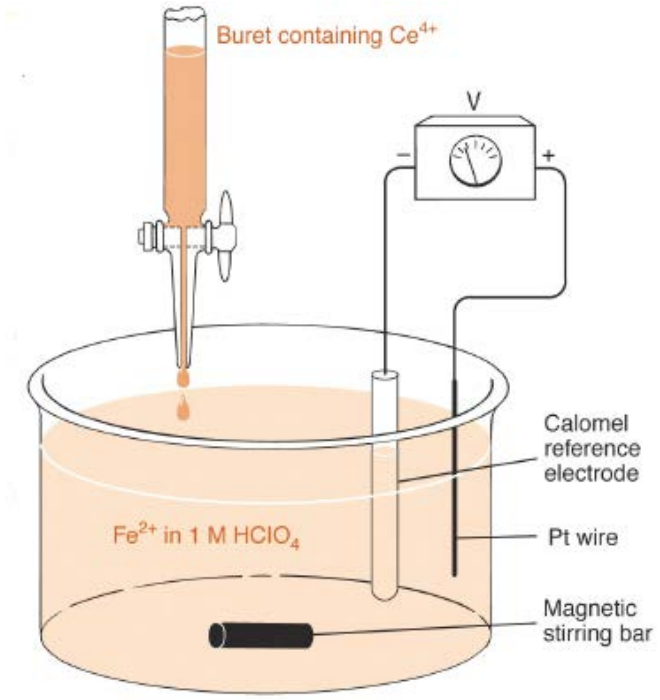
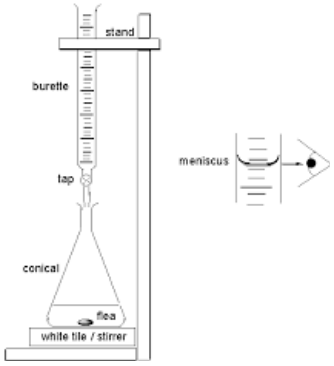
KARNATAKA STATE OPEN UNIVERSITY

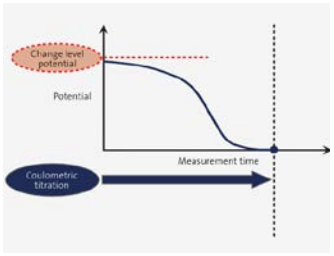
Mukthagangotri, Mysore – 570 006

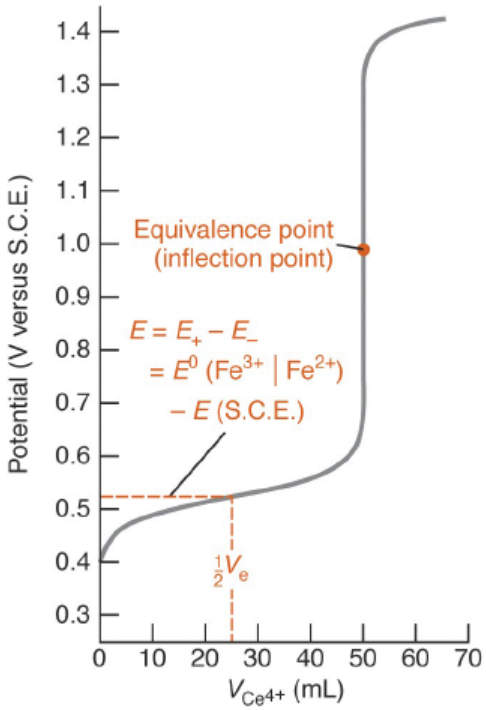
M.Sc. CHEMISTRY

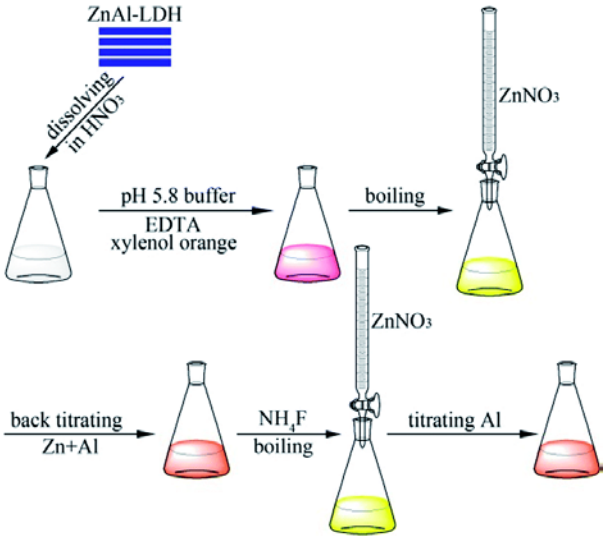
(SECOND SEMESTER)











Course: MCH T 2.4 **Block 1, 2,3 and 4**

ANALYTICAL CHEMISTRY

M.Sc. CHEMISTRY

SECOND SEMESTER

Course: MCHT 2.4

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COURSE INTRODUCTION

Analytical Chemistry is the branch of chemistry which implies to all experimental sciences. This branch guides us to develop and apply new methods and techniques to identify the nature and composition of matter. This is the basis of chemical analysis which is an art of recognizing different substances and determining their constituents. Chemical analysis involves various technologies, develops procedures, provides tools and makes use of variety of instruments and finally the interpretation of the results is obtained. So wherever chemical processes are employed; whether simple problems like analysis of elements and the compounds derived from them using chemical reactions, use of alternative methods of analysis, or the development of high profile instruments; the chemical analysis is indispensable.

It enables us to answer few basic problems like: What is happening in a chemical reaction? Will the course of reaction same every time or everywhere? What are the quantities of reagents required?

Various methods are used to identify the composition of a substance and to determine the exact amounts of the confirmed constituents. Thus the Chemical analysis is subdivided in two broader categories: Qualitative and Quantitative analysis. As the name indicates; Qualitative analysis deals with the identification of elements, ions or compounds present in a sample while Quantitative analysis deals with the determination of how much of one or more constituents is present. Basically it is the answer to two questions; what is it and how much is it? In general qualitative proceeds quantitative. Quantitative analysis mainly includes Gravimetric analysis and Volumetric analysis. In Gravimetric analysis, the substance to be determined which we call analyte, is converted into an insoluble compound using a suitable reagent, which is collected, dried and weighed. The weights of compounds obtained are then

correlated with the amount of substance originally present using stoichiometric equation. It is important that the formulae of the compound should be correctly known. Volumetric analysis is concerned with measurements by volumes. It can be a determination of the concentration of analyte, by volume, in a solution, as by titration which is also called Titrimetric Analysis b. determination of the volume of gases or changes in their volume during combination i.e., with measuring the volumes of gases evolved or absorbed in a chemical reaction.

Unit-1

Structure

1.0 Objectives of the unit

After studying this unit you are able to

- Explain the Classifications of errors
- Identify the characteristics of different errors
- Recognize the difference between indeterminant and determinant errors
- Explain the Gaussian distribution
- Explain the methods of minimising errors in analysis

1.1 Introduction

No other branch of science finds so many extensive applications as analytical chemistry purely for two reasons: Firstly, it finds numerous applications in various disciplines of chemistry such as inorganic, organic, physical and biochemistry and secondly it finds wide applications in other fields of related sciences such as environmental science, agricultural science, biomedical and clinical chemistry, solid state research and electronics, oceanography, forensic science and space research. It would be worthwhile to consider an example in each area of research e.g. in environmental science the monitoring of SO_2, CO, and CO_2, can be done by fluorescence or infrared spectroscopy while analysis of dissolved oxygen or chlorine from water can be carried out by potentiometry or colorimetry. The analysis of pesticides or insecticides from crops by gas chromatography or high performance liquid chromatography, or ascertaining the ratio of potassium to sodium in fertilisers by atomic absorption or flame emission methods. There are instances of the use of analytical chemistry in agricultural sciences. The analysis of micronutrients such as iron, copper, zinc, molybdenum, boron and manganese by spectrophotometry is another example. In the field of biomedical research and clinical chemistry, one can cite several examples like spectral analysis of barbiturates, food poisons, presence of vanadium and arsenic in hair and nails. The spectra of cobalt in vitamin B12, iron in haemoglobin of blood after their isolation by electrophoresis or gel permeation. In the field of electronics the analysis of traces of elements such as germanium in semiconductors and transistors, determination of selenium and caesium in photocells is quite possible by newer methods like spectroscopy or neutron activation analysis. In the field of oceanography, earth sciences and planetary sciences, analytical chemistry is extensively used. The chemical analysis of sea water; or analysis of basaltic rocks for presence of manganese and aluminum or the rapid analysis of elements from lunar samples would not have been possible without spectroscopy. An

array of examples can be cited in support of applications of analytical chemistry in various interdisciplinary areas. All these illustrations amply show that analytical science is truly interdisciplinary in nature.

1.2Error

Analytical science collects enormous amount of data. This data has no value unless one examines how much of it is useful and how much is reproducible. *An error is defined as the numerical difference between the measure value and the true value*. It is important to determine the reliability of a single measurement and the ability of repetitive measurement of the same dimension or property to agree with each other. No measurement is free from absolute error and is always subject to some uncertainty in the final value secured. We can express the measured value to a limited number of digits and the final limiting or significanr figure will reflect the reliability of the method of measurement. In other words the reliability of the result depends upon the magnitude of the difference between the average value and the true value.

The error, observed value and the true value in any analysis are related by an equation E = (O-T) where in E = absolute error, O = observed calue and T = truvalue.

Usually the error of a measurement is an inverse measure of accuracy of that measurement i.e., the smaller the error the greater the accuracy of an analysis. Errors are generally expressed relatively as

Percentage $\left(\frac{E}{T}X100\right)$ = % of error

Per thousand $\left(\frac{E}{T}X1000\right)$ = ppt

From the knowledge of error one can easily differentiate between the two terms generally used in analysis i.e., precision and accuracy. By precision of the measurement of a quantity is meant the reproducibility or the extent of agreement of individual values among themselves. The magnitude of differences between the individual values and the arithmetical mean is the measure of the precision of measurement. In other words the smaller the difference between individual values and the arithmetical mean value, the grater the precision. Secondly, it should be remembered that precise values may not always be accurate values because a more or less constant error can affect them all. Accurate results are those where the individual value matches the actual value of analysis.

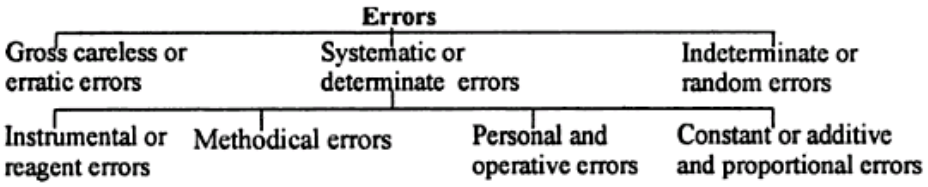
1.3 Classifications of errors

Errors which affect an experimental result or a set of measurement are classified in to three general groups based on the nature of their occurances

i) Gross careless errors or erratic errors

ii) Determinant or systematic 9constant or residual) errors

iii) Indeterminate or random (accidental) errors.



1.4 Gross careless errors or erratic errors

These are mistaken that are not likely to be repeated in similar determinations. Usually these errors are due to one or more of the following reasons.

1) Spilling a portion of the sample
2) Recording the wrong value for one of the weights used
3) Fluctuations in the balance (e.g. wind, shaking of the table, etc…)
4) Reading the rong volume of liquid in a burette
5) Simple mistakes in arithmetic computation- when the decimal points is misplaced in the recording
6) Failure to execute some essential step in the procedure.

This type of error is suspected when a series of careful measurements contain one result that differs makes markedly from the others. These errors are inexcusable and can be very damaging because they can trace by repeating the experiment. They lead to most conceivable large errors or untraceable errors. These errors are always in wide disagreement with a theoretical value or prior result.

Some steps to avoid these erratic errors are

1) Repeating the measurements at least thrice.
2) For excessively complicated measurements a checklist is prepared ahead of time
3) Making sure that label are clear and the scale are clean
4) Keep the experimental set-up as orderly and as uncomplicated as possible.

1.5 Systematic or determinant errors

A systematic error is one that cannot be reduced or eliminated by any number of repetitions of a measurement because it is inherent in the method, the instrumentation or occasionally in the interpretation of data. Determinate errors are those which possess indefinite values and follow no law and invisibly have fluctuating value.

A determinant error may have the same value under a variety of conditions and may remains constant from one measurement to another. Some characteristics of these errors are

- They can be ascribed to definite causes.
- They are unidirectional with respect to the true value.
- Determinate errors are often reproducible.
- They can be predicted by an experimental operator.
- A determinate error is a tangible one, its magnitude can be determined or approximated.
- Sources of these errors can be located or defined
- They occur with definite regularity.

Determinate errors are classified in to four types based on the source of origin of the error. They are 1) Instrumental errors and reagent errors

2) Methodical errors

3) Operative and personal error and

4) Constant and proportional errors.

1.6 Instrumental errors and reagent errors

These errors are primarily due to inaccuracy on the part on the instrument, equipment, glassware and impurities in the chemicals used for analysis. They could be due to

a) Uncertainty in reading an instrument scale or measuring system.
b) Uncalibrated or improperly calibrated and faulty balance weights.
c) Balance arms of unequal length.
d) Poorly or incorrectly graduated glassware, e.g., burette.
e) Impurities in reagent.
f) Poor selection of equipments.
g) Attack of reagents upon glassware and porcelain.
h) Loss of weight of platinum crucibles when strongly heated.
i) Failure to properly establish a “zero” reading of the instrument scale.

These systematic errors can never be detected from the results of a single series of measurements but only by comparing results of separate measurements using different instruments or methods. These errors can be more or less completely corrected by careful calibration of the instruments used.

1.7 Methodical errors

These are errors inherent in a quantitative measurement and are the most serious errors faced in a analysis. They are mostly physical in nature. Basically they originate from incorrect sampling and from incompleteness of reaction. Methodical errors are caused in the following ways

a) In a gravimetric analysis, an error arises owing to the solubility of precipitates, co-precipitations, decompositions, post-precipitations and volatilization of the weighing substance on ignition, etc.

For example, when Ca+2 ions are precipitated as calcium oxalate, washing the precipitate results in a loss of substance due to solubility, leading to an error in the net weight.

b) Simultaneous precipitation, co-precipitation or post-precipitation effects.
c) Leakage of material or of electricity in an electric circuit.
d) Presences of side reactions, auxiliary reactions and induced reactions.
e) Incomplete reaction leading to a failure to proceeds to quantitative completion is common in volumetric estimations.
f) Hydroscopic nature of the weighing substances, i.e., incomplete dehydration of a sample before weighing
g) Difference between the observed end point and the stoichiometric end point of a reaction.
h) Poor selection of desiccant and weight of hot crucible and contents.

These errors can be eliminated or reduced to a very small magnitude by the application of accurate (proper) techniques, because they are mostly hysical in nature.

1.8 Personal errors and operative errors

Personal errors occur very commonly and are always present from a small to an appreciable degree. Personal errors contribute to a high percentage among all kinds of errors. They result from subjective judgments or personal idiosyncrasies on the part of the observer or performer. They are not connected with the method or procedure, but are completely dependent on the individual analyst as they are not followed. These errors are nothing but a reflection of human personal errors. The causes of personal and operative errors are given below

a) Poor colour perception, i.e., inability to distinguish colour or colour blindness, leading to undertitration or overtitration of a solution (overstepping of the end point)
b) Consistent parallax error in reading scales or burettes.
c) Excessive reaction time in actuating a stop-watch.
d) Prejudice in the outcome of an experiment because of the tendency to constrain a result to agree with a preconceived situations, i.e., manipulative errors by the analyst. When the analyst is doubtful about the location of end point, he may be forced to stop the titration at a point that will agree with the previous titration or agree with most other titration values.
e) General lack of laboratory experience.
f) Uncovered beakers and the inadvertent introduction of foreign materials.
g) Spilling and loss of material by bumping during an evaporation process.
h) Over-and under washing during the filtration process.
i) Insufficient drying and ignition.

1.9 Constant or additive proportional errors

When the magnitude of the determinate error is nearly constant in a series of analysis, regardless of the size of the sample or independent of the amount to be determined, it is called a constant or additive error. The significance of constant errors generally decreases as the size of the sample increases.

For example, a constant end point error of 0.1 ml in a series of titrations shows a relative error of 10% for a sample requiring 1 ml of titrant, but only 0.2% if 50 ml of titrant is used.

The absolute value of a proportional error depends on the amount of a constituent used, i.e., the absolute value of the error varies with sample size in such a way that the relative error remains constant. Some examples of constant errors are

a) Errors in weights.
b) Loss of weight of a crucible in which a precipitate is ignited.
c) An impurity in a standard substance interferes in an analytical method, leading to incorrect normality.

For example the iodometric determination of chlorate affected by iodate or bromated impurities, leading to high results

d) Errors may vary with the size of the sample but not in a strictly linear fashion. The estimation of chloride by the use of chloride contaminated nitric acid is a constant volume addition

Systematic or determinate error are usually under the control of the analytical chemist. Generally determinate errors are recognized, corrected and eliminated by running a control experiment, i.e., with a known sample, by calibrating the weights anf glassware to the highest accuracy and by using the purest reagents. The more sound the fundamental chemical knowledge of an analytical chemist, the less likely he is to devise inherently erroneous methods. Careful training in laboratory techniques can eliminate faulty habits or better still, prevent their development. If the determinate error is not controlled, it may lead to an indeterminate error.

1.10 Indeterminate errors (Accidental errors or random errors)

Indeterminate errors manifest themselves by the slight variations that in successive measurement made by the same observe with the greatest care, under as nearly identical conditions as possible. Such errors cannot be attributed to any known cause, nor can they be predicted with regard to magnitude or direction for any single measurement in a series of measurements or for a single measurement standing alone. They can never be completely eliminated with exactitude. They are difficult to assign or define and the operator has little control over them. These errors are commonly due to

a) Limitations of observation.
b) Inherent limitations in the equipment.
c) Lack of care in making the measurement

Some examples are

j) Noise and drift in an electric current.
k) Vibrations in a building caused by moving traffic.
l) Temperature variations.
m) Inability to the eye to detect slight changes in a read-hot device.

The incidents are isolated, random and accidental caused by unpredictable and imperceptible factors. A reduction in the number of manipulative operations decreases the possibility of accident errors

Methodic errors are inherent in the method applied and are the most serious of analytical errors. Such errors have their sources in the chemical properties of the system. Various types of errors

arise in precipitation of aluminium as aluminium hydroxide due to the presence of zirconium, titanium and iron in the solution.

a) Operative errors are those which must be blamed on the analyst and on the method or procedure. There are usually personal errors caused by a variety of reasons such as a colour blindness, incorrect handling of instruments etc..
b) Instrumental errors usually originate in the instrumental itself. They arise from the effect of environmental factors on the instruments e.g., zero error in any reading instrument. Such errors can be minimized by calibration or use of proper blank in cases like spectrometric analysis.
c) Additive and proportional determinate are other types of errors wherein the absolute value of an additive error is independent of the amount of constituent present in a determination, while the absolute value of a proportionate error depends upon the amount of impurity in a sample.

Indeterminate errors are those which either arise from variation in determinate errors or those which are erratic in occurrence. Those belonging to the first kind occur when the analyst cannot control conditions to exclude a determinate error or prevent it varying e.g. in gravimetric analysis of aluminium, aluminiumoxide shows high weight due to the presence of water vapour and can be easily controlled whereas he analyst has no control over the erractic or random type of errors.

1.11 Gaussian distribution

The Gaussian distribution is also referred to as the normal distribution or the bell curve distribution for its bell-shaped density curve. The Gaussian distribution is also the most popularly used distribution model in the field of pattern recognition.

1.12 Standard deviation(S)

It is commonly used as a measure of precision. It is more reliable than mean of average deviation. “Standard deviation is defined as the square root of the sum of the squares of the deviations divided by the degree of freedom for the set of measurements.

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

Where x_i = is the individual measurements,

$\bar{x}$ is the mean or average of measurements and (n-1)= degree of freedom for set of n measurements.

Relative Standard deviation(Coefficient of Variation). It is defined as the standard deviation(S) divided by the mean ($\bar{x}$) of the set.

Thus, relative standard deviation = $= \frac{S}{\bar{x}} \times 100\ ppt$ it can also be expressed in terms of percent and then it is called coefficient of variation, thus

Coefficient of Variation $= \frac{S\tan dard\ deviation}{Mean\ deviation} \times 100$

1.13 Methods of minimising errors

Some methods for the identification and compensation of method error are:

(a) Analysis of standard samples. It involves in conducting analysis of a standard sample prepared in such a way that its composition is exactly the same as that of the material under examination. We know that standard materials containing carefully analysed constituents are available from national bureau of standards. Thus the weight of constituent x in an unknown sample can be calculated by using the relation.

$$\frac{\mathrm{Re}sult\ observed\ \ for\ unknown}{\mathrm{Re}sult\ obtained\ \ for\ s\tan dard} = \frac{x}{Weight\ of\ \ constituent\ in\ s\tan dards}$$

(b) Independent Analysis. It means an entirely different method from the one under study for example the strength of HCl in solution can be determined.

(i) By titration with a standard solution of strong base and

(ii) By precipitation with $AgNO_3$ and weighing AgCl

If the result obtained by both the methods is in good agreement, we say that values are correct within limits. Independent analysis helps in verifying the results.

(c) Running a blank determination. It involves in conducting a blank determination also without a particular sample under similar conditions. It is quit useful for exposing method errors due to contaminations of the reagents and the vessels used the experiment.

1.14 Solved problems

1. The result of an analysis are 26.78% compared to the accepted value 27.0%. what is the relative error in parts per thousand

Solution

Experimental value=26.78%

Accepted value=27.01%

Absolute error = 26.78-27.01=0.23%

Relative error $= -\frac{0.23}{27.01} \times 1000 = 8.52$ *parts per thousand*

2. A chemist analysed the alcoholic content in beer and obtained the following percentage values of the alcoholic content. 5.12, 5.22, 5.25, 5.18, 5.20 calculate mean and median

Solution

The mean value $= \frac{5.12 + 5.22 + 5.25 + 5.18 + 5.20}{5} = \frac{25.97}{5} = 5.19$

Now arranging the values in order of increasing magnitude, we have 5.12, 5.18, 5.20, 5.22, 5.25,

Therefore the median is the middle value i.e 5.20 because there are five i.e odd number of values in the set.

3. In an analytical determination involving six measurements the standard deviation is 0.65 ppm. The mean of the measurement is 20.25 ppm. Calculate the relative standard deviation and the co efficient of variation.

Solution

from the given data we write

$$\text{Re}\,lative\,s\tan dard = \frac{S}{x} \times 1000\ ppt = \frac{0.65}{20.25} \times 1000 = 32.09\ ppt$$

$$Also\,coefficient\,of\ \text{var}\,iation = \frac{S}{x} \times 1000 = \frac{0.65}{20.25} \times 100 = 3.209\%$$

4. Apply the value of the standard deviation obtained from the data given in the example 2.2 to find the 95% confidence interval of the mean (here n=no of observations=6, mean = $\overline{x}$ =19.94)

Standard deviation = S =0.046

For 95% confidence level 't' from table 2.1=2.571

Therefore value $\mu = \bar{x} \pm \frac{tS}{\sqrt{n}} = 19.94 \pm \frac{2.571 \times 0.046}{\sqrt{6}} = 19.94 \pm 0.0482$.

5. Calculate to the appropriate number of significant numbers.

(i) $\frac{3.24 \times 0.08666}{5.006}$, (ii) $0.58 + 324.65$ (iii) $943 \times 0.0345 + 101$

Solution

$$\frac{3.24 \times 0.08666}{5.006} = \frac{0.28077}{5.006} = 0.05608$$

The number of significant figure the answer is four. However the number of significant figures in the least precise value(3.24) is only three. Therefore the correct answer after rounding off is 0.0561

$$0.58 + 324.65 = 325.23$$

The number of decimal places in both the numbers is two. Therefore answer should also have two decimal places. Thus the correct answer is 325.23.

$$943 \times 0.0345 + 101 = 3.25335 + 101 = 104.25335$$

Since 101 contains only number up to decimal place the correct answer is 104

Uncertainties in results involving addition and subtraction

The maximum uncertainty in a result involving addition and substraction is obtained by adding the absolute values of uncertainties in various measurements. For example if the absolute uncertainties in the two data are ± 0.1 and ± 0.3 then the uncertainty in the summation of tha data would be ±(0.1+0.3)=±0.4.

6. Calculate the maximum uncertainty in the following expression: (70.2±0.1) ml + (12.3±0.2) ml.

Solution

The result is obtained by adding up the uncertainties

70.2±0.1

-8.5±0.2

12.3±0.2

74.0±0.5 it may be noted that uncertainties are always added

Uncertainty in result involving Multiplication and Division. The maximum uncertainty in result involving multiplication and division is obtained by adopting the following procedure.

(i) Calculate the percentage uncertainties from the given data

(ii) Add up these percentage uncertainties.

(iii) Convert the percentage uncertainty to cetanity.

7. Calculate the maximum uncertainty in the result of (25.6±0.2) (19.8±0.04)

Solution

(i) (25.6±0.2) (19.8±0.04)= 506.88±?

(ii) Now percentage uncertainties of the two multipliers are:

$$\pm\frac{0.2}{25.6}\times 100 = \pm 0.78 \quad and \pm\frac{0.04}{19.8}\times 100 = \pm 0.20$$

(iii) Summation of percentage uncertainties = ±0.78±0.20=±0.98.

Thus the result is written as : 506.88±0.98%

$\therefore$ uncertainty of the result = $\pm\frac{0.98}{112}\times 506.88 = \pm 4.43$

The required result = 506.88±4.43.

8. The concentration of iron in a sample was found to be (i) 20.17 ppm and (ii) 19.80 ppm in two separate determinations. Taking the accepted value as 20.02 ppm, calculate the absolute error as well as the relative error as percent and also as parts per thousand in the two determinations.

Solution

$(i)\ Measured\ value(x_1) = 20.17\ ppm,\ \ The\ value(x_t) = 20.01\ ppm.$

$\therefore Absolute\ error\ (E) = x_1 - x_t = 20.17 - 20.02 = 0.15\ ppm$

$$Also\ \text{Re}lative\ error\ (E_r) = \frac{0.15}{20.02}\times 100 = 0.749\%$$

$$= \frac{0.15}{20.02}\times 1000 = 7.49\ ppt$$

$(ii)\ Here\ x_1 = 19.8\ ppm \quad x_t = 20.02$

$\therefore Absolute\ error = 19.80 - 20.02 = -0.22\ ppm$

$$\text{Re}lative\ \ error = \frac{-0.22\times 100}{20.02} = -1.09\%$$

$$= \frac{-0.22\times 100}{20.02} = -10.9\ ppt$$

9. Calculate the mean deviation and relative mean deviation of a set of analytical data 16.72, 16.74 and 17.10

Solution

$$\text{Measured mean value}(x_0) = \frac{16.72 + 16.74 + 17.10}{3} = 16.85$$

The deviation are given as :

x_i	$x_i - \bar{x}$
16.72	0.13
16.74	0.11
17.10	0.25

$$\text{Mean deviation} = \frac{0.13 + 0.11 + 0.25}{3} = 0.16$$

$$\therefore \text{Relative Mean deviation} = \frac{0.16}{16.85} = 1000 = 9.49\ ppt$$

1.15 Methods of minimising errors

Some methods for the identification and compensation of method error are:

1) Analysis of standard samples. It involves in conducting analysis of a standard sample prepared in such a way that its composition is exactly the same as that of the material under examination. We know that standard materials containing carefully analysed constituents are available from national bureau of standards. Thus the weight of constituent x in an unknown sample can be calculated by using the relation.

$$\frac{\text{Re}\,sult\ observed\ \ for\ unknown}{\text{Re}\,sult\ obtained\ \ for\ s\,\text{tan}\,dard} = \frac{x}{Weight\ of\ \ constituent\ in\ s\,\text{tan}\,dards}$$

2) Independent Analysis. It means an entirely different method from the one under study for example the strength of HCl in solution can be determined.

i) By titration with a standard solution of strong base and

ii) By precipitation with $AgNO_3$ and weighing AgCl

If the result obtained by both the methods is in good agreement, we say that values are correct within limits. Independent analysis helps in verifying the results.

3) Running a blank determination. It involves in conducting a blank determination also without a particular sample under similar conditions. It is quit useful for exposing method errors due to contaminations of the reagents and the vessels used the experiment.

1.16 Summary of the unit

Error affects the accuracy of results and mainly there are two types of errors they are

(a) Determinate errors

(b) Indeterminate error.

Determinate errors are determinable and that presumably can either be avoided or corrected. Instrumental errors are common to all the instruments as each has a limited accuracy. The manufacture of the instrument generally provides the tables for the reliability of results in the respective ranges. It should be kept in mind that the calibration of the instrument in one range may not be valid for the entire range. Inherent error arises when a theory or a method not well suited to the problem is applied to arrive at a result. For example the applications of an ideal gas law, PV= nRT for a vapour state is approximately only leading to incorporation of inherent error in the result of the experiment.

Operative error is the personal errors which are introduced when an instrument is operated. These can be reduced by experience operations in which these errors occurs include transfer of solutions, effervescence, and bumping during sample dissolution, incomplete drying of samples. Some personal errors are difficult to be removed or corrected like colour blindness which is due to incorrect judgment of colour.

Most of the errors which are discussed above can be minimized or corrected but errors that are inherent in the method cannot be changed unless the conditions of the determination are changed. It is known that Kjeldahl's method is employed for the determination of nitrogen and this method may not give consistent results in certain cases. The reason is that in some organic nitrogen compounds. The digestion with concentrated sulphuric acid may not completely convert the ring nitrogen to ammonium sulphate. This is particularly true in case of pyridine compounds in which the result of nitrogen determination is low. Some other errors include co precipitation of impurities side reaction, slight solubility of precipitate, impure reagents etc. in certain cases we are forced to accept a given method in the absence of better one.

The absolute value of such an error depends upon the size of the sample e.g during iodometric determination of iodate, the presence of a chlorate or a bromate in the sample would increase the result if sample taken has large quantity i.e increase the amount of impurity present in sample would increase the magnitude of a result but not in a strictly linear fashion.

Indeterminate errors are generally accidental or random are indeterminate errors. These are revealed by small difference in successive measurement made by the same analyst under virtually identical conditions. Such errors cannot be determined. The accidental error follow a random distribution, thus a mathematical law of probability can be applied to arrive at some conclusions regarding the most probable result of a series of measurement. As an example, the

analyst reads incorrectly the instrument panel reading in pH meter or spectrophotometer. He notes doen this reading which get multiplied during calculations for the final results,. Such an error is random such an error is high in gravimetric determination because it includes many operations such as filtration, washing ignition, precipitation.

1.17 Key words

Erratic errors; Determinant errors; Instrumental errors; Methodical errors; Personal errors; Additive proportional errors; Indeterminate errors; Standard deviation(S).

1.18 References for further study

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1.19 Questions for self understanding

1) What is error in analysis?
2) How errors are classified? Mention different types of errors.
3) What are eractic errors? Briefly explain
4) What is determinant error?
5) Discuss instrumental errors and reagent errors
6) What are Methodical errors?
7) What is a Personal error?
8) What are additive errors?
9) Explain Gaussian distribution
10) What is Standard deviation(S)?
11) Discuss methods of minimising errors

Unit-2

Structure

2.0 Objectives of unit

After studying this unit you are able to

- Explain the difference between Accuracy and Precision
- Explain the methods of determining the accuracy of analysis
- Explain the methods of improving the accuracy of analysis
- Explain the methods of expressing analysis
- Explain the mean deviation
- Explain the criteria of rejection of data
- Explain the Q-test, F-test and t-test
- Explain the Confidence Limit and confidence level in analysis

2.1 Introduction

We often tend to use the words 'precise' and 'accurate' to mean roughly the same thing. Look the words up in a thesaurus and they both equate to truth or exactness. However, in measurements and statistics, the two words have different meanings, so that we can be precise without being accurate or accurate without being precise.

One of the basic activities in statistics is assessing whether the answer derived from a small number of observations (a sample) is representative of the much larger number of entities (the population) from which the sample has been drawn. It is most unlikely that either the sample or the population will be uniform – each will have an average value (typically a mean) and measurements will vary around this mean. The average and variability define precision and accuracy.

Accuracy describes the nearness of a measurement to the standard or true value, i.e., a highly accurate measuring device will provide measurements very close to the standard, true or known values. Example: in target shooting a high score indicates the nearness to the bull's eye and is a measure of the shooter's accuracy.

Precision is the degree to which several measurements provide answers very close to each other. It is an indicator of the scatter in the data. In many cases, when precision is high and accuracy is low, the fault can lie with the instrument. If a balance or a thermometer is not working correctly, they might consistently give inaccurate answers, resulting in high precision and low accuracy.

2.2 Accuracy

Accuracy is the closeness of an experimental measurement or result to be the true or accepted value. Precision is the closeness of agreement between replicated measurements or results obtained under the same prescribed conditions.

Since correct value is often not known in a chemical analysis (due to limitations of instrument and skill of observer) the errors of measurement which affect the result are estimated. Confidence limits are then set on the experimental values. Accuracy of data is then determined by the agreement between the experimental and the correct value because accuracy is the degree of agreement between accepted true value and measured value. *The inverse of accuracy is called error. Therefore the magnitude of the error in the result measures its accuracy. The smaller the error the greater the accuracy.*

2.3 Methods of expressing accuracy

The precision measurements are not necessarily accurate. The accuracy is expressed in terms of the error called absolute error. It is given by the relation.

Absolute error = Experimentally determined value-Accepted or more probable value.

It is usually reported in the same units as the measurements. If 5.93 gram sample of a material is analysed as 5.83 grm. The absolute error is -0.10 grms. The error is known as mean error in case the measured value is the average of several readings of measurement. The mean value can be determined by taking the average difference, with regarded to sign of the individual test results from the actual value.

Consider that 1% age of water of crystallization in $BaCl_2.2H_2O$ obtained theoretically

$$= \frac{36}{244} \times 100 = 14.75$$

Now if the experimentally its value obtained is 14.70% then absolute error.

=14.70-14.75= -0.05%

Let a physical quantity X by measurement n times and let X_1, X_2, X_3--------X_n be the result of these measurement and A.M is the arithmetic mean of these measurement then

$$A.M = \frac{X_1 + X_2 + X_3 + ... + X_n}{n} \quad or\ A.M = \sum_{i=1}^{n} \times i$$

Since true value of the quantity is not known, A.M is usually taken to be true value. Therefore, absolute error in the measurement of quantity X is given by.

$$\delta_1 = X_1 - A.M$$
$$\delta_2 = X_2 - A.M$$
$$\delta_n = X_i - A.M$$

Mean absolute error. It is the arithmetic mean of all the absolute errors.

$$\delta_{mean} = \frac{\delta_1 + \delta_2 + ... + \delta_n}{n} = \frac{\sum_{i=1}^{n} \delta_1}{n}$$

δ_{mean} *is called final absolute error.*

Accuracy is generally the more important characteristic of quantitative data to be assessed although consistency as measured by precision is of particular concern in some circumstances. Trueness is a term associated with accuracy which describes the closeness of agreement between the average of a large number of results and a true accepted reference value. The degree of accuracy required depends on the context of the analytical problem, results must be to be fit for the propose for which they are intended.

For example, one result may be satisfactory if it is within 0.5%. By repeating an analysis a number of times and computing an average value for the result, the level of accuracy will be improved, provided that no systematic error has occurred.

Accuracy cannot be established with certainty where true or accepted values of a result with a given probability are widely used.

2.4 Determining the accuracy of methods

It is possible to determine the accuracy of quantitative methods by use of a sample containing known amount of the constituent in a synthetic sample or by the use of a sample in which the content of the constituents has been determined by other methods for comparison. The first is called an absolute method while the second is called the comparative method. In the absolute method, the differences between the average of an adequate number of closely agreeing results and the amount of constituent present are measured. In the absence of diverse ions this method is valid. In the comparative method, the sample is determined by two different independent methods and then the accuracy is verified.

Example, analysis of an element by volumetry, gravimetry and colorimetric methods, in most work the allowable error is 1-10 ppt per component. However in a few samples involving no separations the accuracy limit can be increased.

In actual practice, the accuracy of analysis is tested in various ways, namely the replicate determination, summation, charge balance and by independent methods. In replicate determination two or more concurrent parallel determinations are made to gain the results because a single determination is dangerous. A poor agreement in replecates indicates error. In the summation method all results of individual constituents are summed up to see whether the sum is closed to 100 because a good summation is a sufficient criterion of accuracy. The charge balance technique is applicable to solutions of electrolytes as well as solids composed of ions and charge components. Finally in an independent method the analyst checks the accuracy of the result by applying a known method of analysis.

2.5 Improving the accuracy of analysis

The accuracy of the method of analysis is improved upon minimising the errors. The following methods can be used to reduce the magnitude of systematic error.

1. The accuracy of many physical experiments is mainly affected by the accuracy of the instruments which is checked by their calibration. It involves the comparison of the instruments with the appropriate defined quantity of the standard. For highest accuracy all the instruments including burettes, pipettes , flask, weights must be calibrated before use.
2. A blank determination (omitting the sample under examination) must be carried out under exactly the same experimental conditions as being employed in the actual determination of the sample.
3. A control determination using an amount of a standard substance containing the same weight of the constituent (under study) as is contained in the unknown sample should be run employing similar experimental conditions. The amount of the constituent in the unknown is calculated as

$$\frac{Wt.of\ Constituent\ in\ the\ unknown}{Wt.of\ Constituent\ in\ the\ standard} = \frac{\text{Result obtained for unknown}}{\text{Result obtained for standard}}$$

Reference materials can be obtained from the National Bureau of Standards

4. Two difference methods (say, gravimetry and volumetry) of analysis of a constituent say Fe(III) in the unknown substance should be carried out. If these two results are concordant, it is concluded that the values are most probably correct within small limits of error.

2.6 Precision

It is the degree of agreement between replicate measurements of the same quantity i.e it is the repeatability of a result. Good precision does not assure good accuracy because of systematic error in analysis and weight used to measure the sample may be in error. More the measurements made, more reliable will be the measure of precision. To express precision, the arithmetic average of the values is usually taken as the best value. The simplest measure of precision is either average deviation or relative average deviation. The precision of a number can be reportd in many ways. For example when a number is reported as $X \pm x,$ one must qualify the conditions under which one reaches at $\pm x, x$ may represent (s), 2s, (mean), coefficient of deviation etc.

In simple words the precision of a measurement is determined by how closely two or more measurements of the same quantity agree with each other. The closer these values are the more precise the method. In other words, the precision of a measurement is the degree of reproducibility.

2.7 Methods of Expressing Precision

(i) Mean

Mean value is found by adding all the measurements and then dividing the added values by the total number of measurements. For example.

$$\text{Mean (or arithmetic mean)} = \frac{x_1 + x_2 + x_3 + ...x_n}{n}$$

Where $x_1 + x_2 + x_3 + ...x_n$ are the n values of measurements.

(ii) Median

It represents a value about which all the other values are equally distributed. To calculate median, arrange different values in their ascending order. (for example, arrange 7.25, 7.30, 7.28, 7.26, 7.32, and 7.27 as 7.25,7.26,7.27,7.28,7.30, 7.32). if the number of measurements are even the median is the average of two middle values of all the measurements (for example in the

above data median $= \frac{7.27 + 7.28}{2} = 7.28$) if the number of measurement are odd (for example; 10.12, 10.15, 10.14, 10.08 and 10.10) the median is simply the middle value of all the measurements (for example the above data is arranged in ascending order(i.e 10.08,10.10, 10.12, 10.14 and 10.15). So median is 10.12. it may be noted that mean and median may be same. When the number of measurements in the set is high, the mean deviation is obtained by calculating the mean of the results. Then the deviation of each measurement from the mean value is found. Finally the sum of these deviations is divided by the number of measurements.

(iii) Average deviation

Average deviation of a set of single measurements is the mean of the differences of the individual measurements and the mean of the measurement without regard to sign.

$$Average\,deviation = \frac{\sum (x_i - \bar{x})}{N}$$

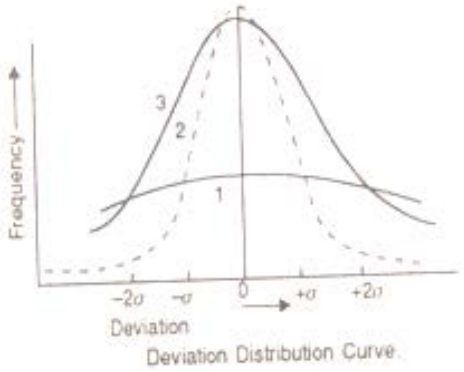
Where x_i = individual measurement ($\bar{x}$ =mean of measurement; N= no of measurement and $\sum$ represents summation. It is also equal to 0.08 X standard deviation when frequency of deviations follows a normal distribution and values of N is large.

2.8 Propagation of error

In an experiment an error introduces in the beginning gets multiplied as we proceed in the further steps, it is called the propagation of error and it becomes significant in the final results.

2.9 Deviation Distribution curve

When the frequency of occurrence is plotted against the indeterminate errors, we get an insight into the magnitude and the effect on the final result. We see that errors lie on a curve. The curve is normal error curve. It indicates that.



Deviation Distribution Curve

(i) The frequency is maximum in the case where indeterminate error is zero.

(ii) Small errors occur more frequently than large errors.

(iii) Positive and negative errors of the same numerical magnitudes are equally likely to occur.

In the above figure

(i) Curve 1 shows a careless operator or an improper measuring method.

(ii) Curve 2 indicates situations opposite to that of curve 1 and

(iii) Curve 3 results when the operator is experienced and the equipment is good.

It may be noted that lower spread of the curve shows greater precision.

2.10 Mean Deviation

The precision may be expressed in terms of mean deviation, standard deviation or the coefficient of variation. We have known that the mean of a set of values can be obtained by dividing the sum of the individual values of a set by the total number of values. Consider a set of values 12, 10, 14,16 and 17. The mean value is $\frac{10+12+14+16+17+18}{6}=\frac{87}{6}=14.5$ and median $\frac{14+16}{2}=15$

Median is the result about which all others are equally distributed, half being numerically greater and half being numerically smaller.

It may be noted that mean median may be same when the number of measurements in the set is high. The mean deviation is obtained by calculating the mean of the results. Then the deviation of each measurement from the mean value is found. Finally the sum of these deviations is divided by the number of measurements.

Difference between Precision and Accuracy

These two terms are different from each other. Some points of difference between precision and accuracy are given below

Accuracy	Precision
It is a measure of the agreement between an experimental result and the true value of the given quantity	It measure the agreement between several experimental results obtained for the same quantity under experimental conditions
It can never be determined exactly. The reason is that it involves the use of absolute or true value of the quantity being measured which is never known	It can be determined almost exactly by replicate measurement of the same quantity .
It is expressed in terms of absolute error or relative error	It is expressed in terms of various types of deviations from the mean.

2.11 Improving the accuracy of analysis

There are several ways of achieving accuracy in the analysis. One can use small blanks along with the sample during determination. The use of large blanks reduces the precision.

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

The precision of results obtained within one working session is known as repeatability or within-run precision. The precision of results obtained over a series of working sessions is known as reproducibility or between-runs precision.

It is sometimes necessary to separate the contributions made to the overall precision by within-run and between-runs variability. It may also be important to establish the precision of individual steps in an analysis.

2.12 Rejection of data

Frequently when a series of replicate analysis are formed one of the results will appear to another markedly from the others. A decision will have to be made whether to reject the result or to main it unfortunately there are no uniform criteria that can be used to decide if a suspect result can be described to accidental error rather than chance variation. The only reliable basis for rejection is when it can be decided that some specific error may have been made in obtaining the doutfull result. No result should be retained in case when a known error has occurred in its collection.

Experience and common sense may serve as just as practical basis for judging the validity of a particular observation as a statistical test would be the experienced analyst will gain good idea of the precision to be expected in a particular method and will recognize when a particular result is expected.

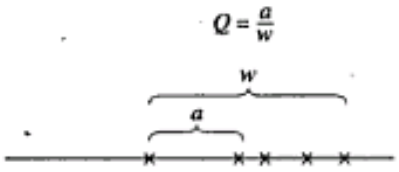
A wide variety of statistical test have been suggested and used to determine whether an observation should be rejected. In all of these range is established with in which statistically significant observations should fall. The difficulty with all of them is in determining with the range should be if it is too small then perfectly good data will be rejected and it is for large then erroneous measurement will be retained.

2.11 Q test

In 1951 Dean and Dixon proposed the Q test for the rejection of data. This test has sound statistical basis and was designed for the use of only three to ten replicate analysis.

The Q test is applicable when one and only one result deviates from the rest of the set. It will not work if there are two results that have considerable distance from the average. Q test is also not applicable when all but one of the pieces of data in the set are identical.

The ratio Q is calculated by arranging the data in decreasing order of numbers. The difference between the suspect numbers and its nearest neighbor (a) is divided by the range (w) i.e the difference between the highest number and the lowest number $Q = a/w$.



This ratio is compared with tabulated values of Q. If it is equal to or greater than the tabulated value the suspected observation can be rejected. The tabulated values of Q, at the 90, 95, 99% confidence levels are given in below table. If Q exceeds the tabulated values for a given number of observations and a given confidence level then the Questionable measurement may be rejected for example

The Q test should be applied to three points it two are identical. In that case the test always indicates rejection of the third value, regard less of the magnitude of the deviation because it is

equal to w and Q case is always equal to 1. The same obviously applies for three identical data points in four measurements and so forth

Rejection Quotient, *Q*, at Different Confidence Limits[a]

No. of Observations	Confidence Level		
	Q_{90}	Q_{95}	Q_{99}
3	0.941	0.970	0.994
4	0.765	0.829	0.926
5	0.642	0.710	0.821
6	0.560	0.625	0.740
7	0.507	0.568	0.680
8	0.468	0.526	0.634
9	0.437	0.493	0.598
10	0.412	0.466	0.568
15	0.338	0.384	0.475
20	0.300	0.342	0.425
25	0.277	0.317	0.393
30	0.260	0.298	0.372

Consider the data set:

0.189, 0.167,0.187, 0.183, 0.186, 0.182, 0.181, 0.184, 0.181, 0.177 ,

Now rearrange in increasing order:

0.167, 0.177, 0.181, 0.181, 0.182, 0.183, 0.184, 0.186, 0.187, 0.189,

We hypothesize 0.167 is an outlier. Calculate Q:

Q= gap\range = {0.177-0.167}/{0.189-0.167}=0.455.

With 10 observations and at 90% confidence, Q = 0.455 > 0.412 = Q_{table}, so we conclude 0.167 is an outlier. However, at 95% confidence, Q = 0.455 < 0.466 = Q_{table} 0.167 is not considered an outlier. This means that for this example we can be 90% sure that 0.167 is an outlier, but we cannot be 95% sure.

2.12 F test

F- test is used to compare the standard deviations inorder to detect random errors of two sets of data.

The F test is used to compare the precision of two set of data. F is the ratio. So set up that it is always greater than one $\left(S_1^2 > S_2^3\right)$. In the F set after the value of F has been calculate from the two sets of data, the value is compared with that given in table of statistical F values. When the calculated F is larger than the tabulated value the data in set 1 are indeed less precise than those in set 2 at the specified confidence level. The data in table gives values for F at 95% confidence

level. V_1 is the number of degree of freedom of data of the set having layer variance and V_2 therefore is the number of degree of freedom of the set of the data having the smaller variance.

Values of F at 95% Confidence Level

V_2 \ V_1	2	3	4	5	6	7	8	9	10	∞
2	19.00	19.16	19.25	19.30	19.33	19.36	19.37	19.38	19.39	19.50
3	9.55	9.28	9.12	9.01	8.94	8.88	8.84	8.81	8.78	8.53
4	6.94	6.59	6.39	6.26	6.16	6.09	6.04	6.00	5.96	5.63
5	5.79	5.41	5.19	5.05	4.95	4.88	4.82	4.78	4.74	4.36
6	5.14	4.76	4.53	4.39	4.28	4.21	4.15	4.10	4.06	3.67
7	4.74	4.35	4.12	3.97	3.87	3.79	3.73	3.68	3.64	3.23
8	4.46	4.07	3.84	3.69	3.58	3.50	3.44	3.39	3.34	2.03
9	4.26	3.86	3.63	3.45	3.37	3.29	3.23	3.18	3.13	2.71
10	4.10	3.71	3.48	3.33	3.22	3.14	3.07	3.02	2.97	2.54
∞	3.00	2.60	2.37	2.21	2.10	2.01	1.94	1.88	1.83	1.00

For example

Consider the data obtained during a determination of the equivalent weight of weak acid. In method A, Phenolphthalein was used to detect the end point, when as in method B, the glass electrode was used as the detection device.

Method A	**Method B**
122.1g	122.0g
122.0	122.2
122.6	122.4
121.8	122.5
Avg 122.2 g/equiv	**122.5 g/equiv**

Now the question is which set of two data is more precise. To set answer we need to use the following equation

$$S^2 = \sum_i (X_i - m)^2 / n - 1$$

The variance S^2 for the data A is $2X10^{-1}$ where as the variance of the data obtained for the data B is $5X10^{-2}$

Applying the F test

$$F = S_1^2 / S_2^2 = 2X10^{-1} / 5X10^{-2} = 4$$

From the table for V_1=3 and V_2=4 we find the value of F=6.59 at 95% confidence level. Since the calculated value if is smaller than this we can conclude that the data obtained using Phenolphthalein are just as precise as those obtained with gloss electrode.

2.13 t-test

In 1908 Gosset derived what he called students t-test in to account the smallest of the usual number of measurement encountered in practice and variations in the number of precise of data used to calculate the average . He defined t as

$$\pm t = (m - \mu)\frac{n^{1/2}}{S} \text{----------(1)}$$

Where m is the average of the set of data μ can be considered as the true value. N is the number of pieces of set of data, and s is the standard deviation for the set

Rearranging the above equation then we get

$$\mu = m \pm + S/n^{1/2} \text{----------(2)}$$

From the equation 2 we can see that the true value will fall within a distance of $\pm + S/n^{1/2}$ (the confidence level), How large this confidence level is well dependent upon the number of measurements taken their standard deviation and confidence level desired. If in practice the true value (known from the same other source) fall outside this interval we can conclude that there is a systematic error in the data in addition to the random errors. When this occurs attempt should be made to scrutinize the analytical method to locate and eliminate the systematic error, values of t are tabulated in most texts on statistics and partial listing is given in below table.

If μ is considered to be known value for the percentage of a constituent in a sample (this value must be obtained from some other source than the method being tested for) and t test can be used

to evaluate the quantity of an analytical method, the performance of an experimenter or the effect of changing parameters, with in the method.

Values of t for Different Confidence Levels

Degree of freedom V	90%	95%	99%
1	6.31	12.71	63.66
2	2.92	4.30	9.93
3	2.35	3.18	5.84
4	2.13	2.78	4.60
5	2.02	2.57	4.03
6	1.94	2.45	3.71
7	1.90	2.37	3.50
8	1.86	2.31	3.46
9	1.83	2.26	3.25
10	1.81	2.23	3.17

If μ is considered to be the average of one set of data and m the average of a second set of data pertaining to the same determination one can use the test to determine variation in the performance of personnel, equipment and the method.

The t test consists of calculating the value of t by means of equation 1 and comparing it with the statistically obtained values of t in tables. This done at a particular confidence level decided up on by the judge of the data. When the calculated t exceeds the statistical value of t there is a real difference (a systematic error) between the mean and the value. When the calculated t is less than the tabulated t the difference between the mean and the true value is acceptable statistically (only to random error) and considered negligible (that is the two values are identical)

Example

In a gravimetric determination of nickel with dimethyl glyoxime, a student truns is the values 3.681, 3.365, 3.593%. The national Bureau standard values is 3.557% then does the average of these value 3.632% of nickel have a difference from the true value i.e real or is it statistically possible to obtain such difference?

The calculated value for the standard deviation of the experimental result s = 0.037%. There are four pieces of data in the test. Thus using equation (1) the student can calculate the value for t for the data.

$$t = (3.632 - 3.557)(4)^{1/2} / 0.037 = 4.02$$

From the table for the three degree of freedom and 95% confidence level the value of t is 3.18. since his calculated t is greater than 3.18 the difference between the students mean and the true value is real, is real one not merely due to statistical fluctuations of indeterminate errors. Note for 99% confidence level the tabulated value of t is 5.84. The student's calculated values are less than this. So that he should suspect a real difference (since the 95% t is exceeded) but have to conclude that it is statistically possible to have this large discrepancy.

2.14 Variance

Variance is a very useful statistics quantity which is the square of the standard deviation,s^2 i.e, Variance = the square of the standard deviation.

2.15 Coefficient of Variation (CV)

Coefficient of variance is also known as the relative standard variation (RSD) which is given by $\frac{100}{\bar{s}x}$ and is a widely used measure of spread σ.

2.16 Confidence Limit and confidence level

For a sample of n measurements, the standard error of mean

$$\text{(s.e.m)} = \frac{\sigma}{\sqrt{n}}$$

The confidence interval for the mean is the range of values within which the population mean, μ, is expected to lie with a certain probability. The boundaries are called confidence limit.

The confidence level is the probability that the true mean lie a certain intervals and is often express in percentage. The confidence intreval for the mean of n measurements can be calculated by

$$\text{CI} = \bar{x} \pm ts\sqrt{N}$$

Where $\bar{x}$ is the sample mean, s is the standard deviation and t is the t-statistic distribution otherwise known as student's t.

For a single measurement with result x,t is given as,

$$t = \bar{x} - \frac{\mu}{s}$$

for N measurement, t is given as, $t = \dfrac{\bar{x} - \mu}{\dfrac{s}{\sqrt{N}}}$

t depends on the desire confidence level,as well as on the number of degree of freedom in the calculation of standard deviation. The value of t is found by consulting the t degree of freedom. The student t level is shown in Table

Values of t for confidence intervals

Degrees of freedom	Values of t for confidence interval of				
	80%	90%	95%	99%	99.9%
1	3.08	6.31	12.7	63.7	637
2	1.89	2.92	4.30	9.92	31.6
3	1.64	2.35	3.18	5.84	12.9
4	1.53	2.13	2.78	4.60	8.60
5	1.48	2.02	2.57	4.03	6.86
6	1.44	1.94	2.45	3.71	5.96
7	1.42	1.90	2.36	3.50	5.40
8	1.40	1.86	2.31	3.36	5.04
9	1.38	1.83	2.26	3.25	4.78
10	1.37	1.81	2.23	3.17	4.59
11	1.36	1.80	2.20	3.11	4.44
12	1.36	1.78	2.18	3.06	4.32
13	1.35	1.77	2.16	3.01	4.22
14	1.34	1.76	2.14	2.98	4.14
∞	1.29	1.64	1.96	2.58	3.29

For large sampless,the confidence limits of the mean are given by,C.I = $\bar{x} \pm zs\sqrt{n}$, where the values of z depends on the degree of confidence required. The values for z at various confidence levels for small and large samples can be found in below Table

Confidence level,%	z
50	±0.67
68	±1.00
80	±1.29
90	±1.64
95	±1.96
96	±2.00
99	±2.58
99.7	±3.00
99.9	±3.29

2.17 Regression analysis

Regression analysis is a statistical tool for the investigation of relationships between variables. Usually, the investigator seeks to ascertain the causal effect of one variable upon another, for example the effect of a price increase upon demand, or the effect of changes in the money supply upon the inflation rate. To explore such issues, the investigator assembles data on the underlying variables of interest and employs regression to estimate the quantitative effect of the causal variables upon the variable that they influence. The investigator also typically assesses the "statistical significance" of the estimated relationships, that is, the degree of confidence that the true relationship is close to the estimated relationship.

Regression analysis measures the degree of influence of the independent variables on a dependent variable. In the case of a single independent variable, the dependent variable could be predicted from the independent variable by the simple equation:

$y = a + bx$ {where a is constant}

2.18 Least squares methods

The term least squares describes a frequently used approach to solving overdetermined or inexactly specified systems of equations in an approximate sense. Instead of solving the equations exactly, we seek only to minimize the sum of the squares of the residuals.

The least squares criterion has important statistical interpretations. If appropriate probabilistic assumptions about underlying error distributions are made, least squares produces what is known as the maximum-likelihood estimate of the parameters. Even if the probabilistic assumptions are not satisfied, years of experience have shown that least squares produce useful results. The computational techniques for linear least squares problems make use of orthogonal matrix

factorizations. The least squares method is specified by an equation with certain parameters to observed data. This method is extensively used in regression analysis and estimation.

2.19 Correlation coefficient

The correlation coefficient of two variables in a data sample is their covariance divided by the product of their individual standard deviations. It is a normalized measurement of how the two are linearly related.

Formally, the sample correlation coefficient is defined by the following formula, where S_x and S_y are the sample standard deviations, and S_{xy} is the sample covariance.

$$r_{xy} = \frac{S_{xy}}{S_x S_y}$$

Similarly, the population correlation coefficient is defined as follows, where σ_x and σ_y are the population standard deviations, and σ_{xy} is the population covariance.

$$P_{xy} = \frac{\sigma_{xy}}{\sigma_x \sigma_y}$$

If the correlation coefficient is close to 1, it would indicate that the variables are positively linearly related and the scatter plot falls almost along a straight line with positive slope. For -1, it indicates that the variables are negatively linearly related and the scatter plot almost falls along a straight line with negative slope. And for zero, it would indicate a weak linear relationship between the variables.

2.20 Summary of the unit

Precision is the degree to which the results of multiple repeat experiments agree with one another. For instance if an experiment is repeated 3 times and the same result is obtained all three times, then the result is considered to be very precise. Accuracy is the degree to which the results of an experiment agree with the true or known value.

The correlation coefficient, or Pearson product-moment correlation coefficient (PMCC) is a numerical value between -1 and 1 that expresses the strength of the linear relationship between two variables.When r is closer to 1 it indicates a strong positive relationship. A value of 0 indicates that there is no relationship. Values close to -1 signals a strong negative relationship between the two variables. You may use the linear regression calculator to visualize this relationship on a graph.

2.21 Key words

Accuracy; Precision; Propagation of error; Deviation Distribution curve; Mean Deviation; Q test; F test; t-test; Variance; Confidence Limit; Confidence level; Regression analysis; Least squares methods; Correlation coefficient.

2.22 References for further study

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2.2Questions for self understanding

1) What is Accuracy in analysis?
2) Discuss the different methods of expressing accuracy
3) How accuracy can be improved in analysis?
4) What is Precision?
5) Explain the difference between Accuracy and Precision.
6) Explain the criteria for the rejection of data.
7) Write a note on
    a) Q-test
    b) F-test
    c) t-test
8) What is mean by Varience?
9) Explain the Coefficient of Variation (CV)
10) Explain Confidence Limit and confidence level in data analysis
11) Write a note on Regression analysis.
12) Explain Least squares methods.
13) Discuss Correlation coefficient.

Unit-3

Structure

3.0 Objectives of the unit

After studying this unit you are able to

- Explain the meaning of gravimetric analysis
- Explain the Principle of Precipitation
- Discuss the different Methods of precipitation
- Write a note on Mechanism of Precipitation
- Explain efficient condition for Precipitation
- Explain the differences between Post-precipitation and Co - precipitation

3.1 Introduction

Gravimetric analysis is based upon the measurement of mass. This analysis can be generalized into two types they are precipitation and volatilization. The quantitative determination of a substance by the precipitation method of gravimetric analysis involves isolation of an ion in solution by a precipitation reaction, filtering, washing the precipitate free of contaminants, conversion of the precipitate to a product of known composition, and finally weighing the precipitate and determining its mass by difference. From the mass and known composition of the precipitate, the amount of the original ion can be determined.

3.2 Principles of gravimetric analysis

The gravimetric analysis is highly technical and requires experimental skill for getting accurate results. The gravimetric estimations involve the following general operation by properly using the apparatus required. Some steps involved in gravimetric estimation are described below.

1. Weighing of the sample
2. Preparation of the solution
3. Precipitation
4. Digestion
5. Filtration
6. Washing
7. Drying
8. Incineration
9. Weighing of the product

a) Weighing of the sample

A clean dry stoppered weighing bottle or a watch glass is first weighed when empty and weighed with a required amount of substance. The difference of the two weights i.e initial and final weights five the weight of the substance.

b) Preparation of the solution

The weighed substance is transferred to the beaker and the weighing bottle of watch – glass is washed, with a spray of distilled water from wash bottle and these washings are also transferred to the sane beaker. The substance is then dissolved by stirring the contents of the beaker. Heating is also done if required. Sometimes, reagents like dil HCl, nitric acid, acetic acid etc., are also added to make the solution clear but their correct choice depends upon the concerned estimation. However generally the substance are supplied with the prepared solutions and asked to take 20 or 25 ml of the solution for gravimetric estimation. A definite volume of the solution or the weight of the substance should give a precipitation or ignited residue preferably weighing between 0.2 to 0.4 gm.

c) Precipitation

The objective of precipitation a compound is to precipitate it in such a way that it should be coarse, granular and dense precipitation as far as possible. For accomplishing such an objective the choice of a suitable precipitation for a given constituent is done keeping in view the factors given as:

(a) The precipitation can be made quantitative by lowering its solubility to the negligible limit.

(b) The precipitation should be convenient to filter and ready to be weighed after prdoper drying and ignition.

(c) The precipitation should be highly specific in nature.

d) Principle of Precipitation

The precipitation of inorganic substance like AgCl, $BaSO_4$, $Al(OH)_3$ etc. is an ionic phenomenon. For the precipitation of such compound the condition of the precipitation is that their respective ionic product must exceed their respective solubility product. In gravimetric analysis the substances having very low solubility products are selected precipitation. This is achieved by adding precipitant solution to the solution of the sample containing sufficiently large concentration of the ion so that their ionic product in the mixture solution exceeds the respective

solubility product of the substance which is precipitated as required for the given gravimetric estimation.

e) Method of precipitation

The solution meant for the precipitation is first diluted to 100 to 150 ml with distilled water and heated suitably by covering it with a clock-glass. It is then precipitated by slowly adding by the side of the beaker the hot solution of the precipitating reagent with constant stirring of the mixture with the round edged glass rod. The precipitate is then digested, allowed to stand for some time and tested for complete precipitation from the neck of the beaker. If this gives any turbidity more drops of the precipitating reagent are added till the completion of precipitation takes place.

f) Mechanism of Precipitation

After the addition of the precipitating agent to the solution of the ion under analysis there is an initial induction period before nucleation occurs. This induction period may range from a very short time period to one which is relatively long, ranging from almost instantaneous to several minutes.

After induction, nucleation occurs, *Nucleation is a process in which a minimum number of atoms, ions, or molecules join together to give a stable solid*. Here small aggregates or nuclei of atoms form and it is from these "clumps" of atoms that the crystals which form the filtrate will grow. These nuclei may be composed of just a few atoms each so there may be up to 10^{10} of the nuclei per mole of precipitating product. As these nuclei form ions from the solution (which at this point are in excess) congregate around them. For example if hydrochloric acid were added very slowly to a solution of silver nitrate, silver chloride nuclei would form and silver ions (which would be in excess relative to Cl^- ions) would congregate around them.

Nucleation is of two kinds one is *Heterogeneous* in which the new phase appears on the walls of the container, at impurity particles, etc. another one is *Homogeneous* in which solid nuclei spontaneously appear within the undercooled phase.

g) The Purity of the Precipitation

The precipitate separated from a solution is not completely pure and may contain greater or less amounts of foreign material as impurity. This is known as defective precipitation and arises due to the process briefly detailed as follows.

(a) Co-precipitation. The contamination of a precipitate by substance which are normally soluble under the conditions of precipitation is known as co precipitation. in this process certain soluble substance present in the mixedx solutions are retained and carried by the precipitate for example when potassium sulphate solution is added to the barium chloride solution, some amount of K_2SO_4 is found to be present in the precipitate of $BaSO_4$.

The process of co precipitation takes place due to :

(i) Adsorption at the surface of particle of the precipitate exposed to the solution.

(ii) Occlusion of a foreign substance during the process of the growth of the crystal from the primary particles.

(iii) Inclusion and

(iv) isomorphous replacement.

The various modes of co precipitation are given in the table

Types of	**Mode of Contamination**	**Precipitation type most**
Isomorphous inclusion	Substitution of the precipitation lattice with	Crystalline
Non-isomorphic inclusion	Solid solution of the impurity with in the precipitate	Crystalline
Occlusion	Physical trapping of impurities with precipitate	Crystalline and colloidal
Surface adsorption	Chemisorption of impurities from the solution onto the	Colloidal

h) Post Precipitation

Post precipitation is the precipitation of an impurity after some appreciable time of the primary precipitation of the main substance. In the absence of a primary precipitate the impurities may precipitate very slowly as they are capable of forming their supersubstrated solution. The primary precipitate actually becomes a nucleus for the post precipitation of impurities from their super saturated solution. For example when an acid solution of ammonium oxalate is added to an acidified solution containing calcium and magnesium ions, calcium oxalate separates as the primary precipitate. The solution becomes supersaturated with respect to magnesium oxalate which now slowly precipitates on the primary precipitate of calcium oxalate. This happens when the concentration of magnesium ions is high. Hence

the primary precipitate of calcium oxalate may have to be filtered redissolved and precipitate of calcium oxalate is past filtered within three hours of its precipitation.

i) Difference between Post-precipitation and Co precipitation

Co precipitation	Post precipitation
Contamination falls with the time if the precipitate is left in contact with the mother liquor	Contamination increases with time if the precipitate is left in contact with the mother liquor
Magnitude of contamination is small	Magnitude of contamination is large
Contamination decreases the faster the solution is agitated by either mechanical or thermal means	Contamination increases the faster the solution is agitated by either mechanical or thermal means

j) Peptization

The process of the redispersion of the coagulated from or the precipitate resulting in the formation of colloidal solution is known as Peptization. The cause for this process is the presence of large amount of water and absence of electrolytes during the process of washing of the precipitate on the filter paper or through sintered crucible. The error due to Peptization can be suitable electrolyte. For example the precipitation of AgCl is washed with distilled water containing few drops of Nitric acid. Similarly the precipitation of $Al(OH)_3$ is washed with 1-2% solution of ammonium Nitrate.

k) Efficient condition for Precipitation

(i) The precipitation should be done in dilute solution in order to avoid error due to co precipitation.

(ii) The precipitation should be done in hot solution unless advised otherwise. This helps in co-agulation, better growth of the precipitate and also avoids error due to post precipitation.

(iii) In order to avoid error due to co precipitation by occlusion the precipitate is digested before filtration.

(iv) Peptization is checked by washing the precipitate with dilute solution of a suitable electrolyte.

(v) Only a small excess of the precipitation reagent should be added to reduce adsorption and Peptization.

(vi) The precipitation should always be carried out in a beaker and not in any other vessel like conical flask as it is easier to transfer the precipitate during filtration.

l) Digestive of the precipitate

In this process the precipitate is generally degested by either heating over a water bath or by allowing it to stand at room temperature for some time or simply heating it at low flame. But all this depends upon the nature of precipitate. This operation helps in bringing about complete precipitation and obtaining a dense granular precipitate which is in readily filterable form. This process is known as digestion of the precipitation. the overall effect of the digestion is to decrease the extent of co precipitation and increasing the size of the particles of the precipitate. But digestion has very little effect on amorphous or gelatinous precipitates.

m) Filtration

The precipitate is isolated from the mother liquor by filtration. Is done by using standard filter paper or sintered glass crucible/

Filtration using filter papers

Choice of the filter paper

For this purpose standard whatman filter papers numbered as 40, 41,42 etc. are generally used. These filter papers have different pore sizes and their choice depends upon the size of the particle of the precipitate of AgCl, $Fe(OH)_3$ and $BaSO_4$ respectively.

Setting the filter paper

A suitable standard filter paper is chosen folded exactly in half and placed in quarters so that the two halves do not coincide exactly. It is then opened in such a way that three quarters are one side and fourth quarter on other side and there after it is placed in funnel at 60^0 C by hnolding it tightly with a finger. The filter paper is wetted with distilled water and pressed so thst it sticks with the wall of the funnel. This funnel is kept in the ring or tripod stand in vertical position and an empty beaker is kept below it in such a way that the stem of the funnel is touching the wall of the beaker but is well above its bottom.

Filtration through sintered crucible

The accurately weighed sintered crucible of suitable porosity is fitted into an adaptor with a rubber ring or collar. This is then fitted in the neck of the filtration flask connected further with the suction pump. Pour the decanted liquid first and start the vacuum pump. Thereafter the

precipitate is transferred to the crucible with the help of a stream of water from the wash bottle and filtration is done as usual.

Filtering of Gelatinous precipitation

The precipitation of $Al(OH)_3$, $Fe(OH)_3$ etc. are gelatinous in nature and colg the pores of the filter paper. This slows the filtration of these gelatinous precipitates. In such cases filtration is speeded up in the presence of filter paper pulp which is prepared by boiling small pieces of the standard filter paper in water. It is also available in readymade tables under the name of whatman accelerators which when dissolved in water give solution.

The required qualification of the washing solution of liquid is to have a minimum solvent effect on the precipitation and of not helping in Peptization of precipitate even after washing off the electrolyte ions.

Washing solution are mainly of three types

Those solutions which do not allow the precipitate to become colloidal and pass through the filter e.g 1% HNO_3 for washing of AgCl precipitate and about 2% Ammonium nitrate solution used for washing $Fe(OH)_3$ precipitate.

Those solutions which can reduce the solubility of the precipitate by having common ion with the precipitate.

Those solutions which can prevent the hydrolysis of salts of weak acids and bases e.g the addition of an acid to the wash solution prevents the hydrolysis of ferric like salts.

However the wash solution must contain any such compound or ions which can interfere with subsequent determination of gravimetric estimations.

n) Drying

The precipitate in the filter paper is dried by making use of the heating device controllable to the desired temperature. It can be done in steam oven or hot air oven or an electric oven. The precipitation in the sintered crucible is dried by keeping it preferably in an electric oven at the required temperature.

Incineration

It is process in which the filter paper is burnt separately. The process is necessary in those cases in which the precipitate like $BaSO_4$, AgCl etc. is reduced by carbon of the filter paper on strong heating or the precipitate is easily fusible or volatile as AgCl.

The filter paper can be incinerated as follows.

(i) When the precipitate and the filterpaper are complex dried up remove the funnel from the cone and cool it on the funnel stand.

(ii) Spread a while and a coloured sheet of glazed paper on the table. Now place a dry watch glass on coloured sheet and tarred crucible on the coloured sheet.

(iii) Now take out the dried filer paper cone from the funnel and loosen the precipitation by pressing its sides gently. Transfer the precipitate to the watch glass. By means of a camel hair brush, remove the precipitate still clinging to the filter paper. After removing the precipitate on the watch glass cover it by means of dry funnel.

(iv) Held the filter paper just out of tarred crucible just kept on glazed paper and light it with non luminous flame. Remove the flame when the paper begins to burn. As the paper burns , tap the ash into the crucible. Also brush down the residue from the tips of the tongs. Some ash which may fall on the paper may also be transferred to the crucible by means of brush.

(v) Now place the crucible on a clay triangle resting on an iron ring. Treat the ash with a few drops to convert any reduced particles of precipitate into the finally desired form. Heat strongly till the ash turns white. Now place the crucible in the desiccators and let it cool.

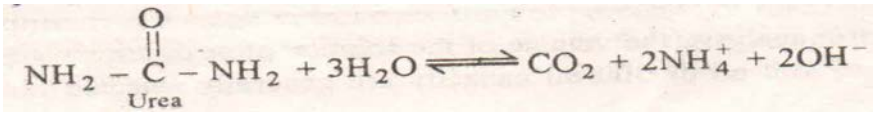
o) Ignition

Take out the crucible from the desiccators and place it on the glazed paper. Transfer the precipitate carefully from the watch glass to the crucible by means of dry camel hair brush. Heat the crucible on low Bunsen flame and increase the size of the flame gradually. After 1.5 minutes. Replace Bunsen burner with a meker burner and ignite for 20 minutes more. The ignition temperature can be regulated by using a muffle furnace.

