Semester-V

16CHU503B ANALYTICAL METHODS IN CHEMISTRY 4H 4C

Instruction Hours/week: L:4 T:0 P:0 Marks: Internal: 40 External: 60 Total:100

Scope

The course deals with various analytical methods in chemistry like Qualitative and quantitative aspects of analysis, Optical methods of analysis, Thermal methods of analysis and electroanalytical methods, separation techniques and various instrumentation methods.

Objectives

This course enable the student to

1. Understand the various Qualitative and quantitative aspects of analysis
2. Understand the different Optical methods of analysis
3. Understand the various methods of Thermal and electroanalytical methods
4. Understand various chromatographic separation techniques
5. Understand the various instrumentation method of analysis.

Methodology

Blackboard teaching, Powerpoint presentation and group discussion.

UNIT I

Qualitative and quantitative aspects of analysis:

Sampling, evaluation of analytical data, errors, accuracy and precision, methods of their expression, normal law of distribution if indeterminate errors, statistical test of data; F, Q and t test, rejection of data, and confidence intervals.

UNIT II

Optical methods of analysis:

Origin of spectra, interaction of radiation with matter, fundamental laws of spectroscopy and selection rules, validity of Beer-Lambert's law.

UV-Visible Spectrometry: Basic principles of instrumentation (choice of source, monochromator and detector) for single and double beam instrument;

Basic principles of quantitative analysis: estimation of metal ions from aqueous solution, geometrical isomers, keto-enol tautomers. Determination of composition of metal complexes using Job's method of continuous variation and mole ratio method.

Infrared Spectrometry: Basic principles of instrumentation (choice of source, monochromator & detector) for single and double beam instrument; sampling techniques.

Structural illustration through interpretation of data, Effect and importance of isotope substitution.

Flame Atomic Absorption and Emission Spectrometry: Basic principles of instrumentation (choice of source, monochromator, detector, choice of flame and Burner designs. Techniques of atomization and sample introduction; Method of background correction, sources of chemical interferences and their method of removal. Techniques for the quantitative estimation of trace level of metal ions from water samples.

UNIT III

Thermal methods of analysis:

Theory of thermogravimetry (TG), basic principle of instrumentation.
Techniques for quantitative estimation of Ca and Mg from their mixture.

Electroanalytical methods:

Classification of electroanalytical methods, basic principle of pH metric, potentiometric and conductometric titrations. Techniques used for the determination of equivalence points. Techniques used for the determination of pKa values.

UNIT IV

Separation techniques:

Solvent extraction: Classification, principle and efficiency of the technique.

Mechanism of extraction: extraction by solvation and chelation. Technique of extraction: batch, continuous and counter current extractions. Qualitative and quantitative aspects of solvent extraction: extraction of metal ions from aqueous solution, extraction of organic species from the aqueous and nonaqueous media. Chromatography: Classification, principle and efficiency of the technique. Mechanism of separation: adsorption, partition & ion exchange. Development of chromatograms: frontal, elution and displacement methods.

Qualitative and quantitative aspects of chromatographic methods of analysis: IC, GLC, GPC, TLC and HPLC.

UNIT V

Separation and analysis: Measurement of optical rotation, calculation of Enantiomeric excess (ee)/ diastereomeric excess (de) ratios and determination of enantiomeric composition using NMR, Chiral solvents and chiral shift reagents. Chiral chromatographic techniques using chiral columns (GC and HPLC). Role of computers in instrumental methods of analysis.

Suggested Readings

Text Books:

1. Christian, G.D. (2014). *Analytical Chemistry*, 6th Ed. New York: John Wiley & Sons.
2. Harris, D. C. (2011). *Exploring Chemical Analysis*. Ed. New York: W.H. Freeman.
3. Khopkar, S.M. (2009). *Basic Concepts of Analytical Chemistry*. New Age, International Publisher.

Reference Books

1. Skoog, D.A., Holler F.J. & Nieman, T.A.(2006). *Principles of Instrumental Analysis*, Cengage Learning India Ed.
2. Mikes, (1979). O. *Laboratory Hand Book of Chromatographic & Allied Methods*, Elles Harwood Series on Analytical Chemistry. John Wiley & Sons.

Karpagam Academy of Higher Education

(Deemed to be University)
(Established Under Section 3 of UGC Act 1956)

DEPARTMENT OF CHEMISTRY

LECTURE PLAN

Name of the Staff : **Dr. M. Gopalakrishnan** Department: Chemistry
Title of the Paper : **ANALYTICAL METHODS IN CHEMISTRY**
Paper Code : **16CHU503B** Class: III-B.Sc Chemistry
Year and Semester : 2016–2019 and V-Semester
Total hours : 48 Hours

S.No	Lecture hour	Topics	Support material
		UNIT-I	
1	1	Sampling, evaluation of analytical data.	T1: 1-8
2	1	Errors, accuracy and precision	T1:13-15; 19-24
3	1	Electrolytic Reduction, methods of their expression	T1: 325; 293-295
4	1	Normal law of distribution if indeterminate errors	T1:14-16
5	1	Statistical test of data; F, Q and t test	T1: 13-18; 31
6	1	Rejection of data	T1: 28-30
7	1	Confidence intervals	T1: 28-30
8	1	Recapitulation and discussion of important questions	
Hours required			**8**

UNIT-II			
1.	1	Fundamental laws of spectroscopy and selection rules	T2: 9-10
2.	1	Basic principles of instrumentation UV-Visible Spectrometry	T2: 12-14, 15
3.	1	Basic principles of quantitative analysis	T2: 36-38
4.	1	Estimation of metal ions from aqueous solution, geometrical isomers and Keto-enol tautomers	W1: 1-2
5.	1	Determination of composition of metal complexes using Job's method	T2:52-54
6.	1	Basic principles of instrumentation of Infrared Spectrometry	T2:69-70
7.	1	Sampling techniques for Infrared Spectrometry	T2:71-76
8.	1	Structural illustration through interpretation of data	T2:83-90
9.	1	Effect and importance of isotope substitution.	T2:88-90
10.	1	Basic principles of instrumentation for Flame Atomic Absorption and Emission Spectrometry	T2:88-90, R1:110
11.	1	Techniques for the quantitative estimation of trace level of metal ions from water samples	T2:268, R1: 171
12.	1	Recapitulation and discussion of important questions	
Hours required			**12**

UNIT-III			
1	1	Introduction of thermogravimetry analysis	T1: 113-118
2	1	Basic principle of instrumentation of thermogravimetry	R2: 143-146
3	1	Techniques for quantitative estimation of Ca and Mg from their mixture	T3:137-138
4	1	Classification of electroanalytical methods.	T1: 307-309, 140
5	1	Basic principle of pH metric, potentiometric and conductometric titrations	T1: 316-318
6	1	Techniques used for the determination of equivalence points	T1: 331-332
7	1	Techniques used for the determination of pKa values	T1:310-316
8	1	Recapitulation and discussion of important questions	
Hours required			**8**
UNIT-IV			
1	1	Introduction of Separation techniques	T1: 279-280
2	1	Solvent extraction: Classification, principle and efficiency of the technique	T1: 280-285
3	1	Mechanism of extraction: extraction by solvation and chelation.	T1: 282-283
4	1	Technique of extraction:Batch, continuous and counter current extractions.	T2: 243-246
5	1	Extraction of metal ions from aqueous solution, extraction of organic species from the aqueous and nonaqueous media.	T1: 280
6	1	Classification, principle and efficiency of the chromatography technique	T1: 285-286
7	1	Mechanism of separation: adsorption, partition and ion exchange	T1: 287

8	1	Development of chromatograms: frontal, elution and displacement methods	T1: 293-295
9	1	Qualitative and quantitative aspects of chromatographic methods of analysis: IC, GLC	T1: 290-293
10	1	Recapitulation and discussion of important questions	
Hours required			**10**
UNIT-V			
1	1	Separation and analysis: Measurement of optical rotation	T2:232-235
2	1	Calculation of Enantiomeric excess (ee)/ diastereomeric excess (de) ratios	T2:235-239
3	1	Determination of enantiomeric composition using NMR, Chiral solvents and chiral shift reagents	T3:524-526
4	1	Chiral chromatographic techniques using chiral columns GC	T2:272-273
5	1	Chiral chromatographic techniques using chiral columns HPLC	T2:263-271
6	1	Role of computers in instrumental methods of analysis	T2:263-271
7	1	Recapitulation and discussion of important questions	
8	1	ESE Question paper discussion	
9	1	ESE Question paper discussion	
10	1	ESE Question paper discussion	
Hours required			**10**

Text Books:

T1. R.M. Verma, 2012, Analytical chemistry theory and Practical, Thired edition..

T2. V. Gopalan, P.S. Subramanian and Ragarajan,2013. Elemental analysis. S.Chand and company.

T3. S. Usharani, 2002, analytical chemistry Mac-Millan Indian Ltd. Chennai

Reference Books:

R1. A.I. Vogel, 2012, Vogel's Quantitative inorganic analysis .

R2. W. Galen,Ewiry, 2000, Intrumental method of Chemical analysis.

Website: W1:https://www.masterorganicchemistry.com/2010/04/12/keto-enol-tautomerism

Qualitative and quantitative aspects of analysis

Sampling, evaluation of analytical data, errors, accuracy and precision, methods of their expression, normal law of distribution if indeterminate errors, statistical test of data; F, Q and t test, rejection of data, and confidence intervals

Qualitative and Quantitative Analysis

There is seldom a unique way to design a measurement process. Even anexplicitly defined analysis can be approached in more than one ways. Different studies have di¤erent purposes, di¤erent financial constraints, and are carried out by sta¤ with di¤erent expertise and personal preferences. The most important step in a study design is the determination of the purpose, and at least a notion of the final results. It should yield data that provide useful information to solve the problem at hand. The objective of an analytical measurement can be qualitative or quantitative. For example, the presence of pesticide in fish is a topic of concern. The questions may be: Are there pesticides in fish? If so, which ones? An analysis designed to address these questions is a qualitative analysis, where the analyst screens for the presence of certain pesticides. The next obvious question is: How much pesticide is there? This type of analysis, quantitative analysis, not only addresses the presence of the pesticide, but also its concentration. The other important category is semiqualitative analysis. the concern is not exactly how much is there but whether it is above or below a certain threshold level. The prostate specific antigen (PSA) test for the screening of prostate cancer is one such example. A PSA value of 4 ng/L (or higher) implies a higher risk of prostate cancer. The goal here is to determine if the PSA is higher or lower then 4 ng/L. Once the goal of the analyses and target analytes have been identified, the methods available for doing the analysis have to be reviewed with an eye to accuracy, precision, cost, and other relevant constraints. The amount of labor, time required to perform the analysis, and degree of automation can also be important.

Table 1.1. Common Instrumental Methods and the Necessary Sample Preparation Steps Prior to Analysis

Analytes	Sample Preparation	Instrument[a]
Organics	Extraction, concentration, cleanup, derivatization	GC, HPLC, GC/MS, LC/MS
Volatile organics	Transfer to vapor phase, concentration	GC, GC-MS
Metals	Extraction, concentration, speciation	AA, GFAA, ICP, ICP/MS
Metals	Extraction, derivatization, concentration, specia-tion	UV-VIS molecular absorp-tion spectrophotometry, ion chromatography
Ions	Extraction, concentration, derivatization	IC, UV-VIS
DNA/RNA	Cell lysis, extraction, PCR	Electrophoresis, UV-VIS, florescence
Amino acids, fats carbohydrates	Extraction, cleanup	GC, HPLC, electrophoresis
Microstructures	Etching, polishing, reac-tive ion techniques, ion bombardments, etc.	Microscopy, surface spectros-copy

SAMPLING AND DATA ANALYSIS

Analysis of the properties of a food material depends on the successful completion of a number of different steps: planning (identifying the most appropriate analytical procedure), sample selection, sample preparation, performance of analytical procedure, statistical analysis of measurements, and data reporting. Most of the subsequent chapters deal with the description of various analytical procedures developed to provide information about food properties, whereas this chapter focuses on the other aspects of food analysis.

A food analyst often has to determine the characteristics of a large quantity of food material, such as the contents of a truck arriving at a factory, a day's worth of production, or the products stored in a warehouse. Ideally, the analyst would like to analyze every part of the material to obtain an accurate measure of the property of interest, but in most cases this is practically impossible. Many analytical techniques destroy the food and so there would be nothing left to sell if it were all analyzed. Another problem is that many analytical techniques are time consuming, expensive or labor intensive and so it is not economically feasible to analyze large

amounts of material. It is therefore normal practice to select a fraction of the whole material for analysis, and to assume that its properties are representative of the whole material. Selection of an appropriate fraction of the whole material is one of the most important stages of food analysis procedures, and can lead to large errors when not carried out correctly.

Once we have selected a sample that represents the properties of the whole population, we must prepare it for analysis in the laboratory. The preparation of a sample for analysis must be done very carefully in order to make accurate and precise measurements.

Making Samples Homogeneous

The food material within the *sample* selected from the *population* is usually heterogeneous, *i.e.*, its properties vary from one location to another. Sample heterogeneity may either be caused by variations in the properties of different units within the sample (*inter-unit* variation) and/or it may be caused by variations within the individual units in the sample (*intra-unit* variation). The units in the sample could be apples, potatoes, bottles of ketchup, containers of milk etc. An example of inter-unit variation would be a box of oranges, some of good quality and some of bad quality. An example of intra-unit variation would be an individual orange, whose skin has different properties than its flesh. For this reason it is usually necessary to make samples *homogeneous* before they are analyzed, otherwise it would be difficult to select a representative *laboratory sample* from the *sample*. A number of mechanical devices have been developed for homogenizing foods, and the type used depends on the properties of the food being analyzed (*e.g.*, solid, semi-solid, liquid). Homogenization can be achieved using mechanical devices (*e.g.*, grinders, mixers, slicers,blenders), enzymatic methods (*e.g.*, proteases, cellulases, lipases) or chemical methods (*e.g.*, strong acids, strong bases, detergents).

Reducing Sample Size

Once the sample has been made homogeneous, a small more manageable portion is selected for analysis. This is usually referred to as a *laboratory sample,* and ideally it will have properties which are representative of the population from which it was originally selected. Sampling plans often define the method for reducing the size of a sample in order to obtain reliable and repeatable results.

Preventing Changes in Sample

Once we have selected our sample we have to ensure that it does not undergo any significant changes in its properties from the moment of sampling to the time when the actual analysis is carried out, *e.g.*, enzymatic, chemical, microbial or physical changes. There are a number of ways these changes can be prevented.

Enzymatic Inactivation. Many foods contain active enzymes they can cause changes in the properties of the food prior to analysis, *e.g.*, proteases, cellulases, lipases, etc. If the action of one of these enzymes alters the characteristics of the compound being analyzed then it will lead to erroneous data and it should therefore be inactivated or eliminated. Freezing, drying, heat treatment and chemical preservatives (or a combination) are often used to control enzyme activity, with the method used depending on the type of food being analyzed and the purpose of the analysis.

Lipid Protection. Unsaturated lipids may be altered by various oxidation reactions. Exposure to light, elevated temperatures, oxygen or pro-oxidants can increase the rate at which these reactions proceed. Consequently, it is usually necessary to store samples that have high unsaturated lipid contents under nitrogen or some other inert gas, in dark rooms or covered bottles and in refrigerated temperatures. Providing that they do not interfere with the analysis antioxidants may be added to retard oxidation.

Microbial Growth and Contamination. Microorganisms are present naturally in many foods and if they are not controlled they can alter the composition of the sample to be analyzed. Freezing, drying, heat treatment and chemical preservatives (or a combination) are often used to control the growth of microbes in foods.

`Physical Changes. A number of physical changes may occur in a sample, *e.g.*, water may be lost due to evaporation or gained due to condensation; fat or ice may melt or crystallize; structural properties may be disturbed. Physical changes can be minimized by controlling the temperature of the sample, and the forces that it experiences.

Sample Identification

Laboratory samples should always be labeled carefully so that if any problem develops its origin can easily be identified. The information used to identify a sample includes: a) Sample

description, b) Time sample was taken, c) Location sample was taken from, d) Person who took the sample, and, e) Method used to select the sample. The analyst should always keep a detailed notebook clearly documenting the sample selection and preparation procedures performed and recording the results of any analytical procedures carried out on each sample. Each sample should be marked with a *code* on its label that can be correlated to the notebook. Thus if any problem arises, it can easily be identified.

Data Analysis and Reporting

Food analysis usually involves making a number of repeated measurements on the same sample to provide confidence that the analysis was carried out correctly and to obtain a best estimate of the value being measured and a statistical indication of the reliability of the value. A variety of statistical techniques are available that enable us to obtain this information about the laboratory sample from multiple measurements.

Measure of Central Tendency of Data

The most commonly used parameter for representing the overall properties of a number of measurements is the *mean*

$$\bar{x} = \frac{x_1 + x_2 + x_3 + \ldots x_n}{n} = \frac{\sum_{i=1}^{n} x_i}{n}$$

Here n is the total number of measurements, x_i is the individually measured values and is the mean value.

The mean is the *best experimental estimate* of the value that can be obtained from the measurements. It does not necessarily have to correspond to the *true* value of the parameter one is trying to measure. There may be some form of systematic error in our analytical method that means that the measured value is not the same as the true value (see below). *Accuracy* refers to how closely the *measured* value agrees with the *true* value. The problem with determining the accuracy is that the true value of the parameter being measured is often not known. Nevertheless, it is sometimes possible to purchase or prepare standards that have known properties and analyze these standards using the same analytical technique as used for the unknown food samples. The absolute errorE_{abs}, which is the difference between the true value (x_{true}) and the measured value

(x_i), can then be determined: $E_{abs} = (x_i - x_{true})$. For these reasons, analytical instruments should be carefully maintained and frequently calibrated to ensure that they are operating correctly

Measure of Spread of Data

The *spread of the data is* a measurement of how closely together repeated measurements are to each other. The *standard deviation* is the most commonly used measure of the spread of experimental measurements. This is determined by assuming that the experimental measurements vary randomly about the mean, so that they can be represented by a normal distribution. The standard deviation *SD* of a set of experimental measurements is given by the following equation:

$$SD = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}}$$

Measured values within the specified range

SD means 68% values within range (x - *SD*) to (x + *SD*)

2*SD* means 95% values within range (x - 2*SD*) to (x + 2*SD*)

3*SD* means >99% values within range (x - 3*SD*) to (x + 3*SD*)

DATA and ERROR ANALYSIS

Performing the experiment and collecting datais only the beginning of the process of completing anexperiment in science. Understanding the results ofany given experiment is always the central goal of theexperiment. Presenting those results in a clear concisemanner completes the experiment. This overview ofthe complete process is as valid in an instructionallaboratory course as in a research environment. Youwill not have learned any physics if you did notunderstand the experiment. Presenting the results ofyour experimental work is fundamentally saying,"This is what I did and this is what I learned."Putting together your presentation of the results shouldhelp you clarify the results to yourself. (If yourinstructor can clearly see what you did and what youlearned, you might get a better grade.)

Data analysis should NOT be delayed untilall of the data is recorded. Take a low point, a highpoint and maybe a middle point, and do a quickanalysis and plot. This will help one avoid theproblem of spending an entire class collecting bad databecause of a mistake in experimental procedure or anequipment failure.

First and foremost, data analysis meansunderstanding what your results mean. Whenanalyzing your data, try to think through the physicalprocesses which are occurring. Write your train ofthought down. Ultimately, the goal is for you tounderstand physics and the world a bit better. Yourunderstanding of your results probably occurs instages, with each stage being a refinement and possiblymore mathematical than the previous stage.

