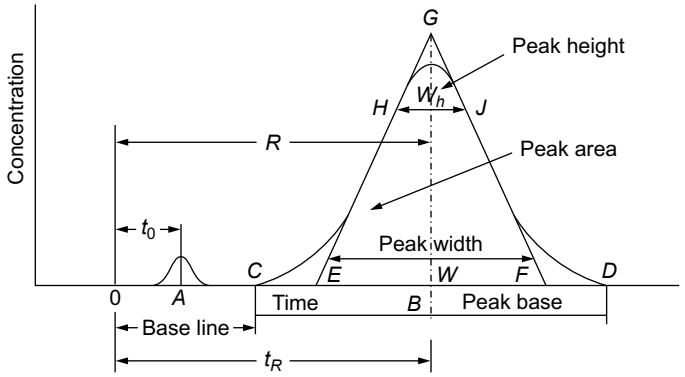


Fig. 16.2 Gaussian error function curve

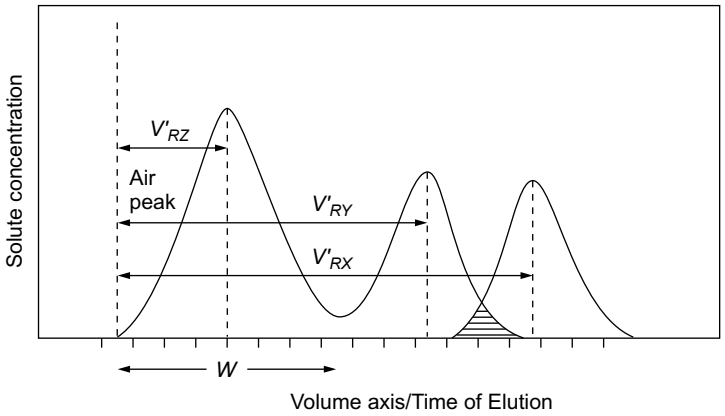


Fig. 16.3 Gaussian curve showing solute concentration versus time of elution

The retention volume V_R^0 at absolute temperature

$$V_R^0 = \frac{V_N}{W_S} \times \frac{273.16}{T} \tag{16.4}$$

where V_N is the adjusted retention volume V_R' such that

$$V_N = jV_R'$$

Partition coefficient K is then

$$K = \frac{t_R - t_0}{t_0} = \frac{V_R - V_0}{V_0} = \frac{KV_S}{V_0} \tag{16.5}$$

where t_R and t_0 are retention times of component and unretained component.

The separation factor α (also referred as relative retention)

$$\begin{aligned} \alpha &= \frac{(t_{R_1} - t_0)}{(t_{R_2} - t_0)} \\ &= \frac{(V_{R_1} - V_0)}{(V_{R_0} - V_0)} \end{aligned} \tag{16.6}$$

$$\alpha = \frac{V'_{R_1}}{V'_{R_2}} = \frac{V^0_{R_1}}{V^0_{R_2}} = \frac{K'_1}{K'_2} = \frac{K_1}{K_2}$$

The number of theoretical plates can also be calculated for GC. The greater is the value of N, higher is the efficiency of separation.

$$N = 16\left(\frac{V_R}{W_V}\right)^2 = 16\frac{t_R}{W_1} \tag{16.7}$$

where W_V and W_1 are the width of elution bands.

Another approach to determining column efficiency is by finding height equivalent to a theoretical plate (HETP). The height equivalent to one theoretical plate is defined by the ratio of column length and number of theoretical plates

$$\text{HETP} = \frac{L}{N}$$

HETP is directly related to the width of a peak in a chromatogram.

Van Deemter equation

$$N = \left(\frac{4t_f}{W}\right)^2$$

where t_R = time between the injection point and middle of the peak

$$W = \text{peak width}$$

Expressed in terms of equivalent HETP the simplest form of Van Deemter equations is

$$H = A + \frac{B}{u_x} + Cu_x$$

where A, B, and C are constants and u_x is the rate of flow of the carrier phase.

Narrow peaks give good separation when there is a reasonable difference of t_R values for different components.

Temperature dependence of molecular adsorption and rate of progression along the column necessitates a careful control of the column temperature. Reducing the temperature produces greater separation and results in successful analysis.

Narrow columns provide higher resolution than wider columns but require higher operating pressure and have less sample capacity.

16.2 Instrumentation

Instrumentation for GC involves a sequence of basic requirements and a typical instrument should consist of the following provisions:

(i) constant flow of carrier gas (mobile phase)
(ii) introduction of sample vapours into the flowing gas stream
(iii) appropriate length of column
(iv) maintain the column at constant and optimum temperature
(v) detectors to detect the components of eluents
(vi) readable signals to measure concentration of every component.

Following block diagram according to the above provisions is found to be suitable (Fig. 16.4).

In today's times, capillary column chromatographic systems are ideal and interface well with detection by mass spectrometry due to the low volume of carrier gas used in capillary columns.

Carrier Gases

The first important component for basic GC system is the carrier gas or mobile phase. Commonly, helium in its purest form is used and sometimes hydrogen, argon, and nitrogen may also be used. Helium is preferred above all due to its inertness, optimal flow rates, and compatibility with most of the detectors. Optimal flow rates are in the order $N_2 < He < H_2$ and so fastest separations can be achieved with H_2 but due to the explosive nature of H_2 and a possibility of reacting with unsaturated hydrocarbon it is not frequently used.

Although both helium and hydrogen give better resolution than N_2 at high flow rates. The more rapidly a solute diffuses between phases, the smaller is the mass transfer. To give maximum efficiency high velocity of carrier gas is chosen although

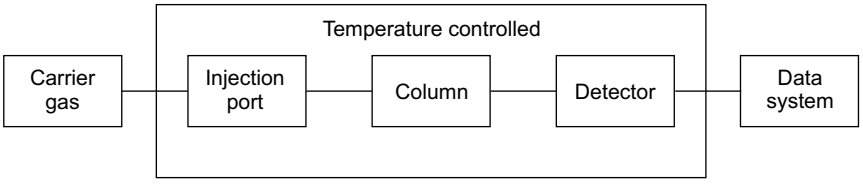


Fig. 16.4 Block diagram of a gas chromatograph

a slight compromise on resolution is required. To maintain constant flow rates of carrier gas, the inlet pressure is kept constant and is controlled by a two-way pressure regulator.

The quality of gas used as carrier gas should be high otherwise the impurities damage the stationary phases. To obtain high quality gas, it should be passed through purifiers to remove traces of O_2, H_2O, and organic compounds.

Sample Injection

After passing through the purification traps, helium enters the injector where it acts as the mobile phase and helps push the analytes through the separation column. To achieve high column efficiency, suitable sample size is chosen and sample injection is done at an optimum rate.

The delivery of sample into the column should be controlled, reproducible, and rapid. The peak width for the GC measurement is basically established by injection step. A variety of injectors are used and various types of injection port liners are designed for split, splitless, and on-column injection. Depending on the concentration of analyte sample and the concentration desired for GC, the type of 6-sample injection port is selected.

The most simple and common technique is using a glass syringe and a metal needle to inject liquid sample into the column. A cleared syringe is rinsed with the solvent and is then filled with air, solvent, and sample one by one. Then these calibrated microsyringes (Fig. 16.5) are used to inject liquid samples through a silicone or rubber diaphragm or septum into a heated sample port located at the head of the column. The heated port causes vapourization of the liquid sample.

An injection port with a silanized glass liner is used through which the career gas sweeps vaporized sample from the port into the chromatography column (Fig. 16.6).

Split Injection

Split Injection is desired when the analyte concentration > 0.1% because small amounts $\leq$1 μL or $\leq$1 ng of each component give best results with high resolution. Gas volume of roughly 0.5 mL is created with a 1 μL liquid injection and so

Fig. 16.5 Design of calibrated microsyringe

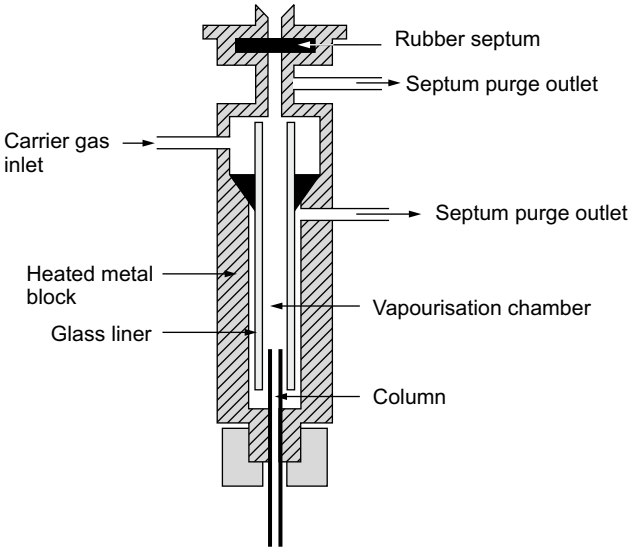


Fig. 16.6 Injection port

a split injection becomes convenient as it delivers only 0.2–2% of the sample to the column. The split injector has a split point through which only a small fraction of vapor enters the chromatography column and most of it goes to a waste vent.

Splitless Injection

Quantitative analysis with split injection cannot provide the same amount to the column in a repeat run so it is not always suitable. Splitless injection is appropriate for trace analysis. A similar port is used and a large volume (2 μL) of dilute solution in a low boiling solvent is injected slowly into the liner keeping the split vent closed. Injector temperature is kept lower in splitless injection (~220 °C) than that in split injection. In splitless almost 80% of the sample is provided to the column. Highly volatile analytes, normally not separated by standard GC conditions can be analysed using a cryogenic focusing injector. Normally, the bottom of the injector contains a cooling fluid (cryogenic liquid N_2) which cools the column and the analyte condenses at the column head. After injection, cryogenic fluid is removed, then the column is heated and analysis is done. For low boiling solutes, such cryogenic treatment is essential.

On Column Injections

On column injections are used for samples that decompose above their boiling points. Such analytes are directly injected into the column and its contact with hot injector is prevented, and the column is warmed to run the chromatographic run and chromatographic run. Micro syringes possessing silica needles are needed for on-column injection.

Therefore, a suitable injection technique can be chosen keeping the following conclusions in mind:

- Split injection is selected for highly concentrated, dirty samples and thermally unstable compounds.
- Splitless injection is required for very dilute solutions but does not give quantitative measurements and also cannot be used for isothermal chromatography.
- On column injection is most suitable for quantitative analysis and for thermally sensitive compounds although it gives low resolution.

Separation Columns

In GC separation columns are the key instruments and are pivotal in the separation method. Two types of columns are used in GC:

(i) Packed columns—Column in GC earlier used were of the packed types of 1.5–1.0 m length and 2–4 mm internal diameter. This is usually made of stainless steel, glass, or teflon and contains a packing of finely divided inert, solid support material (e.g. diatomaceous earth) that is coated with a liquid or solid stationary phase. The efficiency of GC column is inversely proportional to the particle diameter of the packing which also affects the pressure required to maintain the flow rate of the carrier gas. At pressure differences being less than 50 psi, the usual support particles range should be 250 to 170 μm or 170 to 149 μm. A relative table of column dimensions and its applicability can be judged from Table 16.1.

Capillary Column

Capillary column or open tubular columns are more often used in modern days in GC analysis. They are of two types: wall-coated open tubular (WCOT) and support-coated open tubular columns (SCOT).

WCOT are simple capillary sized tubes that were earlier made of stainless steel, aluminium, copper, or plastic and now glass columns etched with gaseous HCl or

Table 16.1 Relative dimensions in a gas chromatography column

Inner diameter film thickness	0.10–0.32 mm ~0.2 μm	0.25–0.32 mm ~1–2 μm	0.53 mm ~2–5 μm
Advantages	High resolution trace analysis, fast separations, low temperatures elute high boiling point compounds	Good capacity good resolution, easy to use, retains volatile compounds, good for mass spectrometry	High capacity (100 ng/solute) good for thermal conductivity and infrared detectors, simple injection technique
Disadvantages	Low capacity (≤1 ng/solute requires highly sensitive detector surface activity exposed silica	Moderate resolution, long retention time for high boiling compounds	Low resolution, long retention time for high boiling compounds

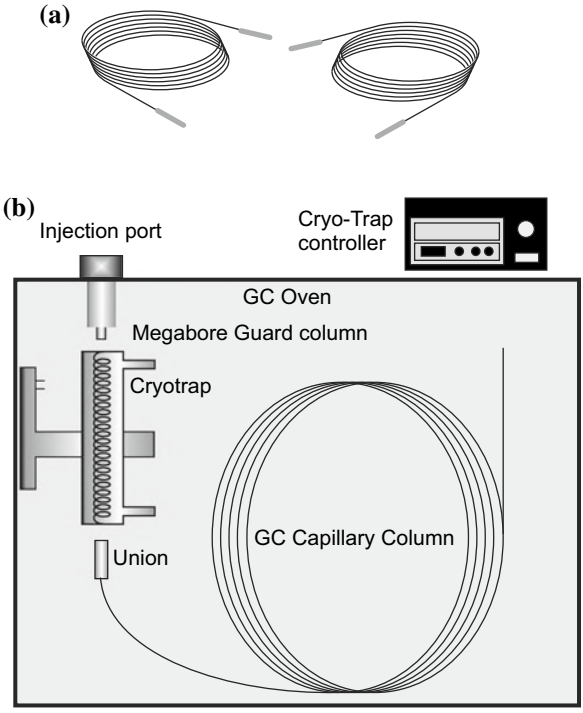


Fig. 16.7 **a** Capillary columns used in GC (WCOT). **b** Capillary column in a GC oven

strong aqueous HCl of potassium hydrogen fluoride to give a rough surface so that the stationary phase is held tightly. In SCOT the inner surface of the capillary is coated with a thin film of support material, i.e. diatomaceous earth. Today most columns are fused silica capillary columns which are widely used and have internal diameters 0.25–0.53 mm (Fig. 16.7b) currently 250 μm capillaries called mega bore columns are available and undoubtedly give better performance than packed columns.

Generally, the following three types of capillary columns are commonly used:

(a) 1/4 inch glass packed GC column
(b) 1/8 inch stainless steel GC column
(c) Fused silica capillary column.

Temperature Control

Apart from column dimensions and the particle size of adsorbents, column temperature is also of great significance. Temperature controlled GC or employing temperature programming for GC columns is essential for successful resolution. Generally, optimal resolution is enabled when GC column is maintained at low temperature. The boiling point of the components of analyte and the kind of separation targeted, decide the appropriate temperature required for the analysis. To keep column temperatures in the range of 40–35 °C, most gas chromatographs are equipped with ovens. Columns

may also be housed in thermostats in which the temperature can be controlled and regulated.

Appropriate temperature programming lead to sharp peaks in the chromatogram and fast response. Even components of a side boiling range can be resolved efficiently by regulating the temperature. The following provisions can facilitate good temperature programming:

(i) Dual column system which makes up for the loss of liquid.
(ii) Thin walls of column prevent further heating and for fast heat transfer their mass should be low.
(iii) Heating of injectors, column ovens, and detectors should be done from different heating arrangements.
(iv) Carrier gas should be used in dry phase.

Detectors

Like all other analytical techniques, detectors are pivotal and contribute maximum to accuracy and precision of GC analysis. Qualitative analysis is enabled in association with the mass spectrometer. Comparison of retention time with an authentic sample is another method of detection. Retention times are compared by spiking in which a known compound is added to an unknown and if the added compound is identical with the unknown, the relative area of that one peak will enhance otherwise another peak will be obtained. Analyte concentrations are indicated by peak area and for narrow peaks, peak heights are rather considered in place of peak area. Quantitative analysis always involves the addition of an internal standard and is calculated by:

$$\frac{A_x}{[x]} = F\left(\frac{A_S}{[s]}\right)$$

A_x = area of analyte signal.
A_S = area of internal standard.
$[x]$ = concentration of analyte.
$[S]$ = concentration of standard.
F = response factor.
An ideal detector should support the following characteristics:

(i) Detector should possess appropriate sensitivity range usually 10–8 to 10–15 g solute/s
(ii) Should give a linear response in a wide range of solute concentration
(iii) Should be highly reliable, reproducible and should be stable enough for handling by different people
(iv) Should be non-destructive so that the solute non-destructive so that the solute does not get destroyed
(v) should give response in short time.

A variety of detection systems are available for GC and the most common and commercially available are listed in Table 16.2.

Table 16.2 Common GC detectors and their characteristics

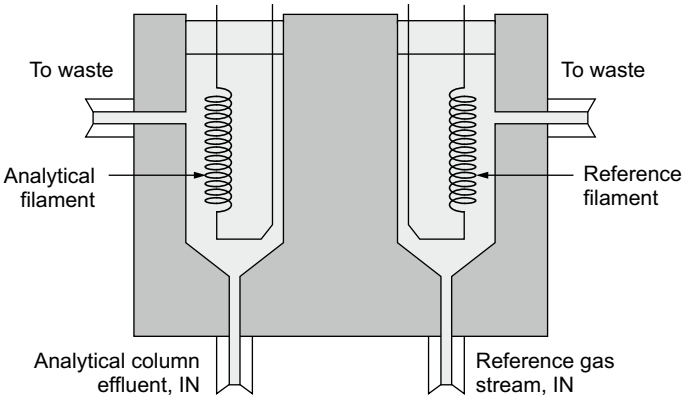
Detector	Mode of operation	Preferred analytes	Detection limits
Thermal conductivity	The ability of a substance to transport heat is measured by measuring change in voltage	All chemicals	400 pg/ml linear range > 10^5
Flame ionisation detector	Burning in O_2/H_2 flame	Organic compounds (hydrocarbons)	2 Pg/S linear range > 10^7
Electron capture detector	Gas is ionized by high energy electrons emitted from ^{63}Ni	Halogen containing molecules, conjugated carbonyls, nitriles, nitro compounds and organometallic compounds	As low as 5 fg/S linear range 10^4
Nitrogen-phosphorus detector (alkali flame detector)	Measures optical emission from P, S, Pb Sn etc. in H_2-air flame	Compounds containing N and P	100 fg/S linear range 10^5
Photoionisation detector	Use vacuum UV source to ionise	Aromatic and unsaturated hydrocarbons	25–50 pg linear range > 10^5
Sulphur chemiluminescence detector	$S \xrightarrow[\text{flame}]{H_2-O_2} SO + \text{products}$ $SO + O_3 \rightarrow SO_2^* + O_2$ $SO_2^* \rightarrow SO_2 + h\nu$ $(SO_2^* = \text{excited state})$	S containing compounds	100 fg/S linear range 10^5
FT–IR		Polar compounds	200 pg to 40 ng linear range 10^4

Detection by Mass Spectrometry

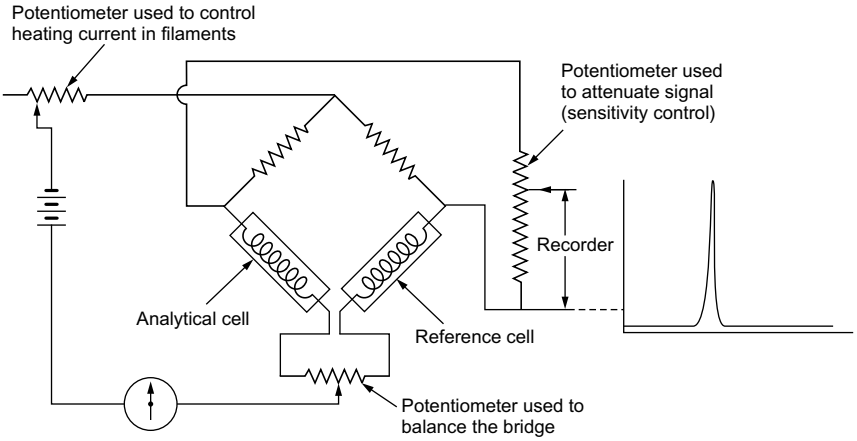
Mass spectrometry (MS) provides a convenient detection method in GC analysis. Both qualitative and quantitative measurements in GC can use mass spectrometry for final detection. After the molecules travel the length of the column, they are made to enter mass spectrometer where the molecules ionize by various methods. Once the sample is fragmented it can then be detected usually by electron multiplier diode, which essentially turns the ionized mass fragment into an electrical signal that is then detected. The typical GC–MS instrument is capable of performing two functions either individually or in combination.: a full scan or a selected ion monitoring (S/M), which leads to high level of accuracy in the detection of even closely resolved components (Fig. 16.8).

16.3 The GC Chromatogram and Its Evaluation

As the components elute from the column they pass into a detector where some physical or chemical property of the analyte produces a response from the detector. This response is amplified and plotted against time giving rise to a chromatogram. Those analytes and sample components that are retained, elute as approximately 'Gaussian' shaped peaks later in the chromatogram. Retention times provide the qualitative aspect of the chromatogram and are same under identical chromatographic conditions. The chromatographic peak height or peak area is related to the quantity of



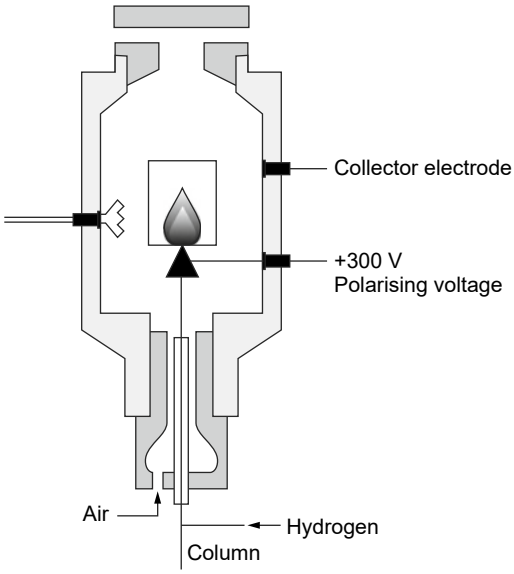
(*a*) Thermal Conductivity Detector



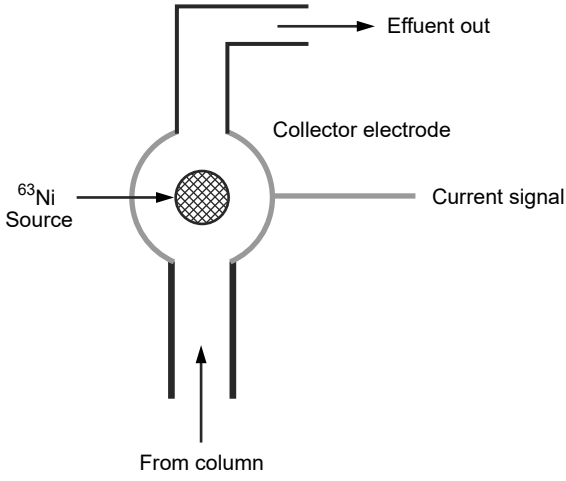
(*b*) Assembling in a thermal conductivity detector

Fig. 16.8 Designs of various detectors

Fig. 16.8 (continued)



(*c*) Flame ionisation detector



(*d*) Electron capture detector

analyte. For determination of the actual amount of the compound, the area or height is compared against standards of known concentration (Fig. 16.9).

Identification is mostly done by the use of position of the peak which accounts for the retention volume and retention time. Retention time is the time from the moment of injection of the analyte to the time of the separation of the components from the column. Equations (16.4) and (16.5) enable the interpretation of the chromatogram.