3.3 Precipitation from homogeneous Solution

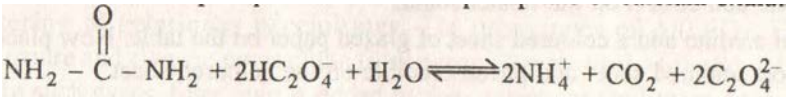
Precipitation from homogeneous solution avoids high local super saturation which is the case when a precipitating reagent is directly added to the solution. One of the species which ultimately forms the precipitate is slowly generate throughout the solution as result of an auxiliary chemical reaction. the solvent remains during the process of precipitation. Co precipitation is also minimized in this case. By changing the rate of chemical reaction producing the precipitant in homogeneous solution, the required particles size if the precipitate can be obtained. Slower the reaction, larger is the particle size of the precipitate. Some example of homogeneous precipitation reactions are given below.

(i) Use of Urea to precipitate Al^{3+}, Fe^{3+}, and Th^{4+} and other metal ions as their hydroxide or basic salts is an example of preparation from homologous solution. urea is weak base and hydrolysis rapidly at 90-100^{0}C



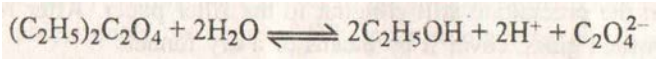
$$NH_2 - \underset{\text{Urea}}{\overset{\overset{O}{\|}}{C}} - NH_2 + 3H_2O \rightleftharpoons CO_2 + 2NH_4^+ + 2OH^-$$

(ii) An acid solution containing hydrogen oxalate ion ($HC_2O_4^-$) can be made to ionize slowly with the addition of urea forming oxalate $C_2O_4^-$ and raising the pH of the solution. under this condition, calcium ions can be precipitated as a dense precipitate of calcium oxalate.



$$NH_2 - \overset{\overset{O}{\|}}{C} - NH_2 + 2HC_2O_4^- + H_2O \rightleftharpoons 2NH_4^+ + CO_2 + 2C_2O_4^{2-}$$

Dimethyl and diethyl exalates can be hydrolysed to form oxalate ions which can be used to precipitate calcium, magnesium, zinc, thorium, as oxalates.



$$(C_2H_5)_2C_2O_4 + 2H_2O \rightleftharpoons 2C_2H_5OH + 2H^+ + C_2O_4^{2-}$$

3.4 Advantages of precipitation from Homogeneous Solution

1. It prevents local high concentration of reagent and thus promoting particle growth as well as minimizing the occlusion of impurities. Thus precipitation from homogeneous solution is an excellent method of enhancing the purity of a precipitation.
2. The filtration method must be selected to fit the treatment of the precipitate. Where the material is merely to be dried and weighed, a sintered glass crucible is generally the most satisfactory. Sintered silica coucibles are also used for these precipitate.

3.5 Weighing

The crucible containing the precipitate is allowed to cool in a desiccators for a few minutes. Then it is weighed accurately on an analytical balance. The operation of heating , cooling and weighing is repeated till a constant weight is obtained. The difference in the weights of the crucible with precipitate and empty crucible gives the weight of the desired substance. From the weight the necessary calculation can be done

3.6 Gravimetric calculations

Gravimetric calculation can be done by using the relation from chemical equations or by using a formula involving gravimetric factor:

$$Persentage\ \ constituent\ in\ a\ sample = \frac{weight\ of\ the\ ppt}{weight\ of\ sample} \times Gravimetric\ \ factor \times 100$$

Table 2.7 Some Gravimetric factors.

Metal/constituent	Form in which precipitated	Gravimetric factor	
Ag	AgCl	Ag/AgCl	0.7526
Al	Al_2O_3	$2Al/Al_2O_3$	0.5291
Ba	$BaSO_4$	$Ba/BaSO_4$	0.5885
Cl^-	AgCl	$Cl^-/AgCl$	0.2474
Cu	$Cu_2(CNS)_2$	$2Cu/Cu_2(CNS)_2$	0.5226
Fe	Fe_2O_3	$2Fe/Fe_2O_3$	0.6994
Mg	$Mg_2P_2O_7$	$2Mg/Mg_2P_2O_7$	0.2185
SO_4^{2-}	$BaSO_4$	$SO_4^{2-}/BaSO_4$	0.4115

3.7 Requirements of gravimetric analysis

The requirement of gravimetric analysis is the proper choice an use of the apparatus required for preparing solution, precipitation, ignition, weighing etc.

j) Apparatus

1. Beaker. In gravimetric analysis the volume of the solution after dilution for precipitation etc. is large and so beaker of 400ml or 500 ml capacity are generally selected for use. These beakers should be made out of right quality which is not affected chemically and can withstand frequent heating and cooling.

k) Precautions

(i) Beaker should be thoroughly washed and dried before use.

(ii) Heat the beaker on wire gauze and not on a direct flame.

(iii) Wipe the outer surface before heating.

(iv) While heating, cover the beaker with a watch glass allowing some space for steam to escape.

(v) When the process of boiling the liquid/ solution is complete the beaker may be kept covered with a watch glass to avoid outside contamination.

ii) Glass rod

A clean glass road of about 4 mm diameter and 20 cm length is cut and its edges are rounded by heating the edges in the flame (of blow pipe burner) to red heat and rolling on the surface of glazed tile. One end of the glass rod is fitted with a rubber cap and is known as police man. This end used for removing particles of a precipitate sticking to the sides of the beaker and transferring these to the filter paper or the sintered crucible or gooch crucible.

Precautions

(i) The glass rod is rested on the sprout and rims of the beaker and not on the working table. It should never be placed on the working table.

(ii) After use the glass rod is washed with stream of water from wash bottle.

(iii) Watch glass (clock glass). It is employed for covering beakers. The suitable size of the water glass is one with which its rim is slightly. Protruded from the tip of the beaker. It is kept with its convex side bugle downwards and its lower surface is washed with a stream of water from the wash bottle into the beaker.

iii) Funnel

It is used for filtration through a filter paper in gravimetric analysis. The standard size ranks from 6 to 9 cm in diameter with long steam of about 10 cm. the funnel is made out of good quality glass and the lower end of the steam is rounded to 40^0. The funnel is properly cleaned and washed well before use.

iv) Wash bottle

The wash bottle is employed for providing a fine stream of distilled water or other solutions if required for use during transfer and washing of the precipitate. The workable size is about 500-750 ml and made out glass or polythene. It is fitted with a jet having orifice of about 1 mm diameter so that a fine stream of water comes out when slight pressure is applied by squeezing its plastic balle or by blowing air in glass wash bottles.

v) Claypipe triangles

The clay pipe triangles are used for supporting a crucible during heating and igniting of the precipitate and keeping it in the desiccators. It is made out of three lengths (5 to 7 cm) of unglazed heat resisting clay tubes through which iron or nickel wire is passed an turned at the ends giving the shape of a triangle. The clay triangle is kept on the tripod stand or rising stand and its wire ends are bent inwardly so that the crucible can be supported safely on it.

vi) Crucible

It is a small vessel consisting of porcelain of fvused silica in which precipitate is heated or ignited to get a constant weight necessitated for the gravimetric estimation. Such a crucible is mainly of two types.

(i) Ignition crucible.

(ii) Filter crucible.

The proper use of these two types of crucible is briefly given below.

(a) Ignition crucible

It has a capacity of about 25 to 30 ml and is preferably made out of fused silica or porcelain with flat bottom and it is glazed on both sides. It is provided with a loose fitting lid to cover it whenever needed. The material of this crucible has very low coefficient of expansion and so it can stand to white heat without cracking. Also this crucible can resist to shock and action of acids even at a high temperature with exception of hydrochloric acid and phosphoric acid.

The use of this crucible is done the way as suggested under.

(i) It should be thoroughly cleaned, dried and ignited before use. The stains are removed by first treating the crucible with acidified concentrated solution of potassium dichromate and then with hot dilute solution of nitric acid followed by washing freely with water however if the stain persists, then it is removed mechanically by rubbing it with moist sand.

(ii) The cleaned crucible is then heated strongly and placed in a desiccator with a pair of tongs so as to get its initial weight. All this is known as taring of the crucible.

Precautions

(i) Never place a wet crucible on flame otherwise it can break.

(ii) Always use a clean, non-luminous flame for heating the crucible.

(iii) Handle the tared crucible only with tongs and not with hands in order to protect it from moisture and dust from the fingers.

(iv) The tared crucible should be kept and carried in a desiccators for weighing etc.

(b) Filter Crucible

These are used whenever the precipitates can not be filtered out easily through the filter paper. Also filtering a precipitate and its subsequent ignition is very lengthy and laborious process and these crucibles serve as cut- short method for gravimetric estimations. These crucibles are manufactured out of porcelain, borosilicate glass, alundum (fused Al_2O_3) etc. with capacity of

20 to 50 ml and provided with a pours mat at the bottom. The filtration of the precipitate directly done by sucation using a filter pump through these cruciblee. Further sintered glass and gooch crucibles are use for this purpose but sintered crucible is chiefly used for convenience as described under.

Using sintered glass crucible. These are manufactured out of good quality resistance – glass such as pyrex, corning etc. and is provided with a permanent porous-disc of sintered ground glass fused at the bottom of the crucible. These crucible are further graded as No 1,2,3, and 4 according to the fineness of the pores in the filter mat. Generally No 3 (G-3) crucible is used for medium sized particle of the precipitate such as $Ni(DMS)_2$, AgCl etc. while No. 4(G-4) crucible is used for very fine precipitates such as $BaSO_4$ etc.

The suitable numbered sintered crucible is taken, cleaned and washed with distilled water and thereafter, it is dried by keeping it in an oven at 120-150^0 C for about half on hour. It is then cooled in desiccators and weighed. The heating is repeated to get a constant weight. The sintered crucible is there after fixed by means of an adapter rubber-collar in the neck to the filtration flask fitted with a filter pump the filtration is done by decantation.

Precautions

(i) Chromic acid is not used as cleansing agent for sintered glass crucible as its use gives coloured stain on the mat of the crucible. Also washing is not done with NaOH or KOH

(ii) The sintered glass mat is not scratched as otherwise it gives defective filtration.

(iii) Sintered glass crucible should not be heated strongly as unglazed glass is softened above 400^{0}C

(iv) Only mild pressure should be observed during filtration under suction with filter pump as otherwise the bursting of the mat can take place.

Cleaning filter mat

The dried precipitate is first thrown out by inverting and tapping the sides of the sintered glass crucible. It is then washed thoroughly with water and diluted ammonia concentrated HCl and again with water and rinsed with acetone to quicken the process of drying of the crucible.

vii) Crucible tongs

A pair of crucible tongs is used for handling the crucible and its lid in the gravimetric estimation. It is made of stainless steel, nick plated metal or any other dust-proof metal. Before using it is checked that its arms are moving freely and the tips meet properly for giving proper

grip of the crucible and its lid. The instructions as given below are kept in mind regarding the proper use of the crucible tongs.

(a) The dirty tips of the tongs are rubbed with sand paper and wiped with a clean cloth so that these are cleaned.

(b) The clean tongs are kept on the table with tips pointing upward so that these do not catch some matter which may ne transferred to the crucible giving results.

(c) The tips of the tongs should be heated in the flame before lifting the hot crucible or its lid as otherwise it can crack due to large difference in temperatures.

(d) During fitting of the crucible the tip of the tongs should not touch the content of the crucible. Also hold the tongs with the tips pointing towards the back of your hand.

viii) Desiccator

The function of the desiccators is to provide a dry atmosphere in which the crucible is kept so that it is protected from dust, atmospheric moisture and laboratory fumes and gases. It is a large vessel with varying sizes from about 10 to 20 cm in diameter and is provided with tightly fitting cover or lid. The top of the desiccators and its cover are ground flat and thin layer of Vaseline or special grease is applied between them to provide an air tight seal. A porceline plate with holes, wire gauze or clay pipe triangle stand is kept in desiccators for supporting the crucible. Under this supporting device is kept the drying agent which is either anhydrous calcium chloride or dry lime CaO

Precautions

(i) Do not keep the cover with the rim resting on the table but instead either hold it in your left hand or place it beside down on the table.

(ii) The desiccators should always be kept covered except when keeping or removing the crucible.

(iii) The desiccators should be handled with both the hands when it is carried from the working table to the balance room.

(iv) When a hot crucible is kept in the desiccators the cover is kept in yhe position after 5-10 seconds so that the inner expanded air is expelled.

(v) The cover of the desiccators should be removed by sliding it sideways by holding the base tightly otherwise the desiccators would topple and break.

ix) Burners

These types of burners are generally used in gravimetric analysis. These are described a sunder.

(i) Bunsen Burner. This burner is most commonly used in the laboratory. The supply of air is regulated by opening the air holes as desired by getting a steady oxidising flame.

(ii) Meker burner. This burner is used for the ignition of precipitation. a temperature upto 1200^0C can be at tamed with sufficient quality of air is allowed to enter through its holes at the base to get large non-luminous flame. It prevents striking back of the flame and provides smooth heating.

(iii) Blow pipe burner. This burner in used to ignite precipitate transferred to the silica crucible. It provides perfect non-luminous tapering and nosing flame by the complete combustion of oil gas with compressed air. The blast of air comes from electric blower. The complete oxidation raises the temperature to 1200^0C which is equal to that of Meker burner. In comparison to this Meker burner is relatively easy to handle.

3.8 Summary of the unit

All Gravimetric analyses rely on some final determination of weight as a means of quantifying an analyte. Since weight can be measured with greater accuracy than almost any other fundamental property, gravimetric analysis is potentially one of the most accurate classes of analytical methods available. These methods are among the oldest of analytical techniques, and they may be lengthy and tedious. Samples may have to be extensively treated to remove interfering substances. As a result, only a very few gravimetric methods are currently used in environmental analysis.

There are four fundamental types of gravimetric analysis: physical gravimetry, thermogravimetry, precipitative gravimetric analysis, and electrodeposition. These differ in the preparation of the sample before weighing of the analyte. Physical gravimetry is the most common type used in environmental engineering. It involves the physical separation and classification of matter in environmental samples based on volatility and particle size (e.g., total suspended solids). With thermogravimetry, samples are heated and changes in sample mass are recorded. Volatile solids analysis is an important example of this type of gravimetric analysis. As the name implies, precipitative gravimetry relies on the chemical precipitation of an analyte. Its most important application in the environmental field is with the analysis of sulfite. Electrodeposition involves the electrochemical reduction of metal ions at a cathode, and simultaneous deposition of the ions on the cathode.

Nucleation is a process in which several ions of the precipitate come together to form a microsize particle called the nucleus. The particle grows with the addition of ions of the precipitate until the system comes to equilibrium

3.9 Key words

Gravimetric analysis; Precipitation; Post Precipitation; Homogeneous Solution; Gravimetric calculations.

3.10 References for further study

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3.11 Questions for self understanding

1) What is gravimetric analysis?
2) Explain the Principle of Precipitation
3) Discuss the Method of precipitation
4) Write a note on Mechanism of Precipitation
5) Discuss the Purity of the Precipitation
6) Explain efficient condition for Precipitation
7) What are the differences between Post-precipitation and Co - precipitation?
8) What is Peptization?
9) Explain Precipitation from homogeneous Solution
10) What is the advantage of precipitation from Homogeneous solution?
11) Discuss about Gravimetric calculations
12) Explain the Requirements of gravimetric analysis
13) What is meant by seeding? Explain its advantage in precipitation
14)

Unit-4

Structure

4.0 Objectives of the unit

After studying this unit you are able to

- Explain the conditions required for Volumetric analysis
- Explain the Theory of acid-base titrations
- Recognize the Indicators Used in Acid-base Titration
- Explain the Common ion effect
- Explain the procedure of an acid-alkali titration
- Explain the titration of poly protic acid

4.1 Introduction

Quantitative analysis involves the determination of the composition by weights of the constituents of a chemical substance. Quantitative analysis is divided in to two branches.

(i) Volumetric analysis

(ii) Gravimetric analysis.

In the unit 3 we have learned about gravimetric analysis. In this unit we will discuss and know about volumetric analysis. Volumetric analysis is also called quantitative analysis refers to the determination of how much of a given component is present in a solution of a sample.

This is achieved or estimated by determining what volume of one substance that reacts with a known volume of another and it is done through titration. Titration is a method employed in volumetric analysis in which one solution is added to another solution such that it reacts under conditions in which added volume may be accurately measured. Titration is an easy and quick way to find the concentration of an element or compound in solution. Not much is required for a titration, needed are pipette, burette, retort stand and conical flask.

There are two common types of titration

Acid-Base titration

4.2 Oxidation-Reduction titration

Principles of Volumetric Analysis

In volumetric analysis the quantities of the constituents present in the given unknown solution are determined by measuring the volume of the solution taking part in the given chemical reaction. A known volume of the given solution is taken in the titration flask. The solution is called the titrants.

The titrant is made to react with another solution called the titre. Titre is added dropwise to titrant. When the reaction is complete the volume of the titre for a known of the titrant is noted. Knowing volumes of both the solution and the strength of one of them, the strength of the other solution can be calculated this process is called titration. The flask used in titration is called titration flask.

4.3 Types of substance

Two types of substance are generally employed for preparing solutions.

(a) Primary standard substance. These are the substance whose standard solutions can be directly prepared by dissolving a known mass of the substance in a known volume of the solution. Primary standard substance can be easily obtained in the pure and crystalline form. Examples are oxalic acid, anhydrous sodium carbonate, potassium dichromate.

Some important characteristics of primary standard substance are

(i) It should be easily obtained in the pure state and should not be hygroscopic.

(ii) It should not decompose at ordinary temperature.

(iii) It should have high equivalent mass and molecular mass. It minimizes the error due to weighing.

(iv) The substance should be soluble in water or in a given solvent.

(v) Its composition should not change when stored or during weighing.

(b) Secondary standard substance. the substance whose standard solutions cannot be prepared directly are called secondary standard substance. These substances are not easily available in the pure form. Examples are sodium hydroxide, potassium hydroxide, potassium permanganate, hydrochloric acid, sulphuric acid etc.

The composition of such substance keeps on changing when stored or during weighing. Solutions of such substance of approximate normality are prepared and then standardized by titrating against a primary standard solution of known molarity or normality.

4.4 Condition of Volumetric analysis

The following conditions are governed in volumetric analysis of the substance.

(i) The reaction between titrate and titrant should be expressed by a chemical equation.

(ii) The reaction should be instantaneous.

(iii) There should be a marked change in some physical or chemical property of the solution at the end point.

4.5 Equivalence point

It is defined as a stage at which the amount of reagent added is exactly and stoichiometrically equivalent to the amount of the reacting substance in the titrated solution. The end point and equivalence point may not be identical. End point is generally detected only after adding a slight excess of titrant. In majority of the cases the difference between the equivalence point and the end point is well within the experimental error.

4.6 Theory of acid-base titrations

The chemical reaction involved in acid-base titration is known as neutralisation reaction. It involves the combination of H_3O^+ ions with OH^- ions to form water. In acid-base titrations, solutions of alkali are titrated against standard acid solutions. The estimation of an alkali solution using a standard acid solution is called acidimetry. Similarly, the estimation of an acid solution using a standard alkali solution is called alkalimetry**.**

(i) Acidimetry. In acidimetry the given alkali solutions is estimated by using a standard acid solution

(ii) alkalimetry. In alkalimetry the given acid solution is estimated by using a standard alkali solution.

Ostwald developed a theory of acid base indicators which gives an explanation for the colour change with change in pH. According to this theory, a hydrogen ion indicator is a weak organic acid or base. The undissociated molecule will have one colour and the ion formed by its dissociation will have a different color.

Let the indicator be a weak organic acid of formulae HB. It has dissociated into H^+ and B^- . The unionized molecule has one colour, say colour (1), while the ion, B^- has a different colour, say colour (2). Since HB and B^- have different colours, the actual colour of the indicator will dependent upon the hydrogen ion concentration $[H^+]$. When the solution is acidic, that is the H^+ ions present in excess, the indicator will show predominantly colour (1). On other hand, when the solution is alkaline, that is, when OH^- ions present in excess, the H^+ ions furnished by the indicator will be taken out to form undissociated water. Therefore there will be larger concentration of the ions, B^-. Thus the indicator will show predominantly colour (2).

4.7 Indicators Used in Acid-base Titration

Consider an unknown solution of an acid is to be titrated against a standard solution of alkali. For this we take acid in the burette. 20ml alkali solution is taken in the titration flask. Now acid

is added in small quantities in the titration flask till alkali solution is just neutralised. To make the neutralization point visible we add a few drops of another solution in the titration flask. This solution helps in locating the end point by understanding a colour change is called an Indicator.

Thus *Indicator is defined as a substance which produces a visible change in colour when the reaction is complete or when equivalence point is reached*

An indicator is defined as a substance whose solution helps in locating the end point by understanding a colour change. An indicator should fulfill the following condition

(i) It should be readily soluble

(ii) It should not affect the actual chemical reaction.

(iii) It should give a particular colour with one type of ions and a different colour with another type of ions.

(iv) One or two drops of it should be sufficient to produce the necessary colour change.

Some indicators can be used to determine pH because of their colour changes somewhere along the change in pH range. Some common indicators and their respective colour changes are given below

Indicator	**Colour on Acidic Side**	**Range of Colour Change**	**Colour on Basic Side**
Methyl Violet	Yellow	0.0 - 1.6	Violet
Bromophenol Blue	Yellow	3.0 - 4.6	Blue
Methyl Orange	Red	3.1 - 4.4	Yellow
Methyl Red	Red	4.4 - 6.2	Yellow
Litmus	Red	5.0 - 8.0	Blue
Bromothymol Blue	Yellow	6.0 - 7.6	Blue
Phenolphthalein	Colourless	8.3 - 10.0	Pink
Alizarin Yellow	Yellow	10.1 - 12.0	Red

4.8 Indicators Used for Various Titrations

1. ***Strong Acid against a Strong Base***

Let us consider the titration of HCl and NaOH. The pH values of different stages of titration shows that, at first the pH changes very slowly and rise to only about 4. Further addition of such a small amount as 0.01 mL of the alkali raises the pH value by about 3 units to pH 7. Now the

acid is completely neutralized. Further of about 0.01 mL of 0.1 M NaOH will amount to adding hydrogen ions and the pH value will jump to about 9. Thus, near the end point, there is a rapid increase of pH from about 4 to 9.

An indicator is suitable only if it undergoes a change of colour at the pH near the end point. Thus the indicators like methyl orange, methyl red and phenolphthalein can show the colour change in the ph range of 4 to 10. Thus, in strong acid- strong base titrations, any one of the above indicators can be used.

2. Weak Acid against Strong Base

Let us consider the titration of acetic acid against NaOH. The titration shows the end point lies between pH 8 and 10. This is due to the hydrolysis of sodium acetate formed. Hence phenolphthalein is a suitable indicator as its pH range is 8-9.8. However, methyl orange is not suitable as its pH range is 3.1 to 4.5.

3. Strong Acid against Weak Base

Let us consider the titration ammonium hydroxide against HCl. Due to the hydrolysis of the salt, NH4Cl, formed during the reaction, the pH lies in the acid range. Thus, the pH at end point lies in the range of 6 to 4. Thus methyl orange is a suitable indicator while phenolphthalein is not suitable.

Some common indicators which are used in acid-alkali titration are

Types of Titration	**Indicator**	**Alkaline solution**	**Neutral solution**	**Acidic Solution**
Strong acid –Strong base say HCl and NaoH	Any indicator with a colour change between pH 3 and 10	-	-	-
Weak acid- Strong base say CH_3COOH and NaOH	Phenolpthalein	Red	Pink	Colorless
Weak base Strong Acid say Na_2CO_3 and HCl	Methyl orange	Yellow	Orange	Red
Weak acid- weak base CH_3COOH and Na_2CO_3	No satisfactory end point with any indicator. Such titrations are avoided	-	-	-

4.9 Choice of indicator

For a titration if a strong acid and strong base are employed any indicator can be used because the range of pH change at the end point is very large. When a weak acid is titrated against a strong base, it is necessary to use an indicator which changes colour on the alkaline side of pH 7 for this phenolphithalein is most suitable. But when a strong acid is titrated against a weak base then that indicator is used which changes colour on the acid side. Methyl orange is the best indicator for such titration.

Types of Titration	Indicator	pH	Solution	Colour change
Strong acid – Strong Base	Phenolphthalein	8.2-10	Alkaline	Pink to colourless
Strong acid – Strong Base	Phenolphthalein	8.2-10	Acid	Colourless to pink
Strong acid- Weak base	Methyl orange	3.0-4.4	Alkali	Yellow to orange
Weak acid –Strong base	Phenolphthalein	8.0-4.4	Alkali	Pink to colourless

4.10 The theory of acid-base indicators

An acid-base indicator is defined as a substance which changes its colour (within limits) with variation in pH value of the solution to which it is added. The pH range differs considerably from one indicator to another. We know that phenolphthalein gives pink colour in alkaline solution and in acidic solution. It is colourless. The pH range of phenolphthalein is found to 8.3-10.0 and the indicator can be used to determine pH values over this range. Similarly another common indicator i.e Methyl orange can be used over a pH range of 3.0 to 4.4.

3. **Ostwald theory**

According to this theory a hydrogen ion indicator is either a weak organic acid or a weak organic base. The unionized compound has one colour and the ion produced from it, has another colour. The indicator (HI_n) ionizes as follows

$$HI_n \Leftrightarrow HI + I_n^-$$

The ionised and unionized parts of the indicator show different colours in solution. Applying the law of chemical equilibrium.

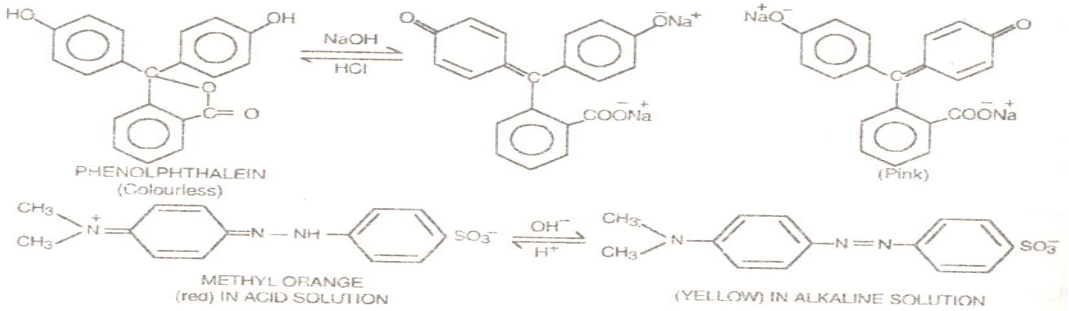
$$K_{\ln} = \frac{[H^+][I_n^-]}{[HI_n]} \quad or\ [H^+] = \frac{K_{\ln}[HI_n]}{[I_n^-]}$$

or

$$\log_{10}[H^+] = \log_{10} k_{\ln} + \log_{10} \frac{[HI_n]}{[I_n^-]} or\ pH = pK_{\ln} + \log_{10} \frac{[I_n^-]}{[HI_n]}$$

Where dK_{in} = indicator constant and $\log_{10}K_{in}=pK_{in}$

Consider the above equilibrium. If the solution is acidic the increase in H^+ ion concentration shifts the equilibrium towards left and hence the colour will be due to unionized molecule (HI_n). on the other hand, if the solution is alkaline then H^+ ions will be taken up by OH^- ions. Then decrease in concentration of hydrogen ion shifts the equilibrium towards right and more of the indicator molecules will ionize. Hence the colour of the solution will be due to I_n^- ions. Thus the indicator has one colour in the acidic solution and different colour in the alkaline solution.



(a) **Action of Phenolphthalein:** it is a colourless organic compound. It dissolves in water and dissociates slightly to form H^+ ions (colourless) and Ph^- ions (pink). The indicator first undergoes a tautomeric reversible change into a substance (HPh^*) of different colour. The substance then ionises into H^+ ions and Ph^- ions.

$$HPh \leftrightarrow HPh^* \leftrightarrow H^+ + Ph^-$$

Case I. if the solution is made acidic by adding HCl then the increase in H^+ ion concentration suppresses the dissociation of phenolphthalein. It is due to common ion effect. The equilibrium shifts towards left and the solution remains colourless.

Case II. The solution is made alkaline by adding a few drops of NaOH the OH^- ions respect with H^+ ions to form unionsed water molecules. The decrease in H^+ ion concentration shifts the

equilibrium towards rifgt. Thus more mof phenolphthalein ionises and the solution becomes pink (due to Ph^- ion) hence the indicator is colourlrss in the acidic solution and pick in the alkaline solution.

(b) **Action of methyl orange**. It is an orange coloured organic compound and acts as a weak base. It is soluble in water and ionises to a very small extent as below.

$$\underset{\text{(Orange)}}{MeOH} \leftrightarrow \underset{\text{(pink)}}{Me^+ + OH^-}$$

Case I. If the solution is made acidic then the hydrogen ions furnished by the acid sombine with OH^- ions from unionized water molecules. Thus the decrease in $OH^—$ ion concentration shifts the equilibrium towards right. Hence the solution acquires a pink colour a pink due to Me^+ ions.

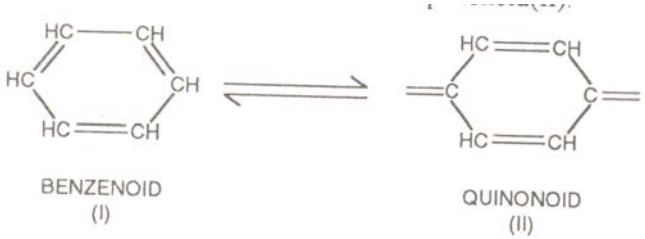
Case II if the solution is made alkaline by adding NaOH solution, then the conc of OH^- ion will increase in the solution. The increase in concentration of OH^- ions suppresses the dissociation of methyl orange. Thus the equilibrium shifts towards the left the colour of the solution will be due to unionized MeOH molecules (yellow). Thus methyl orange is red in acidic solution and yellow in alkaline solution.

Note: Methyl orange is not a suitable indicator for titrating a weak acid say acetic acid against a strong base. We know that acetic acid is a weak acid and the H^+ ions furnished by it are too few to combine with a sufficient number of hydroxyl ions of methyl orange to shift the equilibrium in favor of pink coloured Me^+ ions. Thus, more of weak acid will have to be added to get the pink colour.

2. Quinonoid theory

The following are the main features of the theory

(i) Acid-base indicators are complex aromatic molecules which are capable of existing in two tautomeric forms in equilibrium with each other. One of the two forms is called benzenoid (I) and the other form is known as quinonoid(II).



(ii) On form is stable in acidic medium while the other form is stable in the alkaline medium.

(iii) The two forms have different colours. The colour of form(I) is lighter than that of form(II).

(iv) If the medium of solution changes from acidic to base and vice-versa the colour changes due to change in the form.

Important points

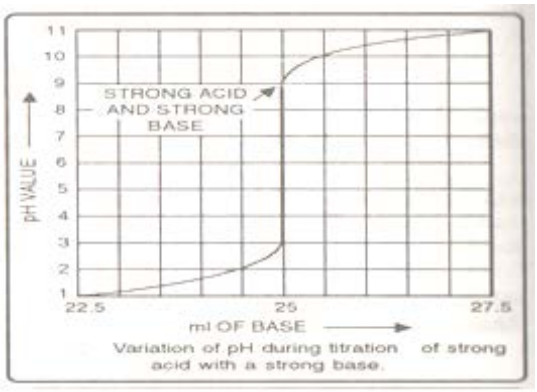
1. An indicator should be added by means of glass dropper or a dropping bottle.
2. Always use one or two drops of the indicator. If a large volume of indicator is used the change in colour at end point will not be sharp.
3. The same number of drops of the indicator should be used for each titration.

4.11 Variation of pH during different types of titrations

In acid alkali titration the variation in pH during the titration is discussed below.

(v) Titration of a strong acid with a strong base

Consider the titration of 25ml of 0.1 N HCl (strong acid) against 0.1 N NaOH (strong base). The base is continuously added and the pH value of the solution plotted against the volume of the base added. As expected the pH of the solution at the neutralization point should be equal to 7.0. It has been found that the pH value of the solution just before neutralization was 3.0 and just after the neutralization, it is 9.5. Hence all the indicators which have pH range between 3.0 to 9.5 are suitable for the titration of a strong acid with a strong base.

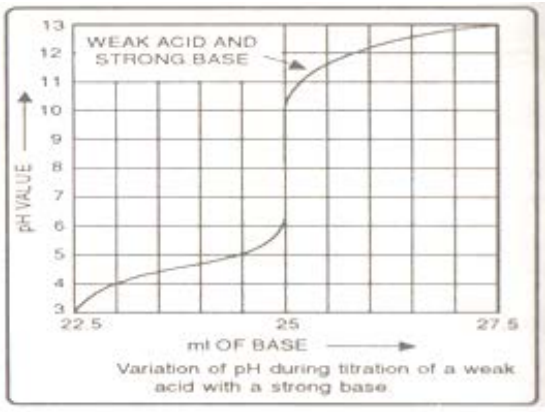


Variation of pH during titration of strong acid with a strong base.

(vi) Titration of a weak acid with strong base

Consider the titration of 25ml of 0.1 N acetic acid (weak acid) with 0.1 N NaOH solution (strong base). Acetic acid reacts with sod. Hydroxide to form sodium acetate and water. Sodium acetate on hydrolysis forms a basic solution. clearly the end point lies at some pH value which is more than 7.0. The base is continuously added and the pH value of the solution is plotted against the volume of the base added. Clearly for such a titration the indicator

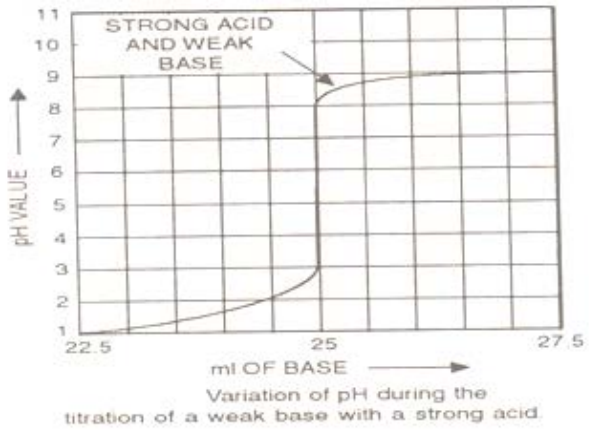
should be selected which has a pH range between 7.0 (neutralization point) to 9.0 phenolpthalein has a pH range between 8 to 9.8 and serves the purpose best.



Variation of pH during titration of a weak acid with a strong base

(vii) Titration of a weak base with a strong acid

Consider the titration of 25ml of 0.1 N ammonium hydroxide solution (weak base) against 0.1 N hydrochloric acid(Strong acid). Ammonium hydroxide reacts with hydrochloric acid to form ammonium chloride and water. Ammonium chloride on hydrolysis forms an acidic solution. the base is continuously added and the pH value of the solution is plotted against the volume of base added. Clearly the end point lies at some pH value which is less than 7.0. here for such a titration the indicator should be selected which has a pH range somewhere below 7. Methyl orange has a pH range between 3.0 to 4.5 and hence serves the purpose best .

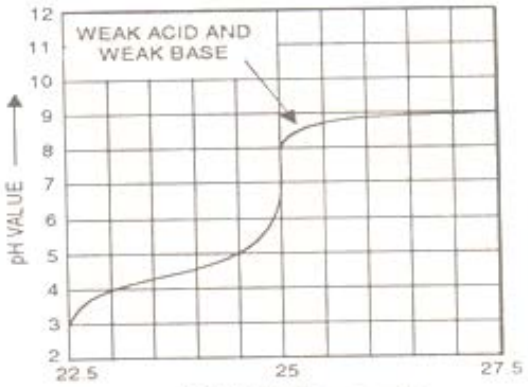


Variation of pH during the titration of a weak base with a strong acid

(viii) Titration of a weak acid with a weak base

Consider the titration of 25 ml of 0.1 N ammonia hydroxide solution (weak base) against 0.1N oxalic acid (weak acid). The base is continuously added and the pH value of the solution is plotted against the volume of the base added. From the curve obtained, we see that in this case

there is no sharp change in the pH value. Thus, such a titration is not possible. Phenol red has a pH range (6.8 to 8.4) and hence by using this indicator, approximate determination of the neutralization point can be made.



Variation of pH during the titration of a weak acid with a weak base

4.12 Universal or Multiple Range Indicators

By suitable mixing certain indicators, the colour change may be made to extend over a considerable portion of the pH range. Such mixtures are usually called universal indicators.

These indicators are not suitable for the quantitative titrations. However these indicators can be used to determine the approximate pH of a solution by noting down the change in colour. One such universal indicator can be obtained by dissolving 0.1 g of phenolphthalein, 0.2 g of methyl red 0.3 g of methyl yellow 0.4 g of bromothymol blue and 0.5 g of thymol blue in 500ml of absolute alcohol and then adding sufficient NaOH solution so that solution acquires yellow colour. The colour changes are as follows:

pH 2, red; pH 4, orange; pH 6, yellow; pH 8 green; pH 10 blue.

4.13 Common ion effect

Weak electrolytes dissociate to a very small extent and there exists a dynamic equilibrium between the unionized electrolyte molecule and the ions from it.

$$AB \rightleftharpoons A^{+}+B^{-}$$

If the solution of a strong electrolyte (AX or YB) is mixed with that of the weak electrolyte having a common ion, the degree of dissociation of both electrolytes in suppressed. However the decrease is much more in case of the weak electrolyte. For example by adding ammonium chloride to ammonium hydroxide solution (weak electrolyte) the degree of ionization of

ammonium hydroxide solution (weak electrolyte) the degree of ionization of ammonium hydroxide is suppressed. This can be explained using Le-chatelier's principle.

Ammonium chloride being a strong electrolyte, ionises almost completely. This leads to a high ceoncentration of ammonium ions in solution. therefore according to Le-chateler's principle NH_4 and OH^- ions combine to decrease the concentration of ammonium ions in solution. Thus dissociation of NH_4 OH is suppressed.

The suppression of the degree of ionization of a weak electrolyte by the addition of a strong electrolyte having an ion common with the weak electrolyte, is known as common ion effect.

4.14 Procedure of an acid-alkali titration

An acid- alkali titration involves a following steps

(a) Cleaning and Rinsing the apparatus

Collect the whole apparatus required for the titration viz two beakers, burette, titration flask, A pipette, A funnel. See that no item is cracked or broken. Clean each piece of apparatus to remove greasy matter. Then wash with free excess of water and then with small quantity of distilled water.

Rinse the burette and funnel with the acid solution. rinse the pipette with the alkali solution. never rinse the titration flask with alkali solution. wash it with water before and after the titration.

It may be noted that a piece of glass apparatus is clean, if no drop of water sticks inside it.

(b) Charging the Burette

Fix the burette in the stand so that it stands vertical. Now fill it with the acid solution and mount the antiparallax card. Now open the stop cock with a jerk so that the acid solution forces out of nipple. No air bubble should remain inside the nozzle. Bring the level of the solution below the zero mark. After a minute take the burette reading. It is called initial reading.

(c) How to do volumetric titration

Wash the titration flask with water. Put 20 ml of alkali solution in it by means of a pipette. Add to it one or two drops of the indicator. Place the titration flask on the glazed tile so that the tip of the burette is inside the neck of the titration flask. Now run the acid solution from the burette in lots. The solution in the titration flask is given a swirling motion after each addition. Always open the stop cock gently by left hand and give a swirling motion to the titration flask with the right hand. When the indicator begins changing colour the acid solution is added more slowly

(one drop at a time). In case phenolphthalein is used the pink colour starts fading. The solution is given swirling motion continuously after each addition. A stage comes when there is an apparent change in colour. Here the addition of acid is recorded. It is called the final reading. The difference of the final reading and the initial reading gives the volume of acid used. This volume of acid is just sufficient for the neutralizatiuon of 20 ml alkali solution. the whole procedure involved for finding the volume of the acid required neutralize 20 ml alkali solutions is called titration. Repeat the titration in the same way till three concordant reading are obtained.

(d) Concordant readings

If the difference in the volume of acid used by repeating a titration is not more than 0.05 ml then two readings are said to be concordant. Consider an acid-alkali titration. Let 20 ml of alkali solution be titrated against a standard acid solution. The titration is repeated four times. The data is recorded as under.

Sl.no	**Initial Reading**	**Final reading**	**Vol of acid used**
01	2.30	22.60	20.30 ml(Rough)
02	6.50	26.75	20.25 ml
03	7.25	27.45	20.20ml
04	5.90	26.10	20.20ml

In this case volumes 20.25 ml, 20.20 ml and 20.20 ml are set of three concordant readings. The average volume is generally taken for making calculations.

4.15 Important Points

(i) Record all the readings directly in your note book shown in table

(ii) Take the rough readings to start with. For this take 20 ml of alkali solution in the titration flask. Add 1 ml of acid from the burette each time with constantly shaking the flask. Suppose after adding 20 ml of acid solution there is no change in colour. But on adding the next 1 ml the colour changes. This shows that the exact volume of acid required for complete neutralization lies between 20 to 21 ml. for repeating the titration take 20 ml alkali solution and add 19.5 ml acid solution rapidly from the burette. Further volume required for the neutralization point is added dropwise. This avoids the wastage of time.

(iii) If a drop of acid sticks with the side of titration flask wash it down with a spray of distilled water.

4.16 Some important Relations in acid- Base titrations

1. ***Calculating Normality***

For calculating the normality of an unknown solution use naormality equation

$N_1V_{1(acid)} = N_2V_{2(base)}$

Where N_1 is the normality of acid V_1 is the volume of the acid used [from concordant burette readings], N_2 is the normality of acid V_1 is the volume of the alkali solution taken for each titration Thus V_2=20 ml. now if the normality of acid is known of alkali can be calculated.

2. ***Calculating the strength***

Strength of a solution is calculated by multiplying its normality with equivalent weight.

Strength = normality x Equivalent Weight

The strength of the solution is written in grams per liter.

If the strength of the solution is given then the Equivalent weight of the substance can be calculated

Equivalent weight=Strength/ normality

3. ***Basicity of an acid***

Knowing the molecular weight of an acid, its basicity can be calculated as f ollows.

$$Basicity = \frac{Molecular\ weight\ of\ the\ acid}{Equivalent\ weight}$$

4. ***Acidity of a base***

Knowing the molecular weight of a base its Acidity can be calculated as follows

$$Acidity = \frac{Molecular\ weight\ of\ the\ base}{Equivalent\ weight}$$

Note-it is important to note that the acidity or basicity is always a whole number. If basicity comes out to be 1.9 take it as 2 i.e nearest whole number.

4.17 Titration of poly protic acid

Polyprotic acids, also known as polybasic acids, are able to donate more than one proton per acid molecule.

The first dissociation constant is typically greater than the second; i.e., $K_{a1} > K_{a2}$.

Although the subsequent loss of each sequential hydrogen ion is increasingly less favorable, all of the conjugate bases are present in solution.

For example, Sulfuric acid (H_2SO_4) can donate a proton to form the bisulfate anion (HSO_4^-), for which K_{a1} is very large. Then it can donate a second proton to form the sulfate anion (SO_4^{2-}), wherein the K_{a2} is intermediate.

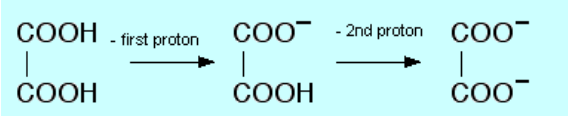
monoprotic acids are acids able to donate one proton per molecule during the process of dissociation (sometimes called ionization) as shown below (symbolized by HA)

$$HA_{(aq)} + H_2O_{(l)} \rightleftharpoons H_3O^+_{(aq)} + A_{(aq)}$$

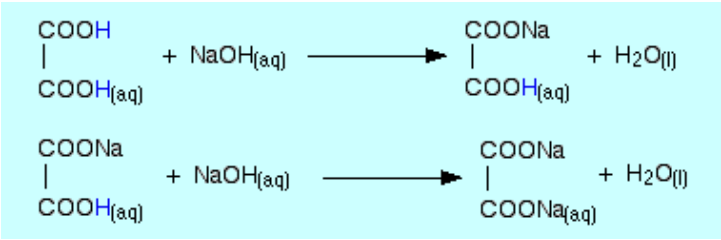
Common examples of monoprotic acids in mineral acids include hydrochloric acid (HCl) and nitric acid (HNO_3). On the other hand, for organic acids the term mainly indicates the presence of one carboxylic acid group and sometimes these acids are known as monocarboxylic acid.

Polyprotic acids, also known as polybasic acids, are able to donate more than one proton per acid molecule, in contrast to monoprotic acids that only donate one proton per molecule. Certain types of polyprotic acids have more specific names, such asdiprotic acid (two potential protons to donate) and triprotic acid (three potential protons to donate).

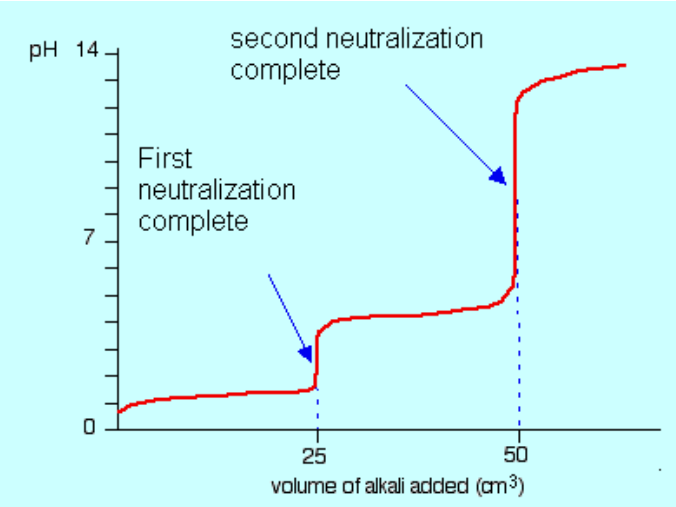
A diprotic acid, oxalic acid, also called ethanedioic acid as two protons to donate.



If a dilute solution of oxalic acid were titrated with a sodium hydroxide solution, the protons would react in a stepwise neutralization reaction



If the pH of this titration were recorded and plotted against the volume of NaOH added, a very clear picture of the stepwise neutralization emerges. Note the very distinct equivalence points on the titration curves.



This oxalic acid is an example of an acid able to enter into a reaction with two available protons

$$H_2A(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + HA^-(aq) \qquad K_{a1}$$
$$HA^-(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + A^{2-}(aq) \qquad K_{a2}$$

Likewise, a triprotic, or tribasic, system can be envisioned. Each reaction proceeds with its unique value of Ka, affected by the pH environment and inductive effects of neutralization.

$$H_3A(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + H_2A^-(aq) \qquad K_{a1}$$
$$H_2A^-(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + HA^{2-}(aq) \qquad K_{a2}$$
$$HA^{2-}(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + A^{3-}(aq) \qquad K_{a3}$$

An inorganic example of a triprotic acid is orthophosphoric acid (H_3PO_4), usually just called phosphoric acid. All three protons can be successively lost to yield $H_2PO_4^-$, then HPO_4^{2-}, and finally PO_4^{3-}, the orthophosphate ion, usually just called phosphate. An organic example of a triprotic acid is citric acid, which can successively lose three protons to finally form the citrate ion. Even though the positions of the protons on the original molecule may be equivalent, the successive K_a values will differ since it is energetically less favorable to lose a proton if the conjugate base is more negatively charged.

Although the subsequent loss of each hydrogen ion is less favorable, all of the conjugate bases are present in solution. The fractional concentration, α (alpha), for each species can be calculated. For example, a generic diprotic acid will generate three species in solution: H_2A, HA,

and A^{2-}. The fractional concentrations can be calculated as below when given either the pH (which can be converted to the $[H^+]$) or the concentrations of the acid with all its conjugate bases.

$$\alpha_{H_2A} = \frac{[H^+]^2}{[H^+]^2 + [H^+]K_1 + K_1K_2} = \frac{[H_2A]}{[H_2A] + [HA^-] + [A^{2-}]}$$

$$\alpha_{HA^-} = \frac{[H^+]K_1}{[H^+]^2 + [H^+]K_1 + K_1K_2} = \frac{[HA^-]}{[H_2A] + [HA^-] + [A^{2-}]}$$

$$\alpha_{A^{2-}} = \frac{K_1K_2}{[H^+]^2 + [H^+]K_1 + K_1K_2} = \frac{[A^{2-}]}{[H_2A] + [HA^-] + [A^{2-}]}$$

4.18 Summary of the unit

Volumetric or titrimetric analyses are quantitative analytical techniques which employ a titration in comparing an unknown with a standard. In a titration, a volume of a standardized solution containing a known concentration of reactant "A" is added incrementally to a sample containing an unknown concentration of reactant "B". The titration proceeds until reactant "B" is just consumed (stoichiometric completion). This is known as the equivalence point. At this point the number of equivalents of "A" added to the unknown equals the number of equivalents of "B" originally present in the unknown. Volumetric methods have the potential for a precision of up to 0.1%.

For volumetric methods to be useful, the reaction must reach 99%+ completion in a short period of time. In almost all cases, a buret is used to meter out the titrant. When a titrant reacts directly with an analyte (or with a reaction the product of the analyte and some intermediate compound), the procedure is termed a direct titration. The alternative technique is called a back titration. Here, an intermediate reactant is added in excess of that required to exhaust the analyte, then the exact degree of excess is determined by subsequent titration of the unreacted intermediate with the titrant. Regardless of the type of titration, an indicator is always used to detect the equivalence point. Most common are the internal indicators, compounds added to the reacting solutions that undergo an abrupt change in a physical property (usually absorbance or color) at or near the equivalence point. Sometimes the analyte or titrant will serve this function (auto indicating). External indicators, electrochemical devices such as pH meters, may also be used. Ideally, titrations should be stopped precisely at the equivalence point. However, the ever-present random and systematic error, often results in a titration endpoint, the point at which a titration is stopped, that is not quite the same as the equivalence point. Fortunately, the

systematic error, or bias may be estimated by conducting a blank titration. In many cases the titrant is not available in a stable form of well-defined composition. If this is true, the titrant must be standardized (usually by volumetric analysis) against a compound that is available in a stable, highly pure form (i.e., a primary standard).

The basic requirements or components of a volumetric method are:

1. A standard solution (i.e., titrant) of known concentration which reacts with the analyte with a known and repeatable stoichiometry (i.e., acid/base, precipitation, redox, complexation)
2. A device to measure the mass or volume of sample (e.g., pipet, graduated cylinder, volumetric flask, analytical balance)
3. A device to measure the volume of the titrant added (i.e., buret)
4. If the titrant-analyte reaction is not sufficiently specific, a pretreatment to remove interferents
5. A means by which the endpoint can be determined. This may be an internal indicator (e.g., phenolphthalein) or an external indicator (e.g., pH meter).

14.19 Key words

Oxidation-Reduction titration; Volumetric analysis; Equivalence point; Indicators; Ostwald theory; Quinonoid theory.

14.20 References for further study

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14.21 Questions for self understanding

1) What are redox reactions? give one example.
2) Explain the Condition of Volumetric analysis
3) What is Equivalence point in titration?
4) Discuss the Theory of acid-base titrations
5) What is indicator? What are the indicators Used in Acid-base titrations?

6) Discuss briefly Choice of indicator in titration reactions

7) Explain the theory of acid-base indicators

1. Ostwald theory
2. Quinonoid theory

8) Explain the Common ion effect

9) Discuss the Variation of pH during following types of titrations

a) Titration of a strong acid with a strong base
b) Titration of a weak acid with strong base
c) Titration of a weak base with a strong acid
d) Titration of a weak acid with a weak base

10) Write a note on Universal or Multiple Range Indicators

11) Explain the procedure follows in acid-alkali titration

12) Explain titration of poly protic acid

Unit-5

Structure

5.0 Objectives of the unit

- After studying this unit you are able to
- Explain the terms involved in the titration analysis
- Explain the titrimetric reactions
- Explain the method of end-point detections
- Explain the Mohr method
- Explain the Volhard method
- Explain the Absorption indicators

5.1 Introduction

A titration is the process of determining the quantity of a substance A by adding measured increments of substance B, with which it reacts with provision for some means of recognizing the point, the end point at which essentially all of A has reacted. If the end point coincides with the addition of the exact chemical equivalence, it is called the equivalence point or stoichiometric or theoretical end point, thus allowing the amount of A to be found from known amount of B added up to this point, the reacting weight ratio of A to B being known from stoichiometry or otherwise. Terms for varieties of titration can reflect the nature of the reaction between A and B. Thus, there are acid-base, complexometric, chelatometric, oxidation-reduction, and precipitation titrations.

Additionally, the term can reflect the nature of the titrant, such as acidimetric, alkalimetric, iodimetric titrations as well as coulometric titrations, in which the titrant is generated electrolytically rather than being added as a standard solution.

A parallel series of terms involves substituting the word titrimetry for titration, or, more economically, eliminating either word and using -metry endings denoting either the chemistry involved, as in acidimetry, alkalimetry, iodimetry, and complexometry, or the means of end point detection, as in amperometric, chronopotentiometric, conductometric, fluorometric, high frequency, nephelometric, potentiometric, radiometric, spectrophotometric, thermometric, and turbidimetric titrations. The titrant solution, containing the active agent with which a titration is made, is standardized, i.e., the concentration of the active agent is determined, usually by titration with a standard solution of accurately known concentration.

5.2 Definitions of the term involved in titration

The definite volume of the analyte (i.e.; the substance to be determined) is allowed to react with a suitable reagent whose standard solution can be prepared and the volume of the solution consumed for complete reaction is used to find out the concentration of analyte solution. At this point, it is necessary to know definitions of some useful terms

e) Titration

Titration is the overall procedure for the determination of the stoichiometric or equivalence point. Or *the process of determining analyte by adding the small increments of standard solution untill the reaction is just complete the reacting ratio of the two being known from stoichiometry or otherwise is called titration.*

A titrimetric method involves the controlled reaction of a standard reagent in known amounts with a solution of the analyte, in order that the *stoichiometric* or *equivalence point* for the reaction between the reagent and the analyte may be located. If the details of the reaction are known and the stoichiometric point is located accurately and precisely, the amount of analyte present may be calculated from the known quantity of standard reagent consumed in the reaction. In most cases a standard reagent solution is prepared and added manually or automatically from a burette; an alternative procedure is coulometric generation of the reagent *in situ*. The stoichiometric point may be detected by use of a visual indicator or by an electrochemical method

f) Titrant and Titrand

Titrant is a solution added or reagent generated in a titration. In Titrimetric analyses, the solution of accurately known concentration i.e.; standard solution is called the Titrant and the substance to be determined is called Titrand or analyt (in other words the solution to which the titrant is added).

g) End Point

A point in the progress of the reaction which may be precisely located and which can be related to the stoichiometric or equivalence point of the reaction; ideally, the two should be coincident. The point in a titration at which the amount of titrant added is chemically equivalent to the amount of substance titrated i. e. The point at which the completion of the reaction occurs is called the equivalence point or theoretical or stoichiometric end point. *The point at which the completion of a reaction is practically observed is called end point.*

h) Indicator

A reagent or device used to indicate when the end point has been reached is called indicator.

5.3 Titrimetric Reactions

It is clear that reactions suitable for use in titrimetric procedures must be stoichiometric and must be fast if a titration is to be carried out smoothly and quickly. Generally speaking, ionic reactions do proceed rapidly and present few problems. On the other hand, reactions involving covalent bond formation or rupture are frequently much slower and a variety of practical procedures are used to overcome this difficulty. The most obvious ways of driving a reaction to completion quickly are to heat the solution, to use a catalyst, or to add an excess of the reagent. In the last case, a *back titration* of the excess reagent will be used to locate the stoichiometric point for the primary reaction. Reactions employed in titrimetry may be classified as acid-base; oxidation-reduction; complexation; substitution; precipitation.

5.4 End-point Detection

A prerequisite for a precise and accurate titration is the reproducible identification of an *end point* which either coincides with the stoichiometric point of the reaction or bears a fixed and measurable relation to it. An end point may be located either by monitoring a property of the titrand which is removed when the stoichiometric point is passed, or a property which can be readily observed when a small excess of the titrant has been added. The most common processes observed in end-point detection are change of colour; change of electrical cell potential; change of electrical conductivity; precipitation or flocculation.

5.5 Visual Indicators

The use of a colour change to indicate the end point is common to a wide variety of titrimetric methods. Visual detection of end points is a major factor in maintaining the simplicity of titrimetry, hence the capability of the human eye to detect colour change plays an important role in these techniques. In general terms a visual indicator is a compound which changes from one colour to another as its chemical form changes with its chemical environment

$$\underset{\text{colour 1}}{\mathrm{In_A}} = \underset{\text{colour 2}}{\mathrm{In_B}} + n\mathrm{X}$$

where X may be H^+, Mn^+ or e^-, and the colour of the indicator is sensitive to the presence of H^+, Mn^+, oxidants or reductants. An *indicator constant* is defined as,

$$K_{In} = \frac{[In_B][X]^n}{[In_A]}$$

whence

$$[X]^n = K_{In}([In_A]/[In_B])$$

and

$$npX = pK_{In} + \log_{10}([In_B]/[In_A])$$

If the indicator is present in an environment where a titration reaction generates or consumes the X species, the indicator will change with the concentration of X in the solution and the colour of the solution will be determined by the ratio $[In_B]/[In_A]$. As a general guide, the eye will register a complete change from one colour to the other when this ratio changes from 10:1 to 1:10. Substitution in equation (5.4) enables the concentration range of X over which the indicator will change colour to be calculated, i.e.

$$npX = pK_{In} \pm 1$$

For example, the acid-base indicator methyl orange has a pK_{In} of 3.7 and will thus change colour over the pH range 2.7–4.7. The ultimate sharpness of the end point will further depend upon the rate at which pX is changing at the end point of the titration. The additional factors involved in determining this rate of change are examined later in the discussions of specific titration methods. Because the indicator competes with the analyte and reagent for X it is obvious that the indicator concentration must be kept as low as possible in order to minimize interference with the analyte-reagent equilibrium. It follows that the colours exhibited by an indicator must be of a high intensity.

5.6 Apparatus for Titrimetric Analysis

In general, the apparatus for titrimetric analysis is simple in construction and operation. A typical analysis procedure would involve measurement of the amount of sample either by mass or volume, and then addition of the titrant from a burette or micro-syringe. Apart from visual indication, the course of a titration may be followed by electrochemical or photometric means; in neither is the equipment required complex. A simple valve voltmeter or conductivity bridge will

suffice on the one hand and a simple spectrophotometer or filter photometer with minor modifications on the other. Varying degrees of automation may be incorporated.

Titrimetric methods are classified into four groups based on the type of reaction involved. These groups are acid–base titrations, in which an acidic or basic **titrant** reacts with an analyte that is a base or an acid; complexometric titrations involving a metal–ligand complexation reaction; redox titrations, where the titrant is an oxidizing or reducing agent; and precipitation titrations, in which the analyte and titrant react to form a precipitate. Despite the difference in chemistry, all titrations share several common features, providing the focus for this section.

5.7 Equivalence Points and End Points

For a titration to be accurate we must add a stoichiometrically equivalent amount of titrant to a solution containing the analyte. We call this stoichiometric mixture the **equivalence point.** Unlike precipitation gravimetry, where the precipitant is added in excess, determining the exact volume of titrant needed to reach the equivalence point is essential. The product of the equivalence point volume, V_{eq}, and the titrant's concentration, C_T, gives the moles of titrant reacting with the analyte.

Moles titrant = $V_{eq} \times C_T$

Knowing the stoichiometry of the titration reaction(s), we can calculate the moles of analyte. Unfortunately, in most titrations we usually have no obvious indication that the equivalence point has been reached. Instead, we stop adding titrant when we reach an **end point** of our choosing. Often this end point is indicated by a change in the color of a substance added to the solution containing the analyte. Such substances are known as **indicators.** The difference between the end point volume and the equivalence point volume is a determinate method error, often called the **titration error.** If the end point and equivalence point volumes coincide closely, then the titration error is insignificant and can be safely ignored. Clearly, selecting an appropriate end point is critical if a titrimetric method is to give accurate results.

5.8 Titration Curves

To find the end point we monitor some property of the titration reaction that has a well-defined value at the equivalence point. For example, the equivalence point for a titration of HCl with NaOH occurs at a pH of 7.0. We can find the end point,

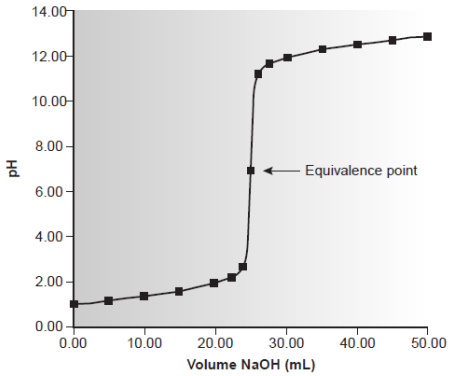


Fig: 1 Acid–base titration curve for 25.0 mL of 0.100 M HCl with 0.100 M NaOH.

Therefore, by monitoring the pH with a pH electrode or by adding an indicator that changes color at a pH of 7.0. Suppose that the only available indicator changes color at a pH of 6.8. Is this end point close enough to the equivalence point that the titration error may be safely ignored? To answer this question we need to know how the pH changes during the titration.

A **titration curve** provides us with a visual picture of how a property, such as pH, changes as we add titrant (Figure 1). We can measure this titration curve experimentally by suspending a pH electrode in the solution containing the analyte, monitoring the pH as titrant is added. As we will see later, we can also calculate the expected titration curve by considering the reactions responsible for the change in pH. However we arrive at the titration curve, we may use it to evaluate an indicator's likely titration error. For example, the titration curve in Figure 9.1 shows us that an end point pH of 6.8 produces a small titration error. Stopping the titration at an end point pH of 11.6, on the other hand, gives an unacceptably large titration error.

The titration curve in Figure 1 is not unique to an acid–base titration. Any titration curve that follows the change in concentration of a species in the titration reaction (plotted logarithmically) as a function of the volume of titrant has the same general sigmoidal shape. Several additional examples are shown in Figure 2.

Concentration is not the only property that may be used to construct a titration curve. Other parameters, such as temperature or the absorbance of light, may be used if they show a significant change in value at the equivalence point. Many titration reactions, for example, are exothermic. As the titrant and analyte react, the temperature of the system steadily increases. Once the titration is complete, further additions of titrant do not produce as exothermic a response, and the change in temperature levels off. A typical titration curve of temperature

versus volume of titrant is shown in Figure 3. The titration curve contains two linear segments, the intersection of which marks the equivalence point

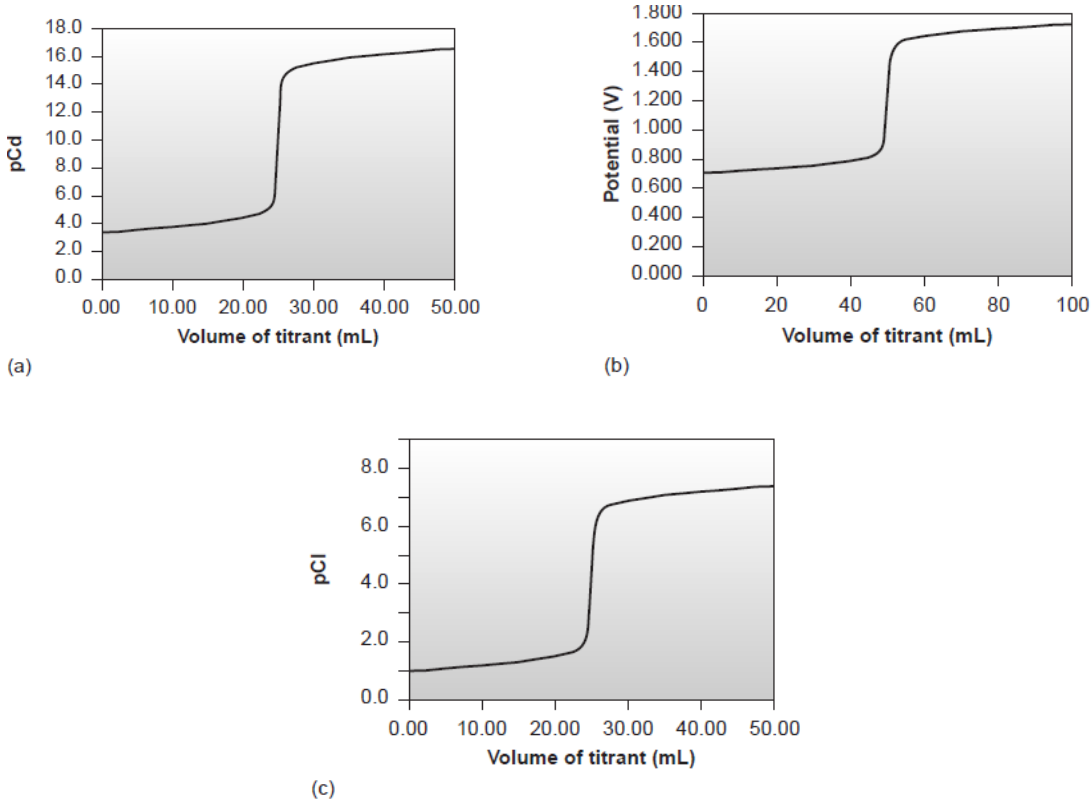


Figure.2 Examples of titration curves for (a) a complexation titration, (b) a redox titration, and (c) a precipitation titration.

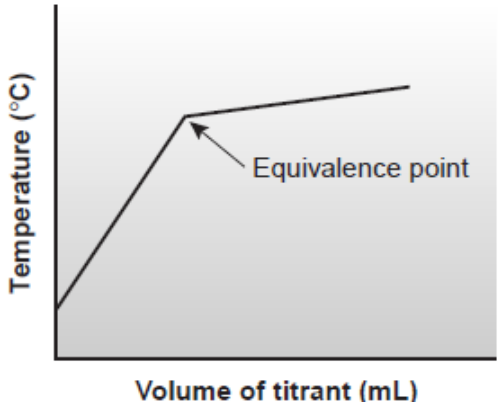


Figure. 3 Example of a thermometric titration curve.

5.9 Acid - Base Titration Curves

In the overview to this chapter we noted that the experimentally determined end point should coincide with the titration's equivalence point. For an acid–base titration, the equivalence point is characterized by a pH level that is a function of the acid–base strengths and concentrations of

the analyte and titrant. The pH at the end point, however, may or may not correspond to the pH at the equivalence point. To understand the relationship between end points and equivalence points we must know how the pH changes during a titration. In this section we will learn how to construct titration curves for several important types of acid–base titrations. We also will learn how to sketch a good approximation to any titration curve using only a limited number of simple calculations.

Titrating Strong Acids and Strong Bases

For our first titration curve let's consider the titration of 50.0 mL of 0.100 M HCl with 0.200 M NaOH. For the reaction of a strong base with a strong acid the only equilibrium reaction of importance is

$$H_3O^+ (aq) + OH^-(aq) \leftrightarrow 2H_2O (l) \ldots\ldots\ldots\ldots\ldots\ldots \textbf{1}$$

The first task in constructing the titration curve is to calculate the volume of NaOH needed to reach the equivalence point. At the equivalence point we know from reaction 1 that

$$\text{Moles HCl} = \text{moles NaOH or } M_aV_a = M_bV_b$$

where the subscript 'a' indicates the acid, HCl, and the subscript 'b' indicates the base, NaOH. The volume of NaOH needed to reach the equivalence point, therefore, is

$$V_{eq} = V_b = \frac{M_aV_a}{M_b} = \frac{(0.100\ \text{M})(50.0\ \text{mL})}{(0.200\ \text{M})} = 25.0\ \text{mL}$$

Before the equivalence point, HCl is present in excess and the pH is determined by the concentration of excess HCl. Initially the solution is 0.100 M in HCl, which, since HCl is a strong acid, means that the pH is

$$\text{pH} = -\log[H_3O^+] = -\log[\text{HCl}] = -\log(0.100) = 1.00$$

The equilibrium constant for reaction 9.1 is (Kw)–1, or 1.00 1014. Sin

value we can treat reaction 9.1 as though it goes to completion. After adding 10.0 mL of NaOH, therefore, the concentration of excess HCl is

$$[\text{HCl}] = \frac{\text{moles excess HCl}}{\text{total volume}} = \frac{M_aV_a - M_bV_b}{V_a + V_b}$$

$$= \frac{(0.100\ \text{M})(50.0\ \text{mL}) - (0.200\ \text{M})(10.0\ \text{mL})}{50.0\ \text{mL} + 10.0\ \text{mL}} = 0.050\ \text{M}$$

giving a pH of 1.30.

At the equivalence point the moles of HCl and the moles of NaOH are equal. Since neither the acid nor the base is in excess, the pH is determined by the dissociation of water.

$$Kw = 1.00 \text{ X } 10^{-14} = [H3_O+][OH–] = [H_3O+]^2$$

$$[H_3O+] = 1.00 \text{ X } 10^{-7} \text{ M}$$

Thus, the pH at the equivalence point is 7.00.

Finally, for volumes of NaOH greater than the equivalence point volume, the pH is determined by the concentration of excess OH^-. For example, after adding 30.0 mL of titrant the concentration of OH^- is,

Data for Titration of 50.00 mL of 0.100 M HCl with 0.0500 M NaOH

Volume (mL) of Titrant	pH
0.00	1.00
5.00	1.14
10.00	1.30
15.00	1.51
20.00	1.85
22.00	2.08
24.00	2.57
25.00	7.00
26.00	11.42
28.00	11.89
30.00	12.50
35.00	12.37
40.00	12.52
45.00	12.62
50.00	12.70

$$[OH^-] = \frac{\text{moles excess NaOH}}{\text{total volume}} = \frac{M_b V_b - M_a V_a}{V_a + V_b}$$

$$= \frac{(0.200 \text{ M})(30.0 \text{ mL}) - (0.100 \text{ M})(50.0 \text{ mL})}{50.0 \text{ mL} + 30.0 \text{ mL}} = 0.0125 \text{ M}$$

To find the concentration of H_3O+, we use the *K*w expression

$$[H_3O^+] = \frac{K_w}{[OH^-]} = \frac{1.00 \times 10^{-14}}{0.0125} = 8.00 \times 10^{-13}$$

giving a pH of 12.10. Table 9.2 and Figure 9.1 show additional results for this titration curve. Calculating the titration curve for the titration of a strong base with a strong acid is handled in

the same manner, except that the strong base is in excess before the equivalence point and the strong acid is in excess after the equivalence point.