For example, one might first note that as timeincreases so does distance. Next a quick graph ofdistance vs time might verify this understanding butthe relationship is NOT linear, i.e. the data does notform a straight line. By further work, one mightdiscover that distance increase linearly with the squareof the time. Or sometimes the mathematicalrelationship may remain hidden.

Relate each successive stage of yourunderstanding and interpretation of your results tothe physical principles that are involved. In theabove example, one might note that the change inposition with time is caused by velocity that is in turncaused by acceleration from the gravitational force.Finally, develop the related mathematics. Equationsare nearly meaninglyunless they are related to thephysical laws. (Remember to identify all the variablesand constants in you equations.)

Sometimes, your results will not supportand may even contradict the physical explanationsuggested by the manual or your instructor. Sayso! But of course then a few suggestions as to thereason for this apparent failure of the physical laws,would be in order. Do NOT just say “The equipmentwas a piece of short Try to explain what went wrongor what competing effects have come into play.

One of the reasons that you are encouraged torecord everything that is going on as it is going on, isthat this information may help explain bad results. Forexample, partly for fun, you note each time your labpartner sneezes. Later while looking at the data, youdiscover that each data point that was being collectedduring a sneeze deviates from the pattern of the rest ofthe data. This may give you good reason for dropping"bad" data.

The quality of the data, determines to agreat extent, what conclusions can be reached fromthem. If you are looking for a small effect, say a totalchange of 1 mm, and the uncertainties in your data is 2mm then you really cannot make any solidconclusions. (See the section on error analysis below.)When one considers the quality of ameasurement there are two aspects to

consider. Thefirst is if one were to repeat the measurement, howclose would new results be to the old, i.e., howreproducible is the measurement? Scientists refer tothis as the precision of the measurement.Secondly, a measurement is considered "good"if it agrees with the true value. This is known as theaccuracy of the measurement. But there is a potentialproblem in that one needs to know the "true value" todetermine the accuracy.

A good measurement must be close to the"true value" and be reproducible. In thisexperiment, if someone made one measurement of g2 and got 9.79 m/s, it would be an accuratemeasurement. But if next time they tried they got 4.12 m/s, no one would believe that they were anything butlucky in the first measurement. Similarly, if one group2 got values of 7.31, 7.30, 7.33, and 7.29 m/s theirresults are reproducible but not really very good.

Mean and standard deviation

One of the best ways to assess the reliability of the precision of a measurement is to repeat the measurement several times and examine the different values obtained.Ideally, all the repeating measurements should give the same value, but in reality the results deviate from each other. Ideally, for a more precise result many replicatemeasurements should be done, however cost and time usually limit the number of replicate measurements possible. Statistics treats each result of a measurement as anitem or individual and all the measurements as the sample. All possible measurements, including those which were not done, are called the population.The basic parameters that characterize a population are the mean, m, and thestandard deviation, s. In order to determine the true m and s, the entire populationshould be measured, which is usually impossible to do.

Terms	Definition*
Absolute standard deviation, s	$s = \sqrt{\frac{\sum_{i=1}^{N}(x_i - \bar{x})^2}{N-1}}$
Relative standard deviation (RSD)	$\text{RSD} = \frac{s}{\bar{x}}$
Standard error of the mean, s_m	$s_m = s/\sqrt{N}$
Coefficient of variation (CV)	$\text{CV} = \frac{s}{x} \times 100\%$
Variance	s^2

In practice, measurement ofseveral items is done, which constitutes a sample. Estimates of the mean and thestandard deviation are calculated and denoted by x and s, respectively. The valuesof x and s are used to calculate confidence intervals, comparison of precisionsand significance of apparent discrepancies. The mean, x, and the standard deviation,s, of the values x1, x2, . . . , xn obtained from n measurements is given by theequations:

$$\bar{x} = \frac{x_1 + x_2 + \ldots + x_n}{n} \tag{2.1a}$$

$$s = \sqrt{\left(\frac{(x_1 - \bar{x})^2 + (x_2 - \bar{x})^2 + \ldots + (x_n - \bar{x})^2}{n-1}\right)} \tag{2.2a}$$

These equation can be written in a shorter way using the Σ notation:

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n} \tag{2.1b}$$

$$s = \sqrt{\left(\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}\right)} = \sqrt{\left(\frac{\sum_{i=1}^{n} x_i^2}{n-1}\right) - \frac{n(\bar{x})^2}{n-1}} = \sqrt{\left(\frac{\sum_{i=1}^{n} x_i^2}{n-1} - \frac{\left(\sum_{i=1}^{n} x_i\right)^2}{n(n-1)}\right)} \tag{2.2b}$$

In some older books the use of the term 'average' instead of 'mean' (Youden1994), can be found, but the common term nowadays is 'mean'. There are differentkinds of 'means' (Woan 2000) (e.g. arithmetic mean, harmonic mean), but if not explicitly written the 'mean' is meant to be the arithmetic mean as defined byEquation (2.1).There are several reasons why the arithmetic mean and not the other ones ischosen. The main reason is because it is the simplest one:The mean of the sum of squares of the deviation of the observed data from the mean is called the variance:The division by (n 1) and not by n is done because we do not know the true value of x, i.e. m, and instead we used the calculated value of x. For the calculation of x,we use one degree of freedom (one unit of information), and this is the reason that we divide by (n 1) (the number of degrees of freedom, i.e. the number of freeunits of information which were left). The dimension of the variance, V, is the square of the dimension of our observation and in order to get the same dimension we take the square root of V, which is called thestandard deviation, s. In many cases the variance is not denoted byV, but is written as s2.

$$\text{Arithmetic mean:} \quad \overline{x}_a = \frac{1}{n}(x_1 + x_2 + \ldots + x_n)$$

$$\text{Geometric mean:} \quad \overline{x}_g = (x_1 \times x_2 \times x_3 \times \ldots \times x_n)^{1/n}$$

$$\text{Harmonic mean:} \quad \overline{x}_h = n\left(\frac{1}{x_1} + \frac{1}{x_2} + \ldots + \frac{1}{x_n}\right)^{-1}$$

$$\sum_{j=1}^{n} (x_j - \overline{x}_a)^2 = \text{minimum}$$

$$\overline{x} = \overline{x}_a = \frac{x_1 + x_2 + \ldots + x_n}{n}$$

$$V = \frac{(x_1 - \overline{x})^2 + (x_2 - \overline{x})^2 + \ldots + (x_n - \overline{x})^2}{n - 1}$$

$$s = \sqrt{\left(\frac{(x_1 - \overline{x})^2 + (x_2 - \overline{x})^2 + \ldots + (x_n - \overline{x})^2}{n - 1}\right)} = \sqrt{\left(\frac{n\Sigma x_i^2 - (\Sigma x_i)^2}{n(n-1)}\right)}$$

The values of x and s can be calculated using a computer program or a calculator.It is important to note that all scientific calculators have two keys, one depicted as sn and the other one as sn1. Equation (2.2) fits the key sn1. The other key usesn instead of (n 1) in Equation (2.2). The key sn1 gives the standard deviation of our sample, but not of the whole population, which can be obtained by doing aninfinite number of repeated measurements. In other words, sn is the standard deviation if the true mean m is known. Otherwise, one degree of freedom is lost on theCalculation of x. For a small number of repetitions, the equation with (n 1) gives a better estimate of the true s, which is unknown. The mean x is a better estimatefor the true value than one measurement alone. The standard deviation s (or its estimate s) represents the dispersion of the measured values around the mean. Thestandard deviation has the same dimension as that of the measured values, xi often; analysts prefer to use a dimensionless quantity to describe the dispersion ofthe results.

In this case they use the relative standard deviation as a ratio (SV) (also called the coefficient of variation, CV) or as a percentage (RSD):Analytical chemistry is a branch of pure chemistry which is very similar to physical chemistry.The main objective of this branch of science is to develop and employ new methods andinstrumentation for the purpose of providing

information on nature and composition of matter.It helps in determination of a compound's total or partial structure in various samples.

Theword chemistry used in "analytical chemistry" clearly signifies the analysis of chemicalelements and their derived compounds. This branch of science is used in all the fields ofsciences.Chemical sciences serve as proof of various advances and evolution of technologies whichhas led to the growth of high performance instruments. This development of instrumentationhas led to increase in more sophisticated and non-destructive method of analysis. Nondestructivetechniques plays the most valuable role in forensic science as they can be conductedon very small samples and does not even require extensive sample preparation before themeasurement.

With these advances techniques quality and precision requirements can be metefficiently. This is an important step in the official recognition of the quality of the laboratory.The use of various instrumental techniques has become an important part of chemical analysisin various fields of science i.e. pure and applied science. Only a single instrument cannot solvean analytical issue, instead numerous instrumental methods are necessary for efficient analysisto a maximum extent.

Statistical test of data

Once an experiment is completed, the resultant data requires statistical analysis in order to interpret the results. There are statistical methods available that allow us to make judgments about the data, its relationship to other experimental data and ultimately its relationship with our hypothesis. These methods also allow us to determine the uncertainty (or error) in our measurements and results. The following are brief descriptions of these methods

Confidence Interval

A confidence interval is an estimated range in which measurements correspond to the given percentile. For example, a 95% confidence interval means that the 95% of the measured values will be within the estimated range. It can also tell precision and stability of the measurements from the uncertainty.

μ interval = $\pm t*s / \sqrt{N}$

μ = true value

= estimated mean

s = estimated standard deviation

N = number of data points

t = student's t

N-1 = degrees of freedom

Student t-Test

The t-test is a convenient way of comparing the mean one set of measurements with another to determine whether or not they are the "same" (statistically). Its main goal is to test the null hypothesis of the experiment. There are assumptions about the data that must be made before being completed. Once the t value is calculated, it is then compared to a corresponding t value in a t-table. The value in the table is chosen based on the desired confidence level. The higher the % confidence level, the more precise the answers in the data sets will have to be. A 95% confidence level test is generally used.

If the calculated t value is greater than the tabulated t value the two results are considered different. However, one must be cautious when using the t-test since different scenarios require different calculations of the t-value. Three examples can be found in the textbook titled "Quantitative Chemical Analysis" by Daniel Harris. The examples are titled "Comparing a Measured Result with a 'Known' Value", "Comparing Replicate Measurements" and "Paired t test for Comparing Individual Differences"

F-test

As the t-test describes whether two numbers, or means, are significantly different from each other, the f-test describes whether two standard deviations are significantly different from each other. The difference between the standard deviations may seem like an abstract idea to grasp. This, however, can be thought of a way to test if the deviation between two values places them as equal.

In order to perform the F test, the quotient of the standard deviations squared is compared to a table value. When entering the S_1 and S_2 into the equation, S_1 is always the larger number

$$F_{calculated} = \frac{S_1^2}{S_2^2}$$

This value is compared to a table value constructed by the degrees of freedom in the two sets of data. The table being used will be picked based off of the % confidence level wanting to be determined. The 95% confidence level table is most commonly used. If the calculated F value is smaller than the F value in the table, then the precision is the same, and the results of the two sets of data are precise. If the calculated F value is larger than the F value in the table, the precision is different.

If Fcalculated > Ftable The standard deviations are significantly different from each other. If Fcalculated < Ftable The standard deviations are not significantly different.

Q Test & Grubb's Test

The Q test is designed to evaluate whether a questionable data point should be retained or discarded. In general, this test can be thought of as a comparison of the difference between the questionable number and the closest value in the set to the range of all numbers. The calculated Q value is the quotient of gap between the value in question and the range from the smallest number to the largest (Qcalculated = gap/range).

This calculated Q value is then compared to a Q value in the table. This table is sorted by the number of observations and each table is based on the percent confidence level chosen.

If Qcalculated > Qtable The number can be discarded If Qcalculated < Qtable The number should be kept at this confidence level
The Grubb test is also useful when deciding when to discard outliers, however, the Q test can be used each time. Both can be used in this case

Rejecting Data

When carrying out an experimental analytical procedure it will sometimes be observed that one of the measured values is very different from all of the other values, *e.g.*, as the result of a blunder in the analytical procedure. Occasionally, this value may be treated as being incorrect, and it can be rejected. There are certain rules based on statistics that allow us to decide whether a

particular point can be rejected or not. A test called the *Q-test* is commonly used to decide whether an experimental value can be rejected or not.

$$Q = \frac{X_{BAD} - X_{NEXT}}{X_{HIGH} - X_{LOW}}$$

Here X_{BAD} is the questionable value, X_{NEXT} is the next closet value to X_{BAD}, X_{HIGH} is the highest value of the data set and X_{LOW} is the lowest value of the data set. If the *Q-value* is higher than the value given in a *Q-test table* for the number of samples being analyzed then it can be rejected:

S.No	Number of Observations	Q-value for Data Rejection
1.	3	0.94
2.	4	0.76
3.	5	0.64
4.	6	0.56
5.	7	0.51
6.	8	0.47
7.	9	0.44
8.	10	0.41

For example, if five measurements were carried out and one measurement was very different from the rest (*e.g.*, 20,22,25,50,21), having a Q-value of 0.84, then it could be safely rejected (because it is higher than the value of 0.64 given in the Q-test table for five observations)

POSSIBLE QUESITONS

PART-B (2 Mark Questions)

1. What is meant by a sample?
2. What are the errors?
3. What is the wavelength range for visible light?
4. Write the principle of Thermogravimetry analysis
5. What is chelation?

PART-C (6 Mark Questions)

1. Describe the evaluation of analytical data
2. Berifly explain the sampling types
3. Discuss the parts of flame emission photometer?
4. Explain the fundamental laws of spectroscopy and selection rules
5. Describe the instrumentation of thermogravimetry?
6. Explain the characteristics of TGA curves for $CaC_2O_4.H_2O$
7. Disscuss the Principle and applications of Thin Layer Chromotography
8. Explain the batch and continuous solvent extractions methods
9. Optical Rotation *vs*. Specific Rotation – What's the Difference?
10. Briefly discuss about Enantiomeric excess

KARPAGAM ACADEMY OF HIGHER EDUCATION
(Deemed to be University)
(Established Under Section 3 of UGC Act 1956)
DEPARTMENT OF CHEMISTRY
UNIT-I (Qualitative and quantitative aspects of analysis)

(Multiple Choice Questions)

S.No	Questions	Option a	Option b	Option c	Option d	Answer
1.	Who produced the electromagnetic waves first ?	Marconi	Maxwell	J.C. Bose	Hertz	Hertz
2.	Which of these is not one of the four main reasons for missing data	The respondent may have missed a question by mistake	The data was not required from the respondent	The respondent did not know the answer or did not have an opinion	The analyst ignored its presence on the data form	The analyst ignored its presence on the data form
3.	Which of the following rays is emitted by a human body?	X-rays	Visible rays	UV-rays	IR-rays	IR-rays
4.	Which of the following procedures do general analytical procedures not include?	Reasonableness tests	Statistical analysis	Trend analysis	Sequence tests	Statistical analysis
5.	Which of the following is *not* an example of a 'unit of analysis'?	Validity	significant actors	Words	Themes	significant actors
6.	Which of the following is not a data mining method?	Classification and prediction	Data description	Dependency analysis	A priori algorithms	A priori algorithms
7.	Which of the following is an advantage of content analysis?	It is flexible and is not resource dependent	It is highly complex and requires rigorous training	It is only suitable for the analysis of interview transcripts	It is not suitable for the analysis of business reports	It is flexible and is not resource dependent

8.	Which of the following is a true statement about classification method in data mining?	It is the process of finding models or functions that map records into one of several discrete prescribed classes	The objective is to provide an overall description of data, either in itself or in each class or concept	The main approaches in obtaining data description are data characterisation and data discrimination	The purpose is to search for the most significant relationship across a large number of variables or attributes	It is the process of finding models or functions that map records into one of several discrete prescribed classes
9.	Which of the following have zero average value in a plane electromagnetic wave?	Electric energy	Magnetic energy	Electric field	None of these	Electric field
10.	Which of the following analytical methods would you choose to investigate whether a compound is a monomer, dimer or trimer?	elemental analysis	NMR spectroscopy	ESI-MS	IR spectroscopy	ESI-MS
11.	When reading is taken at half scale in the instrument, the error is	Exactly equal to half of full scale error	Equal to full scale error	less than full scale error	more than full scale error	more than full scale error
12.	When light enters from vacuum in to glass, it's velocity	varies depending on mass of glass	decreases	remains same	increases	decreases
13.	The systematic errors of an instrument can be reduced by making	The sensitivity of instrument to environmental input as low as possible	The sensitivity of instrument to environmental input as high as possible	Systematic errors does not depend on the sensitivity of instrument	None of these	The sensitivity of instrument to environmental input as low as possible
14.	The steady state error due to a ramp input for a type to system is equal to	Zero	infinite	non-zero number	constant	constant
15.	The reliability of an instrument refers to	The measurement of changes due to temperature variation	The degree to which repeatability continues to remain within specified limits	The life of an instrument	The extent to which the characteristics remain later	The degree to which repeatability continues to remain within specified limits