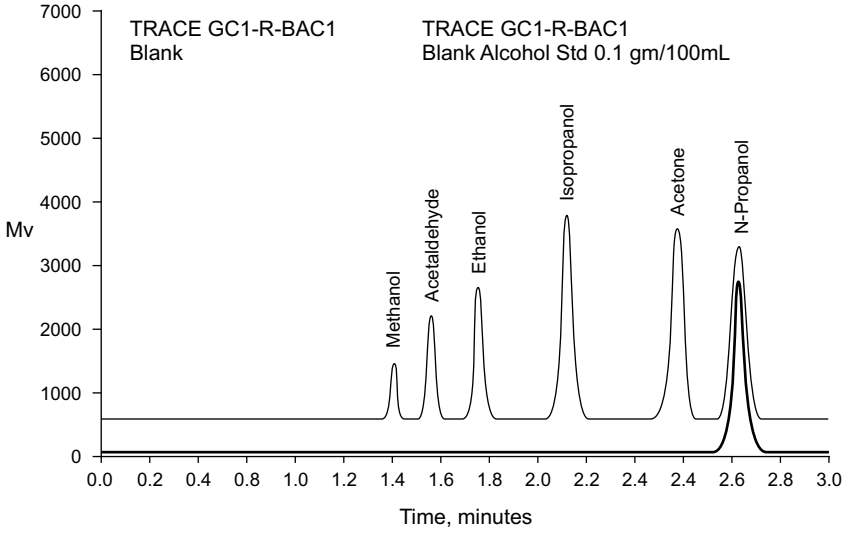


Fig. 16.9 A typical GC chromatogram

16.4 Retention Index

In 1958, E. Kovats proposed the concept of retention index for identifying solutes from chromatograms. The retention index scale is based on normal alkanes and for normal alkane it is equal to 100 times the number of carbons in the compound, whatever be the chromatographic conditions. Retention index of alkanes remains the same, for example, for heptane it is always 700, irrespective of column packings and temperature. Since the retention index is only valid in the case of alkanes, for other compounds it varies largely with temperature and nature of the stationary phase. Retention index (I) for benzene I = 644, hexane I = 600, and heptane I = 700, which indicates benzene is eluted between hexane and heptane when taken together in a mixture analyte. Retention index relates the retention time of a solute to the retention times of linear alkanes. Retention index of some common compounds is known and can be used for comparison to identify the unknown. The use of retention data on two or more GC columns can easily and precisely identify any compound (Fig. 16.10).

16.5 Factors Affecting Separation in Gas Chromatography

There are various factors that directly or indirectly affect the column efficiency in GC which leads to efficiency in resolution. The degree of separation is related to column efficiency which is either measured by the number of theoretical plates (Eq. 16.7) $N = \frac{16t_R}{W_1}$ or the height equivalent of a theoretical plate (HETP). The HETP is directly

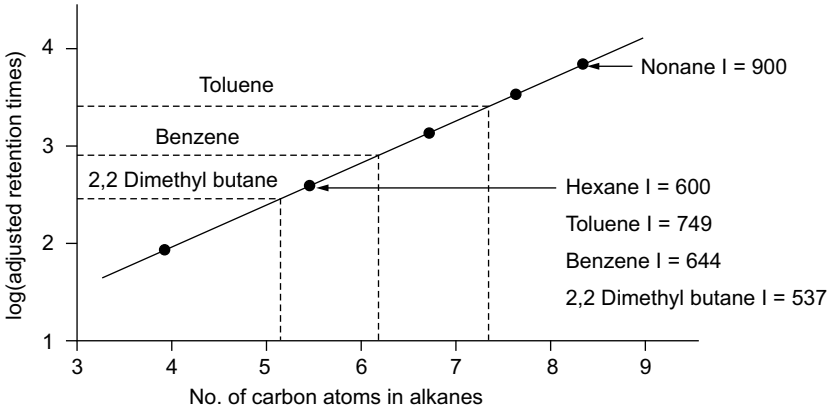


Fig. 16.10 Graphical representation of retention index (I) at 60 °C

related to the width of a peak. For high efficiency short columns with large number of theoretical plates are preferred. Following factors largely affect the separation by GC:

(i) **Column Height**: Although number of theoretical plates which largely affect column efficiency is needed to be kept high and it increases with an increase in length but very long columns are not practical to use. So to keep HETP short, long columns are avoided. Column lengths of 1–10 m are preferred for good efficiency.

(ii) **Column Diameter**: Keeping the inner width of the columns of about 0.1–0.53 mm enhances efficiency. Narrow columns give higher efficiency but pressure increase needs to be checked.

(iii) **Particle Dimensions**: The size of the particles needs to be appropriate and uniform. Too small particles block the column and require higher pressure to flow down the column. Generally, particle diameters of 0.25–0.18 mm, 0.18–0.15 mm, and 0.15–0.13 mm are used in columns to give good separation.

(iv) **The Stationary Phase**: For efficiency in separation, the liquid phase in a gas–liquid chromatography should include (a) low volatility, (b) thermal stability, (c) chemical inertness, and (d) optimum values of distribution constant K and capacity factor α. The retention time of solute which is related to α is crucial for separation and depends largely on the nature of the stationary phase. For an analyte to have an appropriate staying period in the column, it should exhibit some extent of solubility in the stationary phase this can be ensured by matching the polarity of the solute with the stationary phase. The concentration of the substance used as stationary phase is also significant. In order to have a large number of theoretical plates, the concentration of the liquid phase may be high but not beyond a certain limit as too high concentrations of liquid can cause tailing of the peaks in the chromatogram.

Some commonly used liquids for stationary phases for GLC are polydimethyl siloxane, 5% phenyl-polydimethyl siloxane, 50% phenyl-polydimethyl siloxane, 50% cyanoprophl polydimethyl siloxane and polymethylene glycol. All these operate efficiently in ~200–350 °C temperature range.

(v) **Column Temperature**: It is an important variable and depends on the boiling point of the sample and the degree of separation required. The temperature of the column should be kept slightly above the boiling point of the sample to get good results, the effect of temperature on the gas chromatogram can be seen in Fig. 16.11.

(vi) **Flow Rates and Nature of Carrier Gas**: Column efficiency is markedly affected by the rate of flow of carrier gas so it should be neither too high nor too low. When there is rapid flow rate then resolution may not be successful and at very low flow rates broadening of the resolved peak is expected. Helium is preferred for its inertness, greater flow rates, excellent thermal conductivity, and lower density. In the Van Deemter equation:

$$H = \underset{\substack{\text{multiple}\\\text{teams}}}{A} + \underset{\substack{\text{Longitudinal}\\\text{diffusion}}}{\frac{B}{u_x}} + \underset{\substack{\text{Equilibrium}\\\text{time}}}{Cu_x}$$

more rapidly a solute diffuses between phases, the lower is the mass transfer (Cu_x term (Figs. 16.10 and 16.12)).

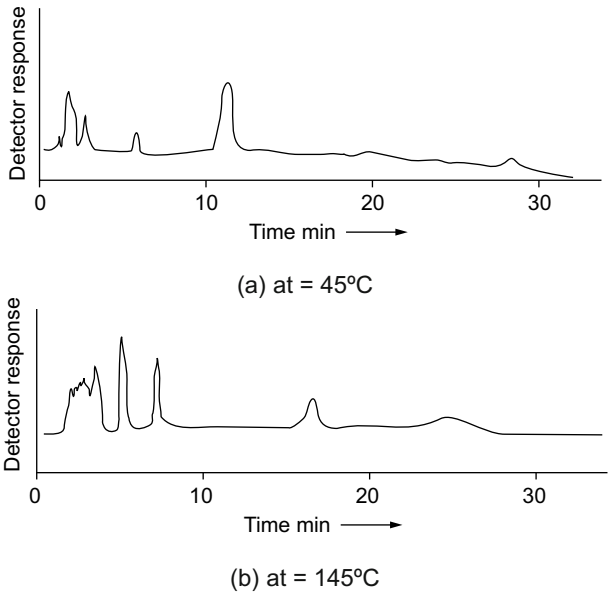


Fig. 16.11 Temperature change affecting the chromatograms

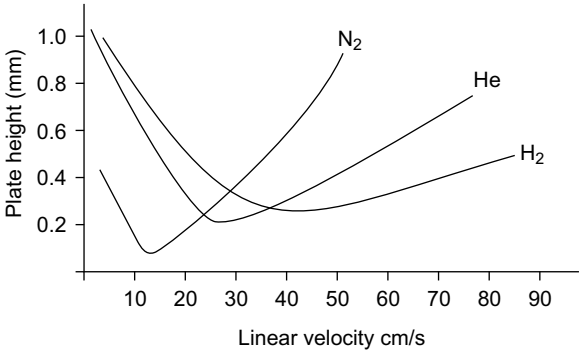


Fig. 16.12 Van Deemter curves for GC of n $C_{17}H_{36}$ at 175 °C

16.6 Applications of Gas Chromatography

(i) Identification and separation of substances can be conveniently performed by gas chromatography. Benzene (353.1 K) and cyclohexane (353.8 K) whose boiling points are so close that they cannot be separated by fractional distillation but can be easily separated by GC.

(ii) In petroleum Industry GC finds a wide application. Crude petroleum products: gasoline, waxes, sulphur, and nitrogen compounds, saturated and unsaturated hydrocarbons are often separated by GC and later identified using IR, UV NMR, and mass spectrometry. Capillary columns of high efficiency have frequently been used to separate components with boiling points <550 °C present in coal tar and petroleum products. Gasoline, LPG, paraffins, N and S compounds have been extracted from petroleum products along with their decomposition into hydrocarbons of different fractions. Separation and characterization of various olefins, alcohols, esters, aromatic hydrocarbons, ketones, and acids are also achieved with high precision.

(iii) In food industry GC is widely used to detect and analyse trace amounts present in food products. At times the contamination of food products can be easily checked. Although food components are quite complex, they can be analysed by GC specially by long capillary or packed columns which are attached to sensitive detectors. Various flavours in food stuff are also detected by GC.

(iv) In the field of biochemistry GC plays a vital role in separating and identifying the components of blood, urine, body fluid, etc.

(v) In industrial products, at various stages GC is employed to check the purity as well as quantity of herbicides, pesticides, drugs, cosmetics, perfumes, etc. Industrial products also include plastic materials, fertilizers, alcoholic beverages, soap, synthetic detergents, and rubber products where GC can be used for separation.

(vi) Moisture determinations in emulsions, pastes, glues, creams, and gases used in industrial and clinical areas depend on GC.

(vii) Mixture of gases uses molecular sieves in gas–solid chromatography to separate the components. Mixture of H_2, CO_2, CO, O_2, CH_4, C_2H_2, C_2H_4, and C_2H_6 can be separated using GC.

(viii) Quantitative measurements by GC are done by measuring peak areas on the chromatogram. Comparisons were done with standard chromatograms of known substances or adding internal standards and then interpreting the chromatogram are commonly used techniques for quantitative determinations by GC.

(ix) Radioactive products can also be traced by GC.

(x) Reaction mechanism and the products formed during the course of any synthetic route are conveniently followed by the GC technique.

(xi) Exhaust gases from automobiles and other environmental pollutants are analysed by GC method.

Solved Problems

1. Two unknown compounds x and y are separated by isothermal gas chromatography with a 2 cm long column having 500 theoretical plates when the flow rate is 15 m/min. A non-absorbing air peak emerges is 30 s peak x emerges in 5 min and peak y emerges in 12 min.

   (a) What is the value of HETP for this column?
   (b) What are the base width of peaks x and y?
   (c) What fraction of time y spends in the mobile phase while passing through the column?

Solution:

(a)
$$\text{HETP} = \frac{L}{N}$$
$$= \frac{2 \times 100}{500} = 0.040\,\text{cm/plate}$$

$$N = \left(\frac{4t_f}{W}\right)$$

$$\therefore \quad W = \frac{4t_f}{\sqrt{N}}$$

(b)
$$W_x = \frac{4 \times 5 \times 60}{\sqrt{5000}} = 17.0\,\text{s}$$

$$W_y = \frac{12}{5} W_x$$
$$= \frac{12}{5} \times 17 = 40.8\,\text{s}$$

Since time taken for the air peak to emerge is 30 s. Hence, the mobile phase for any substance, $T_m = 30$ s, and total time spent by y in the column is 12 min

(720 s). Fraction of time y spends in the mobile phase

$$= \frac{t_m}{720} = \frac{30}{720} = 0.417\,\text{s}$$

2. A gas chromatogram obtained for a mixture of hydrocarbon shows a peak at the following point:

Retention time	60 s
Width (cm)	9.00
Area (cm^2)	0.900

Calculate the number of theoretical plates associated with a given peak:
Solution:

$$N = 16\left(\frac{\text{Retention time}}{\text{Width}}\right)^2$$
$$= 16\left(\frac{60}{9.00}\right)^2$$
$$= 711$$

Unsolved Problems

1. A gas chromatogram shows a peak with retention time 100 s, width 15.00 cm and area 1.2 cm^2. What will be the number of theoretical plates associated with the peak?
2. Underline the principle involved in Gas chromatography.
3. Which are the commonly used carrier gases in Gas chromatography.
4. Describe the principle components of a gas chromatography instrument.
5. What are the main components of gas chromatography?
6. Describe three detectors used in gas chromatography.
7. Explain the nature of peaks obtained in gas–liquid chromatography.
8. Discuss the various applications of gas chromatography.
9. Discuss the factors affecting the separation in gas chromatography.
10. Describe various types of sample injection systems used in gas chromatography.

Answers

1. 711

Chapter 17
High Performance Liquid Chromatography

The great success of gas chromatography inspired scientist to enhance liquid chromatography as GC was not applicable to compounds that are not sufficiently volatile or stable to be separated by GC Classical liquid chromatography gave way to much advanced, powerful, and analytically more accomplished form of high performance liquid chromatography (HPLC).

In 1964, J. Calvin Giddings proposed an improved version of liquid chromatography in which smaller particles with increased flow pressure gave rise to theoretically high plate numbers and enhanced the performance. Today, this high performance liquid chromatography (HPLC) is another name for high pressure liquid chromatography and has emerged as the only substitute for liquid chromatography (LC).

P. Malhotra, *Analytical Chemistry*,
https://doi.org/10.1007/978-3-031-26757-4_17

The greatest advantage of HPLC above GC is that its utility is not limited by sample volatility or thermal stability. HPLC is quite versatile and can separate a wide range of compounds including macromolecules, high molecular weight polyfunctional compounds, polymeric materials, various ionic species, and labile natural products. HPLC also offers adequate separation as compared to GC and allows improved detection limits with the use of fluorescence detection. Currently, ultra-high pressure systems are used in HPLC and their performance can be compared to capillary column GC. The role of HPLC in the pharmaceutical industry is so well acclaimed that further advancements in the system have always been very encouraging.

17.1 Principles Involved in HPLC

Chromatographic separation in HPLC relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. The active component of the column the sorbent, is typically a granular material made of solid particles (e.g. silica, polymers, etc.), 2–50 μm in size. The components of the sample mixture are separated from each other due to their different degrees of interaction with the sorbent particles. Specific interactions between sample molecules with both the stationary and mobile phases result in HPLC separations. HPLC offers a greater variety of stationary phases, which allows a greater variety of these selective interactions leading to wider possibilities of separation. These interactions are physical in nature, such as hydrophobic (dispersive), dipole–dipole, and ionic, most often a combination of more than one interaction. Sample recovery is easily done and separated fractions are collected, usually quantitative, from column outlets. High operational pressures (50–350 bar) and small amounts (2–50 μm in average particle size) desire narrow column dimensions, i.e. 2.1–4.6 mm diameter and 30–250 mm.

HPLC mainly depends on column efficiency and optimization in design and construction of the total system becomes highly significant. The two modes in which HPLC is used are liquid–solid adsorption chromatography and liquid–liquid partition chromatography, the latter being most common. Differences in solute polarity play major roles in rate of adsorption and solubility determinations. Liquid–Liquid partition processes are quite sensitive to small molecular weight differences and so can be conveniently used to separate members of homologous series whereas for isomers having different stearic configurations, adsorption processes are preferred.

Various liquid chromatographic (LC) procedures are available and are complementary to each other in their application. The choice of LC modes is based on the solubility and molecular mass of the solute (Fig. 17.1).

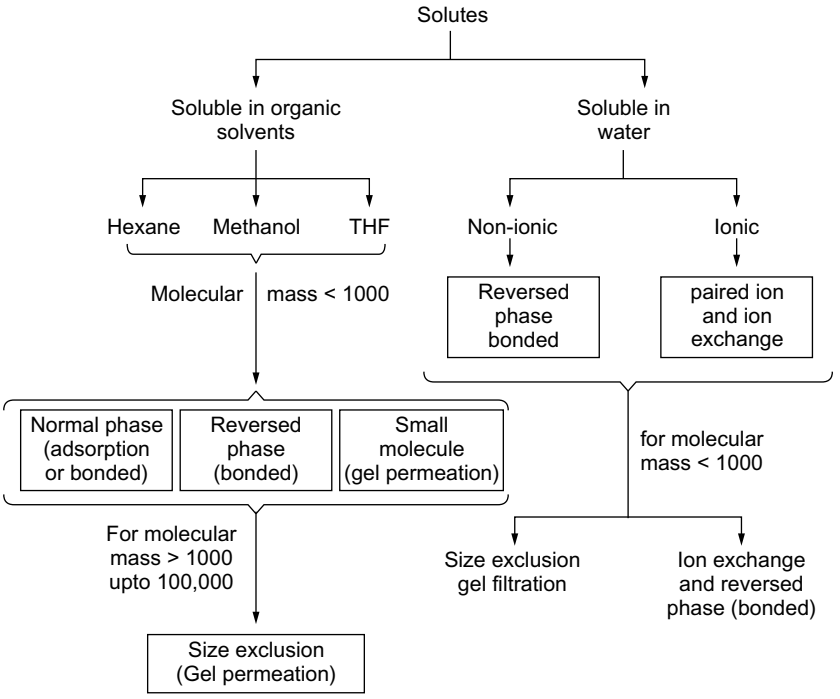


Fig. 17.1 Flow chart of principles involved in HPLC

17.2 Components of HPLC

Stationary Phase and Particle Size of Packings

Irregular shaped porous silica gel or alumina particles of 10 μm were earlier used in HPLC but these days spherical particles of high purity silica, low in trace metal content are used. Pore sizes are in the range 60–100 Å range and for larger biomolecules pore size of 300 Å is used.

HPLC is majorly performed in the liquid–liquid mode driven by partition chromatography and the liquid stationary phases are either coated on the particles or are chemically bonded. The microporous or diffusive particles whose major surface area is within the pores are permeable to the solvent and are commonly used. The mobile phase moves around the particles and solute diffuses into the stagnant mobile phase within the pores to interact with the stationary phase and then diffuses out into the moving mobile phase. The particles used in HPLC are sponge-like structures with 70% porosity and 2.6 Å pore size as shown in Fig. 17.2.

The efficiency of chromatographic columns is given by the expression, similar to Van Deemter equation

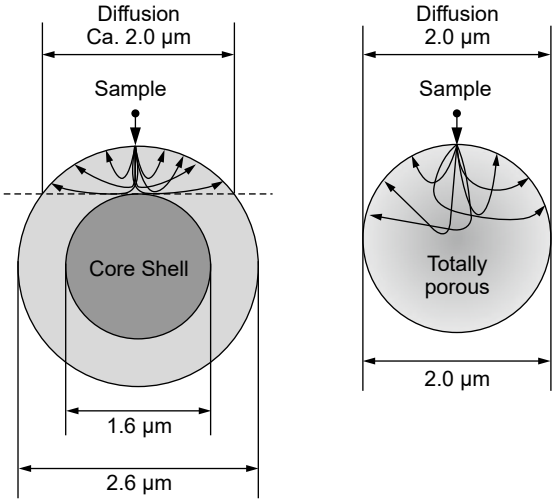


Fig. 17.2 Diffusion of sample in core shell and totally porous silica

$$H = A + \frac{B}{u} + C_S u + C_m u$$

A	coefficient describing multiple path effects
B	Longitudinal diffuse on coefficient
C_S and C_m	are stationary and mobile phase mass transfers, respectively.

In case of immobilized liquid being stationary phase, the mass transfer coefficient is directly proportional to the square of the thickness of the film on support particles d_p^2

$$C_S \propto d_p^2$$

and inversely proportional to the diffusion coefficient D_S of the solute in the film

$$C_S \propto \frac{1}{D_S}$$

The mobile phase mass transfer coefficient C_M is inversely proportional to the diffusion coefficient of the analyte in the mobile phase D_M

$$C_M \propto \frac{1}{D_M}$$

and is proportional to the square of the particle diameter of the packing material d_p

$$C_m \propto d_p^2$$

and for open tubular columns to the square of column diameter d_m

$$C_m \propto d_p^2$$

Therefore, it can be seen that the efficiency of a column should improve with a decrease in size of the particle and also that lowering particle size from 45 to 6 μm can reduce the plate height roughly by ten times.

Band broadening and column height: As the solute is carried through open tubular columns extra column band broadening occurs. This occurs due to the difference in rates of flow of liquids placed in the centre and that towards the wall as the liquid in centre of the column moves faster than the liquid close to the walls. In GC this is equalized by diffusion but diffusion in liquids is much slower and so the difference is significant enough to cause broadening of band. Such extracolumn effects contribute to the total plate height and can be expressed as

$$H_{\text{ex}} = \frac{\pi r^2 u}{24 D_M}$$

where

u is the linear flow velocity
r is the radius of the tube
D_M diffusion coefficient of the solute in the mobile phase cm^2/s.

Reducing the radius of extracolumn components to 0.010 inches or less and shortening the length of extracolumn tubing can reduce the broadening effect to a large extent.

The length of the column can affect not only the resolution of a given separation, but the longer the column the greater the plate number and speed of separation, If a 250 mm column with 5 μm packing gives a certain efficiency, then the same can be obtained by a 150 mm column packed with 3 μm packing.

Column pressure in HPLC: Pressure is one of the most important parameters in successful HPLC. The role of pressure is to drive the mobile phase through the small particles packed into the chromatographic column. For this purpose a high pressure pump is employed that can provide sufficient hydraulic pressure to overcome the resistance of the packed bed. There are two ways to generate pressure in HPLC: first is the pressure generated by going to smaller particles of the stationary phase packed in the column which is the current trend in analytical HPLC and the second is the pressure changes dictated by itself owing to the back pressures created in the column.

The back pressure in a packed bed can be governed by the expression

$$\Delta P = \frac{\eta F L}{K^{\circ} \pi r^2 dp^2}$$

where

η viscosity
F rate of low

L Length
r column radius
dp particle diameter
K^o specific permeability.

As it indicates, lower viscosity solvents are more appropriate and when a choice of organic solvent is available, acetonitrile is preferred over isopropanol due to its lower viscosity. Solvent strength polarity, analyte solubility, and compatibility are other considerations.

Few HPLC measurements are greatly affected by variations in temperature. High temperatures favour because of decrease in the viscosity of the mobile phase, increase in solubility and mass transfer thus leading to better resolution. The sharpness in the peaks also increases with increase in temperature.

A solvent with low viscosity always gives better resolution in HPLC measurements. Increase in viscosity of the solvent always decreases the flow rate of the mobile phase.