Titrating a Weak Acid with a Strong Base

For this example let's consider the titration of 50.0 mL of 0.100 M acetic acid, CH_3COOH, with 0.100 M NaOH. Again, we start by calculating the volume of NaOH needed to reach the equivalence point; thus

Moles CH_3COOH = moles NaOH-

$$MaVa = MbVb$$

$$V_{eq} = V_b = \frac{M_a V_a}{M_b} = \frac{(0.100\ \text{M})(50.0\ \text{mL})}{(0.100\ \text{M})} = 50.0\ \text{mL}$$

Before adding any NaOH the pH is that for a solution of 0.100 M acetic acid. Since acetic acid is a weak acid, we can calculate the pH,

$$CH_3COOH(aq) + H_2O(\ell) \rightleftharpoons H_3O^+(aq) + CH_3COO^-(aq)$$

$$K_a = \frac{[H_3O^+][CH_3COO^-]}{[CH_3COOH]} = \frac{(x)(x)}{0.100 - x} = 1.75 \times 10^{-5}$$

$$x = [H_3O^+] = 1.32 \times 10^{-3}$$

At the beginning of the titration the pH is 2.88. Adding NaOH converts a portion of the acetic acid to its conjugate base.

$$CH_3COOH(aq) + OH–(aq) \leftrightarrow H_2O(l) + CH_3COO–(aq)$$

Any solution containing comparable amounts of a weak acid, HA, and its conjugate weak base, A–, is a buffer. we can calculate the pH of a buffer using the Henderson–Hasselbalch equation,

$$pH = pK_a + \log\frac{[A^-]}{[HA]}$$

The equilibrium constant for reaction 9.2 is large ($K = Ka/Kw = 1.75 \times 10^9$), so we can treat the reaction as one that goes to completion. Before the equivalence point, the concentration of unreacted acetic acid is,

$$[CH_3COOH] = \frac{\text{moles unreacted } CH_3COOH}{\text{total volume}} = \frac{M_a V_a - M_b V_b}{V_a + V_b}$$

and the concentration of acetate is

$$[CH_3COO^-] = \frac{\text{moles NaOH added}}{\text{total volume}} = \frac{M_b V_b}{V_a + V_b}$$

For example, after adding 10.0 mL of NaOH the concentrations of CH_3COOH and $CH_3COO–$ are

$$[CH_3COOH] = \frac{(0.100\ M)(50.0\ mL) - (0.100\ M)(10.0\ mL)}{50.0\ mL + 10.0\ mL} = 0.0667\ M$$

$$[CH_3COO^-] = \frac{(0.100\ M)(10.0\ mL)}{50.0\ mL + 10.0\ mL} = 0.0167\ M$$

giving a pH of

$$pH = 4.76 + \log\frac{0.0167}{0.0667} = 4.16$$

A similar calculation shows that the pH after adding 20.0 mL of NaOH is 4.58. At the equivalence point, the moles of acetic acid initially present and the moles of NaOH added are identical. Since their reaction effectively proceeds to completion, the predominate ion in solution is CH_3COO^-, which is a weak base. To calculate the pH we first determine the concentration of $CH_3COO–$.

$$[CH_3COO^-] = \frac{\text{moles } CH_3COOH}{\text{total volume}} = \frac{(0.100\ M)(50.0\ mL)}{50.0\ mL + 50.0\ mL} = 0.0500\ M$$

The pH is then calculated as shown in Chapter 6 for a weak base

$$CH_3COO^-(aq) + H_2O(\ell) \rightleftharpoons OH^-(aq) + CH_3COOH(aq)$$

$$K_b = \frac{[OH^-][CH_3COOH]}{[CH_3COO^-]} = \frac{(x)(x)}{0.0500 - x} = 5.71 \times 10^{-10}$$

$$x = [OH^-] = 5.34 \times 10^{-6}\ M$$

The concentration of H3O+, therefore, is 1.87 X 10^{-9}, or a pH of 8.73. After the equivalence point NaOH is present in excess, and the pH is determined in the same manner as in the titration of a strong acid with a strong base. For example, after adding 60.0 mL of NaOH, the concentration of OH^- is

$$[OH^-] = \frac{(0.100\ M)(60.0\ mL) - (0.100\ M)(50.0\ mL)}{50.0\ mL + 60.0\ mL} = 0.00909\ M$$

giving a pH of 11.96. Table 9.3 and Figure 9.6 show additional results for this titration. The calculations for the titration of a weak base with a strong acid are handled in a similar manner except that the initial pH is determined by the weak base, the pH at the equivalence point by its conjugate weak acid, and the pH after the equivalence point by the concentration of excess strong acid.

5.10 Selecting and evaluating the end point

analyte is a triprotic weak acid, a titration with NaOH will have three equivalence points corresponding to the addition of one, two, and three moles of OH^- for each mole of the weak acid. An equivalence point, therefore, is a theoretical not an experimental value. An end point for a titration is determined experimentally and represents the analyst's best estimate of the corresponding equivalence point. Any difference between an equivalence point and its end point is a source of determinate error. As we shall see, it is even possible that an equivalence point will not have an associated end point.

We have already learned how to calculate the equivalence point for the titration of a strong acid with a strong base, and for the titration of a weak acid with a strong base. We also have learned to sketch a titration curve with a minimum of calculations. Can we also locate the equivalence point without performing any calculations? The answer, as you may have guessed, is often yes!

It has been shown that for most acid–base titrations the inflection point, which corresponds to the greatest slope in the titration curve, very nearly coincides with the equivalence point. The inflection point actually precedes the equivalence point, with the error approaching 0.1% for weak acids or weak bases with dissociation constants smaller than 10–9, or for very dilute solutions.

The principal limitation to using a titration curve to locate the equivalence point is that an inflection point must be present. Sometimes, however, an inflection point may be missing or difficult to detect. Figure 9.9, for example, demonstrates the influence of the acid dissociation constant, *K*a, on the titration curve for a weak acid with a strong base titrant. The inflection point is visible, even if barely so, for acid dissociation constants larger than 10^{-9}, but is missing when *K*a is 10^{-11}.

Another situation in which an inflection point may be missing or difficult to detect occurs when the analyte is a multiprotic weak acid or base whose successive dissociation constants are similar

in magnitude. To see why this is true let's consider the titration of a diprotic weak acid, H2A, with NaOH. During the titration the following two reactions occur.

$$H_2A\ (aq) + OH–(aq) \leftrightarrow HA–(aq) + H_2O\ (l)\ \text{……}\mathbf{9.3}$$

$$HA–(aq) + OH–(aq) \leftrightarrow A_2–(aq) + H_2O\ (l)\ \text{……..}\mathbf{9.4}$$

Two distinct inflection points are seen if reaction 9.3 is essentially complete before reaction 9.4 begins. Figure 9.10 shows titration curves for three diprotic weak acids. The titration curve for maleic acid, for which *K*a1 is approximately 20,000 times larger than *K*a2, shows two very distinct inflection points. Malonic acid, on the other hand, has acid dissociation constants that differ by a factor of approximately 690. Although malonic acid's titration curve shows two inflection points, the first is not as distinct as that for maleic acid. Finally, the titration curve for succinic acid, for which the two *K*a values differ by a factor of only 27, has only a single inflection point corresponding to the neutralization of $HC_4H_4O_4$ – to $C_4H_4O_4^{2–}$. In general, separate inflection points are seen when successive acid dissociation constants differ by a factor of at least 500 (a ΔpKa of at least 2.7).

5.11 Mohr method

Mohr method of determination of chlorides by titration with silver nitrate is one of the oldest titration methods still in use.

Chlorides are titrated with the silver nitrate solution in the presence of chromate anions. End point is signalled by the appearance of the red silver chromate.

Intense yellow color of chromate may make detection of first signs of formation of red silver chromate precipitation difficult. As some excess of silver must be added before precipitate starts to form, if concentration of titrant is below 0.1M, we may expect singificant positive error. To correct for this error we can determine a blank, titrating a solution of the indicator potassium chromate with standard silver nitrate solution. To make result more realistic we can add small amount of chloride free calcium carbonate to the solution to imitate the white silver precipitate.

Solution during titration should be close to neutral. In low pH silver chromate solubility grows due to the protonation of chromate anions, in high pH silver starts to react with hydroxide anions, precipitating in form of AgOH and Ag_2O. Both processes interfere with the determination accuracy. Exactly the same approach can be used for determination of bromides. Other halides and pseudohalides, like I^- and SCN^-, behave very similarly in the solution, but their precipitate tends to adsorb chromate anions making end point detection difficult.

Reaction taking place during titration is

$Ag^+ + Cl^- \rightarrow AgCl_{(s)}$

End point detection

Before titration small amount of sodium or potassium chromate is added to the solution, making its slightly yellow in color. During titration, as long as chlorides are present, concentration of Ag^+ is too low for silver chromate formation. Near equivalence point concentration of silver cations rapidly grows, allowing precipitation of intensively red silver chromate which signalls end point. See precipitation titration end point detection page for more detailed, quantitative discussion.

In Mohar's method titration of halides with $AgNO_3$ is carried out with Na_2CrO_4 as an indiactor. This is an example of titration which proceeds with the formation of a new coloured precipitate. At the end point excess Ag ions precipitate or brick red Na_2CrO_4. The solution should be neutral or slightly alkaline but not too basic. So as to precipitate Ag or $Ag(OH)_2$. Further with too much acid the end point will be decayed due to lowering of CrO_4^{2-} concentration as per reaction

$$H^+ + CrO_4^{2-} \rightarrow HCrO_4^{2-}$$

Under proper condition Mohar's method is accurate and applicable at low cl^- concentration. In titration of this type the colour indicating precipitate is more soluble than the precipitate formed during titration also it should not be soluble as to need more reagent.

5.12 Volhard method

It is not always possible to use Mohr method to determine concentration of chlorides. For example, Mohr method requires neutral solution, but in many cases solution has to be acidic, to prevent precipitation of metal hydroxides (like in the presence of Fe^{3+}). In such cases we can use Volhard method, which is not sensitive to low pH.

In the Volhard method chlorides are first precipitated with excess silver nitrate, then excess silver is titrated with potassium (or sodium) thiocyanate. To detect end point we use Fe^{3+} cations, which easily react with the thiocyanate, creating distinct wine red complex.

There is a problem though. Silver thiocyanate solutility is slightly lower than solubility of silver chloride, and during titration thiocyanate can replace chlorides in the existing precipitate

$AgCl(s) + SCN^- \rightarrow AgSCN(s) + Cl^-$

To avoid problems we can filtrate precipitated AgCl before titration. However, there exist much simpler and easier procedure that gives the same result. Before titration we add some small volume of a heavy organic liquid that is not miscible with water (like nitrobenzene, chloroform or carbon tetrachloride). These liquids are better at wetting precipitate than water. Once the precipitate is covered with non polar liquid, it is separated from the water and unable to dissolve.

Precipitate solubility is not a problem during determination of I^- and Br^-, as both AgBr and AgI have much lower solubilities than AgSCN.

There are two reactions, as this is a back titration. First, we precipitate chlorides from the solution:

$Ag+ + Cl^- \rightarrow AgCl(s)$

Then, during titration, reaction taking place is

$Ag+ + SCN^- \rightarrow AgSCN(s)$

End point is detected with the use of iron (III) thiocyanate complex, which have very distinct and strong wine color.

The titration of Ag with NH_4SCN with ferric alum as an indicator is an example of the class of titration involving the formation of coloured substance in excess NH_4SCN reacts with Fe(III) to form deep red $[FeSCN]^{2+}$ the amount of thiocyanate which will give a visible colour is very small. Thus the end point error is very small but the solution should be shaken vigorously at the end point as silver ions are absorbed on the precipitate and are then desorbed other wise in basic media Fe^{3+} will hydrolyze. An excess of $AgNO_3$ is added to chloride solution and part of it is unreached. Ag solution is balk titrated with ferric alum as indicator but the method has a source of error.

AgSCN is less soluble than AgCl so

$$AgCl + SCN^- \rightarrow AgSCN + Cl^-$$

This will consume more NH_4SCN and chloride content will appear lower (error). This error can be eliminated by filtering off AgCl precipitate before back titration or it a little nitro benzene is added it will adhere to AgCl and protect it from reaction with thiocyanate however nitrobenzene slow down the reaction. this can be avoided it $Fe(NO_3)_3$ and a small measured amount of

NH_4SCN are added to chloride solution at the start with HNO_3 and mixture titrated with $AgNO_3$ till the red colour disappears.

5.13 Estimation of Fluoride ion

Most type of water contains fluoride ions. The fluoride oncentration of the different water samples ranges from traces to 10mg /dm3. It has been known that low concentration (0.5-1.5mg/dm3) of fluoride in drinking water is beneficial in preventing tooth decay and cavity formation. Therefore fluoridation of potable water is recommended by health authorities and employed in broad scale in practice. Fluoride level of drinking water not higher than 1mg/dm3 is considered optimal, therefore the most often analyzed concentration range of potable water sample is 0.5 to 1.5 mg.dm3.

Wastewater of glass factories and brine waters contain very high fluoride concentration. The water sample collected for fluoride measurement does not require any preservation. It can be stroed in special glass or plastic container for about 28 days.

Detection

No commercial automatic analyzers are available for sample decomposition and subsequent determination of fluoride ion. The main methods of decomposition currently in use are peroxide bome, pyrohydrolytic tube combustion and O2 flask combustion.

The O2 combustion procedure is the popular combustion technique owing to its low cost and simplicity. However if often gives problems with highly fluorinated materials because they tend to combust incompletely even when combustion aids such a sucrose or decan-1-ol are added.

The main methods for determining fluoride ion in solution are acidimetric, colorimetrey, titrimetry and potentiometry using the fluoride ion selective electrode and ion chromatography.

Providing no other acidic species are present and water has been used as the obsorption medium in either the O2 flask pr pyrohydrolytic tube decomposition, the acidometric titration of hydrogen fluoride is the simplest.

Colorimetric methods can be used for detecting fluoride ions in water samples. Most of these methods are based on mixed-lagand complex formation. La(III)-F-alizaline complex-one, Ce(III)-F-alizaline cpmplexone, Zr(IV)-F-xylenolorange, and Zr(IV)-F-semixylenol organe, etc…

In cases, the absorbance of the coloured complex formed in the presence of fluoride ions is detected. Sometimes the presence of the fluoride ions results in fading of the color of the reagent

as fluoride ions replace a ligand in mixed –ligand complex reagent. In these cases, the color of the complex solution prepared with the sample is compared with the colour of a series of complex solutions prepared with known amount of fluoride ion concentrations. Semiquntitative concentration determination can be made in this way.

c) Zirconium-Alizarin Red R method

The complex between zirconium and alizarine red S (sodium 3,4-dihydroxy-9,10-dioxo-2-anthacene sulfonate,) gives red-brown colour in acid solution if alizarine red S is in excess and violet colour if the zirconium is in excess. The complex is decolourized by fluoride ions. Phosphate, arsenate, sulfate, thiosulfate and oxalate as well as organic hydroxyl acids interference with this reaction.

d) Quantitative determination of fluoride content in water samples

Before the introduction of ion-selective fluoride, measuring fluoride concentration was quite a difficult task. Fewer methods are available for this than for the analysis of other halides.

This method is based on the bleaching action of fluoride ion content of the sample. The color of the red Zr-solochrome cyanine R complexe fades as ZrOF2 is formed in the medium. As a matter of fact no simple stiochiometric relationship between the fluoride ion and the zirchonium complex with the dye. Therefore in order to obtain results the reaction conditions need to be controlled very carefully. The absorbance of the reaction media is measured at 540 nm. The fluoride concentration is evaluated using an absorbance-fluoride concentration calibration curve prepared with standard solutions. The method can be used for sample containing 0-0.25µg fluoride.

5.14 Adsorption indicator in precipitate titration

If $AgNO_3$ is added to NaCl containing fluorescein the end point colour changes from light yellow to red pink. On standing the precipitate appears coloured while the solution is colour less due to adsorption of indicator on the precipitation of AgCl. The colour of a substance is modified by adsorption on a surface. The reaction is represented with an ionic indicator like fluoroscein.

If excess Cl: $(Agcl)Cl^- + FL^- \rightarrow$ no reaction (if $FL^- = C_{20}H_{11}O_5$ i.e fluore scent)

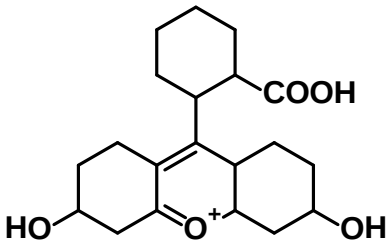
If excess Cl:(AgCl) $Ag^+ + FL^- \rightarrow$ (AgCl) (AgFL) adsorption.

In case of cationic indicator the reaction is

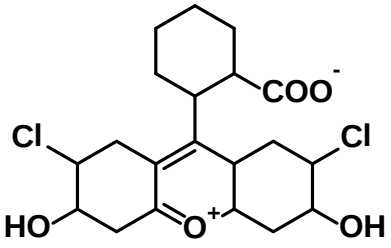
If excess Cl: (AgCl) $Cl^- + (mv)^+ \rightarrow$(AgCl) $(Cl^- V^+)$ adsorption

If excess Cl: (AgCl) $Ag^+ + (MV)^+ \rightarrow$No reaction if MV=methyl violet.

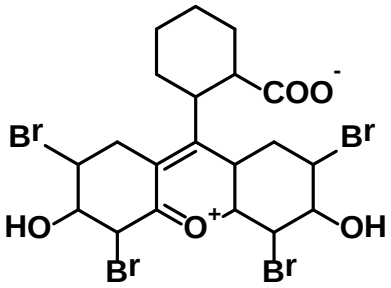
The adsorptivity of fluorosein anions by substitution with bromoriodo group is greately enhanced as shown below



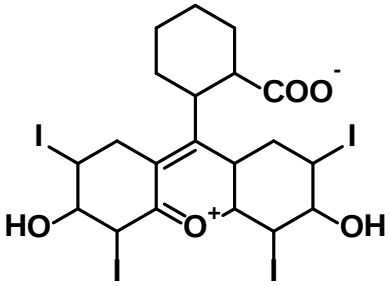
Fluorescein



Di Chlorofluorescein

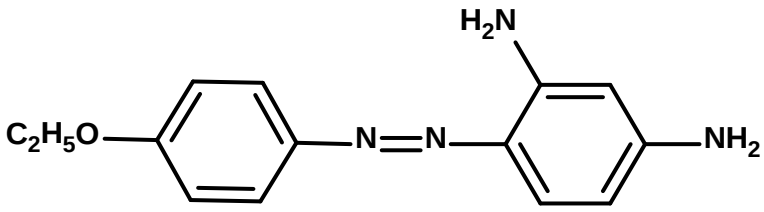


Eosin



This is because halogen atoms make this indicator more polarsiable and therefore they more strongly absorbed. All adsorption indicators are ionic. In addition to these adsorption indicator olizarin and thorin are used as indicators in titration of SO_4^{2-} with $BaClO_4$ in organic solvent media such as acetone.

In addition to above adsorption indicators other indicators used in precipitation titration are clysodine derivatives they are acid base indicator and adsorption redox indicators. They give reversible colour.



Structure of p-ethoxychrysodine

This is used for titration of Iodine against Ag^+ ions it acts in the following manner

(AgI) Ag^++M-$H^+$$\rightarrow$(AgI) Ag^+ M^-+H(yellow in base)

(AgI) M^- +$H^-$$\rightarrow$ $(AgI)^-$ (M^- H^+) (red in acid)

Congo Red is another acid base indicator.

The merits and de merits of adsorption indicator can be summarized as (3) follows

They give very small end point errors, the colour change with adsorption indicators are usually very sharp. Adsorption being a surface phenomenon, these indicators are best suited in precipitates having a large surface area, adsorption indicators lose their value if the precipitates coagulates in the presence of highly charged ions. We cannot use them for Al(III) due to coagulation. Protective colloids can eliminate this problems their limitations of optimum control of P^H or concentration of precipitates during adsorption and desorption procedure.

5.15 Quantitative estimation of Oxalic acid

The quantitative estimation of oxalic acid is best effect by precipitate it form its solution by a solution of lime in the state of octet of lime. The oxalic acid solution is saturated as accurate or possible with ammonia the solution is diluted with water. Then add a solution of neutral calcareous salt, genteelly a solution of chloride of calcium. The resulting oxalate of lime is washed with water the Quantity of the oxalic acid could be calculated from the weight of the oxalate of lime. But it is very difficult expel the water from the oxalate of lime by drying process. Hence it is unsafe to estimate that salt in the hydrous state. So it is best to convert the oxalate of lime to carbonate of lime by ignition and calculate quantity kof the oxalic acid from the weight of the carbonate of lime.

5.16 Estimation of the Carbonic acid

The Quantitative estimation of the carbon or carbonic acid has very frequently to be effected. There are various methods are available. When the carbonic acid is contained in a solid substance its quantitative determination is effected by different process according ast can be separated from the base by ignition most of the compound of carbonic acid when ignited over a sprit lamp with circular wick they completely loose their carbonic acid. the quantity of carbonic acid contained in such compounds can therefore be very accurately estimated from the loss of weight occasioned by ignition provided the cauonic acid will not be accompanied by other volatile constituents. After the ignition the metallic oxide remains behind in a state of purity.

When a metallic oxide is easily reusable then the ignition must be performed in a small weighed porcelain crucible otherwise a platinum crucible can be employed.

If the carbonic acid is in the gaseous state tube standing over mercury and then introduce the tube in to the small sticks of moistened caustic potash. The caustic acid is absorbed by the potash when the volume of the unabsorbed gas is accurately determined the difference in the two measurement of the volume of gas shows the quantity of the carbonic acid.

15.17Summary of the Unit

Titration is a common laboratory method of quantitative chemical analysis that is used to determine the unknown concentration of a known reactant. Because volume measurements play a key role in titration, it is also known as volumetric analysis. A reagent, called the titrant or titrator, of a known concentration (a standard solution) and volume is used to react with a solution of the analyte or titrand, whose concentration is not known. Using a calibrated burette or chemistry pipetting syringe to add the titrant, it is possible to determine the exact amount that has been consumed when the endpoint is reached. The endpoint is the point at which the titration is complete, as determined by an indicator. This is ideally the same volume as the equivalence point—the volume of added titrant at which the number of moles of titrant is equal to the number of moles of analyte, or some multiple thereof (as in polyprotic acids). In the classic strong acid-strong base titration, the endpoint of a titration is the point at which the pH of the reactant is just about equal to 7, and often when the solution takes on a persisting solid color as in the pink of phenolphthalein indicator. There are however many different types of titrations. Many methods can be used to indicate the endpoint of a reaction; titrations often use visual indicators (the reactant mixture changes color). In simple acid-base titrations a pH indicator may be used, such as phenolphthalein, which becomes pink when a certain pH (about 8.2) is reached or exceeded. Another example is methyl orange, which is red in acids and yellow in alkali solutions.

Precipitation titrations are based upon reactions that yield ionic compounds of limited solubility. The most important precipitating reagent is silver nitrate. Titrimetric methods based upon silver nitrate are sometimes termed argentometric methods. Potassium chromate can serve as an end point indicator for the argentometric determination of chloride, bromide and cyanide ions by reacting with silver ions to form a brick-red silver chromate precipitate in the equivalence point region. The Mohr method uses chromate ions as an indicator in the titration of chloride ions with a silver nitrate standard solution. After all the chloride has been precipitated as white silver

chloride, the first excess of titrant results in the formation of a silver chromate precipitate, which signals the end point. By knowing the stoichiometry and moles consumed at the end point, the amount of chloride in an unknown sample can be determined. This report describes experiments aimed at determining the concentration of chloride in a solid sample.

Volhard is uses a back titration with potassium thiocyanate to determine the concentration of chloride ions in a solution. Before the titration an excess volume of a silver nitrate solution is added to the solution containing chloride ions, forming a precipitate of silver chloride. The term 'excess' is used as the moles of silver nitrate added are known to exceed the moles of sodium chloride present in the sample so that all the chloride ions present will react. The indicator Fe^{3+} (ferric ion) is then added and the solution is titrated with the potassium thiocyanate solution. The titrate remains pale yellow as the excess (unreacted) silver ions react with the thiocyanate ions to form a silver thiocyanate precipitate. Once all the silver ions have reacted, the slightest excess of thiocyanate reacts with Fe^{3+} to form a dark red complex.

The concentration of chloride ions is determined by subtracting the titration findings of the moles of silver ions that reacted with the thiocyanate from the total moles of silver nitrate added to the solution. This method is used when the pH of the solution, after the sample has been prepared, is acidic. If the pH is neutral or basic, Mohr's method or the gravimetric method should be used. The method is illustrated below by using the procedure to determine the concentration of chloride (from sodium chloride) in cheese.

Surface of certain indicators possess a property of adsorption. At the equivalence point, certain ions are adsorbed and a typical orientation is developed at the surface which imparts a characteristic colour change at the end point. Due to this phenomenon of adsorption these are termed as adsorption indicators. Hence "adsorption indicators is such a substance on whose surface adsorption takes place at equivalence point resulting in a colour change to detect the end point".

5.18 Key Words

Titration; Titrant and Titrand; End Point; Indicator; Visual Indicators; Titration Curves; Mohr method; Volhard method; Absorption indicators.

5.19 References for further study

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2) Basic Concepts of Analytical Chemistry; S M Khopkar; *New Age International*, **1998**.

3) Analytical Chemistry; Clyde Frank; *Elsevier*, **2012**.

4) Fundamentals of Analytical Chemistry; Douglas Skoog, Donald West, F. Holler, Stanley Crouch; *Cengage Learning*, **2013**.

5) Handbook of Water Analysis, Second Edition; Leo M.L. Nollet, Leen S. P. De Gelder; *CRC Press*, **2007**.

5.20 Questions for self understanding

1) Write the definitions of the following term involved in titration

i) Titration

j) Titrant and Titrand

k) End Point

l) Indicator

2) What are titrimetric reactions?

3) Write a note on end-point detection

4) With example explain the visual Indicators

5) Mention different apparatus used in titrimetric analysis

6) Rite the difference between equivalence points and end points

7) Explain the titration curves

Write a note on Acid - Base titration curves

8) How selecting and evaluating the end point is achived?

9) Explain Mohr method

10) Explain Volhard method

11) Write a procedure for estimation of fluoride ion using following methods

e) Zirconium-Alizarin Red R method

f) Quantitative determination of fluoride content in water samples

12) Explain the adsorption indicator in precipitate titration

13) Write a note on quantitative estimation of Oxalic acid

14) Write a note on estimation of the Carbonic acid

Unit-6

Structure

6.0 Objectives of the unit

After studying this unit you are able to

- Explain the methods involved in redox reactions
- Give the example of redox titration Curves
- Explain the Inorganic Analysis using redox titration
- Explain the Organic Analysis using redox titration

6.1 Introduction

The term oxidation referrers to any chemical change in which there is an increase in oxidation number while the term reduction refers to any change resulting in decrease in oxidation number. Thus oxidation proceeds with loss of electrons while reduction is accompanied by gain of electrons. The oxidizing agent is by definition the substance containing the atom which shows decrease in oxidation number, while a reducing agent is the substance containing the atom which shows an increase on oxidation number. Oxidation and reduction must always occur together and must compensate each other. Th term oxidizing or reducing agent refers to the entire substance and not to just one atom. If a reagent acts as both oxidizing and reducing agent we say it undergoes autooxidation or disproportionation.

Volumetric methods based upon electron transfer reactions are numerous. The separation of oxidation-reduction into its components i.e., half reactions is a suitable way of indicating the species that gain electrons and those that lose electrons as oxidation-reduction reactions result from the direct transfer of electrons from donor to receiver. Many redox reactions could be used in volumetric titrations provided that equilibrium is reached very rapidly following each addition of titrant and also that the indicator is capable of locating the stochiometric equivalence point with reasonable accuracy. Many standard redox titrations are carried out using colour change indicators. Two half reactions for any redoxtitration system are in equilibrium at all points after the start of titration so that reduction potential for the half cell are identical to all points. Thus the cell potential changes during the titration and furnishes a characteristic and relatively large ΔE cell around the equivalence point for all titrations.

6.2 Titrations bases on Redox Reactions

Redox titrations were introduced shortly after the development of acid–base titrimetry. The earliest methods took advantage of the oxidizing power of chlorine. In 1787, Claude Berthollet introduced a method for the quantitative analysis of chlorine water (a mixture of Cl_2, HCl, and

HOCl) based on its ability to oxidize solutions of the dye indigo (indigo is colorless in its oxidized state). In 1814, Joseph Louis Gay- Lussac (1778–1850), developed a similar method for chlorine in bleaching powder. In both methods the end point was signaled visually. Before the equivalence point, the solution remains clear due to the oxidation of indigo. After the equivalence point, however, unreacted indigo imparts a permanent color to the solution.

The number of redox titrimetric methods increased in the mid-1800s with the introduction of MnO_4^-, $Cr_2O_7^{2-}$ and I_2 as oxidizing titrants, and Fe^{2+} and $S_2O_3^{2-}$ as reducing titrants. Even with the availability of these new titrants, however, the routine application of redox titrimetry to a wide range of samples was limited by the lack of suitable indicators. Titrants whose oxidized and reduced forms differ significantly in color could be used as their own indicator. For example, the intensely purple MnO_4^- ion serves as its own indicator since its reduced form, Mn^{2+}, is almost colorless. The utility of other titrants, however, required a visual indicator that could be added to the solution. The first such indicator was diphenylamine, which was introduced in the 1920s. Other redox indicators soon followed, increasing the applicability of redox titrimetry.

6.3 Redox Titration Curves

To evaluate a redox titration we must know the shape of its titration curve. In an acid–base titration or a complexation titration, a titration curve shows the change in concentration of H_3O^+ (as pH) or M*n*+ (as pM) as a function of the volume of titrant. For a redox titration, it is convenient to monitor electrochemical potential.

You will recall from Chapter 6 that the Nernst equation relates the electrochemical potential to the concentrations of reactants and products participating in a redox reaction. Consider, for example, a titration in which the analyte in a reduced state, *A*red, is titrated with a titrant in an oxidized state, *T*ox. The titration reaction is

$$Ar_{ed} + T_{ox} \rightarrow T_{red} + A_{ox}$$

The electrochemical potential for the reaction is the difference between the reduction potentials for the reduction and oxidation half-reactions; thus,

$$E_{rxn} = ET_{ox}/T_{red} - EA_{ox}/A_{red}$$

After each addition of titrant, the reaction between the analyte and titrant reaches a state of equilibrium. The reaction's electrochemical potential, *E*rxn, therefore, is zero, and

$$ET_{ox}/T_{red} = EA_{ox}/A_{red}$$

Consequently, the potential for either half-reaction may be used to monitor the titration's progress. Before the equivalence point the titration mixture consists of appreciable quantities of both the oxidized and reduced forms of the analyte, but very little unreacted titrant. The potential, therefore, is best calculated using the Nernst equation for the analyte's half-reaction

$$E_{A_{ox}/A_{red}} = E^{\circ}_{A_{ox}A_{red}} - \frac{RT}{nF} \ln \frac{[A_{red}]}{[A_{ox}]}$$

Although $E°A_{ox}$ $/A_{red}$ is the standard-state potential for the analyte's half-reaction, a matrix-dependent *formal potential* is used in its place. After the equivalence point, the potential is easiest to calculate using the Nernst equation for the titrant's half-reaction, since significant quantities of its oxidized and reduced forms are present.

$$E_{T_{ox}/T_{red}} = E^{\circ}_{T_{ox}T_{red}} - \frac{RT}{nF} \ln \frac{[T_{red}]}{[T_{ox}]}$$

6.4 Calculating the Titration Curve

As an example, let's calculate the titration curve for the titration of 50.0 mL of 0.100 M Fe^{2+} with 0.100 M Ce^{4+} in a matrix of 1 M $HClO_4$. The reaction in this case is,

$$Fe^{2+}(aq) + Ce^{4+}(aq) \rightleftharpoons Ce^{3+}(aq) + Fe^{3+}(aq)$$

The equilibrium constant for this reaction is quite large (it is approximately 6 ´ 1015), so we may assume that the analyte and titrant react completely. The first task is to calculate the volume of Ce4+ needed to reach the equivalence point. From the stoichiometry of the reaction we know

Moles Fe^{2+} = moles Ce^{4+}

Or

$$M_{Fe}V_{Fe} = M_{C}eV_{Ce}$$

$$V_{Ce} = \frac{M_{Fe}V_{Fe}}{M_{Ce}} = \frac{(0.100\ \text{M})(50.0\ \text{mL})}{(0.100\ \text{M})} = 50.0\ \text{mL}$$

Solving for the volume of Ce4+ gives the equivalence point volume as 50.0 mL. Before the equivalence point the concentration of unreacted Fe^{2+} and the concentration of Fe^{3+} produced by

reaction 9.16 are easy to calculate. For this reason we find the potential using the Nernst equation for the analyte's half-reaction

$$E = E^{\circ}_{Fe^{3+}/Fe^{2+}} - 0.05916 \ln \frac{[Fe^{2+}]}{[Fe^{3+}]}$$

The concentrations of Fe^{2+} and Fe^{3+} after adding 5.0 mL of titrant are

$$[Fe^{2+}] = \frac{\text{moles unreacted } Fe^{2+}}{\text{total volume}} = \frac{M_{Fe}V_{Fe} - M_{Ce}V_{Ce}}{V_{Fe} + V_{Ce}}$$

$$= \frac{(0.100\ \text{M})(50.0\ \text{mL}) - (0.100\ \text{M})(5.0\ \text{mL})}{50.0\ \text{mL} + 5.0\ \text{mL}} = 8.18 \times 10^{-2}\ \text{M}$$

$$[Fe^{3+}] = \frac{\text{moles } Ce^{4+} \text{ added}}{\text{total volume}} = \frac{M_{Ce}V_{Ce}}{V_{Fe} + V_{Ce}}$$

$$= \frac{(0.100\ \text{M})(5.0\ \text{mL})}{50.0\ \text{ml} + 5.0\ \text{mL}} = 9.09 \times 10^{-3}\ \text{M}$$

Substituting these concentrations into equation 9.17 along with the formal potential for the Fe^{3+}/Fe^{2+} half-reaction from Appendix 3D, we find that the potential is

$$E = +0.767\ \text{V} - 0.05916 \log\left(\frac{8.18 \times 10^{-2}}{9.09 \times 10^{-3}}\right) = +0.711\ \text{V}$$

At the equivalence point, the moles of Fe^{2+} initially present and the moles of Ce^{4+} added are equal. Because the equilibrium constant for reaction 9.16 is large, the concentrations of Fe^{2+} and Ce^{4+} are exceedingly small and difficult to calculate without resorting to a complex equilibrium problem. Consequently, we cannot calculate the potential at the equivalence point, *E*eq, using just the Nernst equation for the analyte's half-reaction or the titrant's half-reaction. We can, however, calculate *E*eq by combining the two Nernst equations. To do so we recognize that the potentials for the two half-reactions are the same; thus,

$$E_{eq} = E^{\circ}_{Fe^{3+}/Fe^{2+}} - 0.05916 \log \frac{[Fe^{2+}]}{[Fe^{3+}]}$$

$$E_{eq} = E^{\circ}_{Ce^{4+}/Ce^{3+}} - 0.05916 \log \frac{[Ce^{3+}]}{[Ce^{4+}]}$$

Adding together these two Nernst equations leaves us with

$$2E_{eq} = E^{\circ}_{Fe^{3+}/Fe^{2+}} + E^{\circ}_{Ce^{4+}/Ce^{3+}} - 0.05916 \log \frac{[Fe^{2+}][Ce^{3+}]}{[Fe^{3+}][Ce^{4+}]}$$

At the equivalence point, the titration reaction's stoichiometry requires that

$$[Fe^{2+}] = [Ce^{4+}]$$

$$[Fe^{3+}] = [Ce^{3+}]$$

The ratio in the log term of equation 9.18, therefore, equals one and the log term is zero. Equation 9.18 simplifies to

$$E_{eq} = \frac{E^{\circ}_{Fe^{3+}/Fe^{2+}} + E^{\circ}_{Ce^{4+}/Ce^{3+}}}{2} = \frac{0.767\ V + 1.70\ V}{2} = 1.23\ V$$

After the equivalence point, the concentrations of Ce^{3+} and excess Ce^{4+} are easy to calculate. The potential, therefore, is best calculated using the Nernst equation for the titrant's half-reaction.

$$E = E^{\circ}_{Ce^{4+}/Ce^{3+}} - 0.05916 \log \frac{[Ce^{3+}]}{[Ce^{4+}]}$$

For example, after adding 60.0 mL of titrant, the concentrations of Ce^{3+} and Ce^{4+} are

$$[Ce^{3+}] = \frac{\text{intial moles } Fe^{2+}}{\text{total volume}} = \frac{M_{Fe}V_{Fe}}{V_{Fe} + V_{Ce}}$$

$$= \frac{(0.100\text{ M})(50.0\text{ mL})}{50.0\text{ mL} + 60.0\text{ mL}} = 4.55 \times 10^{-2}\text{ M}$$

$$[Ce^{4+}] = \frac{\text{moles excess } Ce^{4+}}{\text{total volume}} = \frac{M_{Ce}V_{Ce} - M_{Fe}V_{Fe}}{V_{Fe} + V_{Ce}}$$

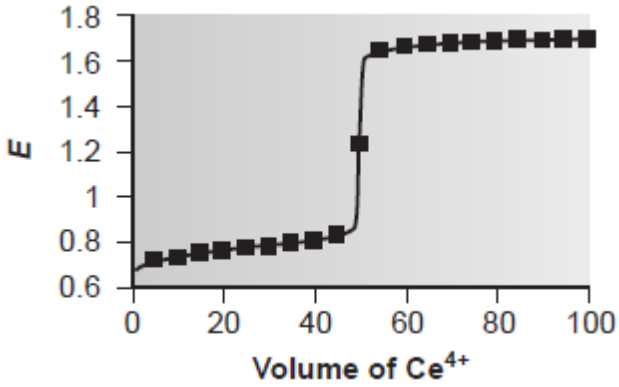
$$= \frac{(0.100\text{ M})(60.0\text{ mL}) - (0.100\text{ M})(50.0\text{ mL})}{50.0\text{ mL} + 60.0\text{ mL}} = 9.09 \times 10^{-3}\text{ M}$$

Substituting these concentrations into equation 9.19 gives the potential as

$$E = +1.70\text{ V} - 0.05916 \log \frac{4.55 \times 10^{-2}}{9.09 \times 10^{-3}} = 1.66\text{ V}$$

Data for Titration of 50.0 mL of 0.100 M Fe^{2+} with 0.100 M Ce^{4+}

Volume Ce^{4+} (mL)	*E* (V)	Volume Ce^{4+} (mL)	*E* (V)
5.00	0.711	55.00	1.64
10.00	0.731	60.00	1.66
15.00	0.745	65.00	1.67
20.00	0.757	70.00	1.68
25.00	0.767	75.00	1.68
30.00	0.777	80.00	1.69
35.00	0.789	85.00	1.69
40.00	0.803	90.00	1.69
45.00	0.823	95.00	1.70
50.00	1.23	100.00	1.70



Redox titration curve for 50.0 mL of 0.100 M Fe^{2+} with 0.100 M Ce^{4+} in 1 M $HClO_4$

Finding the End Point with a Visual Indicator

Three types of visual indicators are used to signal the end point in a redox titration. A few titrants, such as MnO_4^-, have oxidized and reduced forms whose colors in solution are significantly different. Solutions of MnO_4^- are intensely purple. In acidic solutions, however, permanganate's reduced form, Mn2+, is nearly colorless. When MnO_4^- is used as an oxidizing titrant, the solution remains colorless until the first drop of excess MnO_4^- is added. The first permanent tinge of purple signals the end point. A few substances indicate the presence of a specific oxidized or reduced species. Starch, for example, forms a dark blue complex with I_3^- and can be used to signal the presence of excess I_3^- (color change: colorless to blue), or the completion of areaction in which I_3^- is consumed (color change: blue to colorless). Another exampleof a specific indicator is thiocyanate, which forms a soluble red-colored complex,$Fe(SCN)^{2+}$, with Fe^{3+}.The most important class of redox indicators, however, are substances that do not participate in the redox titration, but whose oxidized and reduced formsdiffer in color. When added to a solution containing the analyte, the indicator impartsa color that depends on the solution's electrochemical potential. Since theindicator changes color in response to the electrochemical potential, and not tothe presence or absence of a specific species, these compounds are called general **redox indicators.** The relationship between a redox indicator's change in color and the solution's electrochemical potential is easily derived by considering the half-reaction for the indicator

$$In_{ox} + ne^- \rightleftharpoons In_{red}$$

where Inox and Inred are, respectively, the indicator's oxidized and reduced forms. The Nernst equation for this reaction is

$$E = E^{\circ}_{In_{ox}/In_{red}} - \frac{0.05916}{n} \log \frac{[In_{red}]}{[In_{ox}]}$$

If we assume that the indicator's color in solution changes from that of Inox to that of Inred when the ratio [Inred]/[Inox] changes from 0.1 to 10, then the end point occurs when the solution's electrochemical potential is within the range

$$E = E^{\circ}_{In_{ox}/In_{red}} \pm \frac{0.05916}{n}$$

A partial list of general redox indicators is shown in Table 9.18. Examples of appropriate and inappropriate indicators for the titration of Fe^{2+} with Ce^{4+} are shown in Figure

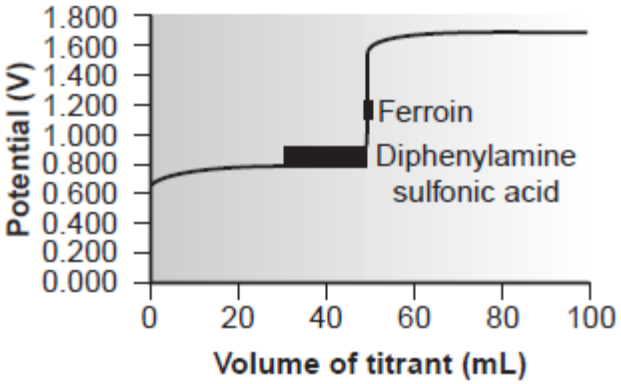


Fig: Titration curve for 50.00 mL of 0.0500 M Fe^{2+} with 0.0500 M Ce^{4+} showing the range of E and volume of titrant over which the indicators ferroin and diphenylamine sulfonic acid are expected to change color.

Selected General Redox Indicators

Indicator	Oxidized Color	Reduced Color	E° (V)
indigo tetrasulfonate	blue	colorless	0.36
methylene blue	blue	colorless	0.53
diphenylamine	violet	colorless	0.75
diphenylamine sulfonic acid	red-violet	colorless	0.85
tris(2,2'-bipyridine)iron	pale blue	red	1.120
ferroin	pale blue	red	1.147
tris(5-nitro-1,10-phenanthroline)iron	pale blue	red-violet	1.25

6.5 Applications of some oxidizing agents

In quantitative work the titrant's concentration must remain stable during the analysis. Since titrants in a reduced state are susceptible to air oxidation, most redox titrations are carried out using an oxidizing agent as the titrant. The choice of which of several common oxidizing titrants is best for a particular analysis depends on the ease with which the analyte can be oxidized.

Analytes that are strong reducing agents can be successfully titrated with a relatively weak oxidizing titrant, whereas a strong oxidizing titrant is required for the analysis of analytes that are weak reducing agents. The two strongest oxidizing titrants are MnO_4^- and Ce_4^+, for which the reduction half-reactions are

$$MnO_4^-(aq) + 8H_3O^+(aq) + 5e^- \rightleftharpoons Mn^{2+}(aq) + 12H_2O(\ell)$$

$$Ce^{4+}(aq) + e^- \rightleftharpoons Ce^{3+}(aq)$$

Solutions of Ce4+ are prepared from the primary standard cerium ammonium nitrate, $Ce(NO_3)_4 \times 2NH_4NO_3$, in 1 M H_2SO_4. When prepared from reagent grade materials, such as $Ce(OH)_4$, the solution must be standardized against a primary standard reducing agent such as $Na_2C_2O_4$ or Fe^{2+} (prepared using Fe wire). Ferroin is a suitable indicator when standardizing against Fe^{2+} (Table 9.20). Despite its availability as a primary standard and its ease of preparation, Ce^{4+} is not as frequently used as MnO_4^- because of its greater expense. Solutions of MnO_4^- are prepared from $KMnO_4$, which is not available as a primary standard. Aqueous solutions of permanganate are thermodynamically unstable due to its ability to oxidize water.

$$4MnO_4^-(aq) + 2H_2O(\ell) \rightleftharpoons 4MnO_2(s) + 3O_2(g) + 4OH^-(aq)$$

This reaction is catalyzed by the presence of MnO_2, Mn^{2+}, heat, light, and the presence of acids and bases. Moderately stable solutions of permanganate can be prepared by boiling for an hour and filtering through a sintered glass filter to remove any solid MnO_2 that precipitates. Solutions prepared in this fashion are stable for 1–2 weeks, although the standardization should be rechecked periodically. Standardization may be accomplished using the same primary standard reducing agents that are used with Ce^{4+}, using the pink color of MnO_4^- to signal the end point Potassium dichromate is a relatively strong oxidizing agent whose principal advantages are its availability as a primary standard and the long-term stability of its solutions. It is not, however, as strong an oxidizing agent as MnO_4^- or Ce^{4+}, which prevents its application to the analysis of analytes that are weak reducing agents. Its reduction half-reaction is,

$$Cr_2O_7^{2-}(aq) + 14H_3O^+(aq) + 6e^- \rightleftharpoons 2Cr^{3+}(aq) + 21H_2O(\ell)$$

Although solutions of $Cr_2O_7^{2-}$ are orange and those of Cr^{3+} are green, neither color is intense enough to serve as a useful indicator. Diphenylamine sulfonic acid, whose oxidized form is

purple and reduced form is colorless, gives a very distinct end point signal with $Cr_2O_7^{2-}$. Iodine is another commonly encountered oxidizing titrant. In comparison with MnO_4–, Ce^{4+}, and $Cr_2O_7^{2-}$, it is a weak oxidizing agent and is useful only for the analysis of analytes that are strong reducing agents. This apparent limitation, however, makes I_2 a more selective titrant for the analysis of a strong reducing agent in the presence of weaker reducing agents. The reduction half-reaction for I_2 is

$$I_2(aq) + 2e^- \rightleftharpoons 2I^-(aq)$$

Because of iodine's poor solubility, solutions are prepared by adding an excess of I^-. The complexation reaction

$$I_2(aq) + I^-(aq) \rightleftharpoons I_3^-(aq)$$

increases the solubility of I_2 by forming the more soluble triiodide ion, I_3^-. Even though iodine is present as I_3^- instead of I_2, the number of electrons in the reduction half-reaction is unaffected.

$$I_3^-(aq) + 2e^- \rightleftharpoons 3I^-(aq)$$

Solutions of I_3^- are normally standardized against $Na_2S_2O_3$ (see Table 9.20) usingstarch as a specific indicator for I_3.

Oxidizing titrants such as MnO_4^-, Ce4+, $Cr_2O_7^{2-}$ and I_3^-, are used to titrate analytes that are in a reduced state. When the analyte is in an oxidized state, it can be reduced with an auxiliary reducing agent and titrated with an oxidizing titrant. Alternatively, the analyte can be titrated with a suitable reducing titrant. Iodide is a relatively strong reducing agent that potentially could be used for the analysis of analytes in higher oxidation states. Unfortunately, solutions of I^- cannot be used as a direct titrant because they are subject to the air oxidation of I^- to I_3^-.

$$3I^-(aq) \rightleftharpoons I_3^-(aq) + 2e^-$$

Instead, an excess of KI is added, reducing the analyte and liberating a stoichiometric amount of I_3^-. The amount of I_3^- produced is then determined by a back titration using $Na_2S_2O_3$ as a reducing titrant.

$$2S_2O_3^{2-}(aq) \rightleftharpoons S_4O_6^{2-}(aq) + 2e^-$$

Solutions of $Na_2S_2O_3$ are prepared from the pentahydrate and must be standardized before use. Standardization is accomplished by dissolving a carefully weighed portion of the primary

standard KIO_3 in an acidic solution containing an excess of KI. When acidified, the reaction between IO_3^- and I^-

$$IO_3^-(aq) + 8I^-(aq) + 6H_3O^+(aq) \rightleftharpoons 3I_3^-(aq) + 9H_2O(\ell)$$

liberates a stoichiometric amount of I_3^-. Titrating I_3^- using starch as a visual indicator allows the determination of the titrant's concentration. Although thiosulfate is one of the few reducing titrants not readily oxidized by contact with air, it is subject to a slow decomposition to bisulfite and elemental sulfur. When used over a period of several weeks, a solution of thiosulfate should be restandardized periodically. Several forms of bacteria are able to metabolize thiosulfate, which also can lead to a change in its concentration. This problem can be minimized by adding a preservative such as HgI_2 to the solution.

Another reducing titrant is ferrous ammonium sulfate, $Fe(NH_4)_2(SO_4)_2 \times 6H_2O$, in which iron is present in the +2 oxidation state. Solutions of Fe^{2+} are normally very susceptible to air oxidation, but when prepared in 0.5 M H_2SO_4 the solution may remain stable for as long as a month. Periodic restandardization with $K_2Cr_2O_7$ is advisable. The titrant can be used in either a direct titration in which the Fe^{2+} is oxidized to Fe^{3+}, or an excess of the solution can be added and the quantity of Fe^{3+} produced determined by a back titration using a standard solution of Ce^{4+} or $Cr_2O_7^{2-}$.

6.6 Inorganic Analysis

Redox titrimetry has been used for the analysis of a wide range of inorganic analytes. Although many of these methods have been replaced by newer methods, a few continue to be listed as standard methods of analysis. In this section we consider the application of redox titrimetry to several important environmental, public health, and industrial analyses. Other examples can be found in the suggested readings listed at the end of this chapter. One of the most important applications of redox titrimetry is in evaluating the chlorination of public water supplies. In Method 9.3 an approach for determining the total chlorine residual was described in which the oxidizing power of chlorine is used to oxidize I^- to I_3^-. The amount of I_3^- formed is determined by a back titration with $S_2O_3^{2-}$. The efficiency of chlorination depends on the form of the chlorinating species. For this reason it is important to distinguish between the free chlorine residual, due to Cl_2, HOCl, and OCl^-, and the combined chlorine residual. The latter form of chlorine results from the reaction of ammonia with the free chlorine residual, forming NH_2Cl, $NHCl_2$, and NCl_3. When a sample of iodide-free chlorinated water is mixed with an excess of

the indicator *N*,*N*-diethyl-*p*phenylenediamine (DPD), the free chlorine oxidizes a stoichiometric portion of DPD to its red-colored form. The oxidized DPD is then titrated back to its colorless form with ferrous ammonium sulfate, with the volume of titrant being proportional to the amount of free residual chlorine. Adding a small amount of KI reduces monochloramine, NH_2Cl, forming I_3^-. The I_3^- then oxidizes a portion of the efficiency of chlorination depends on the form of the chlorinating species. For this reason it is important to distinguish between the free chlorine residual, due to Cl_2, HOCl, and OCl^-, and the combined chlorine residual. The la ter form of chlorine results from the reaction of ammonia with the free chlorine residual, forming NH_2Cl, $NHCl_2$, and NCl_3. When a sample of iodide-free chlorinated water is mixed with an excess of the indicator *N*,*N*-diethyl-*p*phenylenediamine (DPD), the free chlorine oxidizes a stoichiometric portion of DPD to its red-colored form. The oxidized DPD is then titrated back to its colorless form with ferrous ammonium sulfate, with the volume of titrant being proportional to the amount of free residual chlorine. Adding a small amount of KI reduces monochloramine, NH_2Cl, forming I_3^-. The I_3^- then oxidizes a portion of the DPD to its red-colored form. Titrating the oxidized DPD with ferrous ammonium sulfate yields the amount of NH_2Cl in the sample. The amount of dichloramine and trichloramine are determined in a similar fashion the methods described earlier for determining the total, free, or combined chlorine residual also are used in establishing the chlorine demand of a water supply. The chlorine demand is defined as the quantity of chlorine that must be added to a water supply to completely react with any substance that can be oxidized by chlorine while also maintaining the desired chlorine residual. It is determined by adding progressively greater amounts of chlorine to a set of samples drawn from the water supply and determining the total, free, or combined chlorine residual.

Another important example of redox titrimetry that finds applications in both public health and environmental analyses is the determination of dissolved oxygen. In natural waters the level of dissolved O_2 is important for two reasons: it is the most readily available oxidant for the biological oxidation of inorganic and organic pollutants; and it is necessary for the support of aquatic life. In wastewater treatment plants, the control of dissolved O_2 is essential for the aerobic oxidation of waste materials. If the level of dissolved O_2 falls below a critical value, aerobic bacteria are replaced by anaerobic bacteria, and the oxidation of organic waste produces undesirable gases such as CH_4 and H_2S.

One standard method for determining the dissolved O_2 content of natural waters and wastewaters is the Winkler method. A sample of water is collected in a fashion that prevents its exposure to the atmosphere (which might change the level of dissolved O_2). The sample is then treated with a solution of $MnSO_4$, and then with a solution of NaOH and KI. Under these alkaline conditions Mn2+ is oxidized to MnO_2 by the dissolved oxygen.

$$2Mn^{2+}(aq) + 4OH^{-}(aq) + O_2(aq) \rightarrow 2MnO_2(s) + 2H_2O(\ell)$$

After the reaction is complete, the solution is acidified with H_2SO_4. Under the now acidic conditions I– is oxidized to I_3– by MnO_2.

$$MnO_2(s) + 3I^{-}(aq) + 4H_3O^{+}(aq) \rightarrow Mn^{2+}(aq) + I_3^{-}(aq) + 6H_2O(\ell)$$

The amount of I_3^- formed is determined by titrating with $S_2O_3^{2-}$ using starch as an indicator. The Winkler method is subject to a variety of interferences, and several modifications to the original procedure have been proposed. For example, NO_2^- interferes because it can reduce I_3^- to I^- under acidic conditions. This interference is eliminated by adding sodium azide, NaN_3, reducing NO_2^- to N_2. Other reducing agents, such as Fe^{2+}, are eliminated by pretreating the sample with $KMnO_4$, and destroying the excess permanganate with $K_2C_2O_4$. Another important example of a redox titration for inorganic analytes, which is important in industrial labs, is the determination of water in nonaqueous solvents. The titrant for this analysis is known as the Karl Fischer reagent and consists of a mixture of iodine, sulfur dioxide, pyridine, and methanol. The concentration of pyridine is sufficiently large so that I_2 and SO_2 are complexed with the pyridine (py) as py × I_2 and py × SO_2. When added to a sample containing water, I_2 is reduced to I^-, and SO_2 is oxidized to SO_3.

$$py \cdot I_2 + py \cdot SO_2 + py + H_2O \rightarrow 2py \cdot HI + py \cdot SO_3$$

Methanol is included to prevent the further reaction of py × SO3 with water. The titration's end point is signaled when the solution changes from the yellow color of the products to the brown color of the Karl Fischer reagent.

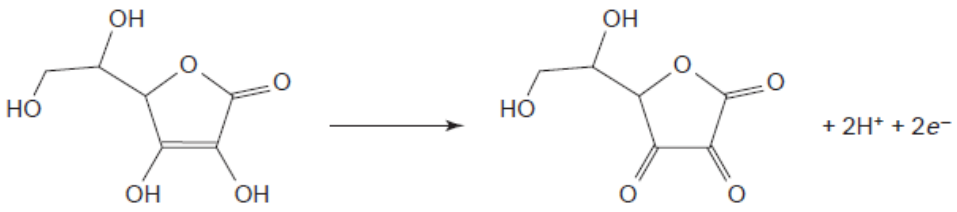
6.7 Organic Analysis

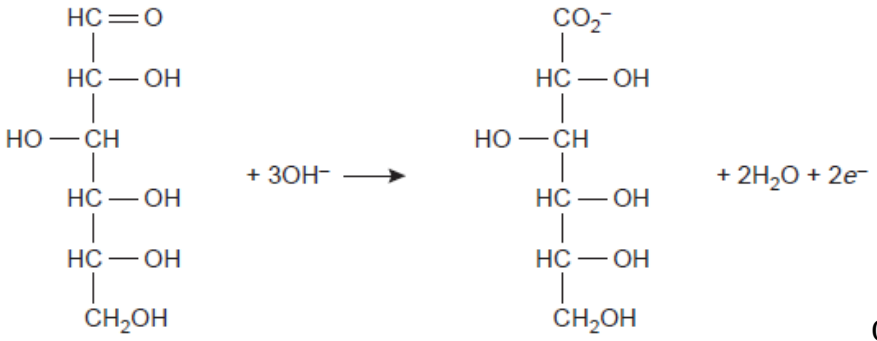
Redox titrimetric methods also are used for the analysis of organic analytes. One important example is the determination of the chemical oxygen demand (COD) in natural waters and

wastewaters. The COD provides a measure of the quantity of oxygen necessary to completely oxidize all the organic matter in a sample to CO2 and H2O. No attempt is made to correct for organic matter that cannot be decomposed biologically or for which the decomposition kinetics are very slow. Thus, the COD always overestimates a sample's true oxygen demand. The determination of COD is particularly important in managing industrial wastewater treatment facilities where it is used to monitor the release of organic-rich wastes into municipal sewer systems or the environment. The COD is determined by refluxing the sample in the presence of excess $K_2Cr_2O_7$, which serves as the oxidizing agent. The solution is acidified with H_2SO_4, and Ag_2SO_4 is added as a catalyst to speed the oxidation of low-molecular-weight

fatty acids. Mercuric sulfate, HgSO4, is added to complex any chloride that is present, thus preventing the precipitation of the Ag+ catalyst as AgCl. Under these conditions, the efficiency for oxidizing organic matter is 95–100%. After refluxing for 2h, the solution is cooled to room temperature, and the excess $Cr_2O_7^{2-}$ is determined by a back titration, using ferrous ammonium sulfate as the titrant and ferroin as the indicator. Since it is difficult to completely remove all traces of organic matter from the reagents, a blank titration must be performed. The difference in the amount of ferrous ammonium sulfate needed to titrate the blank and the sample is proportional to the COD.

Iodine has been used as an oxidizing titrant for a number of compounds of produces the tetrathionate ion, $S_4O_6^{2-}$. The tetrathionate ion is actually a dimer consisting of two thiosulfate ions connected through a disulfide (-S-S-) linkage. In the same fashion, I_3^- can be used to titrate mercaptans of the general formula RSH, forming the dimer RSSR as a product. The amino acid cysteine also can be titrated with I_3^-. The product of this titration is cystine, which is a dimer of cysteine. Triiodide also can be used for the analysis of ascorbic acid (vitamin C) by oxidizing the enediol functional group to an alpha diketone and for the analysis of reducing sugars, such as glucose, by oxidizing the aldehydefunctional group to a carboxylate ion in a basic solution.

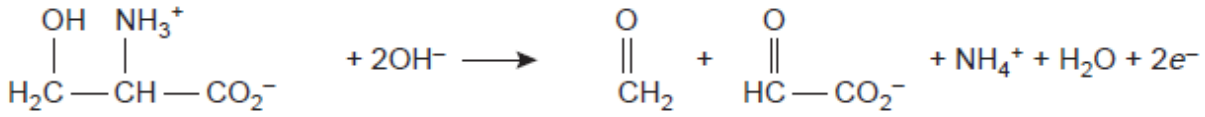




Organic compounds containing a hydroxyl, carbonyl, or amine functional group adjacent to a hydoxyl or carbonyl group can be oxidized using metaperiodate, IO_4^-, as an oxidizing titrant.

$$IO_4^-(aq) + H_2O(\ell) + 2e^- \rightarrow IO_3^-(aq) + 2OH^-(aq)$$

A two-electron oxidation cleaves the C—C bond between the two functional groups, with hydroxyl groups being oxidized to aldehydes or ketones, carbonyl functional groups being oxidized to carboxylic acids, and amines being oxidized to an aldehyde and an amine (ammonia if the original amine was primary). For example, treatment of serine with IO_4^- results in the following oxidation reaction



The analysis is conducted by adding a known excess of IO_4^- to the solution containing the analyte and allowing the oxidation to take place for approximately 1 h at room temperature. When the oxidation is complete, an excess of KI is added, which reacts with the unreacted IO_4^- to form IO_3^- and I_3^-.

$$IO_4^-(aq) + 3I^-(aq) + H_2O(\ell) \rightarrow IO_3^-(aq) + I_3^-(aq) + 2OH^-(aq)$$

The I_3^- is then determined by titrating with $S_2O_3^{2-}$ using starch as an indicator.

The amount of ascorbic acid, $C_6H_8O_6$, in orange juice was determined by oxidizing the ascorbic acid to dehydroascorbic acid, $C_6H_6O_6$, with a known excess of I_3^-, and back titrating the excess I_3^- with $Na_2S_2O_3$. A 5.00-mL sample of filtered orange juice was treated with 50.00 mL of excess 0.01023 M I_3^-. After the oxidation was complete, 13.82 mL of 0.07203 M $Na_2S_2O_3$ was needed to reach the starch indicator end point. Report the concentration of ascorbic acid in milligrams per 100 mL.

SOLUTION

Oxidizing ascorbic acid requires two electrons, and reducing I_3^- to I^- also requires two electrons. Thus

$$(\text{Moles } I_3^-)_{\text{ascorbic acid}} = \text{moles } C_6H_8O_6$$

For the back titration, the stoichiometric relationship between I_3^- and $S_2O_3^{2-}$ is (see Example 9.14)

$$(\text{Moles } I_3^-)_{\text{back titration}} = 0.5 \times \text{moles } S_2O_3^{2-}$$

The total moles of I_3^- used in the analysis is the sum of that reacting with ascorbic acid and $S_2O_3^{2-}$

$$(\text{Moles } I_3^-)_{\text{tot}} = (\text{moles } I_3^-)_{\text{ascorbic acid}} + (\text{moles } I_3^-)_{\text{back titration}}$$

or

$$\text{Moles } I_3^- = \text{moles } C_6H_8O_6 + 0.5 \times \text{moles } S_2O_3^{2-}$$

Making appropriate substitutions for the moles of I_3^-, $C_6H_8O_6$, and $S_2O_3^{2-}$

$$M_{I_3^-} \times V_{I_3^-} = \frac{\text{g } C_6H_8O_6}{\text{FW } C_6H_8O_6} + 0.5 \times M_{S_2O_3^{2-}} \times V_{S_2O_3^{2-}}$$

and solving for the grams of $C_6H_8O_6$ gives

$$(M_{I_3^-} \times V_{I_3^-} - 0.5 \times M_{S_2O_3^{2-}} \times V_{S_2O_3^{2-}}) \times \text{FW } C_6H_8O_6 =$$

$$[(0.01023 \text{ M})(0.0500 \text{ L}) - (0.5)(0.07203 \text{ M})(0.01382 \text{ L})](176.13 \text{ g/mol})$$

$$= 0.00243 \text{ g } C_6H_8O_6$$

6.8 Summary of the unit

Oxidation is defined as the loss of electrons producing a more positive product. The transfer of electrons represents the simplest kind of oxidation – reduction reaction, a reaction frequently referred to as a redox reaction. In such a reaction the number of electrons lost must equal the

number of electrons gained. Atoms of different elements may lose or gain varying numbers of electrons; therefore the oxidation or reduction capacity of any atom or ion must be calculated for a particular reaction. Knowing that information you can determine the stoichiometric relationship between the reactants. Redox titration is based on the redox reaction (oxidation - reduction) between analyte and titrant. Sodium oxalate and potassium permanaganate react in an acid medium according to the following half-cell reactions

$$C_2O_4^{2-} \rightarrow 2CO_2 + 2e^- \quad \text{(oxidation half-cell)} \qquad (1)$$

$$8H^+ + MnO_4^- + 5e^- \rightarrow Mn^{2+} + 4H_2O \quad \text{(reduction half-cell)} \qquad (2)$$

The two half-cell reactions are combined to obtain the complete redox reaction:

$$5C_2O_4^{2-} + 16\,H^+ + 2MnO_4^- \rightarrow 10CO_2 + 2Mn^{2+} + 8H_2O \qquad (3)$$

From this balanced equation, you can see that there is a 5:2 stoichiometric ratio between the moles of oxalate and the moles of permanganate. To facilitate the reduction of Mn+7 (purple) to Mn+2 (colorless), the reaction must be carried out in an acidic medium through the addition of H2SO4. Without the H2SO4, the reaction may stall at Mn^{+4}, resulting in the production of MnO2 (greenish-brown). If this should occur, the reaction would not proceed further and would have to be repeated using the proper conditions.

6.9 Key words

Redox Reactions; Redox Titration Curves; Inorganic Analysis; Organic Analysis.

6.10 References for further study

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6.11 Questions for self understanding

1) Explain the methods involved in redox reactions

2) Give the example of redox titration Curves

3) Explain the Inorganic Analysis using redox titration

4) Explain the Organic Analysis using redox titration

Unit-7

Structure

7.0 Objective of the unit

After studying this unit you are able to

- Explain the Karl Fischer Method
- Identify the Reagents in Karl Fischer Method
- Write the Original formulation according to Karl Fischer
- Recognize the Influence of the water content in Karl Fischer Method
- Recognize the Influence of the organic solvent in Karl Fischer Method
- Explain the Volumetric titration of Karl Fischer Method
- Explain the Coulometric titration Karl Fischer Method
- Analyze the amount of water content in different organic substance using KF Method

7.1 Introduction

Karl Fisher Titration is a technique for the determination of moisture content. The technique was developed by a chemist named Karl Fischer. It is based on a reagent which reacts with water and converts the water into a non conductive chemical. Karl Fisher provides for the specific detection of water. There are two methods used to perform the Karl Fischer titration test. One is known as Volumetric Karl Fischer. With this method the moisture determination is based on the amount, or volume, of reagent used to convert the water. In the Volumetric Method samples are dissolved in a solvent before the titration begins. A reagent is added until the water is removed.

The other method is known as Coulometric Karl Fischer. In this approach, the reagent and solvent are combined in the titration cell. When a sample is introduced into the titration cell and dissolved, reagent is released by the induction of an electrical current. The amount of current required to convert the water is the determinant of the amount of moisture. A Coulometric Karl Fischer instrument is often referred to as a coulometer.

7.2 Karl Fischer Method

The Water Determination Test (Karl Fischer Method) is designed to determine water content in substances, utilizing the quantitative reaction of water with iodine and sulfur dioxide in the presence of a lower alcohol such as methanol and an organic base such as pyridine, as shown in the following formulae

$$H_2O+I_2+SO_2 + 3\ C_5H_5N \rightarrow 2(C_5H_5N+H)I^- + C_5H_5N\cdot SO_3$$
$$C_5H_5N\cdot SO_3 + CH_3OH \rightarrow (C_5H_5N^+H)O\cdot SO_2\cdot OCH_3^-$$

There are two determination methods different in iodine-providing principle: the volumetric titration method and the coulometric titration method. In the volumetric titration method, iodine

required for reaction with water is previously dissolved in water determination TS, and water content is determined by measuring the amount of iodine consumed as a result of reaction with water in a sample.

In the coulometric titration method, first, iodine is produced by electrolysis of the reagent containing iodide ion, and then, the water content in a sample is determined by measuring the quantity of electricity which is required for the electrolysis (i.e., for the production of iodine), based on the quantitative reaction of the generated iodine with water.

7.3 Reagents

When developing his new analytical method Karl Fischer took into account the well-known Bunsen reaction, which is used for the determination of sulfur dioxide in aqueous solutions

$$SO_2 + I_2 + 2\,H_2O \rightarrow H_2SO_4 + 2\,HI$$

This reaction can also be used for the determination of water if sulfur dioxide is present in excess and the acids so produced are neutralized by a base. The selection of pyridine, the base used by Karl Fischer, was completely at random: «pyridine was just standing in the rack». This led to the establishment of the classical KF reagent, a solution of iodine and sulfur dioxide in a mixture of pyridine and methanol.

7.4 Original formulation according to Karl Fischer

254 g iodine is dissolved in 5 liters of anhydrous methanol. This is treated with 790 g pyridine, mixed thoroughly and then 192 g liquid SO_2 is added. 1 mL KFR corresponds to approx. 3 mg H_2O

The titer of this combined reagent was not very stable – titer determinations had to be carried out virtually every day. Help was provided by the use of separate reagents. The titration solution consisted of 30 g iodine in 1 liter methanol. For the reaction solution 60 g SO_2 was introduced into a mixture of 300 mL each of methanol and pyridine. The titer of the titration solution prepared in this way (approx. 1.7 mg H_2O / mL) only decreased by approx 1.3% over a two-month period and could be regarded as being relatively stable.

However, for most applications it is advisable to work with a one-component reagent with a relatively stable titer. In order to obtain such a reagent the methanol was replaced by ethylene glycol monomethyl ether (Cellosolve).

7.5 Chemical reactions

Karl Fischer presented the following equation for water determination with his reagent

$$2\ H_2O\ +\ SO_2\ x\ (C_5H_5N)_2\ +\ I_2\ +\ 2\ C_5H_5N\ \rightarrow\ (C_5H_5N)_2\ x\ H_2SO_4\ +\ 2\ C_5H_5N\ x\ HI$$

This gave a molar ratio of

$$H_2O : I_2 : SO_2 : \text{pyridine}\ =\ 2 : 1 : 1 : 4$$

under the assumption that methanol only functions as a solvent and pyridine forms additive compounds with the acids.

However, this molar ratio was incorrect, as established by the studies of Smith, Bryant and Mitchell (Fischer had assumed an aqueous Bunsen reaction). The three authors determined the molar ratio

$$H_2O\ :\ I_2\ :\ SO_2\ :\ \text{pyridine}\ :\ CH_3OH\ =\ 1 : 1 : 1 : 3\ :\ 1$$

In a first partial reaction water forms the (hypothetical) pyridine sulfur trioxide, which then further reacts with methanol

$$H_2O\ +\ I_2\ +\ SO_2\ +\ 3\ C_5H_5N\ \rightarrow\ 2\ C_5H_5N\ x\ HI\ +\ C_5H_5N\ x\ SO_3$$

$$C_5H_5N\ x\ SO_3\ +\ CH_3OH\ \rightarrow\ C_5H_5N\ x\ HSO_4CH_3$$

The authors subsequently revised this equation by formulating iodine and SO_2 as pyridine adducts.

From the pH-dependency of the reaction constants Verhoef and Barendrecht later became aware that in the KF reagent it is not the SO_2 that functions as the reactive component, but rather the monomethyl sulfite ion that is formed from SO_2 and methanol.

$$2\ CH_3OH\ +\ SO_2\ \rightleftharpoons\ CH_3OH_2^+\ +\ SO_3CH_3^-$$

They also established that pyridine does not take part in the reaction but only acts as a buffer substance (e.g. at the same pH the same reaction rate is achieved with sodium salicylate). Pyridine can therefore be replaced by another suitable base (RN). The added base results in a considerable displacement of the equilibrium to the right

$$CH_3OH\ +\ SO_2\ +\ RN\ \Rightarrow\ [RNH]\ SO_3CH_3$$

This means that the KF reaction in methanolic solution can now be formulated as follows

$$H_2O\ +\ I_2\ +\ [RNH]^+\ SO_3CH_3^-\ +\ 2\ RN\ \rightarrow\ [RNH]^+\ SO_4CH_3^-\ +\ 2\ [RNH]^+\ I^-$$

7.6 Influence of the water content

The water content of the working medium influences the stoichiometry of the KF reaction. If it exceeds 1 mol/L (18 g/L) then the reaction behavior changes to favor the Bunsen reaction for aqueous solutions. This means that 2 H_2O are consumed for one I_2 or one SO_2. It is uneconomic

to titrate such a high water content in a large volume of sample. In this case it is important to dilute the sample and/or to use a small amount of sample. If sepa rate KF reagents are used then the water capacity of the solvent must be taken into consideration, particularly for subsequent titrations. (The water capacity of these solvents is normally about 5...7 mg/mL, for a solvent volume of 25 mL this means max. 125...175 mg H_2O.)

7.7 Influence of the organic solvent

Theoretically non-alcoholic KF reagents can also be used, but in this case the stoichiometry also changes. In protic solvents, e.g. methanol or ethanol, the ratio H_2O : I_2 = 1 : 1, in aprotic solvents, e.g. DMF, the ratio H_2O : I_2 = 2 : 1. Intermediate values (uneven ratios) have been observed, e.g. with propanol, butanol and other long-chain alcohols. This has to do with the reactivity of the alcohol used. (In methanol about 100%, in propanol approx. 80% and in butanol only approx. 50% is present as sulfite ester.) With the KF reagents normally used an alteration in the stoichiometry is not to be expected, because:

– the titer determination and water determination are carried out in the same titration medium, and

– the alcohol fraction in the sample solution continuously increases during the course of the titration.

7.8 Kinetics of the KF reaction

Cedergren was (presumably) the first to thoroughly investigate the KF reaction sequence and establish that the reaction rate increases as the concentrations of SO_2, I_2 and H_2O increase. The reaction is first order referring to each individual component. For the rate constant K he used the equation

$$- d [I_2] / dt = K [I_2] \times [SO_2] \times [H_2O]$$

to obtain the value:

$$K = (1.2 \pm 0.2) \times 10^3 \times l^2 \times mol^{-2} \times s^{-1} \quad \log K = 3.08\ 0.08$$

Verhoef and Barendrecht confirmed the results of Cedergren, but at the same time found that the reaction constant K depends on the pH of the solution:

– Up to pH = 5 log K increases linearly with the pH.