16.	the following types of analytical procedure, which one uses the most variables?	Trend analysis	Data mining	Ratio analysis	Reasonableness test	Data mining
17.	The difference between indicated value and true value of a quantity is	Gross Error	. Absolute Error	Dynamic Error	Relative Error	Absolute Error
18.	The degree of closeness of the measured value of a certain quantity with its true value is known as	Accuracy	Precision	Standard	Sensitivity	Accuracy
19.	The data from each row in a coding schedule can be entered into a quantitative analysis computer program called	Endnote	N-Vivo	Outlook	SPSS	SPSS
20.	Testing the probability of a relationship between variables occurring by chance alone if there really was no difference in the population from which that sample was drawn is known as	chi-squared tests	significance testing	correlation coefficients	multiple regression analysis	significance testing
21.	Systematic errors occur due to	overuse of instruments	careful usage of instruments	human sight	instrument quality	overuse of instruments
22.	Systematic errors occur due to	overuse of instruments	careful usage of instruments	human sight	instrument quality	over use of instruments
23.	Systematic errors can be removed by	buying new instrument	breaking the instrument	dusting the instrument	recalibrating the instrument	recalibrating the instrument
24.	Systematic error of an instrument for measurement can be minimized by	Selecting a proper measuring device for the particular application	Calibrating the measuring device against a standard device	Appllying correction factors for change of ambient	All	All

				conditions		
25.	Systematic error occurred due to the poor calibration of the instrument that can be corrected by	taking several readings	replacing instruments	taking mean of values	taking median of values	replacing instruments
26.	Standard deviation of a sampling distribution is also classified as	standard error	statistic error	sampling error	probability error	standard error
27.	Standard deviation is	inappropriate in management and business research	a way of describing those phenomena that are not the norm	a way of measuring the extent of spread of quantifiable data	a way of illustrating crime statistics	a way of measuring the extent of spread of quantifiable data
28.	Speed of electromagnetic waves is same	for all wavelengths	in all media	for all intensities	for all frequencies	for all intensities
29.	Speed of electromagnetic wave is the same	for all wavelengths	in all media	for all intensities	for all frequencies	for all intensities
30.	Speed of electromagnetic radiation is independent of	wavelength	amplitude	time period	frequency	frequency
31.	Speed at which stars and galaxies are moving away from us is determined by phenomena of	blue shift	yellow shift	red shift	orange shift	red shift
32.	Relative amounts of elements are discussed in	Testing	quantitative analysis	Qualitative analysis	Physical test	quantitative analysis
33.	Regardless to difference in distribution of sample and population, mean of sampling distribution must be equal to	degree of freedom	statistic error	population mean	standard error	population mean
34.	Quantitative data refers to	graphs and tables	numerical data that could usefully be quantified to help you answer your research question(s) and to	any data you present in your report	statistical analysis	numerical data that could usefully be quantified to help you answer your research question(s)

			meet your objectives			and to meet your objectives
35.	Quantitative content analysis is an approach that aims to	objectively and systematically measure the content of a text	reach an interpretive understanding of social action	engage in a critical dialogue about ethical issues in research	provide a feminist alternative to 'male-stream' quantitative methods	objectively and systematically measure the content of a text
36.	If population standard deviation is not known then formula used to calculate standard error is as	n - 1 / sample size square root	s / sample size square root	n + 1 / square root of s	n * 2 / sample size square root	s / sample size square root
37.	How is a variable name different from a variable label?	It is shorter and less detailed.	It is longer and more detailed.	It is abstract and unspecific.	It refers to codes rather than variables.	It is shorter and less detailed.
38.	Errors that occur during measurement of the quantities are of	2 types	3 types	5 types	4 types	2 types
39.	Error that occurs during the measurement of quantities is	random error	systematic error	random and systematic error	random frequent error	random and systematic error
40.	Error that occurs due to equally affected measurements is called	random error	systematic error	frequent error	precision	systematic error
41.	Electromagnetic waves ware transverse in nature is evident by:	polarization	interference	reflection	diffraction	polarization
42.	Electromagnetic waves do not transport:	energy	charge	momentum	information	charge
43.	Electromagnetic waves are produced by:	accelerated charged particle	deaccelerated charged particle	charge in uniform motion	none of the above	accelerated charged particle
44.	Electromagnetic waves are produced by	a static charge	a moving charge	an accelerating charge	chargeless particles	an accelerating charge
45.	Computers are essential for quantitative data analysis because	increasingly data analysis software contain algorithms	they are so powerful.	they enable easy calculation for those of us not	they are fun to use	increasingly data analysis software contain algorithms

		that check the data for obvious errors as it is entered		too good with figures		that check the data for obvious errors as it is entered
46.	Analysis based on study of price fluctuations, production of commodities and deposits in banks is classified as	sample series analysis	time series analysis	numerical analysis	experimental analysis	time series analysis
47.	An electromagnetic radiation has an energy of 13.2 keV. Then, the radiation belongs to the region of	visible light	ultraviolet	infrared	X-ray	ultraviolet
48.	All values in sample distribution that can freely varies in selected random sample from population are indicated as	degree of freedom	degree of error	degree of statistic	degree of possibility	degree of freedom
49.	All of the waves listed below are a part of the electromagnetic spectrum except	sound waves	X rays	gamma rays	radio waves	sound waves
50.	According to Maxwell, a changing electric field produces	emf	Electric current	magnetic field	radiation pressure	magnetic field
51.	A measurement which on repetition gives same or nearly same result is called	accurate measurement	average measurement	precise measurement	estimated measurement	precise measurement
52.	A free electron is placed in the path of a plane electromagnetic wave. The electron will start moving:	along the electric field	along the magnetic field	along the direction of propagation of the wave	Electric and magnetic waves	along the electric field
53.	A basic premise of using analytical procedures is that	There exist plausible relationships	They are a good indicator of fraud and error	There exist plausible relationships	They are essential in the planning process	There exist plausible relationships among data that can

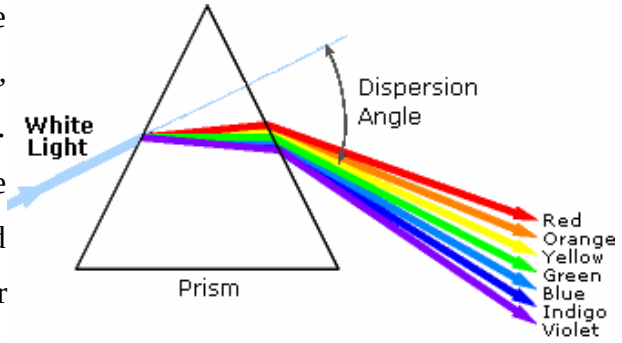
		among data that can reasonably be expected to continue		among data that is highly accurate		reasonably be expected to continue
54.	Which one of the following correctly represents the systematic errors	These errors can be calculaterd from the details of the instruments	These are the residual errors	These errors may occur under controlled conditions	These are error committed by the experimenters	These errors may occur under controlled conditions
55.	The error between mean of finite data set and mean of infinite data set is known as	True error of the mean	Standard error of the mean	Finite error	Infinite error	Standard error of the mean
56.	Random errors in a measurement system are due to	Environmental changes	Use of uncalibrated instrument	Poor cabling practices	Unpredictable effects	Unpredictable effects
57.	Measurement which is close to the true value is	accurate	average	precise	error	accurate
58.	In statistical analysis, sample size is considered large if	n > or = 30	n < or = 30	n > or = 50	n < or = 50	n > or = 30
59.	If the instrument is used in wrong manner while application, then it will results in	Systematic error	Instrument error	Random error	Environmental error	Instrument error
60.	Branch of statistics which deals with development of particular statistical methods is classified as	industry statistics	economic statistics	applied statistics	mathematical statistics	mathematical statistics

Origin of spectra, interaction of radiation with matter, fundamental laws of spectroscopy and selection rules, validity of Beer-Lambert's law.
UV-Visible Spectrometry: Basic principles of instrumentation (choice of source, monochromator and detector) for single and double beam instrument;
Basic principles of quantitative analysis: estimation of metal ions from aqueous solution, geometrical isomers, keto-enol tautomers. Determination of composition of metal complexes using Job's method of continuous variation and mole ratio method.
Infrared Spectrometry: Basic principles of instrumentation (choice of source, monochromator & detector) for single and double beam instrument; sampling techniques.
Structural illustration through interpretation of data, Effect and importance of isotope substitution.
Flame Atomic Absorption and Emission Spectrometry: Basic principles of instrumentation (choice of source, monochromator, detector, choice of flame and Burner designs. Techniques of atomization and sample introduction; Method of background correction, sources of chemical interferences and their method of removal. Techniques for the quantitative estimation of trace level of metal ions from water samples.

Background

An obvious difference between certain compounds is their color. Thus, quinone is yellow; chlorophyll is green; the 2,4-dinitrophenylhydrazone derivatives of aldehydes and ketones range in color from bright yellow to deep red, depending on double bond conjugation; and aspirin is colorless. In this respect the human eye is functioning as a spectrometer analyzing the light reflected from the surface of a solid or passing through a liquid. Although we see sunlight (or white light) as uniform or homogeneous in color, it is actually composed of a broad range of radiation wavelengths in the ultraviolet (UV), visible and infrared (IR) portions of the spectrum. As shown on the right, the component colors of the visible portion can be separated by passing sunlight through a prism, which acts to bend the light in differing degrees according to wavelength.

Electromagnetic radiation such as visible light is commonly treated as a wave phenomenon, characterized by a wavelength or frequency. Wavelength is defined on the left below, as the distance between adjacent peaks (or troughs), and may be designated in meters, centimeters or nanometers (10-9 meters). Frequency is the number

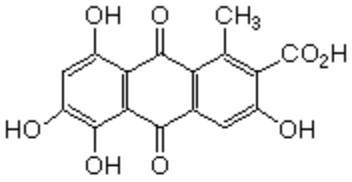


of wave cycles that travel past a fixed point per unit of time, and is usually given in cycles per second, or hertz (Hz). Visible wavelengths cover a range from approximately 400 to 800 nm. The longest visible wavelength is red and the shortest is violet. Other common colors of the spectrum, in order of decreasing wavelength, may be remembered by the mnemonic: ROY G BIV. The wavelengths of what we perceive as particular colors in the visible portion of the spectrum are displayed and listed below. In horizontal diagrams, such as the one on the bottom left, wavelength will increase on moving from left to right

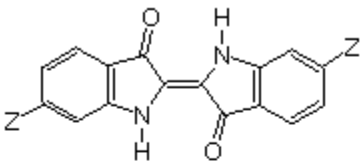
- Violet: 400 - 420 nm
- Indigo: 420 - 440 nm
- Blue: 440 - 490 nm
- Green: 490 - 570 nm
- Yellow: 570 - 585 nm
- Orange: 585 - 620 nm
- Red: 620 - 780 nm

When white light passes through or is reflected by a colored substance, a characteristic portion of the mixed wavelengths is absorbed. The remaining light will then assume the complementary color to the wavelength(s) absorbed. This relationship is demonstrated by the color wheel shown on the right. Here, complementary colors are diametrically opposite each other. Thus, absorption of 420-430 nm light renders a substance yellow, and absorption of 500-520 nm light makes it red. Green is unique in that it can be created by absoption close to 400 nm as well as absorption near 800 nm. Early humans valued colored pigments, and used them for decorative purposes. Many of these were inorganic minerals, but several important organic dyes were also known. These included the crimson pigment, kermesic acid, the blue dye, indigo, and the yellow saffron pigment, crocetin. A rare dibromo-indigo derivative, punicin, was used to color the robes of the royal and wealthy. The deep orange hydrocarbon carotene is widely distributed in plants, but is not sufficiently stable to be used as permanent pigment, other than for food coloring. A common feature of all these colored compounds, displayed below, is a system of **extensively conjugated pi-electrons**.

Some Natural Organic Pigments

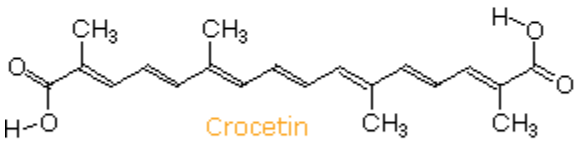


Kermesic Acid
(Carminic Acid)
from the insect *Coccus cacti*

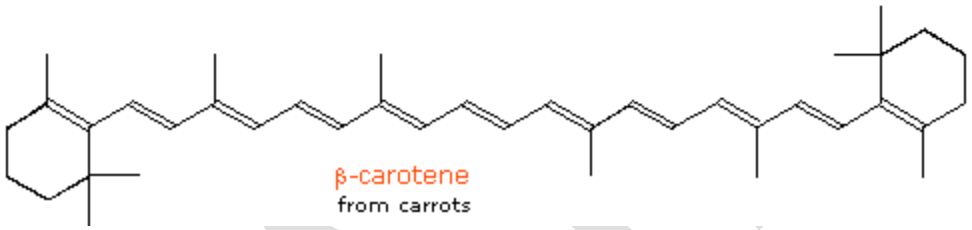


Z=H
Indigo
from *Isatis tinctoria* (woad)

Z=Br
Punicin or Tyrian Purple
from mollusks of the genus *Murex*



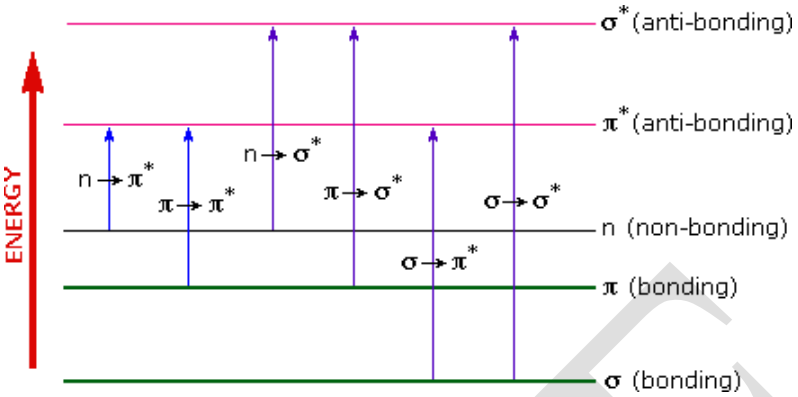
Crocetin
from saffron



β-carotene
from carrots

To understand why some compounds are colored and others are not, and to determine the relationship of conjugation to color, we must make accurate measurements of light absorption at different wavelengths in and near the visible part of the spectrum. Commercial optical spectrometers enable such experiments to be conducted with ease, and usually survey both the near ultraviolet and visible portions of the spectrum

Ultraviolet radiation having wavelengths less than 200 nm is difficult to handle, and is seldom used as a routine tool for structural analysis.



The energies noted above are sufficient to promote or excite a molecular electron to a higher energy orbital. Consequently, absorption spectroscopy carried out in this region is sometimes called "electronic spectroscopy". A diagram showing the various kinds of electronic excitation that may occur in organic molecules is shown on the left. Of the six transitions outlined, only the two lowest energy ones (left-most, colored blue) are achieved by the energies available in the 200 to 800 nm spectrum. As a rule, energetically favored electron promotion will be from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO), and the resulting species is called an excited state. For a review of molecular orbitals . When sample molecules are exposed to light having an energy that matches a possible electronic transition within the molecule, some of the light energy will be absorbed as the electron is promoted to a higher energy orbital. An optical spectrometer records the wavelengths at which absorption occurs, together with the degree of absorption at each wavelength. The resulting spectrum is presented as a graph of absorbance (A) versus wavelength, as in the isoprene spectrum shown below. Since isoprene is colorless, it does not absorb in the visible part of the spectrum and this region is not displayed on the graph. Absorbance usually ranges from 0 (no absorption) to 2 (99% absorption), and is precisely defined in context with spectrometer operation. Because the absorbance of a sample will be proportional to the number of absorbing molecules in the spectrometer light beam (e.g. their molar concentration in the sample tube), it is necessary to correct the absorbance value for this and other operational factors if the spectra of different compounds are to be compared in a meaningful way. The corrected absorption value is called "molar absorptivity", and is particularly useful when comparing the spectra of different

compounds and determining the relative strength of light absorbing functions (chromophores). **Molar absorptivity** (ε) is defined as

If the isoprene spectrum on the right was obtained from a dilute hexane solution (c = 4 * 10^{-5} moles per liter) in a 1 cm sample cuvette, a simple calculation using the above formula indicates a molar absorptivity of 20,000 at the maximum absorption wavelength. Indeed the entire vertical absorbance scale may be changed to a molar absorptivity scale once this information about the sample is in hand. Clicking on the spectrum will display this change in units

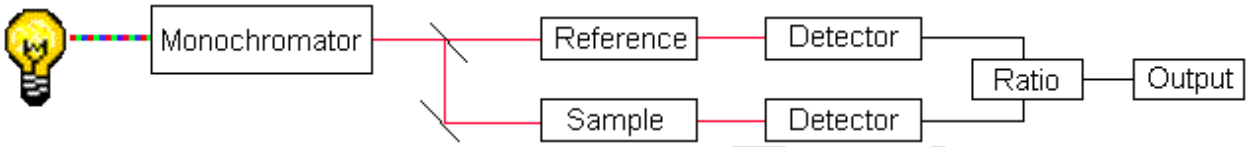
Chromophore	Example	Excitation	λ_{max}, nm	ε	Solvent
C=C	Ethene	π > π*	171	15,000	hexane
C≡C	1-Hexyne	π > π*	180	10,000	hexane
C=O	Ethanal	n _ > π* π > π*	290 180	15 10,000	hexane hexane
N=O	Nitromethane	n > π* π > π*	275 200	17 5,000	ethanol ethanol
C-X X=Br X=I	Methyl bromide Methyl Iodide	n > σ* n > σ*	205 255	200 360	hexane hexane

Chromophores. A list of some simple chromophores and their light absorption characteristics is provided on the left above. The oxygen non-bonding electrons in alcohols and ethers do not give rise to absorption above 160 nm. Consequently, pure alcohol and ether solvents may be used for spectroscopic studies. The presence of chromophores in a molecule is best documented by UV-Visible spectroscopy, but the failure of most instruments to provide absorption data for wavelengths below 200 nm makes the detection of isolated chromophores problematic. Fortunately, conjugation generally moves the absorption maxima to longer wavelengths, as in the case of isoprene, so conjugation becomes the major structural feature identified by this technique. Molar absorptivities may be very large for strongly absorbing chromophores (>10,000) and very small if absorption is weak (10 to 100). The magnitude ofε

reflects both the size of the chromophore and the probability that light of a given wavelength will be absorbed when it strikes the chromophore

Instrumentation

Have a look at this schematic diagram of a double-beam UV-Vis. Spectrophotometer



Instruments for measuring the absorption of U.V. or visible radiation are made up of the following components;

Sources (UV and visible)

Wavelength selector (monochromator)

Sample containers

Detector

Signal processor and readout

Instrumental components

Sources of UV radiation

It is important that the power of the radiation source does not change abruptly over it's wavelength range.

The electrical excitation of deuterium or hydrogen at low pressure produces a continuous UV spectrum. The mechanism for this involves formation of an excited molecular species, which breaks up to give two atomic species and an ultraviolet photon. This can be shown as;

$$D_2 + \text{electrical energy} \text{ ---------- } D_2^* + D' + D'' + h\nu$$

Both deuterium and hydrogen lamps emit radiation in the range 160 - 375 nm. Quartz windows must be used in these lamps, and quartz cuvettes must be used, because glass absorbs radiation of wavelengths less than 350 nm.

Sources of visible radiation

The tungsten filament lamp is commonly employed as a source of visible light. This type of lamp is used in the wavelength range of 350 - 2500 nm. The energy emitted by a tungsten filament lamp is proportional to the fourth power of the operating voltage. This means that for the energy output to be

stable, the voltage to the lamp must be *very* stable indeed. Electronic voltage regulators or constant-voltage transformers are used to ensure this stability.

Tungsten/halogen lamps contain a small amount of iodine in a quartz "envelope" which also contains the tungsten filament. The iodine reacts with gaseous tungsten, formed by sublimation, producing the volatile compound WI_2. When molecules of WI_2 hit the filament they decompose, redepositing tungsten back on the filament. The lifetime of a tungsten/halogen lamp is approximately double that of an ordinary tungsten filament lamp. Tungsten/halogen lamps are very efficient, and their output extends well into the ultra-violet. They are used in many modern spectrophotometers.

Wavelength selector (monochromator)

All monochromators contain the following component parts;

- An entrance slit
- A collimating lens
- A dispersing device (usually a prism or a grating)
- A focusing lens
- An exit slit

Polychromatic radiation (radiation of more than one wavelength) enters the monochromator through the entrance slit. The beam is collimated, and then strikes the dispersing element at an angle. The beam is split into its component wavelengths by the grating or prism. By moving the dispersing element or the exit slit, radiation of only a particular wavelength leaves the monochromator through the exit slit.

Cuvettes

The containers for the sample and reference solution must be transparent to the radiation which will pass through them. Quartz or fused silica cuvettes are required for spectroscopy in the UV region. These cells are also transparent in the visible region. Silicate glasses can be used for the manufacture of cuvettes for use between 350 and 2000 nm.

Detectors

The photomultiplier tube is a commonly used detector in UV-Vis spectroscopy. It consists of a *photoemissive cathode* (a cathode which emits electrons when struck by photons of radiation), several *dynodes* (which emit several electrons for each electron striking them) and an *anode*.

A photon of radiation entering the tube strikes the cathode, causing the emission of several electrons. These electrons are accelerated towards the first dynode (which is 90V more

positive than the cathode). The electrons strike the first dynode, causing the emission of several electrons for each incident electron. These electrons are then accelerated towards the second dynode, to produce more electrons which are accelerated towards dynode three and so on. Eventually, the electrons are collected at the anode. By this time, each original photon has produced 10^6 - 10^7 electrons. The resulting current is amplified and measured.

Photomultipliers are very sensitive to UV and visible radiation. They have fast response times. Intense light damages photomultipliers; they are limited to measuring low power radiation

The linear photodiode array is an example of a *multichannel photon detector*. These detectors are capable of measuring all elements of a beam of dispersed radiation simultaneously.

A linear photodiode array comprises many small silicon photodiodes formed on a single silicon chip. There can be between 64 to 4096 sensor elements on a chip, the most common being 1024 photodiodes. For each diode, there is also a storage capacitor and a switch. The individual diode-capacitor circuits can be sequentially scanned.