The Mobile Phase

In liquid chromatography, the mobile phase is highly critical as it depends upon the interaction of the solutes with the mobile phase and stationary phase. After ensuring moisture free organic solvent and aqueous solvent containing no more than trace amounts of other compounds, the following factors are considered for the choice of an appropriate solvent: The eluting power of the mobile phase, its overall polarity, polarity of the stationary phase and the nature of sample components. For normal phase separations, eluting power increases with increase in polarity of the solvent but for reverse phase separations, eluting power decreases with increasing solvent polarity. Polar materials are best separated using partition chromatography whereas nonpolar materials are separated using adsorption chromatography. The polarity order followed is $RH < RX < RNO_2 < ROR = RCO_2R = RCOR = RCHO = RCONHR < RNH_2 < R_2NH, R_3N, < ROH < H_2O < ArOH < RCOOH <$ nucleotides $< NH_3RCO_2^-$.

In most of the systems, a pure solvent does not provide efficient separation and instead an appropriate mix of solvents is used. An optimum ratio of solvents can give efficient separations. For example, the difference in resolution can be seen in Fig. 17.3, starting from 80% acetonitrile water to 40% acetonitrile water gave better resolution. Mixing 50% MeOH with 40 acetonitrile gave improved resolution but a blend of 20% acetonitrile + 25% MeOH with water gave the best separation.

A polar mobile phase such as methanol, acetonitrile, tetrahydrofuran, water, or usually a mixture of water with one of the organic solvents is more commonly used in reversed phase chromatography. The polarity levels, as required, can be altered by changing the ratio of water content. There is a wide range of organic compounds which can be separated using organic solvent water mixture as a solvent in reversed phase chromatography.

There are two ways in which optimization of mobile phase can take place, either by **isocratic operation** or by **gradient elution methods**.

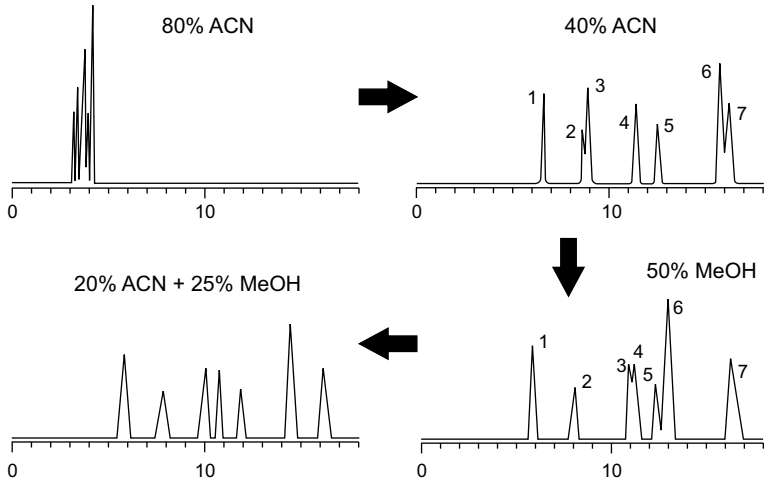


Fig. 17.3 Increase in resolution with mixing of solvents

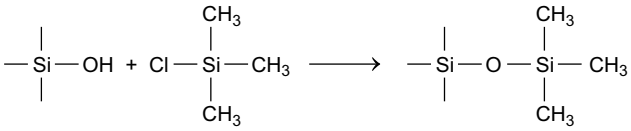
Isocratic mode of HPLC means the composition of the mobile phase remains constant throughout the run and in a single run 2 or 3 compounds are separated. Isoeratic mode is usually preferred where the system and column are equilibrated all the time and do not suffer from fast chemical changes. However, in recent times the gradient elution methods are practised in major chromatographic runs. In gradient elution, with the use of low pressure mixing system, the solvents from separate reservoirs are fed to a mixing chamber and the mixed solvent is then pumped into the column. This kind of arrangement enables changing the composition of the mobile phase during the chromatographic run. For example, in reversed phase chromatography the mobile phase's composition at the start of the run is highly aqueous and the percentage of the organic mixture methanol or acetonitrile) is increased with time, leading to enhanced elution strength. In normal phase chromatography, the initial mobile's phase's composition is usually pure hexane in which gradually a more polar organic solvent such as chloroform, THF, ethanol, or isopropanol is added and chromatographic run is performed. As the gradient develops, it increases the resolution for each chromatogram, reduces the run time, and increases the sensitivity.

Stationary phase: In liquid–liquid chromatography, to make the liquid stationary phase the liquid may be distributed on a solid support which may or may not contribute to the separation process. The liquid may also be chemically bonded to the solid (bonded phase) or immobilized onto the solid.

Silica is the most widely used sorbent however it dissolves above pH 8 therefore as a replacement cross-linked polymeric particles like polystyrene or polymethacrylates are used for the separation of bases.

Silica gel coated with high percentage of stationary phase (when liquid) is often used as it offers a support material to the stationary phase. It is essential for the support material to be inert towards the solute otherwise its interferences with the partition.

To prevent the loss of stationary phase from the support material, the stationary phase may be bonded to the support material. This type of chromatography gives rise to bonded phase chromatography. For example, silylation reaction on the surface of silica gel provides a bonded phase. Reaction of silica with dimethyl chlorosilane produces a monomeric bonded phase

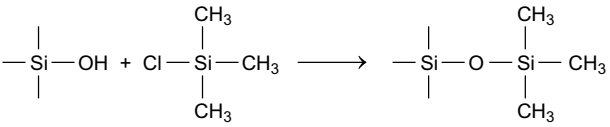


where R is variable.

$R =$ octadecyl hydrocarbon (C_{18}) is the most commonly used bonded phase in HPLC.

The silexane bonds are stable in both acidic and basic mediums (pH 3–8 range).

Uncreated silanol groups sometimes absorb polar groups and distribute the chromatogram which is prevented by rendering silanol groups inactive. This is achieved by its reaction with dimethyl chlorosilane, a process of end-capping.



A list of some typical stationary and mobile phases for normal and reverse phase chromatography is given in Table 17.1 (Fig. 17.4).

Separation mode	Stationary phase (particle)	Mobile phase (solvent)
Normal phase	Polar	Non-polar
Reversed phase	Non-polar	Polar

Table 17.1 Examples of stationary phases and corresponding mobile phases

Stationary phase	Mobile phases
Normal phase chromatography carbowax (400, 600, 750 et) β, β′-oxydipropionitrile ethylene glycol cyanoethyl silicone	Aromatic solvents: xylene, toluene, heptane saturated hydrocarbons, e.g., hexane hydrocarbons mixed with dioxen (10% methanol, ethanol, $CHCl_3$, CH_2Cl_2
Reverse phase Squalane, Zipax-HCP Cyano, ethylsilicone	Water and alcohol water mixtures, acetonitrile and acetonitrile water mixtures

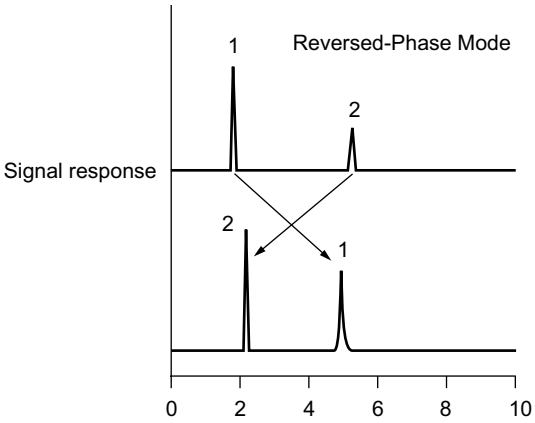


Fig. 17.4 Reversal of polarity of phases in reverse phase HPLC

17.3 Principle of Reversed Phase Chromatography

Reversed phase chromatography employs a polar (aqueous) mobile phase and a nonpolar hydrophobic stationary phase. As a result, hydrophobic molecules in the polar mobile phase tend to adsorb to the hydrophobic stationary phase, and hydrophilic molecules in the mobile phase will pass through the column and are eluted first. Reversed phase chromatography offers a preparative route for the preparation of a wide range of biomolecules, in which the binding of mobile phase solute to an immobilized *n*-alkyl hydrocarbon or aromatic ligand occurs via hydrophobic interaction. Molecules that possess some degree of hydrophobic characters such as proteins, peptides, and nucleic acids can be separated by reversed phase chromatography with excellent recovery and resolution (Fig. 17.5).

The separation mechanism in reversed phase chromatography depends on the hydrophobic binding interaction between the solute molecule in the mobile phase and the immobilized hydrophobic ligand, i.e. the stationary phase. Reversed phase chromatography is an adsorptive process that relies on a partitioning mechanism to effect separation. The solute molecules partition (i.e. an equilibrium is established) between the mobile phase and the stationary phase. The distribution of the solute between the two phases depends on the binding properties of the medium, the hydrophobicity of the solute, and the composition of the mobile phase. Initially solute from the mobile phase is adsorbed on the stationary phase and subsequently the mobile phase composition is modified to favour the desorption of the solute from the stationary phase back into the mobile phase. Thus, whatever is the percentage distribution or adsorption, it just gets reversed, i.e. a 100% solute in the stationary phase becomes a 100% solute in the mobile phase when reversed.

Separation in reverse phase chromatography is due to the difference in binding properties of sample components because of the difference in their hydrophobic properties. The degree of solute molecule binding to the reversed phase medium can be controlled by manipulating the hydrophobic properties of the initial mobile

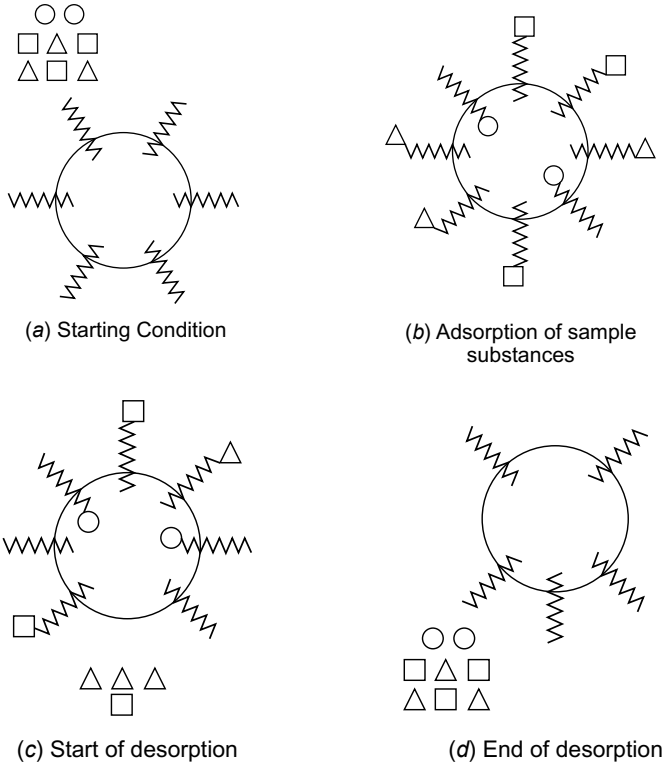


Fig. 17.5 Principle of reversed phase chromatography with gradient elution

phase. Solute which differs even slightly in their hydrophobicity can be separated by reversed phase chromatography. It has an excellent resolving power and can be confidently used for the separation of complex biomolecules.

Reversed phase chromatography of biomolecules generally uses gradient elution instead of isocratic elution. While biomolecules strongly adsorb to the surface of a reversed phase matrix under aqueous conditions, they desorb from the matrix within a very narrow window of organic modifier concentration. A diversity in the adsorption affinities of high molecular weight molecules practically leads to the choice of gradient elution as most appropriate method for reversed phase separation.

17.4 Instrumentation

High pressure liquid chromatography apparatus consists of four principal components: mobile phase supply system, sample injection system, column, and detector (Fig. 17.6).

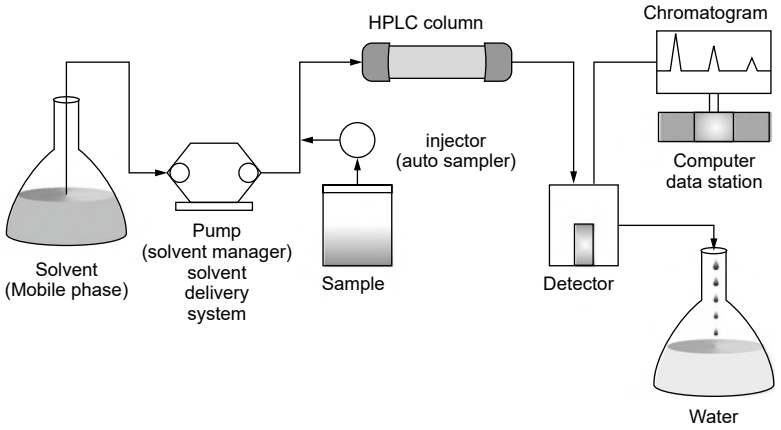


Fig. 17.6 Main components in an HPLC system

Mobile phase supply: This part of the system contains a solvent reservoir which also has a provision for degassing (remove dissolved gases and dust from the liquid) and solvent proportioning valve so that variation in the composition of mobile phase can be made for various chromatographic runs following the gradient elution methodology. The solvent reservoirs can be filled with a range of solvents having different polarities (Fig. 17.7).

Its attachment with a millipore valve ensures the purity of the solvent as well as the purity of water which is mixed in different compositions as required for gradient elution. To maintain a high pressure flow of the mobile phase it is further attached to a pump and in HPLC the most commonly used pump is the reciprocating pump. Such pumps are capable of providing high output pressure and constant flow rates. Even pumps that are screw driven types are used, but modern chromatographs use the reciprocating type pumps only. Pressures up to 414 bar and flow rates in the range of 0.1–10 ml/min with flow reproducibility are more common.

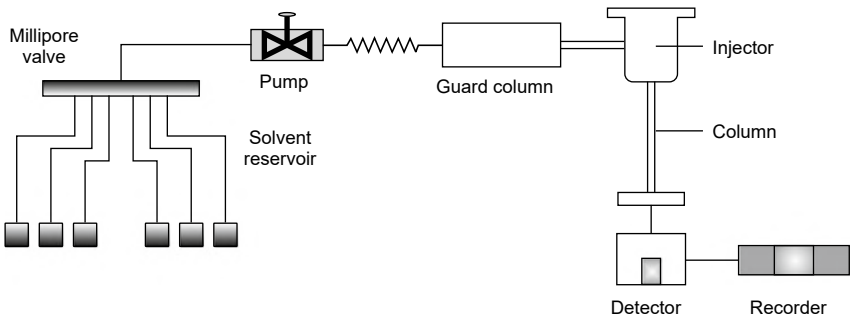


Fig. 17.7 Schematic diagram of HPLC system showing solvent reservoir

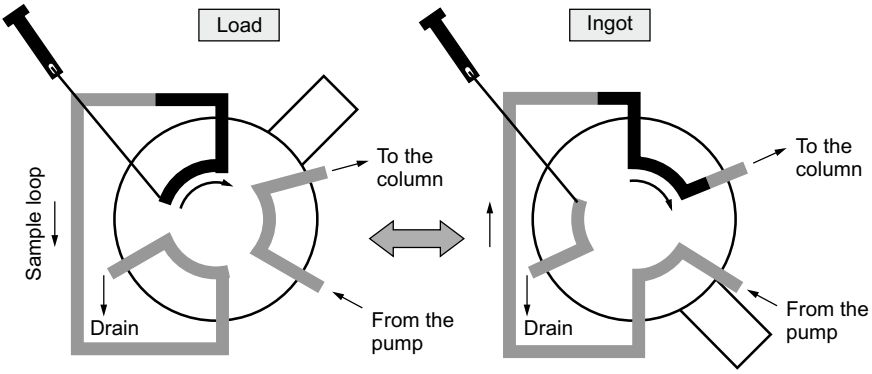


Fig. 17.8 Sample injector

Sample injection systems: The way samples are injected into the column, greatly influences the precision of liquid chromatographic measurements. Since very small volumes 1–50 μL are required to be injected at high pressure (7000 psi). There are two ways sample injections can be carried out, either by syringe injection or through a sampling valve which is most commonly used because valve injection gives higher precision. It consists of a port connected to an external sample loop having a known or fixed volume. The loop is filled with the sample by automated sampling valves which not only maintains constant flow to the columns but also prevents back flow, (Fig. 17.8) and can be operated for varying the volume of the sample injection.

Column

Various kinds of packed columns differing in size and packing are available in the range of 5–25 cm length. Usually the internal diameter of the column is 3–5 mn and the particle size of packings is in the range of 3–5 μm which makes 100,000 plates/m having the advantage of high speed and less solvent requirement. Sometimes, between the injector and the main column a guard column (or a precolumn) of 3–10 cm length is placed in order to collect debris generated by the contaminants of the solvents and strongly adsorbed components of the analyte. This short guardcolumn contains the same stationary phase as the analytical column. It enhances the life of the analytical column and needs to be replaced from time to time.

Column temperatures are normally kept below <150° for which instruments fitted with either water jackets or thermostats for controlling the temperatures are used.

Packing used in the columns consists of porous microparticles (~5 μm) composed of silica, alumina, synthetic resin polystyrene-divinylbenzene or an ion exchange resin.

Detectors

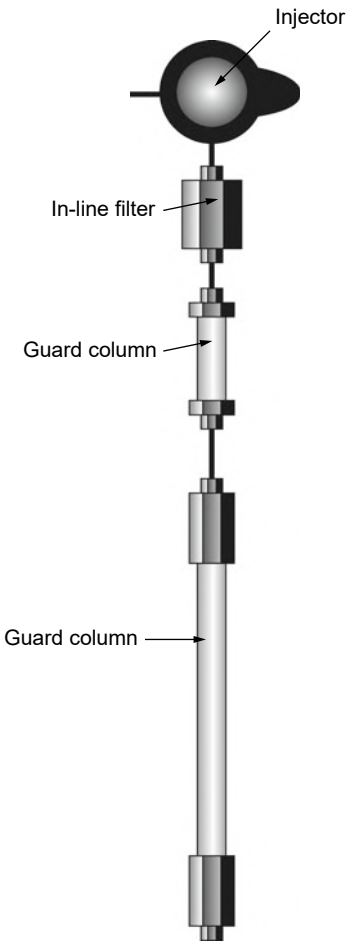
The liquid chromatographic detectors are of two basic types. The first one are **Bulk property detectors** which respond to the properties of the mobile phase. Their detection is based on the variation in density, refractive index, or dielectric constant of the

mobile phase due to the presence of solute. The *other type are solute property* detectors which identify the solutes by their UV absorbance, fluorescence, or diffusion current, as they are not present in the mobile phase.

The essential characteristics required for HPLC detectors are quite similar to those necessary for GC detectors except for the fact that HPLC detectors need not be responsive over a wide temperature range and should have least volume. Thus, an ideal HPLC detector should be sensitive to low concentration of every analyte, provide linear response, and should not broaden the eluted peaks. The detector volume is less than 20% of the volume of the chromatographic bands (Fig. 17.9).

Sensitivity of a detector is rated in terms of noise equivalent. That concentration C_n of the solute which produces a noise level in the detector should be very low. Linear range of a detector is assessed on the basis of the concentration range over which the response of the detector is directly proportional to the concentration of

Fig. 17.9 HPLC column



the solute. A universal detector is always preferred to, as that which can respond to all types of constituents of a sample, whereas a selective detector only responds to a particular category of compounds.

Derivatization

For those type of selective detectors which respond only to a particular type of constituents of analyte, some derivatives are formed either before the analyte enters the column or after it exits the column so that a detector response is available. For example, with compounds lacking any ultraviolet chromophore in a particular region (~254 nm) but possessing a reactive functional group, may be converted to a suitable compound in which a chromophore is introduced so that it can be detected. Such a derivative preparation may generally be carried out before the separation, known as precolumn derivatization, or afterwards, known as postcolumn derivatization. Pre-column derivatization is most commonly used as it does not require any modification in the instrumentation system nor does it impose any limitations. For example, ninhydrin is commonly used for the derivatization of amino acids which show absorption at 570 nm, and reagent dansyl chloride is used to obtain fluorescent derivation of proteins, amines, and phenolic compounds.

One of the most common HPLC detectors is **ultraviolet detector** because many solutes absorb ultraviolet light and high quality detectors are available which provide full-scale absorbance ranges from 0.0005 to 3 absorbance units. The spectrum of each solute as it is eluted is recorded. Ultraviolet detectors are suitable for gradient elution with non-absorbing solvents.

Fluorescence Detectors

Are those which excite the eluate with a laser and measure fluorescence and are very sensitive, however, they respond to very limited analytes. Fluorescent or electroactive groups may be covalently attached to the analyte in order to make good use of fluorescent detectors. This process is also known as **derivatization** which can be either performed on the mixture before adding it to the column or addition of originates to the eluate before entering the detector so that the compound can respond to fluorescence detector. For example, to a mixture of hormones, namely, epinephrine, norepinephrine, and dopamine when separated by HPLC, Tb (EDTA) is added, and all these compounds form complexes. These complexes can be excited at 300 nm and they emit at 500–600 nm (Fig. 17.10).

Evaporative Light Scattering Detector

For those analytes whose boiling points are much higher than the mobile phase, evaporative light scattering detectors can be used. The eluate is mixed with nitrogen gas and after nebulizing it into droplets, the solvent evaporates from the droplets and a fine mist of solid particles is left behind. These particles scatter light from a diode laser to a photo-diode and are detected on this basis. The difference in the scattering is observed on the basis of molecular mass difference of the particles rather than the structural difference. The evaporative light scattering detector's signals give a graph

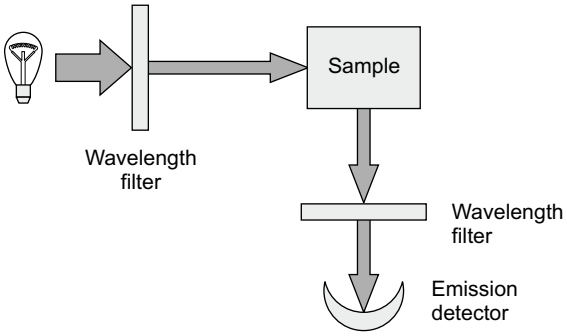


Fig. 17.10 Fluorescence detector

of scattered high intensity versus time. As there is no peak of the solvent, it is quite suitable for gradient elution so there is no interference with various eluting peaks (Fig. 17.11).

Electrochemical Detectors

For compounds that can undergo oxidation and reduction respond to electrochemical detectors. The eluate when containing compounds like phenols, aromatic amines, peroxides, mercaptans, ketones, aldehydes, conjugated nitriles, aromatic halides, aromatic nitro compounds, and electrochemical detectors can be used. The eluate is made to either get oxidized or reduced at the working electrode and the current generated is measured by the detector as the current is proportional to the analyte concentration (Fig. 17.12).