– Between pH = 5.5 and 8 the reaction rate is constant (there is a plateau).

– Above pH 8 the reaction rate again increases slightly (side reactions probably occur).

From the increases in K under acidic conditions the authors conclude that it is not SO2, but the sulfite base that is the reactive component.

The reaction rate is also strongly influenced as a result of the concentration of iodide ions. The iodine in the KF solution reacts with iodide to form triiodide; this strongly displaces the equilibrium to the right

$$I_2 + I^- \Rightarrow I_3^-$$

However, during the oxidation of the methyl sulfite the free iodine reacts far more quickly than the triiodide (Δ K approx. 104). This reaction is also pH-dependent.

7.10 Volumetric titration

Apparatus

Generally, the apparatus consists of an automatic burette, a backtitration flask, a stirrer, and an equipment for amperometric titration at constant voltage or potentiometric titration at constant current.

Because water determination TS is extremely hygroscopic, the titration apparatus should be protected from atmospheric moisture. Silica gel or calcium chloride for water determination is usually used for moisture protection.

Procedure

As a rule, the titration of the sample with water determination TS should be performed at the same temperature as that at the standardization of the TS, while protecting from moisture. The apparatus is equipped with a variable resistor in the circuit, and the resistor is adjusted to apply a definite voltage (mV) between a pair of platinum electrodes immersed in the solution to be titrated. The change in current (μA) is measured during the dropping of water determination TS (Amperometric titration at constant voltage). As titration continues, the abrupt change in current in the circuit occurrs, but returns to the original state within several seconds. At the end of a titration, the change in current persists for a certain time (usually, longer than 30 seconds). The end point of titration is determined at this electric state.

Otherwise, by adjusting the resistor, a definite current is passed between the two platinum electrodes, and the change in potential (mV) is measured during dropping water determination TS (Potentiometric titration at constant current). With the progress of titration, the value indicated by the potentiometer in the circuit decreases suddenly from a polarization state of several hundreds (mV) to the nonpolarization state, but it returns to the original state within several seconds. At the end of titration, the non-polarization state persists for a certain time

(usually, longer than 30 seconds). The end point of titration is determined when this electric state attains.

In the case of back titration, when the amperometric titration method is used at constant voltage, the needle of microammeter is out of scale while an excessive quantity of water determination TS remains. It returns rapidly to the original position when the titration reaches the end point. Similarly, when the potentiometric titration method at constant current is used, the needle of the millivoltmeter is at the original position while an excessive quantity of water determination TS remains. A definite voltage is applied when the titration reaches the end point. Unless otherwise specified, the titration of water with water determination TS is performed by either of the methods below. Usually, the end point of the titration can be observed more clearly in the back titration method than in the direct titration method.

a) Direct titration

Unless otherwise specified, proceed as directed below,

Take 25ml of methanol for water determination in a dried titration flask, and titrate with water determination TS to the end point. Unless otherwise specified, weigh accurately a quantity of the sample containing 10 to 50 mg of water, transfer it quickly into the titration flask, and dissolve by stirring. Titrate the solution with water determination TS to the end point under vigorous stirring. When the sample is insoluble in the solvent, powder the sample quickly, weigh a suitable amount of the sample accurately, and transfer it quickly into the titration vessel, stir the mixture for 30 minutes while protecting it from moisture. Perform a titration under vigorous stirring. When the sample interferes with the Karl Fisher reaction, water in the sample can be removed by heating and under a stream of nitrogen gas, and introduced into the titration vessel by using a water-evaporation device.

$$\text{Water}(H_2O) = \frac{\text{Volume(ml) of TS for Water Determination consumed} \times f\ (\text{mg/mL})}{\text{Weight of the sample(mg)}} \times 100\,(\%)$$

b) Back titration

Unless otherwise specified, proceed as directed below,

Take 20ml of methanol for water determination in the dried titration vessel, and titrate with water determination TS. Weigh accurately a suitable quantity of the sample containing 10－50 mg of water, transfer the sample quickly into the titration vessel, add an excessive and definite volume

$\text{Water}(H_2O)$

of water determination TS, stir for 30 min, protecting from atmospheric moisture, and then titrate the solution with Water－Methanol Standard Solution under vigorous stirring.

7.11 Coulometric titration

Apparatus

Usually, the apparatus is comprised of an electrolytic cell for iodine production, a stirrer, a titration flask, and a potentiometric titration system at constant current. The iodine production device is composed of an anode and a cathode, separated by a diaphragm. The anode is immersed in the anolyte solution for water determination and the cathode is immersed in the catholyte solution for water determination. Both electrodes are usually made of platinum-mesh.

Because water determination TS is extremely hygroscopic, the titration apparatus should be protected from atmospheric moisture. For this purpose, silica gel or calcium chloride for water determination is usually used.

Procedure

Take a suitable volume of an analyte for water determination in a titration vessel, immerse in this solution a pair of platinum electrodes for potentiometric titration at constant current. Then, immerse the iodide production system filled with a catholyte for water determination in the anolyte solution. Switch on the electrolytic system and make the content of the titration vessel anhydrous. Next, take an accurately weighed amount of the sample containing 1－5 mg of water, add it quickly to the vessel, and dissolve by stirring. Perform thetitration to the end point under vigorous stirring. When the sample is insoluble in the anolyte, powder it quickly, and add an accurately weighed amount of the sample to the vessel. After stirring the mixture for 5－30 minutes, while protecting from atmospheric moisture, perform the titration with vigorous stirring.

Determine the quantity of electricity (C) [electric current (A) × time (s)] required for the production of iodine during the titration, and calculate the content

(%) of the water in the sample by the formula below. When the sample interferes with the Karl Fisher reaction, water in the sample can be removed by heating under a stream of nitrogen gas, and introduced into the titration vessel by using a water-evaporation device.

7.12 Carbonates, hydroxides and oxides

During the KF titration methyl sulfuric acid and hydriodic acid are produced. Although these are attached to the base, they still form weak acids that react with the above-mentioned compounds to form salts and release water

$$Na_2CO_3 + 2\,HI \rightarrow 2\,NaI + CO_2 + H_2C$$
$$Ca(OH)_2 + H_2SO_4 \rightarrow CaSO_4 + 2\,H_2O$$
$$MgO + 2\,HI \rightarrow MgI_2 + H_2O$$

Not all oxides take part in an acidic reaction. In addition to alkali oxides and alkaline earth oxides, the following will react: Ag_2O, HgO, MnO_2, PbO, PbO_2 and ZnO.

We recommend that the H_2O in these compounds is determined by the oven method

7.13 Aldehydes and ketones

These two classes of compound combine with the methanol in the KFR to form acetals and ketals respectively with the release of water

$$\text{Aldehyde; } RHC{=}O + 2\,CH_3OH \rightarrow \text{Acetal; } RHC(OCH_3)_2 + H_2O$$
$$\text{Ketone; } R_2C{=}O + 2\,CH_3OH \rightarrow \text{Ketal; } R_2C(OCH_3)_2 + H_2O$$

With aldehydes the reactivity decreases as the chain length increases. Aromatic aldehydes react more slowly than aliphatic aldehydes. Acetaldehyde reacts the quickest and causes the most problems. But there are always exceptions to any rule, formaldehyde and chloral do not form acetals and can be titrated without any problems.

Ketones are less reactive than aldehydes. The reactivity also decreases as the chain length increases. Ketal formation is most rapid with acetone and cyclohexanone. The following are regarded as being stable (no ketal formation): diisopropyl ketone, benzophenone, deoxybenzoin, benzil, benzoin, camphor, alizarin, dibenzalacetone and dichlorotetrafluoroacetone.

Acetal and ketal formation is also influenced by the alcohol present. As the chain length increases the reactivity also decreases. This means that methanol is the quickest to react.

There are several ways of preventing acetal or ketal formation:

a) By using KF reagents specially labeled for this purpose.

b) By using methanol-free KF reagents. Methanol is replaced by a higher alcohol, e.g. 2-methoxyethanol.

c) By making use of the different reaction rates and slowing down acetal or ketal formation so much that they do not interfere with the titration. This can be achieved by carrying out the titration at –10 °C. This also freezes the bisulfite addition.

With aldehydes a second reaction – bisulfite addition – can also interfere. In this case H_2O is consumed, so that the water content determined could be too low

$$RHC{=}O + SO_2 + H_2O + NR' \rightarrow RHC(OH)SO_3HNR'$$

7.14 Summary of the unit

In addition to the determination of the pH, weighing and acid-base titration, the determination of the water content is one of the most frequently used methods in laboratories around the world. In addition to methods requiring complex apparatus, such as infrared reflection spectrometry, gas chromatography or microwave spectroscopy, two methods in particular have been able to establish themselves. KF method is commonly found in various standards, but suffers from the following disadvantages:

– In principle the loss on drying is determined, and not necessarily the water content. Apart from water, other volatile components of the sample and/or decomposition products are also determined. In order to obtain comparable results work must be carried out under strictly maintained predefined conditions (temperature and duration of the drying process).

– It takes a long time to obtain the analytical results (several hours in a drying oven). This can lead to bottlenecks, e.g. in the monitoring of manufacturing processes.

7.15 Key words

Karl Fischer Method; Volumetric titration; Direct titration; Back titration; Coulometric titration

7.16 References for further study

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7.17 Question for self understanding

1) Write a note on Karl Fischer Method

2) What are the reagents used in KF method?

3) Deduce Original formulation according to Karl Fischer

4) Write the Chemical reactions involved in KF methods

5) Explain the Influence of the water content in KF methods

6) Explain the Influence of the organic solvent in KF methods

7) Write a note on Kinetics of the KF reaction

8) Explain volumetric titration of KF methods with respect to

   a) Direct titration

   b) Back titration

9) Explain the Coulometric titration of KF methods

10) How water content is determined in Carbonates, hydroxides and oxides using in KF methods

11) Explain the determination of water content in Aldehydes and ketones in KF methods

Unit-8

Structure

8.0 Objectives of the unit

After studying this unit you are able to

- Explain the importance and current trends of radioanalytical chemistry
- Explain the principles and importance radioanalytical chemistry
- Explain the Isotope dilution analysis
- Explain the direct Isotope dilution dnalysis
- Explain the reverse Isotope Dilution Analysis
- Explain the substoichiometric Isotope Dilution Analysis
- Explain the redox Substoichiometry
- Explain the displacement Substoichiometry

8.1 Introduction

Radio analytical chemistry covers the use of radioactive nuclides and nuclear radiation for analytical purposes. The use of radionuclide techniques in analytical chemistry was first reported in 1913 by G. HEVESY and F. PANETH in a study of the solubility of lead sulfide in water, using the natural lead isotope. Isotope dilution analysis was introduced by HAHN in 1923, using 231Pa to determine the yield of 234Pa. The development of radioreagent methods followed, and further development of radioanalytical chemistry has established a range of analytical methods and techniques. These include the use of artificial radionuclides and labeled compounds, the principles of nuclear activation, and absorption and scattering of radiation.

8.2 General Features

Radiochemical analysis is based on two outstanding features of radioactivity:

1) The high sensitivity and ease of measurement of radioactive radiation

2) The possibility of labeling chemical compounds with radioactive tracers

Radionuclide techniques often have higher sensitivity than other analytical methods. The amounts of nuclides, correlated to an activity of 10OOBq can be derived from the law of radioactive decay. These amounts vary considerably, corresponding to the wide range of radioactive half-lifes. For 90% of the commonly used nuclides, half-lifes range from several minutes to several years, so the corresponding masses are extremely low. Radionuclides are often diluted with inactive isotopes, but specific activities (i,e., activity per total mass of element) are still very high. Since the background in nuclear spectroscopy is very low, and sensitivity is high, activities as low as 0.2 Bq can be readily detected.

Analytical applications of radionuclide techniques rely on the assumption that different isotopes of the same element exhibit the same properties in any macroscopic physical or chemical process, and that radioactive labeling does not influence the other properties of a chemical species. This is generally the case, with deviations below 1 % (with the exception of hydrogen isotopes) owing to isotopic fractionation or radiation effects. For analytical purposes, the radiotracer and the analyte must be present in the same chemical form. This is usually to achieve, but specialized preparative techniques may be necessary for radioactive labeling of more complex organic compounds.

8.3 Importance and Current Trends of Radioanalytical Chemistry

Radionuclides are used in many subdivisions of analytical chemistry. Of major importance are radiotracers in methodological and pathway studies, isotope dilution analysis (IDA), radioimmunoassay, and nuclear activation analysis (AA). They are all especially suited to analyze the extremely small amounts of substances encountered in ultratrace analysis or in trace analysis of microsamples. Over the past two decades, the emphasis has shifted from high detection power in routine analysis toward an independent approach, applying this high detection power to the development of analytical procedures and reference materials. Radiochemical methods for routine analysis have lost ground to other, primarily spectroscopic, methods. Nonradiochemical methods often yield highly reproducible results, but may involve systematic errors. The need for increased reliability and analytical quality control has emphasized the usefulness of radiochemical methods for the certification of standard reference materials (SRM). Radioanalytical methods are often suitable for homogeneity testing and distribution analysis of traces in SRMs. Activation analysis, radiotracer techniques, and isotope dilution analysis are becoming increasingly important for assessment of analytical quality. Radioanalytical methods are well suited to the determination of basic analytical data, such as equilibrium constants, or kinetic data. These data are important for development and optimization of new analytical procedures. The most frequent analytical use of tracers is in the biomedical sciences. Radioimmunoassays, in the form of rapid diagnostic test kits have led to the development of nonradioactive assays based on similar principles]. Many important tracer applications can be substituted by other methods. Even IDA and tracer applications in self-diffusion studies can be replaced by inactive isotope tracer methods using mass spectrometry and other methods for

isotope ratio determination. However, because of the extremely high sensitivity of IDA, radioactive tracers are of unique usefulness in radioimmunoassays,

radiorelease reagents, radiochromatography, AA, and for systematic studies in trace and ultratrace analysis, physiological chemistry, IDA, diffusion, isotope exchange, and physical chemistry of solids.

Radioanalytical chemistry will continue to occupy an important position, despite increasing criticism of nuclear technology. Recently, there has been little innovative work in the field of methodology, but the applications field has continued to expand. Highlights include application of isotope enrichment techniques in environmental studies, improved accuracy in the characterization of SRM, preirradiation chemistry with high-purity reagents under clean room conditions to permit speciation from, derivative AA, accelerator-based dating methods combined with IDA, short-time and pulsed reactor activation, extension of the sub- and superequivalence method, in-vivo analysis by prompt- neutron AA and by short-lived AA and extended use of the single comparator ***(ko)*** standardization method in neutron AA L301.

8.4 Choice of Radionuclide

The choice of radionuclide involves:

1) Measurement, possibly including discrimination between different indicator nuclides or indicator and interfering nuclides (e.g., from byproducts or blank level impurities)
2) Suitable half-life
3) Chemical species

Gamma (γ) *Emitters* are preferred for multitracer experiments (most of all in nuclear AA) for applications where the tracer is dispersed in, or shielded by a material (e.g., in-vivo diagnostics in biology and medicine, measurement from bulky solid material), but also from tracer solutions.

Pure beta β *emitters* are especially useful where the shorter range of their radiation is utilized, e.g., for autoradiography 1391, and for surface and thin film studies, including adsorption, corrosion, and catalysis. Measurements of 8-emitting tracer solutions are performed with high efficiency by liquid scintillation.

Alpha α Emitters are only used as tracers in special cases, either where their very low range is important

(e.g., surface and thin film techniques), or where they represent the only available nuclides of the element (e.g., actinides).

Imperfect trucers are often used when labeling of a specific chemical site is inappropriate with isotopic nuclides. Imperfect tracers are widely used to label gas streams with inert gases (85Kr, " Ar, "'Xe), water with dissolved anionic tracers

(**Br, I3'I, 24Na), hydrocarbon fuels with dissolved "Co-naphthenate or 1,2-**Br-dibromoethane. Similarly, loss of metal ions at ultra-trace levels due to adsorption on the vessel walls and by volatilization have been modeled by "'Au, ""Hg, *'"Co* which are easily measured, and which are available at high specific activity.

8.5 Radiotracers in Methodological Studies

Radio analytical chemistry offers a variety of accurate and highly sensitive methods. Even with the increased performance of other trace analysis methods, including electrothermal atomic absorption spectrometry, atomic emission, and fluorescence spectrometry, coupling methods, and high-performance sample introduction in organic and inorganic mass spectrometry), chromatographic separations and neutron AA, there are still a number of areas where radionuclide applications are unsurpassed.

Picogram amounts of chemical species can be traced through a complete chemical process, and problems in process performance are often revealed and overcome rapidly and simply [%I, WI. lmportant information can be obtained rapidly and cheaply by radioindicators. Specific procedures for quantitatie determinations based on radiotracers involve additional principles.

8.6 Principles and Importance

Radioactive tracer analysis is based on the simple proportionality between the mass *m,* of analyte ***x*** and the radioactivity ***A,*** initially added to the sample:

$$m, = k A,$$

Where ***k*** is proportionality constant, measured for an individual experiment. This is based on the condition that the radioactive substance **ml** added to the sample does not increase the mass of the analyte, i.e., *m,* >> *m,*. Otherwise, for determination of *m,* from ***A,*** the formalism of IDA is applied, but very often the simple proportionality between mass ***(m,*** + ml) and **A,** is sufficient for a methodological investigation.

By adding a radioactive tracer before theindividual process stage, and following it by radioactivity measurements, the recovery, distribution, and loss are measured independently of any additional risk of contamination. By means of nuclear activation, radioactive tracers can be introduced into solid samples and can be used to determine the recovery and loss during

decomposition of solids, and separation from solid samples by volatilization, leaching, and solvent extraction , A variety of examples from the general analytical field and from trace element analysis covers separate stages of combined analytical procedures such as decomposition evaporation, coprecipitation, adsorption, and washout of precipitates: contamination and adsorption, separation by chromatography, ion-exchange, liquid - liquid distribution, and electrodeposition. Contamination and adsorption, separation by chromatography, ion-exchange, liquid - liquid distribution, and electrodeposition

Radioanalytical indicator methods (experienced a revival in the 1960s and early 1970s. Modern direct instrumental methods underemphasized the importance of radioanalytical methods, which are necessary to establish the accuracy of spectroscopic methods, to avoid systematic errors, and to assess trace content in SRMs. An important advantage is the ability to reveal accidental losses, even at the ultra-trace level. The most common areas of analytical applications are:

1) Determination of equilibrium constants, including solubility and complex dissociation constants, phase distribution in solvent extraction, adsorption, ion exchange, coprecipitation: and kinetic data, such as rate constants

2) Tracing the distribution of a chemical species in an analytical system for decomposition, chromatography, evaporation, and separation

3) Determination of recovery, yield, and loss of a chemical species in a process

8.7 Control of Sampling

Inaccuracies in sampling procedures occur, owing to contamination, loss, and nonrepresentative sampling. Sampling, pretreatment, storage, etc., all affect the accuracy.

Extended radioindicator studies on trace element sampling (e.g., of Al, Cr, Mn, Fe, Co, Zn, Sr. Cs, As, Hg, Pb) have been reviewed. In environmental analysis (e.g., solids, water, aerosols, biological tissue), adequate sampling and sample treatment is of primary importance to account for variation in biological activity due to the distribution of chemical species between different phases and solid fractions. Sample homogeneity may be a significant limitation to the quality of analytical results. It is of increasing importance with decreasing concentrations from the pg/g to the ng/g level, and it limits the amounts of subsamples and the number of replicates. Studies on sample homogeneity, and on sampling and storage of very dilute solutions, are conveniently performed with radiotracers for numerous trace elements.

8.8 Control of Contamination and Loss

Contamination generally poses the most important problem in ultra-trace analysis. It causes fluctuations of the blank values, thus defining the lower limits of detection, but also introduces systematic inaccuracies. Tracer techniques can be used to study the sources of contamination: reagent blanks, vessel walls, airborne pollutants, etc.

Contamination cannot be overcome by radiochemical means. Purification procedures for reagents and vessels and a clean working environment are required and SRMs should be used. Tracer techniques are unique tools in its investigation and control. Radioindicator studies have been conducted on reagent purification by electrolysis, sublimation, sub-boiling distillation, liquid- liquid distribution, ion exchange, and on vessel cleaning by rinsing, leaching, and steaming.

In general, the same principles are applied to radiochemical investigations of losses by adsorption (i.e., during storage, pretreatment, precipitation, filtration), by volatilization (during decomposition, ashing, storage, digestion, drying), and by chemical reaction (complexation, ion exchange, photochemical and redox reaction) using radioindicators .

8.9 Separation Procedures

Separation stages and chemical reactions, e.g., for decomposition or phase transformations, are essential components of combined analytical procedures. They should be reproducible, quantitative, selective, and unequivocal. Separation procedures are based on the principles of volatilization, liquid - liquid distribution, adsorption, diffusion, chromatography, ion exchange, electrophoresis, precipitation, coprecipitation, and electrodeposition. In all of these, radiotracers provide the best tool for methodological investigations, determination of equilibrium constants, kinetic data, and optimization of applied analytical data (yield, interference levels, etc.).

8.10 Control of the Determination Stage

Radionuclides in methodological studies of determination methods are sometimes useful. These are the measurement processes associated with the determination stage of a combined analytical procedure. Sources of systematic error in atomic absorption spectroscopy, optical emission spectrometry, and electrochemical methods. As well as optimization of the determination procedures have been examined. Examples are the behavior of trace elements in graphite furnace atomizers and investigations of the double layer structure on analytical electrodes and its exchange reactions with the solution.

8.11 Isotope Dilution Analysis

The principle of isotope dilution analysis (IDA), 1901, 1971, 1981 involves measurement of the change in isotopic ratio when portions of **a** radiolabeled and nonlabeled form of the same chemical species are mixed. To perform a radioisotope IDA, an aliquot of a radioactive spike substance of known specific activity *ai=Ai/tn,* is added to the test sample containing an unknown analyte mass *m,*. This analyte mass is then calculated from a determination of the specific activity *u.*, of the resulting mixture after complete homogenization.

The important advantage of IDA is that the analyte need not be isolated quantitatively. This is often significant at the trace concentration level, where quantitative separation is not feasible or inconvenient, or if interferences occur, e.g., in the analysis of mixtures of chemically similar compounds. Only a portion of the analyte is separated, so that the separation reagent is not used in excess, and this generally improves selectivity. Selective determination of *chemical species* in various states (e.g., tri- and pentavalent **As,** Sb, tri- and tetravalent Ru, Ir) by IDA is favored by the supplementary selectivity gained from the use of substoichiometric amounts of reagent. Losses during purification and other steps are taken into account. A suitable separation procedure is needed to isolate part of the homogenized sample**.**

In more advanced variants of IDA, measurements of masses are substituted by volumetric and complementary activity measurements. The same principle is alw applied to inactive stable iwtope **IDA.** Maw spectrometric determination of isotope ratios then replaces the activity meawrements [82] (- Mass Spectrometry).

8.12 Direct Isotope Dilution Analysis

In this most frequently used version of IDA (single IDA), the mass *m,* of inactive analyte substance can be determined by using the labeled substance of mass ml , radioactivity **Al** , and the specific activity *a,:*

$$a_2 = \frac{A_1}{m_1 + m_x} = \frac{A_2}{m_2}$$

The total radioactivity of the system is unchanged:

$$(m_1 + m_x)a_2 = m_1 a_1$$

The unknown mass ***m,*** is calculated from:

$$m_x = m_1 \left(\frac{a_1}{a_2} - 1 \right)$$

The specific activities al and *a2* are obtained from activities **A,** and *A2* and masses *m,* and ***m2*** of the fractions isolated from the initial labeled substance (Eq. 4) and from the homogenized solution Equation (7) shows that IDA depends on the relative change in specific activity due to isotope dilution. Low-yield separations may be sufficient. For high precision, *a,* should be high, with mass ***m,*** << *m,*. Then Equation (7) reduces to

$$m_x \approx m_2 \frac{A_1}{A_2} = \frac{A_1}{a_2}$$

Above Equation shows that the accuracy of ***m,*** is limited by the physicochemical determination of *m2.*

Example. Determination of glycine in hydrolyzed protein [98]: 152.6 mg hydrolyzed protein homogenized with 5.07 mg 14CC-labelegdl ycine of specific activity u I =(96.2? **I** .2) counts min-' mg-' (relative activities are sufficient). After separation of a portion of glycine from the mixture, specific activity of u2=(51.3?0.9) counts min-' mg-I. Equation (7) gives:

$$m_x = 5.07\,\text{mg}\left(\frac{96.2}{51.3} - 1\right) = (4.44 \pm 0.17)\,\text{mg}$$

The percentage of glycine in the protein is:

$$\frac{4.44}{152.6} \cdot 100 = (2.91 \pm 0.11)\%$$

8.13 Reverse Isotope Dilution Analysis

Another application of IDA is the determination of the activity **A!** of a radioactive substance *y* from a complex mixture of radioactive substances by adding a known mass ***m,.*** of an inactive carrier substance. This technique is also based on Equation (7), reversely solved for activity ***A*** l. Homogenization and separation of a portion *m2* (activity ***Az,*** specific activity *u2)* gives:

$$A_y = a_2 (m_c + m_y)$$

from the specific activity *a,* of substance *y.* If the inactive carrier is used in significant excess (i.e., ***mC>> m,)*** the separation yield is:

$$A_y = A_2 \frac{m_c}{m_2}$$

To obtain ***m,,*** the specific activity *u,* is required:

m, =AJa,.

Reverse IDA (indirect IDA, or dilution with inactive isotopes) is particularly important in organic analysis and biochemistry to test for radiochemical purity and stability of labeled compounds. It is often used in radiochemical nuclear AA for the separation of activated element traces from a variety of interfering radionuclides in the analytical sample, for determination of the isolated activity, in comparison with that of a reference sample (i.e., to determine the radiochemical yield according to above equation).

In contrast to direct IDA, reverse IDA is not limited by the sensitivity of the analytical determination of the isolated mass ***m2,*** but merely by the specific activity *a,*. of the radioactive substance. By adjusting the carrier mass ***mc,*** the sensitivity and accuracy can be increased, **so** reverse IDA is suitable for trace and microanalysis. Losses by absorption from carrier-free solutions must be taken into account. In separations from mixtures of radioactive substances, high-quality purification is important.

8.14 Double (Multiple) IDA

Since the specific activity ***a,*** is often unknown, double IDA can be used to determine *m,*. Two equal aliquots containing the same, but unknown mass *m,* are diluted with different known carrier masses, ***m,*** and ***m',*** . After separation of portions *m2,* ***ml2*** and measurement of specific activities *a*, a'2* one obtains from Equation (9):

$$a_2 = \frac{A_y}{m_c + m_y} = \frac{a_y m_y}{m_c + m_y} \qquad (11)$$

$$a_2' = \frac{a_y m_y}{m_c + m_y} \qquad (12)$$

Thus:

$$m_y = \frac{a_2' m_c - a_2 m_c}{a_2 - a_2'} \qquad (13)$$

$$a_y = \frac{(m_c - m_c')a_2 a_2'}{a_2 m_c - a_2' m_c} \qquad (14)$$

Multiple (direct and reverse) IDA produces a calibration graph ***m,*** =f(*Ilaz).*

8.15 Derivative Isotope Dilution Analysis

If the preparation of labeled substances is too difficult, derivative IDA may be applied. This technique combines a radioreagent and IDA, pref erably of low selectivity, to react with one or

more analytes. It is mainly used with mixtures of complex organic compounds. The basic stages are:

1) Reaction of analyte A with radioreagent B*, of known specific activity, to form a radioactive product AB*. Excess B* may be removed in an optional purification step:

$$A + B^* \longrightarrow AB^* + B^*_{ex} \qquad (15)$$

2) The mass of AB* is determined by mixture with a known mass of inactive AB by reverse IDA:

$$AB^* + B^*_{ex} + AB \longrightarrow (AB^* + AB) + B^*_{ex} \qquad (16)$$

3) Separation of diluted product (AB* + AB) and determination of its specific activity.

The principal requirements for this technique are:

1) Reaction (Eq. 1.5) should be quantitative or of known yield.
2) Isotope exchange between substances AB and B* must be prevented. Since inorganic substances are quite amenable to such exchange, application of derivative IDA to inorganic analytes is impossible.
3) Purification from excess reagent must be quantitative to avoid significant bias, even from minor impurities. As a variant, dilution with A* is applied prior to the first reaction (Eq. 1.5) with radioreagent B*. Determination of the reaction yield and analysis of inorganic substances are possible with this variant technique [2]. Its main importance is in the determination of trace amounts in complex mixtures. It is routinely used in biochemistry and physiological chemistry [2], 1311, 1321, [7.5]. The simpler approaches of radioimmunoassays are generally preferred. Further variants are based on combinations with double derivative IDA, or addition of an inactive derivative prior to direct IDA.

8.16 Substoichiometric Isotope Dilution Analysis

Utilization of conventional IDA for trace determinations is limited by the necessity to isolate macroscopic amounts of the analyte substance for determination of *ml* and *m2* by physicochemical methods. The principle of substoichiometry developed by RUWKA and **STAR+** ***[S7]*** avoids this limitation. Exactly equal (low) masses ***m,*** and *m2* are isolated from the spike solution of initial specific activity *a* , **= Al/m,** and from the solution after homogenization with the analyte ***(a2 =Az/mz).*** Equation (7) reduces to:

$$m_x = m_1\left(\frac{A_1}{A_2} - 1\right) \qquad (17)$$

The masses **ml** and *m2* are isolated using the same amount of the separation reagent, which must be stoichiometrically insufficient and consumed quantitatively (or to exactly the same amount) in this reaction. It must form a product which can easily be separated from the excess of unreacted substance. Separation reagents should have high chemical stability, no tendency to adsorption at very low concentrations, and sufficient selectivity for the analyte. Separations with enrichment factors of **lo4-** lo5 are typical. Since the mass ***m,*** of the added radioactive substance must be accurately known, reverse IDA can be applied. At trace levels below 1 pg, standard deviations of ca. 3 % and accuracies of ca. 6% are typical; precision and accuracy can be I **.5** % or better at higher concentrations.

8.17 Substoichiometric Separation by Liquid - Liquid Distribution

The extraction of metal chelates is suitable for separating equal masses of metals from solutions of different concentrations. Extraction of a metal chelate MA from a metal M"+ by an organic reagent HA is generally described by [47], [S7], [%I:

$$M^{n+} + n\,(HA)_{org} \rightleftharpoons (MA_n)_{org} + nH^+ \qquad (18)$$

with the extraction constant K:

$$K = \frac{[MA_n]_{org}\,[H]^n}{[M^{n+}]\,[HA]^n_{org}} \qquad (19)$$

where subscript org denotes the organic phase. In trace element analysis by substoichiometric IDA, concentrations of I o - -~ I O-' mol/L are typically extracted from aqueous samples of volumes V in the range 10 mL to 100 **pL** into organic phases of smaller volumes (typically one-tenth of the sample volume) of 10^{-5} to 10^{-8} mol/L solutions of chelating reagent.

The dependence of Equation (18) on the pH value of the solution makes it necessary to keep within an optimum pH range. The substoichiometric principle requires a higher than 99 % consumption of the initial concentration **c,~,,** in Equation (IX), so that $[HA]_{conc}$ < O.O1C,HA. Thus the solution must satisfy the condition:

$$pH > -\log\,(0.01c_{,HA}) - \frac{1}{n}\log K \qquad (20)$$

With increasing pH, dissociation of acid **HA** (dissociation constant **KHA)** in the aqueous phase increases. This dissociation is negligible at:

$$pH < pK_{HA} + \log q_{HA} + \log\frac{V_{org}}{V} \qquad (21)$$

where q_{HA} is the distribution coefficient of the reagent, V i h the sample volume, and **Vo,s** is the volume of the organic phase. Hydrolysis of the metal at increasing pH must not interfere with the separation.

The reagent (e.g., diphenylthiocarbazone, diethyldithiocarbamic acid (2-thenoxy)-3,3,3-trifluoroacetone, 8-hydroxyquinoline) must be stable to decomposition, even at very low concentrations in acid and neutral media, and must form an extractable chelate with a sufficiently high value of *K.* Optimum conditions for substoichiometric extraction are calculated from *K,* **KHA,** and **qHA.** Selectivity of the separation can be improved by use of additional masking reagents. Substoichiometric extraction of ion-association complexes is generally restricted to higher concentration levels (e.g., determination of Na, P). Water-soluble complexes of more than 20 metals with ethylenediaminotetraacetic acid can be used for substoichiometric determination at concentrations below **1** ng/g, provided that the excess of unreacted metal is separated by liquid-liquid distribution, ion-exchange, carrier coprecipitation, or other suitable means. Selectivity is high and can be further increased by masking reagents.

8.18 Redox Substoichiometry

Oxidation or reduction by a substoichiometric mass of reagent is followed by separation, extraction, coprecipitation, etc.. $KMn0_4$, $K_2Cr_20_7$, and FeSO, have been used as substoichiometric redox reagents

8.19 Displacement Substoichiometry

Complete extraction of an analyte metal M, with excess complexing reagent is followed by removal of the excess and subsequent displacement of Mi by a substoichiometric amount of another metal M2, provided that M2 has a sufficiently higher extraction constant *KZ.* Further variants are discussed elsewhere.

8.20 Applications

Applications include trace element determination in rocks, soils, biogenic samples, and pure metals by direct IDA, i.e., added radiotracer. Another field of increasing importance is in radiochemical AA for assessment and improvement of accuracy at trace and ultra-trace levels by determination of the radiochemical yield. This applies primarily to biomedical samples, high-purity materials, and environmental materials.

8.21 Isotope Exchange Methods

Isotope exchange methods (IEM) are based on the exchange of isotopes between two different

compounds of the element X, one of which (XA) is nonradioactive, the other (XB) being labeled with a radioactive isotope [21, [611, L871, (971. After isotopic equilibrium is reached

$$XA + {}^{*}XB \rightleftharpoons {}^{*}XA + XB$$

the specific activities of the element X in both compounds are equal

$$A_1/m = A_2/m_x$$

where A_1 and A_2 are the equilibrium radioactivities of XB and **XA,** respectively, and *m,* and m are the amounts of **X** in **XA** and XB, respectively. The value of ***m,*** can be computed from:

$$m_x = mA_2/A_1$$

or from the calibration graph of ***m, = f(A2/A,), m,*** *= f (A 2)* or *tn, = f (A l)* .

Isotopic exchange can be carried out in either a heterogeneous or a homogeneous system. down to 10 pg/mL by liquid-liquid extraction from 3 mol/L aqueous HCI into benzene, and addition of K"'1 to the separated organic phase. Inactive chloride is completely displaced

by ^{131}I:

$$(CH_3HgCl)_{org} + {}^{131}I^- \longrightarrow (CH_3Hg^{131}I)_{org} + Cl^-$$

The method has been used for determination of methylmercury species in fish and in drinking water**.**

Radiorelease Methods

In radiorelease methods the analyte substance A reacts with a radioactive reagent, so that radioactive R* is released into **a** second phase, without being replaced by an inactive analyte. Applications involve either release of radioreagents from solids or liquids into the gas phase, or release from solids into a liquid. Radiorelease methods may be classified according to the type of the radioactive reagents employed, i.e. (1) radioactive kryptonates, (2) radioactive metals, and (3) radioactive salts and other substances.

Radioactive Kryptonates

The term radioactive kryptonates is used for substances into which atoms or ions of krypton-85 (%r) are incorporated (by diffusion of 8sKr; by bombardment with accelerated krypton ions; by crystallization of the kryptonated substances from a melt, or by placing the solution in an atmosphere of ^{85}Kr, etc.). The radionuclide can be released from the solid lattice by any chemical or physical reaction that breaks down the lattice at the solid surface. The released inert gas is

conveniently measured from its main **β** radiation (0.67 MeV). The half life of 10.27 a is suitable for long-term remote sensing devices.

Applications of Radioactive Kryptonates

The determination of *oxygen* is performed by surface oxidation of copper or pyrographite kryptonate, at elevated temperature, resulting in destruction of the surface layer and release of ^{85}Kr proportional to the oxygen mass. Detection limits are at the 10 ng/m3 level. Ozone oxidizes copper kryptonate at temperatures below 100 "C, whereas reaction with oxygen starts well above 200 "C, so this detector can detect O7 and O2 differentially. Determination of *ozone* in air is feasible over **a** concentration range of 10^{-7} to $10^{-3}g/m^3$ with hydroquinone kryptonate:

$$[C_6H_4(OH)_2]_3[^{85}Kr] + O_3 \longrightarrow 3\,C_6H_4O_2 + 3\,H_2O + {}^{85}Kr\uparrow$$

Sulfur dioxide has also been determined by **a** method based on the mechanism of double release. In the first stage, sulfur dioxide reacts with sodium chlorate to release chlorine dioxide which is **a** strong oxidizing agent. The chlorine dioxide then oxidizes radioactive hydroquinone kryptonate and gaseous 85Kr is released. The following reactions are invoved:

$$SO_2 + 2\,NaClO_3 \longrightarrow 2\,ClO_2 + Na_2SO_4$$

$$ClO_2 + [C_6H_4(OH)_2]_3[^{85}Kr] \longrightarrow {}^{85}Kr\uparrow$$

A radioactive kryptonate of silica has been suggested for the determination of *hydrogen fluoride* in air. *Oxygen dissolved in water* can be measured by the use of thallium kryptonate

$$4\,Tl[^{85}Kr] + O_2 + 2\,H_2O \longrightarrow Tl^+ + 4\,OH^- + {}^{85}Kr\uparrow$$

Thallium is oxidized by oxygen, and the amount of ^{85}Kr released or the decrease in the activity of kryptonated thallium is proportional to the dissolved oxygen concentration in the sample (at constant pH) down to 0.3 pg/mL.

Radioactive Metals

Oxidizing agents in solution can react at the surface of a labeled metal, releasing radioactive ions into the solutions which are used for determination of the oxidants. The decisive factors in the choice of the metal are the following: the metal should not react with water, but with oxidizing agents to yield ions which do not form precipitates in aqueous media. The metal should have a radionuclide with suitable nuclear properties. These conditions are met by thallium (^{204}Tl) and silver (^{110m}Ag). Dissolved *oxygen* has been determined in seawater, drinking water, and

wastewater down to the ng/g level with metallic thallium labeled with ^{204}T1. Selectivity requires removal or masking of other oxidizing agents. The *vanadate* ion is assayed by acidifying the corresponding sample (pH ≈ 3) and passing it over a column containing radioactive metallic ^{110m}Ag. The labeled silver is oxidized, dissolved, eluted from the column, and detected. The measurement of *&chromate* ion concentration in natural waters can be carried out similarly.

8.22 Radioactive Salts and Other Radioactive Substances

Sulfur dioxid is determined on the basis of the reaction:

$$5\,SO_2 + 2\,K^{131}IO_3 + 4\,H_2O \longrightarrow K_2SO_4 + 4\,H_2SO_4 + {}^{131}I_2\uparrow$$

This reaction takes place in an alkaline solution through which air containing sulfur dioxide is bubbled. After completion of the reaction, the **so**lution is acidified and the iodine released is extracted. Aclive *hydrogen* in organic substances may be determined by reaction with lithium aluminum hydride labeled with tritium (3H). The activity of released tritium is measured using a proportional counter. A number of determinations are based on the formation of a soluble complex between the analyte in solution with a radioactively labeled precipitate.

In this way it is possible to determine anions forming soluble complexes (e.g., CN^-, S_20_3, I^-, F^-). The principle of the determination are given in the following equations:

$$2\,CN^- + {}^{203}Hg(IO_3)_2\downarrow \longrightarrow {}^{203}Hg(CN)_2 + 2\,IO_3^-$$
(>0.50 µg/mL, ± 55 %)

$$2\,Cl^- + {}^{203}Hg(IO_3)_2\downarrow \longrightarrow {}^{203}HgCl_2 + 2\,IO_3^-$$ (> 1 µg/mL, ± 5 %)

$$n\,S_2O_3^{2-} + m\,{}^{110m}AgSCN\downarrow \longrightarrow {}^{110m}Ag_m(S_2O_3)_2^{(2n-m)-} + m\,SCN^-$$

$$2\,I^- + {}^{203}HgI_2\downarrow \longrightarrow {}^{203}HgI_4^{2-}$$ (> 25 µg/mL, ± 5 %)

8.23 Radiometric Titration

Radiometric titrations follow the relation between the radioactivity of one component or phase of the solution under analysis and the volume of added titrant. The compound formed during the titration must be easily separable from the excess of unreacted ions. This separation is directly ensured only in the case of precipitation reactions. In other types of reactions, the separation can be accomplished using an additional procedure. The endpoint is determined from the change in the activity of the residual solution or of the other phase.

According to the type of chemical reaction used, methods based on the formation of precipitates and methods based on complex formation can be distinguished. Because of the necessity for handling precipitates, precipitation radiometric titrations are difficult to apply to less than milligram amounts and, therefore, have no special advantages over other volumetric methods. The sensitivity of complexometric titrations is limited by the sensitivity of the determination of the endpoint. However, the use of radiometric detection can substantially increase the sensitivity of this type of determination. For the separation of the product from the initial component, liquid-liquid distribution, ion-exchange, electrophoresis, or paper chromatography are most often,.Their main advantage is where classical methods for detection of the endpoint are either impossible or subject to interference from the titration medium.

Radiopolarography offers highly increased sensitivity and selectivity over polarographic current measurement, without interference from major components of the solution. It measures the amount of labeled ions deposited in single drops in a dropping mercury electrode as a function of potential.

8.24 Summary of the unit

Radiometric titration is a volumetric titration method with a special opportunity for the indication of the end-point. It can be used when the titrant gives an insoluble precipitate or a compound can be extracted easily, and when one of the reaction partners can be labeled. During the titration, the reagent is added in different quantities and the activity of the precipitate or the filtrate is measured, or in case of extraction the activity of one phase is determined. There are three methods, depending the way of labeling:

1. The titrant is labeled with its radioactive isotope: the activity of the solution decreases to the equivalent point, and then it remains constant.
2. The reagent is labeled: the activity of the solution increases after the equivalency.
3. Both reaction partners are labeled: the activity of the solution has a minimum.

Since the variation of the activity is linear or the activity is constant, 2-3 points are suitable for the evaluation of the titration curve. For example in Method 1 after the addition of titrant with volume V_t, the activity of the solution decreases from I to I_t, the equivalent volume of the reagent V_e is

$$V_e = V_t - \frac{I}{I - I_t}$$

In Method 2 the volume of reagents at the equivalence point can be calculated from two titration data after the equivalent point

$$V_e = \frac{V_t^1 I_t^2 - V_t^2 I_t^1}{I_t^2 - I_t^1}$$

If the dilution has to be taken into consideration (small amount of titrant, diluted reagent) the activities have to be multiplied with a factor Δt.

$$\Delta = \frac{V + V_t}{V}$$

The concentration of two substances can be determined in one titration if they have differences in the solubility and the complex stability. For this, the ion forming more soluble precipitation or less stable complex has to be labeled. The advantages of the method are that the titration curve is linear, so automation is easy.

8.25 Key Words

radioanalytical chemistry; Isotope dilution analysis; Direct Isotope dilution dnalysis; Reverse Isotope Dilution Analysis; Substoichiometric Isotope Dilution Analysis; Redox Substoichiometry; Displacement Substoichiometry

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8.27 Questions for self understanding

1) Define the general features of radioactive titration?

2) Explain the importance and current trends of radioanalytical chemistry

3) Write a note on choice of radionuclide in radioanalytical chemistry

4) Explain the radiotracers in methodological mtudies

5) What are the principles and importance of radioanalytical chemistry?

6) Explain how control of sampling is made in radioanalytical chemistry?

7) How control of contamination and loss is done in radioanalytical chemistry?

8) Explain the control of the determination stage

9) Write a note on followings

i) Isotope Dilution Analysis

ii) Direct Isotope Dilution Analysis

iii) Reverse Isotope Dilution Analysis

iv) Double (Multiple) IDA

10) What are the derivatives obtained in isotope dilution analysis?

11) Explain Substoichiometric Isotope Dilution Analysis

12) Explain Substoichiometric Separation by Liquid - Liquid Distribution

13) Explain Redox Substoichiometry

14) Write a note on Displacement Substoichiometry

15) What are the Applications of radioanalytical chemistry?

16) Discuss Isotope Exchange Methods

17) Discuss Radioactive Salts and Other Radioactive Substances

18) nDiscuss Radiometric Titration

Unit-9

Structure

9.0 Objectives of the unit

After studying this unit you are able to

- Explain the stability of the complexes
- Derive the expression of stepwise stability constant
- Identify the different chelating agents
- Explain the complexometric titration
- Explain the complexometric titration with EDTA
- Calculate the Metal–EDTA Formation Constants
- Derive the EDTA Titration Curve

9.1 Introduction

Analytical chemistry of complex compounds of metals with organic ligands has developed through three stages. At first intense investigations of new organic reagents for metal ions were carried out. Then researches were directed to the elucidation of the effect of the structure of reagents on their reactivity, taking into account the presence of functional-analytical groups and substituents: simultaneously work on the mechanisms of complex formation in solution and the calculation of different constants was carried out. At last, during the past few years the studies on the structure of complex compounds using quantum-chemical methods have been developed

9.2 Stability of the complexes

The stability of compounds means in a most general sense, the compounds exist under suitable conditions may be stored for a long period of time. However when the formation of complexes in solution is studied, two types of stabilities, thermodynamic stability and kinetic stability are considered. In the language of thermodynamics, the equilibrium constants of a reaction are the measure of the heat released in the reaction and entropy change during reaction.

The greater amount of heat evolved in the reaction, the most stable are the reaction products. Secondly, greater the increase in entropy during the reaction, greater is the stability of products. The kinetic stability of complexes refers to the speed with which transformation leading to the attainment of equilibrium will occur. Here we are mainly concerned with the thermodynamic stability of the complex compound.

9.3 Determination of stability constant of complexes

In complexes the term stability is employed in two ways thermodynamic stability and kinetic stability. Thermodynamic stability deals with the bond energy, stability constant and redox

potential. Kinetic stability deals with the rate of the reaction, mechanism of reaction, formation of intermediate complexes, and activation for the process etc.

The thermodynamic stability of a species is a measure of the extent to which the species will form or be transformed into other species under certain conditions, when the system has reached equilibrium.

Let metal ion (Mn^+) combines with ligand (L) to form complex MLn, then

$$M + nL \leftrightarrows ML_n$$

$$K = \frac{[ML_n]}{[M][L]^n}$$

Thus by knowing the value of [M], [L] and [MLn] the value of K, stability constant of the complex MLn, can be computed.

The knowledge of stability constant is needed for computing quantitatively the concentration of free metal ion, ligand and any of its complexes formed in the system, under different conditions of pH. These data are extensively employed in analytical chemistry, stereochemistry, and biochemistry and in the technology of non ferrous and rare metals, solvent extraction, ion exchange etc. There are so many techniques for the computation of stability constants. Here only two methods are explained known as pH-metric method and spectrophotometric method.

9.4 Stepwise stability constant

As complexing processes are considered as occurring by a series of stages thus it is possible to express the formation (stability) constants referring specially to the addition of ligands in a stepwise manner as follows

$M + L \leftrightarrows ML$	$K_1 = \frac{[ML]}{[M][L]}$	$\therefore [ML] = K_1[M][L]$	(a)
$ML + L \leftrightarrows ML_2$	$K_2 = \frac{[ML_2]}{[ML][L]}$	$\therefore [ML_2] = K_2[ML][L]$	(b)
$ML_2 + L \leftrightarrows ML_3$	$K_3 = \frac{[ML_3]}{[ML_2][L]}$	$\therefore [ML_3] = K_3[ML_2][L]$	(c)
……	……		
……	……		
……	……		
$ML_{n-1} + L \leftrightarrows ML_n$	$K_n = \frac{[ML_n]}{[ML_{n-1}][L]}$	$\therefore [ML_n] = [ML_{n-1}][L]$	(n)

The constants K_1, K_2, K_3,……K_n are called the stepwise stability constants. The stepwise constants are related to the overall stability constant by the simple related:

$\beta_1 = K_1$

$\beta_2 = K_1.K_2$

$\beta_3 = K_1.K_2.K_3$

$\beta_4 = K_1.K_2.K_3.K_4$

Therefore $\beta_n = K_1.K_2.K_3. \ldots\ldots K_n$ (1)

A large number of techniques of great diversity are now being employed for the determination of stepwise stability constants. The most generally utilised and probably the most accurate and reliable method for the determination of stability constant is based on the potentiometric measurement of hydrogen ion concentration. This depends on the fact that pH of the solution is directly affected by complex formation, which is accompanied by the displacement of a proton from the acidic ligand. The magnitude of the observed pH change may be employed to determine the stability constant of the metal complexes by Bjerrum's method, Calvin and Wilson's method.

9.5 Chelating agents

Chelating agents, also known as chelants, complexing, or sequestering agents, are compounds that are able to form stable complexes with metal ions. They achieve this by coordinating with metal ions at a minimum of two sites, thus solubilizing and inactivating the metal ions that would otherwise produce adverse effects in the system on which they are used. Aminopolycarboxylates represent the most widely consumed chelating agents. The chelating agent has a ring like center, which forms a complex with the metal ion/substrate by two or more bindings and the metal ion is bound and excreted. Chelating agents can be used to treat metal poisoning and they may be used for other purposes

9.6 Complexometric titration

Complexometric titration is a type of titration based on complex formation between the analyte and titrant. Complexometric titrations are particularly useful for determination of a mixture of different metal ions in solution. An indicator with a marked color change is usually used to detect the end-point of the titration.

Any complexation reaction can in theory be applied as a volumetric technique provided that

i) The reaction reaches equilibrium rapidly following each addition of titrant.

ii) Interfering situations do not arise (such as stepwise formation of various complexes resulting in the presence of more than one complex in solution in significant concentration during the titration process).

iii) An complexometric indicator capable of locating equivalence point with fair accuracy is available

In practice, the use of EDTA as a titrant is well established

9.7 Complexometric titration with EDTA

Ethylenediamminetetraacetic acid, has four carboxyl groups and two amine groups that can act as electron pair donors, or Lewis bases. The ability of EDTA to potentially donate its six lone pairs of electrons for the formation of coordinate covalent bonds to metal cations makes EDTA a hexadentate ligand. However, in practice EDTA is usually only partially ionized, and thus forms fewer than six coordinate covalent bonds with metal cations. Disodium EDTA, commonly used in the standardization of aqueous solutions of transition metal cations, only forms four coordinate covalent bonds to metal cations at pH values less than or equal to 12 as in this range of pH values the amine groups remain protonated and thus unable to donate electrons to the formation of coordinate covalent bonds.

In analytical chemistry the shorthand "Na_2H_2Y" is typically used to designate disodium EDTA. This shorthand can be used to designate any species of EDTA. The "Y" stands for the EDTA molecule, and the "Hn" designates the number of acidic protons bonded to the EDTA molecule.

EDTA forms an octahedral complex with most 2^+ metal cations, M^{2+}, in aqueous solution. The main reason that EDTA is used so extensively in the standardization of metal cation solutions is that the formation constant for most metal cation- EDTA complexes is very high, meaning that the equilibrium for the reaction

$$M^{2+} + H_4Y \rightarrow MH_2Y + 2H^+$$

lies far to the right. Carrying out the reaction in a basic buffer solution removes H^+ as it is formed, which also drives the reaction to the right. For most purposes it can be considered that the formation of the metal cation-EDTA complex goes to completion, and this is chiefly why EDTA is used in titrations /standardizations of this type.

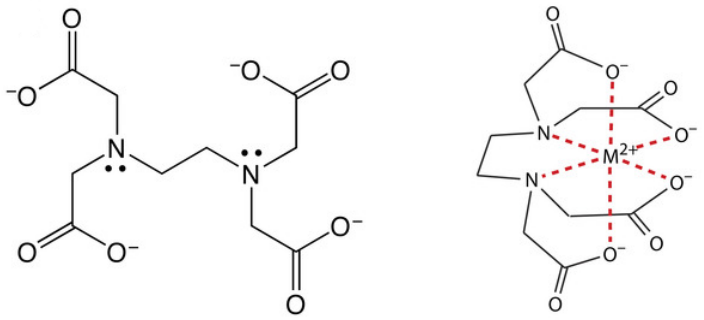
To carry out metal cation titrations using EDTA it is almost always necessary to use a complexometric indicator, usually an organic dye such as Fast Sulphon Black, Eriochrome Black T, Eriochrome Red B or Murexide, to determine when the end point has been reached.

These dyes bind to the metal cations in solution to form colored complexes. However, since EDTA binds to metal cations much more strongly than does the dye used as an indicator the EDTA will displace the dye from the metal cations as it is added to the solution of analyte. A color change in the solution being titrated indicates that all of the dye has been displaced from the metal cations in solution, and that the endpoint has been reached.

Complexometric titration has made it possible for man to be exposed to an advanced method of titration which not only enables us to analyze more ions, but also do them in very small quantities. We've to be aware of the effects of pH on the titration method. Complex ion titration is possible in very minute quantities. The biological use of complexometric titration seems to involve an advanced method of this kind of titration; and we can learn its application on living cells

9.8 Chemistry and Properties of EDTA

Ethylenediaminetetraacetic acid, or EDTA, is an aminocarboxylic acid. EDTA, shown in below figure in its fully deprotonated form, is a Lewis acid with six binding sites four negatively charged carboxylate groups and two tertiary amino groups that can donate six pairs of electrons to a metal ion. The resulting metal–ligand complex, in which EDTA forms a cage-like structure around the metal ion is very stable. The actual number of coordination sites depends on the size of the metal ion, however, all metal–EDTA complexes have a 1:1 stoichiometry.



9.9 Metal–ED TA Formation Constants

To illustrate the formation of a metal–EDTA complex, let's consider the reaction between Cd^{2+} and EDTA

$$Cd^{2+}(aq) + Y^{4-}(aq) \rightleftharpoons CdY^{2-}(aq) \text{---------- (1)}$$

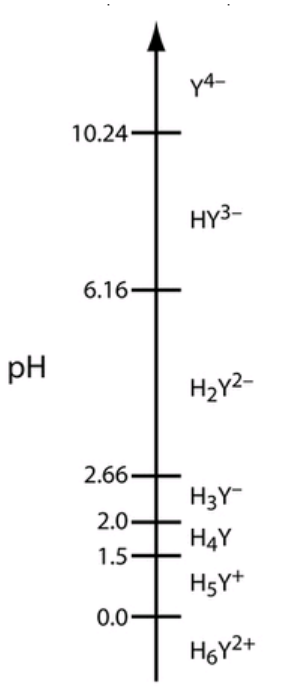
where Y^{4-} is a shorthand notation for the fully deprotonated form of EDTA.

$$K_f = \frac{[CdY^{2-}]}{[Cd^{2+}][Y^{4-}]} = 2.9\times10^{16}\text{----------(2)}$$

is large, its equilibrium position lies far to the right. Formation constants for other metal–EDTA complexes are found in literature.

In addition to its properties as a ligand, EDTA is also a weak acid. The fully protonated form of EDTA, H_6Y^{2+}, is a hexaprotic weak acid with successive pKa values of

pK_{a1}=0.0; pK_{a2}=1.5; pK_{a3} = 2.0; pK_{a4}=2.66; pK_{a5}=6.16; pK_{a6} = 10.24



Ladder diagram for EDTA.

The first four values are for the carboxylic acid protons and the last two values are for the ammonium protons. Above figure shows a ladder diagram for EDTA. The specific form of EDTA in reaction 9.9 is the predominate species only at pH levels greater than 10.17

9.10 Conditional Metal–Ligand Formation Constants

The formation constant for CdY^{2-} in equation 2 assumes that EDTA is present as Y^{4-}. Because EDTA has many forms, when we prepare a solution of EDTA we know it total concentration, C_{EDTA}, not the concentration of a specific form, such as Y^{4-}. To use equation 2, we need to rewrite it in terms of C_{EDTA}.

At any pH a mass balance on EDTA requires that its total concentration equal the combined concentrations of each of its forms.

$C_{EDTA}=[H_6Y^{2+}]+[H_5Y^{+}]+[H_4Y]+[H_3Y^{-}]+[H_2Y^{2-}]+[HY^{3-}]+[Y^{4-}]$

To correct the formation constant for EDTA's acid–base properties we need to calculate the fraction, $\alpha_Y{}^{4-}$, of EDTA present as Y^{4-}.

$$\alpha_Y{}^{4-} = \frac{[Y^{4-}]}{C_{EDTA}} \text{ ---------- (3)}$$

pH	$\alpha_Y{}^{4-}$	pH	$\alpha_Y{}^{4-}$
1	1.9×10^{-18}	8	**5.6×10^{-3}**
2	3.4×10^{-14}	9	5.4×10^{-2}
3	2.6×10^{-11}	10	0.37
4	3.8×10^{-9}	11	0.85
5	3.7×10^{-7}	**12**	0.98
6	2.4×10^{-5}	**13**	1.00
7	5.0×10^{-4}	**14**	**1.00**

Above table provides values of $\alpha_Y{}^{4-}$ for selected pH levels. Solving equation 3 for $[Y^{4-}]$ and substituting into equation 2 for the CdY^{2-} formation constant

$$K_f = \frac{[CdY^{2-}]}{[Cd^{2+}]Y^{4-}C_{EDTA}} \text{ ---------- (4)}$$

and rearranging gives

$$K'_f = K_f \times \alpha_Y{}^{4-} = \frac{[CdY^{2-}]}{[Cd^{2+}]C_{EDTA}} \text{ ---------- (5)}$$

where $K`_f$ is a pH-dependent conditional formation constant. As shown in below table, the conditional formation constant for CdY^{2-} becomes smaller and the complex becomes less stable at more acidic pHs.

pH	K_f'	pH	K_f'
1	5.5×10^{-2}	8	1.6×10^{14}
2	1.0×10^{3}	9	1.6×10^{15}
3	7.7×10^{5}	10	1.1×10^{16}
4	1.1×10^{8}	11	2.5×10^{16}

5	1.1×10^{10}	12	2.9×10^{16}
6	6.8×10^{11}	13	2.9×10^{16}
7	1.5×10^{13}	14	2.9×10^{16}

9.11 Complexometric EDTA Titration Curves

Let's calculate the titration curve for 50.0 mL of 5.00×10^{-3} M Cd^{2+} using a titrant of 0.0100 M EDTA. Furthermore, let's assume that the titrand is buffered to a pH of 10 with a buffer that is 0.0100 M in NH_3.

Because the pH is 10, some of the EDTA is present in forms other than Y^{4-}. In addition, EDTA must compete with NH_3 for the Cd^{2+}. To evaluate the titration curve, therefore, we first need to calculate the conditional formation constant for CdY^{2-}. From above tables we find that $\alpha_{Y}{}^{4-}$ is 0.35 at a pH of 10, and that $\alpha_{Cd}{}^{2+}$ is 0.0881 when the concentration of NH_3 is 0.0100 M. Using these values, the conditional formation constant is

$$K''_f = K_f \times \alpha_{Y}{}^{4-} \times \alpha_{Cd}{}^{2+} = (2.9 \times 10^{16})(0.37)(0.0881) = 9.5 \times 10^{14}$$

Because K_f'' is so large, we can treat the titration reaction

$$Cd^{2+}(aq) + Y^{4-}(aq) \rightarrow CdY^{2-}(aq)$$

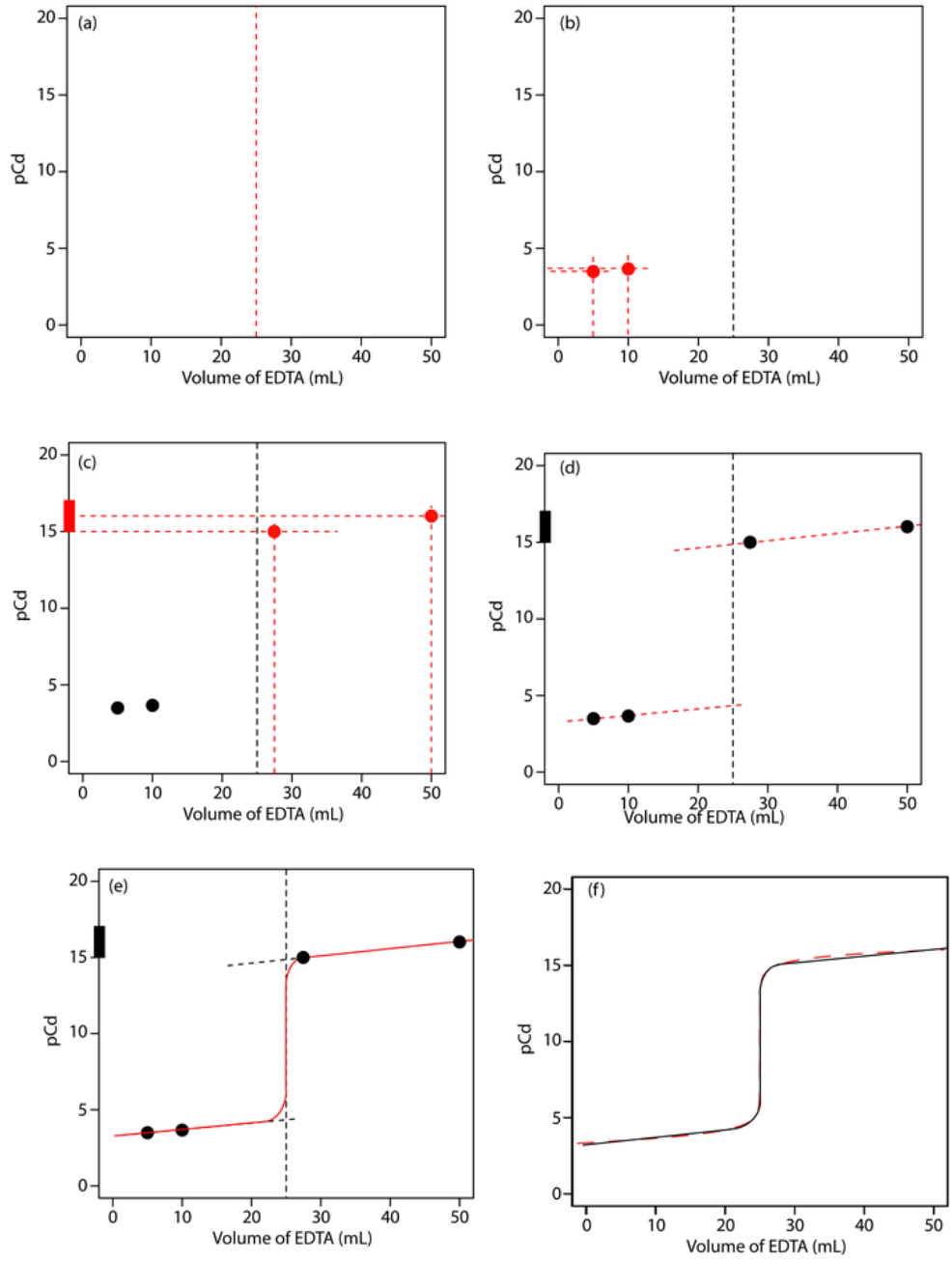
as if it proceeds to completion.

9.12 Derivation of an EDTA Titration Curve

To evaluate the relationship between a titration's equivalence point and its end point, we need to construct only a reasonable approximation of the exact titration curve. In this section we demonstrate a simple method for sketching a complexation titration curve. Our goal is to sketch the titration curve quickly, using as few calculations as possible. Let's use the titration of 50.0 mL of 5.00×10^{-3} M Cd^{2+} with 0.0100 M EDTA in the presence of 0.0100 M NH_3 to illustrate our approach.

We begin by calculating the titration's equivalence point volume, which, as we determined earlier, is 25.0 mL. Next, we draw our axes, placing pCd on the y-axis and the titrant's volume on the x-axis. To indicate the equivalence point's volume, we draw a vertical line corresponding to 25.0 mL of EDTA. Below figure (a) shows the result of the first steps in our sketch.

Before the equivalence point, Cd^{2+} is present in excess and pCd is determined by the concentration of unreacted Cd^{2+}. Because not all the unreacted Cd^{2+} is free some is complexed with NH_3we must account for the presence of NH_3. The calculations are straightforward, as we saw earlier. Below figure (b) shows the pCd after adding 5.00 mL and 10.0 mL of EDTA.



The third step in sketching our titration curve is to add two points after the equivalence point. Here the concentration of Cd^{2+} is controlled by the dissociation of the Cd^{2+}–EDTA complex. Beginning with the conditional formation constant

$$K'_f = \frac{[CdY^{2-}]}{[Cd^{2+}]Y^{4-}C_{EDTA}} = \alpha_{Y^{4-}} \times K_f = (0.37)(2.9\times10^{16}) = 1.1\times10^{16}$$

we take the log of each side and rearrange, arriving at

$$\log K'_f = -\log[Cd^{2+}] + \log\frac{[CdY^{2-}]CEDTA}{C_{EDTA}}$$

$$pCd = \log K'_f + \log\frac{C_{EDTA}}{[CdY^{2-}]}$$

Note that after the equivalence point, the titrand's solution is a metal–ligand complexation buffer, with pCd determined by CEDTA and $[CdY^{2-}]$. The buffer is at its lower limit of pCd = $\log K_f' - 1$ when

$$\frac{C_{EDTA}}{[CdY^{2-}]} = \frac{\text{moles EDTA added} - \text{initial moles } Cd^{2+}}{\text{initial moles } Cd^{2+}} = \frac{1}{10}$$

Making appropriate substitutions and solving, we find that

$$\frac{M_{EDTA}V_{EDTA} - M_{Cd}V_{Cd}}{M_{Cd}V_{Cd}} = \frac{1}{10}$$

$$M_{EDTA}V_{EDTA} - M_{Cd}V_{Cd} = 0.1\times M_{Cd}V_{Cd}$$

$$V_{EDTA} = \frac{1.1\,X\,M_{Cd}\,V_{Cd}}{M_{EDTA}} = 1.1X\,V_{eq}$$

Thus, when the titration reaches 110% of the equivalence point volume, pCd is $\log K_f' - 1$. A similar calculation should convince you that pCd = $\log Kf'$ when the volume of EDTA is 2×Veq. Above figure (c) shows the third step in our sketch. First, we add a ladder diagram for the CdY^{2-} complex, including its buffer range, using its $\log K_f'$ value of 16.04. Next, we add points representing pCd at 110% of Veq (a pCd of 15.04 at 27.5 mL) and at 200% of Veq (a pCd of 16.04 at 50.0 mL).

Next, we draw a straight line through each pair of points, extending the line through the vertical line representing the equivalence point's volume (Figure (d)). Finally, we complete our sketch by drawing a smooth curve that connects the three straight-line segments (Figure (e)). A comparison of our sketch to the exact titration curve (Figure (f))shows that they are in close agreement.

The equivalence point of a complexation titration occurs when we react stoichiometrically equivalent amounts of titrand and titrant. As is the case with acid–base titrations, we estimate the equivalence point of a complexation titration using an experimental end point. A variety of methods are available for locating the end point, including indicators and sensors that respond to a change in the solution conditions.

9.13 Finding the End point with an Indicator

Most indicators for complexation titrations are organic dyes, known as metallochromic indicators that form stable complexes with metal ions. The indicator, In^{m-}, is added to the titrand's solution where it forms a stable complex with the metal ion, MIn^{n-}. As we add EDTA it reacts first with free metal ions, and then displaces the indicator from MIn^{n-}.

$$MIn^{n-}+Y^{4-}\rightarrow MY^{2-}+In^{m-}$$

If MIn^{n-} and In^{m-} have different colors, then the change in color signals the end point.

The accuracy of an indicator's end point depends on the strength of the metal–indicator complex relative to that of the metal–EDTA complex. If the metal–indicator complex is too strong, the change in color occurs after the equivalence point. If the metal–indicator complex is too weak, however, the end point occurs before we reach the equivalence point.

Most metallochromic indicators also are weak acids. One consequence of this is that the conditional formation constant for the metal–indicator complex depends on the titrand's pH. This provides some control over an indicator's titration error because we can adjust the strength of a metal–indicator complex by adjusted the pH at which we carry out the titration. Unfortunately, because the indicator is a weak acid, the color of the uncomplexed indicator also changes with pH. For example below figure, shows the color of the indicator calmagite as a function of pH and pMg, where H_2In^-, HIn^{2-}, and In^{3-} are different forms of the uncomplexed indicator, and $MgIn^-$ is the Mg^{2+}–calmagite complex. Because the color of calmagite's metal–indicator complex is red, its use as a metallochromic indicator has a practical pH range of approximately 8.5–11 where the uncomplexed indicator, HIn^{2-}, has a blue color.

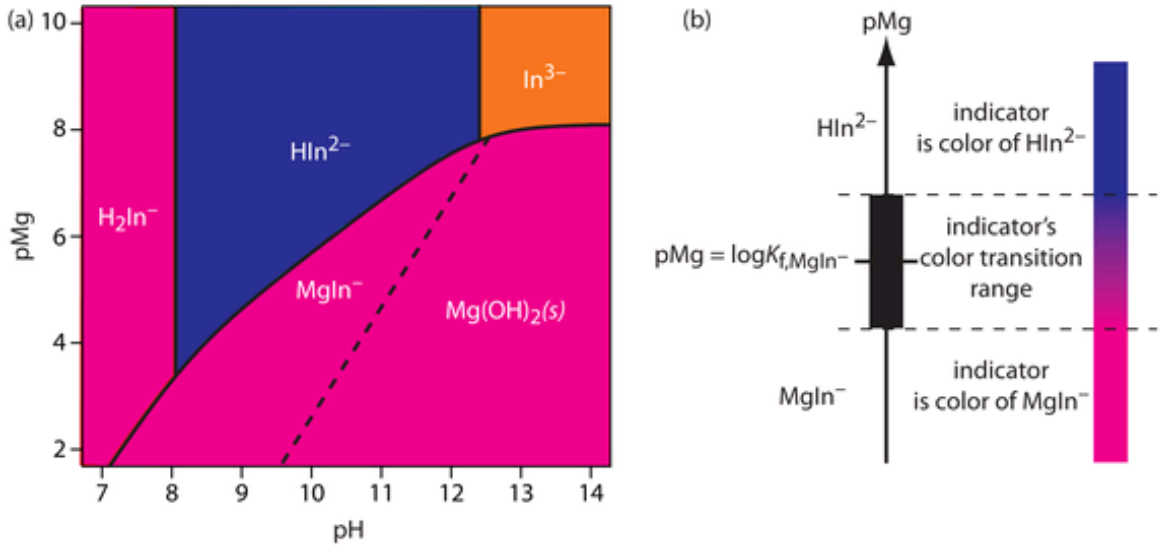


Table given below provides examples of metallochromic indicators and the metal ions and pH conditions for which they are useful. Even if a suitable indicator does not exist, it is often possible to complete an EDTA titration by introducing a small amount of a secondary metal–EDTA complex, if the secondary metal ion forms a stronger complex with the indicator and a weaker complex with EDTA than the analyte. For example, calmagite gives poor end points when titrating Ca^{2+} with EDTA. Adding a small amount of Mg^{2+}–EDTA to the titrand gives a sharper end point. Because Ca^{2+} forms a stronger complex with EDTA, it displaces Mg^{2+}, which then forms the red-colored Mg^{2+}–calmagite complex. At the titration's end point, EDTA displaces Mg^{2+} from the Mg^{2+}–calmagite complex, signaling the end point by the presence of the uncomplexed indicator's blue form.