In use, the photodiode array is positioned at the focal plane of the monochromator (after the dispersing element) such that the spectrum falls on the diode array. They are useful for recording UV-Vis. absorption spectra of samples that are rapidly passing through a sample flow cell, such as in an HPLC detector.

Charge-Coupled Devices (CCDs) are similar to diode array detectors, but instead of diodes, they consist of an array of photocapacitors.

Molecular spectroscopy:

Infrared spectroscopy:

Interactions with molecules: absorption and scattering. Means of excitation (light sources), separation of spectrum (wavelength dispersion, time resolution), detection of the signal (heat, differential detection), interpretation of spectrum (qualitative, mixtures, resolution), advantages of Fourier Transform (FTIR). Samples and results expected. Applications: Issues of quality assurance and quality control, Special problems for portable instrumentation and rapid detection. *UV-Visible/ Near IR* – emission, absorption, fluorescence and photoaccoustic. Excitation sources (lasers, time resolution), wavelength dispersion (gratings, prisms, interference filters, laser, placement of sample relative to dispersion, resolution), Detection of signal (photocells, photomultipliers, diode arrays, sensitivity and S/N), Single and Double Beam instruments,

Interpretation (quantification, mixtures, absorption vs. fluorescence and the use of time, photoaccoustic, fluorescent tags).

Infrared Spectroscopy

Infrared spectroscopy comprises of interaction of matter and infrared radiation. It is also known as vibrational spectroscopy. It is also used to identify and analyse chemicals. Infraredspectrometer is the main instrument used produces an infrared spectrum irrespective of the nature of matter i.e. solid, liquid or gas. When the wavelength of IR radiation is identical tovibrational frequency then absorption takes place. Similar to absorption spectroscopy, the examination of transmitted light provides information about the amount of energy absorbed.

Scattering and Absorption

At its most basic, the interaction of light with matter entails the interaction of a single atom with a single quantum of light, called a photon. When an atom interacts with a photon, one of two things happen: it either absorbs (or later re-emits) the photon or it scatters the photon. For the atom to absorb thephoton, the energy of the photon must exactly match the gap between two of the energy states of one of the atom's electrons (two so-called "electronic states"). This is what is called an "electronic transition" and is usually between the ground state and an excited state of the atom. This same condition is not true of scattering: the photon may be scattered regardless of its energy (which is directly proportional to its frequency), although different energies will lead to different types of scattering. If the energy of the incident photon is high enough, it can knock an electron completely out of an atom, thereby ionising it.At its most complicated, the interaction of light with matter entails many photons interacting not only with atoms, but with molecules and agglomerations of molecules and atoms, be they in solid, liquid, or gas form. The photons may be absorbed or scattered, or they may not interact with the material and pass straight through it.Similarly to an atom, a molecule will only absorb a photon and be promoted to an excited state if the energy of the incident photon corresponds to the gap between the ground state and an excited state of the molecule. However, the gaps between the ground and excited states of a molecule are more complicated than the electronic transitions in an atom, as as a molecule will have not only electronic but also vibrational and rotational sub-levels. In other words, a molecule is composed of two or more atoms, each with their own electronic states, which rotate and vibrate with respect to each other such that the energy stored

in the molecule is a sum of electronic, rotational and vibrational energy. As was also the case with an atom, it is possible for a photon to interact with a molecule and scatter from it, with no need for the photon to have an energy which matches the difference between two energy levels of the molecule.

Nonlinear optical processes:

Getting the colours necessary for optical spectroscopy Amplified pulses from Ti:Sapphire lasers can be readily used for time-resolved spectroscopyexperiments. One question, however, remains unsolved: what do we do if the system we want to investigate absorbs the light outside the spectral range accessible by Ti: Sapphire lasers (around800 nm). How do we get light, at, say 670 nm? It is clearly impractical to construct a new laser each time you get a new sample in your lab. Wavelength tuning and getting colours other than 800 nmwavelength is done employing the phenomena of nonlinear optics. Nonlinear optics is the entire branch of science investigating optical phenomena occurring when the electric field of light iscomparable to that holding the electrons of atoms at the nuclei (roughly 1010 V/m). There are entire books on nonlinear optics; here we just depict several phenomena employed in getting the rightcolours for time-resolved spectroscopy.

Dispersion

Light dispersion is the phenomenon of linear optics, but it is important for the furtherdiscussion, therefore we will say a few words about it. In optics, dispersion is the dependence of refractive index on the frequency (or wavelength) of light. Transparent materials exhibit so-called

normal dispersion, i.e. refractive index increases with frequency (or decreases with wavelength). The speed of light in the material is reversely proportional to its refractive index; therefore the bluephotons (shorter wavelength) travel more slowly than the red ones. This means that if a transform limited pulse passes through a slab of transparent medium (e.g. a piece of glass),its duration will increase because the photons of different colours spread out and do not reach the observer all at the same time. If the medium exhibits normal dispersion, the red photons will arriveearlier, and the blue ones – later. Such pulse is called chirped and resembles the pulses in the cavity of the regenerative amplifier. Dispersion broadening of theultrashort pulses is usually

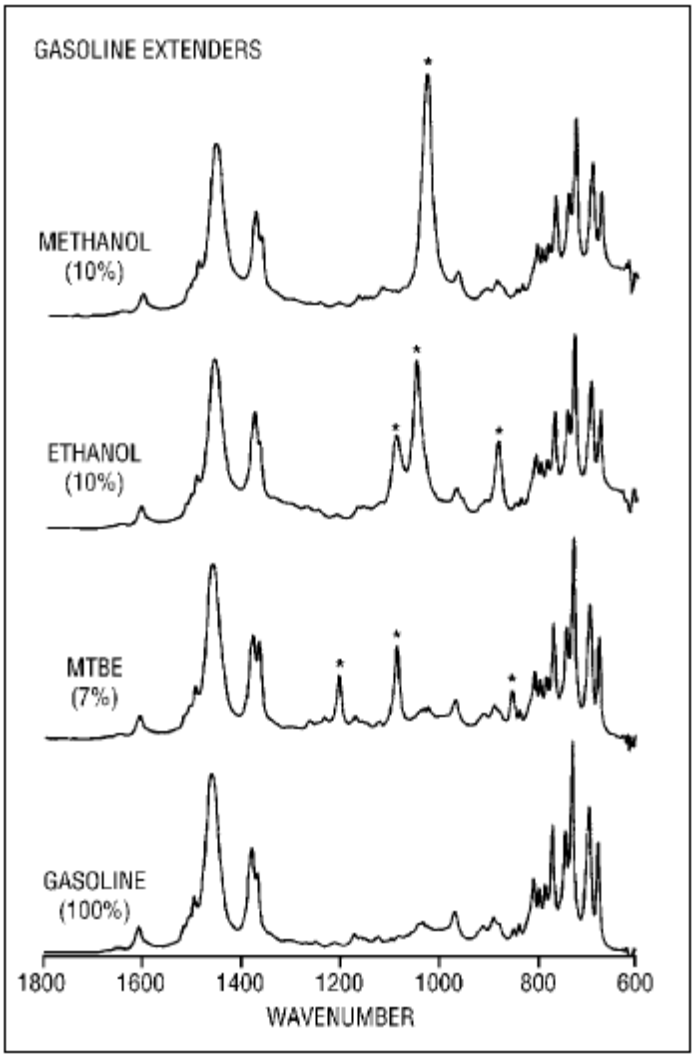
an undesirable effect in the time-resolves spectroscopic experiments. It makes the pulses longer and decreases the time resolution of the experiments. To prevent it as muchas possible, optical components of femtosecond beam lines (like lenses, wave plates or polarisers) are made as thin as possible. This also prevents transporting femtosecond pulses using optical fibers: inseveral meters of fiber a femtosecond pulse will stretch to tens of picoseconds, and different frequency components will scatter in time killing any dreams of good time resolution.

Fourier transforms infrared (FT-IR) spectrometers for use in quality assurance (QA)

The growth in popularity and acceptance of Fourier transform infrared (FT-IR) spectrometers for use in quality assurance (QA) laboratories and on manufacturing floors is one of the major developments affecting industrialenvironments in recent years. FT-IR spectroscopy offers almost unlimited analytical opportunities in many areas of production and quality control. It covers a wide range of chemical applications, especially in the analysis of organic compounds. In addition to its more classical role in qualitative analysis, its use in quantitative determinationshas grown due to the improvements in signal-to-noise performance coupled with the development of advanced statistical analysis algorithms.We offer a wide range of FT-IR spectrometers that address the needs of quality control (QC) and quality assurance (QA) laboratories. Nicolet FT-IR spectrometers offer thefull advantages of FT-IR technology combined with the simplicity of push-button operation. Rugged instrument construction and design allow reliable, uninterrupted operation in many industrial laboratory environments.Depending on the sampling interface, the spectrometers can be used for gas, liquid, or solid sample evaluations.

Analysis of Oxygenated Extenders in Gasoline

The use of organic extenders in gasoline for octane rating improvement and emission control is increasing. Owing to their unique spectral features, oxygenated extenders areeasily detected and quantified in gasoline.



Toluene Diisocyanate in Pre-polymer Mixtures

Toluene diisocyanate (TDI) is used in various resin blends in the manufacture of polymeric foams. The TDI concentration in the pre-polymer mixture affects the quality of the final product. Attenuated total reflectance (ATR) FT-IR spectroscopy can be used to quantitatively determine the TDI concentration in resin blends prior to polymerization to ensure product quality. Once the calibrated method is developed, analysis can be performed with a single keystroke.

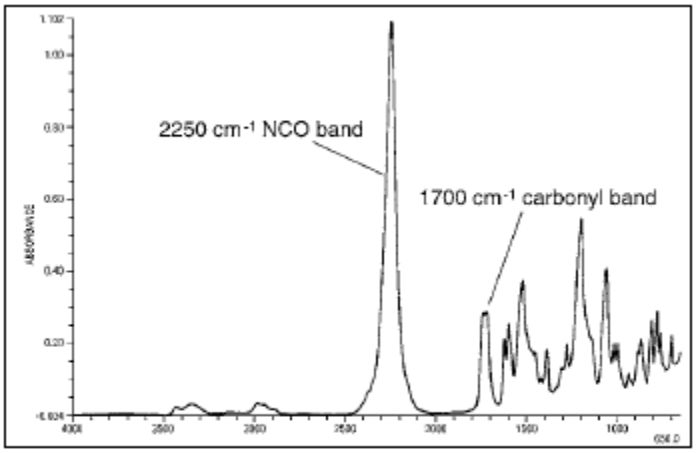


Figure 2: Toluene diisocyanate pre-polymer spectrum obtained on a horizontal ATR accessory. Bands used for quantitative determination are indicated.

Monitoring of Fluorination Level of Polyethylene

Chemically reinforced polyethylene is used in many industrial applications. Fluorination of the polyethylene surface is one of the processes for improving its performance.The fluorination level can be conveniently monitored using a Nicolet FT-IR spectrometer, offering price and performance advantages over currently used neutron activation analysis (NAA) and electron scatteranalysis (ESCA).

Corn Syrup

Rapid measurement of dextrose equivalent (DE) and dry substance (DS) at intermediate steps of corn syrup processing allows for better control of syrup production.The parameters are calibrated against the referencemethod using Partial Least Squares quantitative analysis software, providing a powerful yet rapid monitoring process for product quality.

Lubricating Oil Blend

Lubricating oils are blended from a number of different components, including base oil, additives, pour point depressants, and viscosity enhancers. FT-IR can be used to measure the levels of these components in the finished product.

Lubricating Oil Condition Monitoring

FT-IR analysis of used lubricating fluids followed by subtraction of the appropriate new oil reference is an effective tool for monitoring changes in the lubricant.These changes are the result of oxidation processes or contamination from other parts of the mechanical system.

Hydroxyl Number in Glycols

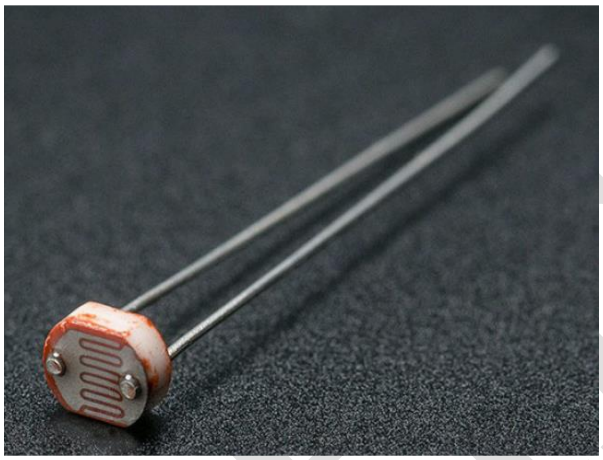
Knowledge of the hydroxyl group content of glycols is important for predicting the functional characteristics of the products. The hydroxyl value relates to molecular weight, viscosity, extent of reaction, and other parameters important to and dependent on the final application. Assessment of this value can be quickly and easily doneusing FT-IR.

Fluorescence Excitation and Emission

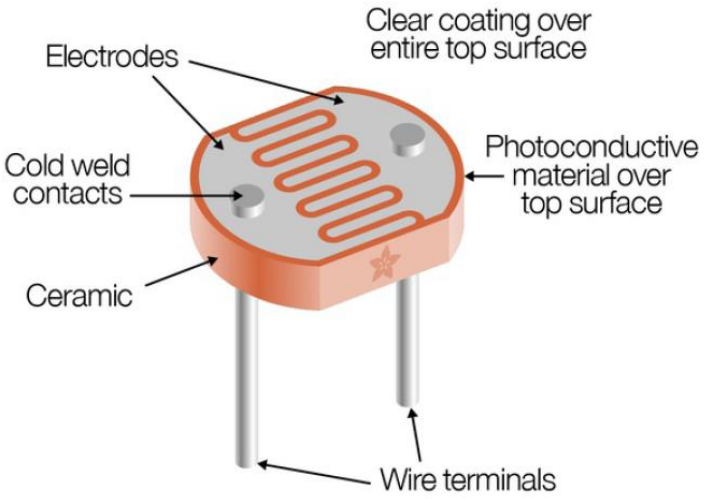
Fluorescence is a member of the ubiquitous luminescence family of processes in which susceptible molecules emit light from electronically excited states created by either a physical (forexample, absorption of light), mechanical (friction), or chemical mechanism. Generation of luminescence through excitation of a molecule by ultraviolet or visible light photons is aphenomenon termed photoluminescence, which is formally divided into two categories,fluorescence and phosphorescence, depending upon the electronic configuration of theexcited state and the emission pathway. Fluorescence is the property of some atoms and molecules to absorb light at a particular wavelength and to subsequently emit light of longer wavelength aftera brief interval, termed the fluorescence lifetime. The process of phosphorescence occurs in a manner similar to fluorescence, but with a much longer excited state lifetime.The categories of molecules capable of undergoing electronic transitions that ultimately result in fluorescence are known as fluorescent probes, fluorochromes, or simply dyes. Fluorochromesthat are conjugated to a larger macromolecule (such as a nucleic acid, lipid, enzyme, or protein) through adsorption or covalent bonds are termed fluorophores. In general, fluorophores aredivided into two broad classes, termed intrinsic and extrinsic. Intrinsic fluorophores, such as aromatic amino acids, neurotransmitters, porphyrins, and green fluorescent protein, are those thatoccur naturally. Extrinsic fluorophores are synthetic dyes or modified biochemical's that are added to a specimen to produce fluorescence with specific spectral properties.

Photocells

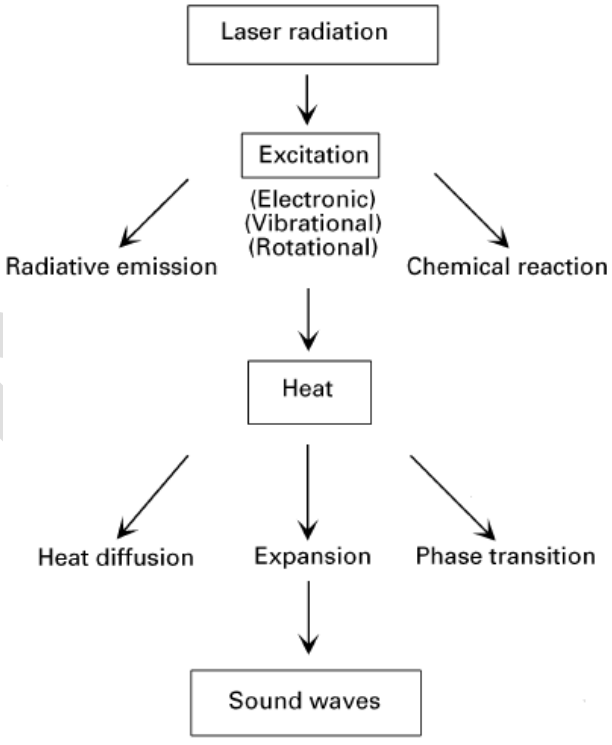
Photocells are sensors that allow you to detect light. They are small, inexpensive, low-power, easy to use and don't wear out. For that reason they often appear in toys, gadgets and appliances. They are often referred to as CdS cells (they are made of Cadmium-Sulfide), light-dependent resistors (LDR), and photoresistors.



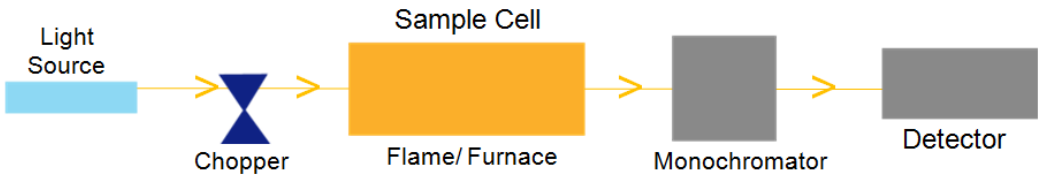
Photocells are basically a resistor that changes its resistive value (in ohms Ω) depending on how much light is shining onto the squiggly face. They are very low cost, easy to get in many sizes and specifications, but are very inaccurate.Each photocell sensor will act a little differently than the other, even if they are from the same batch. The variations can be really large, 50% or higher! For this reason, they shouldn't be used to try to determine precise light levels in lux ormillicandela. Instead, you can expect to only be able to determine basic light changes.For most light-sensitive applications like "is it light or dark out", "is there something in front of the sensor (that would block light)", "is there something interrupting a laser beam" (break-beam sensors), or "which of multiple sensors hasthe most light hitting it", photocells can be a good choice!



Photoaccoustic

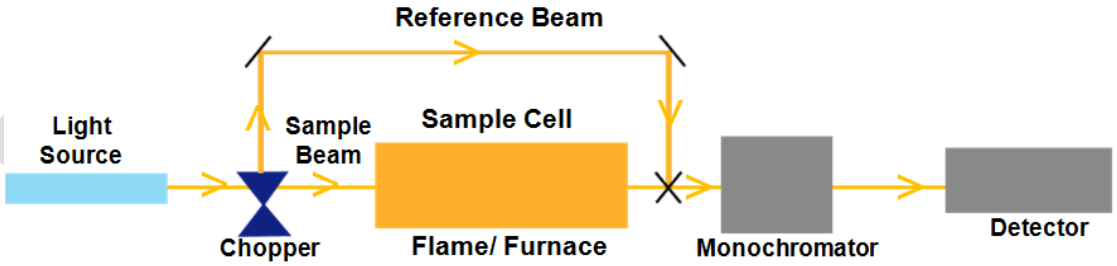


Single Beam spectrometer



Double Beam Spectrometer

The purpose of this instrument is to determine the amount of light of a specific wavelength absorbed by an analyte in a sample. Although samples can be gases or liquids, an analyte dissolved in a solvent is discussed here. [In the infrared, solid pellets using an IR. transparent matrix (like a high purity salt such as Kr) can be used for solid analytes. Thin disks are made using a pellet press and the disksuspended in the sample cell through which the sample beam passes.