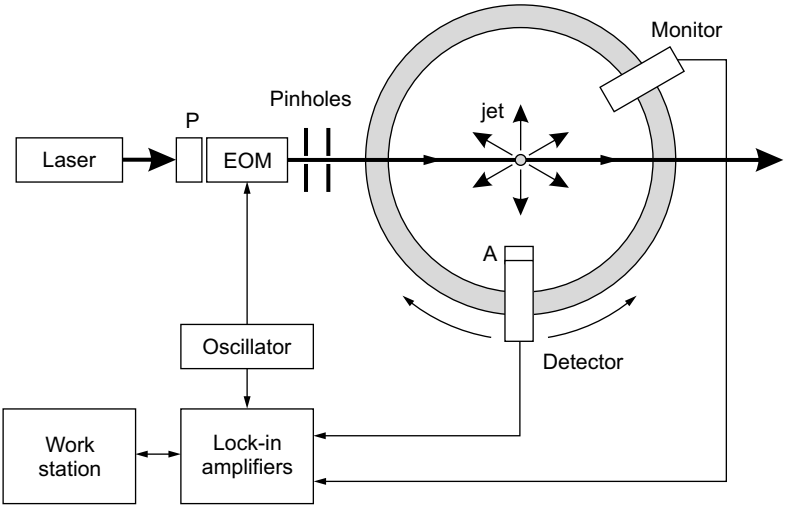


Fig. 17.11 Evaporative light scattering detector

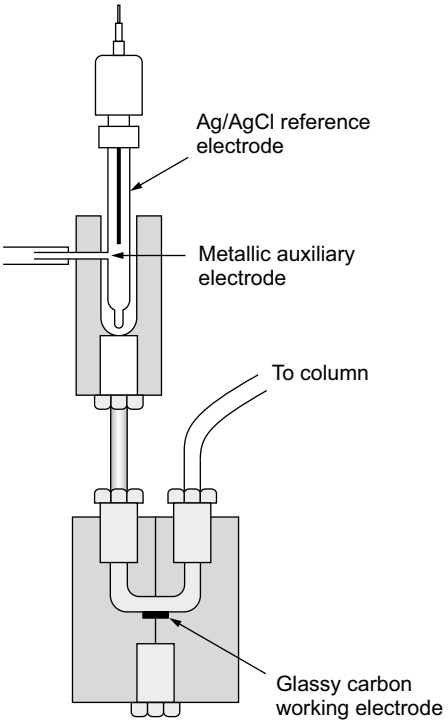


Fig. 17.12 An electrochemical detector (double beam type)

Charged Aerosol Detector

It is an universal detector that is highly sensitive to non-volatile analytes and indicates the molecular mass of the components of analyte from the peak area of the peaks in the chromatograph.

Refractive Index Detectors

This is a deflection type detector in which there are two triangular 5 to 10 μL compartments through which pure solvent or eluate are allowed to pass. Monochromatic light free from infrared radiation which causes heating effect, passes through the compartments and is directed to a photo-diode array. When solute with a different refractive index enters the cell, the deflected beam irradiates different pixels. Even those solutes which do not respond to ultraviolet absorption can be used with this detector. However, due to its low sensitivity, a refractive index detector is not useful for trace analysis and cannot be used for gradient elution we cannot match the reference and the sample for changing solvent composition (Fig. 17.13).

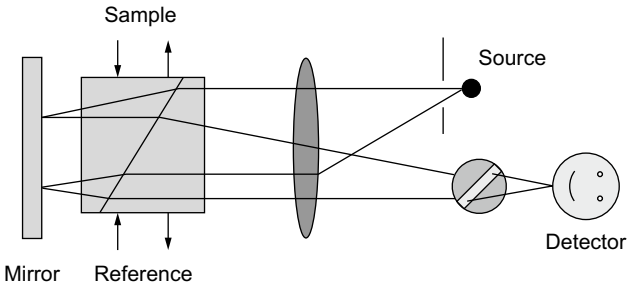


Fig. 17.13 Refractive index detector

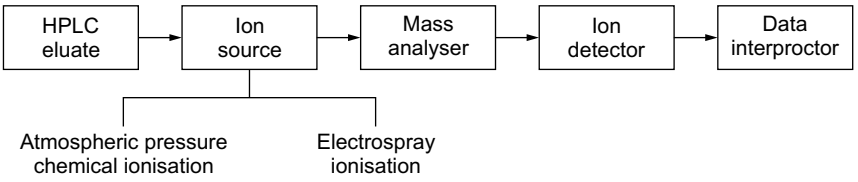


Fig. 17.14 Block diagram of HPLC/MS system

Importance of Mass Spectrometric Detection

Mass spectrometre can also be coupled with HPLC for detection as in GC measurements. Since a gas phase sample is needed for mass spectrometry, therefore, the eluents need to be vaporized which produces voluminous vapours. To restrict the volume most of the solvent is removed.

The most common liquid chromatography-mass spectrometry interfaces are atmospheric pressure chemical ionization and electrospray ionization. Atmospheric pressure chemical ionization creates ions from analyte and the solute present in eluate electrospray requires that analyte be charged in solution. The ions produced are then sorted by the mass analyser and detected by the ion detector (Fig. 17.14).

The combination of HPLC and mass spectrometry gives high selectivity and it is advantageous because it relies on eluate fingerprinting instead of its retention time in the column. The combination provides molecular mass, structural information, and accurate quantitative analysis.

17.5 Applications of HPLC

HPLC has proven to be an optimal technology most widely used in the pharmacy, medicine, and food industry. The advent of HPLC has resulted in many new applications whose no alternatives were previously available. Its major contribution has been to the pharmaceutical and pesticide industry. Since in HPLC polar and moderately

polar substances can be separated, but in combination with ion exchange chromatography on porous layer leads, a wide range of separations have been successfully performed.

HPLC systems are also used in controlling and automating other chromatography instrumentations.

Increasingly, food analysis methods are built around high performance liquid chromatography for detecting and/or quantifying the vast majority of food analytes. Environmentalists highly depend upon HPLC method to determine trace amount pollutants in water from various sources. Ions including 4^{+}, Na^{+}, K^{+},Mg^{+2}, $NH_4^{+}$and Ca^{+2}.

HPLC can also be prominently used in the following areas:

(a) Water purification
(b) Preconcentration of trace components
(c) Ligand exchange chromatography
(d) Ion exchange chromatography of proteins
(e) High-pH anion exchange chromatography of carbohydrates and oligosaccharides
(f) Steroid analysis
(g) Widely used in biochemistry for identification, separation, preparation of proteins peptides and aminoacids.
(h) HPLC—MS is used in the study of the metabolism of drugs and other xenobiotics.
(i) HPLC—MS is used for the identification of drug degradation products in pharmaceutical formulations including herbal medicines.
(j) Mixture of sugars is efficiently separated using 70% aqueous acetonitrile as the mobile phase and zorbax NH_2 as the stationary phase.

Quantitative Analysis

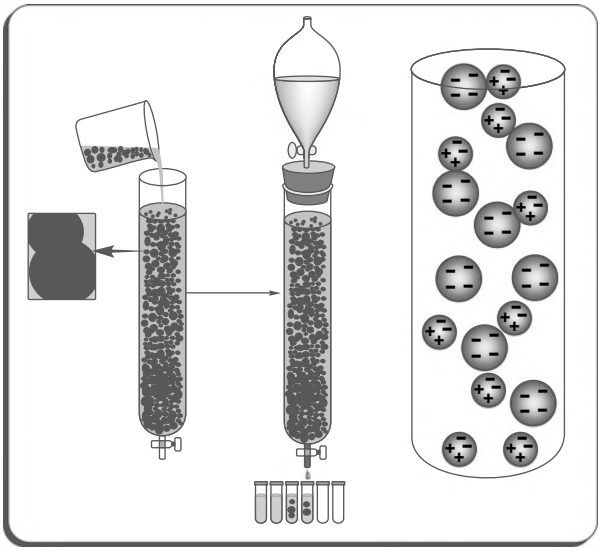
HPLC provides a reliable quantitative precision and accuracy along with a high linear dynamic range achieved by a single run. Quantitative analysis of flavonols, flavonels, and flavanones in fruits, vegetables, and beverages has been carried out using photo-diode array and mass spectrometre detection, various phospholipids and sphingolipids have been quantitatively analysed by HPLC. Bioanalytical fields rely highly on HPLC for quantitative analysis of all alkylated DNA specially methylated and ethylated DNA. Recently quantitative reversed phase high performance liquid chromatographic analysis of wheat storage proteins has acquired much importance as a potential quality prediction tool.

Unsolved Problems

1. What are the basic principles of HPLC?
2. How is reversed phase chromatography different from the normal phase?
3. Describe gradient elution used in HPLC.
4. How does temperature affect HPLC separations?
5. Enlist few applications of HPLC.

6. What is the importance of mass spectrometry in HPLC detection?
7. Describe some commonly used detectors in HPLC.
8. What is the difference between isocratic and gradient elution?
9. Why does eluent strength increase in normal phase chromatography when a more polar solvent is added?
10. Why does eluent strength increase in reversed phase chromatography when the fraction of organic solvent is increased?

Chapter 18
Ion Exchange Chromatography



Ion exchange chromatography (or ion chromatography) is a process that allows the separation of ions and polar molecules based on their affinity to the ion exchanger. It can be used for almost any kind of charged molecules including large proteins, small nucleotides, and amino acids. It is often used in protein purification, water analysis, and quality control.

Experiments on Ion exchange chromatography (IC) began between 1935 and 1950 and got established in 1975 since then it is being commercially used. Today IC is of great importance in water analysis and in solving environmental problems. IC is a fast, economical, and versatile technique for the effective separation of ions, amino acids, peptides, nucleotides, nucleic acids, etc.

P. Malhotra, *Analytical Chemistry*,
https://doi.org/10.1007/978-3-031-26757-4_18

18.1 Basic Principles of Ion Exchange Chromatography

Ion exchange chromatography is a process that allows the separation of ions and polar molecules based on the charge on the surface of molecules. It is a type of solid–liquid chromatography in which the stationary phase is an ion exchange resin. It is the most popular method used for the proteins and is based on charge-charge interactions between the proteins.

Ion exchange chromatography separates proteins or other molecules on the basis of the differences in their accessible surface charges. The analyte molecules are retained on the column on the basis of coulombic interactions. Basically, it is the reversible exchange of analyte ions bound to solid support with similar ions generated from the liquid phase. Many biological molecules such as proteins, aminoacids, nucleotides, and other ions have ionizable groups which carry a net charge (+ve or −ve) dependent on their pKa and on the pH of the solution and is utilized in the separation of such a mixture of molecules. IC experiments are carried out mainly in columns *packed* with ion *exchangers*. On the basis of type of exchanger used for separation, IC is divided into two types: **Cation exchange** and **Anion exchange**.

Most of the ion exchange resins consist of an insoluble polystyrene organic polymer cross-linked with divinyl benzene with a large number of ionic functional groups (acidic or basic groups) covalently attached to free phenyl group present in it. These ionic functional groups are called active groups. The ionic active groups can exchange with other ions of similar charge of the mobile phase. This exchange may be cationic or anionic as shown below (Fig. 18.1).

$$\text{Cation exchange}: x^+ + Ry^+\rceil \rightleftharpoons Rx^+ + y^+ \quad \text{both } x \text{ and } y \text{ are active groups}$$
$$\text{Anione exchange}: x^- + Ry^- \rightleftharpoons Rx^- + y^-$$

where

x sample ion
y mobile phase ion

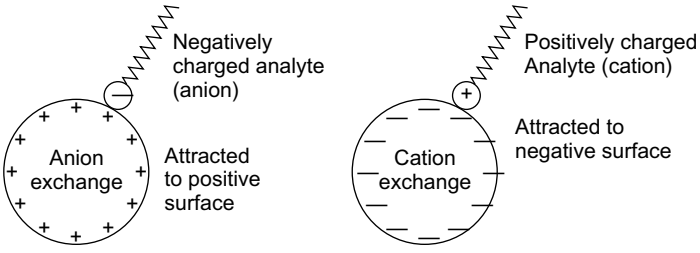


Fig. 18.1 Ion exchange on surface

R ionic site of the exchanger.

Based upon such exchanges, *IC* is categorized as cation exchange and anion exchange.

The Basic process of chromatography using ion exchange can be represented in five steps:

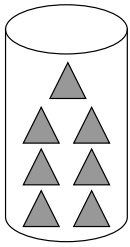
(i) eluent loading
(ii) sample injection
(iii) separation of sample
(iv) elution of analyte *A*
(v) elution of analyte *B*.

Elution is a process where the compound of interest is moved through column. This happens because the eluent which is the solution used as the solvent in chromatography, is constantly pumped through the column.

The chemical reaction explained below is for an anion exchange process.

Key: Eluent ion =  Ion A =  Ion B = 

Step I. The eluent loaded onto the column displaces any anions bonded to the resin and saturates the resin surface with the eluent anion.



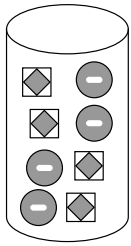
Resin surface loaded with eluent

(Eluent ion $E^- =$ )

This process of the eluent ion (E^-) displacing an anion (x^-) bonded to the resin can be expressed by the following chemical reaction

$$\text{Resin}^+ - x^- + E^- \Leftrightarrow \text{Resin}^+ - E^- + x^-$$

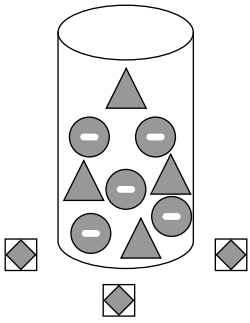
Step II. A sample containing anion A and anion B is injected into the column. The sample may contain many ions but in this illustration, only two ions A and B are being considered for simplicity and are injected as shown below:



After injecting sample A and B onto the column

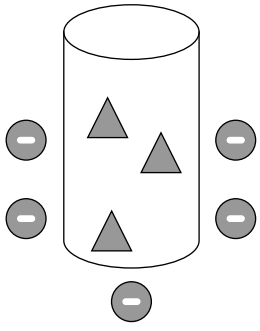
Step III. After the sample has been injected, the continued addition of eluent causes a flow through the column. As the sample elutes (or moves through the column), anion *A* and anion *B* adhere to the column surface differently. The sample zones move through the column as the eluent gradually displaces the analytes.

Step IV.



Sample B getting eluted

Step V.



Sample A getting eluted

Mechanism of ion exchange: The actual ion exchange mechanism is considered to be going through the following stages:

(i) Diffusion of the ion to the surface of the resin which is quite fast in a homogeneous solution.
(ii) Diffusion of the ion through the matrix structure of the resin to the site of exchange on the surface. This depends on the concentration of the solution and controls the whole ion exchange process.
(iii) Exchange of ions at the exchange site and higher is the charge, more difficult is its exchange.
(iv) Diffusion of the exchanged ions through the exchanger to the surface.
(v) Selective desorption by the eluent and diffusion of the species into the external solution driven strongly by pH and ionic concentration.

To optimize binding of all charged molecules, the mobile phase is generally of a low to medium conductivity (i.e. low to medium salt concentration) solution. The adsorption of the molecules to the solid support is driven by the ionic interaction between the oppositely charged ionic groups in the sample molecule and in the functional ligand on the support. The strength of the interaction is determined by the number and location of the charges on the molecule and on the functional group. By increasing the salt concentration (generally by using a linear salt gradient) the molecules with the weakest ionic interactions start to elute from the column first. Molecules that have a stronger ionic interaction require a higher salt concentration and elute later in the gradient. The binding capacities of ion exchange resins are generally quite high.

Buffer pH: As a rule, the pH of the mobile phase buffer must be between the pI (isoelectric point) or pKa (acid dissociation constant) of the charged molecule and the pKa of the charged group on the solid support. For example, in cation exchange chromatography, using a functional group on the solid support with a pKa of 1.2, a sample molecule with a pI of 8.2 may be run in a mobile phase buffer of pH 6.0. In anion exchange chromatography a molecule with a pI of 6.8 may be run in a mobile phase buffer at pH 8.0 when the pKa of the solid support is 10.3.

Varying pH: Changes in pH drastically affect a separation. In cation exchange chromatography, raising the pH of the mobile phase buffer will cause the molecule to become less protonated and hence less positively charged. The result is that the protein no longer can form an ionic interaction with the negatively charged solid support, which ultimately results in the molecule to elute from the column. In anion exchange chromatography, lowering the pH of the mobile phase buffer will cause the molecule to become more protonated and hence more positively (and less negatively) charged. As a result, the protein no longer forms an ionic interaction with the positively charged solid support which causes the molecule to elute from the column.

Components of Ion Exchange Media

Chromatography media for ion exchange are made from porous or non-porous matrices, chosen for their physical stability. The matrices are substituted with functional groups that determine the charge of the medium. High porosity, inert matrix,

high physical and chemical stability, and suitable particle size provide the basis of selection of matrix beads for ion exchange chromatography.

An ion exchange resin is a polymer (normally styrene) with electrically charged sites at which one ion may replace another. Natural soils contain solids with charged sites that exchange ions, and certain minerals called zeolites are quite good exchangers. Ion exchange also takes place in living materials because cell walls, cell membranes, and other structures have charges. In natural waters and in wastewaters, there are often undesirable ions and some of them may be worth recovering. For example, cadmium ion is dangerous to health but is not present usually at concentrations that would justify recovery. On the other hand, silver ion in photographic wastes is not a serious hazard but its value is quite high. In either case, substitution by sodium ion in wastewater is advantageous.

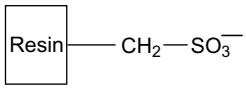
Synthetic ion exchange resins are usually cast as porous beads with considerable external and pore surface where ions can attach. Whenever there is a great surface area, adsorption plays a role. If a substance is adsorbed to an ion exchange resin, no ion is liberated. Testing for ions in the effluent will distinguish between removal by adsorption and removal by ion exchange.

While these are numerous functional groups that have charge, only a few are commonly used for man-made ion exchange resins. These are

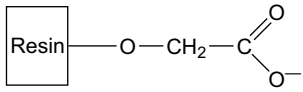
- –COOH which is weakly ionized to COO^-
- $–SO_3H^-$ which is strongly ionized to $SO_3{}^{-2}$
- $–NH_2$ that weakly attracts protons from NH_3^+
- –Secondary and tertiary amines that also attract protons weakly
- $–NR_3{}^+$ that has a strong, permanent charge (R stands for the organic group).

18.2 Cation Exchangers

Cation exchangers are positively charged molecules that are attracted to a negatively charged solid support. Commonly used cation exchange resins are *S*-resin, sulphate derivatives; and CM resins, carboxylate derived ions



S—cation exchanger



CM—cation exchanger

The adsorbents in ion exchange chromatography consist of beads made of a polystyrene polymer cross-linked with divinylbenzene (Fig. 18.2). The cross-linked polymer is namely resin and has free phenyl groups attached to it which can be treated to add ionic functional groups. Cation exchange resins contain acidic functional groups added to the aromatic ring of the resin. In a cation exchange resin all the sites are negatively charged, so that only positive ions can be separated. The acid

cation exchangers have sulphonic, carboxylic, and phenolic groups as an integral part of the resin and equivalent number of cations as (Res A^-) B^+. To the basic polymer of resin the anion is attached and the cation B^+ is mobile. The cation can either be H^+, or Na^+ which are among the most mobile cations. When a cation exchange resin is placed in a solution, the mobile or active cations of the resin are exchanged with cations of the solution. The strong acid cation exchange resin has sulphonic, $–SO_3H$ group attached to polymeric matrix, whereas weak acid cation exchange resin has carboxylic (–COOH) group attached to the polymeric matrix. From strong acids H^+ are completely ionized whereas from weak acids H^+ ions are partially dissociated.

$$nR_xSO_3^-H^+ + M^{+n} \rightleftharpoons (R_xSO_3)_nM + nH^+$$

and $$nR_xCO_2^-H^+ + M^{+n} \rightleftharpoons (R_xCO)_nM + nH^+$$

With the increase or decrease in the concentration of $[H^+]$ and $[M^{+2}]$ the equilibrium can be shifted to left or right. The strong acid cation exchange resins are usually taken in the form of their sodium salts and then treated with concentrated HCl.

$$\left[\text{Res } A^-\right]Na^+ + H^+ \rightleftharpoons \left[Res\ A^-\right]H^+ + Na^+$$

The weak acid cation exchange resins are highly influenced by pH changes and can be used only in the pH range of 5–14 whereas the strong acids resins can be

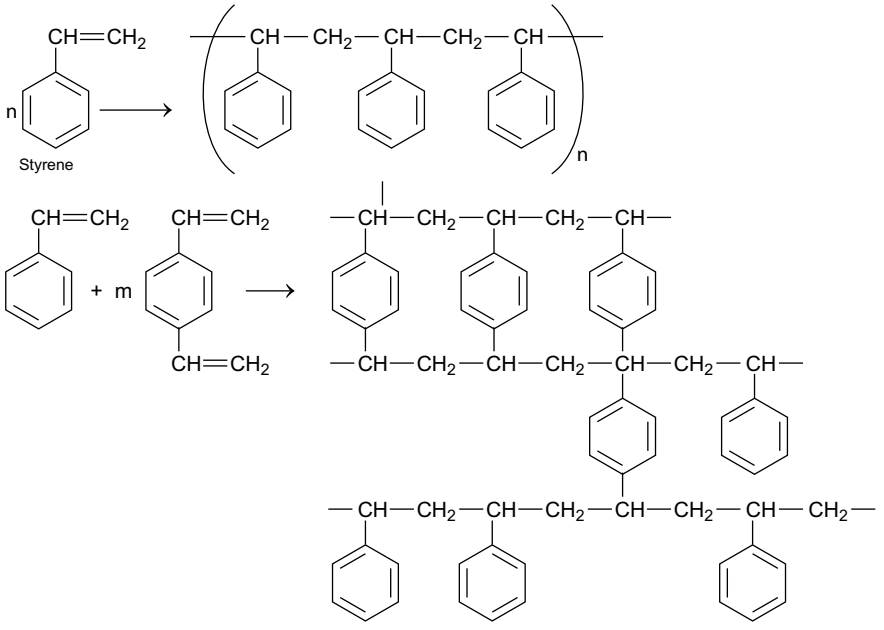
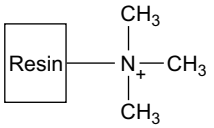


Fig. 18.2 Crosslinking of polystyrene polymer with divinyl benzene

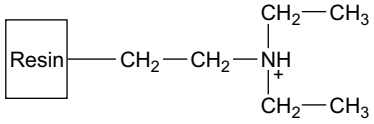
used in a wider pH range of 1–14. For separating strongly basic or bifunctional ionic compounds such as proteins or peptides, weak acid resins are generally preferred whereas strong acid resins are preferred for complex mixtures.

18.3 Anion Exchangers

Anion exchangers are negatively charged molecules that are attracted to a positively charged solid support. Commonly used anion exchange resins are Q-resin, a Quaternary amine; and DEAE resin (Diethylamino ethane)



Q-anion exchanger



DEAE-anion exchanger

Cross linked high molecular mass polymers having $-N^+(R)_3$, $-N^+H(R)_2$ – $N^+H_2(R)$, $-NH_3$ groups as basic resin to which anionic groups such as Cl^-, SO_4^{-2}, OH^- are attached, are common anion exchangers. A variety of weak base anion exchangers containing primary, secondary, or tertiary amine groups and mixtures of these have been prepared.

Thus, an anion exchange resin can be represented as (Res B^+) A^- where Res represents high molecular mass polymer acting as a resin to which cation B^+ and a mobile anion A^- are attached. In the solution of another anion, the mobile anion A^- is exchanged for anions of the solution.

$$\left(\text{Res } B^+\right)A^- + C^- \rightleftharpoons \left[\text{Res } B^+\right]C^- + A^-$$

The tertiary and quaternary amines are strongly basic and like their cationic counterpart, are pH independent (range 0–12) whereas the weak base type anion exchanger resin cationing $-N^+H\,(R)_2OH^-$, $-N^+H_2(R)OH^-$, $-N^+H_3OH^-$, highly depend on the pH (0–9) of the solution

$$R'NR_3^+OH^- + A^- \rightleftharpoons R'NR_3^+A^- + OH^-$$
$$R'NRH_3^+OH^- + A^- \rightleftharpoons R'NRH_3A^- + OH^-$$

where $R' =$ resin and $R =$ alkyl group.