Indicator	pH Range	Metal Ions[a]
calmagite	8.5–11	Ba, *Ca*, Mg, Zn
eriochrome Black T	7.5–10.5	Ba, *Ca*, Mg, Zn
eriochrome Blue Black R	8–12	*Ca*, Mg, Zn, Cu
murexide	6–13	Ca, Ni, Cu
PAN	2–11	Cd, Cu, Zn
salicylic acid	2–3	Fe

9.14 Summary of the unit

EDTA is the most commonly used chelating agents as it can form complexes with a wide range

of metals. The ability of EDTA to complex is dependent on its form. The most desirable state is the Y4- form. As the pH increases, more EDTA becomes Y^{4-}. Equilibrium constants are referred to as formation constants, K_f. For simple complexes (1:1) we can make some similar assumptions and generate similar equations as we did for monoprotic acids For more complex systems we must deal with step-wise formations and step-wise formation constants. Before the equivalence point there is excess M in solution. At the equivalence point, treated as dissolving pure MY complex. After equivalence there is excess EDTA. The most common indicator is the metal ion indicator

To be useful must bind less strongly than EDTA

The most common indicator is Eriochrome black T.

EBT binds to metal ions to give a red color. Upon release of the metal to EDTA, it becomes blue Can use ion specific electrodes and/or mercury electrodes. Both of these are more expensive and time consuming. Sometimes there is not a strong reaction between EBT and the metal. This can be overcome by a displacement titration. The solution begins with the Mg^{2+} complexed with EDTA. The analyte is added (assuming higher binding constant and lower concentration) and the Mg^{2+} is displaced. The Mg^{2+} is titrated with EBT. A second way to overcome titrations with weak end points is to do a back titration. In a back titration, excess EDTA is added to the sample solution. The excess is then titrated with a standard Mg or Zn solution.

9.15 Key words

Stability constant; Stepwise stability constant; Chelating agents; Complexometric titration; Metal–ED TA Formation Constants; EDTA Titration Curves; End point.

9.16 References for further study

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9.17 Questions for self under standing

1) What is stability of the complexes?

2) Explain determination of stability constant of complexes

3) Write a note on stepwise stability constant

4) What are chelating agents? Give two examples

5) Explain complexometric titration

6) Write a note on complexometric titration with EDTA

7) Explain the chemistry and Properties of EDTA

8) Derive the expression of Metal–EDTA Formation Constants

9) Explain conditional Metal–Ligand Formation Constants

10) Write a note on complexometric EDTA Titration Curves

11) Derivation of an EDTA Titration Curve

12) Explain the finding the End point with an Indicator

Unit-10

Structure

10.0 Objectives of the unit

After studying this unit your are able to

- Explain the EDTA competes with other ligands
- Draw the shape of the complexometric titration curve
- Explain the Direct titration method
- Explain the Back-titration method
- Explain the Replacement or substitution titration
- Explain the theory of the visual use of metal ion indicators

10.1 Introductions

A titration curve is produced when you measure the change in some parameter of a reaction, such as pH while the reactants are being added in a controlled way. The shape of the resulting graph can provide a lot of insight into what substances were being reacted, and the extent of reaction. In this activity you will learn about the different features of an acid-base titration curve that is produced during a neutralization reaction.

10.2 EDTA Competes With Other Ligands

To maintain a constant pH during a complexation titration we usually add a buffering agent. If one of the buffer's components is a ligand that binds Cd^{2+}, then EDTA must compete with the ligand for Cd^{2+}. For example, an NH_4^+/NH_3 buffer includes NH_3, which forms several stable Cd^{2+}–NH_3 complexes. Because EDTA forms a stronger complex with Cd^{2+} it will displace NH3, but the stability of the Cd^{2+}–EDTA complex decreases.

We can account for the effect of an auxiliary complexing agent, such as NH_3, in the same way we accounted for the effect of pH. Before adding EDTA, the mass balance on Cd^{2+}, C_{Cd}, is

$C_{Cd}=[Cd^{2+}]+[Cd(NH_3)^{2+}]+[Cd(NH_3)_2{}^{+2}]+[Cd(NH_3)_3{}^{+2}]+[Cd(NH_3)_4{}^{+2}]$

and the fraction of uncomplexed Cd^{2+}, $\alpha_{Cd}{}^{2+}$, is

$$\alpha_{Cd}{}^{2+} = \frac{[Cd^{2+}]}{C_{Cd}} \text{-------- (1)}$$

The value of $\alpha_{Cd}{}^{2+}$ depends on the concentration of NH_3. Contrast this with $\alpha_Y{}^{4-}$, which depends on pH. Solving equation 1 for $[Cd^{2+}]$ gives

$$K_f' = K_f \times \alpha_{Y^{4-}} = \frac{[CdY^{2-}]}{\alpha_{Cd^{2+}} C_{Cd} C_{EDTA}}$$

Because the concentration of NH_3 in a buffer is essentially constant, we can rewrite this equation

$$K_f'' = K_f \times \alpha_{Y^{4-}} \times \alpha_{Cd^{2+}} = \frac{[CdY^{2-}]}{C_{Cd}C_{EDTA}}$$

to give a conditional formation constant, K_f'', that accounts for both pH and the auxiliary complexing agent's concentration. Below table provides values of α_M^{2+} for several metal ion when NH_3 is the complexing agent.

$[NH_3](M)$	α_{Ca}^{2+}	α_{Cd}^{2+}	α_{Co}^{2+}	α_{Cu}^{2+}	α_{Mg}^{2+}	α_{Ni}^{2+}	α_{Zn}^{2+}
1	5.50×10^{-1}	6.09×10^{-8}	1.00×10^{-6}	3.79×10^{-14}	1.76×10^{-1}	9.20×10^{-10}	3.95×10^{-10}
0.5	7.36×10^{-1}	1.05×10^{-6}	2.22×10^{-5}	6.86×10^{-13}	4.13×10^{-1}	3.44×10^{-8}	6.27×10^{-9}
0.1	9.39×10^{-1}	3.51×10^{-4}	6.64×10^{-3}	4.63×10^{-10}	8.48×10^{-1}	5.12×10^{-5}	3.68×10^{-6}
0.05	9.69×10^{-1}	2.72×10^{-3}	3.54×10^{-2}	7.17×10^{-9}	9.22×10^{-1}	6.37×10^{-4}	5.45×10^{-5}
0.01	9.94×10^{-1}	8.81×10^{-2}	3.55×10^{-1}	3.22×10^{-6}	9.84×10^{-1}	4.32×10^{-2}	1.82×10^{-2}
0.005	9.97×10^{-1}	2.27×10^{-1}	5.68×10^{-1}	3.62×10^{-5}	9.92×10^{-1}	1.36×10^{-1}	1.27×10^{-1}
0.001	9.99×10^{-1}	6.09×10^{-1}	8.84×10^{-1}	4.15×10^{-3}	9.98×10^{-1}	5.76×10^{-1}	7.48×10^{-1}

10.3 Shape of the complexometric titration curve

The general shape of titration curves obtained by titrating 10.0 mL of a 0.01M solution of a metal ion M with a 0.01 M EDTA solution. The apparent stability constants of various metal-EDTA complexes are indicated at the extreme right of the curves. It is evident that the greater the stability constant, the sharper is the end point provided the pH is maintained constant.

In acid-base titrations the end point is generally detected by a pH-sensitive indicator. In the EDTA titration a metal ion sensitive indicator (metal indicator or metal-ion indicator) is often employed to detect changes of pM. Such indicators (which contain types of chelate groupings and generally possess resonance systems typical of dyestuffs) form complexes with specific metal ions, which differ in colour from the free indicator and produce a sudden colour change at the equivalence point. The end point of the titration can also be evaluated by other methods including potentiometric, amperometric, and spectrophotometric techniques.

F. Direct titration

The solution containing the metal ion to be determinedis buffered to the desired pH (e.g. to pH = 10 with NH:-aq. NH,) and titrated directly with the standard EDTA solution. It may be necessary

to prevent precipitation of the hydroxide of the metal (or a basic salt) by the addition of some auxiliary complexing agent, such as tartrate or citrate or triethanolamine. At the equivalence point the magnitude of the concentration of the metal ion being determined decreases abruptly. This is generally determined by the change in colour of a metal indicator or by amperometric, spectrophotometric or potentiometric methods.

G. Back-titration

Many metals cannot, for various reasons, be titrated directly thus they may precipitate from the solution in the pH range necessary for the titration, or they may form inert complexes, or a suitable metal indicator is not available. In such cases an excess of standard EDTA solution is added, the resulting solution is buffered to the desired pH, and the excess of the EDTA is back-titrated with a standard metal ion solution; a solution of zinc chloride or sulphate or of magnesium chloride or sulphate is often used for this purpose. The end point is detected with the aid of the metal indicator which responds to the zinc or magnesium ions introduced in the back-titration.

H. Replacement or substitution titration

Substitution titrations may be used for metal ions that do not react (or react unsatisfactorily) with a metal indicator, or for metal ions which form EDTA complexes that are more stable than those of other metals such as magnesium and calcium. The metal cation Mn+ to be determined may be treated with the magnesium complex of EDTA, when the following reaction occurs.

$$M^{n+} + MgY^{2-} \rightleftharpoons (MY)^{(n-4)+} + Mg^{2+}$$

The amount of magnesium ion set free is equivalent to the cation present and can be titrated with a standard solution of EDTA and a suitable metal indicator.

An interesting application is the titration of calcium. In the direct titration of calcium ions, solochrome black gives a poor end point; if magnesium is present, it is displaced from its EDTA complex by calcium and an improved end point results.

I. Alkalimetric titration

When a solution of disodium ethylenediaminetetraacetate, Na_2H_2Y, is added to a solution containing metallic ions, complexes are formed with the liberation of two equivalents of hydrogen ion.

$$M^{n+} + H_2Y^{2-} \rightleftharpoons (MY)^{(n-4)+} + 2H^+$$

The hydrogen ions thus set free can be titrated with a standard solution of sodium hydroxide using an acid-base indicator or a potentiometric end point, alternatively, an iodate-iodide mixture is added as well as the EDTA solution and the liberated iodine is titrated with a standard thiosulphate solution.

The solution of the metal to be determined must be accurately neutralised before titration; this is often a difficult matter on account of the hydrolysis of many salts, and constitutes a weak feature of alkalimetric titration.

J. Miscellaneous methods

Exchange reactions between the tetracyanonickelate(I1) ion [Ni(CN),I_2- (the potassium salt is readily prepared) and the element to be determined, whereby nickel ions are set free, have a limited application. Thus silver and gold, which themselves cannot be titrated complexometrically, can be determined in this way.

These reactions take place with sparingly soluble silver salts, and hence provide a method for the determination of the halide ions Cl^-, Br^-, 1^-, and the thiocyanate ion SCN^-. The anion is first precipitated as the silver salt, the latter dissolved in a solution of [Ni(CN),I_2^-, and the equivalent amount of nickel thereby set free is determined by rapid titration with EDTA using an appropriate indicator (murexide, bromopyrogallol red).

Fluoride may be determined by precipitation as lead chlorofluoride, the precipitate being dissolved in dilute nitric acid and, after adjusting the pH to 5-6, the lead is titrated with EDTA using xylenol orange indicator.

Sulphate may be determined by precipitation as barium sulphate or as lead sulphate. The precipitate is dissolved in an excess of standard EDTA solution, and the excess of EDTA is back-titrated with a standard magnesium or zinc solution using solochrome black as indicator.

Phosphate may be determined by precipitating as Mg(NH) PO $6H_2O$ dissolving the precipitate in dilute hydrochloric acid, adding an excess of standard EDTA solution, buffering at pH = 10, and back-titrating with standard magnesium ion solution in the presence of solochrome black.

10.4 Titration of mixtures, selectivity, masking and demasking agents

EDTA is a very unselective reagent because it complexes with numerous doubly, triply and quadruply charged cations. When a solution containing two cations which complex with EDTA is titrated without the addition of a complex-forming indicator, and if a titration error of 0.1 per

cent is permissible, then the ratio of the stability constants of the EDTA complexes of the two metals M and N must be such that $K_M/K_N > 10^6$ if N is not to interfere with the titration of M.

Strictly, of course, the constants KM and KN considered in the above expression should be the apparent stability constants of the complexes. If complex-forming indicators are used, then for a similar titration error $K_M/K_N > 10^8$. The following procedures will help to increase the selectivity.

(a) Suitable control of the pH of the solution

This, of course, makes use of the different stabilities of metal-EDTA complexes. Thus bismuth and thorium can be titrated in an acidic solution (pH = 2) with xylenol orange or methylthymol blue as indicator and most divalent cations do not interfere.

A mixture of bismuth and lead ions can be successfully titrated by first titrating the bismuth at pH 2 with xylenol orange as indicator, and then adding hexamine to raise the pH to about 5, and titrating the lead.

(b) Use of masking agents

Masking may be defined as the process in which a substance, without physical separation of it or its reaction products, is so transformed that it does not enter into a particular reaction. Demasking is the process in which the masked substance regains its ability to enter into a particular reaction.

By the use of masking agents, some of the cations in a mixture can often be 'masked' so that they can no longer react with EDTA or with the indicator. An effective masking agent is thecyanide ion; this forms stable cyanide complexes with the cations of Cd, Zn, Hg(II), Cu, Co, Ni, Ag, and the platinum metals, but not with the alkaline earths, manganese, and lead.

$$M^{2+} + 4CN^- \rightarrow [M(CN)_4]^{2-}$$

It is therefore possible to determine cations such as Ca^{2+}, Mg^{2+}, Pb^{2+}, and Mn^{2+} in the presence of the above-mentioned metals by masking with an excess of potassium or sodium cyanide. A small amount of iron may be masked by cyanide if it is first reduced to the iron(II) state by the addition of ascorbic acid. Titanium(IV), iron(III), and aluminium can be masked with triethanolamine; mercury with iodide ions; and aluminium, iron(III), titanium(IV), and tin(II) with ammonium fluoride (the cations of the alkaline-earth metals yield slightly soluble fluorides).

Sometimes the metal may be transformed into a different oxidation state: thus copper(II) may be reduced in acid solution by hydroxylamine or ascorbic acid. After rendering ammoniacal, nickel or cobalt can be titrated using, for example, murexide as indicator without interference from the copper, which is now present as Cu(I). Iron(III) can often be similarly masked by reduction with ascorbic acid.

(c) Selective demasking

The cyanide complexes of zinc and cadmium may be demasked with formaldehyde-acetic acid solution or, better, with chloral hydrate.

$$[Zn(CN)_4]^{2-} + 4H^+ + 4HCHO \rightarrow Zn^{2+} + 4HO \cdot CH_2 \cdot CN$$

The use of masking and selective demasking agents permits the successive titration of many metals. Thus a solution containing Mg, Zn, and Cu can be titrated as follows:

1. Add excess of standard EDTA and back-titrate with standard Mg solution using solochrome black as indicator. This gives the sum of al1 the metals present.

2. Treat an aliquot portion with excess of KCN (Poison) and titrate as before. This gives Mg only.

3. Add excess of chloral hydrate (or of formaldehyde-acetic acid solution, 3:1) to the titrated solution in order to liberate the Zn from the cyanide complex, and titrate until the indicator turns blue. This gives the Zn only. The Cu content may then be found by difference.

(d) Classical separation

These may be applied if they are not tedious; thus the following precipitates may be used for separations in which, after beingre-dissolved, the cations can be determined complexometrically CaC2O4, nickel dimethylglyoximate, $Mg(NH_2)P0_4,6H_2O$, and CuSCN.

(e) Solvent extraction

This is occasionally of value. Thus zinc can be separated from copper and lead by adding excess of ammonium thiocyanate solution and extracting the resulting zinc thiocyanate with 4-methylpentan-2-one (isobutyl methyl ketone); the extract is diluted with water and the zinc content determined with EDTA solution.

(f) Choice of indicators

The indicator chosen should be one for which the formation of the metal-indicator complex is sufficiently rapid to permit establishment of the end point without undue waiting, and should preferably be reversible.

(g) Removal of anions

Anions, such as orthophosphate, which can interfere in complexometric titrations may be removed using ion exchange resins. For the use of ion exchange resins in the separation of cations and their subsequent EDTA titration.

(h) Kinetic masking

This is a special case in which a metal ion does not effectively enter into the complexation reaction because of its kinetic inertness. Thus the slow reaction of chromium(III) with EDTA makes it possible to titrate other metal ions which react rapidly, without interference from Cr(III); this is illustrated by the determination of iron(III) and chromium(III) in a mixture.

Dyestuffs which form complexes with specific metal cations can serve as indicators of pM values; 1:1-complexes (metal dyestuff = 1:1) are common, but 1:2-complexes and 2:1-complexes also occur.

The metal ion indicators, like EDTA itself, are chelating agents; this implies that the dyestuff molecule possesses several ligand atoms suitably disposed for coordination with a metal atom. They can, of course, equally take up protons, which also produces a colour change; metal ion indicators are therefore not only pM but also pH indicators.

Metal ion indicators

The success of an EDTA titration depends upon the precise determination of the end point. The most common procedure utilises metal ion indicators. The requisites of a metal ion indicator for use in the visual detection of end points include

(a) The colour reaction must be before the end point, when nearly all the metal ion is complexed with EDTA, the solution is strongly coloured.

(b) The colour reaction should be specific or selective.

(c) The metal-indicator complex must possess sufficient stability, otherwise, due to dissociation, a sharp colour change is not attained. The metal-indicator complex must, however, be less stable than the metal-EDTA complex to ensure that, at the end point, EDTA removes metal ions from the metal indicator-complex. The change in equilibrium from the metal indicator complex to the metal-EDTA complex should be sharp and rapid.

(d) The colour contrast between the free indicator and the metal-indicator complex should be readily observed.

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

(f) The above requirements must be fulfilled within the pH range at which the titration is performed.

10.5 Theory of the visual use of metal ion indicators

The use of a metal ion indicator in an EDTA titration may be written as

$$M-In + EDTA \rightarrow M-EDTA + In$$

This reaction will proceed if the metal-indicator complex M-In is less stable than the metal-EDTA complex M-EDTA. The former dissociates to a limited extent, and during the titration the free metal ions are progressively complexed by the EDTA until ultimately the metal is displaced from the complex M-In to leave the free indicator (In). The stability of the metal-indicator complex may be expressed in terms of the formation constant (or indicator constant) Kin

$$K_{In} = [M-In]/[M][In]$$

The indicator colour change is affected by the hydrogen ion concentration of the solution, and no account of this has been taken in the above expression for the formation constant. Thus solochrome black, which may be written as H_2In^-, exhibits the following acid-base behavior.

$$\underset{\text{Red}}{H_2In^-} \underset{5.3-7.3}{\overset{pH}{\rightleftharpoons}} \underset{\text{Blue}}{HIn^{2-}} \underset{10.5-12.5}{\overset{pH}{\rightleftharpoons}} \underset{\text{Yellow-orange}}{In^{3-}}$$

In the pH range 7–11, in which the dye itself exhibits a blue colour, many metal ions form red complexes; these colours are extremely sensitive, as is shown, for example, by the fact that $10^{-6} - 10^{-7}$ molar solutions of magnesium ion give a distinct red colour with the indicator. From the practical viewpoint, it is more convenient to define the apparent indicator constant K'_{In}, which varies with pH, as:

$$K'_{In} = [MIn^-]/[M^{n+}][In]$$

where

$[MIn^-]$ = concentration of metal–indicator complex,
$[M^{n+}]$ = concentration of metallic ion, and
$[In]$ = concentration of indicator not complexed with metallic ion.

(This, for the above indicator, is equal to $[H_2In^-] + [HIn^{2-}] + [In^{3-}]$.)

The equation may be expressed as:

$$\log K'_{In} = pM + \log[MIn^-]/[In];$$

$\log K'_{In}$ gives the value of pM when half the total indicator is present as the metal ion complex. Some values for $\log K'_{In}$ for $CaIn^-$ and $MgIn^-$ respectively (where H_2In^- is the anion of solochrome black) are: 0.8 and 2.4 at pH = 7; 1.9 and 3.4 at pH = 8; 2.8 and 4.4 at pH = 9; 3.8 and 5.4 at pH = 10; 4.7 and 6.3 at pH = 11; 5.3 and 6.8 at pH = 12. For a small titration error K'_{In} should be large ($> 10^4$), the ratio of the apparent stability constant of the metal–EDTA complex K'_{MY} to that of the metal–indicator complex K'_{In} should be large ($> 10^4$), and the ratio of the indicator concentration to the metal ion concentration should be small ($< 10^{-2}$).

The visual metallochromic indicators discussed above form by far the most important group of indicators for EDTA titrations and the operations subsequently described will be confined to the use of indicators of this type; nevertheless there are certain other substances which can be used as indicators.

10.6 Practical considerations

1) Adjustment of pH

For many EDTA titrations the pH of the solution is extremely critical often limits of 11 unit of pH, and frequently limits of 10.5 unit of Ph must be achieved for a successful titration to be carried out. To achieve such narrow limits of control it is necessary to make use of a pH meter while adjusting the pH value of the solution, and even for those cases where the latitude is such that a pH test-paper can be used to control the adjustment of pH, only a paper of the narrow range variety should be used.

2) Concentration of the metal ion to be titrated

Most titrations are successful with 0.25 millimole of the metal ion concerned in a volume of 50 150 mL of solution. If the metal ion concentration is too high, then the end point may be very difficult to discern, and if difficulty is experienced with an end point then it is advisable to start with a smaller portion of the test solution, and to dilute this to 100-150 mL before adding the buffering medium and the indicator, and then repeating the titration.

3) Amount of indicator

The addition of too much indicator is a fault which must be guarded against: in many cases the colour due to the indicator intensifies considerably during the course of the titration, and further, many Indicators exhibit dichroism, i.e. there is an intermediate colour change one to two drops before the real end-point. Thus, for example, in the titration of lead using xylenol orange as indicator at pH = 6, the initial reddish-purple colour becomes orange-red, and then with the addition of one or two further drops of reagent, the solution acquires the final lemon yellow colour. This end point anticipation, which is of great practical value, may be virtually lost if too much of the indicator is added so that the colour is too intense. In general, a satisfactory colour is achieved by the use of 30-50 mg of a 1 per cent solid mixture of the indicator in potassium nitrate.

4) Attainment of the end point.

In many EDTA titrations the colour change in the neighbourhood of the end point may be slow. In such cases, cautious addition of the titrant coupled with continuous stirring of the solution is advisable; the use of a magnetic stirrer is recommended. Frequently, a sharper end point may be achieved if the solution is warmed to about 40 ^{0}C.

Titrations with CDTA are always slower in the region of the end point than the corresponding EDTA titrations.

5) Detection of the colour change.

With the entire metal ion indicators used in complexometric titrations, detection of the end point of the titration is dependent upon the recognition of a specified change in colour; for many observers this can be a difficult task, and for those affected by colour blindness it may be virtually impossible. These difficulties may be overcome by replacing the eye by a photocell which is much more sensitive, and eliminates the human element.

To carry out the requisite operations it is necessary to have available a colorimeter or a spectrometer in which the cell compartment is large enough to accommodate the titration vessel (a conical flask or a tall form beaker). A simple apparatus may be readily constructed in which light passing through the solution is first allowed to strike a suitable filter and then a photocell; the current generated in the latter is measured with a galvanometer.

6) Alternative methods of detecting the end point.

In addition to the visual and spectrophotometric detection of end points in EDTA titrations with the aid of metal ion indicators, the following methods are also available for end point detection.

i) Potentiometric titration using a mercury electrode.

ii) Potentiometric titration using a selective ion electrode responsive to the ion being titrated.

iii) Potentiometric titration using a bright platinum-saturated calomel electrode system; this can be used when the reaction involves two different oxidation states of a given metal.

iv) By amperometric titration.

v) By coulometric analysis.

vi) By conductimetric titration.

10.7 Complexometric and Medicine

Complexometric is widely used in the medical industry because of the microliter-size sample involved. The method is efficient in research related to the biological cell. i) Ability to titrate the amount of ions available in a living cell. ii) Ability to introduce ions into a cell in case of deficiencies. Complexometric tiration involves the treatment of complex ions such as magnesium, calcium, copper, iron, nickel, lead and zinc with EDTA as the complexing agent.

The titration usually depends on pH stability and performed at high (basic) pH, i.e. pH 10 for Ca^{+2} or Mg^{+2}. The titration of metal ion yield high formation constant, K_f. Diffusional microburette is used to deliver the EDTA in microscopic droplets, as low as 6 fmol/s, observed under microscope.

10.8 EDTA titration techniques

EDTA titrations can be performed in many ways, since several of these techniques are pretty specialized and you won't see them again I will just talk about the first and last, Direct titrations and Masking

e) Direct Titrations

In direct titrations you simply add an indicator to a solution of the metal ion and titrate with EDTA. Before you start the titration yo need to check that the pH of the solution gives a good Kf' and that the pH is consistent with you indicator color change as well. auxiliary complexing agents like ammonia, tartarate, or citrate may be added to block formation of insoluble OH complexes

f) Back Titrations

In a back titration an excess of EDTA is added to the metal ion solution, and the excess EDTA is titrated with a known concentration of a second metal ion. The second metal ion must form a weaker complex with EDTA than the analyte ion so the second
metal does not displace the analyte ion from its complex with EDTA.
Back titration are used when the metal ion blocks the indicator (see above),
when the metal-EDTA complex forms too slowly, or when the metal precipitates in the
absence of EDTA.

g) Displacement Titrations

For metal ions that do not have a good indicator a second titration method is the displacement titration. Here the analyte is treated with an excess of a second metal bound to EDTA. The analyte ion displaces the second metal from the EDTA complex, and then the second metal is titrated with EDTA. A typical displacement titration involves Hg^{2+} as the analyte and MgEDTA at the displacement titrant.

h) Indirect Titrations

With a little clever thought EDTA can be used as a titrant for anions like SO_4^{2-} $BaSO_4$ is insoluble to one way to determine SO4 is to precipitate with Ba^{2+} filter and wash the ppt, then

boil in excess EDTA to complex all the Ba. Back titrate to determine how much Ba you had, and that, in turn, tells you how much SO_4^{2-} you had.

10.9 Summary of the unit

The use of auxiliary complexing agents is a little trick, because you have a competition between the metal-complexing agent and Metal-EDTA complex. The K_f for the metal-EDTA complex must be many orders of magnitude larger than the Kf for the metal-auxiliary complex agent so that the EDTA can remove the metal from the complexing agent. The most common way to follow complexometric titration is either with a potentiometer and electrode designed to sense the metal involved, or by using a metal ion indicator. Like Acid-Base indicators that were simply another acid or base, that had a color change between forms, Metal Ion indicators are metal complexing agents that have one when it binds a metal ion but it may have several different colors when it is not bound to the metal. All of the indicators have several acid base functionalities and the ionization state of these functional groups affects the color of the unbound indicator. Thus the color of the unbound indicator will vary with the pH of the solution. The choice of proper indicator at the right pH is tied to the K_{ass} of both indicator and EDTA. Since we are doing our titration in EDTA, K of the metal ion complex must be large enough that the complex will form, but it must bind the metal more weakly than EDTA. At the start of the titration the metal and the indicator form the color complex.

The titration proceeds until all the free metal is complexed with EDTA and only the small amount complexed with the indicator is left. Now with one more drop of EDTA, the EDTA must remove the metal from the indicator complex, so it changes back to its free form and you can see the endpoint. If a metal and the indicator complex is too strong the metal is said to block the indicator. The color of the indicators is frequently a function of pH as well, so you have to be careful of pH of the indicator as well as the EDTA.

10.10 Key words

Direct titration; Back-titration; Replacement or substitution titration; masking agents; demasking;

10.11 References for further study

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10.12 Questions for self understanding

1) Explain the EDTA competes with other ligands

2) Deduce the shape of the complexometric titration curve

3) Explain the Direct titration method

4) Explain the Back-titration method

5) Explain the Replacement or substitution titration method

6) Explain the Alkalimetric titrationmethod

7) Write a note on Selective demasking

8) Write a note on Choice of indicators

9) Explain Kinetic masking

10) Explain the Theory of visual use of metal ion indicators

Unit-11

Structure

11.0 Objectives of the unit

After studying this unit you are able to

- Explain the basic concept of Complexometric titrations
- Identify the different masking agent used for EDTA titration
- Identify the different demasking agents used for EDTA titration
- Explain the necessary of use of masking and demasking reagents used in EDTA titration
- Recognize he different metal indicators used for EDTA titration
- Calculate and recognize the water Hardness used for EDTA titration

11.1 Introduction

Many principles of acid-base titrations are used in complexometric titration. In complexometric titration, the free metal ions disappear as they are changed into complex ions. In acid-base titrations, the end point is marked by sudden change in pH. Similarly, in EDTA titration, if we plot pM (negative log of metal ion concentration) v/s volume of titrant, we will find that at the end point, the pM rapidly increases (Fig. 1). This sudden pM raise results from removal of traces of metal ions from solution by EDTA.

Any method, which can determine this disappearance of free metal ions, can be used to detect end point in complexometric titrations. End point can be detected usually with an indicator or instrumentally by potentiometric or conductometric (electrometric) method.

There are three factors that are important in determining the magnitude of break in titration curveat end point.

1. The stability of complex formed: The greater the stability constant for complex formed, larger the charge in free metal concentration (pM) at equivalent point and more clear would be the end point.

2. The number of steps involved in complex formation: Fewer the number of steps required in the formation of complex, greater would be the break in titration curve at equivalent point and clear would be the end point.

3. Effect of pH: During a complexometric titration, the pH must be constant by use of a buffer solution. Control of pH is important since the H+ ion plays an important role in chelation. Most ligands are basic and bind to H+ ions throughout a wide range of pH. Some of these H+ ions are frequently displaced from the ligands (chelating agents) by the metal during chelate formation.

11.2 Titration Selectivity, Masking and Demasking Agents

EDTA is a very unselective reagent because it complexes with numerous doubly, triply and quadruply charged cations. When a solution containing two cations which complex with EDTA is titrated without the addition of a complex-forming indicator, and if a titration error of 0.1% is permissible, then the ratio of the stability constants of the EDTA complexes of the two metals M and N must be such that $K_M/K_N \geq 10^6$ if N is not to interfere with the titration of M. strictly, of course, the constants K_M and K_N considered in the above expression should be the apparent stability constants of the complexes. If the complex-forming indicators are used, then for a similar titration error $K_M/K_N \geq 10^8$.

The following procedures will help to increase the selectivity:

a) Use of masking and demasking agents
b) pH control.
c) Use of selective metal indicators.
d) Classical separation
e) Solvent extraction
f) Removal of anions
g) Kinetic masking

11.3 Use of masking and demasking agents

Masking agents act either by precipitation or by formation of complexes more stable than the interfering ion-EDTA complex.

a) ***Masking by Precipitation***

Many heavy metals e.g.- Co, Cu and Pb, can be separated either in the form of insoluble sulphides using Sodium sulphide, or as insoluble complexes using thioacetamide. These are filtered, decomposed and titrated with disodium EDTA. Other common precipitating agents are sulphate for Pb and Ba, oxalate for Ca and Pb, fluoride for Ca, Mg and Pb, ferrocyanide for Zn and Cu, and 8-hydroxy quinoline for many heavy metals. Thioglycerol ($CH_2SH.CHOH.CH_2OH$) is used to mask Cu by precipitation in the assay of lotions containing Cu and Zn.

b) ***Masking by Complex formation***

Masking agents form more stable complexes with the interfering metal ions. The most important aspect is that the masking agent must not form complexes with the metal ion under analysis. The different masking agents used are enlisted below:

Ammonium fluoride will mask aluminium, iron and titanium by complex formation.

Ascorbic acid is a convenient reducing agent for iron(III) which is then masked by complexing as the very stable hexacyanoferrate(II) complex. This latter is more stable and less intensely coloured than the hexacyanoferrate(III) complex.

Dimercaprol(2,3-Dimercaptopropanol);($CH_2SH.CHSH.CH_2OH$). Cations of mercury, cadmium, zinc, arsenic, tin, lead and bismuth react with dimercaprol in weakly acidic solution to form precipitates which are soluble in alkaline solution.

All these complexes are stronger than the corresponding edetate complexes and are almost colourless. Cobalt, copper and nickel form intense yellowish-green complexes with the reagent under the above conditions. Cobalt and copper, but not nickel, are displaced from their edetate complexes by dimercaprol.

Potassium cyanide reacts with silver, copper, mercury, iron, zinc, cadmium, cobalt and nickel ions to form complexes in alkaline solution which are more stable than the corresponding edetate complexes, so that other ions, such as lead, magnesium, manganese and the alkaline earth metals can be determined in their presence. Of the metals in the first group mentioned, zinc and cadmium can be demasked from their cyanide complexes by aldehydes, such as formaldehyde or chloral hydrate (due to the preferential formation of a cyanohydrin), and selectively titrated.,

Potassium iodide is used to mask the mercury(II) ion as $(HgI_4)^{2-}$ and is specific for mercury. It can be used in the assay of mercury(II) chloride

Tiron (disodium catechol-3,5-disulphonate) will mask aluminium and titanium as colourless complexes. Iron forms highly coloured complexes and is best masked as its hexacyanoferrate(II) complex.

Triethanolamine [$N(CH_2.CH_2.OH)_3$] forms a colourless complex with aluminium, a yellow complex with iron(III), the colour of which is almost discharged by adding sodium hydroxide solution, and a green manganese(III) complex which oxidizes mordant black II. For these reasons, if murexide is used in the presence of iron and manganese, it is best to mask them with triethanolamine; similarly, mordant black II can be used in the presence of triethanolamine-aluminium complex.

Demasking

It is the process in which the masked substance regains its ability to enter into a particular reaction. This enables to determine a series of metal ions in one solution containing many cations.

Example of using masking and demasking agents in complexometry is the analysis of 3 metals Cu, Cd and Ca. the following method of analysis is followed:

1. Direct titration of the mixture with the EDTA gives the sum of the 3 metals.
2. Cu and Cd may be masked with the addition of cyanide to the solution, leaving only Ca ion.
3. When formaldehyde or chloral hydrate is added to the cyanide containing mixture, only Cd is demasked and the EDTA titrates the sum of Ca and Cd. In this manner, the concentration of three ions is determined by 3 individual titrations.

Step 1. All three metals are titrated.

Ca + EDTA ⇌ Ca-EDTA
Cd + EDTA ⇌ Cd-EDTA
Cu + EDTA ⇌ Cu-EDTA

Step 2. Only Ca is titrated.

Cu + Cyanide ion → Cu-Cyanide complex

Cd + Cyanide ion → Cd-Cyanide complex

Ca + Cyanide ion → No reaction $\xrightarrow{\text{EDTA}}$ Ca-EDTA

Step 3. Cd and Ca are titrated.

Cd-Cyanide complex + HCHO ⇌ Cd^{+} + Demasking agent

Cu-Cyanide complex + HCHO ⇌ No reaction

Cd + EDTA → Cd-EDTA
Cu + EDTA → Cu-EDTA

11.4 pH control Method

The formation of a metal chelate is dependent on the pH of the reaction medium. In weakly acid solution, the chelates of many metals are completely dissociated such as alkaline earth metals, whereas chelates of Bi, Fe^{3+} or Cr are readily formed at this pH. Thus, in acidic solution, Bi can

be effectively titratedwith a chelating agent in the presence of alkaline earth metals. This method is based upon the differences in stability of the chelates formed between the metal ions and the chelating agent.

11.5 Use of selective metal indicators

These indicators are the metal complexing agents which react with different metal ions under various conditions. Several selective metal indicators have been used and they are specific for a particular ion.

11.6 Classical separation

These may be applied if they are not tedious; thus the following precipitates may not be used for separations in which, after being re-dissolved, the cations can be determined complexometrically: CaC_2O_4, nickel dimethylglyoximate, $Mg(NH_4)PO_4$, $_6H_2O$, and CuSCN.

Solvent extraction: This is occasionally of value. Thus, Zinc can be separated from copper and lead by adding excess of ammonium thiocyanate solution and extracting the resulting zinc thiocyanate with 4-methylpentan-2-one (isobutyl methyl ketone); the extract is diluted with water and the zinc content determined with EDTA solution.

11.7 Removal of Anions

Anions, such as orthophosphate, which can interfere in complexometric titrations, may be removed using ion exchange resins.

11.8 Kinetic masking

This is a special case in which a metal ion does not effectively enter into the complexation reaction because of its kinetic inertness. Thus the slow reaction of chromium (III) with EDTA makes it possible to titrate other metal ions which react rapidly, without interference from Cr (III); this is illustrated by the determination of iron (III) and chromium (III) in a mixture.

11.9 Complexometric Titration and Water Hardness

Complexometric titration is an efficient method for determining the level of hardness of water. Hardness of water is caused by accumulation of mineral ions as a result the pH of water is increased. The K_f during the titration of hard water is reduced because of the reduced amount of EDTA added. Softening of hard water is done by altering the pH of the water which alternatively reducing the concentration of the metal ions present. Which could be performed in two phases and by maintaining basic pH for ions with high K_f e.g. Ca^{+2} and Mg^{+2}.

11.10 Zinc in Water

The traces of zinc in water can be determined with complexometric titration. Results from these analysis show that 5 bottles of water daily for minimum zinc quantity. The amount of Zn in soil water has been increased by bio-activities. The EDTA could also be performed for complex metal ions at lower pH. Zinc has a low $K_f = 3.16 \times 10^{-17}$. Zn^{+2} can be titrated in acidic pH 5.5

11.11 Zinc in Food

Certain foods contain zinc in small amounts. Oysters and other seafood, meat, liver, eggs, milk and brewer's yeast. Zinc deficiency is rare. It occurs when there is

i) Excess alcohol

ii) Excess exercisec as sweat depletes zinc stores

iii) A strict vegetarian diet

11.12 Applications of Complexometric Titrations

Complexometric titrations have been employed with success for determination of various metals like Ca, Mg, Pb, Zn, Al, Fe, Mn, Cr etc. in different formulations that are official in I.P., and also for the determination of Hardness of water.

11.13 Determination of Calcium in different formulations

Calcium can be determined in almost every formulation by EDTA-titrations. e.g.- Five membered heterocyclic rings are formed with EDTA, which are stain-free, and thus highly stable.

Assay of $CaCO_3$

Accurately weighed amount of $CaCO_3$ is dissolved in water and then acidified with HCl. A mixture of naphthol green and murexide is then added and titrated with EDTA, kept in burette.

1ml of M/20 disodium EDTA ≡ 0.005005 gm of $CaCO_3$

Calcium Lactate tablets

20 tablets are finely powdered and an accurately weighed amount of the powder, representing about 0.5gm of calcium lactate, is transferred to a crucible, ignited until free from carbon and then cooled. 10 ml water is added and the residue is dissolved by adding dropwise dil. HCl

solution. This solution is then transferred to a container, diluted to 150 ml with water and the assay is completed as is given under general procedure.

1ml of M/20 disod. EDTA ≡ 0.01542gm of Ca lactate

Calcium Lactate injection

Measure out a suitable volume of the injection, equivalent to about 0.5gm of Ca lactate. Transfer to the titration flask and proceed as given under general procedure.

Calcium Gluconate

An accurately weighed quantity (0.8gm) is dissolved in water (150ml) containing dil HCl (5ml). To the acidified solution is added, solution of NaOH (15ml), murexide indicator (4mg), solution of naphthol green (3ml). The reaction mixture is titrated with M/20 disod. EDTA until the solution is deep blue in colour.

1ml of M/20 disod. EDTA ≡ 0.02242gm of Ca gluconate

Calcium Gluconate injection

An accurately measured volume of the injection, equivalent to 0.8gm of Calcium gluconate is taken in a titration flask and preceded as above.

Calcium Gluconate tablet

20 Tablets are finely powdered. An accurately weighed amount of the powder, equivalent to 0.8gm of Calcium gluconate is transferred to a crucible and proceeded as described under Calcium lactate tablets.

11.14 Determination of Magnesium

Dissolve an accurately weighed sample (75mg) of Mg in sufficient water to make 100ml. pipette out 50ml of this solution in a titration flask, add 50ml water, 5ml of NH_3 buffer solution and a few drops of eriochrome blackT as indicator. Titrate it to a deep blue colour.

Each ml of M/20 disodium EDTA ≡ 0.02432 gm of Mg

This method could be used for the assay of Mg stearate and Mg sulphate**.**

11.15 Determination of Hardness of Water

Water hardness due to Ca and Mg is expressed as the amount of Ca and Mg ions in ppm. Actually, the hardness is due to both Ca and Mg salts but he two are determined together in the titration. The total Ca and Mg is titrated with standard EDTA solution using eriochrome blackT as indicator.

Method

Disodium salt of EDTA has the general formula: $Na_2H_2Y_2.H_2O$, where Y is the tetravalent anion of EDTA. When Ca is titrated with H_2Y^{2-}, a very stable complex is formed.

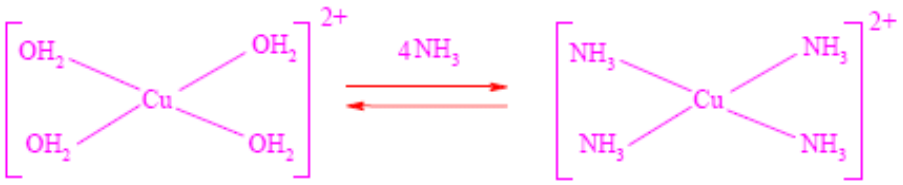
$$Ca^{2+} + H_2Y^{2-} \rightleftharpoons CaY^{2-} + 2H^+$$

Mg^{2+} forms a similar complex which is far less stable than the Ca complex. When a sample containing Ca and Mg ions is titrated with a solution of EDTA, the Ca^{2+} are first complexed as CaY^{2-}. As more reagent is added, all the Ca^{2+} is combined as complex. Mg ion forms MgY^{2-}. The desired end point if the titration is the point at which all the Ca and Mg ions of the solution have combined with the complexing agent.

11.16 Summary of the unit

The technique involves titrating metal ions with a complexing agent or chelating agent (Ligand) and is commonly referred to as complexometric titration. This method represents the analytical application of a complexation reaction. In this method, a simple ion is transformed into a complex ion and the equivalence point is determined by using metal indicators or electrometrically. Various other names such as chilometric titrations, chilometry, chilatometric titrations and EDTA titrations have been used to describe this method. All these terms refer to same analytical method and they have resulted from the use of EDTA (Ethylene diamine tetra acetic acid) and other chilons. These chilons react with metal ions to form a special type of complex known as chelate.

Metal ions in solution are always solvated, i.e. a definite number of solvent molecules (usually 2, 4 or 6) are firmly bound to the metal ion. However, these bound solvent molecules are replaced by other solvent molecules or ions during the formation of a metal complex or metal co-ordination compound. The molecules or ions which displace the solvent molecules are called Ligands. Ligands or complexing agents or chelating agents can be any electron donating entity, which has the ability to bind to the metal ion and produce a complex ion. An example of a complexation reaction between Cu (II) ion and four ammonium molecules in an aqueous solution may be expressed by the following equation.



11.17 Key words

Masking Agent; Demasking Agents; Metal indicators; Water Hardness; Complexometric titrations

11.18 References for further study

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11.19 Questions for self understanding

1) Explain the followings

i) Titration Selectivity

ii) Masking Agents

iii) Demasking Agents

2) Write a note on use of masking and demasking agents

3) Explain the pH control Method

4) Describe briefly the use of selective metal indicators

5) Write a note on followings

i) Classical separation

ii) Removal of Anions

iii) Kinetic masking

6) Explain the Complexometric Titration and Water Hardness

7) Write a note on Zinc in Water

8) Write a note on Zinc in Food

9) Explain different Applications of Complexometric Titrations

10) Explain the process of determination of Calcium in different formulations

11) Explain the process of determination of Magnesium

12) Explain the process of determination of Hardness of Water

Unit-12

Structure

12.0 Objectives of the unit

After studying this unit you are able to

- Explain the theory of Non aqueous titrations
- Identify the different non-aqueous solvents used for titration
- Explain the Procedure for titration in acetic acid
- Explain the Leveling and differentiating solvents
- Explain the advantages of non aqueous solvent over aqueous solvent
- Identify the different applications of non-aqueous titrations
- Explain the Non–aqueous titrations of weakly acidic substances

12.1 Introduction

Acids and bases have long been defined as substances that furnish, when dissolved in water, hydrogen and hydroxyl ions, respectively. This definition, introduced by Arrhenius, fails to recognize the fact that properties characteristic of acids orbases may be developed also in other solvents. A more generalized definition is that of Bro¨nsted, who defined an acid as a substance that furnishes protons, and a base as a substance that combines with protons. Even broader is the definition of Lewis, who defined an acid as any material that will accept an electron pair, a base as any material that will donate an electron pair, and neutralization as the formationof a coordination bond between an acid and a base.

The apparent strength of an acid or a base is determined by the extent of its reaction with a solvent. In water solutionall strong acids appear equally strong because they react with the solvent to undergo almost complete conversion to oxonium ion and the acid anion (leveling effect). In a weakly protophilic solvent such as acetic acid the extent of formation of the acetate acidium ion shows that the order of decreasing strength for acids is perchloric, hydrobromic sulfuric, hydrochloric, and nitric (differentiating effect).

This leveling effect is observed also for bases. In sulfuric acid almost all bases appear to be of the same strength. As the acid properties of the solvent decrease in the series sulfuric acid, acetic acid, phenol, water, pyridine, and butylamine, the bases become progressively weaker until all but the strongest have lost their basic properties. In order of decreasing strength, the strong bases are sodium 2-aminoethoxide, potassium methoxide, sodium methoxide, and lithium methoxide.

12.3 Non aqueous titrations

The earliest titrimetric applications involving metal–ligand complexation were the determinations of cyanide and chloride using, respectively, Ag^+ and Hg^{2+} as titrants. Both methods were developed by Justus Liebig (1803–1873) in the 1850s. The use of a monodentate ligand, such as Cl^- and CN^-, however, limited the utility of **complexation titrations** to those metals that formed only a single stable complex, such as $Ag(CN)^{2-}$ and $HgCl_2$. Other potential metal–ligand complexes, such as CdI_4^{2-}, were not analytically useful because the stepwise formation of a series of metal– ligand complexes (CdI^+, CdI_2, CdI_3^-, and CdI_4^{2-}) resulted in a poorly defined end point.

The utility of complexation titrations improved following the introduction by Schwarzenbach, in 1945, of aminocarboxylic acids as multidentate ligands capable of forming stable 1:1 complexes with metal ions. The most widely used of these new ligands was ethylenediaminetetraacetic acid, EDTA, which forms strong 1:1 complexes with many metal ions. The first use of EDTA as a titrant occurred in 1946, when Schwarzenbach introduced metallochromic dyes as visual indicators for signaling the end point of a complexation titration.

12.4 Titrations in Nonaqueous Solvents

Thus far we have assumed that the acid and base are in an aqueous solution. Indeed, water is the most common solvent in acid–base titrimetry. When considering the utility of a titration, however, the solvent's influence cannot be ignored. The dissociation, or autoprotolysis constant for a solvent, SH, relates the concentration of the protonated solvent, SH_2^+, to that of the deprotonated solvent, S^-. For amphoteric solvents, which can act as both proton donors and proton acceptors, the autoprotolysis reaction is,

$$2SH \rightleftharpoons SH_2^+ + S^-$$

with an equilibrium constant of

$$K_s = [SH_2^+][S^-]$$

You should recognize that *K*w is just the specific form of *K*s for water. The pH of a solution is now seen to be a general statement about the relative abundance of protonated solvent

$$pH = -\log[SH_2^+]$$

where the pH of a neutral solvent is given as

$$pH_{neut} = \frac{1}{2} pK_s$$

Perhaps the most obvious limitation imposed by *K*s is the change in pH during a titration. To see why this is so, let's consider the titration of a 50 mL solution of 10–4 M strong acid with equimolar strong base. Before the equivalence point, the pH is determined by the untitrated strong acid, whereas after the equivalence point the concentration of excess strong base determines the pH. In an aqueous solution the concentration of H_3O+ when the titration is 90% complete is,

$$[H_3O^+] = \frac{M_a V_a - M_b V_b}{V_a + V_b}$$

$$= \frac{(1 \times 10^{-4}\ M)(50\ mL) - (1 \times 10^{-4}\ M)(45\ mL)}{50 + 45} = 5.3 \times 10^{-6}\ M$$

corresponding to a pH of 5.3. When the titration is 110% complete, the concentration of OH– is

$$[OH^-] = \frac{M_b V_b - M_a V_a}{V_a + V_b}$$

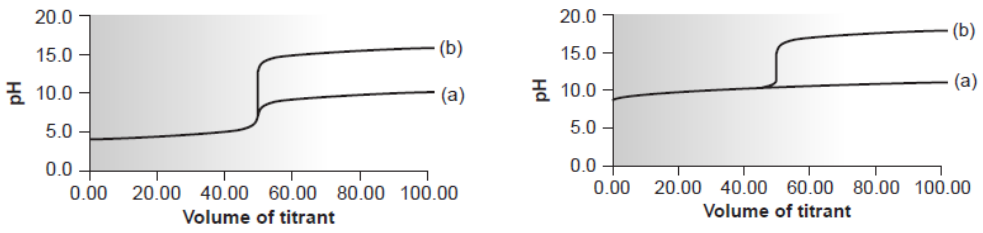
$$= \frac{(1 \times 10^{-4}\ M)(55\ mL) - (1 \times 10^{-4}\ M)(50\ mL)}{50 + 55} = 4.8 \times 10^{-6}\ M$$

or a pOH of 5.3. The pH, therefore, is

$$pH = pK_w - pOH = 14.0 - 5.3 = 8.7$$

The change in pH when the titration passes from 90% to 110% completion is,

$$\Delta pH = 8.7 - 5.3 = 3.4$$



If the same titration is carried out in a nonaqueous solvent with a *K*s of 1.0 ´ 10–20, the pH when the titration is 90% complete is still 5.3. However, the pH when the titration is 110% complete is now,

$$\text{pH} = \text{p}K_s - \text{pOH} = 20.0 - 5.3 = 14.7$$

In this case the change in pH of

$$\Delta\text{pH} = 14.7 - 5.3 = 9.4$$

is significantly greater than that obtained when the titration is carried out in water. Figure 9.16 shows the titration curves in both the aqueous and nonaqueous solvents. Nonaqueous solvents also may be used to increase the change in pH when titrating weak acids or bases (above Figure) Another parameter affecting the feasibility of a titration is the dissociation constant of the acid or base being titrated. Again, the solvent plays an important role. In the Brønsted–Lowry view of acid–base behavior, the strength of an acid or base is a relative measure of the ease with which a proton is transferred from the acid to the solvent, or from the solvent to the base. For example, the strongest acid that can exist in water is H_3O+. The acids HCl and HNO_3 are considered strong because they are better proton donors than H_3O+. Strong acids essentially donate all their protons to H_2O, "**leveling**" their acid strength to that of H_3O+. In a different solvent HCl and HNO_3 may not behave as strong acids. When acetic acid, which is a weak acid, is placed in water, the dissociation reaction,

$$CH_3COOH(aq) + H_2O(\ell) \rightleftharpoons H_3O^+(aq) + CH_3COO^-(aq)$$

does not proceed to a significant extent because acetate is a stronger base than water and the hydronium ion is a stronger acid than acetic acid. If acetic acid is placed in a solvent that is a stronger base than water, such as ammonia, then the reaction

$$CH_3COOH + NH_3 \rightleftharpoons NH_4^+ + CH_3COO^-$$

proceeds to a greater extent. In fact, HCl and CH3COOH are both strong acids in ammonia. All other things being equal, the strength of a weak acid increases if it is placed in a solvent that is more basic than water, whereas the strength of a weak base increases if it is placed in a solvent that is more acidic than water. In some cases, however, the opposite effect is observed. For example, the p*K*b for ammonia is 4.76 in water and 6.40 in the more acidic glacial acetic acid. In contradiction to our expectations, ammonia is a weaker base in the more acidic solvent. A full

description of the solvent's effect on a weak acid's p*K*a or on the p*K*b of a weak base is beyond the scope of this text. You should be aware, however, that titrations that are not feasible in water may be feasible in a different solvent.

All other things being equal, the strength of a weak acid increases if it is placed in a solvent that is more basic than water, whereas the strength of a weak base increases if it is placed in a solvent that is more acidic than water. In some cases, however, the opposite effect is observed. For example, the p*K*b for ammonia is 4.76 in water and 6.40 in the more acidic glacial acetic acid. In contradiction to our expectations, ammonia is a weaker base in the more acidic solvent. A full description of the solvent's effect on a weak acid's p*K*a or on the p*K*b of a weak base is beyond the scope of this text. You should be aware, however, that titrations that are not feasible in water may be feasible in a different solvent.

12.5 Leveling effect or solvent leveling

Leveling effect or solvent leveling refers to the effect of solvent on the properties of acids and bases. The strength of a strong acid is limited ("leveled") by the basicity of the solvent. Similarly the strength of a strong base is leveled by the acidity of the solvent. When a strong acid is dissolved in water, it reacts with it to form hydronium ion (H_3O^+) in the following reaction

$$HCl + H_2O \rightarrow Cl^- + H_3O^+$$

Any acid that is stronger than H_3O^+ reacts with H_2O to form H_3O^+. Therefore, no acid stronger than H_3O^+ exists in H_2O. Similarly, when ammonia is the solvent, the strongest acid is ammonium (NH_4^+), thus HCl and a super acid exert the same acidifying effect.

The same argument applies to bases. In water, OH^- is the strongest base. Thus, even though sodium amide ($NaNH_2$) is an exceptional base (pKa of NH_3 ~ 33), in water it is only as good as sodium hydroxide. On the other hand, $NaNH_2$ is a far more basic reagent in ammonia than is NaOH.

12.6 Leveling and differentiating solvents

In a differentiating solvent, various acids dissociate to different degrees and thus have different strengths. In a leveling solvent, several acids are completely dissociated and are thus of the same strength. A weakly basic solvent has less tendency than a strongly basic one to accept a proton. Similarly a weak acid has less tendency to donate protons than a strong acid. As a result a strong acid such as perchloric acid exhibits more strongly acidic properties than a weak acid such as acetic acid when dissolved in a weakly basic solvent. On the other hand, all acids tend to become

indistinguishable in strength when dissolved in strongly basic solvents owing to the greater affinity of strong bases for protons. This is called the leveling effect. Strong bases are leveling solvents for acids, weak bases are differentiating solvents for acids. Because of the leveling effect of common solvents, studies on super acids are conducted in solvents that are very weakly basic such as sulfur dioxide (liquefied) and SO_2ClF.

12.7 Titrations in glacial acetic acid

Acids and bases have long been defined as substances that, when dissolved in water, furnish hydrogen and hydroxyl ions, respectively. This definition, introduced by Arrhenius, fails to recognize the fact that properties characteristic of acids or bases may also be developed in other solvents. A more generalized definition is that of Brönsted, who defined an acid as a proton donor, and a base as a proton acceptor. Even broader is the definition of Lewis, who defined an acid as any material that will accept an electron pair, a base as any material that will donate an electron pair, and neutralization as the formation of a coordination bond between an acid and a base.

The apparent strength of an acid or base is determined by the extent of its reaction with a solvent. In aqueous solution all strong acids appear equally strong because they react with the solvent to undergo almost complete conversion to hydronium ion (H_3O^+) and the acid anion. In a weakly protophilic solvent such as acetic acid, the extent of formation of the acetonium ion ($CH_3COOH_2^+$) due to the addition of a proton provides a more sensitive differentiation of the strength of acids and shows that the order of decreasing strength for acids is perchloric, hydrobromic, sulfuric, hydrochloric, and nitric.

Acetic acid reacts incompletely with water to form hydronium ion and is, therefore, a weak acid. In contrast, it dissolves in a base such as ethylenediamine, and reacts so completely with the solvent that it behaves as a strong acid.

This so-called levelling effect is observed also for bases. In sulfuric acid almost all bases appear to be of the same strength. As the acid properties of the solvent decrease in the series sulfuric acid, acetic acid, phenol, water, pyridine and butylamine, bases dissolved in them become progressively weaker and the differences between bases are accentuated. In order of decreasing strength, strong bases of value for non-aqueous titrations are potassium methoxide, sodium methoxide, lithium methoxide, and tetrabutylammonium hydroxide.

Many water-insoluble compounds acquire enhanced acidic or basic properties when dissolved in organic solvents. Thus the choice of the appropriate solvent permits the determination of a variety of such materials by non-aqueous titration. Further, depending upon which part of a compound is physiologically active, it is often possible to titrate that part by proper selection of solvent and titrant. Pure compounds can be titrated directly, but it is often necessary to isolate the active ingredient in pharmaceutical preparations from interfering excipients and carriers.

The types of compounds that may be titrated as acids include acid halides, acid anhydrides, carboxylic acids, amino acids, enols such as barbiturates and xanthines, imides, phenols, pyrroles, and sulfonamides. The types of compounds that may be titrated as bases include amines, nitrogen-containing heterocyclic compounds, quarternary ammonium compounds, alkali salts of organic acids, alkali salts of inorganic acids, and some salts of amines. Many salts of halogen acids may be titrated in acetic acid or acetic anhydride after the addition of mercuric acetate, which removes halide ion as the unionized mercuric halide complex. In the case of hydrochlorides of weak bases not containing acetylatable groupings it is also possible to titrate in acetic anhydride without the addition of mercuric acetate and using an indicator such as malachite green or crystal violet. Titrations carried out in the presence of an excess of acetic anhydride must be applied cautiously, however, since any reaction of the anhydride with the substance being titrated may give rise to low results.

In the titration of a basic compound, a volumetric solution of perchloric acid in glacial acetic acid is usually used, although perchloric acid in dioxan may be useful in special cases. In the titration of an acidic compound, a volumetric solution of lithium methoxide in a methanol-toluene solvent is often used. For many applications it is convenient to use a solution of tetrabutylammonium hydroxide in toluene; sodium methoxide, formerly in wide use, may often give rise to troublesome gelatinous precipitates.

Because of interference by carbon dioxide, solvents for acidic compounds must be protected from excessive exposure to the atmosphere by a suitable cover or by an inert atmosphere during the titration. A blank determination should be carried out and the volume generally should not exceed 0.01 mL of a 0.1 mol/l titrant for each mL of solvent.

The end-point may be determined visually by colour change, or potentiometrically. If the calomel reference electrode is used, it is advantageous to replace the aqueous potassium chloride solution in the salt bridge with lithium perchlorate/acetic acid TS for titrations in acidic solvents,

or potassium chloride in methanol for titrations in basic solvents. It should be recognized that certain indicators in common use (crystal violet, for example) undergo a series of colour changes and, in establishing a non-aqueous titration method for a particular use, care should be taken to ensure that the colour change specified as the end-point of the titration corresponds to the maximum value of dE/dV (where E is the electromotive force and V the volume of titrant) in a potentiometric titration of the substance under consideration.

When using titrants prepared with solvents that may have a relatively high coefficient of expansion, for example, glacial acetic acid, toluene, etc., care should be taken to compensate for differences in temperature that may exist between the time the titrant is used and that at which it was standardized.

12.8 Procedure for titration in acetic acid

Method A (for bases and their salts)

Prepare a solution as specified in the monograph or dissolve the substance being examined in a suitable volume of glacial acetic acid R1, previously neutralized to crystal violet/acetic acid TS, warming and cooling if necessary. Alternatively the titration blank for the solvent and indicator may be established in a separate determination. When the substance is a salt of a hydrohalic acid, add 10 mL of mercuric acetate/acetic acid TS. When the end-point is determined visually by colour change, add 2-3 drops of crystal violet/acetic acid TS, and titrate with perchloric acid of the specified concentration (mol/l) to the appropriate colour change of the indicator. When a different indicator is specified in the monograph, this indicator should also be used for the neutralization of the glacial acetic acid R1, and mercuric acetate/acetic acid TS, and the standardization of the titrant.

When the equivalence point is determined potentiometrically, the indicator is omitted and neutralization of the solution and standardization of the titrant are also carried out potentiometrically. A glass electrode and a saturated calomel cell (containing potassium chloride (350 g/l) TS) as reference electrode, are used. The junction between the calomel electrode and the titration liquid should have a reasonably low electrical resistance and there should be a minimum of transfer of liquid from one side to the other. Serious instability may result unless the connections between the potentiometer and the electrode system are in accordance with the manufacturer's instructions.

When the temperature (t_2) at which the titration is carried out differs from the temperature (t1) at which the titrant was standardized, multiply the volume of the titrant required by [1 + 0.001(t1 - t_2)] and calculate the result of the assay from the corrected volume.

Method B (for acids)

The titrant, solvent and (in the case where the end-point is determined visually) the indicator to be used for each substance, are specified in the monograph.

Protect the solution and titrant from carbon dioxide of the atmosphere throughout the determination. This may conveniently be done by replacing the air above the titration liquid with nitrogen.

Dissolve the substance being examined in a suitable volume of the solvent previously neutralized to the indicator, warming and cooling if necessary, or prepare a solution as specified in the monograph. Titrate to the appropriate colour change of the indicator. Carry out a blank determination and make any necessary corrections. The titrant is standardized using the same solvent and indicator as specified for the substance.

When the equivalence point is established potentiometrically, the indicator is omitted and neutralization of the solution and standardization of the titrant are also carried out potentiometrically.

A glass electrode and a saturated calomel reference electrode in which the aqueous potassium chloride (350 g/l) TS has been replaced by a saturated solution of potassium chloride R in methanol R are used. The junction between the calomel electrode and the titration liquid should have a reasonably low electrical resistance and there should be a minimum of transfer of the liquid from one side to the other. Serious instability may result unless the connections between the potentiometer and the electrode system are made in accordance with the manufacturer's instruction.

12.9 Types of solvents

1. Aprotic solvents
2. Protogenic solvents
3. Protophilic solvents
4. Amphiprotic solvents
5. Levelling solvents

1. Aprotic Solvents

Aprotic solvents include those substances, which may be consideredchemically neutral, and virtually un-reactive under the conditions employed. Carbon tetrachloride andtoluene come in this group; they possess low dielectric constants, do not cause ionization in solutesand do not undergo reactions with acids and bases. Aprotic solvents are frequently used to dilutereaction mixture.

2. Protophilic Solvents

Protophilic solvents are the substances that possess a high affinity forprotons. The overall reaction can be represented as: -

$HB + S \leftrightarrow SH^+ + B^-$

The equilibrium in this reversible reaction will be generally influenced by the nature of the acid and the solvent. Weak acids are normally used in the presence of strongly protophilic solvents as their acidic strengths are then enhanced and then become comparable to these of strong acids; this is known as the levelling effect.

3. Protogenic Solvents

Protogenic solvents are acidic in nature and readily donate protons. Anhydrous acids such as hydrogen fluoride and sulphuric acid fall in this category, because of their strength and ability to donate protons, they enhance the strength of weak bases.

4. Amphiprotic Solvents

Amphiprotic solvents consist of liquids, such as water, alcohols and weakorganic acids, which are slightly ionized and combine both protogenic and protophillic properties in being able to donate protons and accept protons ethanoic acid displays acidic properties indissociating to produce protons

$$CH_3\text{-}COOH \leftrightarrow CH_2\text{-}COO^- + H^+$$

But in the presence of perchloric acid, a far stronger acid, it will accept a proton:

$$CH_3\text{-}COOH + HClO_4 \leftrightarrow CH3COOH_2^+ + ClO_4^-$$

The $CH3COOH_2^+$ ion can very readily give up its proton to react with a base, so basic properties of a base is enhanced, so titrations between weak base and perchloric acid can often be accuratelycarried out using ethanoic acid as solvent.

5. Levelling Solvents

In general, strongly protophilic solvents are important to force equilibriumequation to the right. This effect is so powerful that, in strongly protophillic solvents, all acids act as ofsimilar

strength. The converse occurs with strongly protogenic solvents, which cause all bases to actas they were of similar strength. Solvents, which act in this way, are known as Levelling Solvents.

12.10 Interference due to water in non –aqueous titrations

When a weakly basic drug is present, water (OH^-) acts as stronger base as compared to theformer one and preferentially accepts proton from an acid. Thus there is interference in thereaction of weak base with an acid.

Similarly when a weakly acidic drug is present, water (H^+) behaves like a strong acid ascompared to the former one and preferentially donates proton to the base. Thus there is interference in the reaction of weak acid with a base.

Hence in the presence of water, titration of either weakly acidic substances with stronger base or weakly basic substances with stronger acid is not possible.

12.11 Basic concept of non –aqueous titrations

The Bronsted Lowery theory of acid and bases can be applied equally well to reactions occurringduring acid base titrations in non-aqueous solvents. This is because this approach considers an acidas any substance, which will tend to donate a proton, and a base as any substance, which will accepta proton. Substances which give poor end points due to being weak acids or bases in aqueoussolution will frequently give far more satisfactory end point when titrations are carried out in non-aqueous media. An additional advantage is that many substances, which are insoluble in water, aresufficiently soluble in organic solvents to permit their titrations in these non-aqueous media.In the Bronsted Lowery theory, any acid, (HB) is considered to dissociate in solution to give a proton(H^+) and a conjugate base (B^-):- where as any base (B) will combine with a proton to produce aconjugate acid (HB^+):

$$HB \leftrightarrow H^+ + B^-$$

$$B + H^+ \leftrightarrow HB^+$$

The ability of substances to act as acids or bases will very much depend on the choice of solvent system.

12.12 Advantages of non aqueous solvent over aqueous solvent

1) Organic acids and bases that are insoluble in water are soluble in non-aqueous solvent.

2) Organic acid, which is of comparable strength to water, can be titrated easily in non aqueous solvent. Bases also follow the same rules.

3) A non-aqueous solvent may help two are more acids in mixture. The individual acid can give separate end point in different solvent.

4) By the proper choice of the solvents or indicator, the biological ingredients of a substance whether acidic or basic can be selectively titrated.

5) Non aqueous titrations are simple and accurate, examples of non aqueous titration are: Ephedrine preparations, codeine phosphate in APC, tetracycline, teramycin, Antihistamines andvarious piprazine preparations.

12.13 Some examples of non-aqueous solvents

Glacial Ethanoic Acid

Glacial ethanoic acid is the most frequently used non-aqueous solvent.Before it is used it is advisable to check the water content. This may be between 0.1% and 1.0%.

Dimethylformamide

Dimethylformamide (DMF) is a protophillic solvent, which is frequentlyemployed for titrations between, for instance, benzoic acid and amides, although end points maysometimes be difficult to obtain.

Acetonitrile

Acetonitrile (methyl cyanide, cyanomethane) is frequently used with other solvents suchas chloroform and phenol and especially with ethanoic acid. It enables very sharp end points to beobtained in the titration of metal ethanoates when titrated with perchloric acid.

Dioxane

Dioxane is another popular solvent, which is often used in place of glacial ethanoic acidwhen mixtures of substances are to be quantified. Unlike ethanoic acid, dioxane is not a levellingsolvent and separate end points are normally possible, corresponding to the individual components inthe mixtures.

Alcohol

Salts of organic acids, especially of soaps are best determined in mixtures of glycols andalcohols or mixtures of glycols and hydrocarbons. The most common combinations are ethyleneglycol (dihydroxyethane) with propan-2-ol or butan-1-ol. The combinations provide admirable solventsfor both the polar and non-polar ends of the molecules.

12.14 Applications of non-aqueous titrations

Although indicators may be used to establish individual end points, as in traditional acid-basetitrations, potentiometric methods of end point detection are also used extensively, especially forhighly coloured solutions. Non aqueous titration have been used to quantify the mixtures of primary,secondary and tertiary amines, for studying sulphonamides, mixture of purines and for many otherorganic amino compounds and salts of organic acid. And also it is used for the titration of Halogenacid salts of weak bases.

(i) titrations of weak bases with perchloric acid

Principle

weak bases are dissolved in acetic acid and are titrated with acetous perchloric acid. Thevarious reactions which occur are given as follow:Acetic acid alone behaves as a weak acid, because of poor dissociation into H^+

$$CH_3\text{-}COOH \leftrightarrow CH_3\text{-}COO^- + H^+$$

But when a strong acid (Perchloric acid) is added to acetic acid, there is formation of Onium ions,which has more tendency to donate protons.

$$HClO_4 \leftrightarrow H^+ + ClO_4^-$$

$$CH_3COOH + H+ \leftrightarrow CH_3COOH^{2+}$$

(Onium ion)

When weak bases like pyridine are dissolved in acetic acid, equivalent amount of acetates ions are produced which have more tendency to accept protons.

$$C_5H_5N + CH_3\text{-}COOH \leftrightarrow C_5H_5NH^+ + CH_3COO^-$$

Ultimately, the titration of weakly basic drug in acetic acid against acetous perchloric acid yieldsaccurate end point. The series of reactions are given as follows:

$$HClO_4 + CH_3COOH \rightarrow CH_3COOH^{2+} + ClO_4^-$$

(Onium ions)

$$C_5H_5N + CH_3COOH \rightarrow C_5H_5NH^+ + CH_3COO^- \text{ (Acetate ions)}$$

$$CH_3\text{-}COOH^{2+} + CH_3COO^- \rightarrow 2CH_3COOH$$

(Burette) (Conical flask)

The net reaction is given as

$$HClO_4 + C_5H_5N \rightarrow C_5H_5NH^+ + ClO_4^-$$

Thus we have seen that, on one hand the tendency of acid to donate proton is increased and on the other hand, the tendency of base to accept proton is increased .These leads to the sharp endpoint in non–aqueous titrations.

Table: Acidimetric Assays: Non-aqueous Titrations with Perchloric Acid using Mercuric Acetate and different Indicators

S.No.	Name of Substance	Indicator Employed
1.	Amantadine hydrochloride	Crystal violet
2.	Chlorpromazine hydrochloride	Methyl orange
3.	Clonidine hydrochloride	α -Naphthol benzein
4.	Cyproheptadiene.HCl	Crystal violet
5.	Dehydroemetine.HCl	-do-
6.	Ephedrine hydrochloride	-do-
7.	Imipramine hydrochloride	-do-
8.	Isoprenaline hydrochloride	Crystal violet
9.	Lignocaine hydrochloride	-do-
10.	Morphine hydrochloride	-do-
11.	Morphine sulphate	-do-
12.	Phenylephrine hydrochloride	-do-
13.	Phenytoin sodium	α -Naphthol benzein
14.	Promethazine hydrochloride	Methyl orange
15.	Thiabendazole	Crystal violet

Requirements for the titration of weak bases with perchloric acid

Following points should be considered:

- Titrant used.
- Preparation of 0.1N ($HClO_4$) and it standardization.
- Solvent used.
- Practical examples of Indicators.

Titrant used

Solution of $HClO_4$in either glacial acetic acid or dioxane solution is used for titration ofweak bases. Generally $HClO_4$ with a normality of 0.1N to 0.05N is used.

Preparation of 0.1N solution of $HClO_4$ and its standardization

Dissolve 8.5 ml of 72% $HClO_4$ inabout 900 ml glacial acetic acid with constant stirring, add about 30 ml acetic anhydride and make upthe volume (1000 ml) with glacial acetic acid and keep the mixture for 24 hour. Acetic anhydride absorbed all the water from $HClO_4$ and glacial acetic acid and renders the solution virtually anhydrous. $HClO_4$ must be well diluted with glacial acetic acid before adding acetic anhydride because reaction between $HClO_4$ and acetic anhydride results in the formation of acetyl perchloratewhich is an explosive.

Standardization of 0.1N Perchloric acid

Weigh accurately 0.7g of potassium hydrogen phthalate (primary standard), previously powdered lightand dried for 2 hours and dissolve it in 50 ml of glacial acetic acid. Add few drops of crystal violetsolution as indicator and titrate with perchloric acid solution until the violet colour changes to emeraldgreen. Perform a blank titration by using 50 ml of glacial acetic acid and subtract the volume ofperchloric acid consumed (crystal violet -0.5% w/v in acetic acid).

Each ml of 0.1N Perchloric acid ≡ 0.02042 g of Potassium hydrogen Phthalate

$$\text{Strength of 0.1N Perchloric acid} = \frac{\text{wt. of potassium hydrogen phthalate taken}}{\text{Vol. of Perchloric acid X 0.02042}}$$

204.2g of $C_8H_5O_4K$ ≡ 1N 1000 ml $HClO_4$

0.02042g of $C_8H_5O_4K$ ≡ 1 ml of 0.1 N $HClO_4$

Solvent used

Glacial acetic acid alone or sometimes in combination with some aprotic solvents isoften used. The other solvents are $CHCl_3$, benzene, chloro benzene, acetic anhydride and variouscombinations of these sometime glycohydrocarbon mixtures are also used.