The starting point in our movie is the light source. Depending on the wavelength of interest, this can be an electrically powered ultraviolet, visible, or infrared lamp. Notshown in the animations that accompany this page is the spectrophotometer'smonochromator which selects the analytical wavelength from the source lamp'sbroad spectrum containing many wavelengths of light. The analytical wavelength ischosen based on the absorbance characteristics of the analyte. Monochromator areinstruments whose sole purpose is to allow polychromatic (that is many wavelengthcontaining) light into the entrance slit of the monochromator and only allow a

single(or at least very few) wavelength (monochromatic light) out via the exit slit. Thisexiting, well-shaped, narrowly-defined beam now contains a small region of theelectromagnetic spectrum. The spread, or band-pass, of the wavelengths dependson the slit settings of the monochromator (usually adjustable) and the quality of thelight dispersing element in the monochromator (usually a grating in most modernmonochromators).In the instrumental design shown schematically in the animation and below here thesource lamp's beam is alternately diverted at right angles by a rotating disk withthree distinct panels. One sector allows the beam to past straight through the disk,another has a mirror surface, and a third is black. When the beam passes thoughthe disk it shines directly into the sample cell. If the sample is a liquid then this cellcontains a cuvette and is made of a transparent material, such as quartz, that doesnot absorb light in the spectral region of interest. The analyte is dissolved in asolvent held in the cuvette. When the source light is reflected at 90 degrees by therotating disk instead of striking the sample cuvette it passes through a cuvette inthe reference cell which contains ONLY solvent.During the third sequence, when the black sector blocks the source beam, NO lightpasses through the disk. And as can be seen below, therefore no light arrives at thephototransducer. This part of the cycle is used for the computer to digitize andmeasure the dark current--the amount of light produced by the phototransducercircuit when no light impinges on the phototransducer. The dark current can besubtracted from the overall light measurements made by the system.After travelling through either the sample cell or reference cell the light that was notabsorbed--by far, most of the beam-- is directed onto the phototransducer or lightdetector. This component converts the arrival of photons into an electrical signal. Bythe way, the light path through the spectrophotometer need not be in a straight linesince the light beam can be redirected using mirrors as can be seen here. Sometimes,lenses are also used to collect and collimate the light.The alternating light signals, from either the reference beam or sample beamgenerate alternating electrical phototransducer signals. A computer, sampling thosesignals, can now determine the analyte absorption in two ways. Some instrumentsmerely subtract the sample beam signal's digitized light intensity from that of thereference beam. The difference is a measure of the amount of light absorbed by theanalyte.Since phototransducers-based system are relatively poor at measuring the absolutedifference in two different light intensitiesespecially if that difference is large, lightabsorbance’s determined in this manner can contain unacceptable amounts of

error.Phototransducer are, however, good at generating signals from light intensities thatare close together in intensity; therefore, an alternate means of determining theanalyte absorption is used by some instruments:Some spectrophotometer design uses the digitized reference-minus-sample signaldifference to activate a servor motor connected to the computer and a device calledan optical wedge. The servor motor slides the optical wedge into the brighterreference beam's path somewhere after the reference cell but before thephoto transducer. Remember that since the reference cell does not have any lightabsorbing analyte, the light exiting the reference cell will always be brighter thanthat from the sample cell even if the solvent itself absorbs some at the analytewavelength since both cuvettes contain solvent. The optical wedge is made of amaterial that absorbs light so that the more the wedge intersects the reference beamthe more of that beam will be absorbed by the wedge and the less will be thedifference between the sample and reference signals. The wedge is automaticallyfed into the reference beam until the reference and sample beam signals are ofexactly identical intensity as measured by the phototransducer (remember thesystem is good at this). When the signals are equal the amount of wedge needed toproduce this 0 signal difference is a measure of the analyte absorption. Since thecomputer controls the wedge it converts wedge position to an absorbance readingof the analyte.

Photodiode array

The main advantage of employing photodiode array (PDA) detection is thatmultiwavelength spectral information can be obtained. The spectral informationcan be used to aid in the identification of unknown compounds. Furthermore,peak-purity check, and absorbance ratio at different wavelengths can be performedto confirm whether there is any overlapping of peaks in a single chromatogram.

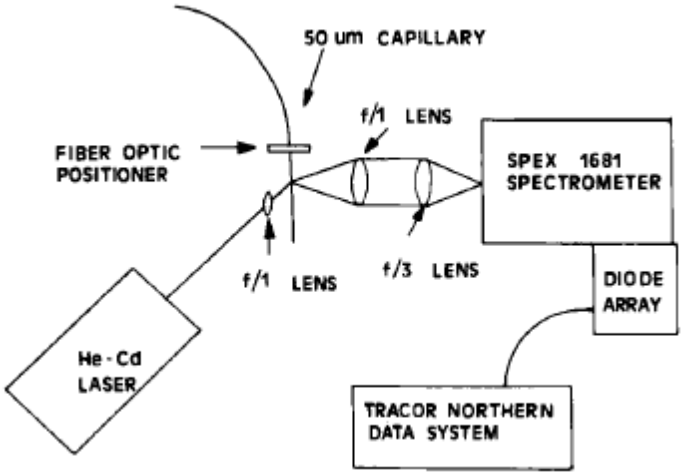
Laser-induced fluorescence detection

Lasers are superior excitation sources for use with small-diameter capillaries[51-751. Advantages over arc lamp sources include better focusing capabilities which allow the excitation energy to be more effectively applied to very smallsample volumes, and better monochromicity which reduces stray light levels. Laserare particularly useful for sensitive detection on capillaries having inside diameterof less than 50 pm because of the ability to be focused into smaller

volume thanare possible with arc lamp excitation. The disadvantages are that the wavelengthsavailable from current types of laser sources are rather limited, and that there arepossibilities of photodegradation of the analytes caused by the high light intensity.

Fluorometric photodiode array detector

A fluorometric photodiode array detector for CE was developed by Swaileand Sepaniak. A schematic diagram of the instrument is shown in Fig. 3.47.Fluorescence excitation was performed normal to the capillary using a He-Cd laser(30 mW, 442 nm), which is focused onto the on-column flow cell, made by removinga small section of the capillary polyimide coating near its outlet. Emission wascollected at a 90" angle from excitation and collimated by a 4 cm diameter, f / lquartz lens, then focused by a 4 cm diameter, f / 3 quartzlenses onto the entranceslits of a spectrometer that dispersed the emission across the diode array. Theentrance slit of the spectrometer was set at 1-2 mm, which prevented secularscatter from the sides of the capillary from reaching the photodiode. Detectionwas accomplished using a photodiode array with 1024 diodes. The diode arraywas operated a t the lowest possible resolution to reduce memory requirementwhich resulted in a spectral resolution of 4 nm per channel. Calibration data werecollected in the histogram mode in order to further reduce memory requirement.Linearity in response over 3 orders of magnitude and detection limits of less than60 fg (10-7 M)were obtained for sodium fluorescein.



Flame emission spectroscopy

A flame (from Latin flamma) is t he visible, gaseous part o f a fire. It is caused by a highly exothermic reaction taking place in a thin zone. Some flames, such as the flame of a burning candle, are hot enough to have ionized gaseous components and can be considered plasma. This subject is, however, hotly debated.

Mechanism

Color and temperature of a flame are dependent on the type of fuel involved in the combustion, as, for example, when a lighter is held to a candle. The applied heat causes the fuel molecules in the candle wax to vaporize. In this state they can then readily react with oxygen in the air, which gives off enough heat in the subsequent exothermic reaction to vaporize yet more fuel, thus sustaining a consistent flame. The high temperature of the flame causes the vaporized fuel molecules to decompose, forming various incomplete combustion products and free radicals, and these products then react with each other and with the oxidizer involved in the reaction. Sufficient energy in the flame will excite the electrons in some of the transient reaction intermediates such as CH and C2, which results in the emission of visible light as these substances release their excess energy (see spectrum below for an explanation of which specific radical species produce which specific colors). As the combustion temperature of a flame increases (if the flame contains small particles of unburnt carbon or other material), so does the average energy of the electromagnetic radiation given off by the flame (see Black body).

Other oxidizers besides oxygen can be used to produce a flame. Hydrogen burning in chlorine produces a flame and in the process emits gaseous hydrogen chloride (HCl) as the combustion product. Another of many possible chemical combinations is hydrazine and nitrogen

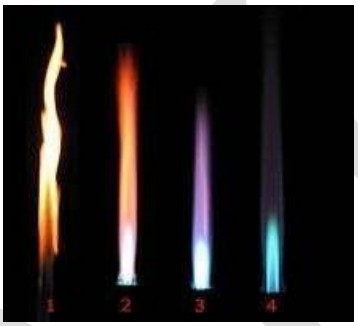
tetroxide which is hypergolic and commonly used in rocket engines. Fluoropolymers can be used to supply fluorine as an oxidizer of metallic fuels, e.g. in themagnesium/teflon/vitoncomposition.

The chemical kinetics occurring in the flame is very complex and involves typically a large number of chemical reactions and intermediate species, most of them radicals. For instance, a

well-known chemical kinetics scheme, GRI-Mech, uses 53 species and 325 elementary reactions to describe combustion ofbiogas.

There are different methods of distributing the required components of combustion to a flame. In a diffusion flame, oxygen and fuel diffuse into each other; where they meet the flame occurs. In apremixed flame, the oxygen and fuel are premixed beforehand, which results in a different type of flame. Candle flames (a diffusion flame) operate through evaporation of the fuel which rises in alaminar flow of hot gas which then mixes with surrounding oxygen and combusts.

Flame Color



premixed flame produces no soot and the flame color is produced by molecular radicals, especially CH and C2 band emission. The purple color is an artifact of the photographic process.

Flame color depends on several factors, the most important typically being black-body radiation and spectral band emission, with both spectral line emission and spectral line absorption playing smaller roles. In the most common type of flame, hydrocarbon flames, the most important factor determining color is oxygen supply and the extent of fuel-oxygen pre-mixing, which determines the rate of combustion and thus the temperature and reaction paths, thereby producing different color hues.

In a laboratory under normal gravity conditions and with a closed oxygen valve, a Bunsen burner burns with yellow flame (also called a safety flame) at around 1,000

°C (1,800 °F). This is due to incandescence of very fine soot particles that are produced in the flame. With increasing oxygen supply, less black body-radiating soot is produced due to a more complete combustion and the reaction creates enough energy to excite and ionize gas molecules in the flame, leading to a blue appearance. The spectrum of a premixed (complete combustion) butane flame on the right shows that the blue color arises specifically due to emission of excited molecular radicals in the flame, which emit most of their light well below ~565 nanometers in the blue and green regions of the visible spectrum. The colder part of a diffusion (incomplete combustion) flame will be red, transitioning to orange, yellow, and white as the temperature increases as evidenced by changes in the black- body radiation spectrum. For a given flame's region, the closer to white on this scale, the hotter that section of the flame is. The transitions are often apparent in fires, in which the color emitted closest to the fuel is white, with an orange section above it, and reddish flames the highest of all. A blue-colored flame only emerges when the amount of soot decreases and the blue emissions from excited molecular radicals become dominant, though the blue can often be seen near the base of candles where airborne soot is less concentrated.

Specific colors can be imparted to the flame by introduction of excitable species with bright emission spectrum lines. In analytical chemistry, this effect is used in flame tests to determine presence of some metal ions. In pyrotechnics, the pyrotechnic colorants are used to produce brightly colored fireworks.

FLAME PHOTOMETRY

Objective:

To determine the concentration of alkali and alkaline earth metals in various samples. Introduction

Atomic spectroscopy is thought to be the oldest instrumental method for the determination of elements. These techniques are introduced in the mid of 19th Century during which Bunsen and Kirchhoff showed that the radiation emitted from the flames depends on the characteristic element present in the flame. The potential of atomic spectroscopy in both the qualitative as well as quantitative analysis were then well established. The developments in the instrumentation

area led to the widespread application of atomic spectroscopy. Atomic spectroscopy is an unavoidable tool in the field of analytical chemistry. It is divided into three types which are absorption, emission, and luminescence spectroscopy. The different branches of atomic absorption spectroscopy are (1) Flame photometry or flame atomic emission spectrometry in which the species is examined in the form of atoms (2) Atomic absorption spectrophotometry, (AAS), (3) Inductively coupled plasma- atomic emission spectrometry (ICP-AES).

Theory:

Photoelectric flame photometry, a branch of atomic spectroscopy is used for inorganic chemical analysis for determining the concentration of certain metal ions such as sodium, potassium, lithium, calcium, Cesium, etc. In flame photometry the species (metal ions) used in the spectrum are in the form of atoms. The International Union of Pure and Applied Chemistry (IUPAC) Committee on Spectroscopic Nomenclature has recommended it as flame atomic emission spectrometry (FAES). The basis of flame photometric working is that, the species of alkali metals (Group 1) and alkaline earth metals (Group II) metals are dissociated due to the thermal energy provided by the flame source. Due to this thermal excitation, some of the atoms are excited to a higher energy level where they are not stable. The absorbance of light due to the electrons excitation can be measured by using the direct absorption techniques. The subsequent loss of energy will result in the movement of excited atoms to the low energy ground state with emission of some radiations, which can be visualized in the visible region of the spectrum. The absorbance of light due to the electrons excitation can be measured by using the direct absorption techniques while the emitting radiation intensity is measured using the emission techniques. The wavelength of emitted light is specific for specific elements.

Parts of a flame photometer

1. Source of flame:

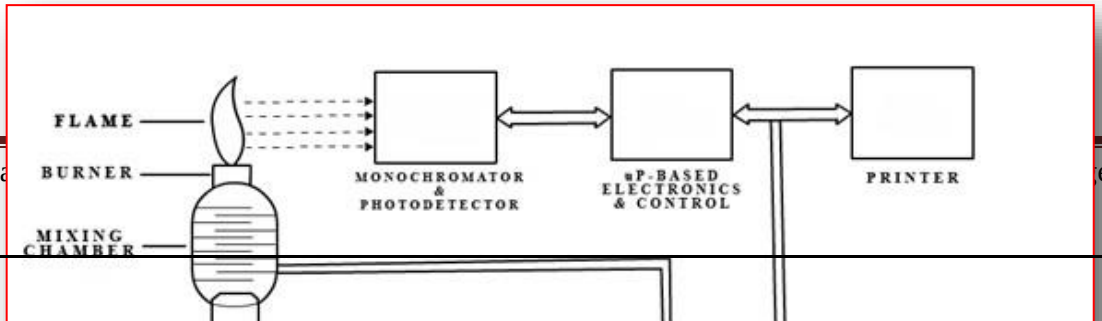
A burner that provides flame and can be maintained in a constant form and at a constant temperature.

2. Nebuliser and mixing chamber:

Helps to transport the homogeneous solution of the substance into the flame at a steady rate.

3. Optical system (optical filter):

The optical system comprises three parts: convex mirror, lens and filter. The convex mirror helps to transmit light emitted from the atoms and focus the emissions to the lens. The convex lens help to focus the light on a point called slit. The reflections from the mirror pass through the slit and reach the filters. This will isolate the wavelength to be measured from that of any other extraneous emissions. Hence it acts as interference type color filters.



Events occurring in the flame

Flame photometry employs a variety of fuels mainly air, oxygen or nitrous oxide (N2O) as oxidant. The temperature of the flame depends on fuel-oxidant ratio. The various processes in the flame are discussed below:

Desolvation: The metal particles in the flame are dehydrated by the flame and hence the solvent is evaporated.

Vapourisation: The metal particles in the sample are dehydrated. This also led to the evaporation of the solvent.

Atomization: Reduction of metal ions in the solvent to metal atoms by the flame heat.

Excitation: The electrostatic force of attraction between the electrons and nucleus of the atom helps them to absorb a particular amount of energy. The atoms then jump to the exited energy state.

Emission process: Since the higher energy state is unstable the atoms jump back to the stable low energy state with the emission of energy in the form of radiation of characteristic wavelength, which is measured by the photo detector.

The intensity of the light emitted could be described by the Scheibe-Lomakin equation:

$I = k. c$

Where:

I=Intensity of emitted light

K=the concentration of the element

c=constant of proportionality (at the linear part of the calibration curve)

That is the intensity of emitted light is directly related to the concentration of the sample. The comparison of emission intensities of unknown samples to either that of standard solutions (plotting calibration curve), or to those of an internal standard (standard addition method), helps in the quantitative analysis of the analyte metal in the sample solution. Flame photometer has both quantitative and qualitative applications. Flame photometer with monochromators emits radiations of characteristic wavelengths which help to detect the presence of a particular metal in the sample.

Alkaline earth metals which are critical for soil cultivation. In agriculture, the fertilizer requirement of the soil is analyzed by flame test analysis of the soil. In clinical field, Na+ and K+ ions in body fluids, muscles and heart can be determined by diluting the blood serum and aspiration into the flame. Analysis of soft drinks, fruit juices and alcoholic beverages can also be analyzed by using flame photometry.

Atomic Emission Spectroscopy

The focus of this section is on the emission of ultraviolet and visible radiation following the thermal excitation of atoms. Atomic emission spectroscopy has a long history. Qualitative applications based on the color of flames were used in the smelting of ores as early as 1550 and were more fully developed around 1830 with the observation of atomic spectra generated by flame emission and spark emission. Quantitative applications based on the atomic emission from electric sparks were developed by Lockyer in the early 1870 and quantitative applications based on flame emission were pioneered by Lundegardh in 1930. Atomic emission based on emission from plasma was introduced in 1964.

Atomic Emission Spectra

Atomic emission occurs when a valence electron in a higher energy atomic orbital returns to a lower energy atomic orbital. Figure shows a portion of the energy level diagram for sodium, which consists of a series of discrete lines at wavelengths corresponding to the difference in energy between two atomic orbitals.

Equipment

An atomic emission spectrometer is similar in design to the instrumentation for atomic absorption. In fact, it is easy to adapt most flame atomic absorption spectrometers for atomic emission by turning off the hollow cathode lamp and monitoring the difference in the emission

intensity when aspirating the sample and when aspirating a blank. Many atomic emission spectrometers, however, are dedicated instruments designed to take advantage of features unique to atomic emission, including the use of plasmas, arcs, sparks, and lasers as atomization and excitation sources, and an enhanced capability for multielemental analysis.

Atomization and Excitation

Atomic emission requires a means for converting a solid, liquid, or solution analyte into a free gaseous atom. The same source of thermal energy usually serves as the excitation source. The most common methods are flames and plasmas, both of which are useful for liquid or solution samples. Solid samples may be analyzed by dissolving in a solvent and using a flame or plasma atomizer.