The strong-base anion exchanger resins are usually available in the chloride form, which can be converted into a hydroxide form with concentrated sodium hydroxide solution. Diphenyl ether is the basic structural unit for a wide variety of recently developed anion exchange resins and they possess remarkable chemical stability (Fig. 18.3).

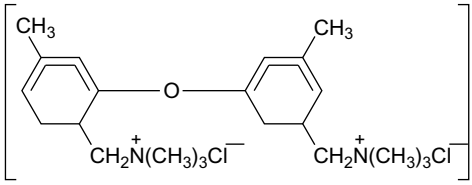


Fig. 18.3 Structure of a typical anion exchange resin

Table 18.1 Some cation exchangers

Commercial name	Acidic groups	Basic unit
1. Dowex	– SO_3H	Styrene + divinyl benzene (copolymers) (homoporous)
2. Amberlite IR-120	– SO_3H	Styrene + divinyl benzene (copolymers, homoporous)
3. Zerolit	– SO_3H	Styrene + divinyl benzene (copolymers, homoporous)
4. Amberlite –200	– SO_3H	Styrene + divinyl benzene (heteroporous)
5. SE cellulose	– C_2H_4, $–SO_3H$	Cellulose
6. Amberlite IRC –50	– COOH	Methacrylic acid
7. CM (carboxymethyl)	– CH_2COOH	Cellulose, fibrous

$$\left[\text{Res. N(CH3)}_3^+\right]\text{OH}^- + \text{H}^+\text{Cl}^- \rightleftharpoons \left[\text{Res. N(CH}_3)_3^+\right]\text{Cl}^- + \text{H}_2\text{O}$$

$$\left[\text{Res. N(CH}_3)_3^+\right]\text{Cl}^- + \text{OH}^- \rightleftharpoons \left[\text{Res. N(CH}_3)_3^+\right]\text{OH}^- + \text{Cl}^-$$

$$2\left[\text{Res N(CH}_3)_3^+\right]\text{Cl}^- + \text{SO}_4^{-2} \rightleftharpoons \left[\text{ Res. N(CH}_3)_3\right]_2\text{SO}_4^{-2} + 2\text{Cl}^-$$

Some commercially available popular cation exchangers are given in Table 18.1. Some commercially available anion exchangers are given in Table 18.2.

18.4 Ion Exchange Equilibria

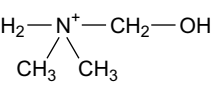
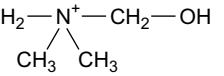
In the recent past lot of attention is being given to ion exchange reactions. Its reversible nature leads to the study of the equilibrium constant and factors affecting it. For a typical ion exchange reaction

$$A^{b+} + B_R^{a+} \rightleftharpoons A_R^{b+} + B^{a+}$$

equilibrium constant K can be given as

$$K_B^A = \frac{[A_R]^{b+}[B]^{a+}}{[A]^{b+}[B_R]^{a+}}$$

Table 18.2 Some anion exchangers

Commercial name	Alkaline groups	Basic unit
1. Amberlite IRA –400	—CH_2—$N^+(CH_3)_3$	Styrene + divinyl benzene copolymers (homoporous)
2. Amberlite IRA –410		Styrene + divinyl benzene copolymers (homoporous)
3. Zerolit FF–IP	—CH_2—$N^+(CH_3)_3$	Styrene + divinyl benzene (heteroporous)
4. Zerolite N–IP		Styrene + dioxy (benzene) is porous
5. QAE Sephadex A 25		Dextran

If $K_B^A > 1$ then 'A' has more affinity for resin but if $K_B^A < 1$ it indicates that B is bonded strongly to the resin. Thus, K_B^A refers to selectivity and $K_B^A = 1$ indicates no selectivity for both ions, in column.

A cation exchange equilibrium can be set up by

$$\underset{\text{solid}}{x\text{RSO}_3\text{H}^+} + \underset{\text{solution}}{\text{M}^{+x}} \rightleftharpoons \underset{\text{solid}}{\left(\text{RSO}_3^-\right)_x\text{M}^{+x}} + \underset{\text{solution}}{x\text{H}^+}$$

where $\text{RSO}_3{}^-\text{H}^{-1}$ represents sulphonic acid group attached to a large polymer, and a strong-base anion exchange reaction is set up by

$$x\text{RN}(\text{CH}_3)_3\text{OH}^- + \text{A}^{x-} \rightleftharpoons \left[\text{RN}(\text{CH}_3)_3^+\right]\text{A}^{x-} + x\text{OH}^-$$

If a singly charged cation is held by a cation exchange resin in a column as,

$$\text{RSO}_3\text{H}^+(s) + \text{B}^+(aq) \rightleftharpoons \text{RSO}_3^-\text{B}^+(s) + \text{H}^+(aq)$$

then with elution from dilute hydrochloric acid solution, the equilibrium is shifted to left.

Equilibrium exchange constant

$$K_{ex} = \frac{\left[\text{RSO}_3^-\text{B}^+\right]_S\left[\text{H}^+\right]_{aq}}{\left[\text{RSO}_3^-\text{H}^+\right]_S\left[\text{B}^+\right]_{aq}} \tag{18.1}$$

which can be rearranged to give

$$\frac{\left[\mathrm{RSO_3^-B^+}\right]_S}{\left[\mathrm{B^+}\right]_{aq}} = \frac{\mathrm{K}_{aq}\left[\mathrm{RSO_3^-H^+}\right]_S}{\left[\mathrm{H^+}\right]_{aq}}$$

The aqueous concentration of H^+ ions is higher during the elution than the concentration of B^+ ions in the mobile phase.

Thus, it can be interpreted that there are more number of exchange sites, as compared to the number of B^+ ions being retained on the column and the overall concentrations of $[RSO_3{}^-H^+]S$ and $[H^+]_{aq}$ are constant throughout, so that

$$\frac{\left[\mathrm{RSO_3^-B^+}\right]_S}{\left[\mathrm{B^+}\right]_{aq}} = \mathrm{K} = \frac{C_S}{C_M}$$

Now, here K is a constant similar to the distribution constant described earlier for other chromatographic concepts.

K_{ex} in Eq. (18.1) indicates the affinity of the resin for the ion B^+ and its higher value represents a strong tendency for B^+ to get retained on the solid phase. This value is also a measure of the distribution ratio of the ion B^+ with respect to H^+. When measured for various cations through different experiments, it was observed that highly charged ions have stronger affinity for solid phase than the monovalent ions, although the size of the ion and its hydrated species is also influential. For univalent ions the affinity series (also known as Bonner Affinity series) is in the following order:

$$\mathrm{Li^+ > H^+ > Na^+ > NH_4^+ > K^+ > Rb^{-1} > Cs^+ > Ag^+ > Tl^+},$$

on resin with 8% DVB, whereas, for divalent ions on Dowex, 50 W –8, resin is:

$$\mathrm{UO_2^{+2} > Mg^{+2} > Zn^{+2} > Co^{+2} > Cu^{+2} > Cd^{+2} > Ni^{+2} > Ca^{+2}} \\ \mathrm{> Sr^{+2} > Pb^{+2} > Ba^{+2}}.$$

Donnan Equilibrium

The equilibrium set up between ions in the solution and ions inside the resin is called the Donnan equilibrium. This is an ionic equilibrium reached in a solution of an electrolyte whose ions are diffusible through a selective layer of resin and is set up due to the presence of non-diffusible ions in the resin, and higher concentration of electrolyte ions outside the resin. Donnan equilibrium forms the basis of many ion exchange processes and dictates the separation of ions through ion exchange columns while also magnifying the role of pH or H^+ ion concentration.

18.5 Factors Affecting Ion Exchange Equilibria

Ion exchange equilibrium is greatly affected by the nature of resin, its exchange capacity, and nature of ion including its affinity for the solid phase and the mobile phase. The major factors influencing ion exchange equilibria are as follows:

1. **Ion exchange capacity**: The ion exchange capacity of the resin, expressed as multi-equivalents per gram of the resin, is a major factor. A standard solution of sodium chloride of volume V is passed through a column containing known amount of resin and the acid eluted from the column is titrated with a standard alkali solution of normality N, the ion exchange capacity is given by

$$\text{Ion exchange capacity} = \frac{V \times N}{W}$$

where V = volume of alkali used for titrating all acid eluent from the column.

Similarly, the ion capacity of an anion exchanger is determined by passing a standard solution of sodium nitrate through the column and titrating the eluent with standard $AgNO_3$ for Cl^- ion and standard acid for OH^- ion.

Greater is the ion exchange capacity of the resin, higher is the ion exchange in the chromatographic column.

2. **Ionic charge**: For hydrated ions of the same size, the extent of exchange with H^+ ions among cations decreases in the order $Th^{+4} > Al^{+3} > Ca^{+2} > Na^+$.

Thus, higher is the charge, higher is the extent of exchange.

Similarly, for anions of same size the order of exchange is $PO_4^{-3} > SO_4^{-2} > I^-$.

3. **Size of the ion**: For ions having same charge, the size of hydrated ion plays a significant role in their exchange capacity and the order for alkali metal is

$$Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$$

which indicates that the smallest hydrated ion Cs^+ is most strongly held by the resin and the largest hydrated Li^+ ion is least strongly bonded.

Among anions of same charge the order is

$$I^- > N_3^- > Br^- > CN^- > Cl^- > OH^- > F^-$$

4. **Concentration of the mobile phase**: The concentration of ions in the mobile phase also affects the exchange. When an active polyvalent ion in a resin is to be exchanged with an ion of lower valency, higher concentration of ion in the solution is preferred.
5. **Nature of Resin**: The ion exchange capacity of the resin is also governed by the degree of cross-linking present in the polymer. Higher is the cross-linking in the polymer more efficient is the resin (Fig. 18.4).

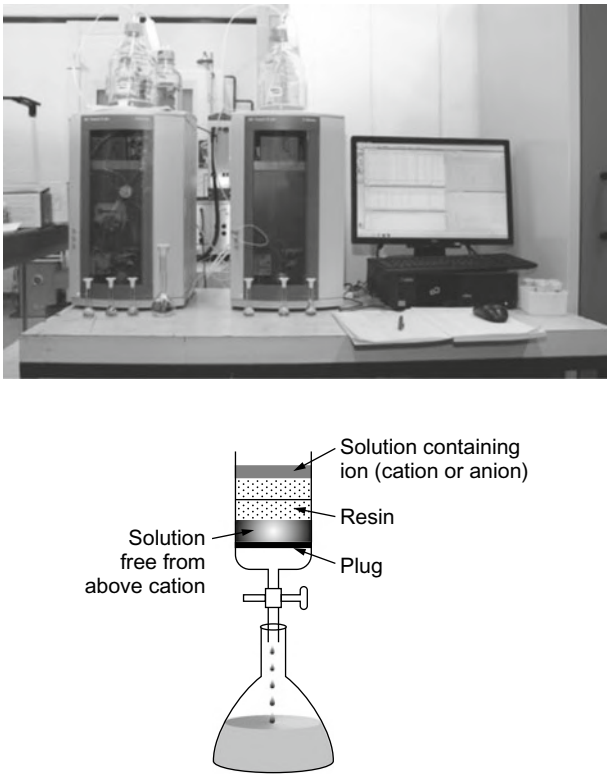


Fig. 18.4 **a** A commercial IC instrument. **b** An ion exchange column

18.6 Experimental Conditions

Experimental conditions and setup for ion exchange chromatography requires the following optimization of the various components at various stages.

For Final Resolution

1. Particle size
2. Porosity
3. Column packing.

Separation Criteria

1. Net surface charge of a protein at a particular pH
2. The pH of the buffer
3. Ionic strength of the buffer
4. Elution conditions.

Sample Preparation

1. The buffer of the sample should be the same as the starting buffer.

2. For small sample volume diluting the sample in the start buffer in order to lower ionic strength and adjust pH.
3. If the pI of the protein is known the pH of the starting buffer should be 0.5 to 1 pH unit away from pI (where I = ion exchanger).

Column

Ion exchange chromatography is carried out in columns whose length depends on the separation factor. The usual ratio of the column length to height is maintained at 10:1 or 20:1.

The packing of the column consists of ion exchange resin of optimum size of the particle so that a flow rate that is neither too fast nor too slow is produced. The normal range of particle size is 50–100 mesh or 100–200 mesh which is packed uniformly in the column. The resin should be either filled in the slurry form into the column or filled in the dry form and tapped down so that it is uniformly packed.

Development of chromatogram: The analyte mixture is dissolved in a suitable solvent and the same solvent is run down the packed column so that the same liquid is present at the top bed. The sample solution is added to the top bed in a small amount (0.1–2 ml) carefully. The mobile phase is allowed to run through the column at a desired flow rate after the sample solution has traversed steadily through the column. As the developing solvent which is the mobile phase, percolates through the column the various components of the sample mixture get separated. If the components are colored, different colored zones are observed in the column. With the continuous flow, the separation becomes more distinct. On eluting the most strongly adsorbed ions displace less strongly adsorbed ions which further displace the least absorbed ions. The separation of the components of the mixture depends upon their distribution coefficients and on the nature of the adsorption isotherm for each component.

Eluate Analysis: The eluates obtained from the column are collected as distinct fractions and are analysed using spectrophotometric, polarographic, conductometric, and radiochemical methods.

18.7 Applications of Ion Exchange Chromatography (IEC)

Ion exchange chromatography can be applied for the separation and purification of many charged or ionizable molecules such as proteins, peptides, enzymes, nucleotides, DNA, antibiotics, and vitamins from natural sources or synthetic origin. Examples in which ion exchange is used for nonchromatographic applications are as also included and they have significant commercial importance. The main areas where ion exchange chromatography finds its applications can be summarized as follows:

(i) **Softening of hard water**: Both anion and cation exchange methods are successfully used for deionizing hard water and removing its permanent hardness present due to calcium and magnesium sulphates and chlorides.

When passing the hard water through a cation exchanger (R_2H) calcium and magnesium ions are removed from hard water samples.

$$CaCl_2 + H_2R \rightarrow CaR + 2HCl$$
$$MgCl_2 + H_2R \rightarrow MgR + 2HCl$$
$$CaSO_4 + H_2R \rightarrow CaR + H_2SO_4$$
$$MgSO_4 + H_2R \rightarrow MgR + H_2SO_4$$

During anion exchange, the following reactions take place.

$$HCl + RNH_3 - OH \rightarrow RNH_3 - Cl + H - OH$$
$$H_2SO_4 + RNH_3 - OH \rightarrow RNH_3 - SO_4 + H - OH$$

Regeneration of the cation exchange resin is the biggest advantage.

$$CaR + 2HCl \rightarrow H_2R + CaCl_2$$
$$MgR + 2HCl \rightarrow H_2R + MgCl_2$$

and also the regeneration of anion exchange resin gives it an added advantage.

$$RNH_3 - Cl + NH_4OH \rightarrow RNH_3 - OH + NH_4Cl$$
$$RNH_3 - SO_4 + NH_4OH \rightarrow RNH_3 - OH + (NH_4)_2SO_4$$

This causes a complete deionization of water which is commercially used in many testing laboratories.

(ii) **Determination of total salt concentration**: Total salt concentration of any solution can be easily determined by ion exchange chromatography. For example, a standard solution of a mixture of sodium sulphate and copper sulphate is analysed for its sulphate ion concentration by passing its known volume through the column containing cation exchange resin. The acid content present in the effluent is determined by titrating it with standard alkali. This measures the sulphate ion concentration of the sample solution.

(iii) **Conversion of salt**: Ion exchange can be used to convert one salt into another. For example, preparation of tetrapropylammonium hydroxide from a tetrapolyammonium salt of some other anion can be easily done using ion exchange columns.

$$\underset{\text{tetrapropyl ammonium iodide}}{(CH_3CH_2CH_2)_4N^+I^-} \xrightarrow[OH^- \text{form}]{\text{anion exchanger}} \underset{\text{Tetrapropyl ammonium hydroxide}}{(CH_3CH_2CH_2)_4N^+OH^-}$$

(iv) **Preconcentration of trace components**: To characterize geographic and depth distributions of trace metals and their isotopes whose amounts are not sufficient for identification, they need to be concentrated so that there is enough for analysis. Ion exchange is used for such a preconcentration exercise of trace components of a solution. For example, in oceans the metals Al, Mn, Fe, Cu, Zn, and Cd are present in parts per trillions of levels as compared to the levels of Na^{+}, Ca^{+2}, and Mg^{+2} ions. Therefore the trace metals are preconcentrated and separated from alkali and alkaline earth elements by passing through the chelate: PA 1 ion exchange resin. The presence of EDTA group on the resin strongly binds the trace metals, whereas it binds weakly to Na, Mg, and Ca ions at pH 6 and so they are washed away thereby increasing the concentration of trace elements in the eluate. Analysing the eluate by mass spectrometry—inductively coupled plasma—reveals that the trace metals get concentrated by 8–10 times of the original level.

(v) **Separation of metals, separation of lanthanoids**: Ion exchange methods have the most significant and remarkable use in separating metals, separation of alkali metals, alkaline earth metals, and lanthanoids which have been successfully performed using ion exchange chromatography.

Example:

- Separation of alkali metals was done using ammonium chloride for gradient elution and final elution with hydrochloric acid Fig. 18.5.
- Alkaline earth metals were separated on Dowex 50 using ammonium lactate as eluent at pH 7 where with increase in volume of eluant, the strongly bound cation is easily eluted from the column.
- Separation of transition metals is based on the gradient elution of the complexes formed by the transition metals, several transition metals like Ni, Mn, Co, Fe, Cu, and Zn have been separated using anion exchange resin, and different concentrations of HCl are used for elution of these metals.

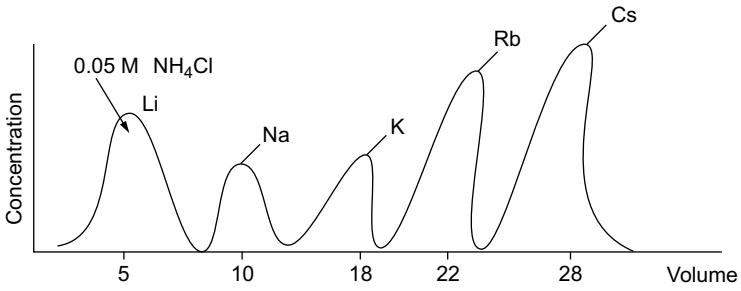


Fig. 18.5 Separation of alkali metals

- Separation of lanthanoids by anion exchange chromatography in sulphate medium further extends its applications in the separation.

(vi) **Separation of anions**: Separation of anions like chloride and bromide is carried out with anion exchange resin (De-Acidite FF) and is treated with concentrated sodium nitrate. The nitrate ions of the resin exchange with halide ions and on elution with Na NO_3, chloride ions are eluted first and then bromide ions. The eluate can be estimated for the chloride and bromide ion concentrations volumetrically.

(vii) **Industrial Applications**: Ion exchange chromatography is frequently used in almost every industry and it substantially contributes to the manufacture as well as testing organizations. Both separations and purifications during industrial processes highly rely on ion exchange chromatography. Some examples are:

- Ion exchange chromatography is being widely used for the separation and purification of substances in the food processing industry using amberlite FP and Dowex ion exchange resins and adsorbents. Sweeteners, food additives, nutritionals, beverages, and juices (for deacidification) often use IEC.
- Purification of proteins that are charged with trace metals. Metals like thorium uranium and europium form anionic sulphate complex with 0.1 – 1.5 M sulphuric acid.

Anion exchange separation of some lanthanoid metal ions from uranium (VI) has been performed in sodium nitrite and acetone, sodium nitrite media using Dowex 1 × 8, in nitrate form of resin. Separations of La(III) –U(VI), Ce(III) –U(VI), and Sm(II) –U(VI) have been successfully carried out by this method.

Cation exchangers are generally used for the separation of trivalent ions of lanthanoids by forming their complex with the chelating agents such as α-hydroxyisobutyric acid (HIBA). The analytical column used for this method is the IonPac CS3.

Molecules, efficiently used polystyrene and cross-linked agarose (Sepharose CL–6B) and their derivatives and separation as adsorbents Fig. 18.6.

- Pharmaceutical industry also relies majority on IEC for its drug analysis before finalizing any methodology for its synthesis before its biological trial.
- Ion exchange is a very versatile solution to overcoming several purification challenges within bio-based chemical industries. Enzymes possess a net charge in solution and their structure is pH dependent. Ion exchange cellulose and large pore gels are designed for chromatographic beds during the purification of enzymes.

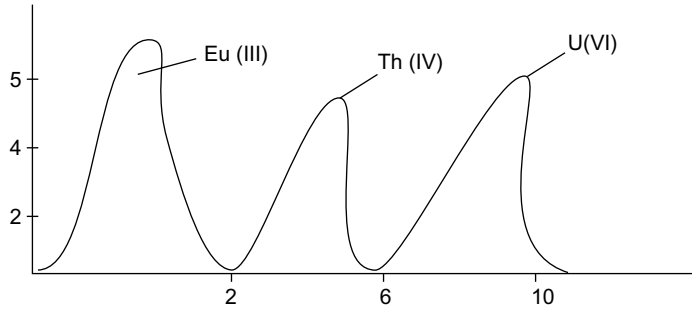


Fig. 18.6 Separation of lanthanoids

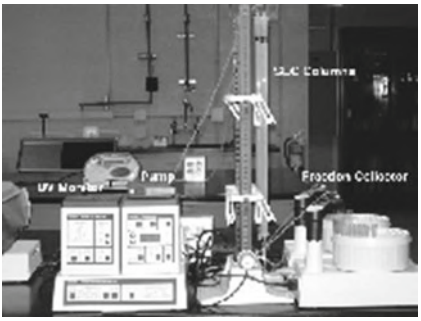
(viii) **Preparation of deionized water**: Deionized water is prepared by passing water through an anion exchange resin loaded with OH^- and a cation exchange resin loaded with H^+. For example, deionizing a water sample containing copper nitrate $Cu(NO_3)_2$ the cation exchange resin binds Cu^{+2} ion and replaces it with $2H^+$. The anion exchange resin binds $NO_3{}^-$ and replaces it with OH^-. The H^+ and OH^- combine, so bring pure water in the eluate.

$$\left.\begin{array}{r} Cu^{+2} \xrightarrow{H^+ \text{ion exchange}} 2H^+ \\ 2NO_3^- \xrightarrow{OH^- \text{ion exchange}} 2OH^- \end{array}\right\} 2H^+ + 2OH^- \rightarrow \underset{\text{(pure)}}{2H_2O}$$

Unsolved Problems

1. What are cation exchangers? Explain giving example.
2. What are anion exchangers? Explain giving example.
3. Explain the basic principles of ion exchange chromatography.
4. What is the mechanism of the ion exchange chromatography?
5. Illustrate the factors affecting ion exchange equilibrium.
6. How is ion exchange applied to softening of hard water?
7. What is deionized water and how is it obtained?
8. Describe the factors that affect the selectivity of ion exchange resins?
9. How is the ion exchange capacity of a resin determined?
10. List some industrial applications of IC.