Indicators used

Crystal violet 0.05% w/v in glacial acetic acid, methyl red 0.1% w/v in anhydrous methanol, oracet blue 0.5% w/v in glacial acetic acid.

(ii) Titration of halogen acid salts of bases with perchloric acid

In general, the halide ions, namely: chloride, bromide and iodide are very weakly basic in characterso much so that they cannot react quantitatively with acetous perchloric acid. In order to overcomethis problem, mercuric acetate is usually added (it remains undissociated in acetic acid

solution) to ahalide salt thereby causing the replacement of halide ion by an equivalent amount of acetate ion,which serves as a strong base in acetic acid as shown below

$$2R.NH_2.HCl \leftrightarrow 2RNH_3^+ + 2Cl^-$$

$$(CH_3COO)_2Hg + 2Cl^- \rightarrow HgCl_2 + 2CH_3COO^-$$

(Undissociated) (Undissociated)

$$2CH_3COOH_2^+ + 2CH_3COO^- \leftrightarrow 4\,CH_3COOH$$

12.15 Non–aqueous titrations of weakly acidic substances

There are several drugs which are weakly acidic. Such substances can be titrated against strongbases like potassium methoxide, sodium methoxide, lithium methoxide, tetra butyl ammoniumhydroxide, etc in solvents like toluene- methanol. The principle is similar to the titration of weak basesagainst perchloric acid.

Table: Alkalimetric Assays: Non-Aqueous Titrations using Lithium Methoxide/Sodium Methoxide either Potentiometrically or Titrimetrically

S.No.	Name of Substance	Indicator Employed
1.	Acetazolamide	Potentiometric determination
2.	Bendrofluazide	Azo violet
3.	Allopurinol	Thymol blue
4.	Mercaptopurine	-do-
5.	Amylobarbitone	Quinaldine Red
6.	Nalidixic acid	Thymolphthalein

Preparation of 0.1 N Sodium Methoxide

It is prepared exactly in a similar manner as for

Procedure

Add into a dry flask, a mixture of methanol (40 ml) and dry toluene (50 ml) and cover itloosely. Carefully add freshly cut pieces of potassium metal (5.6 gm) to the above mixture graduallywith constant shaking. After complete dissolution of potassium metal, add enough absolute methanolto yield a clear solution. Toluene 50 ml is added with constant shaking until the mixture turns hazy inappearance. The process is repeated by the alternate addition of methanol and benzene until 1 litreof solution is obtained, taking care to add a minimum volume of methanol to give a visible clearsolution.0.1 N Potassium Methoxide, using 2.3g of freshly cut sodium in place of potassium.

Preparation of 0.1 N Lithium Methoxide

It is prepared as for 0.1 N Potassium Methoxide, but using0.7 g of lithium in place of potassium.

Standardization of 0.1 N Methoxide Solution

Material Required

Dimethylformamide (DMF): 10 ml; thymol blue (0.3% in MeOH); 0.1 N lithiummethoxide in toluene methanol; benzoic acid: 0.6 g.

Procedure

Transfer 10 ml of DMF in a conical flask and add to it 3 to 4 drops of thymol blue and firstneutralize the acidic impurities present in DMF by titrating with 0.1 N lithium methoxide in toluene-methanol. Quickly introduce 0.06g of benzoic acid and titrate immediately with methoxide in toluene-methanol.

Equations

The various equations involved in the above operations are summarized as stated below:

(i) $$Na + CH_3OH \rightarrow CH_3ONa + H\uparrow$$

Interaction between sodium metal and methanol is an exothermic reaction and hence, special caremust be taken while adding the metal into the dry solvent in small lots at intervals with adequatecooling so as to keep the reaction well under control.

(ii) $$H_2O + CH_3ONa \rightarrow CH_3OH + NaOH$$

$$H_2CO_3 + 2CH_3ONa \rightarrow 2CH_3OH + Na_2CO_3$$

The clear solution of sodium methoxide must be kept away from moisture and atmospheric CO_2 as far as possible so as to avoid the above two chemical reactions that might ultimately result into the formation of turbidity.

(iii) $$C_6H_5COOH + H{-}CON(CH_3)_2 \leftrightarrow HCON^{+}H(CH_3)_2 + C_6H_5COO^{-} \quad \text{------1}$$

(DMF)

$$CH_3ONa \leftrightarrow CH_3O^{-} + Na^{+} \quad \text{----------2}$$

$$HCON^{+}H(CH_3)_2 + CH_3O^{-} \rightarrow HCON(CH_3)_2 + CH_3OH \quad \text{----------3}$$

Summing up: $C_6H_5COOH + CH_3ONa \rightarrow C_6H_5COONa + CH_3OH$

Step 1 It shows the solution of benzoic acid (primary standard) in DMF,

Step 2 It depicts ionization of sodium methoxide,

Step 3 It illustrates the interaction between the solvated proton and the methylated ion. In summing up, the net reaction between the water in the solvent (DMF) and the titrant is equivalent to the volume of sodium methoxide consumed by DMF or may be considered as a blank determination.

N/10 KOH in Methanol

Dissolve 5.6 gm of anhydrous KOH in 1000 ml of anhydrous methanol. This titrant is not as powerfulas others. Its main disadvantage is that it reacts with acidic functional groups and produces amolecule of water, which would affect the sensitivity of titration.

Standardisation

All these titrants are usually standardized against standard benzoic acid AR-Grade. A sufficientamount of benzoic acid which would give a titrate value of 20-30 ml is transferred in a dry flask anddissolved in 25 ml dimethylformamide, 2-3 drops of 0.5% thymol blue indicator in dry methanol is added to the solution. A blank titration is also per formed in the solvent to account acidic impurity indimethylformamide and the correction is made accordingly.

Tetrabutylammonium Hydroxide

The alkalimetry in non-aqueous titrations may also be carried out efficiently by usingtetrabutylammonium hydroxide along with an appropriate indicator.

Preparation of 0.1 N Tetrabutylammonium Hydroxide in Toluene-Methanol

Materials Required

Tetrabutylammonium iodide: 40 g; absolute methanol: 90 ml; silver oxide: 25 g;dry toluene: 150 ml.

Procedure

Carefully dissolve 40 g of tetrabutylammonium iodide (Bu_4NI) in 90 ml of absolutemethanol, add to it 20 g of finely powdered purified silver oxide and finally shake the mixturethoroughly for 1 hour. Centrifuge about 2-3 ml of the resultant mixture and test for iodide in thesupernatant liquid. In case, it gives a positive test, add about 2 g more of silver oxide and shake foran additional period of 30 minutes. The said method may be repeated until the supernatant liquidobtained is completely free from iodide. The mixture thus obtained is filtered through a fine sinteredglass filter and finally rinse the container with 3 portions, each of 50 ml of dry toluene. Thesewashings may be added to the filtrate and the final volume is made up to 1 litre

with dry toluene. Theclear solution should be kept duly protected from both CO_2 and moisture during storage.

Equation

$$2Bu_4NI + Ag_2O + H_2O \rightarrow 2Bu_4NOH + 2AgI$$

Tetra butyl-Ammonium Iodide — *Tetra butyl ammonium hydroxide*

Precautions

Following points should be considered:-Moisture and CO_2 have to be excluded, water being weakly basic would compete with perchloric acidand sharpness of end point would be lost, therefore, moisture contents should be less that 0.05%.The presence of CO_2 affects basic solvent like Dimethyleformamide, ethylene diamine and pyridine asthey adsorb CO_2 from air.

During preparation of Perchloric acid it must be well diluted with acetic acid before adding the acetic anhydride to prevent the formation of explosive acetyl per chlorate.

Do not use a solvent until fully acquainted with its hazards and how to use it safely.

12.16 Summary of the unit

The substances, which are either to weakly acidic or too weakly basics to give sharp end point in aqueous solutions, can easily be titrated with accuracy is non-aqueous solvent.

Advantages of Non Aqueous Solvent over Aqueous Solvent

1) Organic acids and bases that are insoluble in water are soluble in non-aqueous solvent.

2) Organic acid, which is of comparable strength to water, cannot be titrated easily non-aqueous solvent. Bases also follow the same rules.

3) A non-aqueous solvent may help two are more acids in mixture. The individual acid can give separate end point in different solvent.

4) By the proper choice of the solvents or indicator, the biological ingredients of a substance whether acidic or basic can be selectively titrated.

5) Non aqueous titrations are simple and accurate, examples of non aqueous titration are : Ephedrine preparations, codeine phosphate in APC, tetracycline, teramycin, Anti- histamines and various piprazine preparations.

Strongly protophilic solvents are important to force equilibrium equation to the right. This effect is so powerful that, in strongly protophillic solvents, all acids act as of similar strength. The

converse occurs with strongly protogenic solvents, which cause all bases to act as they were of similar strength. Solvents, which act in this way, are known as Leveling Solvents.

Determination in non-aqueous solvents is important for substances which may give poor end points in normal aqueous titration and for substances which are not soluble in water. They are particularly valuable for determining the properties of individual components in mixtures of acids or mixture of bases. These differential titrations are carried out in solvents, which do not exert a leveling effect.

Although indicators may be used to establish individual end points, as in traditional acid-base titrations, potentiometric methods of end point detection are also used extensively, especially for highly coloured solutions. Non aqueous titration have been used to quantify the mixtures of primary, secondary and tertiary amines, for studying sulphonamides, mixture of purines and for many other organic amino compounds and salts of organic acid.

12.17 Key words

Non aqueous titrations; leveling effect; differentiating solvents

16.18 References for further study

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12.19 Questions for self understanding

1) Write a note on titrations in nonaqueous Solvents

2) What is leveling effect or solvent leveling?

3) Explain leveling and differentiating solvents?

4) Describe Titrations in glacial acetic acid

5) Write the procedure for titration in acetic acid

6) With example each explain the types of different solvents used for titration

7) Write a note on interference due to water in non –aqueous titrations

8) Explain the basic concept of non –aqueous titrations

9) What are the advantages of non aqueous solvent over aqueous solvent

10 Mention five examples of non-aqueous solvents

11) Explain the followings

(i) Titrations of weak bases with perchloric acid

(ii) Titration of halogen acid salts of bases with perchloric acid

12) Explain the non–aqueous titrations of weakly acidic substances

Unit -13

Structure

13.0 Objectives of the Unit

After studying this unit you are able to

- Explain the method of solvent extraction in purification of substance
- Explain the distribution Law of solvent extraction
- Calculate the percent extraction in different solvent
- Explain the chemical interactions in the aqueous phase
- Explain the Ion association complexes
- Identify the better solvent to extract particular substance

13.1 Introduction

Solvent extraction is a method of separation long been known to the chemists. In recent years it has achieved recognition among analysts as a powerful separation technique. Liquid-liquid extraction, mostly commonly used in analysis, is a technique in which a solution is brought into contact with a second solvent, essentially immiscible with the first, in order to bring the transfer of one or more solutes into the second solvent. The separations that can be achieved by this method are simple, convenient and rapid to perform. They are clean as much as the small interfacial area certainly precludes any phenomena analogous to the undesirable co-precipitation encountered in precipitation separations.

Solvent extraction is one of the most extensively studied and most widely used techniques for the separation and pre-concentration of elements. The technique has become more useful in recent years due to the development of selective chelating agents for trace metal determination. With proper choice of extracting agents, this technique can achieve group separation or selective separation of trace elements with high efficiencies.

13.2 Solvent extraction

Solvent extraction is a process whereby two immiscible liquids are vigorously shaken in an attempt to disperse one in the other so that solutes can migrate from one solvent to the other. When the two liquids are not shaken the solvent to solvent interface area is limited to the geometric area of the circle separating the two solvents. However as the two liquids are vigorously shaken the solvents become intimately dispersed in each other.

In analytical applications solvent extraction may serve the following three purposes

i) Preconcentration of trace elements

ii) Elimination of matrix interference

iii) Differentiation of chemical species.

The procedure is applicable to both, trace and macro levels. A further advantage of solvent extraction method lies in the convenience of subsequent analysis of the extracted species. If the extracted species are coloured, as is the case with many chelates, spectrophotometric methods can be employed.

13.3 Distribution Ratio (D)

The distribution of a solute between two immiscible solvents can be described by the distribution ratio "D".

$$D = \frac{[A_1]}{[A_2]}$$

Where [A] represents the stoichiometric or formal concentration of a substance A and the subscripts 1 and 2 refer to the two phases. Since in most cases, two-phase system is of analytical interest, an organic solvent and aqueous are involved, D will be understood to be

$$D = \frac{[A_{org}]}{[A_{Aqe}]}$$

The subscripts org and Aq refer to the organic and aqueous phases respectively.

13.4 Percent Extraction (E)

The more commonly used term for expressing the extraction efficiency by analytical chemist is the percent extraction "E", which is related to "D" as

$$\%\ \text{Extraction(E)} = \frac{100[\text{A}]_{\text{Org}}\,\text{V}_{\text{Org}}}{[\text{A}]_{\text{Org}}\,\text{V}_{\text{Org}} + [\text{A}]_{\text{Aq}}\,\text{V}_{\text{Aq}}} = \frac{100D}{D + V_{Aq}/V_{Org}}$$

Where V represent solvent volume and the other quantities remain as previously defined. The percent extraction may be seen to vary with the volume ratio of the two phases as well as with D. It may also be seen from equation (2.3) that at extreme values of "D", "E" becomes less sensitive to changes in "D". For example, at a phase volume ratio of unity, for any value of D below 0.001, the solute may be considered quantitatively retained in the aqueous phase whereas for D values from 500 to 1000, the value of "E" changes only from 99.5 to 99.9%.

13.5 Separation Factor (γ)

Since solvent extraction is used for the separation of different elements and species from each other, it becomes necessary to introduce a term to describe the effectiveness of separation of two solutes. The separation factor γ is related to the individual distribution ratios as follows

$$\gamma = \frac{[A]_{Org}/[B]_{Org}}{[A]_{Aq}/[B]_{Aq}} = \frac{[A]_{Org}/[A]_{Aq}}{[B]_{Org}/[B]_{Aq}} = \frac{D_A}{D_B}$$

where A and B represent the respective solutes.

In those systems where one of the distribution ratios is very small and the other relatively large, complete separations can be quickly and easily achieved. If the separation factor is large but the smaller distribution ratio is sufficiently large then less separation of both components occurs. It is then necessary to apply various techniques to suppress the extraction of the undesired component.

13.6 Distribution Law

In the simplest extraction case, the distribution ratio is constant in accordance with the classical Nernst distribution law, a solute will distribute itself between two essentially immiscible solvents so that at equilibrium the ratio of the concentrations of the solute in the two phases at a particular temperature will be constant, provided the solute is not involved in chemical interactions in either phase. For such a solute, then

$$K_d = \frac{[A]_{Org}}{[A]_{Aq}} = D$$

where "Kd" is termed as the distribution coefficient.

Deviations from the distribution law arise from two sources they are

(a) Neglect of activity corrections and

(b) Participations of the distributing solute in chemical interactions in either or both of the two solvent phases. Although the distribution law, as described in above equation is not thermodynamically rigorous, variation in K_d due to variation in activity coefficients is likely to be under one order of magnitude for most extraction systems of interest to analysts. Far more important are the changes in extraction characteristics of solute because of chemical changes, which occur. Such changes do not represent failure of the law. Rather, they add complexity to

the distribution expressions, which can be properly accounted for by using appropriate equilibrium expressions.

The distribution of acetic acid between benzene and water may serve as an illustration of the effects of chemical interactions of the solute. The distribution of acetic acid itself may be described as follows

$$(CH_3COOH)_{Aq} \Leftrightarrow (CH_3COOH)_{Org}$$

$$K_D = \frac{[CH_3COOH]_{Org}}{[CH_3COOH]_{Aq}}$$

However, acetic acid dissociates in aqueous phase

$$CH_3COOH \leftrightarrow CH_3COO^- + H^+$$

$$K_A = \frac{[H^+][CH_3COO]^-}{[CH_3COOH]_W}$$

and forms a dimmer in benzene

$$2CH_3COOH \leftrightarrow (CH_3COOH)_2$$

$$K_P = \frac{[(CH_3COOH)_2]_O}{[CH_3COOH]_O^2}$$

The overall distribution of acetic acid is described bv “D”. which is

$$D = \frac{[CH_3COOH]_O}{[CH_3COOH]_W} = \frac{[CH_3COOH]_O + [(CH_3COOH)_2]^2_O}{[CH_3COOH]_W + [CH_3COO^-]}$$

Upon incorporation of all the equilibrium expression we get

$$D = \frac{K_D[1 + 2K_P[CH_3COOH]_O]}{1 + K_A/[H^+]}$$

This shows how the distribution of acetic acid varies as a function of pH and acetic acid concentration.

13.7 Process of Extraction

From the above equations, it is clear that three essential aspects are involved in the extraction of acetic acid:

i) Chemical interaction in the aqueous phase.

ii) Distribution of extractable species.

iii) Chemical interactions in the organic phase.

These three aspects are shared by almost all extraction systems and serve as the basis of a useful organizational pattern.

13.8 Chemical interactions in the aqueous phase

A major point of differentiation between extraction of organic and inorganic materials is the extent to which the formation of an uncharged extractable species depends on chemical interactions in the aqueous phase. Most organic compounds are already uncharged and extractable. Such aqueous phase reactions if do occur might well transform these to charged non-extractable species, e.g. $RCOOH + H_2O \leftrightarrow RCOO^- + H_3O^+$

$$RNH_2 + H_2O \leftrightarrow R\overset{+}{N}H_3 + OH^-$$

In contrast, most of the inorganic compounds are dissociated, so that in order to extract a species of interest into organic solvent, reactions in the aqueous phase leading to the formation of an uncharged, extractable complex must be utilized.

For example, in order to extract aluminum-III from an aqueous solution of aluminum nitrate, one must bring about the reaction of the aluminum-III cation with a reagent such as 8-quinolinol to form aluminum-8-quinolinate, which may be extracted into a variety of organic solvents such as chloroform or benzene. Therefore, the formation of an uncharged complex is very important in the extraction of metals and other inorganic species that makes it convenient to classify such extractions according to the nature of the complexes.

13.9 Distribution of extractable species

Although the ratio of solubilities of a solute in each of two solvents may not be critically equated to the distribution coefficient of the solute between the two solvents, the underlying factors affecting relative solubility and distribution are undoubtedly similar. It is, therefore, useful to discuss solubility characteristics of various types of substances and to note structural effects in both solvent and solute on the solubility. In solutions where specific chemical forces are not active, the classical principle of “like dissolves like” is of great help in predicting solubility. This principle may be expressed in modern terms as Hildebrand’s theory of regular solutions from which, the solubility is seen to increase as values of the solubility parameter “δ” of solute and solvent approach each other.

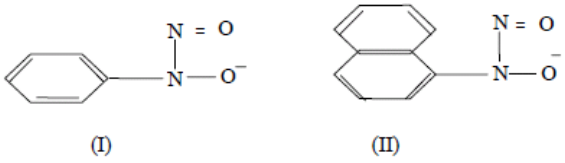
The solubility parameter, defined as the square root of the heat of vaporization per milliliter, is a measure of cohesive energy density. Comparison of solubility parameters should be of maximum assistance of dealing with those organic extraction systems in which specific chemical or associative forces are inoperative. Burrell has successfully used solubility parameters to rationalize the solubility behavior of various polymers.

In systems, where hydrogen bonding may be present, particularly those involving an aqueous phase, the solubility parameter is inadequate in predicting solubility. This might be expected in as much as this concept is, strictly speaking, applicable only in regular solutions. Collander has been able to observe regularities in distribution characteristics in systems involving hydrogen bonding. On the basis of the determination of K_d values for two hundred organic compounds in the ethyl ether-water system, he noted that low K_d values were obtained for compounds having groups capable of hydrogen bonding, such as alcohol, amines, carboxylic acids, and acid amides. Increasing the molecular weight of the organic portion of the molecule would increase the K_d value about two to four times for each additional methylene group in the homologous series. The effect to the oxygen in the molecule seemed to be about the same for alcohols, ketones, aldehydes and carboxylic acids. Increase in Kd resulting in replacing alcoholic or carboxylic hydrogen with methyl group seemed to be little more than would be expected upon the increase in molecular weight. Increase in K_d were observed with the introduction of a halogen atom.

Pasquinelli has been able to correlate the mutual solubilities of a pair of liquids with the electric and magnetic properties of the pure components. The relation, that has been used to predict solubilities with probable absolute error of about ± 3% for 100 pairs of liquids, involves the dipole moments, dielectric constant, specific magnetic susceptibility and molar volume. In as much as the prediction may be made for systems involving hydrogen bonding, the relation may be more generally applicable than the comparison of solubility parameters.

Solubility of metal salts in aqueous media can be explained on the basis of two special properties of water. First, its high dielectric constant permits dissociation of ionic species relatively easily. Even more important, the high basic character of water results in the solvation of cations (and anions), which gives these ions a solvent sheath serving to reduce electrostatic interaction and to make the ions more “solvent-like”. The role of the complex forming extraction agent is largely to replace the coordinated water from around the metal ion to give a species that is more likely to be soluble in organic solvents. The solubility characteristics of metal chelates in organic solvents

in general terms are not at all unlike those of conventional organic compounds. For example, hydrocarbon substituents will increase the solubility of chelates in organic solvents. Although the neodymium chelate of cupferron-(I) is not soluble in chloroform where as the corresponding neocupferron (II) is soluble in chloroform.



(I) (II)

Polar substituents will of course reduce solubility in organic solvents. The chelates of 8-quinolinol-5-sulphonic acid are not at all soluble in organic solvents but are quite soluble in water.

Among the ion association complexes, the oxonium type is noteworthy, since in most cases the solvent participates directly in complex formation. The ability of the oxonium solvent to replace water from the coordination sphere of the metal would depend upon the basicity of solvent, which in turn would reflect the electron density and steric availability of the electron pair in the oxygen of the solvent molecule. Many ion association extractions are aided by the use of salting-out agents, electrolytes used in high concentrations to

(a) Produce a mass action effect by adding a common ion,

(b) Reduce water activity greatly,

(c) Lower the dielectric constant so as to favor ion-pair formation.

The use of salting-out agents in organic extractions is also well known.

13.10 Chemical interactions in organic phase

Chemical interactions of the extracted species in the organic phase would naturally lower its concentration in this phase and hence, improve extractability. If, in the case of a carboxylic acid extraction, the organic solvents is one in which the acid dimerizes, this would result in a higher D value than if the reaction does not occur. Ion association complexes, being dipoles, tend to form higher aggregates in organic solvents at higher concentrations. Where there is a polymerization reaction of any type, the value of D will be found to vary with the concentration of the extracted material

Types of inorganic extractable complexes

Most salts are strong electrolytes whose solubility in water can be attributed to the high dielectric constant of water which greatly reduced the work of dissociation and solvating tendency of water since hydrated ions experience less inter ionic attraction and resemble more closely the medium in which they are dispersed. In fact, for a metal to form an extractable complex it is necessary to remove some or all of the water molecules associated with the metal ion. Complexing of metal ions leading to the formation of uncharged species falls into two main categories, one involving coordination and the other ion association.

13.11 Coordination complexes

A coordination complex, as the term implies, is formed by coordinate bonds in which a previously unshared pair of electrons on donor atom or ion is now shared with an acceptor atom or ion. Three types of coordination complexes are of interest here

C) Simple or monodentate complexes

In simple or monodentate complexes, central metal ion acting as acceptor having a coordination number "n", accepts 'n' pairs of electrons from 'n' individual donor groups,

e.g. $Ge^{4+} + 4:Cl \rightarrow GeCl_4$

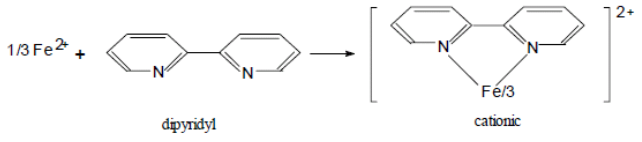
$Fe^{3+} + 4:Cl \rightarrow FeCl_4^-$

$Cu^{2+} + 4:NH_3 \rightarrow Cu(NH_3)_4^{2+}$

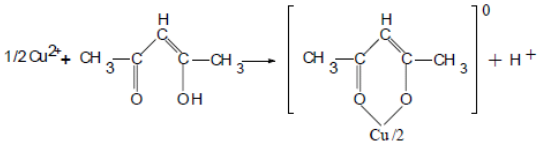
From the above examples, only the first one gives the neutral, extractable complex.

D) Chelate or polydentate complexes

Chelate or polydentate complexes with the central metal atom or ion having coordination number n, combines with no more than n/2 molecules of a specie having at least two donor atoms per molecule; these being so located as to permit the formation of a relatively strain-free (i.e. , 5-6 membered) ring, e.g.



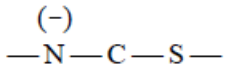
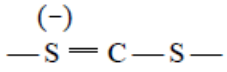
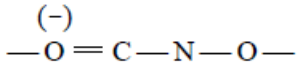
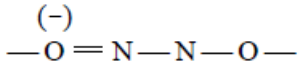
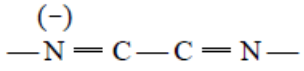
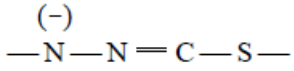
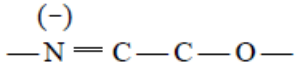
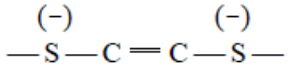
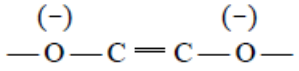
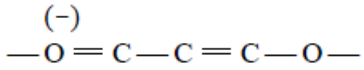
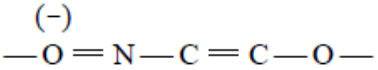
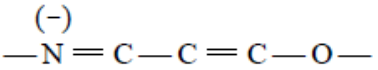
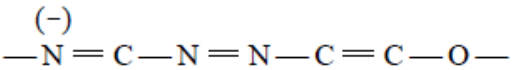
dipyridyl cationic



acetylacetone uncharged

Chelates have relatively large stability constants, so their formation greatly lowers the concentration of hydrated metal ion. Those chelating agents such as acetylacetone, cupferron, dithizone, and 8-quinolinol form uncharged, essentially covalent compounds, which are readily soluble in organic solvents. Chelating agents such as dipyridyl or ethylene diamine tetra acid (EDTA) which form charged chelates are useful as metal masking agents.

Metal Extraction Systems

Chelate Systems	
A. 4-*Membered ring systems*	***Reactive Grouping***
1. Dialkyl dithiocarbamates	
2. Xanthates	
B. *5-Membered ring systems*	
1. Benzoylphenylhydroxylamine	
2. Cupferron	
3. *a*-Dioximes	
4. Dithizone	
5. 8-Quinolinols	
6. Toluene-3:4-dithiol	
7. Catechol	
C. 6-*Membered ring systems*	
1. β-Diketones and Hydroxycarbonyls a) Acetylacetone b) Thenoyltrifluoracetone c) Morin d) Quinalizarine	
2. Nitrosonaphthols	
3. Salicylaldoxime	
1. Pyridyl-azo-naphthol (PAN)	

13.12 Ion association complexes

Ion association complexes are uncharged species formed by the association of ions because of purely electrostatic attraction. The extent of such association increases sharply as the dielectric constant of the solvent decreases below 40 to 50. This condition not only exists in all of the commonly used organic solvents but also in highly concentrated aqueous solutions of strong electrolytes. Ion-pairs, which preferentially dissolve in the organic phase, are those, which resemble the solvent. Ion association complexes are capable of forming clusters larger than just pairs with increasing concentration, particularly in organic solvents. In some cases, aggregates large enough to be described as micelles are encountered. Two categories of ion association complexes may be recognized. The first includes those ion-pair formed from a reagent having large organic ion such as tetraphenylarsonium ion, tribenzylammonium ion or perfluorobutyrate ion. These reagents combine with a suitable metal-containing ion to give a large organic solvent-like ion-pair. The second type of ion-pair is essentially like that of the first with the exception that solvent molecules are directly involved in its formation. Thus in the extraction of uranyl nitarate with isobutyl alcohol, the extractable complex is probably $UO_2(BuOH)_6.(NO_3)_2$ in which the coordinated solvent molecules contribute both to the size of cation and the resemblance of the complex to the solvent. Most of the solvents which participate directly in the formation of ion association complexes are containing oxygen. The term oxonium complex is used here to describe such a complex, since the solvent molecules from coordinate linkages to the metal atoms through their oxygen atoms.

Ion Association Systems

A. Metals contained in cationic number of ion-pair

1. Alkylphosphoric Acids
2. Carboxylic Acids
3. Cationic chelates
   a. Phenanthrolines
   b. Polypyridyls
4. Nitrate
5. Trialkylphosphine oxide

B. Metal contained in anionic number of ion-pair

1. Halides ($GaCl_4^-$)
2. Thiocynate [$Co(CNS)_4^{2-}$]
3. Oxyanions (MnO_4^-)
4. Anionic Chelates [Co (Nitroso R salt)$_3^3$

Chelate extraction systems include only those involving neutral chelates, since charged chelates must pair with oppositely charged ions to form extractable species. It will be noted that the chelate systems are ordered with respect to the size of the chelate ring.

Differentiation of ion association extraction systems is based on the sign of the charge of the metal-containing ion. In those systems in which the metal is part of theanion, a further classification on the basis of the nature of the cation is helpful. These cations are usually varieties of “onium” ions such as oxonium, ammonium, arsonium, etc.,

13.13 Extraction of metal chelates

The equation describing the extraction of metal chelates may be derived by considering the reactions occurring when an aqueous phase containing a metal ion is contacted with an organic phase containing a chelate extractant. The steps leading to theextraction may be conveniently visualized as follows. The chelating agent distributes between the two phases. Since the majority of chelating extractants exhibit an acid dissociation, the symbol HR will serve as a general formula for the reagent.

$$HR_{aq} \leftrightarrow HR_{org}$$

$$K_{D_R} = \frac{[HR]_{Org}}{[HR]_{aq}}$$

The regent will dissociate in the aqueous phase

$$HR \leftrightarrow H^+ + R^-$$

$$K_a = \frac{[H^+][R^-]}{[HR]}$$

To give a chelating anion R^-, which reacts with the metal ion and forms the extractable chelate

$$M^{n+} + nR^- \leftrightarrow MR_n$$

$$K_f = \frac{[MR_n]}{[M^{n+}][R^-]^n}$$

Which, in turn distributes between the phases

$$MR_{n(aq)} \leftrightarrow MR_{n(org)}$$

$$K_{D_X} = \frac{[MR_n]_{Org}}{[MR_n]_{aq}}$$

The distribution ratio "D" can be evaluated from these equilibrium expressions if the metal chelate MR_n, may be assumed to be the only metal-containing species in the organic phase and the metal ion, M^+, essentially the only metal-containing species in the aqueous phase. Thus

$$D \equiv \frac{[M]_{Org}}{[M]_{Aq}} = \frac{[MR_n]_{Org}}{[M^{n+}]_{Aq}} = \frac{K_f K_a{}^n K_{D_X}}{K_{D_R}{}^n} \frac{[HR]_{Org}{}^n}{[H^+]^n_{Aq}}$$

The validity of this equation was first verified by Kolthoff and Sandell for dithizone extractions and later by Furman et al. for cupferron extraction, extends to many chelate extraction systems.

13.14 Selectivity in chelate extractions

The separation of two metals with a particular reagent-solvent system may be evaluated with above equation. The separation factor "γ" defined as the ratio of D values of the metals in question, is seen to be

$$\gamma = \frac{D_1}{D_2} = \frac{K_{f_1} . K_{D_{X_1}}}{K_{f_2} . K_{D_{X_2}}}$$

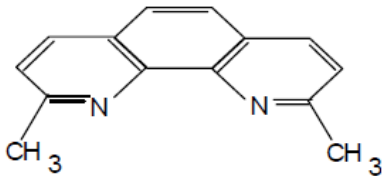
The ease of separation of two metals is seen to be depending not only on the difference in the stability of their chelates but also on the relative solubility of these chelates in the organic solvent. A sufficiently great difference in solubility may result in an extraction sequence that differs from the stability sequence. For instance, although nickel-II and cobalt-II form more stable acetylacetonates than does zinc II, the latter is extractable whereas the former two are not.

With regard to chelate stability, the order of stability of a number of metal ions has been shown to be fairly independent of the nature of the chelating reagent employed. Mellor and Maley list the following stability sequence for bivalent metal ions:

Pd > Cu > Ni > Pb > Co > Zn > Cd > Fe > Mn > Mg

Despite the adherence of the behavior of many reagents to the "natural stability sequence for metals" a number of interesting exceptions are noteworthy. One such example involves the exceptionally high stability of the tris-phenanthroline-iron-II complex. The fact that, this complex is more stable than of the corresponding nickel or cobalt chelats has been attributed to resonance stabilization. Steric hindrance in a chelating agent can result increased selectivity. For example, 2,9-dimethylphenanthroline (neocuproine) no longer gives the typical phenanthroline

like complex with iron-II since the methyl groups greatly hinder the attachment of three reagent molecules around the iron-II ion.



This hindrance is minimum in the tetrahedral geomety of two reagent molecules about a univalent tetracoordinated ion such as copper. Steric hindrance of the 2-methyl groups to chelate formation is the reason for the non-reactivity of 2-methyl-8-quinolinol with the small aluminium ion. This reagent offers a distinct advantage over 8-quinolinol in the determination of many metals in the presence of aluminium. It is also possiblethat reagents containing the mercapto functionality may exhibit a different metal stability sequence than do those containing oxygen.

A more generally applicable approach to increasing selectivity in chelate extractions than that of depending on “unusual” reagents may be based on the use of competing complexing agents, called masking agents. These masking agents, illustrated by cyanide ion or EDTA, form water-soluble complexes with some metals and thus alter the extraction characteristics of these metals. The use of two competing reagents will tend, in favorable cases, to exaggerate even small differences in the stability order to the point where dramatic changes may be observed. For example, copper-II gives more stable chelates with both 8-quinolinol and EDTA than does uranyl ion and hence in the presence of EDTA only uranyl ion may be extracted with 8-quimolinol.

13.5 Batch extraction

The simplest extraction procedure possible and the technique most employed in the laboratory for analytical separations involves the bringing of a given volume of solution into contact with a given volume of solvent until equilibrium has been attained, followed by separation of the liquid layers. If necessary, the procedure may be repeated after the addition of fresh solvent. This batch extraction process provides rapid, simple, and clean separations, and is more beneficial when the distribution ratio of the solute of interest is larg. In such instances, a few extractions will effect quantitative separation.

The most commonly employed apparatus for performing a batch extraction is a separatory funnel, since it is a relatively simple matter to add and withdraw the respective liquid phases. When extracting from a heavier liquid to a lighter solvent, it is necessary to remove the lower

phase from the funnel after each extraction before removing the extracting solvent as in the case of ethyl ether extractions from aqueous solutions.

When performing a batch extraction, it is important to follow a few simple steps to separate the phases for sampling for subsequent processing or estimation. Most batch extractors are separatory funnels taper off into a narrow bottom with a sealed stopcock. Thus, it is a relatively easy task to separate the two phases on withdrawal for further processing. It is, of course, essential to wait until the phases have completely settled after agitation. Usually this will occur in a matter of minutes.

If only aliquots of the phases are to be used, it is necessary to notice any volume changes of the phases due to mutual solubility of the solvents. The extraction and sampling must be performed at a constant temperature, since both the distribution ratio and the volumes of the solvents are influenced by temperature changes. A useful methodof withdrawing the phases for sampling involves the use of three graduates. Most of the heavier phase is withdrawn into the first graduate and then the remainder of the heavier phase and a little of the lighter phase are withdrawn into the second graduate. The remaining portion of the lighter phase is run into the third graduate, and the volumes of the three are noted. The second graduate can now be discarded and aliquots of the other two taken without danger of contamination of one by the other.

If droplets of aqueous phase are entrained in the organic extract, it is possible to remove them by filtering the extract through a dry filter paper. The aqueous droplets will be absorbed by the paper, which should be washed several times with fresh organic solvent. Another method commonly used in extractions is the addition of a drying agent, such as sodium sulphate, to the organic extract.

13.16 Summary of the unit

Solvent extraction is a process whereby two immiscible liquids are vigorously shaken in an attempt to disperse one in the other so that solutes can migrate from one solvent to the other. The picture on the right shows the shape of a traditional separatory funnel. When the two liquids are not shaken the solvent to solvent interface area is limited to the geometric area of the circle separating the two solvents. However as the two liquids are vigorously shaken the solvents become intimately dispersed in each other. Solvent extraction involves many different basic chemical principles. Solvent extraction is one of the most commonly used laboratory purification methods, particularly in organic chemistry labs. Solvent extractions done in chemistry labs are

usually small-scale, batch-mode operations using a separatory funnel. Solvent extraction is also widely used in industrial operations. Some industrial applications use batch-mode extraction, albeit usually on a much larger scale than laboratory solvent extractions. Other industrial applications use continuous-mode solvent extractions, often on a gigantic scale, where the two solvents are continuously added to and removed from a large reaction vessel.

In some solvent extractions, the desired product migrates from the original liquid phase to the second liquid phase. In other solvent extractions, impurities migrate from the original liquid phase to the second liquid phase, leaving the product in the original liquid phase. The phase that contains the desired product is called the product layer. The phase that contains impurities, excess reactants, and other undesirable compounds is called the waste layer. Many first-year organic chemistry students come to grief by discarding what they think is the waste layer, only to learn later that they actually discarded the product layer.

13.17 Key words

Distribution Ratio (D); Percent Extraction (E); Separation Factor (γ); Distribution Law; Extraction; Aqueous phase; Arganic phase; Coordination complexes; Polydentate complexes; Ion association complexes

13.18 References for further study

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13.19 Questions for self understanding

1) What is distribution ratio (D) in solvent extraction?

2) Write a note on percent Extraction (E)?

3) What is separation Factor (γ)? How it will be calculated?

4) Discuss distribution Law

5) What is extraction? Explain the process of extraction

6) Discuss the chemical interactions in the aqueous phase

7) Explain extraction of coordination complexes

8) Discuss extraction of ion association complexes

9) Explain the extraction of metal chelates

10) Write a note on selectivity in chelate extractions

11) Discuss advantage of multiple extractions over single extraction

Unit-14

Structure

14.0 Objective of unit

After studying this unit you are able to

- Classify different Analytical Separations methods
- Explain the theory of Liquid chromatography
- Explain the theory of column chromatography
- Explain the different factor responsible for column selectivity
- Identify the conditions for good chromatographic resolution
- Identify the Optimizing conditions for chromatographic separations

14.1introduction

Some materials appear homogenous, but are actually a combination of substances. For example, green plants contain a mixture of different pigments. In addition, the black ink in the pens that are used in this experiment is a mixture of different colored materials. In many instances, we can separate these materials by dissolving them in an appropriate liquid and allowing them to move through an absorbent matrix, like paper. Chromatography is a method used by scientists for separating organic and inorganic compounds so that they can be analyzed and studied. By analyzing a compound, a scientist can figure out what makes up that compound. Chromatography is a great physical method for observing mixtures and solvents. The word chromatography means "color writing" which is a way that a chemist can test liquid mixtures. While studying the coloring materials in plant life, a Russian botanist invented chromatography in 1903. His name was M.S. Tswett. Chromatography is such an important technique that two nobel prizes have been awarded to chromatographers. Over 60% of chemical analysis worldwide is currently done with chromatography or a variation thereon.

14.2 Chromatography

Chromatographic separations are accomplished by continuously passing one sample-free phase, called a mobile phase**,** over a second sample-free phase that remains fixed, or stationary. The sample is injected, or placed, into the mobile phase. As it moves with the mobile phase, the sample's components partition themselves between the mobile and stationary phases. Those components whose distribution ratio favors the stationary phase require a longer time to pass through the system. Given sufficient time, and sufficient stationary and mobile phase, solutes with similar distribution ratios can be separated. Chromatography can also be defined as a

method of analysis in which a flowing liquid (mobile phase) brings about the separations of substances by differential migration from a narrow initial zone in an adsorptive medium.

The history of modern chromatography can be traced to the turn of the century when the Russian botanist Mikhail Tswett (1872–1919) used a column packed with a stationary phase of calcium carbonate to separate colored pigments from plant extracts. The sample was placed at the top of the column and carried through the stationary phase using a mobile phase of petroleum ether. As the sample moved through the column, the pigments in the plant extract separated into individual colored bands. Once the pigments were adequately separated, the calcium carbonate was removed from the column, sectioned, and the pigments recovered by extraction. Tswett named the technique chromatography, combining the Greek words for "color" and "to write." There was little interest in Tswett's technique until 1931 when chromatography was reintroduced as an analytical technique for biochemical separations. Pioneering work by Martin and Synge in 1941 established the importance of liquid–liquid partition chromatography and led to the development of a theory for chromatographic separations; they were awarded the 1952 Nobel Prize in chemistry for this work. Since then, chromatography in its many forms has become the most important and widely used separation technique. Other separation methods, such as electrophoresis, effect a separation without the use of a stationary phase

14.3 Classifying Analytical Separations

Analytical separations may be classified in three ways: by the physical state of the mobile phase and stationary phase; by the method of contact between the mobile phase and stationary phase; or by the chemical or physical mechanism responsible for separating the sample's constituents. The mobile phase is usually a liquid or a gas, and the stationary phase, when present, is a solid or a liquid film coated on a solid surface. Chromatographic techniques are often named by listing the type of mobile phase, followed by the type of stationary phase. Thus, in gas–liquid chromatography the mobile phase is a gas and the stationary phase is a liquid. If only one phase is indicated, as in gas chromatography, it is assumed to be the mobile phase. Two common approaches are used to bring the mobile phase and stationary phase into contact.

In **column chromatography,** the stationary phase is placed in a narrow column through which the mobile phase moves under the influence of gravity or pressure. The stationary phase is either a solid or a thin, liquid film coating on a solid particulate packing material or the column's walls. In **planarchromatography** the stationary phase coats a flat glass, metal, or plastic plate and is

placed in a developing chamber. A reservoir containing the mobile phase is placed in contact with the stationary phase, and the mobile phase moves by capillary action. The mechanism by which solutes separate provides a third means for characterizing a separation (Figure 12.3). In adsorption chromatography, solutes separate based on their ability to absorb to a solid stationary phase. In partition chromatography, a thin liquid film coating a solid support serves as the stationary phase. Separation is based on a difference in the equilibrium partitioning of solutes between the liquid stationary phase and the mobile phase. Stationary phases consisting of a solid support with covalently attached anionic (e.g., $–SO_3^{–)}$ or cationic (e.g., $–N(CH_3)_3+$) functional groups are used in ion-exchange chromatography. Ionic solutes are attracted to the stationary phase by electrostatic forces. Porous gels are used as stationary phases in size-exclusion chromatography, in which separation is due to differences in the size of the solutes. Large solutes are unable to penetrate into the porous stationary phase and so quickly pass through the column. Smaller solutes enter into the porous stationary phase, increasing the time spent on the column. Not all separation methods require a stationary phase. In an electrophoretic separation, for example, charged solutes migrate under the influence of an applied potential field. Differences in the mobility of the ions account for their separation.

Mobile Phase: *In chromatography, the extracting phase that moves through the system.*

Stationary Phase: *In chromatography, the extracting phase that remains in a fixed position.*

Chromatography: *A separation in which solutes partition between a mobile and stationary phase.*

Column Chromatography: *A form of chromatography in which the stationary phase is retained in a column.*

Planar Chromatography: *A form of chromatography in which the stationary phase is immobilized on a flat surface.*

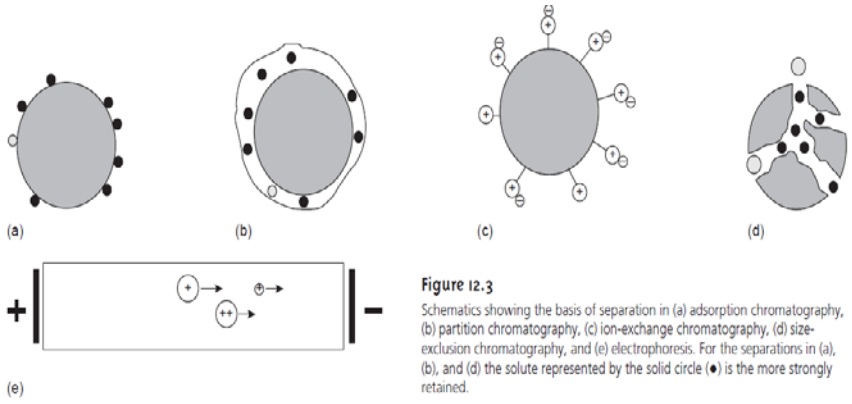

Figure 12.3
Schematics showing the basis of separation in (a) adsorption chromatography, (b) partition chromatography, (c) ion-exchange chromatography, (d) size-exclusion chromatography, and (e) electrophoresis. For the separations in (a), (b), and (d) the solute represented by the solid circle (●) is the more strongly retained.

Different forms of chromatography are named according to the physical state of mobile and stationary phase employed. Generally, Liquid chromatography and Gas chromatography are the two major sub divisions of chromatography based on the mobile phase used. Further classification according to stationary phase employed results in four divisions namely,

14.4 Liquid – Liquid chromatography

Ex; column partition chromatography, paper partition chromatography

Liquid – Solid chromatography

Ex; Thin layer chromatography, HPLC

Gas – Liquid chromatography

Gas –Solid chromatography

14.5 General Theory of Column Chromatography

Of the two methods for bringing the stationary and mobile phases into contact, the more important is column chromatography. In this section we develop a general theory that we may apply to any form of column chromatography. With appropriate modifications, this theory also can be applied to planar chromatography. A typical column chromatography experiment is outlined in Figure 12.4. Although the figure depicts a liquid–solid chromatographic experiment similar to that first used by Tswett, the design of the column and the physical state of the

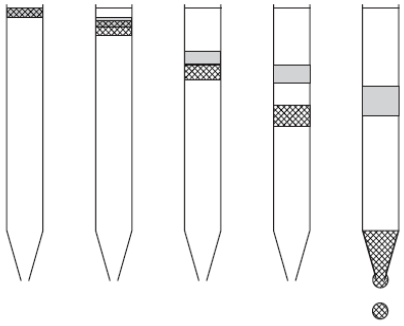

Figure 12.4: Progress of a column chromatographic separation showing the separation of two solute bands

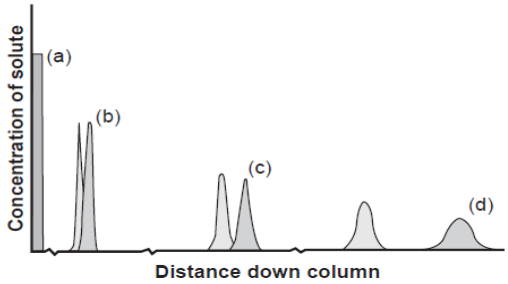

Figure 12.5

Figure 12.5 is an another view of the progress of a column chromatographic separation showing the separation of two solute bands stationary and mobile phases may vary. The sample is introduced at the top of the column as a narrow band. Ideally, the solute's initial concentration profile is rectangular (Figure 12.5a). As the sample moves down the column the solutes begin to separate, and the individual solute bands begin to broaden and develop a Gaussian profile (Figures 12.5b, c). If the strength of each solute's interaction with the stationary phase is sufficiently different, then the solutes separate into individual bands (Figure 12.5d). The progress of a chromatographic separation is monitored with a suitable detector situated at the end of the column. A plot of the detector's signal as a function of time or volume of eluted mobile phase is known as a **chromatogram** (Figure 12.6) and consists of a peak for each of the separated solute bands.

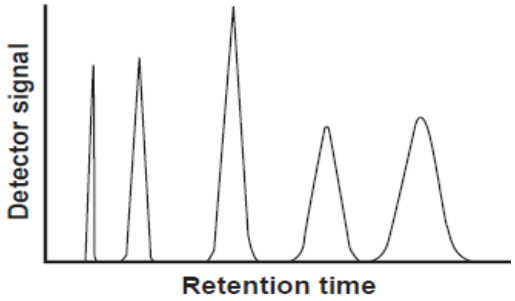

Figure 12.6 Typical chromatogram of detector response as a function of retention time

A chromatographic peak may be characterized in many ways, two of which are shown in Figure 12.7. The **retention time,** *t*r, is the elapsed time from the introduction of the solute to the peak maximum. The retention time also can be measured indirectly as the volume of mobile phase eluting between the solute's introduction and the appearance of the solute's peak maximum. This is known as the **retention volume,** *V*r. Dividing the retention volume by the mobile phase's flow rate, *u*, gives the retention time. The second important parameter is the chromatographic peak's width at the baseline, *w*. As shown in Figure 12.7, **baseline width** is determined by the intersection with the baseline of tangent lines drawn through the inflection points on either side of the chromatographic peak. Baseline width is measured in units of

time or volume, depending on whether the retention time or retention volume is of interest.

Besides the solute peak, Figure 12.7 also shows a small peak eluted soon after the sample is injected into the mobile phase. This peak results from solutes that move through the column at the same rate as the mobile phase. Since these solutes do not interact with the stationary phase,

they are considered nonretained. The time or volume of mobile phase required to elute nonretained components is called the column's **void time,** *t*m, or **void volume.**

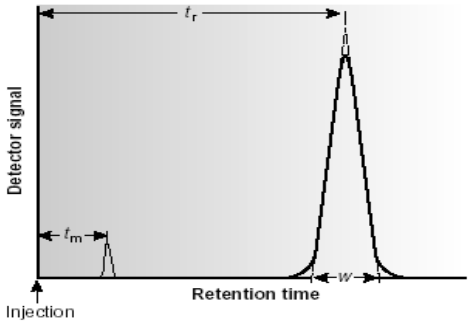

Figure 12.7: Measurement of the column's void time, *t*m, and the retention time, *t*r, and baseline width, *w*, for a solute.

14.6 Chromatographic Resolution

The goal of chromatography is to separate a sample into a series of chromatographic peaks, each representing a single component of the sample. **Resolution** is a quantitative measure of the degree of separation between two chromatographic peaks, A and B, and is defined as **12.1**

$$R = \frac{t_{r\,B} - t_{r\,A}}{\left(W_b + W_A\right)/2} = \frac{2\Delta t_r}{W_B + W_A}$$

As shown in Figure 12.8, the degree of separation between two chromatographic peaks improves with an increase in *R.* For two peaks of equal size, a resolution of 1.5 corresponds to an overlap in area of only 0.13%. Because resolution is a quantitative measure of a separation's success, it provides a useful way to determine if a change in experimental conditions leads to a better separation

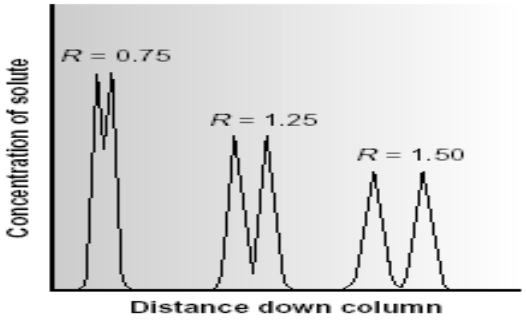

Figure 12.8
Three examples of chromatographic resolution.

Retention Time: *The time a solute takes to move from the point of injection to the detector (t_r).*

Retention Volume: *The volume of mobile phase needed to move a solute from its point of injection to the detector (V_r).*

Baseline Width: *The width of a solute's chromatographic band measured at the baseline (w).*

Void Volume: The volume of mobile phase needed to move an unretained solute from the point of injection to the detector.

Resolution: The separation between two chromatographic bands (*R*).

In a chromatographic analysis of lemon oil a peak for limonene has a retention time of 8.36 min with a baseline width of 0.96 min. g-Terpinene elutes at 9.54 min, with a baseline width of 0.64 min. What is the resolution between the two peaks?

SOLUTION

Using equation 12.1, we find that the resolution is,

$$R = \frac{2\Delta t_r}{W_B + W_A} = \frac{2(9.54 - 8.36)}{0.64 + 0.96} = 1.48$$

From equation 12.1 it is clear that resolution may be improved either by increasing

D*t*r or by decreasing *w*A or *w*B (Figure 12.9). We can increase D*t*r by enhancing the interaction of the solutes with the column or by increasing the column's selectivity for one of the solutes. Peak width is a kinetic effect associated with the solute's movement within and between the mobile phase and stationary phase. The effect is governed by several factors that are collectively called column efficiency. Each of these factors is considered in more detail in the following sections.

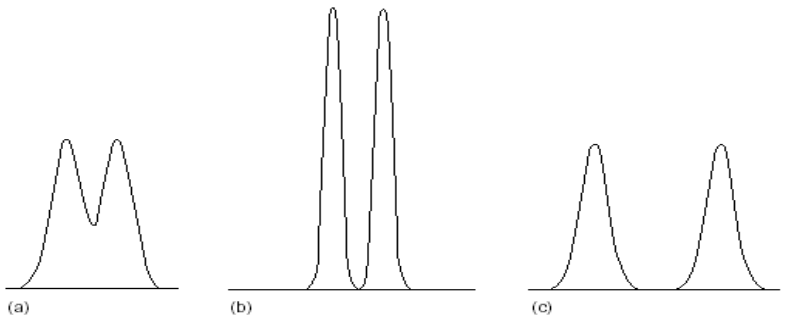

Figure 12.9. Two methods for improving chromatographic resolution: (a) Original separation showing a pair of poorly resolved solutes; (b) Improvement in resolution due to an increase in column efficiency; (c) Improvement in resolution due to a change in column selectivity.

14.7 Capacity Factor

The distribution of a solute, S, between the mobile phase and stationary phase can be represented by an equilibrium reaction $S_m \leftrightarrow Ss$ and its associated partition coefficient, *K*D, and distribution ratio, *D*,

$$KD = \frac{[S_S]}{[S_M]}$$

$$D = \frac{[S_S]_{tot}}{[S_M]_{tot}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \textbf{12.2}$$

Where the subscripts m and s refer to the mobile phase and stationary phase, respectively.

As long as the solute is not involved in any additional equilibria in either the mobile phase or stationary phase, the equilibrium partition coefficient and the distribution ratio will be the same.

Conservation of mass requires that the total moles of solute remain constant throughout the separation, thus

(Moles S) tot = (moles S) m + (moles S) s……. **12.3**

Solving equation 12.3 for the moles of solute in the stationary phase and substituting into equation 12.2 gives

$$D = \frac{\{(molesS)_{tot} - (molesS)_m\} / V_S}{\frac{(molesS)_m}{V_m}} = \frac{(molesS)_{tot} V_m - (molesS)_m V_m}{(molesS)_m V_s}$$

where *V*m and *V*s are the volumes of the mobile and stationary phases. Rearranging and solving for the fraction of solute in the mobile phase, *f*m, gives

$$f_m = \frac{(molesS)_m}{(molesS)_{tot}} = \frac{V_m}{V_m + DV_s} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \textbf{12.4}$$

Note that this equation is identical to that describing the extraction of a solute in a liquid–liquid extraction (equation 7.25 in Chapter 7). Since the volumes of the stationary and mobile phase may not be known, equation 12.4 is simplified by dividing both the numerator and denominator by *V*m; thus

$$fm = \frac{1}{1 + D(V_s + V_m)} = \frac{1}{1 + K^1} \text{.....12.5}$$

$$K^1 = D\frac{V_s}{V_m} \text{.....12.6}$$

is the solute's **capacity factor.**

A solute's capacity factor can be determined from a chromatogram by measuring the column's void time, *t*m, and the solute's retention time, *t*r (see Figure 12.7). The mobile phase's average linear velocity, *u,* is equal to the length of the column, *L,* divided by the time required to elute a nonretained solute.

A measure of how strongly a solute is retained by the stationary phase (k^1).

$$u = \frac{L}{t_m} \text{.....12.7}$$

By the same reasoning, the solute's average linear velocity, v, is

$$v = \frac{L}{t_r} \text{.....12.8}$$

The solute can only move through the column when it is in the mobile phase. Its average linear velocity, therefore, is simply the product of the mobile phase's average linear velocity and the fraction of solute present in the mobile phase.

$$v = uf_m \text{.....12.9}$$

Substituting equations 12.5, 12.7, and 12.8 into equation 12.9 gives

$$\frac{L}{t_r} = \frac{L}{t_m}\left(\frac{1}{1 + K^1}\right)$$

Finally, solving this equation for k¢ gives

$$K^1 = \frac{t_r - t_m}{t_m} = \frac{t_r^1}{t_m} \text{.....12.10}$$

where t_r^1 is known as the adjusted retention time.

adjusted retention time; The difference between a solute's retention time and column's void time t_r^1

In a chromatographic analysis of low-molecular-weight acids, butyric acid elutes with a retention time of 7.63 min. The column's void time is 0.31 min. Calculate the capacity factor for butyric acid.

SOLUTION

$$K^1 = \frac{t_r - t_m}{t_m} = \frac{7.63\text{min} - 0.31\text{min}}{0.31\text{min}} = 23.6\,.$$

14.8 Column Selectivity

The relative selectivity of a chromatographic column for a pair of solutes is given by the **selectivity factor,** α which is defined as

$$\alpha = \frac{K_B^1}{K_A^1} = \frac{t_{rB} - t_m}{t_{rA} - t_m} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 12.11$$

The identities of the solutes are defined such that solute A always has the smaller retention time. Accordingly, the selectivity factor is equal to 1 when the solutes elute with identical retention times, and is greater than 1 when *t*r,B is greater than *t*r,A.

Problem: In the same chromatographic analysis for low-molecular-weight acids considered in Example 12.2, the retention time for isobutyric acid is 5.98 min. What is the selectivity factor for isobutyric acid and butyric acid?

Solution:

First we must calculate the capacity factor for isobutyric acid. Using the void time from Example 12.2, this is

$$K^1 = \frac{t_r - t_m}{t_m} = \frac{5.98\text{min} - 0.31\text{min}}{0.31\text{min}} = 18.3$$

The selectivity factor, therefore, is

$$\alpha = \frac{K_{buty}^1}{K_{iso}^1} = \frac{12.36}{18.3} = 1.29$$

14.9 Column Efficiency

At the beginning of a chromatographic separation the solute occupies a narrow band of finite width. As the solute passes through the column, the width of its band continually increases in a process called **band broadening.** Column efficiency provides a quantitative measure of the extent of band broadening. In their original theoretical model of chromatography, Martin and Synge2 treated the chromatographic column as though it consists of discrete sections at which partitioning of the solute between the stationary and mobile phases occur. They called each section a **theoretical plate** and defined column efficiency in terms of the number of theoretical plates, *N*, or the height of a theoretical plate,

H; where

$$N = \frac{L}{H} \quad \ldots\ldots\ldots\ldots\ldots\ldots 12.12$$

A column's efficiency improves with an increase in the number of theoretical plates or a decrease in the height of a theoretical plate. Assuming a Gaussian profile, the extent of band broadening is measured by the variance or standard deviation of a chromatographic peak. The height of a theoretical plate is defined as the variance per unit length of the column

$$H = \frac{\sigma^2}{L} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots 12.13$$

where the variance, σ^2, has units of distance squared. Because retention time and peak width are usually measured in seconds or minutes, it is more convenient to express the standard deviation in units of time, τ, by dividing σ by the mobile phase's average linear velocity.

$$\tau = \frac{\sigma}{u} = \frac{\sigma t_r}{L} \quad \ldots\ldots\ldots\ldots\ldots\ldots 12.14$$

When a chromatographic peak has a Gaussian shape, its width at the baseline, *w,* is four times its standard deviation, τ.

$$w = 4\tau \quad \ldots\ldots\ldots\ldots\ldots\ldots 12.15$$

Combining equations 12.13 through 12.15 gives the height of a theoretical plate in terms of the easily measured chromatographic parameters *t*r and *w*.

$$H = \frac{Lw^2}{16t_r^2} \quad \ldots\ldots\ldots\ldots\ldots\ldots 12.16$$

The number of theoretical plates in a chromatographic column is obtained by combining equations 12.12 and 12.16.

$$N = 16\left(\frac{t_r}{w}\right)^2 \text{ ……..12.17}$$

Alternatively, the number of theoretical plates can be approximated as

$N = 5.545\left(\frac{t_r}{w_{1/2}}\right)^2$ where $w1/2$ is the width of the chromatographic peak at half its height.

Problem: A chromatographic analysis for the chlorinated pesticide Dieldrin gives a peak with a retention time of 8.68 min and a baseline width of 0.29 min. How many theoretical plates are involved in this separation? Given that the column used in this analysis is 2.0 meters long, what is the height of a theoretical plate?

solution

Using equation 12.17, the number of theoretical plates is,

$$N = 16\left(\frac{t_r}{w}\right)^2 = 16\left(\frac{8.68\,\text{min}}{0.29}\right)^2 = 14{,}300\,plates$$

Solving equation 12.12 for H gives the average height of a theoretical plate as

$$H = \frac{L}{N} = \frac{(2.0m)(1000mm/m)}{14{,}300\,plates} = o.14mm/\,plate$$

It is important to remember that a theoretical plate is an artificial construct and that no such plates exist in a chromatographic column. In fact, the number of theoretical plates depends on both the properties of the column and the solute. As a result, the number of theoretical plates for a column is not fixed and may vary from solute to solute.

14.10 Peak Capacity

Another important consideration is the number of solutes that can be baseline resolved on a given column. An estimate of a column's **peak capacity,** n_c is,

peak capacity: *The maximum number of solutes that can be resolved on a particular column (n_c).*

$$n_c = 1 + \frac{\sqrt{N}}{4}\ln\frac{V_{\max}}{V_{\min}} \text{ ……..12.18}$$

where *V*min and *V*max are the smallest and largest volumes of mobile phase in which a solute can be eluted and detected.3 A column with 10,000 theoretical plates, for example, can resolve no more than

$$nc = 1 + \frac{\sqrt{10{,}000}}{4} \ln \frac{30mL}{1mL} = 86 solutes.$$

if the minimum and maximum volumes of mobile phase in which the solutes can elute are 1 mL and 30 mL. This estimate provides an upper bound on the number of solutes that might be separated and may help to exclude from consideration columns that do not have enough theoretical plates to separate a complex mixture. Just because a column's theoretical peak capacity is larger than the number of solutes to be separated, however, does not mean that the separation will be feasible. In most situations the peak capacity obtained is less than the estimated value because the retention characteristics of some solutes are too similar to effect their separation. Nevertheless, columns with more theoretical plates, or a greater range of possible elution volumes, are more likely to separate a complex mixture

14.11 Nonideal Behavior

The treatment of chromatography outlined in Section 12B assumes that a solute elutes as a symmetrical band, such as that shown in Figure 12.7. This ideal behaviour occurs when the solute's partition coefficient, *K*D, is constant for all concentrations of solute. In some situations, chromatographic peaks show nonideal behavior, leading to asymmetrical peaks, similar to those shown in Figure 12.10. The chromatographic peak in Figure 12.10a is an example of "**fronting**" and is most often the result of overloading the column with sample. Figure 12.10b, which is an example of "**tailing,**" occurs when some sites on the stationary phase retain the solutes more strongly than other sites.

Fronting: *A tail at the beginning of a chromatographic peak, usually due to injecting too much sample.*

Tailing: *A tail at the end of a chromatographic peak, usually due to the presence of highly active sites in the stationary phase.*

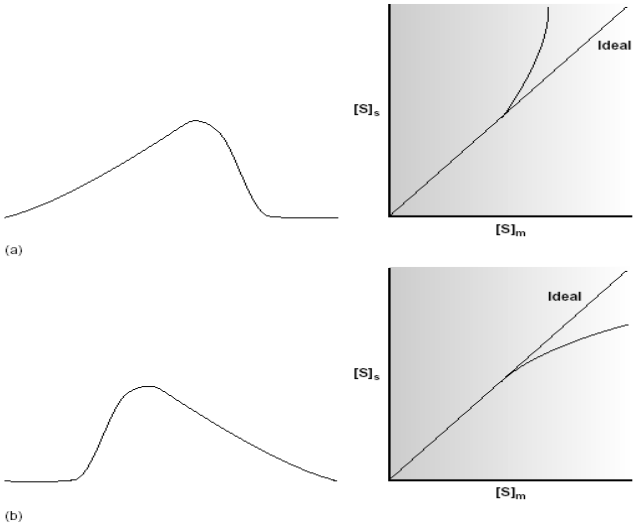

Figure 12.10: Nonideal asymmetrical chromatographic bands showing (a) fronting and (b) tailing. Also depicted are the corresponding sorption isotherms showing the relationship between the concentration of solute in the stationary phase as a function of its concentration in the mobile phase.

14.12 Optimizing Chromatographic Separations

Now that we have defined capacity factor, selectivity, and column efficiency we consider their relationship to chromatographic resolution. Since we are only interested in the resolution between solutes eluting with similar retention times, it is safe to assume that the peak widths for the two solutes are approximately the same. Equation 12.1, therefore, is written as

$$R = \frac{t_{r.B} - t_{r,A}}{w_B} \text{ ……..12.19}$$

Solving equation 12.17 for w_B and substituting into equation 12.19 gives

$$R = \frac{1}{4}\sqrt{N_B}\left(\frac{t_{r,B} - t_{r,A}}{t_{r,B}}\right) \text{ ……..12.20}$$

The retention times for solutes A and B are replaced with their respective capacity factors by rearranging equation 12.10

$$tr = K^1 t_m + t_m$$

and substituting into equation 12.20.

$$R = \frac{1}{4}\sqrt{N_B}\left(\frac{K_B^1 - K_A^1}{1 + K_B^1}\right)$$

Finally, solute A's capacity factor is eliminated using equation 12.11. After rearranging, the equation for the resolution between the chromatographic peaks for solutes A and B is

$$R = \frac{1}{4}\sqrt{N_B}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{K_B^1}{1 + K_B^1}\right) \quad \text{.....12.21}$$

Besides resolution, another important factor in chromatography is the amount of time required to elute a pair of solutes. The time needed to elute solute B is

$$tr, B = \frac{16R^2H}{4}\left(\frac{\alpha}{\alpha - 1}\right)^2 \frac{\left(1 + K_B^1\right)^3}{\left(K_B^1\right)^2} \quad \text{.....12.22}$$

Equations 12.21 and 12.22 contain terms corresponding to column efficiency, column selectivity, and capacity factor. These terms can be varied, more or less independently, to obtain the desired resolution and analysis time for a pair of solutes. The first term, which is a function of the number of theoretical plates or the height of a theoretical plate, accounts for the effect of column efficiency. The second term is a function of a and accounts for the influence of column selectivity. Finally, the third term in both equations is a function of kB^1, and accounts for the effect of solute B's capacity factor. Manipulating these parameters to improve resolution is the subject of the remainder of this section

14.13 Using the Capacity Factor to Optimize Resolution

One of the simplest ways to improve resolution is to adjust the capacity factor for solute B. If all other terms in equation 12.21 remain constant, increasing kB^1 improves resolution. As shown in Figure 12.11, however, the effect is greatest when the original capacity factor is small.

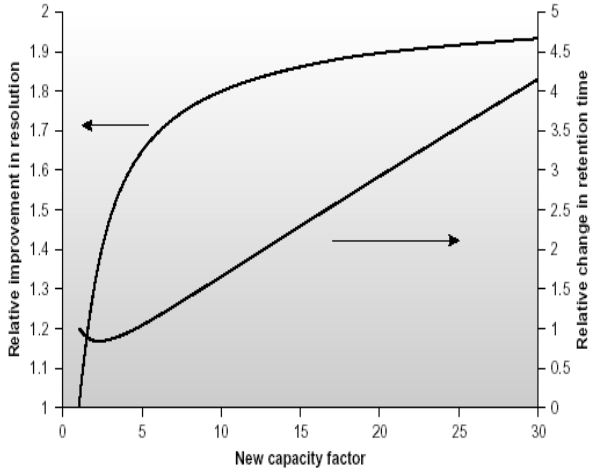

Figure 12.11: Effect of a change in k_B^1 on resolution and retention time. The original value of kB¢ is assumed to be 1.

Furthermore, large increases in kB ^{1} do not lead to proportionally larger increases in resolution. For example, when the original value of kB^1 is 1, increasing its value to 10 gives an 82% improvement in resolution; a further increase to 15 provides a net improvement in resolution of only 87.5%.

Any improvement in resolution obtained by increasing kB^1 generally comes at the expense of a longer analysis time. This is also indicated in Figure 12.11, which shows the relative change in retention time as a function of the new capacity factor. Note that a minimum in the retention time curve occurs when kB^1 is equal to 2, and that retention time increases in either direction. Increasing kB^1 from 2 to 10, for example, approximately doubles solute B's retention time.

The relationship between capacity factor and analysis time can be advantageous when a separation produces an acceptable resolution with a large kB^1. In this case it may be possible to decrease kB^1 with little loss in resolution while significantly shortening the analysis time.

A solute's capacity factor is directly proportional to its distribution ratio (equation 12.6), which, in turn, is proportional to the solute's equilibrium distribution constant. To increase kB^1 without significantly changing α, which also is a function of kB^1, it is necessary to alter chromatographic conditions in a way that leads to a general, nonselective increase in the capacity factor for both solutes. In gas chromatography, this is usually accomplished by decreasing the column's temperature. At a lower temperature a solute's vapor pressure decreases, ensuring that it spends more time in the stationary phase increasing its capacity factor. In liquid chromatography, changing the mobile phase's solvent strength is the easiest way to change a solute's capacity factor. When the mobile phase has a low solvent strength, solutes spend proportionally more time in the stationary phase, thereby increasing their capacity factors. Additionally, equation 12.6 shows that the capacity factor is proportional to the volume of stationary phase. Increasing the volume of stationary phase, therefore, also leads to an increase in kB^1.

Adjusting the capacity factor to improve resolution between one pair of solutes may lead to an unacceptably long retention time for other solutes. For example, improving resolution for solutes with short retention times by increasing kB^1 may substantially increase the retention times for later eluting solutes. On the other hand, decreasing kB^1 as a means of shortening the overall analysis time may lead to a loss of resolution for solutes eluting with shorter retention times. This difficulty is encountered so frequently that it is known as the general elution

problem (Figure 12.12). One solution to the general elution problem is to make incremental adjustments to the capacity factor over time. Thus, initial chromatographic conditions are adjusted to enhance the resolution for solutes with short retention times. As the separation progresses, chromatographic conditions are changed in a manner that increases the elution rate (decreases the retention time) for later eluting solutes. In gas chromatography this is accomplished by **temperature programming.** The column's initial temperature is selected such that the first solutes to elute are fully resolved. The temperature is then increased, either continuously or in steps, to bring off later eluting components with both an acceptable resolution and a reasonable analysis time. In liquid chromatography the same effect can be obtained by increasing the solvent's eluting strength. This is known as a **gradient elution.**

temperature programming: *The process of changing the column's temperature to enhance the separation of both early and late eluting solutes.*

gradient elution: *The process of changing the mobile phase's solvent strength to enhance the separation of both early and late eluting solutes*.

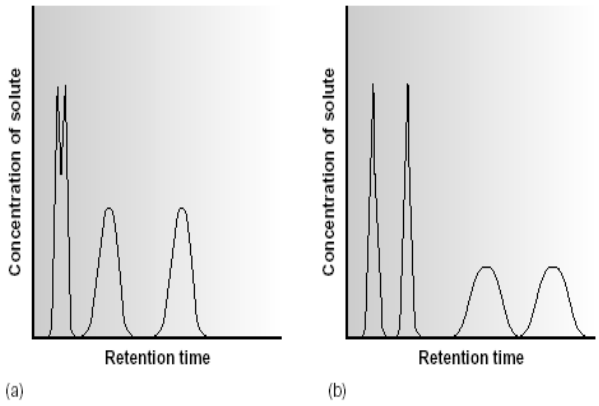

Figure 12.12 The general elution problem in chromatography. Improving the resolution of the overlapping bands in chromatogram (a) results in a longer analysis time for chromatogram (b).

14.14 Using Column Selectivity to Optimize Resolution

A second approach to improving resolution is to adjust alpha, α. In fact, when α is nearly 1, it usually is not possible to improve resolution by adjusting $k\text{B}^1$ or *N*. Changes in α often have a more dramatic effect on resolution than $k\text{B}^1$. For example, changing α from 1.1 to 1.5 improves resolution by 267%.