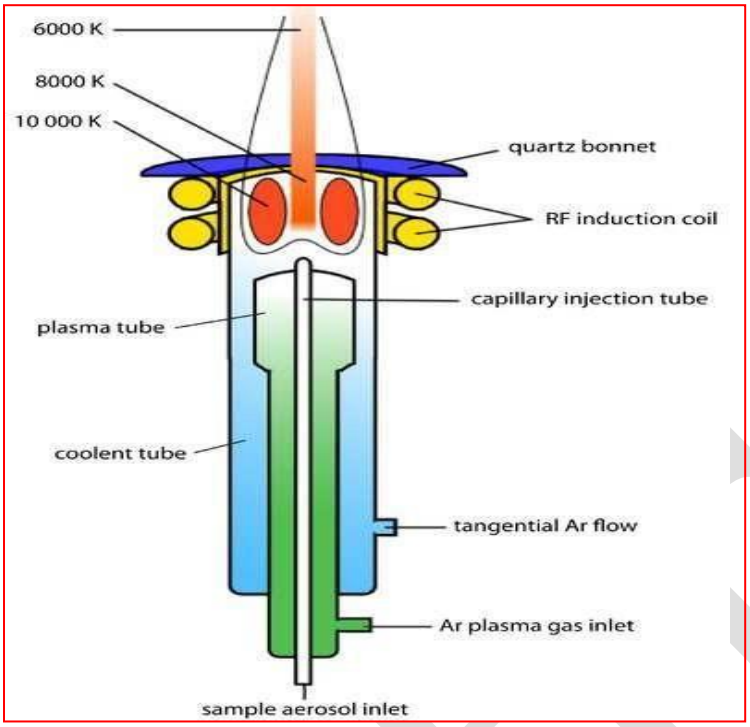
Flame Sources

Atomization and excitation in flame atomic emission is accomplished using the same nebulization and spray chamber assembly used in atomic absorption (Figure). The burner head consists of single or multiple slots, or a Meker style burner. Older atomic emission instruments often used a total consumption burner in which the sample is drawn through a capillary tube and injected directly into the flame.

Plasma Sources

A plasma is a hot, partially ionized gas that contains an abundant concentration of cations and electrons. The plasmas used in atomic emission are formed by ionizing a flowing stream of argon gas, producing argon ions and electrons. A plasma's high temperature results from resistive heating as the electrons and argon ions move through the gas. Because plasmas operate at much higher temperatures than flames, they provide better atomization and a higher population of excited states.

A schematic diagram of the inductively coupled plasma source (ICP) is shown in Figure . The ICP torch consists of three concentric quartz tubes, surrounded at the top by a radio- frequency induction coil. The sample is mixed with a stream of Ar using a nebulizer, and is carried to the plasma through the torch's central capillary tube. Plasma formation is initiated by a spark from a Tesla coil. An alternating radio-frequency current in the induction coils creates a fluctuating magnetic field that induces the argon ions and the electrons to move in a circular path. The resulting collisions with the abundant unionized gas give rise to resistive heating, providing temperatures as high as 10 000 K at the base of the plasma, and between 6000 and 8000 K at a height of 15–20 mm above the coil, where emission is usually measured. At these high temperatures the outer quartz tube must be thermally isolated from the plasma. This is accomplished by the tangential flow of argon shown in the schematic diagram

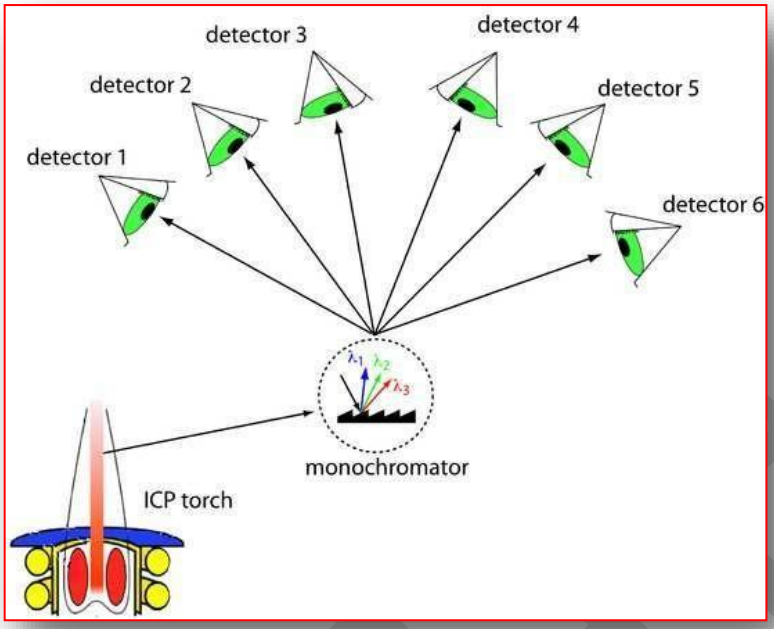


Multi elemental Analysis

Atomic emission spectroscopy is ideally suited for multielemental analysis because all analytes in a sample are excited simultaneously. If the instrument includes a scanning monochromator, we can program it to move rapidly to an analyte's desired wavelength, pause to record its emission intensity, and then move to the next analyte's wavelength. This sequential analysis allows for a sampling rate of 3–4 analytes per minute.

Another approach to a multi elemental analysis is to use a multichannel instrument that allows us to simultaneously monitor many analytes. A simple design for a multichannel spectrometer couples a monochromator with multiple detectors that can be positioned in a semicircular array around the monochromator at positions corresponding to the wavelengths for the analytes.

Figure Schematic diagram of a multichannel atomic emission spectrometer for the simultaneous analysis of several elements. The ICP torch is modified from Xvlun. Instruments may contain as many as 48–60 detectors.



Text Books:

1. Gopalan, V., Subramanian, P. S., & Rangarajan, K. (2010). *Elements of Analytical Chemistry*. New Delhi: S. Chand and Sons.

2. Usharani, S. (2012). *Analytical Chemistry*. Chennai: MacMillan India Ltd.

3. Sharma, B. K. (2010). *Instrumental Methods of Chemical Analysis* (24th Edition). Meerut: Krishna Prakashan Media (P) Ltd.

4. Ewing, G. W. (1988). *Instrumental Methods of Chemical Analysis* (III Edition). Singapore: McGraw Hill International Edition.

POSSIBLE QUESTIONS

PART-B (2 Mark Questions)

1. State the principle of the UV-Visible Spectroscopy
2. Calculate the Fundamental modes of Vibration in CO_2 molecules
3. What is the difference between precision and accuracy?
4. What are the non-polar solvents?
5. What are the Phases in chromatography?

PART-c (6 Mark Questions)

1. (i)Explain the electronic transitions involved in UV spectroscopy?
   (ii). Explain the various parts and functions of a UV-visible spectrophotometer.
2. Explain the Woodward-Fieser rules for calculating absorption maximum for α,β- unsaturated carbonyl compounds with examples
3. i).Explain the description of double beam UV spectrophotometer.
   (ii). How will you determine the structure of α, β – unsaturated compounds and conjugated dienes by UV spectroscopy?
4. (i). Explain the various applications of UV spectroscopy. (ii). Explain the absorption laws in detail.
5. Explain the Woodward-Fieser rules for calculating absorption maximum in dienes with examples.
6. Explain the instrumentation of UV spectrophotometer.
7. (i). Explain the various applications of UV spectroscopy. (ii). Explain the absorption laws in detail
8. Explain the applications of UV spectroscopy?
9. Discuss the following terms:
   (a)(i)Bathochromic shift (ii) hypochromic shift (iii) A chromophore (iv)Hyper chromic effect
10. (i). Explain absorption and intensity shifts in detail.
    (ii). Explain the keto enol tautomerism in UV spectroscopy?

KARPAGAM ACADEMY OF HIGHER EDUCATION
(Deemed to be University)
(Established Under Section 3 of UGC Act 1956)
DEPARTMENT OF CHEMISTRY

UNITII(Optical methods of analysis)
(Multiple Choice Questions)

S.No	Questions	Option a	Option b	Option c	Option d	Answer
1	A frequencyof 1000 cm-1 is in the ------ region	a.UV	b. IR	c.visible	d. microwave	b. IR
2	According to Woodward-Fieser rule, the value of absorbance depend on	a.no of alkyl sustituents	b. no of double bond	c.presence of polar group	d.all the above	d.all the above
3	Addition in unsaturation with the increase in the number of double bonds shifts the absorption to------- wavelength	a.shorter	b.longer	c.no change	d.maximum	b.longer
4	Among the following electromagnetic radiations, which has the maximum wavelength?	a. IR	b.UV	c.radio wave	d. X-ray	c.radio wave
5	As the number of double bonds in conjucation increases, lmax --------	a. increases	b. decreases	c. remains zero	d.zero	a. increases
6	Bathochromic shift also called	a.blue shift	b.red shift	c.yellow shift	d.orange shift	b.red shift
7	Beer-Lamberts law implies the fractional change in light intensity is proportional to	a.concentration of solution and thickness of solution	b.concentration only	c.thickness of the solution only	d.intensity of emitted light.	a.concentration of solution and thickness of solution
8	Cycles/sec is unit for	a. Wavelength	b.frequency	c.wave number	d.energy	d.energy
9	Greater value of Molar extinction coefficient indicates	a.less	b.more	c. nil	d.zero	b.more

	that the probability of transition-------					
10	If a molecule exists in two tautomeric form,the preference of one form to another can be detected by ------ spectroscopy	a.UV	b.IR	c,NMR	d.ESR	a.UV
11	In predissociation, some molecules dissociation occur at ------ energy	a.Higher	b.lower	c.zero	d.without	b.lower
12	In the case of alcohol lmax small, the effect due to	a.Covalent bond	b.H-bonding	c.co ordinate bonding.	D.none	b.H-bonding
13	In π- π* transition, solvent polarity results in---------- shift	a. Bathochromic	b. hypsochromic	c. both	d.none	a. Bathochromic
14	Increase of solvent polarity results in --------- of lmax of absorption.	a.increase	b.decrease	c. increase and decrease	d.zero	b.decrease
15	Methyl chloride is an example for ------- type of transition	a. .n-π*	b.π- π*	c. .s- s*	d. n-s*	d. n-s*
16	Molar extinction coefficient value less than ------- is called forbidden transition	a.1	b.10	c.100	d.1000	c.100
17	Of the following radiations, which represents the visible region?	a. 0-100 nm	b. 100-200 nm	c.200-400 nm	d.400-800 nm	d.400-800 nm
18	One electron- volt of energy is equivalent to a photon with a wave lengthof about	a. 300 A°	b. 30 A°	c. 3000A°	d.12000A°	d.12000A°
19	One nm is equal to	a. 10A°	b. 0.1 A°	c. 10-9 cm	d. 10-8 cm	a. 10A°
20	Parent value for Buta diene system is	a.217mμ	b.218 mμ	c.219 mμ	d.220 mμ	a.217mμ
21	Radiation source used in UV instrumentation is	a.Hydrogen	b.deuterium lamp	c. xenon discharge lamp	d.Hydrogen, deutrium and	d.Hydrogen, deutrium and

					xenon lamp	xenon lamp
22	Radiation which has the least energy (among the following)	a.alpha rays	b.beta rays	c.gammarays	d. electrica; waves	d. electrica; waves
23	Reciprocal of transmittance is called	a. Absorbance	b. Opacity	c. Incidence	d.Molar exinction coefficient	b. Opacity
24	Saturated aldehyde exhibit ------ type of transition	A.both .n-π* and π- π*	b. both .s- s* and π- π *	c. .both .s- s* and n-π*	d.only n- π *	A.both .n-π* and π- π*
25	Very important requirement for a molecule to show an I.R.spectrum is that	a)change in dipolemoment	b)change in force constant	c)change in electronic energy	d)change in wave number	a)change in dipolemoment
26	The rotational energy of a rigid rotar is	a)Erot =h2 / 4л2IJ(J+1)	b.Erot-=h2/8 л2IJ(J+1)	c. Erot=h/8 лIJ(J+1)	d.h/2I(J+1)	b.Erot-=h2/8 л2IJ(J+1)
27	The IR spectrum od rigid rotator consist of ------- equally spaced lines with a spacing of each side of band origin	a)B	b)2B	c)4B	d)3B	a)B
28	The ΔJ=0 transition gives rise to a new group of lines called	a)R branch	b)S branch	c)Qbranch	d)Pbranch	c)Qbranch
29	To get parallel band in IR forCO2,the oscillating dipole moment is ------- to the molecular axis is	a)parallel	b)perpendicular	c)angular	d)linear	a)parallel
30	To get parallel band in IR the selection rule is	a)ΔV=+1, ΔJ=+1	b) ΔV= 1, ΔJ =+1	c) ΔV=+1, ΔJ =_1	d) ΔV=+2, ΔJ=+3	a)ΔV=+1, ΔJ=+1
31	The selection rule to get the perpendicular band in spectrum is	a) ΔV=+1, ΔJ = - 1	b) ΔV=+1, ΔJ = - 1,O	c) ΔV=O, ΔJ =+ - 1	d) ΔV=+2 ΔJ =_1 2	b) ΔV=+1, ΔJ = - 1,O
32	The wave length of 1000 A° is in the ------ region	a. far UV	b.Visible	c.near UV	d. IR	a. far UV
33	The wave length of x-rays is of the order of	a.10-8m	b.10-8 cm	c. 10-23 cm	d. 40000A°	b.10-8 cm
34	Transition in UV absorption	a.size of the	b.Electronegativity	c. H-bonding	d.all the	d.all the above

	depend on	atom			above	
35	Upon irradiation with UV-radiation, benzene displaces ---- bonds due to its transition	a. 1	b.2	c. 3	d.4	c. 3
36	UV absorption spectroscopy is powerful tool for ---------- analysis	a. Quantitative	b. qualitative	c.physical	d environmental	a. Quantitative
37	Water has the electronic transition	a. .n-π*	b.π- π*	c. .s- s*	d. n-s*	d. n-s*
38	What is the calculated value for cyclohexa 1,3-diene	a. 261 mμ	b. 262 mμ	c.263 mμ	d.264 mμ	c.263 mμ
39	What is the forbidden transition in the following	a.s- s*	b.n-π*	c.n-s*	d. π- π *	b.n-π*
40	What is the lmax for the 2,4-hexa diene	a. 217 mμ	b.227 mμ	c.272 mμ	d.271 mμ	b.227 mμ
41	What will be the theoritical number of vibrational degrees of freedom in benzene,CO2, SO2 respectively	a. 12,4,3	b. 3,4,12	c. 30,4,3	d. 30,3,3	c. 30,4,3
42	In IR C—H s tretching vibration occur at the region	a)1470—1430cm-1	b)2960-2850cm-1-	c)1300-1800cm-1	d)1000-1300cm-1	b)2960-2850cm-1-
43	In IR,C=C stretching has the frequency in the region	a)970—980cm-1	b)650-610cm-1	c)1680-1620cm-1	d)995-985cm-1	c)1680-1620cm-1
44	Absorbance is defined as	a)A=log10(1\T)	b)A=e-1\T	c)A=1\T	d)A=2\T	a)A=log10(1\T)
45	For a non linear molecule there are ------degrees of vibrational degree of freedom	a)3n-2	b)3n-6	c)3n-3	d)3n-4	b)3n-6
46	Position of C—O stretching band for primary alcohol occur at ----in IR	a)1050cm-1	b)1500cm-1	c)1800cm-1	d)200cm-1	a)1050cm-1
47	The O-H stretching of Phenol exibit a strong broad band in the range in IR	a)3600-3200cm-1	b)1600-1700cm-1	c)2300-2500cm-1	d)900-100cm-1	a)3600-3200cm-1

48	The potential energy of an anhormonic oscillator is	a)Vr=De[1-expa(r-re)]2	b)Vr=De[1-a(ro-r)]2	c)Vr=De[1\ro-r]2	d)Vr=a[Do-(r-ro)]2	a)Vr=De[1-expa(r-re)]2
49	µ/For a molecule to be IR active	a)dµ/dr=o	b) dr/dµ =o	c) dµ/dr not equal to 0	d) d¥/dc not equal to 0	c) dµ/dr not equal to 0
50	>C=O stretching of aldehudes occur in the region in the IR region.	a)2770-2700 cm-1	b)1740-1720cm-1	c)700-970cm-1	d)3300-3400-1cm	b)1740-1720cm-1
51	The type of H-bonding which give rise to broad lines in IR Techinque is -----	a)Intermolecular	b)Intramolecular	c)C-H stretching	d)C-H bending	b)Intramolecular
52	The type of H-bonding which give rise to sharp lines in IR Techinque is ------	a)Sym.stretching	b)Anti.sym.stretching	c)Intramoleclulear	d)Intercular	a)Sym.stretching
53	In which region we get absorbtiion bands and shoulders	a)Far infradredf	b)near infrared	c)mid-infrared	d)finger print region	d)finger print region
54	N-H bending vibration for primary amines occurs in the region	a)700-900cm-1	b)800-700cm-1	c)1600-1500cm-1	d)800-700cm-1	c)1600-1500cm-1
55	In a double beam instrument ,the beamspilts into-----parts	a)one	b)three	c)two	d)none of the above	c)two
56	The radiation used to study the vibrational spectra of a molecule is	a. microwave	b.radiowave	c.UV	d.IR	d.IR
57	The number of vibrational degrees of freedom of watermolecule is	a. 4	b.9	c.2	d. 3	d. 3
58	Which of the following molecules has the smallest spacing in thefine structureof Irspectrum?	a. HCl	b.HBr	c.CO	d.HI	d.HI
59	Carbonyl group shows a characteristic intenseband in the IR. This high intensity is	a. high reactivity of the carbonyl group	b. the presence of lone pair of electrons which areeasily	c. high polarity of the group	d. largeforce constant of thegroup	c. high polarity of the group

	due to		excited			
60	The vibrational frequency of HD is less than that of H2becausce	a. H2 has a higher force constant	b. H2has a lower force constant	c. HD has a higher mass	d. HD has a higher mass and lower force constant	c. HD has a higher mass

Thermal methods of analysis:
Theory of thermogravimetry (TG), basic principle of instrumentation.
Techniques for quantitative estimation of Ca and Mg from their mixture.

Electroanalytical methods:
Classification of electroanalytical methods, basic principle of pH metric, potentiometric and conductometric titrations. Techniques used for the determination of equivalence points.
Techniques used for the determination of pKa values.

Thermogravimetric analysis:

• The instrument used for TGA analysis is a programmed precision balance for a rise in temperature (called as Thermobalance, see Figure 23.01). Thermobalance consists of an electronic microbalance (important component), a furnace, a temperature programmer and a recorder.

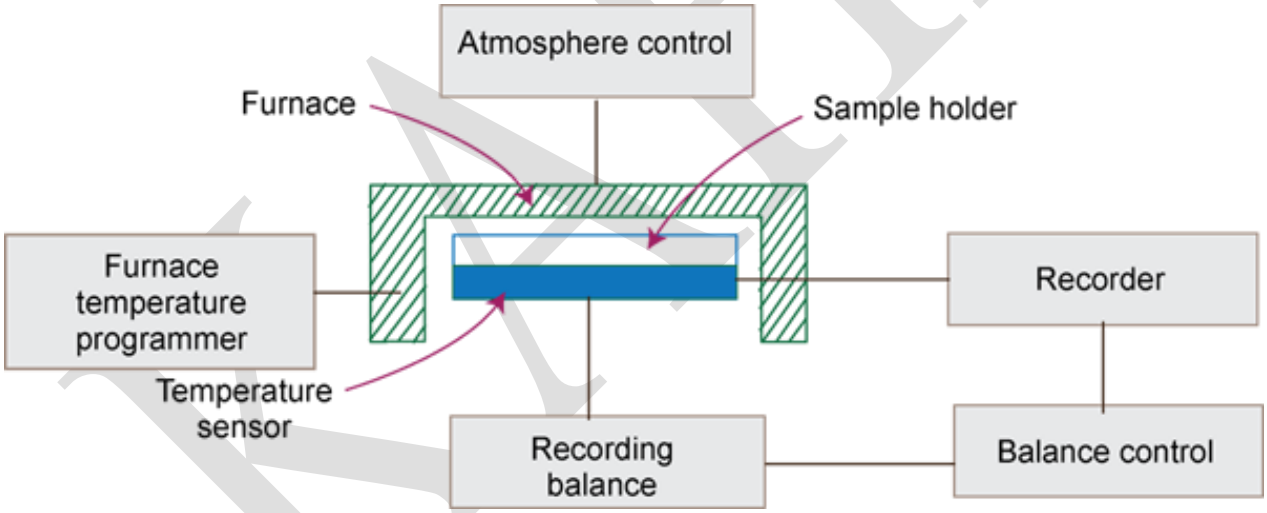


Figure 23.01: Block Diagram of a Thermobalance.