Chapter 19
Exclusion Chromatography



Exclusion chromatography separates molecules on the basis of size. It thus justifies why it is also known as Size Exclusion Chromatography (SEC). Size exclusion chromatography is a chromatographic method in which molecules in solution are separated by their size, not by molecular weight. It is usually applied to large molecules or macromolecular complexes such as proteins and industrial polymers. Various synthetic polymers and biomolecules are the common analytes used for separation by SEC. There are two types of size exclusion chromatography *gel filtration chromatography* and *gel permeation chromatography*. When an aqueous solution is used to transport the sample through the column, the technique is known as gel filtration chromatography, whereas, when an organic solvent is used as a mobile phase, the technique is known as gel filtration chromatography. The main application of gel filtration chromatography is the fractionation of proteins and other water soluble polymers, while gel permeation chromatography is used to analyse the molecular weight distribution of organic-soluble polymers.

Both these methods are capable of good separation of large molecules from the small molecules with a minimal, volume of eluate. Various solutions can be applied without interfering with the filtration process, while also preserving the biological activity of the particles to be separated. The technique is generally combined with

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others that further separate molecules by other characteristics such as acidity, basicity, charge, and affinity for certain compounds. With size exclusion chromatography, there are short and well-defined separation times and narrow bands, which lead to good sensitivity. There is also no sample loss because solutes do not interact with the stationary phase. However, only a limited number of bands can be accommodated because the time scale of the chromatogram is short, and in general, there has to be a 10% difference in molecular mass to have a good resolution.

This technique was invented by Grant Henry Lathe and Colin R Ruthven, working at Queen Charlotte's Hospital, London. While Lathe and Ruthven used starch gels as the matrix, later dextran gels were used. Other gels with size fractionation properties include agarose and polyacrylamide.

Unlike ion exchange chromatography, gel filtration does not depend on any chemical interaction with protein, rather it is based on a physical property of the protein: that being the effective molecular radius (which relates to mass for most globular proteins).

19.1 Principle

Size exclusion chromatography (SEC) is the separation of mixtures based on the molecular size, also referred to as their hydrodynamic volume, of the components. Separation is achieved by the differential exclusion or inclusion of solutes as they pass through stationary phase consisting of heterosporous (pores of different sizes) cross-linked polymeric gels or leads. The process is based upon different permeation rates of each solute molecule into the interior of gel particles. Size exclusion chromatography involves gentle interaction with the sample, enabling high retention of biomolecular activity. For the separation of biomolecules in aqueous systems, SEC is referred to as gel-filtration chromatography (GFC), while the separation of organic polymers in non-aqueous systems is called gel permeation chromatography.

Gel filtration resins can be imagined as leads that contain pores of a defined size range. Large proteins which cannot enter these pores pass around the outside of the leads. Therefore, the volume of the column appears smaller to a large molecule. Smaller proteins that can enter the pores of the beads have a larger volume that they can explore, thus the volume of the column appears larger to a small molecule. Both large and small molecules experience the same flow rate of the mobile phase (*i.e.* L/m). Thus, a sample of proteins passing through a gel filtration column will separate depending on their molecular size, the big ones will elute first and the smallest ones will elute last (and 'middle' sized proteins will elute in the middle (Fig. 19.1).

The basic principle of size exclusion chromatography is quite simple. A column of gel particles or porous matrix is in equilibrium with a suitable mobile phase for the molecules to be separated. Large molecules are completely excluded from the pores and will pass through the space in between the gel particles or matrix and will come first in the effluent. Smaller molecules will get distributed in between the mobile phase of in and outside the molecular sieve and will then pass through the column

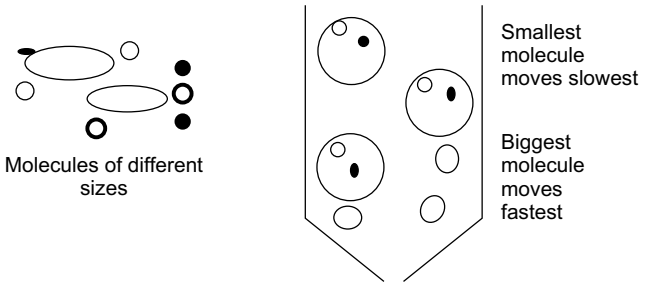


Fig. 19.1 Theory of size exclusion chromatography

at a slower rate, and hence appear later in effluent. There are two extremes in the separation profile of a gel filtration column. There is a critical molecular mass (large mass) that will be completely excluded from the gel filtration beads. All solutes in the sample which are equal to, or larger, than this critical size will behave identically, they will all be eluted in the excluded volume of the column. There is a critical molecular mass (small mass) that will be completely included within the pores of the gel filtration leads. All solutes in the sample which are equal to, or smaller, than this critical size will behave identically; they will all be eluted in the included volume of the column. Solutes between these two ranges of molecular mass will elute between the excluded and included volumes (Fig. 19.2). Thus while deciding a size exclusion matrix.

for protein purification, included and excluded range should be considered. For example, Sephadex G 75 matrix has a fractionation range 3–80, *i.e.* the matrix has included volume range 3 KDa and excluded volume range 80 KDa. (KDa stands for kilo Daltons 64 kg $mol^{-1} = 64$ KDa).

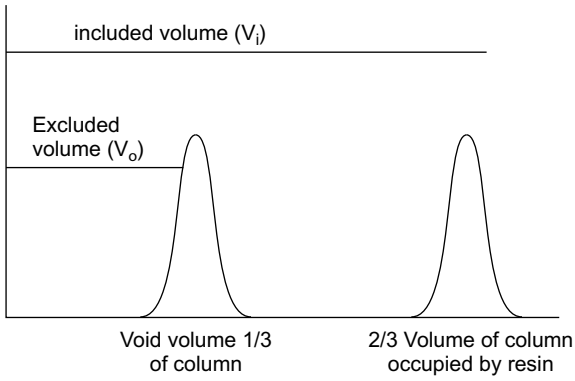


Fig. 19.2 Basic principle of SEC

19.2 Mechanism of Size Exclusion Chromatography

Size exclusion chromatography with reference to gel filtration chromatography is based on liquid–liquid partition chromatography in which the solute molecules get distributed in between two liquid phases. There is liquid in the gel pores and there is liquid outside the gel. The stearic exclusion mechanism can well explain the mechanism of the size exclusion phenomenon, with the availability of a range of sizes in solute, the small molecules enter the gel pores in large number whereas the large molecules will enter in small number of pores in which they can enter. This leads to different fractions of total pore volume which is accessible to molecules of different sizes. As the small molecules can enter in more pores while larger molecules can enter in fewer pores, the molecules with different sizes will differ in distribution coefficient between the two liquid phases.

If the total volume (V_t) of a column packed with a gel that has been swelled by the solvent is given by V_t, then it may be expressed as

$$V_t = V_g + V_i + V_0$$

where V_g is the volume occupied by the solid matrix of gel. V_i is the volume of solvent held in the pores or interstices and V_0 is the free volume outside the gel particles. When mixing or diffusion occurs, the diffusion equilibrium and the retention volume (V_R) of the given species is given by

$$V_R = V_{(\text{int})} + K_d V_{(\text{int})}$$

where distribution coefficient K_d is given by

$$K_d = \frac{V_{i(\text{acc})}}{V_{\text{total}}}$$

where $V_{i(\text{acc})}$ is the accessible pore volume. $V_{(\text{total})}$ is the total pore volume and $V_{(\text{int})}$ is the interstitial volume.

In the later proposed secondary exclusion mechanism, the small molecules in the sample will diffuse rapidly into the pores of the gel whereas the large molecules will find relatively few unoccupied pores and move further down the column till they find the unoccupied pores. This results in the enhancement of the separation of small and large molecules.

Size exclusion chromatography thus employs a stagnant liquid present in the pores of leads as the stationary phase, and a flowing liquid as the mobile phase. The mobile phase can therefore flow between the beads and also in and out of the pores in the beads.

Ideally, the separation of proteins and other compounds by SEC is based on the size of the analytes which allows a calibration curve derived from a set of known analytes. Typical calibration curves are based on proteins or polymers of known

molecular weight. By plotting log M V_s the retention volume, one typically obtains a third-order polynomial, with a linear region that provides the highest resolution and molecular weight accuracy. For example, Fig. 19.3 shows a typical calibration curve for various protein analytes.

The molecular weight range and the slope of the calibration curve are highly dependent on the pore size of the packing matrix. The largest proteins, which are excluded from the pores, elute first. Subsequent proteins elute in order of decreasing size. (Fig. 19.4)

Calibration curves with a steeply sloped portion are obtained using packing materials with large pores intended to measure larger molecules. Small pores give more gradual slopes. The lower the slope, the larger the difference in elution volume

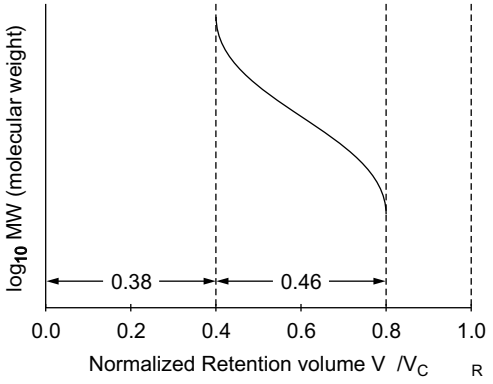


Fig. 19.3 Typical SEC calibration curve: SEC Analysis of Biotherapeutics and Aggregates

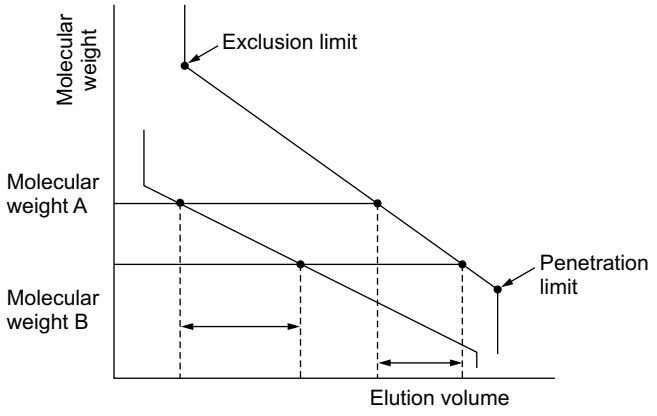


Fig. 19.4 Slope of calibration curve and Differences in elution volume

between small differences in molecular weight. Therefore, if two columns are available that can measure the target sample molecules, the one with the less sloped calibration curve will provide more accurate molecular weights.

19.3 Column Dimensions and Column Packings

A common approach to method development in any chromatographic method also includes the effect of varying column length and inner diameter. Increasing column length provides a means of improving resolution in isocratic separations as in SEC. Most SEC columns are 30 cm in length to provide optimum resolution, additional column length can be attained by linking multiple columns in series. Increasing the inner diameter of SEC columns can significantly improve peak capacity and resolution by minimizing the system contribution to band broadening. In cases where system dispersion is significant, 7.5 mm internal diameter. SEC columns may be required in order to maximize peak capacity. However, with the introduction of newer low dispersion, instrumentation such as (ultra HPLC), smaller width of columns (4.6 mm) can be used to achieve a comparable resolution to SE-HPLC.

Efficiency in SEC is greatly affected by the particle size of the chromatographic stationary phase. Smaller particles give better resolution than larger particles. Generally, particles in SEC are of two types: polymer beads and silica-based particles whose diameters fall in the range of 5–10 μm.

Improved resolution and higher efficiencies were observed for sub–2 μm SEC columns at higher flow rates resulting in shorter run times.

Among the two types of particles being commonly used, the silica particles have greater stability, rapid equilibration ability with a wide variety of solvents, and thermal stability. However, they have a tendency to retain solutes by adsorption and sometimes they also catalyze solute degradation.

Polymeric packings have also been commonly used, their best example is cross-linked polystyrene-divinyl benzene. Their pore sizes are regulated by the extent of cross-linking and are marketed in different pore sizes. Originally, styrene–divinylbenzene gels were hydrophobic and were suitable for gel permeation chromatography. However, the hydrophilic gels are sulfonated divinyl benzenes or polyacrylamides and are suitably used for gel filtration.

Apart from column and particle sizes, other optimizations involve mass load and volume load. Overloading the column deteriorates the resolution between the analytes.

19.4 Instrumentation

An SEC instrument consists of a pump to push the solvent through the instrument, an injection port to introduce the test sample onto the column, a column to hold the stationary phase, one or more detectors to detect the components as they leave the column, and software to control the different parts of the instrument and calculate and display the results.

The analyte is first dissolved in a solvent and is an important step in case of polymeric samples which coil up to form large spheres in solution. (Fig. 19.5)

The selection of solvent is critical because the solvent must not induce any other interaction between the sample and the stationary phase, so that the separation is solely on the basis of sample size. The solvent container should be made of clear glass or amber glass for solvents affected by sunlight.

Thermostatically controlled ovens help to maintain high temperatures, up to 220 °C which is necessary because at high temperatures the viscosities of the solvent are reduced. Lowering of solvent viscosity reduces column back pressure while enhancing the solubility and efficiency both.

Injectors introduce the polymer sample into the flowing solvent stream without interrupting the flow of the mobile phase. Injection volumes are generally in the range of 20–200 μL.

The pump takes the solvent and delivers it to the system at a constant, reproducible, and accurate flow rate with no pulses in the flow.

Detectors: For SEC analysis, UV continues to be the predominant mode of detection. Near UV or longer wavelengths give greater response for aromatic amino acids

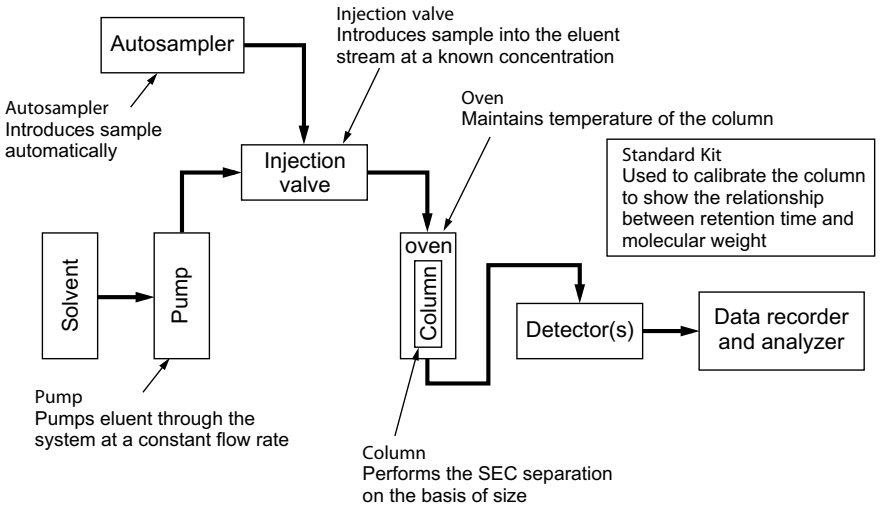


Fig. 19.5 The main components of SEC system

such as tryptophan and are commonly used for protein measurement. Higher sensitivity is provided by detection using for UV or low wavelengths (214 or 220 nm) where the amide peptide bond has a strong absorbance both wavelength ranges can be quantitatively inaccurate due to either scattering from particles at lower wavelengths or the presence of other chromophores absorbing at 280 nm. However, each wavelength range has its advantages. At lower wavelength improved sensitivity allows for analysis of samples of low concentration while higher wavelength provide a greater linear dynamic range. Some assays have also used fluorescence detectors for improved sensitivity and selectivity. Fluorescence detectors have been known to show sensitivity for recombinant human growth hormone.

19.5 Application of Size Exclusion Chromatography

The highlights of the application of size exclusion chromatography are as follows:

1. Purification
2. Desalting
3. Protein–ligand binding studies
4. Protein folding studies
5. Concentration of sample
6. Copolymerisation studies
7. Relative molecular mass determination.

Among the numerous fields where SEC techniques can be applied, the biopharmaceutical has maximum applications. The primary application of SEC in the biopharmaceutical industry is the routine monitoring of protein or modified protein such as protein-drug conjugates and the quaternary proteins. SEC is often used as a tool to aid in the manufacturing process and formulation development and is extensively used to guide the purification process for biopharmaceuticals.

19.6 Advantages and Disadvantages of Size Exclusion Chromatography

The advantages of SEC include good separation of large molecules from the small molecules with a minimal volume of eluate. Various solutions can be applied without interfering with the filtration process, while preserving the biological activity of the particles to be separated. The technique is generally combined with others that further separate molecules by other characteristics, such as acidity, basicity, charge, and affinity for certain compounds. With size exclusion chromatography, there are short and well-defined separation times and narrow bands, which lead to good sensitivity. There is also no sample loss because solutes do not interact with the stationary phase. The only disadvantage is that only a limited number of bands can be accommodated

because time scale of the chromatogram is short, and, in general there has to be a 10% difference in molecular mass to have a good resolution.

Molecular mass determination: Molecular mass determinations by gel filtration columns are done by measuring retention volume (V_R). Retention volume is the volume of the mobile phase required to elute a particular solute from the column. Each stationary phase has a range over which there is a logarithmic relation between molecular mass and retention volume. The molecular mass of unknown is measured by comparing its retention volume with those of standards. For proteins, eluents with high ionic strength, such as 0.05 M NaCl are preferred because it eliminates electrostatic adsorption of solute by occasional charged sites on the gel.

Unsolved Problems

1. Polystyrene standards of known molecular mass gave the following calibration data in a molecular exclusion column. Prepare a plot of log (molecular mass) versus retention time t_r and find the molecular mass of an unknown with a retention time of 13.00 min.

Molecular	Retention time, t_r (min)
8.50×10^6	9.28
3.04×10^6	10.07
1.03×10^6	10.88
3.30×10^5	11.67
1.56×10^5	12.14
6.60×10^4	12.74
2.85×10^4	13.38
9.20×10^3	14.20
3.25×10^3	14.96
?	13.00

2. How many types of size exclusion chromatography are there? Distinguish between them.
3. What is the basic principle of size exclusion chromatography?
4. State the mechanism of size exclusion chromatography.
5. How do column dimensions and column packings affect resolution by size exclusion chromatography?
6. List various factors governing the efficiency in SEC.
7. Discuss the advantages and disadvantages of SEC.
8. What are the highlights of the application of SEC?

Answers

1. 4.8×10^4

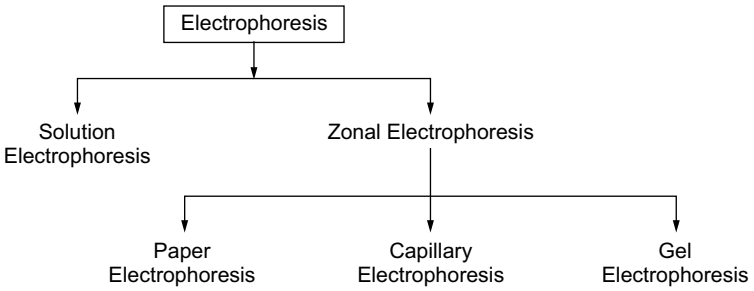
Chapter 20
Electrophoresis

20.1 Introduction

The process of migration of charged molecules through solutions in an applied electric field is known as Electrophoresis. Formally, the process is similar to sedimentation. In both cases a field, which is the gradient of a potential, produces the transport of matter. However, since electrophoresis depends on the charge of a macromolecule rather than its mass, it provides an additional tool for the analysis and separation of mixtures. Electrophoresis of positively charged particles cation) is called cataphoresis and of negatively charged particles (anions) is called anaphoresis. Depending upon, the nature of medium or the supporting matrix which enables the movement of charged molecules, electrophoresis is generally classified into different categories.

P. Malhotra, *Analytical Chemistry*,
https://doi.org/10.1007/978-3-031-26757-4_20

20.2 Types of Electrophoresis



Solution electrophoresis systems employ an aqueous buffer in the absence of a solid support medium. However, it suffers sample mixing due to diffusion of the charged molecules. To prevent this, stabilizing agents which stabilize the aqueous solutions in the electrophoresis cells are employed. To minimize the diffusional mixing of materials being separated during electrophoresis, soluble gradient electrophoresis systems use non-ionic solute, for *example,* sucrose or glycerol (Fig. 20.1).

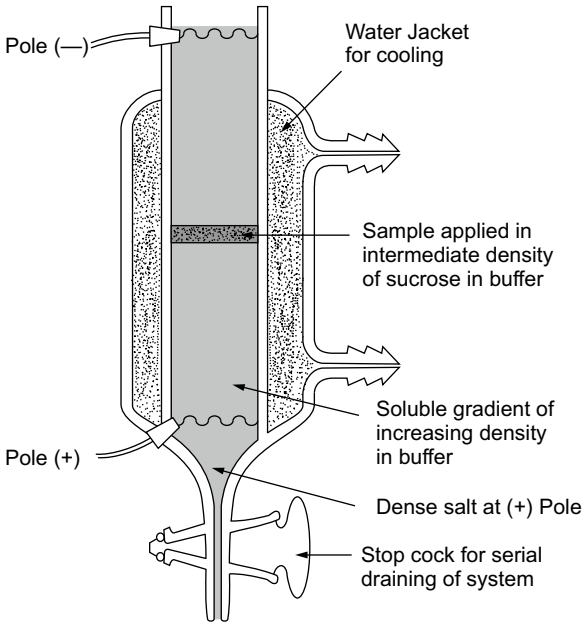


Fig. 20.1 A solution electrophoresis system

Zonal electrophoresis is most commonly employed technique in biochemistry in which the aqueous ionic solution is carried in a solid support and samples are applied as spots or bands of material.

Paper electrophoresis which uses cellulose acetate strip and cellulose nitrate strip, and gel electrophoresis are all examples of zonal electrophoresis systems. Such systems are most frequently used for analytical separations and not for preparation purposes (Fig. 20.2).

Paper electrophoresis is a commonly used electrophoretic method for the analysis and resolution of small molecules but not for resolving macromolecules (*e.g.* proteins) which get denatured by the surface tension. Two different methods are routinely used to apply samples to the electrophoretic paper: the dry application procedure and the wet application procedure.

In the dry application procedure, a sample of solutes dissolved in distilled water, or a volatile buffer, is applied as a small spot or thin stripe on the 'origin line' on the paper along with appropriate standard on other locations on the same line. After the solvent containing the samples has evaporated, the paper is dampened with the electrophoresis buffer, either by uniform spraying or dipping in it. In the wet application procedure, samples dissolved as concentrated solutions in distilled water are applied

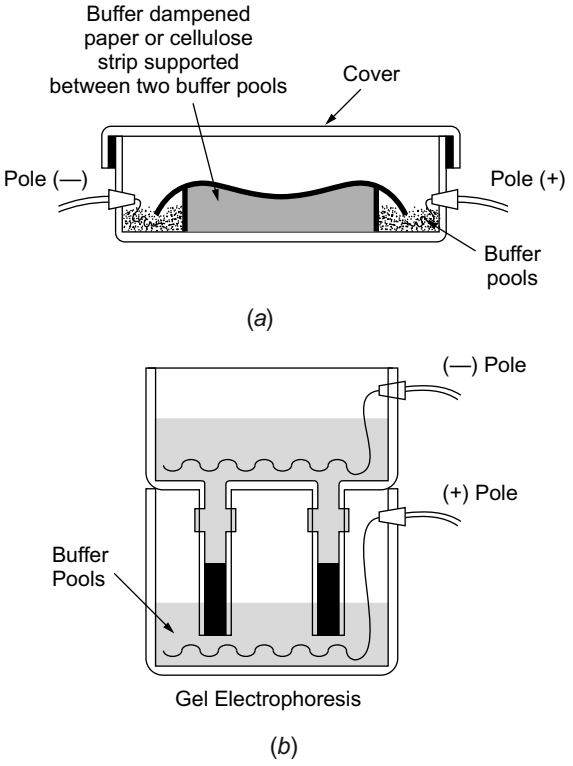


Fig. 20.2 ***a*** Paper or cellulose strip electrophoresis, ***b*** Gel electrophoresis

to paper predampened with electrophoresis buffer. The dry application procedure has the advantage of allowing small initial sample spots and better resolution of similarly mobile compounds whereas the wet application procedure usually yields larger spots and poorer resolution because of sample diffusion.