A change in α is possible if chromatographic conditions are altered in a manner that is more selective for one of the solutes. If a solute participates in a secondary equilibrium reaction in

either the stationary or mobile phase, then it may be possible to alter that phase in a way that selectively changes the solute's capacity factor. For example, Figure 12.13a shows how the pH of an aqueous mobile phase can be used to control the retention times, and thus the capacity factors, for two substituted benzoic acids. The resulting change in α is shown in Figure 12.13b. In gas chromatography, adjustments in α are usually accomplished by changing the stationary phase, whereas changing the composition of the mobile phase is used in liquid chromatography.

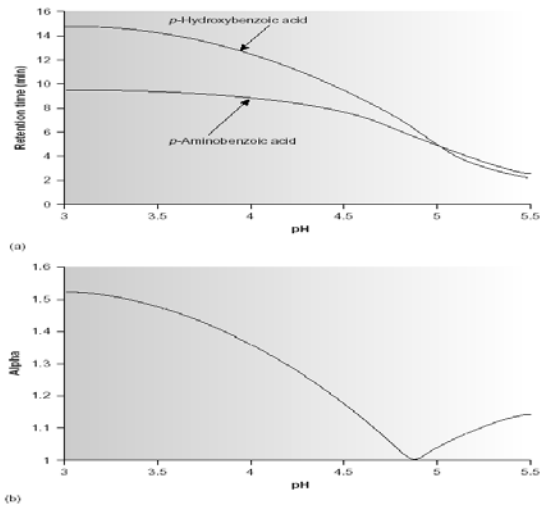

Figure 12.13: Use of column selectivity to improve chromatographic resolution showing: (a) the variation in retention time with mobile phase pH, and (b) the resulting change in alpha with mobile phase pH

14.15 Using Column Efficiency to Optimize Resolution

If the capacity factor and α are known, then equation 12.21 can be used to calculate the number of theoretical plates needed to achieve a desired resolution (Table 12.1). For example, given α= 1.05 and kB^1= 2.0, a resolution of 1.25 requires approximately 24,800 theoretical plates. If the column only provides 12,400 plates, half of what is needed, then the separation is not possible. How can the number of theoretical plates be doubled? The easiest way is to double the length of the column; however, this also requires a doubling of the analysis time. A more desirable approach is to cut the height of a theoretical plate in half, providing the desired resolution without changing the analysis time. Even better, if *H* can be decreased by more than 50%, it also may be possible to achieve the desired resolution with an even shorter analysis time by decreasing kB ^{1}or α.

To determine how the height of a theoretical plate can be decreased, it is necessary

to understand the experimental factors contributing to the broadening of a solute's chromatographic band. Several theoretical treatments of band broadening have been proposed. We will consider one approach in which the height of a theoretical plate is determined by four contributions: multiple paths, longitudinal diffusion, mass transfer in the stationary phase, and mass transfer in the mobile phase.

Table 12.1 Number of Theoretical Plates Needed to Achieve Desired Resolution for Selected Values of k'_B and α

	$R = 1.00$		$R = 1.25$		$R = 1.50$	
k'_B	$\alpha = 1.05$	$\alpha = 1.10$	$\alpha = 1.05$	$\alpha = 1.10$	$\alpha = 1.05$	$\alpha = 1.10$
0.5	63,500	17,400	99,200	27,200	143,000	39,200
1.0	28,200	7,740	44,100	12,100	63,500	17,400
1.5	19,600	5,380	30,600	8,400	44,100	12,100
2.0	15,900	4,360	24,800	6,810	35,700	9,800
3.0	12,500	3,440	19,600	5,380	28,200	7,740
5.0	10,200	2,790	15,900	4,360	22,900	6,270
10.0	8,540	2,340	13,300	3,660	19,200	5,270

Multiple Paths Solute molecules passing through a chromatographic column travel separate paths that may differ in length. Because of these differences in path length, solute molecules injected simultaneously elute at different times. The principal factor contributing to this variation in path length is a nonhomogeneous packing of the stationary phase in the column. Differences in particle size and packing consistency cause solute molecules to travel paths of different length. Some solute molecules follow relatively straight paths through the column, but others follow a longer, more tortuous path (Figure 12.14a). The contribution of multiple paths to the height of a theoretical plate, *H*p, is

$$H_p = 2\lambda d_p \quad \text{.....12.23}$$

where *d*p is the average diameter of the particulate packing material, and l is a constant accounting for the consistency of the packing. A smaller range of particle sizes and a more consistent packing produce a smaller value for l. Note that for an open tubular column, which does not contain packing material, *H*p is 0.

Longitudinal Diffusion The second contribution to band broadening is the result of the solute's ***longitudinal diffusion*** in the mobile phase. Even if the mobile phase velocity is 0, solute molecules are constantly in motion, diffusing through the mobile phase. Since the concentration of solute is greatest at the center of a chromatographic band, more solute diffuses toward the band's forward and rear edges than diffuses toward the band's center. The net result is an

increase in the band's width (Figure 12.14b). The contribution of longitudinal diffusion to the height of a theoretical plate, *H*d, is

$$H_d = \frac{2\gamma D_m}{u} \text{.....12.24}$$

where D_m is the solute's diffusion coefficient in the mobile phase, u is the mobile phase velocity, and g is a constant related to the column packing. The effect of H_d on the height of a theoretical plate is minimized by a high mobile-phase velocity. Because a solute's diffusion coefficient is larger in a gaseous mobile phase than in a liquid mobile phase, longitudinal diffusion is a more serious problem in gas chromatography.

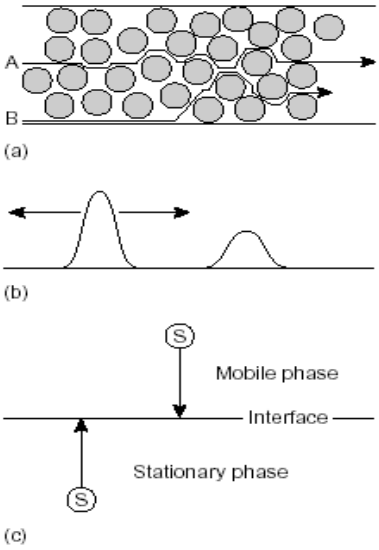

Figure 12.14: Schematics illustrating the contributions to band broadening due to (a) multiple paths, (b) longitudinal diffusion, and (c) mass transfer.

Mass Transfer The final two contributions to band broadening result from the finite time required for a solute molecule to diffuse through the stationary phase and mobile phase. A chromatographic separation occurs because solutes move between the stationary and mobile phases. For a solute to move from one phase to the other, it must first diffuse to the interface between the two phases (Figure 12.14c)—a process called **mass transfer.** A contribution to band broadening occurs whenever the solute's movement to the interface is not fast enough to maintain a true equilibrium distribution of solute between the two phases. Thus, solute molecules in the mobile phase move farther down the column than expected before passing into the stationary phase. Solute molecules in the stationary phase, on the other hand, take longer than

expected to cross into the mobile phase. The contributions of mass transfer in the stationary phase, *H*s, and mass transfer in the mobile phase, H_m, are given by

$$H_s = \frac{qK^1 d_f^2}{\left(1+K^1\right)^2 D_s} u \quad \text{.....12.25}$$

$$H_m = \frac{f_n\left(d_p^2, d_c^2\right)}{D_m} u \quad \text{.....12.26}$$

mass transfer: *One contribution to band broadening due to the time required for a solute to move from the mobile phase or the stationary phase to the interface between the two phases*

where d_f is the thickness of the stationary phase, *d*c is the column's diameter, *D*s is the solute's diffusion coefficient in the stationary phase, *q* is a constant related to the column packing material, and the remaining terms are as previously defined. As indicated in equation 12.26, the exact form of H_m is unknown, although it is a function of particle size and column diameter. The contribution of mass transfer to the height of a theoretical plate is smallest for slow mobile-phase velocities, smaller diameter packing materials, and thinner films of stationary phase.

Putting It All Together The net height of a theoretical plate is a summation of the contributions from each of the terms in equations 12.23–12.26; thus,

$$H = H_p + H_d + H_s + H_m \quad \text{.....12.27}$$

An alternative form of this equation is the **van Deemter equation**

$$H = A + \frac{B}{u} + Cu \quad \text{.....12.28}$$

which emphasizes the importance of the mobile phase's flow rate. In the van-Deemter equation, *A* accounts for multiple paths (H_p), *B*/*u* for longitudinal diffusion (H_d), and *Cu* for the solute's mass transfer in the stationary and mobile phases (H_s and H_m).

van Deemter equation: *An equation showing the effect of the mobile phase's flow rate on the height of a theoretical plate*

There is some disagreement on the correct equation for describing the relationship between plate height and mobile-phase velocity.4 In addition to the van-Deemter equation (equation 12.28), another equation is that proposed by Hawkes

$$H = \frac{B}{u} + \left(C_s + C_m\right)u$$

where C_s and Cm are the mass transfer terms for the stationary and mobile phases respectively. A third equation was devised by Knox.

$$H = Au^{1/3} + \frac{B}{u} + C_u$$

All three equations, and others, have been used to characterize chromatographic systems, with no single equation providing the best explanation in every case.5 To increase the number of theoretical plates without increasing the length of the column, it is necessary to decrease one or more of the terms in equation 12.27 or equation 12.28. The easiest way to accomplish this is by adjusting the velocity of the mobile phase. At a low mobile-phase velocity, column efficiency is limited by longitudinal diffusion, whereas at higher velocities efficiency is limited by the two mass transfer terms. As shown in Figure 12.15 (which is interpreted in terms of equation 12.28), the optimum mobile-phase velocity corresponds to a minimum in a plot of *H* as a function of *u.*

The remaining parameters affecting the height of a theoretical plate are determined by the construction of the column and suggest how the column's design may be used to improve efficiency. For example, both *H*p and *H*m are a function of the size of the particles used for the packing material. Decreasing particle size, therefore, is one approach to improving efficiency. A decrease in particle size is limited, however, by the need for a greater pressure to push the mobile phase through the column.

One of the most important advances in column construction has been the development of open tubular, or **capillary columns** that contain no packing material (*d*p = 0). Instead, the interior wall of a capillary column is coated with a thin film of the stationary phase. The absence of packing material means that the mobile phase can move through the column with substantially less pressure. As a result, capillary columns can be manufactured with much greater lengths than is possible with a packed column. Furthermore, plate height is reduced because the H_p term in equation 12.27 disappears and the H_m term becomes smaller. The combination of a smaller height for a theoretical plate and a longer column leads to an approximate 100-fold increase in the number of theoretical plates. Capillary columns are not without disadvantages. Because capillary columns are much narrower than packed columns, they require a significantly smaller amount of sample. A difficulty with reproducibly injecting small samples complicates the use of capillary chromatography for quantitative work.

Another approach to improving resolution is to use thin films of stationary phase. Capillary columns used in gas chromatography and the bonded phases commonly used in HPLC provide a significant decrease in plate height due to the reduction of the H_s term in equation 12.27.

capillary column: *A narrow bored column that usually does not contain a particulate packing material*

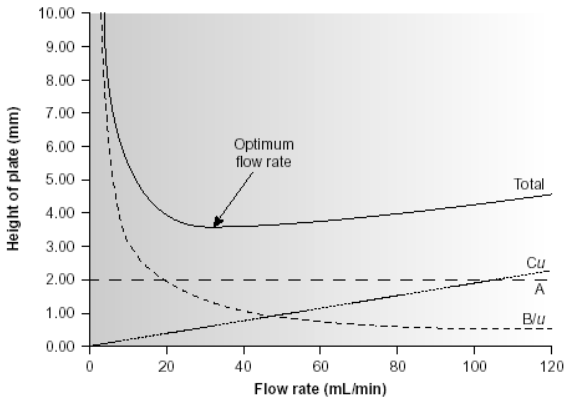

Figure 12.15: Plot of the height of a theoretical plate as a function of mobile-phase velocity using the van Deemter equation. The contributions to the terms *A, B/u,* and *Cu* also are shown.

14.16 Summary of the unit

Chromatography is an analytical technique that separates components in a mixture. Chromatographic columns are part of the instrumentation that is used in chromatography. Five chromatographic methods that use columns are gas chromatography (GC), liquid chromatography (LC), Ion exchange chromatography (IEC), size exclusion chromatography (SEC), and chiral chromatography. The basic principals of chromatography can be applied to all five methods**.**

The process of separating and analyzing a group of substances according to the differences in their absorption affinities for a given absorbent as evidenced by pigments deposited during filtration through the same absorbent contained in a glass cylinder or tube. The substances are dissolved in a liquid that is passed through the absorbent. The absorbates move down the column at different rates and leave behind a band of pigments that is subsequently washed with a pure solvent to develop discrete pigmented bands that constitute a chromatograph. The cylinder of absorbent is then pushed from the tube, and the individual bands are either separated with a knife or further diluted with the pure solvent and collected in the bottom of the tube for analysis. Effective column chromatography depends on the selection of the appropriate absorbent and solvent and a flow rate that is slow enough to allow complete diffusion of the absorbates from

the solvent to the absorbent and the retardation of the absorbates according to their different affinities for the absorbent.

Column chromatography the technique in which the various solutes of a solution are allowed to travel down a column, the individual components being adsorbed by the stationary phase. The most strongly adsorbed component will remain near the top of the column; the other components will pass to positions farther and farther down the column according to their affinity for the adsorbent. If the individual components are naturally colored, they will form a series of colored bands or zones.

14.17 Key words

Chromatography; Analytical separations; Liquid chromatography; Chromatographic Resolution; Column Chromatography; Capacity factor; Column Efficiency; Optimize Resolution.

14.18 References for further study

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14.19 Questions for self under standing

1) What is Chromatography?

2) What is the different chromatography techniques used for separation?

3) What is Liquid chromatography?

4) Explain the general theory of column chromatography

5) Write a note on chromatographic Resolution

6) What is Capacity factor in column chromatography?

7) Discuss Column selectivity

8) What is Column Efficiency?

9) Write a note on Peak Capacity

10) Explain Nonideal Behaviour

11) How Chromatographic separations can be optimized in following conditions

a) Using the Capacity Factor to Optimize Resolution

b) Using Column Efficiency to Optimize Resolution

Unit-15

Structure

15.0 Objectives of the unit

After studying this unit you are able to

- Explain the theory of Gas–liquid chromatography
- Recognize the stationary and mobile phases used in Gas–liquid chromatography
- Identify the different Detectors for Gas Chromatography
- Explain the theory of High-performance Liquid Chromatography
- Identify the different column used in HPLC
- Identify the differences between Isocratic versus Gradient Elution in HPLC

15.1 Introduction

"Chromatography" represents a separation technique; whereas a "chromatograph" is a system for performing chromatography. The chart displaying the time-dependent change in signal intensity as a result of the separation is called a "chromatogram".

As shown in Table 1, gas and liquid chromatography are common classifications that are based upon the mobile and stationary phases utilized for the separation. The speed of a migrating sample component depends on whether the component has an affinity for the stationary or mobile phase. This affinity appears via various actions: adsorption, partition, ion exchange, etc. As shown in Figure 2, components that have a higher affinity for the mobile phase compared with the stationary phase migrate more rapidly, while components that have a higher affinity for the stationary phase are eluted from the column later. The order and resolution of the components emerging from the column depend on the type of selected stationary and mobile phases. Chromatography is based on the principal that under the same conditions, the time between the injection of a component into the column and the elution of that component is constant. This characteristic is used to perform qualitative or quantitative analysis. Such analyses are explained here using the measurement of aspartame, a synthetic sweetener contained in beverages. HPLC is short for the High Performance LC. HPLC is an analysis method that yields high performance and high speed compared with traditional column chromatography because of the forcibly pumped mobile phase.

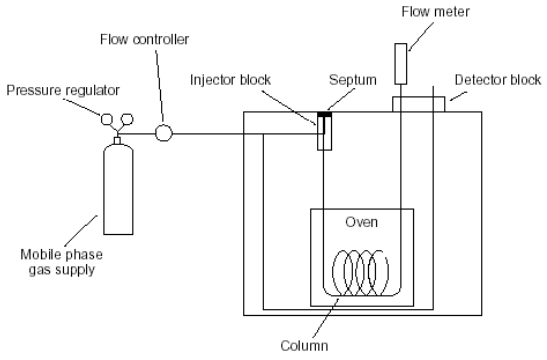
15.2 Gas Chromatography

In **gas chromatography** (GC) the sample, which may be a gas or liquid, is injected into a stream of an inert gaseous mobile phase (often called the carrier gas). The sample is carried through a packed or capillary column where the sample's components separate based on their ability to

distribute themselves between the mobile and stationary phases. A schematic diagram of a typical gas chromatograph is shown in Figure 12.16.

15.3 Mobile Phase

The most common mobile phases for GC are He, Ar, and N_2, which have the advantage of being chemically inert toward both the sample and the stationary phase. The choice of which carrier gas to use is often determined by the instrument's detector. With packed columns the mobile-phase velocity is usually within the range of 25–150 mL/min, whereas flow rates for capillary columns are 1–25 mL/min. Actual flow rates are determined with a flow meter placed at the column outlet.



Gas chromatography: A chromatographic technique in which the mobile phase is a gas.

15.4 Chromatographic Columns

A chromatographic column provides a location for physically retaining the stationary phase. The column's construction also influences the amount of sample that can be handled, the efficiency of the separation, the number of analytes that can be easily separated, and the amount of time required for the separation. Both packed and capillary columns are used in gas chromatography.

15.5 Packed Columns

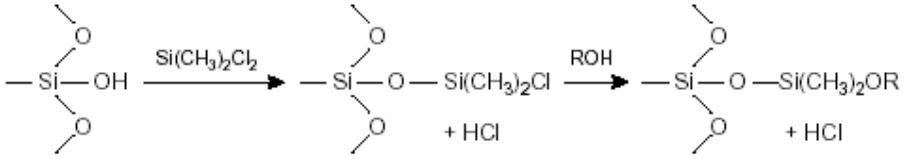
A **packed column** is constructed from glass, stainless steel, copper or aluminum and is typically 2–6 m in length, with an internal diameter of 2–4 mm. The column is filled with a particulate solid support, with particle diameters ranging from 37–44 μm to 250–354 μm. The most widely used particulate support is diatomaceous earth, which is composed of the silica skeletons of diatoms. These particles are quite porous, with surface areas of 0.5–7.5 m^2/g, which provides ample contact between the mobile phase and stationary phase. When hydrolyzed, the surface of a diatomaceous earth contains silanol groups (–SiOH), providing active sites that absorb solute molecules in gas–solid chromatography.

15.6 Gas–liquid chromatography

In **gas–liquid chromatography** (GLC), separation is based on the partitioning of solutes between a gaseous mobile phase and a liquid stationary phase coated on the solid packing material. To avoid the adsorption of solute molecules on exposed packing material, which degrades the quality of the separation, surface silanols are deactivated by silanizing with dimethyldichlorosilane and washing with an alcohol (typically methanol) before coating with stationary phase.

Gas–liquid chromatography is a chromatographic technique in which the mobile phase is a gas and the stationary phase is a liquid coated either on a solid packing material or on the column's walls.

Packed column is a wide-bore column containing a particulate packing material.



More recently, solid supports made from glass beads or fluorocarbon polymers have been introduced. These supports have the advantage of being more inert than diatomaceous earth. To minimize the multiple path and mass transfer contributions to plate height (equations 12.23 and 12.26), the packing material should be of as small a diameter as is practical and loaded with a thin film of stationary phase (equation 12.25). Compared with capillary columns, which are discussed in the next section, packed columns can handle larger amounts of sample. Samples of 0.1–10 μL are routinely analyzed with a packed column. Column efficiencies are typically several hundred to 2000 plates/m, providing columns with 3000–10,000 theoretical plates. Assuming *V*max/*V*min is approximately 50,3 a packed column with 10,000 theoretical plates has a peak capacity (equation 12.18) of

$$n_c = 1 + \frac{\sqrt{10,000}}{4}\ln(50) \approx 100$$

15.7 Capillary Columns

Capillary, or **open tubular columns** are constructed from fused silica coated with a protective polymer. Columns may be up to 100 m in length with an internal diameter of approximately 150–300 □. Larger bore columns of 530 □m, called m

(Figure 12.17)

are available.

Open tubular column A capillary column that does not contain a particulate packing material.

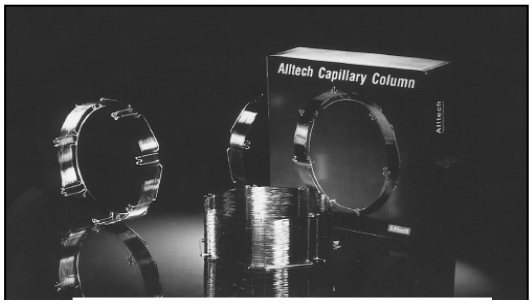


Photo of a capillary column

Capillary columns are of two principal types. **Wall-coated open tubular columns** (WCOT) contain a thin layer of stationary phase, typically 0.25 μm thick, coated on the capillary's inner wall. In **support-coated open tubular columns** (SCOT), a thin layer of a solid support, such as a diatomaceous earth, coated with a liquid stationary phase is attached to the capillary's inner wall. Capillary columns provide a significant improvement in separation efficiency. The pressure needed to move the mobile phase through a packed column limits its length. The absence of packing material allows a capillary column to be longer than a packed column. Although most capillary columns contain more theoretical plates per meter than a packed column, the more important contribution to their greater efficiency is the ability to fashion longer columns. For example, a 50-m capillary column with 3000 plates/m has 150,000 theoretical plates and, assuming *V*max/*V*min is approximately 50,3 a peak capacity of almost 380. On the other hand, packed columns can handle larger samples. Due to its smaller diameter, capillary columns require smaller samples; typically less than 10–2 μL.

Wall-coated open tubular column is an open tubular column in which the stationary phase is coated on the column's walls.

Support-coated open tubular column is an open tubular column in which the stationary phase is coated on a solid support that is attached to the column's walls.

15.8 Stationary Phases

Selectivity in gas chromatography is influenced by the choice of stationary phase. Elution order in GLC is determined primarily by the solute's boiling point and, to a lesser degree, by the solute's interaction with the stationary phase. Solutes with significantly different boiling points are easily separated. On the other hand, two solutes with similar boiling points can be separated only if the stationary phase selectively interacts with one of the solutes. In general, nonpolar

solutes are more easily separated with a nonpolar stationary phase, and polar solutes are easier to separate using a polar stationary phase. The main criteria for selecting a stationary phase are that it should be chemically inert, thermally stable, of low volatility, and of an appropriate polarity for the solutes being separated. Although hundreds of stationary phases have been developed, many of which are commercially available, the majority of GLC separations are accomplished with perhaps five to ten common stationary phases.

Table 12.2 Selected Stationary Phases for Gas–Liquid Chromatography

Stationary Phase	Polarity	Trade Names	Temperature Limit (°C)	Applications
squalane	nonpolar	squalane	150	low-boiling aliphatic hydrocarbons
Apezion L	nonpolar	Apezion L	300	amides fatty acid methyl esters high-boiling aliphatic hydrocarbons terpenoids
polydimethyl siloxane	slightly polar	SE-30	300–350	alkaloids amino acid derivatives drugs pesticides phenols steroids
50% methyl-50% phenyl polysiloxane	moderately polar	OV-17	375	alkaloids drugs pesticides polyaromatic hydrocarbons polychlorinated biphenyls
50% trifluoropropyl-50% methyl polysiloxane	moderately polar	OV-210	275	alkaloids amino acid derivatives drugs halogenated compounds ketones phenols
50% cyanopropyl-50% phenylmethyl polysiloxane	polar	OV-225	275	nitriles pesticides steroids
polyethylene glycol	polar	Carbowax 20M	225	aldehydes esters ethers phenols

Several of these are listed in Table 12.2, in order of increasing polarity, along with their physical properties and typical applications. Many stationary phases have the general structure shown in Figure 12.18a. A stationary phase of polydimethyl siloxane, in which all the –R groups are methyl groups (–CH3), is nonpolar and often makes a good first choice for a new separation. The order of elution when using polydimethyl siloxane usually follows the boiling points of the solutes, with lower boiling solutes eluting first. Replacing some of the methyl groups with other substituents increases the stationary phase's polarity, providing greater selectivity. Thus, in 50% methyl-50% phenyl polysiloxane, 50% of the –R groups are phenyl groups (–C6H5), producing a slightly polar stationary phase. Increasing polarity is provided by substituting trifluoropropyl (–C3H6CF3) and cyanopropyl (–C3H6CN) functional groups or using a stationary phase based on

polyethylene glycol (Figure 12.18b). An important problem with all liquid stationary phases is their tendency to "**bleed**" from the column. The temperature limits listed in Table 12.2 are those that minimize the loss of stationary phase. When operated above these limits, a column's useful lifetime is significantly shortened. Capillary columns with bonded or cross-linked stationary phases provide superior stability. Bonded stationary phases are attached to the capillary's silica surface. Crosslinking, which is done after the stationary phase is placed in the capillary column, links together separate polymer chains, thereby providing greater stability. Another important characteristic of a gas chromatographic column is the thickness of the stationary phase. As shown in equation 12.25, separation efficiency improves with thinner films. The most common film thickness is 0.25 μm. Thicker films are used for highly volatile solutes, such as gases, because they have a greater capacity for retaining such solutes. Thinner films are used when separating solutes of low volatility, such as steroids.

A few GLC stationary phases rely on chemical selectivity. The most notable are stationary phases containing chiral functional groups, which can be used for separating enantiomers.

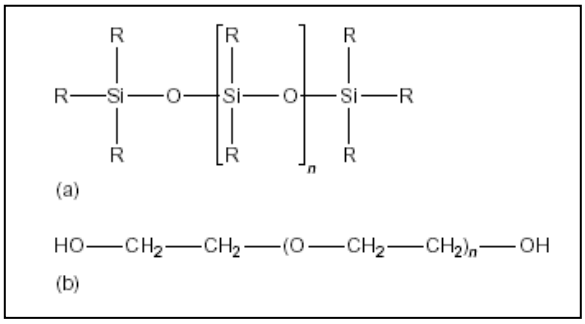


Figure 12.18 General structures of common stationary phases for gas chromatography.

Bleed: The tendency of a stationary phase to elute from the column.

Sample Introduction

Three considerations determine how samples are introduced to the gas chromatograph. First, all constituents injected into the GC must be volatile. Second, the analytes must be present at an appropriate concentration. Finally, injecting the sample must not degrade the separation.

Preparing a Volatile Sample

Gas chromatography can be used to separate analytes in complex matrices. Not every sample that can potentially be analyzed by GC, however, can be injected directly into the instrument. To move through the column, the sample's constituents must be volatile. Solutes of low volatility may be retained by the column and continue to elute during the analysis of subsequent samples.

Nonvolatile solutes condense on the column, degrading the column's performance. Liquid–liquid extractions, in which analytes are extracted from an aqueous matrix into methylene chloride or other organic solvent, are commonly used. Solid-phase extractions also are used to remove unwanted matrix constituents. An attractive approach to isolating analytes is a **solid-phase microextraction** (SPME). In one approach, which is illustrated in Figure 12.19, a fused silica fiber is placed inside a syringe needle. The fiber, which is coated with a thin organic film, such as polydimethyl siloxane, is lowered into the sample by depressing a plunger and is exposed to the sample for a predetermined time. The fiber is then withdrawn into the needle and transferred to the gas chromatograph for analysis. Volatile analytes also can be separated from a liquid matrix using a purge and trap or by headspace sampling. In a purge and trap an inert gas, such as He or N_2, is bubbled through the sample, purging the volatile compounds. These compounds are swept through a trap packed with an absorbent material, such as Tenax, where they are collected. Heating the trap and back flushing with carrier gas transfers the volatile compounds to the gas chromatograph. In **headspace sampling** the sample is placed in a closed vial with an overlying air space. After allowing time for the volatile analytes to equilibrate between the sample and the overlying air, a portion of the vapor phase is sampled by syringe and injected into the gas chromatograph.

solid-phase microextraction A solid-phase extraction in which the solid adsorbent is coated on a fusedsilica fiber held within a syringe needle.

headspace sampling The sampling of the vapor phase overlying a liquid phase.

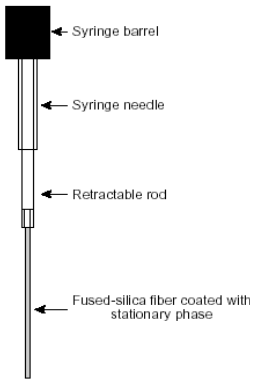


Figure 12.19 Schematic diagram of a device for solidphase microextractions.

Thermal desorption is used to release volatile analytes from solids. A portion of the solid is placed in a glass-lined, stainless steel tube and held in place with plugs of glass wool. After

purging with carrier gas to remove O_2 (which could lead to oxidation reactions when heating the sample), the sample is heated. Volatile analytes are swept from the tube by the carrier gas and carried to the GC. To maintain efficiency the solutes often are concentrated at the top of the column by cooling the column inlet below room temperature, a process known as **cryogenic focusing.** Nonvolatile analytes must be chemically converted to a volatile derivative before analysis. For example, amino acids are not sufficiently volatile to analyze directly by gas chromatography. Reacting an amino acid with 1-butanol and acetyl chloride produces an esterfied amino acid. Subsequent treatment with trifluoroacetic acid gives the amino acid's volatile *N*-trifluoroacetyl-*n*-butyl ester derivative.

Cryogenic focusing The process of concentrating volatilesolutes by cooling the column's inlet below room temperature.

15.9 Adjusting the Analyte's Concentration

Analytes present at concentrations too small to give an adequate signal need to be concentrated before analyzing. A side benefit of many of the extraction methods outlined earlier is that they often concentrate the analytes. Volatile organic materials isolated from aqueous samples by a purge and trap, for example, can be concentrated by as much as 1000-fold. When an analyte is too concentrated, it is easy to overload the column, thereby seriously degrading the separation. In addition, the analyte may be present at a concentration level that exceeds the detector's linear response. Dissolving the sample in a volatile solvent, such as methylene chloride, makes its analysis feasible.

15.10 Injecting the Sample

To avoid any precolumn loss in resolution due to band broadening, a sample of sufficient size must be introduced in a small volume of mobile phase. An example of a simple injection port for a packed column is shown in Figure 12.20. Injections are made through a rubber septum using a microliter syringe.

The injector block is heated to a temperature that is at least 50 °C above the sample component with the highest boiling point. In this way rapid vaporization of the entire sample is ensured. Capillary columns require the use of a special injector to avoid overloading the column with sample. Several capillary injectors are available, the most common of which is a split/splitless injector.7 When used for a **split injection** only about 0.1–1% of the sample enters the column, with the remainder carried off as waste. In a **splitless injection,** which is useful for trace

analysis, the column temperature is held 20–25 °C below the solvent's boiling point. As the solvent enters the column, it condenses, forming a barrier that traps the solutes. After allowing time for the solutes to concentrate, the column's temperature is increased, and the separation begins. A splitless injection allows a much higher percentage of the solutes to enter the chromatographic column. For samples that decompose easily, an **on-column injection** may be necessary. In this method the sample is injected on the column without heating. The column temperature is then increased, volatilizing the sample with as low a temperature as is practical.

15.11 Temperature Control

As noted earlier, control of the column's temperature is critical to attaining a good separation in gas chromatography. For this reason the column is located inside a thermostated oven. In an isothermal separation the column is maintained at a constant temperature, the choice of which is dictated by the solutes. Normally, the tem- perature is set slightly below that for the lowest boiling solute so as to increase the solute's interaction with the stationary phase. One difficulty with an isothermal separation is that a temperature favoring the separation of low-boiling solutes may cause unacceptably long retention times for higher boiling solutes. Ovens capable of temperature programming provide a solution to this problem. The initial temperature is set below that for the lowest boiling solute. As the separation progresses, the temperature is slowly increased at either a uniform rate or in a series of steps.

split injection A technique for injecting samples onto a capillary column in which only a small portion of the sample enters the column.

splitless injection A technique for injecting a sample onto a capillary column that allows a higher percentage of the sample to enter the column.

on-column injection The direct injection of thermally unstable samples onto a capillary column.

15.12 Detectors for Gas Chromatography

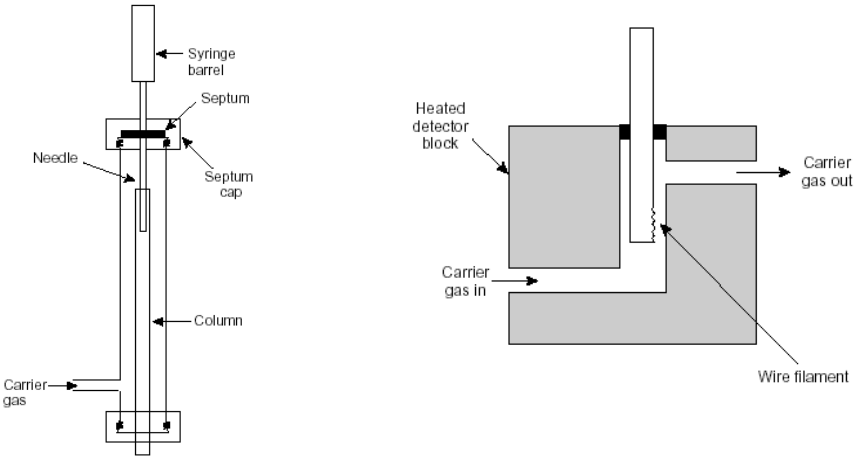
The final part of a gas chromatograph is the detector. The ideal detector has several desirable features, including low detection limits, a linear response over a wide range of solute concentrations (which makes quantitative work easier), responsiveness to all solutes or selectivity for a specific class of solutes, and insensitivity to changes in flow rate or temperature.

15.13 Thermal Conductivity Detector

One of the earliest gas chromatography detectors, which is still widely used, is based on the mobile phase's thermal conductivity (Figure 12.21). As the mobile phase exits the column, it

passes over a tungsten–rhenium wire filament. The filament's electrical resistance depends on its temperature, which, in turn, depends on the thermal conductivity of the mobile phase. Because of its high thermal conductivity, helium is the mobile phase of choice when using a **Thermal conductivity detector** (TCD). When a solute elutes from the column, the thermal conductivity of the mobile phase decreases and the temperature of the wire filament, and thus its resistance, increases. A reference cell, through which only the mobile phase passes, corrects for any time-dependent variations in flow rate, pressure, or electrical power, all of which may lead to a change in the filament's resistance. A TCD detector has the advantage of universality, since it gives a signal for any solute whose thermal conductivity differs from that of helium. Another advantage is that it gives a linear response for solute concentrations over a range of 104–105 orders of magnitude. The detector also is nondestructive, making it possible to isolate solutes with a postdetector cold trap. Unfortunately, the thermal conductivity detector's detection limit is poor in comparison with other popular detectors.

Thermal conductivity detector A universal GC detector in which the signal is a change in the thermal conductivity of the mobile phase.



Schematic diagram of an injector for packed column gas chromatography detector for gas chromatography. Schematic diagram of a thermal conductivity.

15.14 Flame Ionization Detector

Combustion of an organic compound in an H_2/air flame results in a flame rich in electrons and ions. If a potential of approximately 300 V is applied across the flame, a small current of roughly 10–9–10–12 A develops. When amplified, this current provides a useful analytical signal. This is the basis of the popular **flame ionization detector** (FID), a schematic of which is shown in

Figure 12.22. Most carbon atoms, except those in carbonyl and carboxylic groups, generate a signal, making the FID an almost universal detector for organic compounds. Most inorganic compounds and many gases, such as H2O and CO2, cannot be detected, making the FID detector ideal for the analysis of atmospheric and aqueous environmental samples. Advantages of the FID include a detection limit that is approximately two to three orders of magnitude smaller than that for a thermal conductivity detector and a linear response over 106–107 orders of magnitude in the amount of analyte injected. The sample, of course, is destroyed when using flame ionization detector.

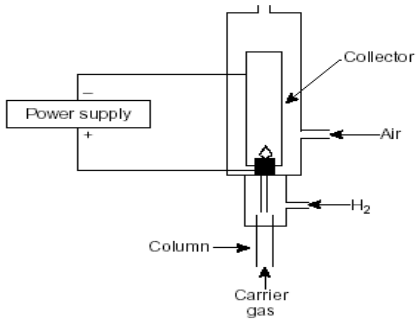


Figure 12.22 Schematic diagram of a flame ionization detector for gas chromatography.

15.15 Electron Capture Detector

The **electron capture detector** is an example of a selective detector. The detector consists of a beta emitter (a beta particle is an electron) such as 63Ni. The emitted electrons ionize the mobile phase, which is usually N2, resulting in the production of additional electrons that give rise to an electric current between a pair of electrodes (Figure 12.23). When a solute with a high cross section for the capture of electrons elutes from the column, the electric current decreases. This decrease in electric current serves as the signal. The ECD is highly selective toward solutes with electronegative functional groups, such as halogens, and nitro groups and is relatively insensitive to amines, alcohols, and hydrocarbons. Although its detection limit is excellent, its linear range extends over only about two orders of magnitude.

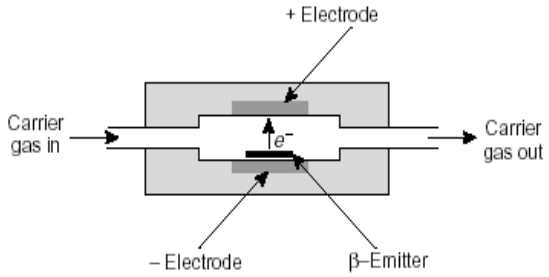
15.16 Other Detectors

Two additional detectors are similar in design to a flame ionization detector. In the flame photometric detector optical emission from phosphorus and sulfur provides a detector selective for compounds containing these elements. The thermionic detector responds to compounds containing nitrogen or phosphorus. Two common detectors, which also are independent

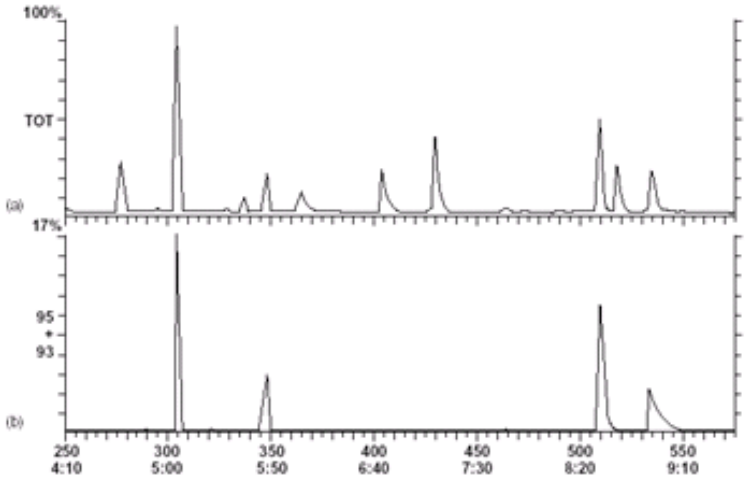
instruments, are Fourier transform infrared spectrophotometers (FT–IR) and mass spectrometers (MS). In GC–FT–IR, effluent from the column flows through an optical cell constructed

Flame ionization detector A nearly universal GC detector in which the solutes are combusted in an H2/air flame, producing a measurable current.

Electron capture detector A detector for GC that provides selectivity for solutes with halogen and nitro functional groups.



Schematic diagram of an electron capture detector for gas chromatography.



(a) Total ion chromatogram for a ten-component mixture;

(b) Chromatogram recorded using selective ion monitoring for mass-to-charge ratios of 93 and 95, which are characteristic ions for themonoterpenes α-pinene

(*t*r = 5.08 min), β-pinene (*t*r = 5.81 min), camphor (*t*r = 8.51 min), and menthol (*t*r = 8.93 min). from a 10–40-cm Pyrex tube with an internal diameter of 1–3 mm. The cell's interior surface is coated with a reflecting layer of gold. Multiple reflections of the source radiation as it is transmitted through the cell increase the optical path length through the sample. In GC–MS effluent from the column is introduced directly into the mass spectrometer's ionization chamber

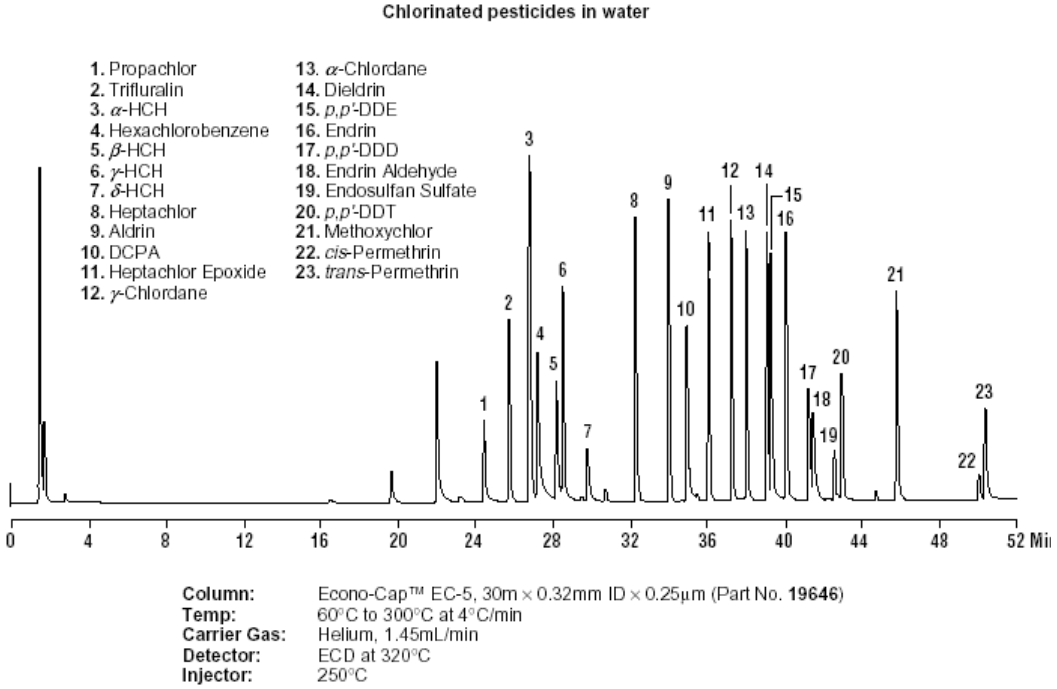
in a manner that eliminates the majority of the carrier gas. In the ionization chamber all molecules (remaining carrier gas, solvent, and solutes) are ionized, and the ions are separated by their mass-to-charge ratio. Because each solute undergoes a characteristic fragmentation into smaller ions, its **mass spectrum** of ion intensity as a function of mass-to-charge ratio provides qualitative information that can be used to identify the solute. As a GC detector, the total ion current for all ions reaching the detector is usually used to obtain the chromatogram (Figure 12.24a). Selectivity can be achieved by monitoring only specific mass-to-charge ratios (Figure 12.24b), a process called selective ion monitoring. A mass spectrometer provides excellent detection limits, typically 25 fg to 100 pg, with a linear range spanning five orders of magnitude.

15.17 Quantitative Applications

Gas chromatography is widely used for the analysis of a diverse array of samples in environmental, clinical, pharmaceutical, biochemical, forensic, food science, and petrochemical laboratories. Examples of these applications are discussed in the following sections.

a) Environmental Analysis

One of the most important environmental applications of gas chromatography is for the analysis of numerous organic pollutants in air, water, and wastewater. The analysis of volatile organics in drinking water, for example, is accomplished by a purge and trap, followed by their separation on a capillary column with a nonpolar stationary phase. A flame ionization, electron capture, or



Examples of the application of gas chromatography to the analysis of (a) chlorinated pesticides in water, (b) blood alcohols, (c) Scotch whiskey, and (d) unleaded gasoline. (Chromatograms courtesy of Alltech Associates, Inc. Deerfield, IL). Examples of the application of gas chromatography to the analysis of (a) chlorinated pesticides in water, (b) blood alcohols, (c) Scotch whiskey, and (d) unleaded gasoline. (Chromatograms courtesy of Alltech Associates, Inc. Deerfield, IL). mass spectrometer can be used as a detector. Figure 12.25a shows a typical chromatogram for the analysis of chlorinated pesticides in water.

b) Clinical Analysis

Clinical, pharmaceutical, and forensic labs make frequent use of gas chromatography for the analysis of drugs. Because the sample's matrix is often incompatible with the GC column, analytes generally must be isolated by extraction. Figure 12.25b shows how gas chromatography can be used in monitoring blood alcohol levels.

c) Consumer Goods

Many flavors, spices, and fragrances are readily analyzed by GC, using headspace analysis or thermal desorption. Foods and beverages are analyzed either directly or following a suitable extraction. Volatile materials, such as those found in spices and fragrances, often can be obtained by headspace sampling. Figure 12.25c shows a typical analysis of a sample of Scotch whiskey.

d) Petroleum Industry

Gas chromatography is ideally suited for the analysis of petroleum products, including gasoline, diesel fuel, and oil. A typical chromatogram for the analysis of unleaded gasoline is shown in Figure 12.25d.

e) Quantitative Calculations

In a quantitative analysis, the height or area of an analyte's chromatographic peak is used to determine its concentration. Although peak height is easy to measure, its utility is limited by the inverse relationship between the height and width of a chromatographic peak. Unless chromatographic conditions are carefully controlled to maintain a constant column efficiency, variations in

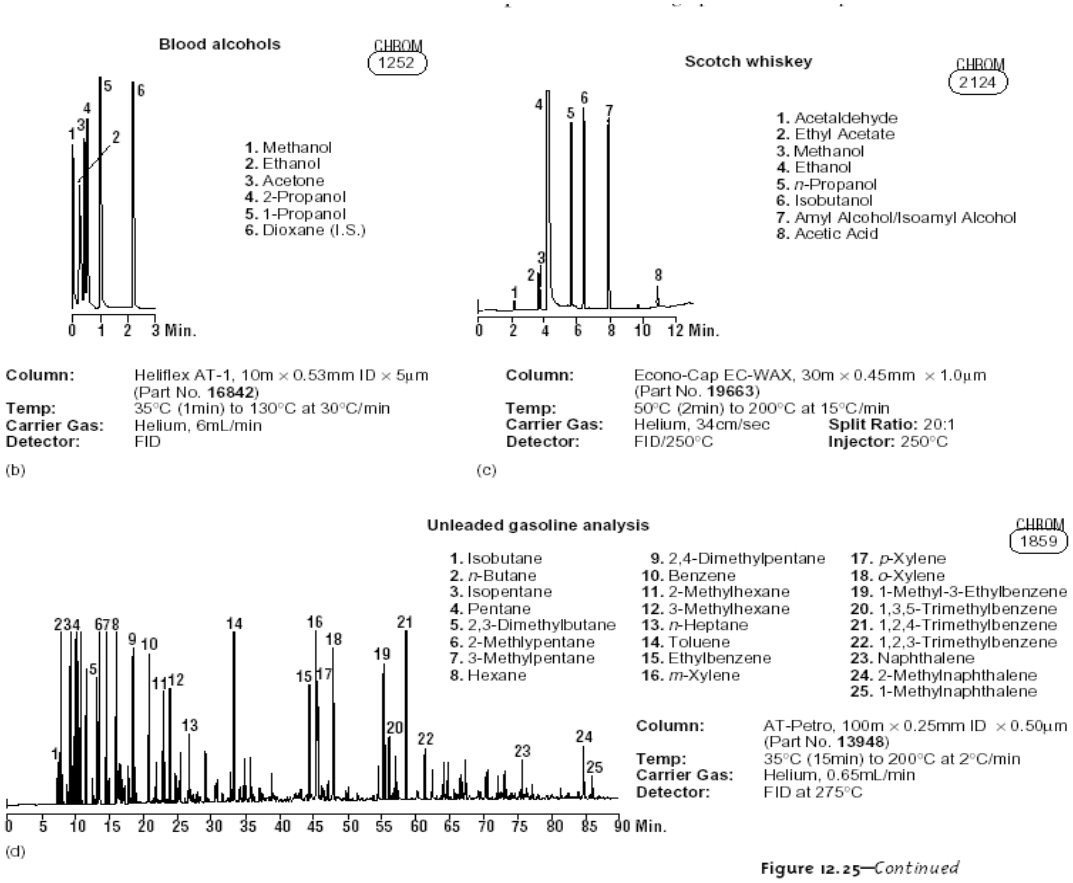


Figure 12.25—*Continued*

peak height may decrease the accuracy and precision of the quantitative analysis. A better choice is to measure the area under the chromatographic peak with an integrating recorder. Since peak area is directly proportional to the amount of analyte that was injected, changes in column efficiency will not affect the accuracy or precision of the analysis. Calibration curves are usually constructed by analyzing a series of external standards and plotting the detector's signal as a function of their known concentrations. As long as the injection volume is identical for every standard and sample, calibration curves prepared in this fashion give both accurate and precise results. Unfortunately, even under the best of conditions, replicate injections may have volumes that differ by as much as 5% and often may be substantially worse. For this reason, quantitative work requiring high accuracy and precision is accomplished using an internal standard.

Kovat's retention index A means for normalizing retention times by comparing a solute's retention time with those for normal alkanes.

15.18 Qualitative Applications

Gas chromatography also can be used for qualitative purposes. When using an FT–IR or a mass spectrometer as the detector, the available spectral information often can be used to identify

individual solutes. With conventional nonspectroscopic detectors, other methods must be used to identify the solutes. One approach is to spike the sample by adding an aliquot of a suspected analyte and looking for an increase in peak height. Retention times also can be compared with values measured for standards, provided that the operating conditions are identical. Because of the difficulty of exactly matching such conditions, tables of retention times are of limited utility.

15.19 Kovat's retention index

This provides one solution to the matching of retention times. Under isothermal conditions, the adjusted retention times of normal alkanes increase logarithmically. Kovat defined the retention index, *I*, for a normal alkane as 100 times the number of carbon atoms; thus, the retention index is 400 for butane and 500 for pentane. To determine the retention index for another compound, its adjusted retention time is measured relative to that for the normal alkanes eluting just before and after. For example, a compound eluting between butane and pentane has a retention index between 400 and 500. The exact value for the compound's retention index, *I*cpd, is given as where *x* is the normal alkane eluting before the compound, and x + 1 is the normal alkane eluting just after the compound.

15.20 Evaluation

i) Scale of Operation

Analytes present at levels from major to ultratrace components have been successfully determined by gas chromatography. Depending on the choice of detector, samples with major and minor analytes may need to be diluted before analysis. The thermal conductivity and flame ionization detectors can handle larger amounts of analyte; other detectors, such as the electron capture detector or a mass spectrometer, require substantially smaller amounts of analyte. Although the volume of sample injected is quite small (often less than a microliter), the amount of available material from which the injection volume is taken must be sufficient to be a representative sample. For trace analytes, the actual amount of analyte injected is often in the picogram range. Using the trihalomethane analysis described in Method 12.1 as an example, a 3.0-μL injection of a water sample containing 1 μg/L of $CHCl_3$ corresponds to 15 pg of $CHCl_3$ (assuming a complete extraction of $CHCl_3$).

ii) Accuracy

The accuracy of a gas chromatographic method varies substantially from sample to sample. For routine samples, accuracies of 1–5% are common. For analytes present at very low concentration

levels, for samples with complex matrices, or for samples requiring significant processing before analysis, accuracy may be substantially poorer. In the analysis for trihalomethanes described in Method 12.1, for example, determinate errors as large as ±25% are possible.

iii) Precision

The precision of a gas chromatographic analysis includes contributions from sampling, sample preparation, and the instrument. The relative standard deviation due to the gas chromatographic portion of the analysis is typically 1–5%, although it can be significantly higher. The principal limitations to precision are detector noise and the reproducibility of injection volumes. In quantitative work, the use of an internal standard compensates for any variability in injection volumes.

iv) Sensitivity

In a gas chromatographic analysis, sensitivity (the slope of a calibration curve) is determined by the detector's characteristics. Of greater interest for quantitative work is the detector's linear range; that is, the range of concentrations over which a calibration curve is linear. Detectors with a wide linear range, such as a thermal conductivity detector and flame ionization detector, can be used to analyze samples of varying concentration without adjusting operating conditions. Other detectors, such as the electron capture detector, have a much narrower linear range.

v) Selectivity

Because it combines separation with analysis, gas chromatography provides excellent selectivity. By adjusting conditions it is usually possible to design a separation such that the analytes elute by themselves. Additional selectivity can be provided by using a detector, such as the electron capture detector, that does not respond to all compounds.

15.21 High-performance Liquid Chromatography (HPLC)

Although gas chromatography is widely used, it is limited to samples that are thermally stable and easily volatilized. Nonvolatile samples, such as peptides and carbohydrates, can be analyzed by GC, but only after they have been made more volatile by a suitable chemical derivatization. For this reason, the various techniques included within the general scope of liquid chromatography are among the most commonly used separation techniques. Although simple column chromatography, first introduced by Tswett, is still used in large-scale preparative work, the focus of this section is on **high-performance liquid chromatography** (HPLC).

In HPLC, a liquid sample, or a solid sample dissolved in a suitable solvent, is carried through a chromatographic column by a liquid mobile phase. Separation is determined by solute/stationary-phase interactions, including liquid–solid adsorption, liquid–liquid partitioning, ion exchange and size exclusion, and by solute/mobile-phase interactions. In each case, however, the basic instrumentation is essentially the same. A schematic diagram of a typical HPLC instrument is shown in Figure 12.26.

15.22 HPLC Columns

An HPLC typically includes two columns: an analytical column responsible for the separation and a guard column. The guard column is placed before the analytical column, protecting it from contamination. **Analytical Columns** The most commonly used columns for HPLC are constructed from stainless steel with internal diameters between 2.1 mm and 4.6 mm, and lengths ranging from approximately 30 mm to 300 mm. These columns are packed with 3–10 μm porous silica particles that may have an irregular or spherical shape. Typical column efficiencies are 40,000–60,000 theoretical plates/m. Assuming *V*max/*V*min is approximately 50,3 a 25-cm column with 50,000 plates/m has 12,500 theoretical plates and a peak capacity of 110. Microcolumns use less solvent and, because the sample is diluted to a lesser extent, produce larger signals at the detector. These columns are made from fused silica capillaries with internal diameters of 44–200 μm and lengths of up to several meters. Microcolumns packed with 3–5-μm particles have been prepared with column efficiencies of up to 250,000 theoretical plates.10 Open tubular microcolumns also have been developed, with internal diameters of 1–50 μm and lengths of approximately 1 m. These columns, which contain no packing material, may be capable of obtaining column efficiencies of up to 1 million theoretical plates. The development of open tubular columns, however, has been limited by the difficulty of preparing columns with internal diameters less than 10 μm.

15.23 Guard Columns

Two problems tend to shorten the lifetime of an analytical column. First, solutes binding irreversibly to the stationary phase degrade the column's performance by decreasing the available stationary phase. Second, particulate material injected with the sample may clog the analytical column. To minimize these problems, a **guard column** is placed before the analytical column. Guard columns usually contain the same particulate packing material and stationary phase as the analytical column, but are significantly shorter and less expensive; a length of 7.5

mm and a cost one-tenth of that for the corresponding analytical column is typical. Because they are intended to be sacrificial, guard columns are replaced regularly.

High-performance liquid chromatography A chromatographic technique in which the mobile phase is a liquid.

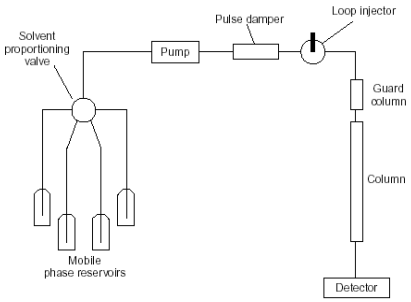
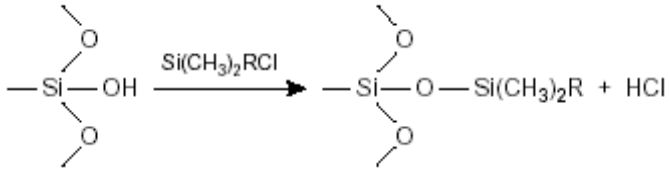


Figure 12.26 Schematic diagram of a high-performance liquid chromatograph.

15.24 Stationary Phases

In liquid–liquid chromatography the stationary phase is a liquid film coated on a packing material consisting of 3–10 μm porous silica particles. The stationary phase may be partially soluble in the mobile phase, causing it to "bleed" from the column over time. To prevent this loss of stationary phase, it is covalently bound to the silica particles. **Bonded stationary phases** are attached by reacting the silica particles with an organochlorosilane of the general form $Si(CH_3)_2RCl$, where R is an alkyl or substituted alkyl group.

Guard column An inexpensive column used to protect a more expensive analytical column.



To prevent unwanted interactions between the solutes and any unreacted –SiOH groups, the silica frequently is "capped" by reacting it with $Si(CH_3)_3Cl$; such columns are designated as end-capped. The properties of a stationary phase are determined by the nature of the organosilane's alkyl group. If R is a polar functional group, then the stationary phase will be polar. Examples of polar stationary phases include those for which R contains a cyano ($–C_2H_4CN$), diol ($–C_3H_6OCH_2CHOHCH_2OH$), or amino ($–C_3H_6NH_2$) functional group. Since the stationary phase

is polar, the mobile phase is a nonpolar or moderately polar solvent. The combination of a polar stationary phase and a nonpolar mobile phase is called **normal-phase chromatography.**

In **reverse-phase chromatography,** which is the more commonly encountered form of HPLC, the stationary phase is nonpolar and the mobile phase is polar. The most common nonpolar stationary phases use an organochlorosilane for which the R group is an *n*-octyl (C8) or *n*-octyldecyl (C18) hydrocarbon chain. Most reversephase separations are carried out using a buffered aqueous solution as a polar mobile phase. Because the silica substrate is subject to hydrolysis in basic solutions, the pH of the mobile phase must be less than 7.5.

15.25 Mobile Phases

The elution order of solutes in HPLC is governed by polarity. In a normal-phase separation the least polar solute spends proportionally less time in the polar stationary phase and is the first solute to elute from the column. Retention times are controlled by selecting the mobile phase, with a less polar mobile phase leading to longer retention times. If, for example, a separation is poor because the solutes are eluting too quickly, switching to a less polar mobile phase leads to longer retention times and more opportunity for an acceptable separation. When two solutes are adequately resolved, switching to a more polar mobile phase may provide an acceptable separation with a shorter analysis time. In a reverse-phase separation the order of elution is reversed, with the most polar solute being the first to elute. Increasing the polarity of the mobile phase leads to longer retention times, whereas shorter retention times require a mobile phase of lower polarity.

15.26 Choosing a Mobile Phase

Several indices have been developed to assist in selecting a mobile phase, the most useful of which is the **polarity index**. Mobile phases of intermediate polarity can be fashioned by mixing together two or more of the mobile phases. For example, a binary mobile phase made by combining solvents A and B has a polarity index, P'_{AB}, of

normal-phase chromatography Liquid chromatography using a polar stationary phase and a nonpolar mobile phase.

reverse-phase chromatography Liquid chromatography using a nonpolar stationary phase and a polar mobile phase.

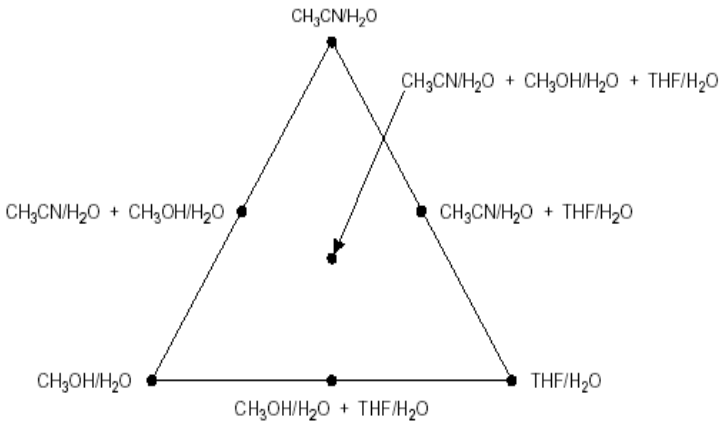
polarity index A quantitative measure of a solvent's polarity.

bonded stationary phase A liquid stationary phase that is chemically bonded to a particulate packing material.

P' AB = ϕAP'□A + ϕBP'B

where P'A and P'B are the polarity indexes for solvents A and B, and ϕA and ϕB are the volume fractions of the two solvents. A useful guide when using the polarity index is that a change in its value of 2 units corresponds to an approximate tenfold change in a solute's capacity factor. Thus, if k' is 22 for the reverse-phase separation of a solute when using a mobile phase of water (P' = 10.2), then switching to a 60:40 water–methanol mobile phase (P'□= 8.2) will decrease k' to approximately 2.2. Note that the capacity factor decreases because we are switching from a more polar to a less polar mobile phase in a reverse-phase separation.

Changing the mobile phase's polarity index, by changing the relative amounts of two solvents, provides a means of changing a solute's capacity factor. Such changes, however, are not very selective; thus, two solutes that significantly overlap may continue to be poorly resolved even after making a significant change in the mobile phase's polarity.



To effect a better separation between two solutes it is often necessary to improve the selectivity factor, α. Two approaches are commonly used to accomplish this improvement. When a solute is a weak acid or a weak base, adjusting the pH of the aqueous mobile phase can lead to significant changes in the solute's retention time. At more acidic pH levels, both weak acids are present as neutral molecules. Because they partition favorably into the stationary phase, the retention times for the solutes are fairly long. When the pH is made more basic, the solutes, which are now present as their conjugate weak base anions, are less soluble in the stationary phase and elute more quickly. Similar effects can be achieved by taking advantage of metal–ligand complexation

and other equilibrium reactions. A second approach to changing the selectivity factor for a pair of solutes is to change one or more of the mobile-phase solvents. In a reverse-phase separation, for example, this is accomplished by changing the solvent mixed with water. Besides methanol, other common solvents for adjusting retention times are acetonitrile and tetrahydrofuran (THF). A common strategy for finding the best mobile phase is to use the solvent triangle shown in above figure. The separation is first optimized using an aqueous mobile phase of acetonitrile to produce the best separation within the desired analysis time (methanol or THF also could be chosen first). These mobile phases are then adjusted, if necessary, establishing the three points of the solvent triangle. Four additional mobile phases are prepared using the binary and ternary mobile phases. From these seven mobile phases it is possible to estimate how a change in the mobile-phase composition might affect the separation.

15.27 Isocratic versus Gradient Elution

When a separation uses a single mobile phase of fixed composition it is called an **isocratic elution.** It is often difficult, however, to find a single mobile-phase composition that is suitable for all solutes. Recalling the general elution problem, a mobile phase that is suitable for early eluting solutes may lead to unacceptably long retention times for later eluting solutes. Optimizing conditions for late eluting solutes, on the other hand, may provide an inadequate separation of early eluting solutes. Changing the composition of the mobile phase with time provides a solution to this problem. For a reverse-phase separation the initial mobile-phase composition is relatively polar. As the separation progresses, the mobile phase's composition is made less polar. Such separations are called gradient elutions.

Isocratic elution The use of a mobile phase whose composition remains constant throughout the separation.

15.28 HPLC Plumbing

An important feature of HPLC instrumentation is the presence of several solvent reservoirs. As discussed in the previous section, controlling the mobile phase's polarity plays an important role in improving a liquid chromatographic separation. The availability of several solvent reservoirs allows the mobile phase's composition to be quickly and easily varied. This is essential when using a gradient elution, for which the mobile-phase composition is systematically changed from a weaker solvent to a stronger solvent. Before they are used, mobile-phase solvents must be treated to remove dissolved gases, such as N_2 and O_2, and small particulate matter, such as dust.

Dissolved gases often lead to the formation of gas bubbles when the mobile phase enters the detector, resulting in a distortion of the detector's signal. Degassing is accomplished in several ways, but the most common are the use of a vacuum pump or sparging with an inert gas, such as He, which has a low solubility in the mobile phase. Particulate material capable of clogging the HPLC tubing or column is removed by filtering. If the instrument is not designed to do so, degassing and filtering can be completed before the solvents are placed in their reservoirs. The mobile-phase solvents are pulled from their reservoirs by the action of a pump. Most HPLC instruments use a reciprocating pump consisting of a piston whose back-and-forth movement is capable both of maintaining a constant flow rate of up to several milliliters per minute and of obtaining the high output pressure needed to push the mobile phase through the chromatographic column. A solvent proportioning valve controls the mobile phase's composition, making possible the necessary change in the mobile phase's composition when using a gradient elution. The back and forth movement of a reciprocating pump results in a pulsed flow that contributes noise to the chromatogram. To eliminate this problem a pulse damper is placed at the outlet of the pump.

15.29 Sample Introduction

The typical operating pressure of an HPLC is sufficiently high that it is impossible to inject the sample in the same manner as in gas chromatography. Instead, the sample is introduced using a **loop injector**. Sampling loops are interchangeable, and available with volumes ranging from 0.5 μL to 2 mL. In the load position the sampling loop is isolated from the mobile phase and is open to the atmosphere. A syringe with a capacity several times that of the sampling loop is used to place the sample in the loop. Any extra sample beyond that needed to fill the sample loop exits through the waste line. After loading the sample, the injector is turned to the inject position. In this position the mobile phase is directed through the sampling loop, and the sample is swept onto the column.

15.30 Detectors for HPLC

As with gas chromatography, numerous detectors have been developed for use in monitoring HPLC separations. To date, the majority of HPLC detectors are not unique to the method, but are either stand-alone instruments or modified versions of the same.

a) Spectroscopic Detectors

The most popular HPLC detectors are based on spectroscopic measurements, including UV/Vis absorption, and fluorescence. These detectors range from simple designs, in which the analytical

wavelength is selected using appropriate filters, to essentially a modified spectrophotometer equipped with a flow cell. When using a UV/Vis detector, the resulting chromatogram is a plot of absorbance as a function of elution time. Instruments utilizing a diode array spectrophotometer record entire spectra, giving a three-dimensional chromatogram showing absorbance as a function of wavelength and elution time. Figure 12.29a shows a typical flow cell for HPLC when using a UV/Vis spectrophotometer as a detector. The flow cell has a volume of 1–10 μL and a path length of 0.2–1 cm. One limitation to using absorbance is that the mobile phase must not absorb strongly at

Loop injector A means for injecting samples in which the sample is loaded into a short section of tubing and injected onto the column by redirecting the mobile phase through the loop.

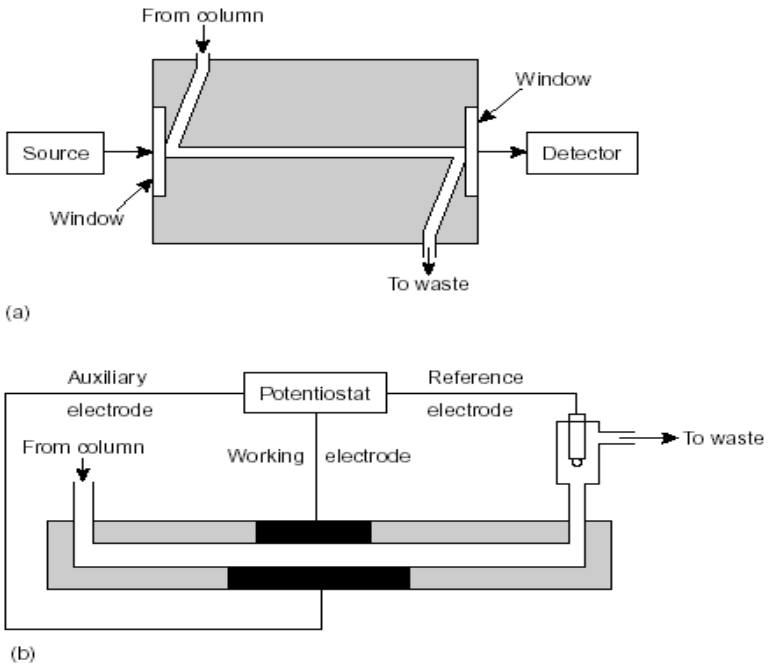


Figure 2.29: Schematic diagrams of flow cell detectors for HPLC using (a) UV/Vis absorption spectrophotometry and (b) amperometry.

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pg^{-1} ng of injected analyte. Fluorescence detectors provide additional selectivity since fewer solutes are capable of fluorescing. The resulting chromatogram is a plot of fluorescence intensity as a function of time. Detection limits are as little as 1–10 pg of injected analyte.

b) Electrochemical Detectors

Another common group of HPLC detectors are those based on electrochemical measurements such as amperometry, voltammetry, coulometry, and conductivity. Figure 12.29b, for example,

shows an amperometric flow cell. Effluent from the column passes over the working electrode, which is held at a potential favorable for oxidizing or reducing the analytes. The potential is held constant relative to a downstream reference electrode, and the current flowing between the working and auxiliary electrodes is measured. Detection limits for amperometric electrochemical detection are 10 pg–1 ng of injected analyte.

c) Other Detectors

Several other detectors have been used in HPLC. Measuring a change in the mobile phase's refractive index is analogous to monitoring the mobile phase's thermal conductivity in gas chromatography. A refractive index detector is nearly universal, responding to almost all compounds, but has a poorer detection limit of 100 ng–1 μg of injected analyte. Furthermore, a refractive index detector is not useful for a gradient elution unless the mobile-phase components have identical refractive indexes. Another useful detector is a mass spectrometer. The advantages of using a mass spectrometer in HPLC are the same as for gas chromatography. Detection limits are quite good, typically 100 pg–1 ng of injected analyte, with values as low as 1–10 pg in some situations. In addition, a mass spectrometer provides qualitative, structural information that can help identify the analytes. The interface between the HPLC and mass spectrometer is technically more difficult than that in a GC–MS because of the incompatibility of a liquid mobile phase with the mass spectrometer's high vacuum requirement. Recent developments in mass spectrometry, however, have led to a growing interest in LC–MS.

15.31 Quantitative Applications

HPLC is routinely used for both qualitative and quantitative analyses of environmental, pharmaceutical, industrial, forensic, clinical, and consumer product samples. Below figure shows several representative examples.

i) Preparing Samples for Analysis

Samples in liquid form can be analyzed directly, after a suitable clean-up to remove any particulate materials or after a suitable extraction to remove matrix interferents. In determining polyaromatic hydrocarbons (PAH) in wastewater, for example, an initial extraction with CH_2Cl_2 serves the dual purpose of concentrating the analytes and isolating them from matrix interferents. Solid samples must first be dissolved in a suitable solvent, or the analytes of interest must be brought into solution by extraction. For example, an HPLC analysis for the active ingredients and degradation products in a pharmaceutical tablet often begins by extracting the powdered

tablet with a portion of mobile phase. Gases are collected by bubbling through a trap containing a suitable solvent. Organic isocyanates in industrial atmospheres can be determined in this manner by bubbling the air through a solution of 1-(2- methoxyphenyl)piperazine in toluene. Reacting the isocyanates with 1-(2-methoxyphenyl)piperazine serves the dual purposes of stabilizing them against degradation before the HPLC analysis while also forming a derivative that can be monitored by UV absorption.

ii) Quantitative Calculations

Quantitative analyses are often easier to conduct with HPLC than GC because injections are made with a fixed-volume injection loop instead of a syringe. As a result, variations in the amount of injected sample are minimized, and quantitative measurements can be made using external standards and a normal calibration curve.