• The plot of mass change in percentage versus temperature or time (known as TGA curves) is the typical result of TGA analysis as shown in Figure 23.02.

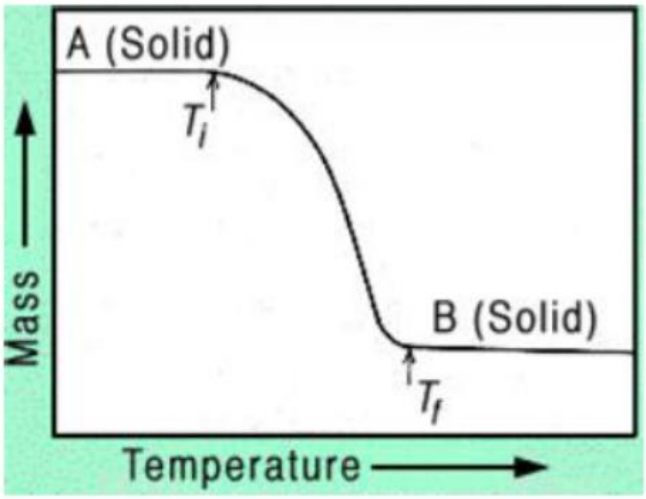
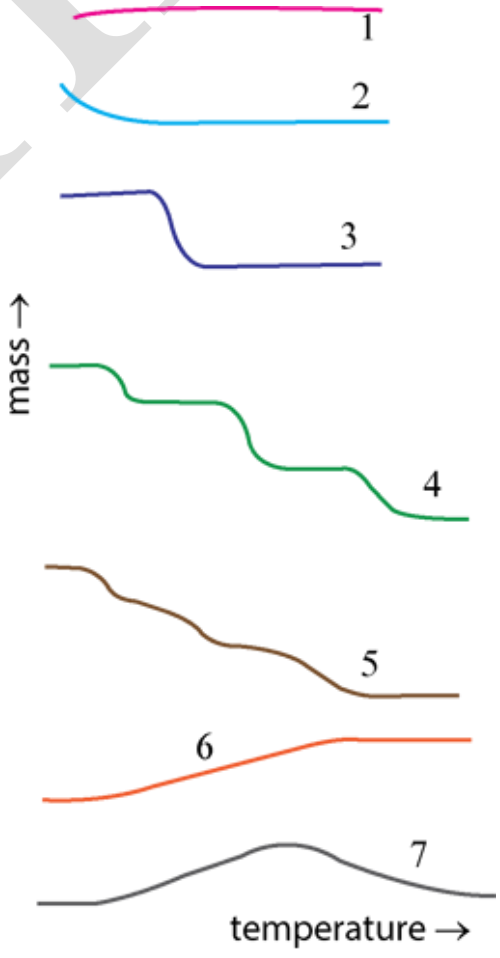


Figure 23.02: The plot of mass change with temperature.

• There are two temperatures in the reaction: T_i (starting of decomposition temperature) and T_f(final temperature) representing the lowest temperature at which the onset of a mass change is seen and the lowest temperature at which the process has been completed, respectively. The reaction temperature and interval (T_f - T_i) strongly depend on the conditions of the experiments. Hence, they cannot have any fixed values.



Interpretation of TGA Curves:

TGA curves are typically classified into seven types according to their shapes. Figure 23.03 shows schematic of various types of TGA curves.

• Curve 1: No change: This curve depicts no mass change over the entire range of temperature, indicating that the decomposition temperature is greater than the temperature

range of the instrument.

• Curve 2: Desorption / drying: This curve shows that the mass loss is large followed by mass plateau. This is formed when evaporation of volatile product(s) during desorption, drying or polymerization takes place. If a non-interacting atmosphere is present in the chamber, then curve 2 becomes curve 1.

• Curve 3: Single stage decomposition: This curve is typical of single-stage decomposition temperatures having T_i and T_f.

• Curve 4: Multistage decomposition: This curve reveals the multi-stage decomposition processes as a result various reactions.

• Curve 5: Similar to 4, but either due to fast heating rate or due to no intermediates.

• Curve 6: Atmospheric reaction: This curve shows the increase in mass. This may be due to the reactions such as surface oxidation reactions in the presence of an interacting atmosphere.

• Curve 7: Similar to curve 6, but product decomposes at high temperatures. For example, the reaction of surface oxidation followed by decomposition of reaction product(s).

Processes that leads to Weight Gain and loss in TGA experiment:

Process	Weight gain	Weight loss
Adsorption or absorption	✓	
Desorption, drying		✓
Dehydration, desolvation		✓
Sublimation		✓
Vaporization		✓
Solid-state reactions (some cases)		✓
Solid-gas reactions	✓	✓
Magnetic transitions	✓	✓

Applications of TGA:

a) Thermal stability of the related materials can be compared at elevated temperatures under the required atmosphere. TGA curve helps to explicate decomposition mechanisms.

b) Materials Characterization: TGA curves can be used to fingerprint materials for identification or quality control.

c) Compositional analysis: By a careful choice of temperature programming and gaseous environment, many complex materials/ mixtures can be analyzed by decomposing or removing their components. For example: filler content in polymers; carbon black in oils; ash and carbon in coals, and the moisture content of many substances.

d) Kinetic studies: A variety of methods can be used to analyze the kinetic features of weight loss or gain through controlling the chemistry or predictive studies.

e) Corrosion studies: TGA provides a means of studying oxidation or some reactions with other reactive gases or vapors.

Examples of TGA curves:

Figure 23.04 shows the heat decomposition mass curve of Whewellite (calcium oxalate monohydrate) [1]

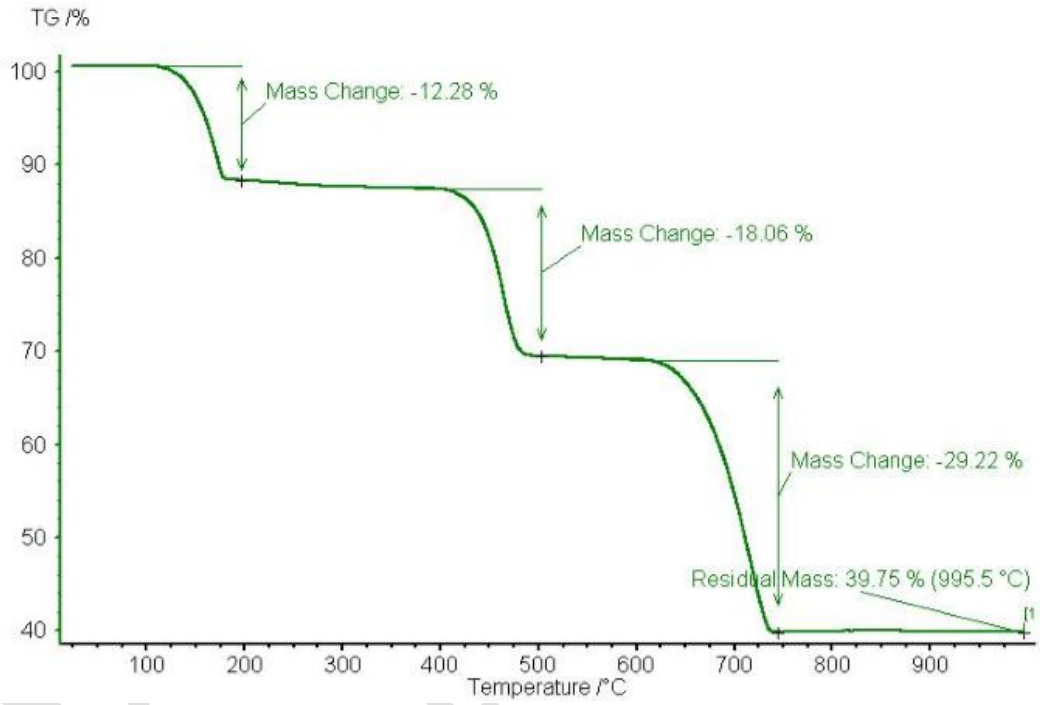


Figure 23.04: TGA curve of Whewellite.

Examples of TGA curves:

Thermal decomposition of calcium oxalate monohydrate studied by TGA

(a) $Ca(COO)_2\ H_2O \rightarrow [200°C] \rightarrow Ca(COO)_2 + H_2O$ (g);

(b) $Ca(COO)_2 \rightarrow [500°C] \rightarrow CaCO_3 + CO$ (g); and,

(c) $CaCO_3 \rightarrow [800°C] \rightarrow CaO + CO_2$ (g).

Figure 23.05 depicts the mass change corresponding to each reactions of calcium oxalate monohydrate.

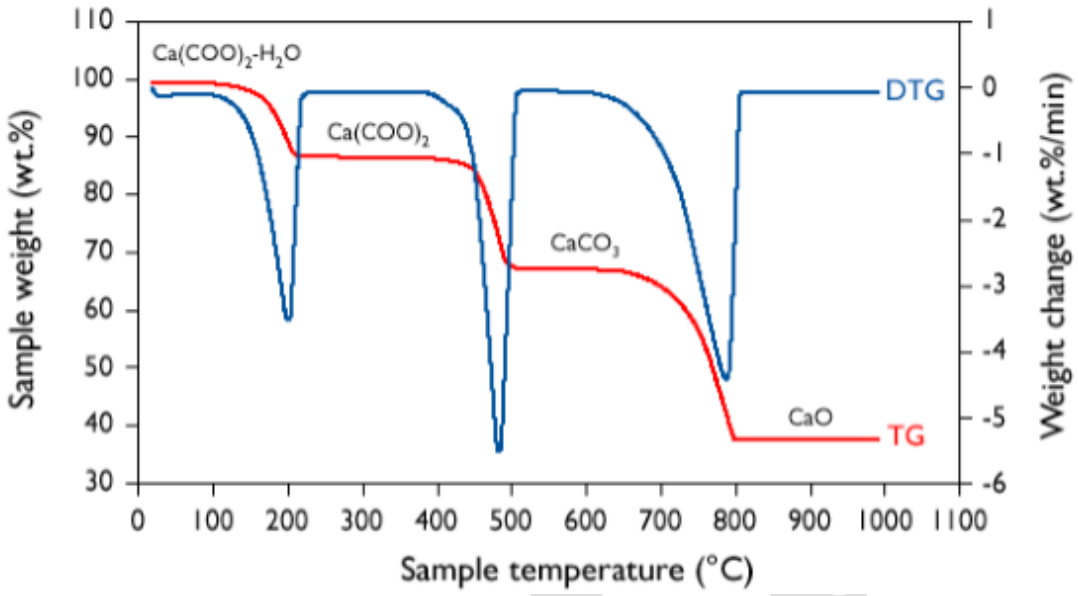
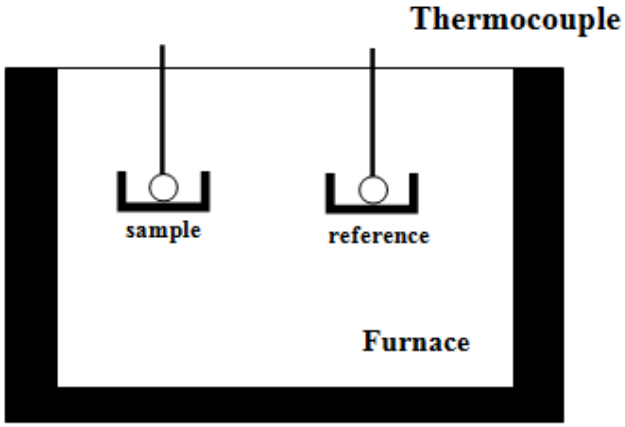


Figure 23.05: TGA curve of calcium oxalate monohydrate [2].

Differential Thermal Analysis (DTA)

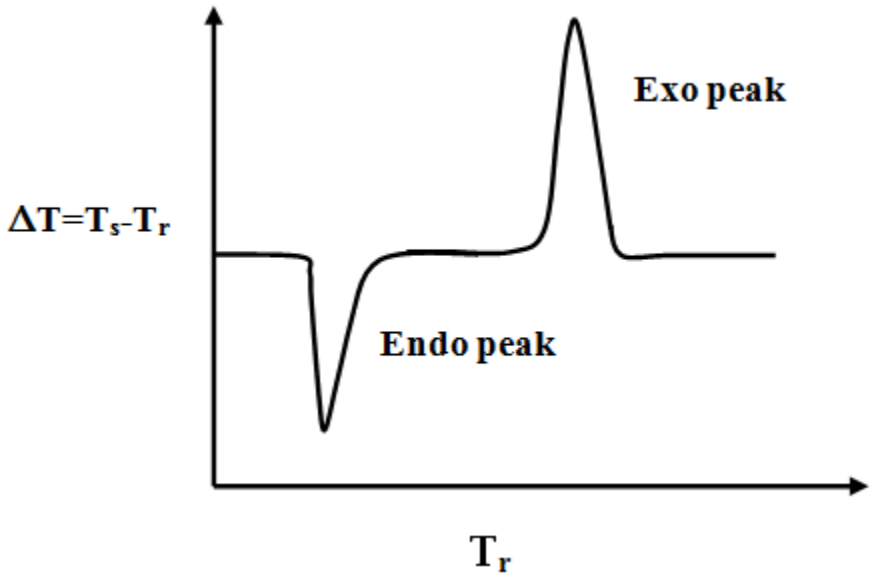
DTA consists of heating a sample and reference material at the same rate and monitoring the temperature difference between the sample and reference. In this method, the sample is heated along with a reference standard under identical thermal conditions in the same oven. The temperature difference between the sample and reference substance is monitored during the period of heating. As the samples undergo any changes in state, the latent heat of transition will be absorbed/ evolved and the temperature of the sample will differ from that of the reference material. This difference in temperature is recorded. Hence, any change in state can be detected along with the temperature at which it occurs.



Schematic diagram for differential thermal analysis technique.

When an endothermic process occurs (ΔH positive) in the sample, the temperature of sample (T_s)lags behind the temperature of reference (T_r). The temperature difference $\Delta T = (T_s - T_r)$ is recorded against reference temperature T_r and the corresponding plot is shown in Fig 12. In DTA, by convention, endothermic response is represented as negative that is by downward peaks. When an exothermic process (ΔH negative) occurs in the sample, the response will be in the reverse direction and the peaks are upward. Since the definition of $\Delta T = T_s - T_r$ is rather arbitrary, the DTA curves are usually marked with endo or exo direction.

It is essential that reference sample must not undergo any change in state over the temperature range used and both the thermal conductivity and heat capacity of reference must be similar to those of samples. Both sample and reference materials should be also inert towards sample holder or thermocouples. Alumina or silicon carbide are most commonly used standard reference samples. DTA profiles are affected by heating rate, sample size and thermocouple position within the sample.



Typical exo and endo peak in a DTA profile.

Application:

Any change associated with enthalpy change can be studied by DTA. In general DTA curves are used to get informations about temperature and enthalpy changes for decomposition, crystallization, melting, glass transition etc. In solid catalysis it is particularly useful to detect phase changes associated with calcination process. For example change of aluminum hydroxide to alumina can be easily detected by DTA.

Differential scanning calorimetry:

• Differential scanning calorimetry (DSC) technique was developed by E.S. Watson and M. J. O'Neill in 1962 and commercial introduction was done at 1963 in Pittsburgh conference.

• DSC is a thermo-analytical technique in which the differences in the amount of heat required to increase the temperature of a sample and reference are measured as a function of temperature.

• Both the sample and reference are maintained at nearly the same temperature throughout the experiment. The reference sample should have a well defined heat capacity over the range of temperatures to be scanned and analyzed.

• In general, the temperature program of the DSC is designed to increase the sample holder temperature linearly as a function of time.

• The main application of DSC is in studying phase transitions such as melting point, glass transitions, or exothermic decompositions. These transitions involve energy changes or heat capacity changes that can be detected by DSC with great sensitivity.

Description of DSC:

There are two types of DSC commercially available: Heat Flux (HF) Type and Power Compensation (PC) Type. Figure 22.01 shows the block diagram of HF and PC types.

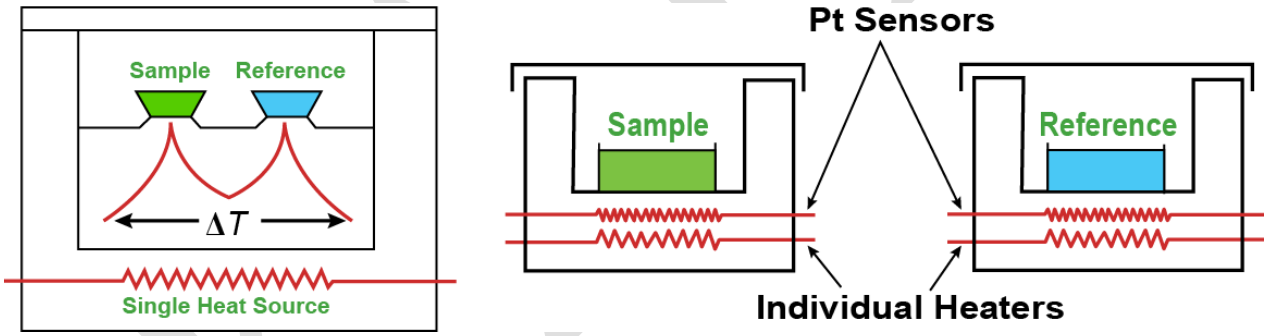


Figure 22.01: Schematic diagram of HF and PC types DSC.

In HF type DSC:

• Both sample and reference pans are heated by a single furnace through heat sink and heat resistor. Heat flow is proportional to the heat difference of heat sink and holders. The temperature versus time profile through a phase transition in a heat flux instrument is not linear.
• At a phase transition, there is a large change in the heat capacity of the sample, which leads to a difference in temperatures between the sample and reference pan.

• A set of mathematical equations convert the signal into heat flow information. By calibrating the standard material, the unknown sample quantitative measurement is achievable.

In PC type DSC:

Both sample and reference pans are heated by a different furnaces. When an event occurs in the sample, sensitive Platinum Resistance Thermometer (PRT) detects the changes in the sample, and power (energy) is applied to or removed from the sample furnace to compensate for the change in heat flow to or from the sample. As a result, the system is maintained at a "thermal null" state at all times. The amount of power required to maintain system equilibrium is directly proportional to the energy changes occurring in the sample. No complex heat flux equations are necessary with a power compensation DSC because the system directly measures energy flow to and from the sample.

In addition, PC type DSC has enhanced modulated temperature DSC (StepScan) technique and fast scan DSC (HyperDSC) for dramatic improvements in productivity, as well as greater sensitivity.

Furthermore, the heating and cooling rate of PC types DSC can be as high as 500°C/min.

Detection of phase transitions:

The underlying principle is that when the sample undergoes a physical transformation (phase transitions, etc), more or less heat will be needed to flow to it as compared to the reference to maintain both of them at the same temperature. This certainly depends on whether the process is exothermic or endothermic.