Capillary electrophoresis is a new method of analytical electrophoresis that is rapidly finding increasing application in biochemical research. In this method, the material to be analysed and the electrophoresis medium (a conducting liquid, usually aqueous) are placed in a long, fine-bore capillary tube, typically 50–100 cm long and 25–100 μm inside diameter. A very small sample (in nanoliter range) is placed at one end of the capillary and subjected to electrophoresis under fields up to 20 to 30 kV. Capillary electrophoresis offers the advantages of extremely high resolution, speed, and high sensitivity for the analysis of extremely small samples. It can be very useful for the separation of polynucleotides in various designs of DNA inspite of their minimal difference in size. Capillary electrophoresis can also be adapted to the separation of uncharged molecules by including charged micelles of a detergent in the aqueous electrophoresis medium.

Gel electrophoresis is a method of electrophoresis in which molecules are separated in aqueous buffers supported within a polymeric gel matrix. Gel electrolysis is quite distinct from other procedures of electrophoresis and its advantages are remarkable. They can accommodate larger samples than most electrophoresis systems and can also be used for preparative methods of macromolecules. Suitable alterations in the gel matrix can be done for a particular application. Low concentrations of matrix material or a low degree of cross-linking of the monomers in polymerized gel systems allow them to be used largely as a stabilizing or a medium to allow faster migration of macromolecules. On the other hand, higher concentrations of matrix composed of highly cross-linked monomers generate great friction leading to molecular sieving. Migration of solutes and their electrophoretic mobility are both affected by the viscosity and pore size of the gel matrix which together contribute to the molecular sieving. As a result, molecular weights of the macromolecules also start affecting their migration.

Many gel-like agents are used in electrophoretic systems. Agarose, which is a poly-galactose polymer, has proved to be a successful gel used for the separation of larger macromolecules such as nucleic acids, lipoproteins, and others. Another versatile gel is polyacrylamide gel which is used for the electrophoretic separation of proteins and nucleic acids.

20.3 General Principles

All types of electrophoresis are based on a single set of general principles which focus on the mobility of a molecule. It can be expressed as follows:

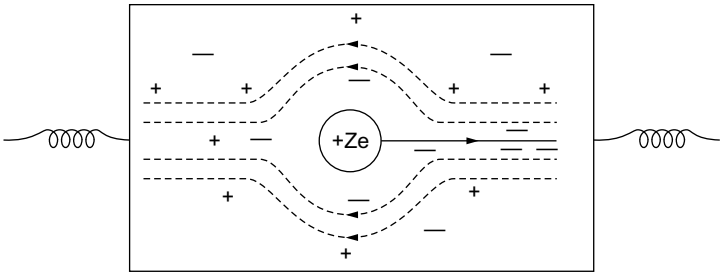


Fig. 20.3 Distortion of ion atmosphere by the field and macromolecule

$$\text{Mobility of a molecule} = \frac{\text{(applied voltage) (net charge on molecule)}}{\text{friction of the molecule}}$$

The mobility or rate of migration of a molecule increases with increased applied voltage and increased net charge on the molecule, whereas the mobility of a molecule decreases with increased molecular friction, or resistance to flow through the viscous medium, caused by molecular size and shape.

Electrophoresis is almost always carried out in aqueous solutions containing buffer ions and salts in addition to the macromolecule of interest. In a more realistic model, a macromolecule is subjected to an electric field in an aqueous salt solution. The small ions form an ion atmosphere around the macromolecule in which ions of opposite charge to that of the macromolecule predominate. The ion atmosphere is distorted by the field and by the motion of the macromolecule (Fig. 20.3).

The force experienced by a particle in an electric field is given by Coulomb's law:

$$F = zeE$$

where z is the number of electron units of charge ($e = 4.8 \times 10^{-10}$ *esu*) and E is the electric field. Then the velocity v is given by

$$f\mathrm{v} = zeE$$
$$u = \frac{\mathrm{v}}{E} = \frac{ze}{f}$$

For spherical particles, Stoke's Law may be applied to give the equation:

$$u = \frac{ze}{6\pi\eta R}$$

where R is the particle radius and η the solvent viscosity. In principle, this equation could be generalized to take into account hydration and deviations from spherical shape, as are done in the cases of sedimentation and diffusion.

20.4 Factors Affecting Electrophoresis

The various factors which influence the migration of molecules during electrophoresis are reflected by the following:

(a) **Charge**: The characteristics of electrophoresis greatly depend upon the net charge, size, and molecular mass of the migrating ions. The difference in charge-size ratio enables separation of solute molecules but for molecules having same charge-size (Q/R) ratio require sieving gels which are used as support matrix, *e.g.* agarose and polyacrylamide.

(b) **Nature of electrophoretic system**: For zonal electrophoresis, the kind of supporting material used depends on the type of molecules to be separated and the desired basis of separation, *i.e.* charge, molecular weight, or both. Almost all electrophoresis of biological macromolecules is presently carried out on either polyacrylamide or agarose gels. While applying gel electrophoresis the pore size of the gel molecules is quite important as it also depends on the size of the macromolecule to be separated. Pore size is also determined by the conditions of polymerization and can be easily altered by changing the monomer concentration.

(c) **The pH of the buffer**: Molecules like proteins, which are amphoteric molecules, can carry positive, negative, or zero net charges depending on the pH of their proximal environment. For every protein, there is a specific pH at which its net charge is zero. This pH is called isoelectric point or pI. For pH < pI, the protein is positively charged, and when pH > pI it will bear a negative charge. The pH of the buffer used greatly affects the separation of protein molecules of the same size and shape at a constant temperature.

(d) **Ionic concentration of buffer**: The electrophoretic migration of solute is of great importance and should not be hindered by the movement of any other ion especially those of the buffer solution. Therefore, the ionic concentration of the buffer solution should be low so that the ions do not interfere with the migration of solute ions.

(e) **Potential gradient**: The potential difference between the two electrodes highly affects the migration of ions as it is the main driving force. At constant current the movement of ions also depends upon the resistance of the medium as well as the solute molecules, their size, and molecular mass.

(f) **Temperature**: The movement of the ions is not directly affected by temperature. It is the viscosity of the medium which is affected by temperature and also its density that further regulates the migration of ions of the solute molecules. Maintaining a constant temperature is essential for electrophoresis.

20.5 Isoelectric Focussing

Isoelectric focussing is an electrophoretic technique that separates macromolecules on the basis of their isoelectric points, which is the pH at which they carry no net charge. A pH gradient is established in the polyacrylamide gel with the aid of ampholytes, which are small polymers containing random distributions of weakly acidic and weakly basic functional groups (*e.g.* carboxyls, imidazoles, amines, etc.). A polyacrylamide gel containing these ampholytes is connected to an electrophoresis apparatus that contains dilute acid solution (H^+) in the anode chamber and a dilute base (OH^-) solution in the cathode chamber, when voltage is applied to the system, the current flow will be due largely to migration of the charged ampholyte species present in the gel. The ampholytes migrate towards either pole. Ampholytes with lower isoelectric points (*e.g.* those that contain more carboxylic groups carrying a net negative charge) migrate to the anode and those with higher isoelectric points (*e.g.* those that contain more amine groups and have a net positive charge) migrate towards the cathode. This terminates in a situation where the ampholytes reach isoelectric pH and they stop migrating in the gel. After a sufficient period of electrophoresis, the ampholytes act as local buffers to maintain a stable pH gradient in the polyacrylamide gel. This pH gradient forms the basis for the separation of macromolecules that are present in the system (Fig. 20.4).

The proteins present in the system act like ampholytes which leads to generate pH = pI and reach a situation where they no longer migrate. This causes their separation and once the proteins and ampholytes no longer migrate the current (i) in the system decreases dramatically. Fairly low concentrations of ampholytes (2–4%) enable high voltage potential (150 V/cm^2) to be applied which in turn generates high current with heating effects. Since this method is designed to separate proteins on the basis of isoelectric point, the pore size of the gel should be large enough so that all of the macromolecules in the system can migrate freely to their approximate isoelectric points.

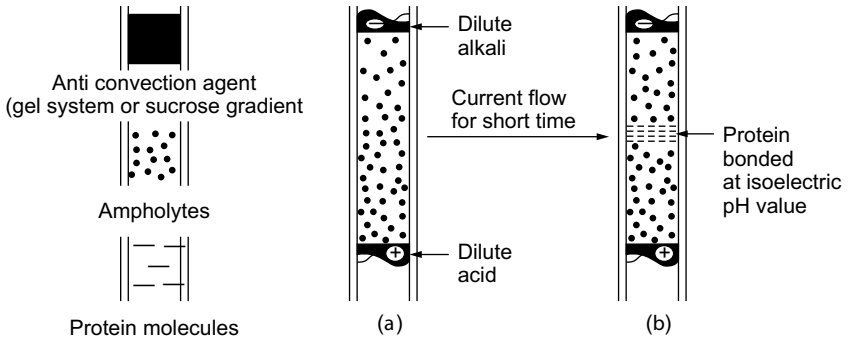


Fig. 20.4 Stepwise isoelectric focusing procedure

Agarose Gel Electrophoresis: It is the principle technique used to determine the size of high molecular weight nucleic acids (DNA and RNA). Agarose is a long polymer of galactose and 3, 6,-anhydrogalactose linked via 1 → 4 glycosidic bonds. It is isolated from sea weed and contain upon 100 monomeric units with an average molecular weight of around 10,000 Da (Dalton's unit for atomic mass). At room temperature, hydrogen bonding within and poly-galactose units in the solution will cause the formation of a rigid gel with a relatively uniform pore size. As with polyacrylamide gels, the pore size of the gel can be controlled by the percentage of the agarose dissolved in the solution.

The nucleic acid samples are loaded into the wells of the gel, along with a sample of DNA fragments of known molecular weight in one of the wells. The tracking dye, bromophenol blue added to DNA sample and is used to monitor the progress of the electrophoresis as it migrates at the same rate as DNA molecule having 500 base pairs. The nucleic acid samples are loaded into the wells of the gel, along with a sample of DNA fragments of known molecular weight in one of the wells. These standards are used to follow the electrophoretic separation and determination of the molecular weight of the unknown sample.

Moving Boundary Electrophoresis

Also known as free boundary electrophoresis, is electrophoresis in a free solution. It was developed by Arne Tiselius in 1937 for the separation of colloids particles through a stationary liquid under the influence of an electric field.

The apparatus used for free boundary electrophoresis includes a U-shaped cell filled with buffer solution and electrodes immersed at its ends. Analytes are detected in the free solution as discrete boundaries throughout the tube. Electrophoresis is then applied to hydrated gel supports (*e.g.* polycrylamide gel electrophoresis (PAGE) and later to micron-diameter capillary tubes (*i.e.* capillary electrophoresis or CE). The two primary electromotive forces in electrophoresis in a confined separation medium (*i.e.* tubular, capillary, or microfluidic channel) are electrophoretic mobility and electroosmotic flow (EOF) which arises when a surface charge is present along the separation column. In glass-based separations with solutions above pH > 2, the surface silanol groups exhibit net negative charges. Positively charged ions in the solution media, *i.e.* typically a buffered electrolyte solution are drawn toward the negative surface forming an electrical double layer that can be mobilized axially under an applied voltage along the length of the separation media. The mobile cations additionally cause movement of the bulk solution through viscous drag which is referred to as electroosmotic flow EOF.

SDA-PAGE System

For proteins, sodium dodecyl sulphate (SDS) is an anionic detergent applied to protein samples to linearize proteins and to impart a negative charge to linearized proteins. This procedure is called SDS-PAGE. The purpose of SDS-PAGE is to separate proteins according to their size and no other physical feature. It is a very common method for separating proteins by electrophoresis using discontinuous polyacrylamide gel as a support medium and sodium dodecyl sulphate (SDS) to denature

the proteins. The method SDS-PAGE is called sodiumdocyl sulphate polyacrylamide gel electrophoresis and is used to determine the number and size of protein chains or protein subunit chains in a protein preparation. SDS binds to all regions of the proteins and disrupts most noncovalent intermolecular and intramolecular protein interactions, thus causing unfolding or denaturation to yield highly anionic polypeptide chain.

SDS-PAGE is widely used in biochemistry forensics, genetics, molecular biology, and biotechnology to separate biological macromolecules, usually proteins or nucleic acids, according to their electrophoretic mobility as mobility is a function of the length, conformation, and charge of the molecule.

20.6 Applications of Electrophoresis

Electrophoresis is one of the widely used techniques in molecular biochemistry, microbiology, and also for disease diagnosis. It is one of the highly efficient techniques for analysis but it is slightly time consuming, expensive, and also needs technical skills. It is a both qualitative and quantitative analysis technique. Applications of electrophoresis include areas in forensics, molecular biology, genetics, microbiology, and biochemistry. Some of its applications can be discussed as follows:

Preparative electrophoresis has blossomed in recent years, however, their sample sizes are larger than analytical electrophoresis. Therefore, preparative electrophoresis systems require scaling up from the respective analytical systems and modifications to the instrumentation technique. The problem of thermal convection arising due to migration of ions can be overcome by using anticonvective media such as filter paper, agarose, starch, glass powder, or polyacrylamide. Preparative free-flowing electrophoresis has been specially designed at low temperatures (*e.g.* at 5 °C) so that degradation of heat sensitive samples is prevented.

Separation of biological products such as peptides, proteins, glycerol, lipoproteins, enzymes, nucleic acid, nucleotides, nucleosides have been frequently carried out using electrophoresis.

Biological activities of enzymes and other biological fluids can be traced by the use of electrophoresis technique. The high sensitivity of antibodies to agarose gel is well exploited for their separation from antigens by electrophoresis.

Separation of alkali metals or other metals in their cationic form is successfully performed by paper electrophoresis or gel electrophoresis.

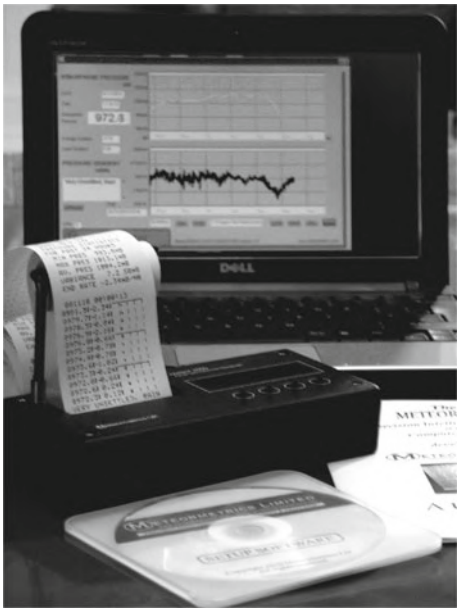
In identification and separation of metal complexes including metal chelates, capillary electrophoresis has been employed very frequently. Complexes of nickel, silver, and other transition metals have been separated by capillary electrophoresis separation of many.

Organic compounds such as aliphatic amines, dyes, hydrolyzed products of aminoacids, and nucleic acids are commonly achieved by the electrophoresis technique. In the separation of nucleic acid containing nucleotides or bases, simple paper electrophoresis or gel electrophoresis is used.

Electrophoretic deposition of graphene has been exploited to fabricate graphene and graphene-based nanostructures which have a wide range of applications in energy storage materials, field emission devices, supports for fuel cells, dye-sensitized solar cells, supercapacitors, and sensors. In general, electrophoretically deposited graphene layers show excellent properties, *e.g.* high electrical conductivity, large surface area, good thermal stability, high optical transparency, and robust mechanical strength.

1. Define electrophoresis. List the various types of electrophoresis.
2. Discuss Gel electrophoresis.
3. Explain (*a*) Paper electrophoresis (*b*) Capillary electrophoresis
4. What is the advantage of a narrow capillary in electrophoresis?
5. Predict which one of these: whether fumarate or maleate will have greater electrophoretic mobility and why?
6. How does electrophoretic mobility affect resolution by electrophoresis?
7. What are the main applications of electrophoresis?
8. Discuss the factors affecting the migration of molecules during electrophoresis.
9. Explain isoelectric focussing as a technique used in electrophoresis.
10. Write a short note on moving boundary electrophoresis.

Chapter 21
Role of Computers in Instrumental Methods of Analysis



21.1 Introduction

All types of chemical analysis engage some type of chemical instrumentation to obtain information from the substance being analysed. Microprocessors have grown to become essential components of nearly every type of modern instrumentation. Virtually all modern chemical instrumental analysis use devices operated by a

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computer interface. Most advanced analytical instruments are intimately controlled by computers and use digital computers for recording, interpreting, storing, and displaying of output signals. Data collected from the instrument is transferred to a suitable input medium and then programmed by the computer. After being processed by the computer, the final results appear on the computer.

The role of the computer and its interaction with any instrumental design generally follows four types of combinations:

(i) Off-line interaction
(ii) On-line interaction
(iii) In-line, interaction
(iv) Intra-line interaction

In an **off-line** arrangement the computer collects the data from the instrument and the data is processed by the computer in FORTRAN or BASIC languages and displayed at the output device (line printer) (Figs. 21.1 and 21.2).

An **on-line** computer is linked directly to instruments through an electronic interface so that there is rapid or instantaneous response from the computer. Since the series of computation, control functions, and information data analysis occur rapidly the instrument operations can be adjusted suitably to acquire accuracy in the results.

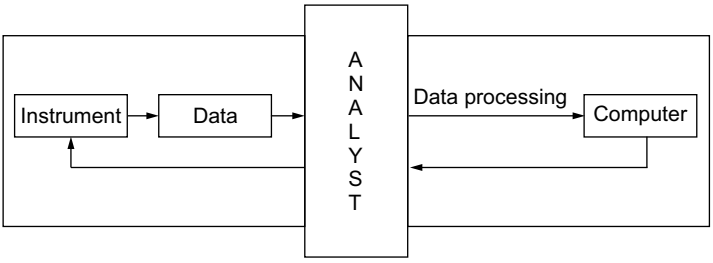


Fig. 21.1 An off-line arrangement of computer

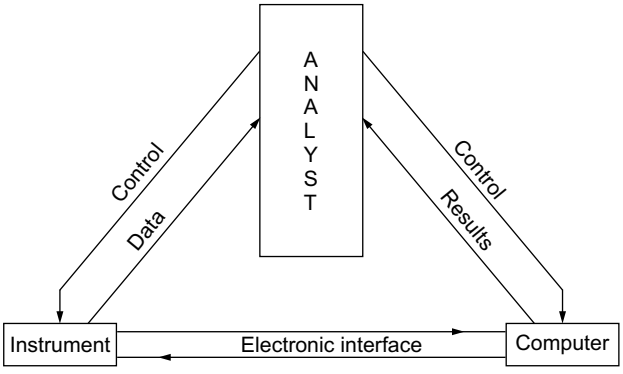


Fig. 21.2 An on-line arrangement of computer

For example, gas chromatography and spectroscopic results are more concise and accurate when there is on-line computer linking of the instruments. Its advantages can also be witnessed in high resolution mass spectrometry and Fourier transform Infrared spectroscopy. Almost instant effects of applying different window functions to an NMR spectrum are also seen commonly.

21.2 Role of Computers in Instrumentation

Recent advances in computing technology and related drops in the cost of computing capabilities have spawned a new generation of analytical instrumentation. The vast development in microprocessors (or microcomputers) and automation systems is changing the way an analyst works, thus simplifying a tedious and iterative task and rather allowing more creativity. Furthermore, automation permits to carry out new types of experiments with the acquisition of more data and controlling a large number of parameters or long time periods for completion. Thus, the computers play a significant role in the emerging instrumentation methods.

In-line microcomputers are present day demands for almost every analytical instrument especially in the competitive global economy. Microprocessor-based instrumentation systems are in great demand. In-line microcomputers which were earlier designed for chromatographs and spectrophotometers, not only handle data analysis but can even enable the instrument for repetitive performance with equal accuracy. The in-line microcomputers can be controlled by programmes stored in Read Only Memory (ROM) (Figs. 21.3, 21.4).

Intra-line assembly in any instrument emphasizes several microcomputers to be distributed within a single instrument so that various functions like instrument control, database management, spreadsheet analysis, and graphics display can be

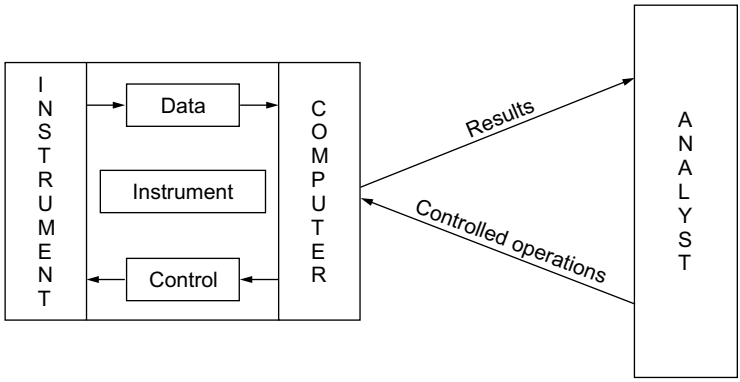


Fig. 21.3 An In-line arrangement of computer

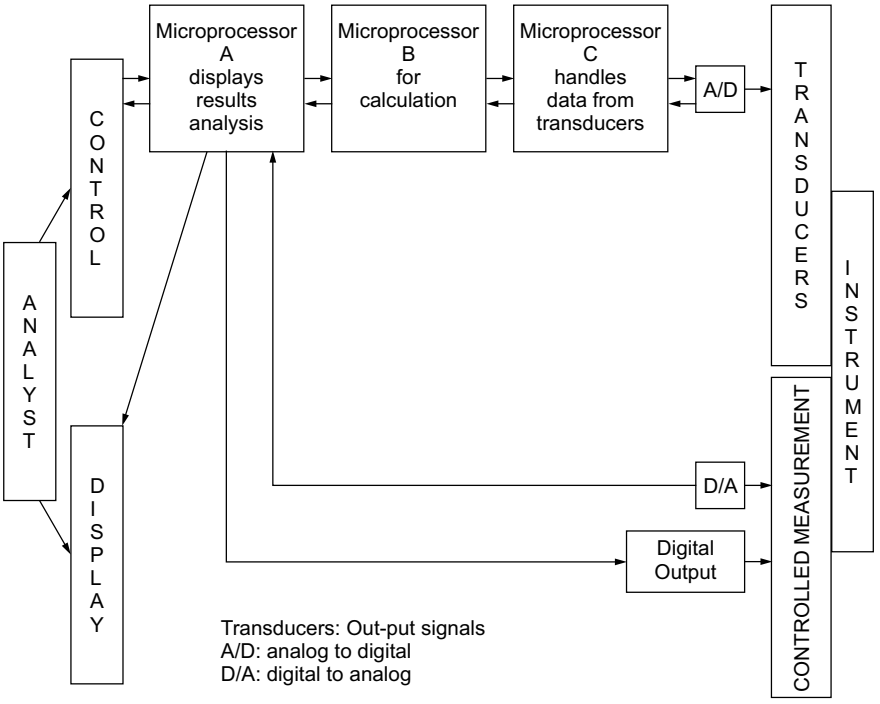


Fig. 21.4 Intra-line computer arrangement

performed simultaneously. The intra-line assembly is capable of handling input from control panels, performing calculations (e.g. Fourier transformation), controlling measurement parameters, and giving a digital output to the analyst.