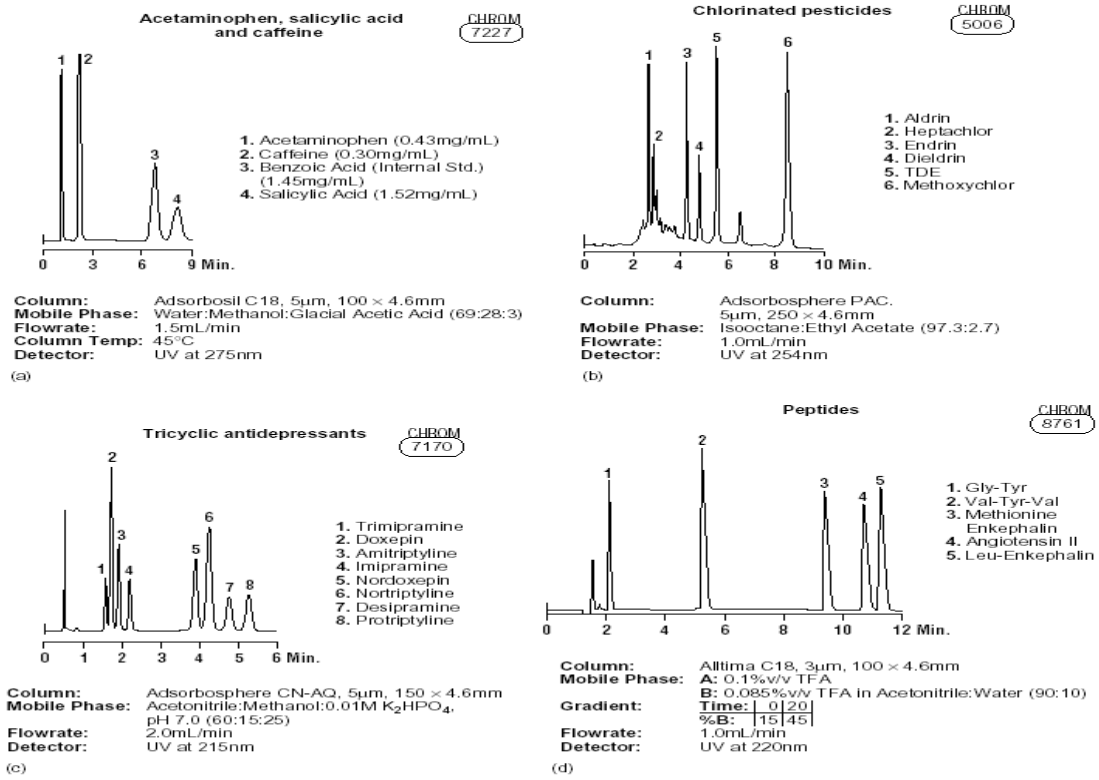

Figure 12.30
Examples of the application of HPLC to the analysis of (a) acetaminophen, salicylic acid, and caffeine; (b) chlorinated pesticides; (c) tricyclic antidepressants; and (d) peptides. (Chromatograms courtesy of Alltech Associates, Inc. Deerfield, IL).

15.32 Sumary of the unit

Gas chromatography specifically gas-liquid chromatography involves a sample being vapourised 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 column itself contains a liquid stationary

phase which is adsorbed onto the surface of an inert solid. The carrier gas must be chemically inert. Commonly used gases include nitrogen, helium, argon, and carbon dioxide. The choice of carrier gas is often dependant upon the type of detector which is used. The carrier gas system also contains a molecular sieve to remove water and other impurities.

For optimum column efficiency, the sample should not be too large, and should be introduced onto the column as a "plug" of vapour - slow injection of large samples causes band broadening and loss of resolution. The most common injection method is where a microsyringe is used to inject sample through a rubber septum into a flash vapouriser port at the head of the column. The injector can be used in one of two modes; split or splitless. The injector contains a heated chamber containing a glass liner into which the sample is injected through the septum. The carrier gas enters the chamber and can leave by three routes (when the injector is in split mode). The sample vapourises to form a mixture of carrier gas, vapourised solvent and vapourised solutes. A proportion of this mixture passes onto the column, but most exits through the split outlet. The septum purge outlet prevents septum bleed components from entering the column. There are two general types of column, packed and capillary (also known as open tubular). Packed columns contain a finely divided, inert, solid support material (commonly based on diatomaceous earth) coated with liquid stationary phase. Most packed columns are 1.5 - 10m in length and have an internal diameter of 2 - 4mm.

High performance liquid chromatography is basically a highly improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster. It also allows you to use a very much smaller particle size for the column packing material which gives a much greater surface area for interactions between the stationary phase and the molecules flowing past it. This allows a much better separation of the components of the mixture. The other major improvement over column chromatography concerns the detection methods which can be used. These methods are highly automated and extremely sensitive.

15.33 Key words

Gas Chromatography; Capillary Columns; Mobile Phase; Packed Columns; Stationary Phases; Detectors; Kovat’s retention index; High-performance Liquid Chromatography; Gradient Elution.

15.34 Reference for further study

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15.35 Questions for self understanding

1) What is gas chromatography?

2) What are the different mobile Phases used in gas chromatography?

3) Write a note on chromatographic columns

4) What are packed columns?

5) What are capillary columns?

6) Write a note on

   a) Stationary Phases

   b) Mobile phases

7) Explain detectors for gas chromatography

8) How thermal conductivity detector works?

9) How flame ionization detector work?

10) Explain the theory of electron capture detector

11) Write a note on Kovat's retention index

12) What is High-performance Liquid Chromatography (HPLC)?

13) Write a notes on columns used in HPLC

14) What are guard columns? What their roles?

15) What are the stationary phases and mobile phases used in HPLC?

16) What are the criteria follows to choosing a mobile phase in HPLC?

17) Explain Isocratic and Gradient Elution in HPLC?

18) What is HPLC Plumbing?

19) Discuss different detectors used for HPLC

Unit-16

Structure

16.0 Objectives of the unit

After studying this unit you are able to

- Explain the different electrochemical techniques
- Explain the importance of reference Electrodes
- Explain the theory and applications of Potentiometry
- Explain the importance of diffusion currents
- Explain the theory of stripping voltammetry
- Explain the theory of amperometric titrations
- Explain the theory of coulometric titrations
- Explain the theory of cyclic voltammetry

16.1 Introduction

The focus of this unit is on analytical techniques that use a measurement of potential, charge, or current to determine an analyte's concentration or to characterize an analyte's chemical reactivity. Collectively we call this area of analytical chemistry electrochemistry because its originated from the study of the movement of electrons in an oxidation–reduction reaction. Despite the difference in instrumentation, all electrochemical techniques share several common features. Before we consider individual examples in greater detail, let's take a moment to consider some of these similarities. As you work through the chapter, this overview will help you focus on similarities between different electrochemical methods of analysis. You will find it easier to understand a new analytical method when you can see its relationship to other similar methods.

16.2 Electrochemical Techniques

If a solution forms part of an electrochemical cell, the potential of the cell, the current flowing through it and its resistance are all determined by the chemical composition of the solution. Quantitative and qualitative information can thus be obtained by measuring one or more of these electrical properties under controlled conditions. Direct measurements can be made in which sample solutions are compared with standards; alternatively, the changes in an electrical property during the course of a titration can be followed to enable the equivalence point to be detected. Before considering the individual electrochemical techniques, some fundamental aspects of electrochemistry will be summarized in this section.

16. 3 Definitions

a) Electrochemical Cell

A pair of electrodes (metallic or otherwise) in contact with an electrolyte solution.

b) Galvanic or Voltaic Cell

An electrochemical cell which spontaneously produces current (or energy) when the electrodes are connected externally by a conducting wire.

c) Electrolysis or Electrolytic Cell

An electrochemical cell through which current is forced by a battery or some other external source of energy.

d) Half-cell Reactions

e) Oxidation or reduction reaction occurring at an electrode (Table 6.1).

f) Anode

The electrode at which oxidation occurs.

g) Cathode

The electrode at which reduction occurs.

Typical half-cell reactions

Reductions	Oxidations
deposition of metals	dissolution of metals
e.g. $Ag^+ + e^- = Ag$	e.g. $Cd = Cd^{2+} + 2e^-$
formation of hydrogen gas	formation of oxygen gas
$2H^+ + 2e^- = H_2$	$2H_2O = O_2 + 4H^+ + 4e^-$
	oxidation of halogens
	e.g. $2Cl^- = Cl_2 + 2e^-$
change of oxidation state	
e.g. $Fe^{3+} + e^- = Fe^{2+}$	$Sn^{2+} = Sn^{4+} + 2e^-$

h) Reversible Cell

One in which the half-cell reactions are reversed by reversing the current flow; such a cell is said to be in thermodynamic equilibrium.

i) Standard Hydrogen Electrode (SHE)

This consists of a platinum electrode coated with platinum black to catalyse the electrode reaction and over the surface of which hydrogen at 760 mm of mercury is passed. The electrode

is in contact with a solution of hydrogen ions at unit activity (1.228 M HCl at 20°C) and its potential is arbitrarily chosen to be zero at all temperatures.

j) Electrode Potential E

The potential of an electrode measured relative to a standard, usually the SHE. It is a measure of the driving force of the electrode reaction and is temperature and activity dependent (p. 230). By convention, the half-cell reaction must be written as a reduction and the potential designated positive if the reduction proceeds spontaneously with respect to the SHE, otherwise it is negative. If the sign of the potential is reversed, it must be referred to as an oxidation potential.

k) Standard Electrode Potential,

Electrode potential measured in solutions where all reactants and products are at unit activity.

l) Theoretical Cell Potential

The algebraic sum of the individual electrode potentials of an electrochemical cell at zero current, i.e.

*E*cell = *E*cathode + *E*anode. In practice, when current flows in a cell or a liquid junction is present (*vide infra*), and for certain electrode systems or reactions, the cell potential departs from the theoretical value.

m) Liquid-junction Potential

A potential developed across a boundary between electrolytes differing in concentration or chemical composition. It is caused by different rates of migration of cations and anions across the boundary thereby leading to a charge separation. Its value is often several hundredths of a volt and variable, but it can be minimized by using a *salt bridge* connection, e.g. an agar gel saturated with KCl or NH_4NO_3 for which the potential is only 1–2 mV.

n) Ohmic Drop, IR

A potential developed when a current *I* flows in an electro-chemical cell. It is a consequence of the cell resistance *R* and is given by the product *IR*. It is always subtracted from the theoretical cell potential and therefore reduces that of a galvanic cell and increases the potential required to operate an electrolysis cell.

o) Activation Overpotential (Overvoltage)

The additional potential required to cause some electrode reactions to proceed at an appreciable rate. The result of an 'energy barrier' to the electrode reaction concerned, it is substantial for gas evolution and for electrodes made of soft metals, e.g. Hg, Pb, Sn and Zn. It increases with

current density and decreases with increasing temperature, but its magnitude is variable and indeterminate. It is negligible for the deposition of metals and for changes in oxidation state.

p) Concentration Overpotential or Concentration Polarization

The additional potential required to maintain a current flowing in a cell when the concentration of the electroactive species at the electrode surface is less than that in the bulk solution. In extreme cases, the cell current reaches a limiting value determined by the rate of transport of the electroactive species to the electrode surface from the bulk solution. The current is then independent of cell potential and the electrode or cell is said to be completely polarized. Concentration overpotential decreases with stirring and with increasing electrode area, temperature and ionic strength.

16.4 Activity Dependence of Electrode Potentials – The Nernst Equation

Electrode and therefore cell potentials are very important analytically as their magnitudes are determined by the activities of the reactants and products involved in the electrode reactions. The relation between such activities and the electrode potential is given by the *Nernst equation*. For a general half-cell reaction written as a reduction, i.e. aA + bB + . . . ne = xX + yY + . . . , the equation is of the form

$$E = E^{\ominus} - \frac{RT}{nF} \ln \frac{[\mathrm{X}]^x [\mathrm{Y}]^y \ldots}{\mathrm{A}^a [\mathrm{B}]^b \ldots}$$

where E is the electrode potential, the standard potential, the activities of reactants and products at the electrode surface, R the gas constant, T the thermodynamic temperature, F the Faraday constant and n the number of electrons involved in the electrode reaction. At 298.15 K RT/F In 10= 0.059 158 V thus

$$E = E^{\ominus} - (0.059\mathrm{V}/n) \log_{10} \frac{[\mathrm{X}]^x [\mathrm{Y}]^y}{[\mathrm{A}]^a [\mathrm{B}]^b}$$

If the activities of all reactants and products are unity. Theoretical cell potentials can be calculated using tabulated values. For dilute solutions (< 10–1 M) concentrations can be used in place of activities, the error becoming insignificant below 10–3 M.

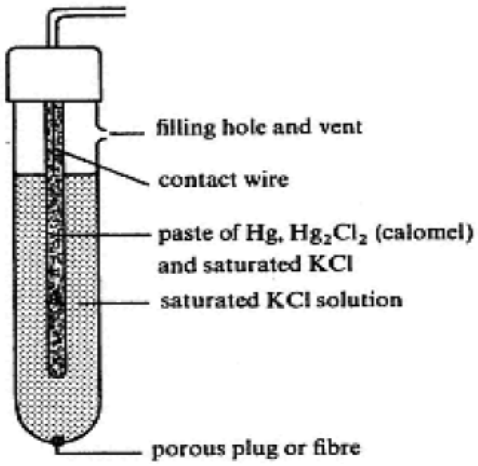
16.5 Reference Electrodes

Electroanalytical measurements relating potential or current to concentration rely on the response of one electrode only, the other ideally being independent of solution composition and

conditions. The latter is known as a reference electrode; two such electrodes having these properties and in common uses are based on calomel and silver–silver chloride respectively.

i) Calomel Electrode

The electrode consists of two concentric glass tubes, the inner one of which contains mercury in contact with a paste of mercury, mercury(I) chloride (calomel), and potassium chloride. This is in contact with a solution of potassium chloride in the outer tube which itself makes contact with the sample solution via a porous frit, fibre or ground-glass sleeve



Saturated calomel reference electrode (SCE)

The half-cell and its associated electrode reaction may be represented as,

$$Hg|KCl, Hg_2Cl_2(saturated)$$

$$Hg_2Cl_2 + 2e^- = 2Hg + 2Cl^-,\ E^\ominus = 0.242\,V$$

The electrode potential is given by and therefore depends on the chloride ion activity. The saturated calomel electrode (SCE) is the easiest to prepare, although it is more temperature-sensitive than versions employing 1 or 0.1 M potassium chloride.

$$E = 0.267\,6\,V - (0.059/2\,V)\log_{10}[Cl^-]^2$$

ii) Silver-silver Chloride Electrode

This consists of a silver wire, coated with silver chloride and in contact with a solution of potassium chloride saturated with silver chloride. The solution is contained in a tube, the end of which is sealed with a porous plug or disc to facilitate contact with the sample solution. The half-cell and associated electrode reaction is represented by

$$Ag|KCl, AgCl(saturated)$$

$$AgCl + e^- = Ag + Cl^-, E = 0.222\,V$$

The electrode potential is given by

$$E = 0.222\,V - 0.059\,V \log_{10}[Cl^-]$$

Like the calomel electrode, the saturated KCl version of this electrode is the most convenient to prepare.

16.6 Potentiometry

Principles

Measurement of the potential of a galvanic cell, usually at zero current; cell potential governed by the potential of an indicator electrode which responds to changes in the activity of the species of interest.

Instrumentation

Indicator and reference electrodes; potentiometer, pH meter or millivoltmeter; electronics and recorder for automated systems.

Applications

Quantitative determination and monitoring of many species in solution over a wide range of concentrations (10–7 – 1 M); relative precision 0.1–5%. Titrations are especially useful for coloured or turbid samples or for mixtures.

Disadvantages

Titrations are slow and time-consuming unless automated. Potentiometry is the most widely used electroanalytical technique. It involves the measurement of the potential of a *galvanic cell* , usually under conditions of zero current, for which purpose potentiometers are used. Measurements may be 'direct' whereby the response of samples and standards are compared, or the change in cell potential during a titration can be monitored.

16.7 Electrode Systems

The cell consists of an *indicator* and a *reference* electrode, the latter usually being the calomel or silversilver chloride type. The potential of the indicator electrode is related to the activities of one or more of the components of the solution and it therefore determines the overall cell potential. Ideally, its response to changes of activity should be rapid, reversible and governed by the Nernst equation. There are two types of indicator electrode which possess the desired characteristics – metallic and membrane.

a) Metallic Indicator Electrodes

Metals such as silver, copper, mercury, lead and cadmium respond to variations in the activities of their own ions in a *Nernstian* and reproducible manner, e.g. for silver, the electrode reaction is Ag+ + e– = Ag, and the electrode potential is given by

$$E = E^{\ominus} - 0.059\ \mathrm{V} \log_{10} \frac{1}{[\mathrm{Ag}^+]}$$

Iron, nickel, cobalt, tungsten and chromium do not behave reproducibly due to crystal strain or oxide coatings. Metal electrodes which respond directly to solutions of their own ions are called 'Class I' or 'first order'. Metals which form sparingly soluble salts will also respond to changes in the activity of the relevant anion provided the solution is saturated with the salt, e.g. for silver in contact with a saturated solution of silver chloride and containing solid silver chloride the electrode reaction is AgCl + e– = Ag + Cl–, and the electrode potential is given by:

$$E = E^{\ominus} - 0.059\ \mathrm{V} \log_{10}[\mathrm{Cl}^-]$$

Such electrodes are described as 'Class II' or 'second order'.

For titrations involving a change in oxidation state (redox systems) an inert electrode material such as platinum is used. The electrode potential is determined by the proportions of oxidized and reduced forms present, e.g.

$$\mathrm{Fe}^{3+} + \mathrm{e}^- = \mathrm{Fe}^{2+}$$

and

$$E = E^{\ominus} - 0.059\ \mathrm{V} \log_{10} \frac{[\mathrm{Fe}^{2+}]}{[\mathrm{Fe}^{3+}]}$$

b) Membrane or Ion-selective Electrodes

These can be subdivided into (a) glass electrodes, (b) solid-state electrodes, (c) liquid-membrane electrodes, (d) gas-sensing electrodes

The construction and mechanism for the development of activity-dependent potentials is similar for all types, although gas-sensing electrodes are constructed slightly differently from the others and incorporate an internal pH-sensitive glass electrode. The first three types consist of a tube into one end of which is sealed an electrically-conducting membrane. The tube contains a solution or gel incorporating the ion to which the electrode is to respond, and another electrolyte, usually potassium or sodium chloride. The latter, together with a silver wire in contact with the solution or gel, constitutes an internal silver-silver chloride reference electrode. The cell is completed with a second or 'external' reference electrode. On immersion of both electrodes into a solution containing the ion to be monitored, a potential develops across the membrane the magnitude of which is related to the activities of the ion of interest in the internal and external

(sample) solution. The response of many membranes is highly selective in that the *membrane potential* is a function of the activity of only one ion or a small number of ions.

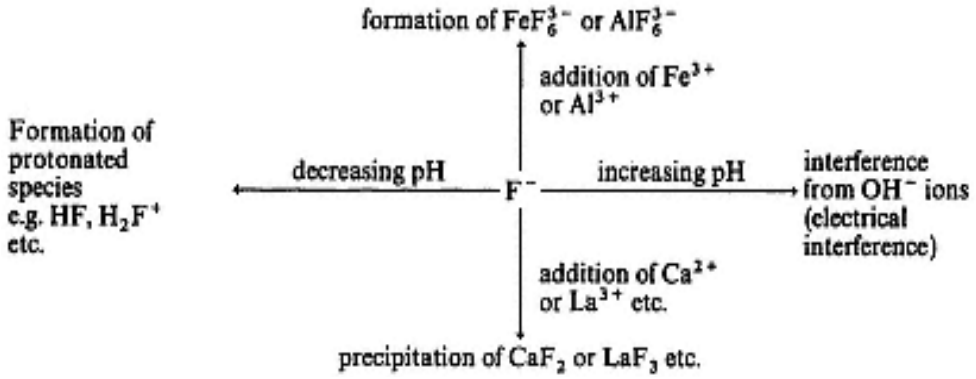
In essence, the cell comprises two reference electrodes, whose potentials are constant, separated by the membrane whose potential governs the overall cell potential. Ideally, the response will be Nernstian, and at 298.15 K the cell potential is given by

$$E_{cell} = k - \frac{0.059\,V}{n}\log_{10}\frac{a_1}{a_2}$$ where *k* is a constant including the external and internal reference electrode potentials, and *a*1 and *a*2 are the activities of the ion to be measured in the external and internal solutions respectively. As *a*2 is also constant, then

$$E_{cell} = k' - \frac{0.059\,V}{n}\log_{10} a_1$$ where *k*' includes log10 *a*2

c) Electrode Response and Selectivity

The term '*ion-selective*' is to be preferred to '*ion-specific*' in discussing membrane electrodes as most if not all are subject to the influence of ions other than the one to which they nominally respond. Interference from such ions and other species in solution may be either chemical or electrical in origin. As electrode response is a function of the *activity* of the ion to be monitored rather than concentration, reactions resulting in its partial or complete complexation, precipitation or other chemical changes will reduce the activity of the ion and consequently the electrode response, i.e. the electrode responds only to the "*free*' ion in solution. These effects are illustrated for the fluoride ion as follows:



Ions which affect the membrane potential directly will produce an apparent increase in activity of the ion to which the electrode nominally responds. In these circumstances the cell potential is more accurately given by the expression

$E_{cell} = k' - \frac{0.059}{n}\log_{10}(a_1 + k_{1,2}a_2^{n/z})$ where $a2$ is the activity of the interfering ion of charge Z, and $k1,2$ is the selectivity ratio for ion 1 over ion 2. If $k1,2$ is zero, the electrode is truly specific for ion 1, whereas values greatly in excess of one indicate that the electrode would be subject to severe interference from the second ion. As selectivity ratios are influenced by overall solution composition they should be regarded as approximations only. Furthermore, confusion can arise because reciprocal values may be quoted, e.g. if $k1,2 = 0.005$ then $k_{1,2}^{-1} = 200$

d) Glass Electrodes for pH Measurements

The membrane consists of a thin envelope of soft glass sealed into the end of a hard-glass tube. The tube is filled with a dilute solution of hydrochloric acid in which a silver wire is immersed thus forming a silver-silver chloride reference electrode. The acid also provides a solution of hydrogen ions of constant activity $a2$. The chemical composition and physical characteristics of the glass membrane are critical in determining the electrode response. Soda-glasses are highly hygroscopic, have a high electrical conductivity and show a good response whereas Pyrex glass or quartz is virtually insensitive. Typically, the composition of a suitable soda glass is 72% SiO2, 22% Na2O, and 6% CaO. The surface of the glass *must* be hydrated for the membrane to be pH sensitive. This is achieved by soaking the electrode in water or an aqueous buffer solution for several hours when the following ion-exchange reaction occurs

$$H^+(\text{solution}) + Na^+(\text{glass}) = Na^+(\text{solution}) + H^+(\text{glass})$$

The equilibrium is driven far to the right by this prolonged treatment, and results in the formation of hydrated gel layers at the inner and outer surfaces of the membrane. A diagrammatic representation of a cross-section of the membrane is shown in below Figure.

external solution $[H^+] = a_1$	hydrated gel $\leftarrow \sim 10^{-4}$ mm $\rightarrow$ $H^+ + Na^+$	dry glass layer $\leftarrow \sim 10^{-1}$ mm $\rightarrow$ Na^+ only	hydrated gel $\leftarrow \sim 10^{-4}$ mm $\rightarrow$ $Na^+ + H^+$	internal solution $[H^+] = a_2$

Cross-section of a glass membrane

Although the surfaces of the gel layers contain only hydrogen ions, the ratio [H+]/[Na+] decreases towards the dry glass layer which contains sodium ions only. Differences in hydrogen ion activity between the internal and external solutions result in a potential developing across the membrane by virtue of ion-exchange and a migration of charge. The charge is carried by sodium and hydrogen ions within the gel layers, by proton-proton exchange across the gel -solution

interfaces and by the movement of sodium ions between vacant lattice sites (defects) in the dry glass layer. The potential developed is given by an equation similar to equation E_{cell}

$$E_{membrane} = k'' - 0.059\,V \log_{10} a_{H}+$$

where *a*H+ is the activity of hydrogen ions in the external solution and *k*" contains the logarithm of the activity of the internal solution and an *asymmetry potential* which is caused by strains in the curved membrane structure. The asymmetry potential changes with time primarily because of changes in the external gel surface with use or because of contamination. The electrode therefore requires frequent calibration when used for direct measurements.

e) Errors in the Use of a Glass Electrode

Because soda-glass membranes contain a high proportion of sodium ions, they exhibit a marked response to sodium ions in solution. The effect becomes increasingly significant as the hydrogen ion activity decreases, i.e. at high pH, and it is sometimes referred to as the *alkaline error*. At pH 12, the error is about 0.3 of a pH unit if the solution is 0.1 M with respect to sodium ions, and 0.7 of a pH unit if the solution is 1 M in sodium ions. Other monovalent cations such as lithium and potassium have a similar but smaller effect. By replacing the sodium in the glass with lithium, and the calcium with strontium or barium, the error can be reduced by about one order of magnitude. So-called 'wide range' glass electrodes which have alkaline errors as low as 0.1 of a pH unit in a 2 M sodium solution are commercially available. Below pH 1, the electrode is unlikely to have a Nernstian or a reproducible response, the effect being known as the *acid error*.

f) Glass Electrodes for the Determination of Cations Other Than Hydrogen

The alkaline error shown by a soda-glass membrane has been exploited in developing membranes with a high selectivity for sodium and other cations. For example, a study of glass composition has shown that the presence of A12O3 or B2O3 can enhance the response to sodium ions relative to hydrogen ions. The membrane potential is then given by

$$E_{membrane} = k'' - 0.059\,V \log_{10}\left([H^+] + K\frac{\mu_{Na^+}}{\mu_{H^+}}[Na^+]\right)$$

where *K* is the equilibrium constant for the reaction

$$H^+(glass) + Na^+(solution) = Na^+(glass) + H^+(solution)$$

and is known as the *selectivity ratio*, μNa+ and μH+ being the mobilities of the sodium and hydrogen ions within the gel layers. For large values of *K* and at high pH, such an electrode exhibits a Nernstian response to sodium ions.

Sodium and other cation-sensitive glass electrodes are available commercially; those with a high selectivity for H+, Na+, Ag+ or Li+ have proved to be the most satisfactory.

g) Solid-state Electrodes

These incorporate membranes fabricated from insoluble crystalline materials. They can be in the form of a single crystal, a compressed disc of micro-crystalline material or an agglomerate of micro-crystals embedded in a silicone rubber or paraffin matrix which is moulded in the form of a thin disc. The materials used are highly insoluble salts such as lanthanum fluoride, barium sulphate, silver halides and metal sulphides. These types of membrane show a selective and Nernstian response to solutions containing either the cation or the anion of the salt used. Factors to be considered in the fabrication of a suitable membrane include solubility, mechanical strength, conductivity and resistance to abrasion or corrosion.

In all cases the mechanism by which the membrane responds to changes in the activity of the appropriate ion in the external solution is one of ionic conduction. Lattice defects in the crystals allow small ions with a low formal charge a degree of mobility within the membrane. It is this shift in charge centres which gives rise to the membrane potential, the process resembling the movement of sodium ions in the dry glass layer of a glass membrane. A fluoride electrode, in which the membrane is a single crystal of lanthanum fluoride doped with europium to increase the conductivity, is one of the best ion-selective electrodes available. Conduction through the membrane is facilitated by the movement of F– ions between anionic lattice sites which in turn is influenced by the F– ion activities on each side of the membrane. If the electrode is filled with a standard solution of sodium fluoride, the membrane potential is a function of the fluoride activity in the sample solution only. Thus, $E_{\text{cell}} = k' - 0.059\log_{10} a_{F^-}$ where *k* incorporates the logarithm of the activity of the internal fluoride solution. The electrode has a Nernstian response down to 10–5 M F– (0.19 ppm) and selectivity ratios of less than 0.001 for all other anions except OH–.

h) Liquid Membrane Electrodes

Two types have been developed. One utilizes liquid ion-exchangers with a selective response to certain polyvalent cations or anions. The other involves the selective complexing ability of univalent cations by neutral macro-cyclic antibiotics and cyclic polyethers. In earlier versions of both types, a waterimmiscible liquid supported on an inert porous plastic disc formed the membrane but gradual leakage and dissolution of the membrane liquid necessitated the frequent replenishment of a reservoir inside the electrode body. Later versions employ PVC-gelled

membranes incorporating the appropriate liquid and these behave more like a solid-state membrane, no replenishment of the liquid being required during use.

i) Gas-sensing Electrodes

The concentration of gases such as CO2, NH3, SO2 and NO2 in aqueous solutions can be measured with an electrode consisting of a glass electrode/reference electrode pair inside a plastic tube which is sealed with a thin gas-permeable membrane and containing an appropriate electrolyte solution. The membrane consists of a microporous hydrophobic plastic film through which only gases can diffuse. On immersion of the electrode in a solution containing a dissolved gas (CO2, NH3, SO2 or NO2), rapid diffusion through the membrane occurs until an equilibrium is established in which the gas concentrations are the same in the internal electrolyte and the sample solution. Any change of the gas concentration in the internal electrolyte solution results in a change in its pH which in turn is sensed by the glass/reference pair. Such changes are directly proportional to the concentration of the gas in the sample solution.

The following equations summarize the equilibria involved for a CO2- sensing electrode which utilizes a sodium hydrogen carbonate solution as the internal electrolyte:

$$CO_2\ (\text{sample}) \rightleftharpoons CO_2\ (\text{membrane})$$
$$CO_2\ (\text{membrane}) \rightleftharpoons CO_2\ (\text{internal electrolyte})$$
$$CO_2\ (\text{internal electrolyte}) + 2H_2O \rightleftharpoons H_3O^+ + HCO_3^-$$

The overall reaction may be represented as:

$$CO_2(\text{sample}) + 2H_2O \rightleftharpoons H_3O^+ + HCO_3^-\ (\text{internal elecrolyte})$$

whence

$$K_e = \frac{[H_3O^+][HCO_3^-]}{CO_2\ (\text{sample})]}$$

By using a high concentration of HCO_3^- in the internal electrolyte $[HCO_3^-]$, can be considered constant

$\therefore CO_2\ (\text{sample})] = k'[H_3O^+] = k'a_{H^+}$ where k' includes Ke and the response of the electrode is given by

$$E_{\text{membrane}} = k'' - 0.059V \log_{10} [CO_2\ (\text{sample})]$$

and k'' includes k'.

Thus, the pH response of the glass/reference pair is a function of the CO2 content of the sample.

Similar equations for an ammonia-sensing electrode whose internal electrolyte is an ammonium salt lead to the relation $[NH_3\ (\text{sample})] = k'[OH^-]$ and the pH response of the glass/electrode pair

is a function of the NH3 concentration in the sample. Gas-sensing electrodes differ from ion-selective electrodes in that no species in solution can interfere with the electrode response as only gases can diffuse through the membrane. However, it should be noted that any gas which causes a pH change in the internal electrolyte solution will affect electrode response.

16.8 Polorography

Principles

Measurement of the diffusion-controlled current flowing in an electrolysis cell in which one electrode is polarizable. The current is directly proportional to the concentration of an electroactive species.

Instrumentation Micro-electrode (dropping mercury carbon or platinum), reference electrode, variable dc source, electronics and recorder.

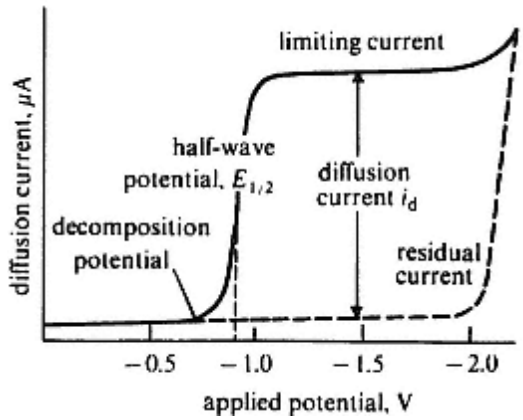
Applications Quantitative and qualitative determination of metals and organic compounds at trace levels (10–4 to 10^{-8} M); relative precision 2–3%. Amperometric titrations are more versatile and more precise than polarography.

Disadvantages

Measurements very sensitive to solution composition, dissolved oxygen and capillary characteristics. Impurities in background electrolyte limit sensitivity.

The study of current–potential relations in an electrolysis cell where the current is determined solely by the rate of diffusion of an electroactive species is called *voltammetry*. To obtain diffusion-controlled currents, the solution must be unstirred and the temperature of the cell thermostatically controlled so as to eliminate mechanical and thermal convection. In addition, a high concentration of an electrochemically inert *background* or *supporting* electrolyte is added to the solution to suppress the migration of electroactive species towards the electrodes by electrostatic attraction. Typically, the cell comprises a mercury or platinum micro-electrode, which is readily polarizable, and a calomel or mercury-pool reference electrode, which is non-polarizable. By using a small polarizable electrode, conditions can readily be attained wherein the diffusion current is independent of applied potential and directly proportional to the concentration of electroactive species in the bulk solution. Measurement of such *limiting currents* forms the basis of quantitative analysis. The polarizable micro-electrode is usually made the cathode at which the electroactive species is reduced. The most widely used electrode is the dropping mercury electrode DME and the technique involving its use is known as *polarography*.

A plot of current flowing in the cell as a function of the applied potential is called a *polarogram* or a *polarographic wave* (Figure 6.9). At small applied potentials, only a *residual current* flows in the cell caused by the reduction of trace impurities in the sample solution and by charging of the mercury drops. The charging effect is analogous to the behaviour of a condenser. Above the *decomposition potential* , at which point reduction of an electroactive species is initiated, the current increases with applied potential, until it levels off at a limiting value. The difference between the limiting value and the residual current is known as the diffusion current, *i*d. If, on increasing the applied potential further, other species in the solution are reduced, additional polarographic waves will be observed. Finally, the current will increase due to reduction of the supporting electrolyte or of the electrode



Polarographic wave or polarogram

material. In each polarographic wave, the potential at which the diffusion current reaches half the limiting value is known as the half-wave potential *E*1/2 and is characteristic of the particular electroactive species involved. It is therefore useful for qualitative identification.

16.9 Diffusion Currents

When the potential applied to a polarographic cell exceeds the decomposition potential of an electroactive species, its concentration at the surface of the mercury drop is immediately diminished. A concentration gradient is thereby established and more of that species diffuses from the bulk solution to the electrode surface (Fick's law of diffusion). The resulting current flow is proportional to the rate of diffusion which in turn is determined by the concentration gradient, i.e. $i = k(C - C_o)$

where *C* and *C*o are the concentrations of the electroactive species in the bulk solution and at the surface of the DME respectively. By progressively increasing the applied potential, reduction

occurs more rapidly, *C*o eventually becomes virtually zero, and the concentration gradient reaches a maximum. At this point, the rate of diffusion, and therefore the current flowing in the cell, reaches a limiting value, i.e. $i_d = kC$ Further increases in the applied potential do not increase the current and the cell is said to be completely polarized or operating under conditions of high concentration overpotential. The diffusion current *i*d is hence directly proportional to the bulk concentration of the electroactive species.

16.10 Half-wave Potentials

Electroactive species are characterized by their $E_{1/2}$ values which are constants related to the standard electrode potentials and given by the equation

$$E_{\frac{1}{2}} = E^{\ominus} - \frac{0.059\,\text{V}}{n} \log \frac{k}{k_r} - E_{\text{reference}}$$

where *k* and *k*r are proportionality constants relating cell current to the rates of diffusion of oxidized and reduced forms of the electroactive species. Values are independent of the bulk concentration *C* but depend on the composition of the supporting electrolyte which can affect if complexes are formed. The components of a mixture will give separate polarographic waves if the $E_{1/2}$ values differ by at least 0.1 V. Often, if two waves overlap, by careful choice of a complexing agent, the and hence the $E_{1/2}$ value of one component can be changed by up to a volt or more. For example, in a potassium chloride solution Fe(III) and Cu(II) waves overlap whereas in fluoride medium, the value of $E_{1/2}$ for the anion is 0.5 V more negative, the Cu(II) wave being unaffected. Alkali metals which are reduced at potentials around –2.0 V can be determined using a tetraalkylammonium salt as the supporting electrolyte. Some other examples of the effect of complexing are given in below Table.

Ion	No complexing agent $E_{1/2}$/V	KCN $E_{1/2}$/V	NH_3 $E_{1/2}$/V
Cu^{2+}	+0.02	*	–0.24 and –0.51
Zn^{2+}	–1.00	*	–1.35
Cd^{2+}	–0.59	–1.18	–0.81
Pb^{2+}	–0.40	–0.72	—

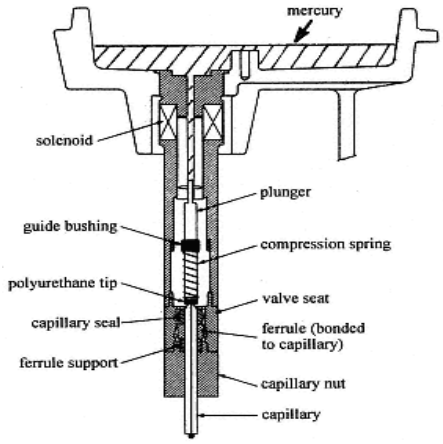
* Not reducible

16.11 Characteristics of the DME

A diagrammatic representation of a DME is shown in Figure 6.10. Mercury from a reservoir is forced through a narrow-bore glass capillary (~ 0.06 mm bore) by gravity. A succession of identical drops is formed which are detached at regular intervals (3–8s), timing and reproducibility of drop dimensions being ensured by electrical control. This characteristic of the DME results in an oscillating cell current, the average of which is given by the Ilkovic equation

$$i_d = AnD^{\frac{1}{2}}m^{\frac{2}{3}}t^{\frac{1}{6}}C$$

when n denotes the number of farads, D is the diffusion coefficient for the electroactive species, m is the rate of flow of mercury, t is the drop time, C is the concentration in the bulk solution, and A is a constant. The equation is useful in comparing diffusion currents from electrodes with different capillary characteristics, i.e. different values of $m^{\frac{2}{3}}t^{\frac{1}{6}}$. The diffusion coefficient D is temperature sensitive to the extent of about 2.5% per kelvin; so



Dropping mercury electrode. that it is essential to control the cell temperature to ± 0.1 K if the highest precision is required.

In practice, a DME has certain advantages over other micro-electrodes:

(1) The surface is continually and reproducibly renewed so that its past history is unimportant.

(2) A reproducible average current is produced instantly on changing the applied potential.

(3) Mercury has a large activation overpotential for hydrogen formation which facilitates the reduction of many species in acid solution. Its use is restricted to the determination of reducible or easily oxidized species as at positive applied potentials greater than 0.4 V with respect to the SCE, mercury dissolves to give an anodic polarographic wave.

Current Maxima Polarographic waves often show a peak followed by a sharp fall to the limiting current plateau, the cause of which is related to streaming of the solution past the mercury drop. Known as a 'current maximum', it can be eliminated by adding a surfactant such as gelatin or methyl-red to the sample solution.

Oxygen Waves

Two waves caused by the reduction of dissolved oxygen can interfere with the waves of other electroactive species unless the solution is purged with nitrogen prior to obtaining the polarogram

$$\left.\begin{array}{l} O_2 + 2H^+ + 2e^- = H_2O_2 \\ \text{or} \quad O_2 + 2H_2O + 2e^- = H_2O_2 + 2OH^- \end{array}\right\} \begin{array}{l} E_{\frac{1}{2}} = -0.05\,\text{V} \\ \text{with respect to SCE} \end{array}$$

$$\left.\begin{array}{l} H_2O_2 + 2H^+ + 2e^- = 2H_2O \\ \text{or} \quad H_2O_2 + 2e^- = 2OH^- \end{array}\right\} \begin{array}{l} E_{\frac{1}{2}} = -0.9\,\text{V} \\ \text{with respect to SCE} \end{array}$$

The reactions have been utilized for the determination of dissolved oxygen or hydrogen peroxide.

16.12 Quantitative Analysis

Either calibration graphs prepared from standards or the method of standard addition (p. 30) can be used. For the former, the standards should be as similar as possible in overall chemical composition to that of the samples so as to minimize errors caused by the reduction of other species or by variation in diffusion rates. Often, the limiting factor for quantitative work is the level of impurities present in the reagents used.

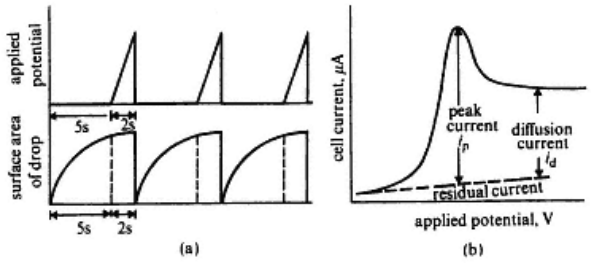
16.13 Modes of Operation Used in Polarography

The earliest types of polarograph involved the manual changing of a dc applied potential stepwise and plotting it against corresponding current values. Later versions automated this process so that a chart recording could be obtained. More recently, *oscillographic* or *rapid scan* polarography, *pulse* polarography, *stripping voltammetry* and *ac* polarography were introduced primarily to increase sensitivity and to facilitate the resolution of closely spaced polarographic waves. The first three of these techniques have found more application than the last.

a) Linear Sweep Oscillographic Polarography

A repetitive dc potential sweep of 0.5 to 1.0 V is synchronized with the growth of each mercury drop and the resulting current-potential curve displayed on the screen of a cathode-ray oscilloscope. Because the current increases with the surface area of the drop, the potential sweep is timed to occupy the last 2 s of a 7 s drop-life where the relative change of surface area is

minimal. Exact synchronization is achieved mechanically by tapping the capillary every 7 s to detach the drop. Figure 6.11(a) shows the variation of the drop area and applied potential as a function of time, and Figure 6.11(b) the current– potential curve as seen on the oscilloscope screen. The peak current *i*p is not a polarographic maximum but is caused by the rapid potential sweep; it is however directly proportional to the concentration of the electroactive species. A twin-cell version of this type of instrument enables the polarogram of a blank solution to be automatically subtracted



Oscillographic polarography.

(a) Variation of drop area and applied potential with time.

(b) Oscillographic display of a polarogram

from that of the sample solution before display on the oscilloscope screen. Alternatively, by having the same sample solution in both cells and using sophisticated electronic circuitry, first and second derivative curves can be displayed. This enables components with *E*1/2 values as little as 25 mV apart to be separated, but some sensitivity is sacrificed. Twin-cell operation suffers from the difficulty of maintaining a pair of exactly matched capillaries.

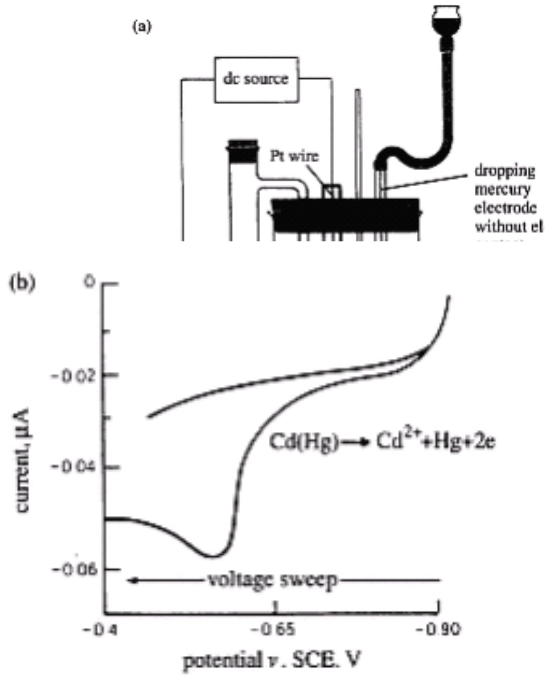
b) Differential Pulse Polarography

Pulse polarography seeks to minimize the contribution to measured cell currents arising from charging of the mercury drops (p. 248). This is achieved by superimposing a 10–100 mV pulse of short duration (40–60 ms) onto the usual increasing dc potential. The pulse is applied during the last quarter of the growth of each drop and the diffusion current is measured just before the pulse and again during the last 20 ms. The difference between the two measured currents for each drop is plotted as a function of the dc potential. The timing sequence of operations and the resulting differential polarogram are shown in above Figure. Synchronization of the pulse with each drop is achieved in a similar manner to that used in *linear sweep oscillographic polarography*. The height of the peak maximum is directly proportional to the concentration of

the electroactive species and it is possible to discriminate between species with half-wave potentials differing by only 0.05 V. Apart from suppression of charging current, sensitivity in pulse polarography is enhanced relative to conventional dc polarography because the application of a voltage pulse to each drop produces higher currents than are obtained when diffusion alone controls the electrochemical reaction.

16.14 Stripping Voltammetry

This is a modification of the polarographic technique whereby extremely low levels of certain metals electrode by cathodic deposition then determined by *anodic stripping*. The sample solution is electrolysed for an accurately measured period of time during which reducible species are deposited at a stationary mercury drop or mercury-coated solid electrode. The polarity of the cell is then reversed and a linear potential sweep, sometimes pulsed, is applied to produce anodic polarographic waves of the sample components concentrated in the mercury electrode. The initial pre-concentration or preelectrolysis step must take place under carefully controlled and reproducible conditions, as only a small fraction of the electroactive species is deposited at the micro-electrode. Thus, variations in preelectrolysis time, stirring rate, temperature, electrode potential and current density between samples and standards must be eliminated as far as possible. The ultimate sensitivity of the method is determined by the length of the pre-electrolysis time which can be up to an hour or more. However, by employing a differential pulse technique during the subsequent anodic stripping rather than a linear potential sweep, equivalent sensitivities can be obtained with pre-electrolysis times of a few minutes or less, and with better reproducibility. A diagram of an anodic stripping instrument and a typical stripping trace are shown in Figure 6.13. Metals which form amalgams with mercury, e.g. Pb, Cd, Cu, Zn and Sn, are readily determined by this technique but the ultimate sensitivity is limited by the level of impurities present in reagents and the background electrolyte solution. Nevertheless, concentrations in the range 10–8–10–9M (ppb) can be determined under favourable conditions and methods have been devised for Pb in blood and traces of Cu, Cd, Pb and Zn in drinking water are first pre-concentrated at a stationary,



(a) Apparatus for stripping analysis.

(b) Curve A: Current–voltage curve for anodic stripping of cadmium. Curve B: Residual current curve for blank.(Reprinted with permission and adapted from R. D. DeMars and I. Shain, Anal. Chem. ***29****, 1825 (1957). Copyright by the American Chemical Society)*

16.15 The Dissolved Oxygen Electrode and Biochemical Enzyme Sensors

There is a longstanding demand for a simple and portable instrument for the detection and measurement of oxygen dissolved in water. Suitable electrodes have been developed and more recently have been ingeniously used as the basis for a range of biochemical sensors.

a) Principles of Dissolved Oxygen Measurement

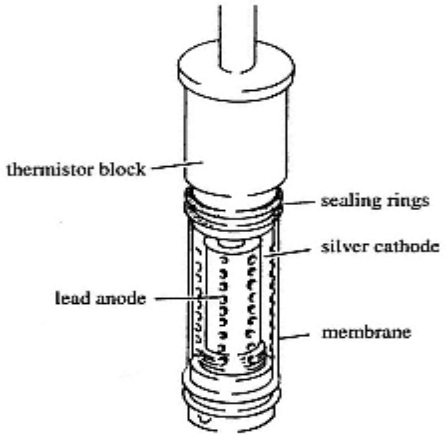
A typical commercial electrode is based on a galvanic cell comprising a lead anode and a silver cathode. In an alkaline electrolyte (e.g. 1 M KOH) the following reaction will occur if a reducible species is present,

$$Pb(s) + 4OH^-(aq) = PbO_2^{2-}(aq) + 2H_2O + 2e^-$$

If the electrodes are surrounded by a semipermeable membrane through which only oxygen can diffuse from the external solution, reduction of oxygen will take place

$$O_2(aq) + 4e^- + 2H_2O = 4OH^-(aq)$$

The current flowing in the cell will depend upon the rate of diffusion of oxygen to the electrode surface and ultimately on the concentration of oxygen in the external solution. The construction of such an electrode is shown in below Figure.



Construction of an EIL temperature compensated oxygen electrode (from EIL advertising material).

b) Applications of the Dissolved Oxygen Electrode

Electrodes are capable of measurement at around the 1 ppm level. They are simple, robust and portable. As such there is considerable potential for their employment in ecological studies and they can provide constant monitoring of dissolved oxygen, e.g. in river waters. The potential for their employment in monitoring industrial liquors is clear, as is their applications in biological investigations of oxygen transport.

c) Principles of Enzyme Sensors

The biochemical oxidation of organic molecules is accomplished by enzymes known as oxidases. Specific oxidases exist for the oxidation of each compound. Oxidases require oxygen to react, and so decrease the amount of oxygen in their immediate environment. The degree of oxygen depletion is hence related to the enzyme activity, which is in turn determined by the amount of the oxidizable species present. Use of a dissolved oxygen electrode to measure the degree of oxygen depletion will thus provide a basis for the measurement of the amount of the oxidizable species which is present. Specially designed electrodes in which the electrode tip is covered in a gel containing the appropriate oxidase are commercially available. A semipermeable membrane retaining the gel, and permitting oxygen diffusion, completes the assembly.

d) Applications of Enzyme Sensors

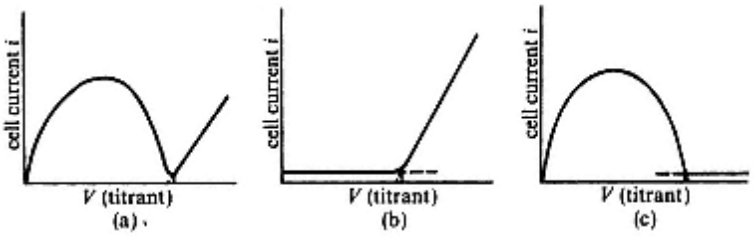
The type of enzyme sensor described above is highly selective and can be sensitive in operation. There are obvious applications for the determination of small amounts of oxidizable organic compounds. However, it is perhaps too early to give a realistic assessment of the overall importance of enzyme sensors to analytical chemistry. This is especially so because of parallel developments in other biochemical sensors which may be based upon a quite different physical principle. Commercially available kits allow for the determination of compounds such as ethyl alcohol, glucose, lactic acid and lactose.

16.16 Amperometric Titrations

If the limiting current flowing in a polarographic cell is measured during a titration in which any of the reactants or products are reducible at the micro-electrode, the equivalence point can readily be detected. The choice of applied potential is not critical provided it corresponds to a point on the limiting current plateau. Values of *i*d are simply plotted as a function of the volume of titrant added. Three types of titration curve may be observed, as shown in Figure 6.15(a), (b) and (c). In each case, the equivalence point is located at the intersection of two straight lines. Curvature in this region is due to partial dissociation of the products of the titration but as current readings can be taken well away from the equivalence point, accuracy is not impaired (cf. potentiometric and visual indicator methods). Titrations which produce curves similar to that shown in Figure 6.15(a) are sometimes referred to as 'dead-stop' methods because the current falls virtually to zero at the equivalence point. The most accurate determinations can be made if the curve is V-shaped (Figure 6.15(c)). Unless the titrant is at least twenty times as concentrated as the analyte solution, current readings must be corrected for volume changes by multiplying each one by the ratio $(V + \upsilon)/V$, where V is the solution volume before adding an increment of titrant of volume υ. An alternative to the DME is the rotating platinum micro-electrode. Steady currents can be obtained by rotating the electrode at a constant speed of 600 min^{-1} or more. These are generally some twenty times larger than the currents derived from a DME because of mechanical convection created by the rotation. The rotating electrode is particularly useful for redox titrations involving such species as Br_2 and Fe(III) but the low hydrogen activation overpotential for platinum limits its applications to alkaline or weakly acid conditions. It is very sensitive to the presence of dissolved oxygen.

16.17 Bi-amperometric Titrations

Two identical stationary micro-electrodes (usually platinum) across which a potential of 0.01–0.1 V is applied can be used in place of either the DME or the rotating platinum micro-electrode. The equivalence point is marked by a sudden rise in current from zero, a decrease to zero, or a minimum at or near zero (Figures 6.16(a), (b) and (c)). The shape of the curve depends on the reversibility of the redox reactions involved. The two platinum electrodes assume the roles of anode and cathode, and in all cases a current flows in the cell only if there is a significant concentration of both the oxidized and reduced forms of one of the reactants. In general, two types of system can be envisaged:



Bi-amperometric titration curves.

(a) Both electrode reactions reversible, e.g. Fe^{2+} against Ce^{4+}.

(b) Only titrant reaction is reversible, e.g. against I_2.

(c) Only substance titrated is reversible, e.g. I_2 against $Na_2S_2O_3$.

(1) *Both reactants behave reversibly*. The curve is as shown in Figure (a) and is exemplified by the titration of Fe(II) with Ce(IV) when a potential of 0.1 V is applied to the cell. At the outset, no current flows because only Fe(II) is present, the only electrode reaction possible being

$Fe^{2+} \rightarrow Fe^{3+} + e^-$ at the

anode. Upon the addition of the first increment of titrant and up to the equivalence point, the concentration of Fe(II) diminishes, whilst that of Fe(III) increases, and a current proportional to the smaller of the two concentrations flows in the cell, resulting in a maximum at the half-way stage. At the equivalence point only Fe(III) and Ce(III) are present and no current flows because neither electrode reaction Fe(III) $\rightarrow$ Fe(II) nor Ce(III) $\rightarrow$ Ce(IV) can proceed at the potential of 0.1 V. After the equivalence point, the current increases linearly with the rise in concentration of Ce(IV), that of Ce(III) being constant.

(2) *Only one reactant behaves reversibly*. If the titrant alone behaves reversibly, no current can flow until it is in excess (Figure (b)). This is the case if is titrated with I_2 at an applied potential

of 0.1 V. After the equivalence point the current is linearly related to the concentration of excess I_2, that of I– being constant. In cases where only the analyte forms a reversible couple, e.g. I_2 titrated with $Na_2S_2O_3$, the current before the equivalence point follows a similar path to that in the Fe(II)/Ce(IV) system, but afterwards remains at zero (Figure (c)).

A 'dead-stop' titration curve is produced if Ag+ is titrated with a halide using a pair of identical silver electrodes. Only whilst both Ag+ and Ag are present will a current flow in the cell, and this is linearly related to the Ag+ concentration. Bi-amperometric titrations require only simple equipment but generally give poorer precision because the currents measured are not necessarily on the limiting current plateau. Amperometric titrations are inherently more precise than polarography and are more generally applicable because the analyte need not itself be electroactive. Titrations involving the DME are not affected by changes in capillary characteristics as are conventional polarographic determinations, whilst working at a predetermined temperature is unnecessary provided that it remains reasonably constant throughout the titration.

16.18 Applications of Polarography

Most metal ions are reducible at the DME, and multicomponent mixtures can often be analysed by selecting an appropriate supporting electrolyte and complexing agent. Polarography is used for the determination of trace metals in alloys, ultra-pure metals, minerals, foodstuffs, beverages and body fluids although recently it has been largely superseded by atomic absorption spectrometry. It is ideally suited to the determination of metal impurities in AnalaR and other high-purity salts where the sample matrix can act as its own supporting electrolyte. Reducible anions such as can also be determined provided solutions are well buffered. Organic applications are restricted by an inability to distinguish between members of a homologous series and by the irreversibility of many reductions at the DME. Solutions must be well buffered as many electrode reactions are pH dependent. The limited aqueous solubility of most organic compounds necessitates the use of polar solvents such as glycols, dioxane, formamide and methyl cellosolve with lithium or tetraalkylammonium salts as the supporting electrolyte. Reducible organic functional groups include ketones, aldehydes, alkenes, nitriles, azo, peroxy, disulphide, nitro, nitroso and nitrite. Examples of the applications of polarography are given in the below table.

Table : Applications of polarography

Element or compound determined	Type of sample
Cu, Pb, Sn, Zn	foodstuffs
Ga, Zn, Cd, Ni	high-purity aluminium
Cu, Pb, Ni, Co	steels
Mo, Ge, As, Sb	minerals and ores
Sn, Pb	beer and soft drinks
transition metals	high-purity salts
free sulphur	petroleum fractions
antioxidants	fuels
riboflavin	milk, pharmaceuticals
antibiotics, steroids	body fluids
vitamin C	fruit and vegetables
oxygen	seawater, gases

16.19 COULOMETRY

Principles

Measurement of the quantity of electricity used in an electrochemical reaction at constant potential or constant current.

Instrumentation

Working and counter-electrodes; source of constant current and timer, or a potentiostat and integrator; equivalence point detection system.

Applications

Quantitative determination of many species in solution, especially at trace levels (micrograms or less); relative precision 0.2–5%. Useful for unstable titrants and easy to automate.

Disadvantages

Constant potential methods can be lengthy; constant current methods have similar disadvantages to other titrimetric techniques. Coulometric methods of analysis involve measuring the quantity of electricity required to effect a quantitative chemical or electrochemical reaction and are based on Faraday's laws of electrolysis:

(1) The quantity of a substance liberated at an electrode during electrolysis is proportional to the quantity of electricity passing through the solution.

(2) A given quantity of electricity liberates different substances in the ratio of their molar masses divided by the number of electrons involved in the corresponding electrode reactions.

The electrolysis cell consists of a *working-electrode*, at which the species to be determined is reduced or oxidized or at which a chemically reactive species is formed, and a *counter-electrode*. In practice electrolysis may be at *constant potential*, in which case the current diminishes to zero as the reaction goes to completion, or at *constant current*. The quantity of electricity involved in the former is measured by means of a chemical coulometer or by integrating the area under the current-time curve. Constant current methods involve the generation of a titrant for a measured length of time, the completion of the reaction with the species to be determined being indicated by any of the methods used in titrimetric analysis. For this reason, such procedures are described as *coulometric titrations*. In both constant potential and constant current coulometry, the current efficiency must be 100%, i.e. all the electricity passing through the cell must be utilized in a reaction involving the species to be determined, either directly or indirectly.

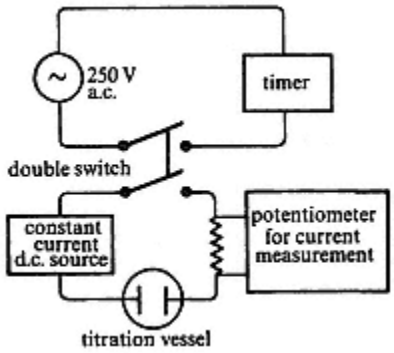
16.20 Coulometry at Constant Potential

The technique is similar to electrogravimetry at constant cathode potential , but is much more versatile because the substance to be determined need not form a stable, adherent deposit on the electrode. The quantity of electricity consumed in the reaction is determined by connecting a chemical coulometer in series with the cell or by using an electronic or electro-mechanical integrator. Chemical coulometers take the form of electrolysis cells where the total amount of product(s) liberated at the electrode(s) can readily be measured. The simplest to use are gas coulometers, in which the amount of hydrogen and oxygen (or nitrogen) liberated from a suitable electrolyte solution (e.g. sodium sulphate) is measured in a gas burette. During the analysis close control of the cathode (or anode) potential with a potentiostat (p. 261) is essential to avoid electrode reactions other than that related to the species of interest. A convenient working-electrode is a mercury cathode at which numerous metals and organic compounds can be reduced. Examples include the determination of lead in cadmium, nickel in cobalt and trichloroacetic acid in the presence of the mono- and dichloro-derivatives. Precisions of better than 1% are easily attained, but analyses can take up to an hour or more

16.21 Coulometric Titrations

The titrant is generated at a working electrode by the passage of a constant current until the equivalence point is indicated by potentiometric, amperometric or, less commonly, visual or

photometric means. An accurate timer is required to enable the total quantity of electricity used to be calculated. A schematic diagram of a coulometric titrator is shown in Figure 6.17. Constant-current sources, often just a battery, should be capable of delivering currents of 10–100 mA with an accuracy of 0.5% or better. Electric timers with solenoid-operated brakes or electronic timers should be used to eliminate cumulative errors arising from starting and stopping the motor, a sequence which may be repeated many times as the equivalence point is approached.



Circuit diagram for coulometric titrations. Device for external generation of H^+ and OH^-.

The titrant is usually generated directly in the solution whence it can immediately react with the species to be determined. The electrodes are typically made of platinum coils or sheets. The counter-electrode is normally isolated from the bulk solution by enclosing it in a tube sealed with a sintered-glass disk to prevent contamination of the solution with undesirable electrolysis products. An alternative arrangement, especially useful for acid-base titrations, is to generate the reagent externally before adding it to the titration vessel. An apparatus for generating and separating hydrogen and hydroxyl ions using an electrolyte solution such as sodium sulphate is shown in the below Figure. To eliminate errors due to impurities in reagents used in the preparation of the sample, a 'pre-titration' method is sometimes used. A blank solution is first titrated, the sample is added to the titrated solution and the solution titrated again. This procedure is particularly advantageous for determinations at very low levels.

16.22 Applications of Coulometric Titrations

The coulometric generation of titrants is widely applicable to redox, precipitation, acid-base and complexing reactions. Of particular value is the determination of many organic compounds with bromine and of mercaptans and halides with the silver ion. Amperometric equivalence point detection is the most common. An attractive feature of the technique is that the need to store standard and possibly unstable reagent solutions is obviated. In fact many applications involve

the use of electrogenerated reagents such as halogens and chromium(II) which are difficult or impossible to store. The technique is especially useful for the determination of very small amounts, i.e. in the microgram or nanogram ranges. Relative precisions of 0.2–5% can be attained at trace levels, which is better than most other Titrimetric procedures. Some examples of titrations with electrogenerated titrants are given in the below Table.

Applications of coulometric titrations at constant current

Element or compound determined	Titrant generated	Means of equivalence point detection	Example of application
Water	I_3^- (Karl–Fischer reagent)	amperometric	organic solvents, petroleum products
Cr, V	Fe^{2+}	amperometric	steels, oils, asphalt
Ag	I^-	amperometric	lubricating oils
phenols, olefins	Br_2	amperometric	petroleum products
thymol	Br_2	amperometric	herbs
mercaptans, chloride	Ag^+	amperometric	fuels
aromatic amines	H^+	potentiometric	organic chemicals
oxygen	Cr^{2+}	amperometric	seawater, gases

16.23 Cyclic Voltammetry (CV)

Cyclic Voltammetry (CV) is an electrochemical technique which measures the current that develops in an electrochemical cell under conditions where voltage is in excess of that predicted by the Nernst equation. CV is performed by cycling the potential of a working electrode, and measuring the resulting current.

The potential of the working electrode is measured against a reference electrode which maintains a constant potential, and the resulting applied potential produces an excitation signal such as that of figure 1. In the forward scan of figure 1, the potential first scans negatively, starting from a greater potential (a) and ending at a lower potential (d). The potential extrema (d) is call the switching potential, and is the point where the voltage is sufficient enough to have caused an oxidation or reduction of an analyte. The reverse scan occurs from (d) to (g), and is where the potential scans positively. Figure 1 shows a typical reduction occurring from (a) to (d) and an oxidation occurring from (d) to (g). It is important to note that some analytes undergo oxidation

first, in which case the potential would first scan positively. This cycle can be repeated, and the scan rate can be varied. The slope of the excitation signal gives the scan rate used.

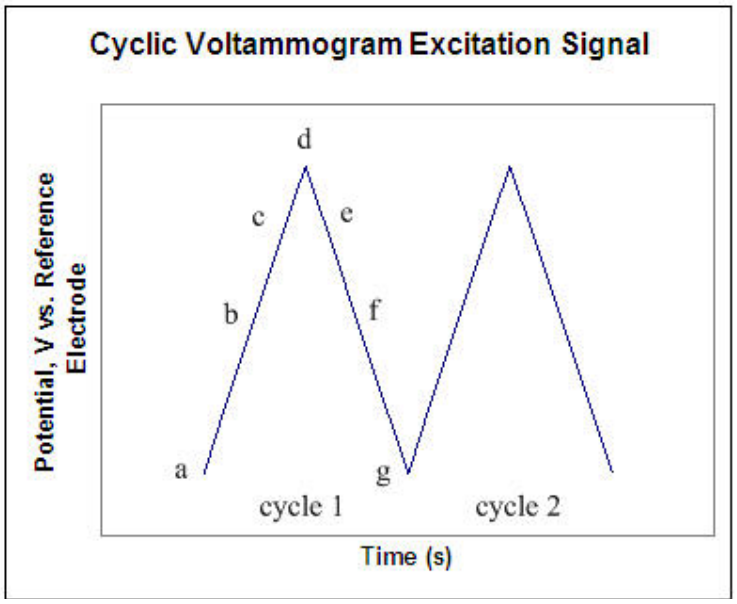


Figure 1: CV Excitation Signal

A cyclic voltammogram is obtained by measuring the current at the working electrode during the potential scans.[2] Figure 2 shows a cyclic voltammogram resulting from a single electron reduction and oxidation. Consider the following reversible reaction:

$$M^{+} + e^{-} \rightleftharpoons M$$

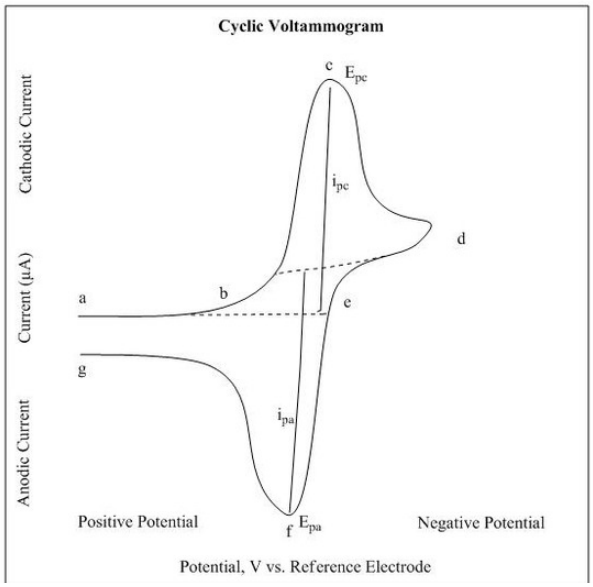


Figure 2: Voltammogram of a Single electron oxidation-reduction

In Figure 2, the reduction process occurs from (a) the initial potential to (d) the switching potential. In this region the potential is scanned negatively to cause a reduction. The resulting current is called cathodic current (i_{pc}). The corresponding peak potential occurs at (c), and is called the cathodic peak potential (E_{pc}). The Epc is reached when all of the substrate at the surface of the electrode has been reduced. After the switching potential has been reached (d), the potential scans positively from (d) to (g). This results in anodic current (I_{pa}) and oxidation to occur. The peak potential at (f) is called the anodic peak potential (E_{pa}), and is reached when all of the substrate at the surface of the electrode has been oxidized.

Electrode potential (*E*)

$$E = E_i + vt \text{ ---------(1)}$$

where

E_i is the initial potential in volts,

v is the sweep rate in volts/s, and

t is the time in seconds.

When the direction of the potential sweep is switched, the equation becomes,

$$E = E_s - vt\text{----------(2)}$$

Where E_s is the potential at the switching point. Electron stoichiometry (*n*)

$$E_p - E_{\frac{P}{2}} > \frac{0.056}{n} \quad \text{----------} \quad \text{-(3)}$$

where

E_{pa} is the anodic peak potential,

E_{pc} is the cathodic peak potential, and

n is the number of electrons participating in the redox reactions.

Formal Reduction Potential (E°') is the **mean** of the E_{pc} and E_{pa} values:

$$E^{0'} = \frac{E_{pa} + E_{pc}}{2}$$

In an unstirred solution, mass transport of the analyte to the electrode surface occurs by diffusion alone.[1] Fick's Law for mass transfer diffusion relates the distance from the electrode (x), time (t), and the reactant concentration (CA) to the diffusion coefficient (DA).

$$\frac{\partial cA}{\partial t} = D_A \frac{\partial^2 cA}{\partial x^2} \text{ ---------- (4)}$$

During a reduction, current increases until it reaches a peak: when all M^+ exposed to the surface of an electrode has been reduced to M. At this point additional M^+ to be reduced can travel by diffusion alone to the surface of the electrode, and as the concentration of M increases, the distance M+ has to travel also increases. During this process the current which has peaked, begins to decline as smaller and smaller amounts of M+ approach the electrode. It is not practical to obtain limiting currents Ipa, and Ipc in a system in which the electrode has not been stirred because the currents continually decrease with time.[1]

In a stirred solution, a Nernst diffusion layer ~10^{-2} cm thick, lies adjacent to the electrode surface. Beyond this region is a laminar flow region, followed by a turbulent flow region which contains the bulk solution.[1] Because diffusion is limited to the narrow Nernst diffusion region, the reacting analytes cannot diffuse into the bulk solution, and therefore Nernstian equilibrium is maintained and diffusion-controlled currents can be obtained. In this case, Fick's Law for mass transfer diffusion can be simplified to give the peak current

$$i_p = \left(2.69x10^5\right) n^{\frac{3}{2}} S D_A^{\frac{1}{2}} V^{\frac{1}{2}} C_A^* \text{ ----------(5)}$$

Here, (n) is equal to the number of electrons gained in the reduction, (S) is the surface area of the working electrode in cm^2, (DA) is the diffusion coefficient, (v) is the sweep rate, and (CA) is the molar concentration of A in the bulk solution.

Instrumentation

A CV system consists of an electrolysis cell, a potentiostat, a current-to-voltage converter, and a data acquisition system. The electrolysis cell consists of a working electrode, counter electrode, reference electrode, and electrolytic solution. The working electrode's potential is varied linearly with time, while the reference electrode maintains a constant potential. The counter electrode conducts electricity from the signal source to the working electrode. The purpose of the electrolytic solution is to provide ions to the electrodes during oxidation and reduction. A potentiostat is an electronic device which uses a dc power source to produce a potential which can be maintained and accurately determined, while allowing small currents to be drawn into the system without changing the voltage. The current-to-voltage converter measures the resulting current, and the data acquisition system produces the resulting voltammogram.

Applications

Cyclic Voltammetry can be used to study qualitative information about electrochemical processes under various conditions, such as the presence of intermediates in oxidation-reduction reactions, the reversibility of a reaction. CV can also be used to determine the electron stoichiometry of a system, the diffusion coefficient of an analyte, and the formal reduction potential, which can be used as an identification tool. In addition, because concentration is proportional to current in a reversible, Nernstian system, concentration of an unknown solution can be determined by generating a calibration curve of current vs. concentration.

16.23 Summary of the unit

Electrochemical analyses can be thought of in terms of two broad classes of measurement, one in which the potential that develops between two electrodes is measured (potentiometry) and another in which the current that flows between two electrodes is measured (amperometry). In potentiometry, it often proves helpful to arrange things such that the current is very low (e.g., by placing a high-resistance voltmeter in series between two electrodes). The electrochemical potential of one electrode (the reference electrode) is usually fixed, so the measured cell potential can be interpreted in terms of an equilibrium half-cell reaction involving an analyte species in contact with the other electr ode (the working electrode). In favorable cases, one can use data from potentiometric measurements to calculate analyte concentrations directly from the Nernst equation. Potentiometry is a simple and straightforward analytical method, and is routinely used to solve many problems in the analysis of electrochemically active and/or charged analytes.

16.24 Keywords

Electrochemical Techniques; Reference Electrodes; Potentiometry; Polorography; Diffusion Currents; Half-wave Potentials; Stripping Voltammetry; Amperometric Titrations; Amperometric Titrations; Coulometric Titrations; Cyclic Voltammetry (CV).

16.25 References for further study

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6) Handbook of Electrochemistry; Cynthia G. Zoski; *Elsevier*, **2007**.

16.26 Questions for self understanding

1) What are the different electrochemical techniques used in analytical chemistry?

2) Write a note on Activity Dependence of Electrode Potentials -The Nernst Equation

3) What are reference electrodes? Give two examples

4) Discuss Potentiometry method

5) Discuss Polorography method

6) What is diffusion Currents?

7) What is Half-wave Potentials?

8) What are the characteristics of the DME?

9) Explain modes of operation used in Polarography

10) Discuss stripping voltammetry method

11) Write a note on

a) The Dissolved Oxygen Electrode

b) Biochemical Enzyme Sensors

12) Expalin Amperometric Titrations

13) Discuss Bi-amperometric Titrations

14) Explain the applications of Polarography

15) What is coulometry?

16) Explain coulometry at constant potential

17) Discuss coulometric Titrations

18) What are the applications of coulometric Titrations?

19) Discuss cyclic voltammetry (CV)