21.3 Computer Basics

A computer is composed of hardware and software. Computer hardware includes all the electrical, mechanical, and the electronic parts of a computer whereas the functioning of the computer is based on its software.

Hardware of every computer comprises five basic functional units: the central processing unit, the arithmetic-logic unit, input, output, and memory.

Arithmetic logic unit performs logic operations on the basis of data provided to it. An Arithmetic Logic Unit (ALU) represents the fundamental building block of the Central Processing Unit (CPU) of a computer and is a major component of CPU.

Control Unit (CU) handles all processor control signals. It directs all input and output flow, fetches code for instructions from microprograms, and directs other

units and models by providing control and timing signals. It is considered as the processor brain of CPU because it issues orders to just about everything and ensures correct instruction execution.

Central Processing Unit (CPU) of a computer is a piece of hardware that carries out the instructions of a computer programme. It performs the basic, arithmetical, logical, and input/output operations of a computer system. It takes the output and writes it into the computer memory. Its functions can be described in four steps; Fetch, Decode, Execute, and Writeback. The critical parameters that are needed to evaluate CPU operation are the minimum time required to carry out specific types of instructions and the number of bits in the instructions, memory addresses, and data processed by the CPU.

Memory units are used by the computer to store information. It holds data and instructions for processing. There are two types of memory units: RAM, i.e. random access memory and ROM, *i.e.* read only memory. Its units are

$$\begin{aligned} 1 \text{ Bit} &= \text{Binary digit} \\ 8 \text{ Bits} &= 1 \text{ Byte} \\ 1024 \text{ Bytes} &= 1 \text{ KB} \\ 1024 \text{ KB} &= 1 \text{ MB} \\ 1024 \text{ MB} &= 1 \text{ GB}. \end{aligned}$$

RAM is volatile and ROM is non-volatile. Once a programme is entered into ROM, it may never be altered. An erasable, programmable ROM, EPROM may be erased and reprogrammed.

Input/Output Units provide the computer with its links to the outer world and are used to enter information and instructions into a computer for storage or processing and to deliver the processed data to a user. Input/output devices are required by the users to communicate and are also known as peripherals since they surround the CPU and memory of a computer system.

Buses, in computing, is a set of physical connections (cables, printed circuits, etc.) which can be shared by multiple hardware components in order to communicate with one another.

The purpose of Buses is to reduce the number of pathways needed for communication between the components, by carrying out all communications over a single data channel.

Buses are very essential to create an interface between the analytical instrument and the computer.

Microprocessors and Microcomputers are both generally the same as large computers. Microprocessors, also known as the Central Processing Unit (CPU), is the

brain of all computers and many household and electronic devices. Multiple microprocessors, working together, are the hearts of data centers, supercomputers, communications products, and other digital devices. It is a multipurpose programmable device that accepts digital data as inputs, processes it according to instruction stored in its memory, and provides the result as output. It has an internal memory in the form of sequential digital logic. A microcomputer is a computer with a microprocessor as its central processing unit. Earlier microcomputers were constructed from large-scale integrated chips, including the microprocessor chip.

21.4 Interfacing Instruments with Computers

With a perfect linking of computers with the analytical instruments, the complete process of analysis can be dictated by the analyst. The analyst computer interaction engages a suitable hardware along with peripheral devices. Main focus of a computerized instrument system is the interface between the computer's buses and the instrument's control and output signal lines. For an efficient interfacing the following characteristics are required:

(a) The interface should be capable of transmitting digital information in both directions: from the analyst to the computer and from the computer to the analyst.
(b) The analog data from the instrument when obtained should be instantly digitalized and displayed. The analog to digital convertors become an essential feature of such an interfacing.
(c) Each electronic component of a smart instrument should be linked to a communication bus (Fig. 21.5*a*).

Some important components of the interface include multiplexers, demultiplexers or decoders, digital to analog converter (DAC), and analog to digital converter (ADC).

Multiplexers: A multiplexer is a device allowing one or more low speed analog or digital input signals to be selected and functions as a multiple input, single output switch. It is a type of selector that allows the selection of inputs, so as to appear at the output. Time division multiplexing is often required as it is more efficient than frequency division multiplexing because it does not need Guardbands, selecting the transmission speed for the multiplexed circuit is based on a statistical analysis of the usage requirements of the circuits to be multiplexed. Multiplexers can also be used to connect communication lines to another multiplex and are generally used in pairs (Fig. 21.5b).

The demultiplexer or decoder is the reverse of multiplexer as its input and output functions are reversed. The decoders are used to accept data signals from the instrument and spread it on a communication or display device for the analyst (Fig. 21.5c).

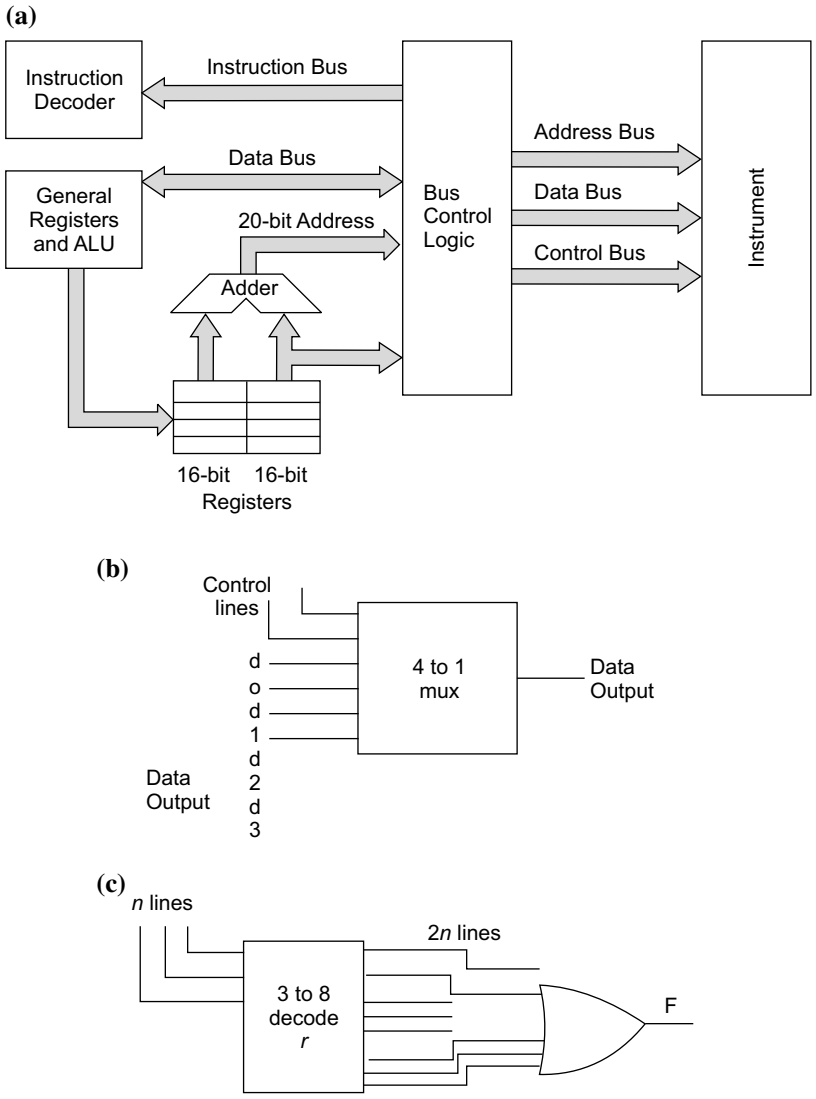


Fig. 21.5 **a** Communication bus, **b** Digital design of multiplexer, **c** Decoder (has n input lines and $2n$ output lines)

Digital to Analog Converter (DAC) involves transforming the computer's binary output in 0's and 1's which means that it converts a digital signal to an analog voltage or current output (Figs. 21.6, 21.7).

Analog to Digital Converters represent digital instrumentation as it converts the inputs from transducers to digital signals. Speed and accuracy of conversion is a critical a parameter in all ADC. The main component of an ADC is the voltage

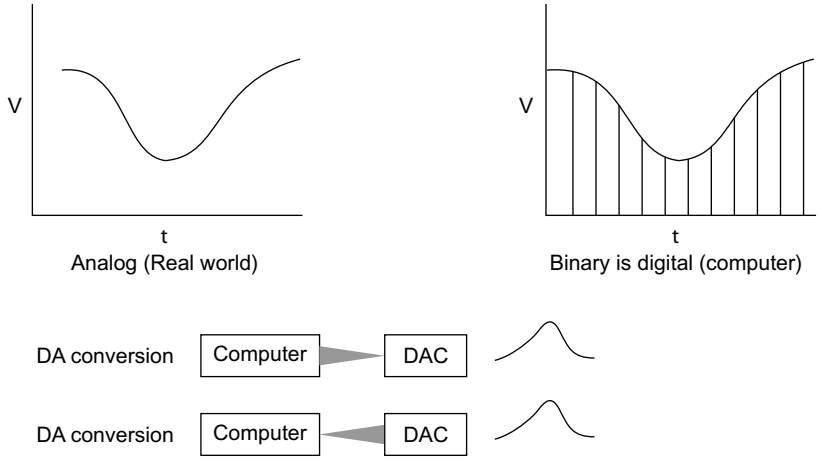


Fig. 21.6 Representation of analog and digital signals

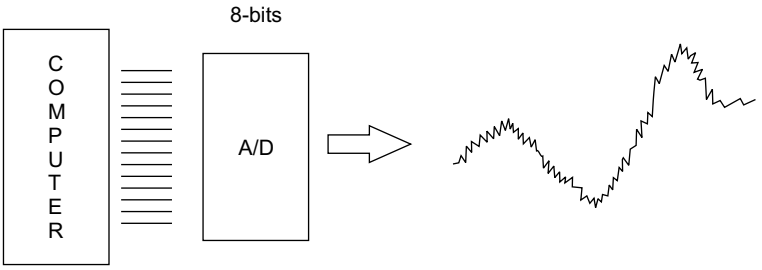


Fig. 21.7 Showing an Analog to digital conversion

comparator which needs to convert the analog voltage and compare it with a reference voltage. The comparator produces a digital output when the two voltages are equal.

Software : The functioning of the computer is not dependent on hardware alone. It requires a set of instructions that tells the computer what is to be done with the input data. In computer terminology, this set of instructions is called a programme, and one or more programmes is termed as software. Software used for computers may be treated as two different types (Fig. 21.8):

(a) Application Software
(b) System Software

Application software is a programme or group of programmes designed for end users, whereas system software consists of low-level programmes that interact with the computer at a very basic level. System software serves as the interface between a user, the application software, and the computer's hardware (Fig. 21.9).

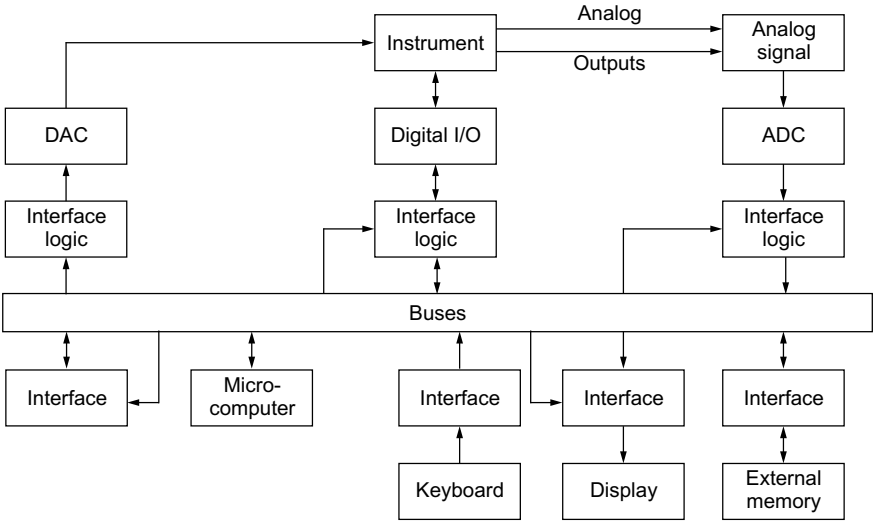
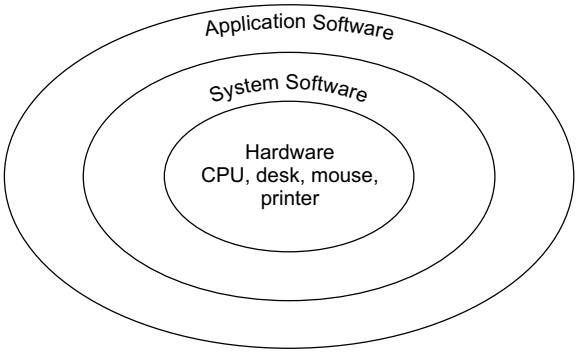


Fig. 21.8 Block diagram of computer in the instrument system

Fig. 21.9 Basic design of computer



Many commercial software are available for interfacing with various analytical instruments. Examples are ASYST from Data Translation, MEASURE from LOTUS, LABSOFT from Cyborg Corp, and ADLAB from Interactive Microware. However, an analytical chemist need not bother about software packages because automated Smart Instruments are freely available (Fig. 21.10).

Fig. 21.10 A computer interfaced potentiometer

21.5 Advantages of Microprocessors in Analytical Instruments

Since data acquisition is the target of every instrumental analysis, it is best facilitated by the combined use of microprocessors with almost every instrument. There is an increased reliability, improved accuracy, and easier maintenance as well as handling of the instrument when combined with microcomputers. The instruments get calibrated automatically and there is improved precision in the results due to the use of microprocessors. The digital signal processing enables the analyst to perform conveniently and run greater number of analysis in short duration. Analytical determinations by various technologies in conjunction with microcomputers show the following advantages:

(i) Automation of potentiometric titration with microprocessors has proved to be very useful and reliable. The data sampling and data acquisition from pH/mV meter to the computer enable convenient analysis in terms of accuracy and precision. The analog-to-digital conversion further enhances the number of experiments and brighter scope for all titrations.

For example, PC automated potentiometric titration of iron (11) sulphate by potassium dichromate solution allows the measurement of iron (11) concentration of 0.00015 M with a relative error of less than 2% error.

(ii) Conductometric titrations and the conductometric determinations of stability constants by computer simulation have led to great accuracy and enormous data collection and storage.

(iii) FTIR spectrometers use computers as an integral part of their system, required for carrying out Fourier transform. In FTIR spectrometers the output of the detector signal is transferred to a computer for mathematical calculations. They are also available in computer readable format for rapid searching and spectrum matching in IR and other spectroscopic techniques.

(iv) Microprocessors and/or computer control have taken much of the drudgery out of the data processing in *UV*–visible spectroscopy and have made sample handling more convenient. Customized softwares are available for logical measurements, 3D spectrum analysis, and performing the additional function in quantitative *UV*–visible measurements.

(v) Ensembling quantum computing with NMR spectroscopy enables an analyst to generate reliable structural determinations along with its three-dimensional structure. A novel PASCAL programme has been written to provide for the interactive analysis and display of the high resolution NMR spectra. Computer aided molecular modelling by NMR techniques has opened new frontiers in biomolecular studies.

(vi) A computer generated data system in atomic absorption spectroscopy converts the change in intensity into an absorbance-based interpretation and it can store information for many elements. In recent advanced instruments, the computer provides control over the instrument variables and can set variables to optimum conditions for a particular determination. With computer optimization programme even more sophisticated background correction and interference reduction technology has emerged. Automatic sample changer, diluter, and all other types of peripheral control by a central processor enhance the performance of an AAS. Modern microprocessor technology and stepper motors for wavelength drive have made possible fully automatic element change whenever simultaneous multielement determination and time-dependent absorbance changes are required.

(vii) A computer controlled scanning monochromator system has been specifically designed for the rapid, sequential determination of the elements. Such a monochromator is combined with an inductively coupled plasma excitation source so that elements at major, minor, trace, and ultratrace levels can be determined by their atomic emission spectra in sequence without changing excitation source parameters. Automation of the entire analytical cycle is particularly influential with ICP-AES.

(viii) The detector in HPLC is wired to the computer so that the chromatogram is displayed and the identification along with the quantification job is automatically done. The optimization of gradient elution separations has been widely supported by computer-simulation systems. In gas chromatography analysis also the integration of chromatographic peaks, the collection and processing of results, as well as the quality control tests involve the use of computer-aided techniques.

Unsolved Problems

1. How many types of combinations of computers and instruments are generally used?
2. Discuss the role of computers in instrumentation.
3. What are the main advantages of microprocessors in analytical instrument?
4. Give a block diagram of computer interfaced instrument.
5. Discuss the advantages when a computer interfaced potentiometer is used.
6. What are the functions of analog to digital and digital to analog converter in computer interfacing of instruments?
7. How many types of softwares are used for computers, explain?
8. How are multiplexers used in interfacing the instrument with computers?

National Ambient Air Quality Standards (NAAQS)

EPA has set National Ambient Air Quality Standards for six principal pollutants, which are called 'criteria' pollutants. They are listed below. Units of measure of the standards are parts per million (ppm) by volume, parts per billion (ppb) by volume, and micrograms per cubic meter of air ($\mu g/m^3$) (Table 1).

The US EPA currently lists 188 substances as hazardous air pollutants under the US Clean Air Act (1990). The HAPs are listed in Tables 2 and 3.

Table 1 Six principal pollutants

Pollutant [final rule cite]		Primary/secondary	Averaging time	Level
Carbon monoxide		Primary	8-h	9 ppm
			1-h	35 ppm
Lead		Primary and secondary	Rolling 3 month average	0.15 $\mu g/m^3$ (1)
Nitrogen dioxide		Primary Primary and secondary	1-h Annual	100 ppb 53 ppb (2)
Ozone		Primary and secondary	8-h	0.075 ppm (3)
Particle pollution	$PM_{2.5}$	Primary	Annual	12 $\mu g/m^3$
		Secondary	Annual	15 $\mu g/m^3$
		Primary and secondary	24-h	35 $\mu g/m^3$
	PM_{10}	Primary and secondary	24-h	150 $\mu g/m^3$
Sulfur Dioxide		Primary	1-h	75 ppb (4)
		Secondary	3-h	0.5 ppm

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Table 2 List of Hazardous air pollutants

1,2-Dibromo-3-chloropropane	Styrene oxide	Ethylene thiourea
1,3-Butadiene	Methylene chloride (Dichloromethane	Cadmium Compounds
1,3-Dichloropropene	Toluene	Dimethyl phthalate
1,4-Dichlorobenzene(p)	1,1,2,2-Tetrachloroethane	Formaldehyde
2,4-D, salts and esters	1,2-Propylenimine (2-Methyl aziridine)	2,4-Toluene diisocyanate
2-Acetylaminofluorene	Hydrogen sulfide	4-Nitrophenol
2-Chloroacetophenone	Phosgene	Triethylamine
3,3-Dichlorobenzidene	Tetrachloroethylene (Perchloroethylene)	Heptachlor
3,3-Dimethoxybenzidine	Ethyl chloride (chloroethane)	1,2-Diphenylhydrazine
3,3'-Dimethyl benzidine	Ethylene dichloride (1,2-Dichloroethane)	1,2-Epoxybutane
4-Aminobiphenyl	Methoxychlor	Hexamethylphosphoramide
Acetaldehyde	Hexane	4,4-Methylenedianiline
Acetamide	Hydrazine	Naphthalene
Acetonitrile	Hydrochloric acid	Nitrobenzene
Acetophenone	Hydrogen fluoride (Hydrofluoric acid)	4-Nitrobiphenyl
Acrolein	Hydroquinone	Hexachlorobenzene
Acrylamide	Isophorone	Hexachlorobutadiene
Acrylic acid	Lindane (all isomers)	Hexachlorocyclopentadiene
Acrylonitrile	Maleic anhydride	Hexachloroethane
Allyl chloride	Methanol	Hexamethylene-1,6-diisocyanate
Aniline	Methyl bromide (Bromomethane)	Selenium Compounds
Asbestos	Methyl chloroform (1,1,1-Trichloroethane)	Manganese compounds
Benzene (including benzene from gasoline)	Methyl ethyl ketone (2-butanone)	Mercury Compounds
Benzidine	Methyl hydrazine	Fine mineral fibers
Benzotrichloride	Methyl iodide (Iodoethane)	Nickel Compounds
Benzyle chloride	Methyl isobutyl ketone (Hexone)	**Polycyclic Organic Matter**
Biphenyl	Methyl isocyanate	Radionuclides (including radon)
Bis(2-ethylhexyl)phthalate (DEHP)	Methyl methacrylate	Arsenic compounds (inorganic including arsine)
Bis(chloromethyl)ether	Methyl tert butyl ether	Antimony Compounds
Caprolactam	2-Nitropropane	Cobalt Compounds

Table 3 List of elements with their detection limits (3 sigma) for particulate matter on the air filter using EDXRF

S. No	Name of the element	Symbol	Detection Limit in $\mu g/cm^2$
1	Sodium	Na	3.2599
2	Magnisium	Mg	1.5188
3	Aluminium	Al	0.0824
4	Silicon	Si	0.0509
5	Phosphorus	P	0.0068
6	Sulphur	S	0.0169
7	Chlorine	Cl	0.0152
8	Potassium	K	0.0048
9	Calcium	Ca	0.0037
10	Scandium	Sc	0.0031
11	Titanium	Ti	0.0047
12	Vanadium	V	0.0014
13	Chromium	Cr	0.0023
14	Manganese	Mn	0.0048
15	Iron	Fe	0.0061
16	Cobalt	Co	0.0018
17	Nickel	Ni	0.0022
18	Copper	Cu	0.0034
19	Zinc	Zn	0.0045
20	Gallium	Ga	0.0134
21	Germanium	Ge	0.0083
22	Arsenic	As	0.0012
23	Selenium	Se	0.0019
24	Bromine	Br	0.0048
25	Rubidium	Rb	0.0020
26	Strontium	Sr	0.0129
27	Yttrium	Y	0.0080
28	Molybdenum	Mo	1.0054
29	Rhodium	Rh	0.0219
30	Palladium	Pd	0.0227
31	Silver	Ag	0.0170
32	Cadmium	Cd	0.0258
33	Tin	Sn	0.0223
34	Antimony	Sb	0.0274

(continued)

Table 3 (continued)

S. No	Name of the element	Symbol	Detection Limit in $\mu g/cm^2$
35	Tellurium	Te	0.0297
36	Iodine	I	0.0161
37	Cesium	Cs	0.0395
38	Barium	Ba	0.0362
39	Lanthanum	La	0.0671
40	Tungsten	W	0.0514
41	Gold	Au	0.0173
42	Lead	Pb	0.0054

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