For example:

When a solid sample melts into a liquid, then it requires more heat flowing to the sample to increase its temperature at the same rate as the reference. This is due to the absorption of heat by the sample as it undergoes the endothermic phase transition from solid to liquid. Similarly, when

the sample undergoes exothermic processes (such as crystallization) less heat is required to raise the sample temperature.

By observing the difference in heat flows between the sample and reference, DSC is able to measure the amount of heat absorbed or released during such transitions. DSC may also be used to observe more subtle phase changes, such as glass transitions.

Information about the DSC curves:

In general, the result of a DSC experiment is a curve of heat flux versus temperature or versus time. This curve can be used to calculate enthalpies of transitions, i.e., $\Delta H = kA$ (where, H is the enthalpy of transition, k is the calorimetric constant, and A is the area under the curve) , which is done by integrating the peak corresponding to a given transition.

The value of k is typically given by the manufacturer for an instrument or can generally be determined by analyzing a well-characterized sample with known enthalpies of transition.

Applications of DSC:

DSC technique can be used to obtain glass transition, melting points, crystallization times and temperatures, heats of melting and crystallization, percentage of crystallinity, oxidative stabilities, heat capacity, completeness of cure, purities, thermal stabilities, polymorphism, recyclates or regrinds

Evaluation and interpretation of DSC curves:

Figure 22.02 shows the typical DSC curve for a sample exhibiting endotherm of melting at a particular heating rate.

The onset of melting (122.8°C) and peak temperature of melting (123.66°C) can be determined by extrapolation technique and peak values, respectively.

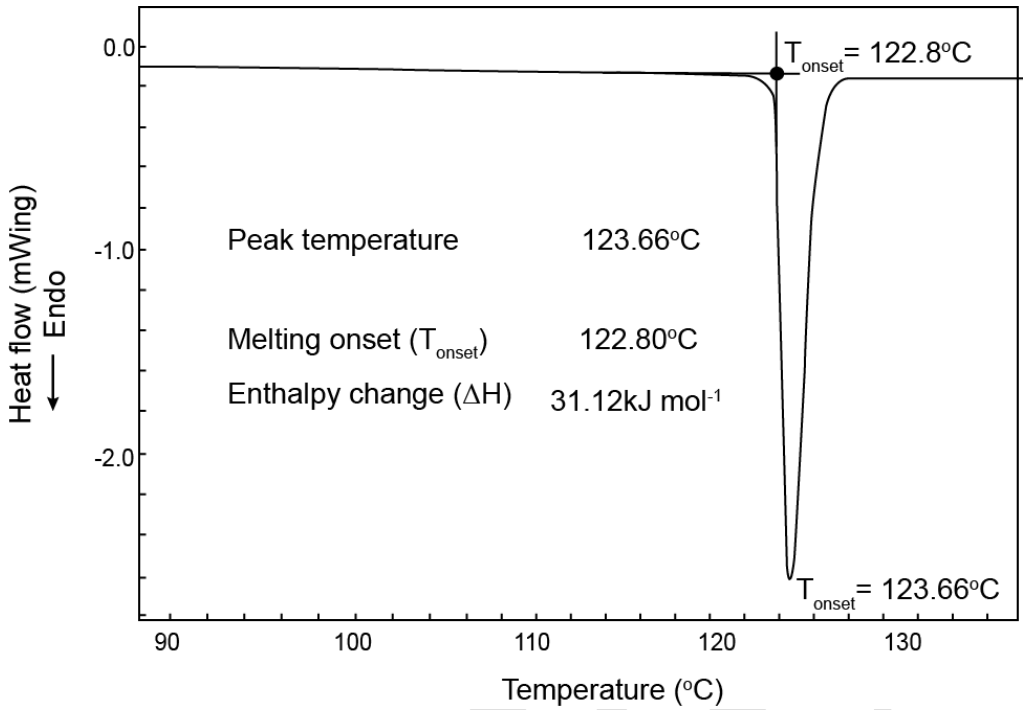


Figure 22.02: Typical DSC curve of a sample.

The enthalpy change can be calculated by integrating the area under the curve. The unit can be either J/g or J/mole depending on the nature of the sample.

Effect of heating rate:

Heating rate affects the melting point and enthalpy of melting. Figure 22.03 shows the typical DSC curves taken at different heating rate.

With increasing heating rate, the onset of the melting does not change significantly, but the peak point of melting shifts slowly to higher temperature.

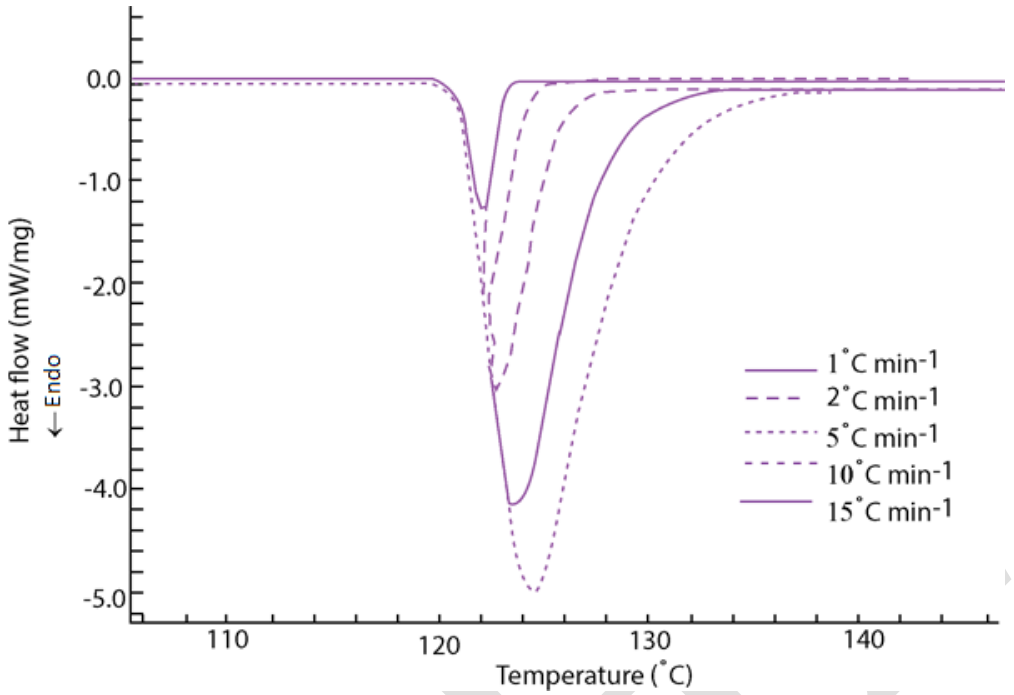


Figure 22.03: Typical DSC curves taken at different heating rates.

Effect of sample weight:

The sample weight also affects the thermal properties significantly. Figure 22.04 shows the typical DSC curves taken at a constant heating rate for different mass of the samples.

It could be clearly seen that the onset of melting, peak point of melting and enthalpy undergo small variations when the sample mass is changed.

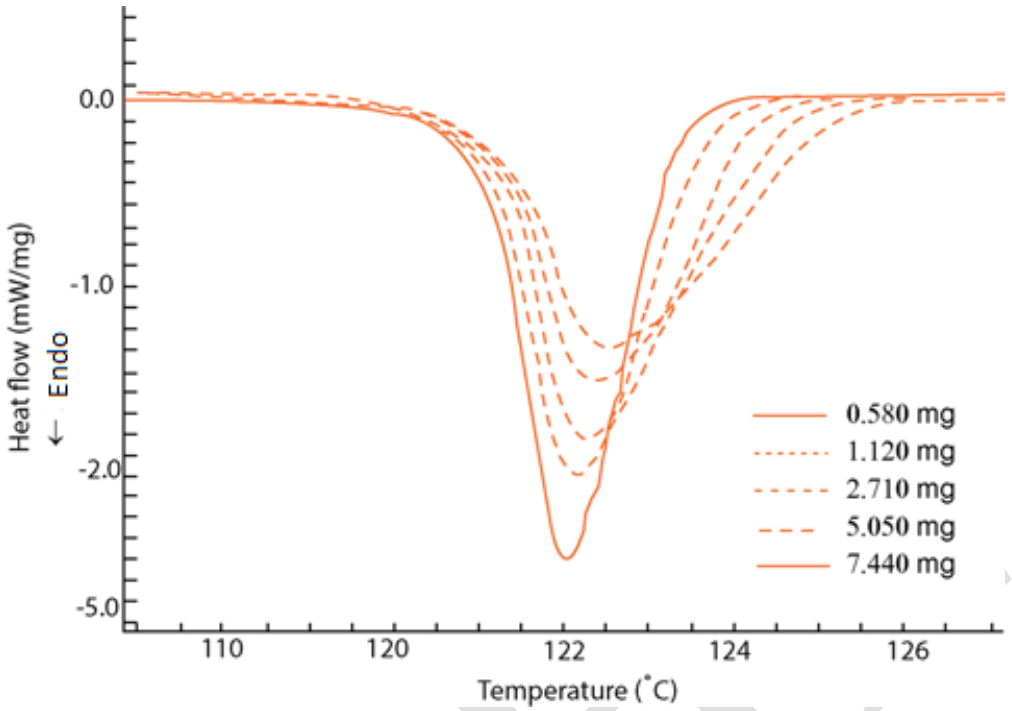


Figure 22.04: Typical DSC curves taken for different weighed samples.

Thermogravimetric analysis:

• Thermogravimetric (TG) is a branch of thermal analysis examining the mass changes of a sample as a function off temperature (in the scanning mode) or as a function of time (in the isothermal mode).

• Thermal gravimetric analysis or thermogravimetric analysis (TGA) is a method of thermal analysis in which changes in physical and chemical properties of materials are measured as a function of increasing temperature (with constant heating rate), or as a function of time (with constant temperature and/or constant mass loss).

• Changes in the mass of a sample due to various thermal events (desorption, absorption, sublimation, vaporization, oxidation, reduction and decomposition) are studied while the sample

is subjected to a program of change in temperature. Therefore, it is used in the analysis of volatile products, gaseous products lost during the reaction in thermoplastics, thermosets, elastomers, composites, films, fibers, coatings, paints, etc.

• There are different types of TGA available:

i. Isothermal or Static TGA: In this case, sample is maintained at a constant temperature for a period of time during which change in weight is recorded.

ii. Quasi-static TGA: In this technique, the sample is heated to a constant weight at each of a series of increasing temperature.

iii. Dynamic TGA: In this type of analysis, the sample is subjected to condition of a continuous increase in temperature at a constant heating rate, i.e., usually linear with time.

Electroanalytical methods

Electroanalytical methods are a class of techniques in analytical chemistry which study an analyte by measuring the potential (volts) and/or current (amperes) in an electrochemical cell containing the analyte. These methods can be broken down into several categories depending on which aspects of the cell are controlled and which are measured. The three main categories arepotentiometry (the difference in electrode potentials is measured), coulometry (the cell's current is measured over time), and voltammetry (the cell's current is measured while actively altering the cell's potential).

Potentiometry passively measures the potential of a solution between two electrodes, affecting the solution very little in the process. One electrode is called the reference electrode and has a constant potential, while the other one is an indicator electrode whose potential changes with the composition of the sample. Therefore, the difference of potential between the two electrodes gives an assessment of the composition of the sample. In fact, since potentiometric measurement is a non-destructive measurement, assuming that the electrode is in equilibrium with the solution we are measuring the potential of the solution. Potentiometry usually uses indicator electrodes

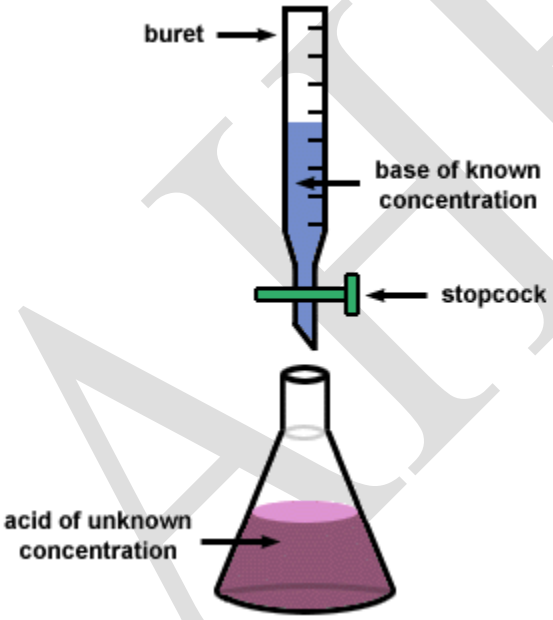
made *selectively* sensitive to the ion of interest, such as fluoride in fluoride selective electrodes, so that the potential solely depends on the activity of this ion of interest. The time that takes the electrode to establish equilibrium with the solution will affect the sensitivity or accuracy of the measurement. In aquatic environments, platinum is often used due to its high electron transfer kinetics, although an electrode made from several metals can be used in order to enhance to electron transfer kinetics. The most common potentiometric electrode is by far the glass-membrane electrode used in a PH METER. A variant of potentiometry is chronopotentiometry which consists in using a constant current and measurement of potential as a function of time. It has been initiated by Weber.

Coulometry uses applied current or potential to completely convert an analyte from one oxidation state to another. In these experiments, the total current passed is measured directly or indirectly to determine the number of electrons passed. Knowing the number of electrons passed can indicate the concentration of the analyte or, when the concentration is known, the number of electrons transferred in the redox reaction. Common forms of coulometry include bulk electrolysis, also known as *Potentiostatic coulometry* or *controlled potential coulometry*, as well as a variety of coulometric titrations.

Voltammetry applies a constant and/or varying potential at an electrode's surface and measures the resulting current with a three electrode system. This method can reveal the reduction potential of an analyte and its electrochemical reactivity. This method in practical terms is nondestructive since only a very small amount of the analyte is consumed at the two-dimensional surface of the workingand auxiliary electrodes. In practice the analyte solutions is usually disposed of since it is difficult to separate the analyte from the bulk electrolyte and the experiment requires a small amount of analyte. A normal experiment may involve 1–10 mL solution with an analyte concentration between 1 and 10 mmol/L. Chemically modified electrodes are employed for analysis of organic and inorganic samples.

Principle of pH metric Titration

In an acid-base titration, the important information to obtain is the equivalence point. If there are a given number of moles of acid in the titration flask, the equivalence point is reached when that same number of moles of base have been added from the buret. The molarity of the base can then be calculated since the number of moles of base added is the same as the number of moles of acid in the flask, and the volume of the base added is also known. Similarly, if the number of moles of acid in the titration flask is unknown, it can be calculated for the equivalence point if the molarity of the base and the volume of base added are known.



Often the pH of the solution will change dramatically at the equivalence point. An acid-base indicator works by changing color over a given pH range. If an indicator which changes color near the equivalence point is chosen, there is also a dramatic change in the color of the indicator at the equivalence point because the pH changes so rapidly.

In a potentiometric acid-base titration, an indicator is not necessary. A pH meter is used to measure the pH as base is added in small increments (called aliquots) to an acid solution. A graph is then made with pH along the vertical axis and volume of base added along the

horizontal axis. From this graph the equivalence point can be determined and the molarity of the base calculated.

OBJECTIVES

1. To perform a potentiometric titration of an acidic solution of known molarity.

2. To graph the volume of base added vs the pH and to determine the equivalence point.

3. To calculate the molarity of the basic solution.

SAFETY

1. Wear your goggles and apron at all times during this experiment.

2. The HCl solution is corrosive. If any is spilled, you should neutralize it with sodium bicarbonate solution. If you should get some on your skin, neutralize it , and then wash it off with plenty of water. In either case, notify your teacher immediately.

3. The NaOH solution is caustic. If you get any on your skin, flush the affected area with plenty of water. Notify your teacher immediately.

Conductometric titration

A chemical reaction in which there is a significant change in the number or mobilities of ionic species can be followed by monitoring the change in conductance. Many acid-base reactions fall into this category. Inconductometric titration, conductometry is employed to detect the end-point of a titration.

Consider, for example, the titration of the strong acid HCl by the strong base NaOH. In ionic terms, the process can be represented as

$H^+ + Cl^- + Na^+ + OH^- \rightarrow H_2O + Na^{++} Cl^-$

At the end point, only two ionic species remain, compared to the four during the initial stages of the titration, so the conductivity will be at a minimum. Beyond the end point, continued addition of base causes the conductivity to rise again. The very large mobilities of the H+and OH– ions cause the conductivity to rise very sharply on either side of the end point, making it quite easy to locate.

Theory, introduced in 1887 by the Swedish scientist Svante Arrhenius, that acids are substances that dissociate in water to yield electrically charged atoms or molecules, called ions, one of which is a hydrogen ion (H^+), and that bases ionize in water to yield hydroxide ions (OH^-). It is now known that the hydrogen ion cannot exist alone in water solution; rather, it exists in a combined state with a water molecule, as the hydronium ion (H_3O^+). In practice the hydronium ion is still customarily referred to as the hydrogen ion.

The acidic behaviour of many well-known acids (*e.g.,* sulfuric, hydrochloric, nitric, and acetic acids) and the basic properties of well-known hydroxides (*e.g.*, sodium, potassium, and calcium hydroxides) are explained in terms of their ability to yield hydrogen and hydroxide ions, respectively, in solution. Furthermore, such acids and bases may be classified as strong or weak acids and bases depending on the hydrogen ion or hydroxide ion concentration produced in solution. The reaction between an acid and a base leads to the formation of a salt and water; the latter is the result of the combination of a hydrogen ion and a hydroxide ion.

Requirements

Buffer stock solution (concentrated), pH meter, distilled water, wash bottle, volumetric flasks, measuring

cylinders, beakers, and pipettes.

Glycine-NaOH buffer system:

1. 20 mM glycine

2. 200 mM NaOH

Glycine-HCl buffer system

1. 20 mM glycine

2. 200 mM HCl

Theory

The observation that partially neutralized solutions of weak acids or weak bases are resistant to pH changes on addition of small amounts of strong acid or strong base leads to the concept of "buffering". Buffers consist of an acid and its conjugate base, such as carbonate and bicarbonate, or acetate and acetic acid. The quality of a buffer is dependent on its buffering capacity (resistance to change in pH by addition of strong acid or base) and ability to maintain a stable pH upon dilution or addition of neutral salts. Because of the following equilibria, addition of small amounts of strong acid and strong base result in removal of only small amounts of the weakly acidic or basic species, therefore there is little change in the pH:

$$HA(\text{acid}) \$ H+ + A-(\text{conjugate base})$$

$$B\ (\text{base}) + H+ \$ BH+(\text{conjugate acid})$$

The pH of a solution of a weak acid or base may be calculated from the Henderson-Hasselbalch equation

$$\text{pH} = \text{pKa} + \log [\text{basic species}]/[\text{acidic species}]$$

The pKa of a buffer is that pH where the concentrations of basic and acidic species are equal, and this basic form of equation is accurate between the pH ranges of 3 to 11. Below pH 3 and above pH 11 the concentration of the ionic species of water must be included in the equation. Since the pH range of interest to the biochemical engineer is 3 - 11 ranges, this can be ignored. From the Henderson-Hasselbalch equation an expression for buffer capacity ($= d[A-]/d[pH]$) may be deduced

Procedure

1. pH Measurement: Mix all solutions thoroughly. The pH measurement may be made in original beakers. Do not change any control on the pH meter except as directed. With the meter on stand

by,rinse the electrode with deionized water, gently shake off the excess water, and immerse the electrode in the sample solution. Switch the meter to the pH mode, allow the reading to stabilize, and record the pH. Switch the meter back to the standby mode, rinse the electrode again, and leave the electrode immersed in deionized water. Repeat this procedure for all samples.

2. Glycine-NaOH buffer system: Prepare 50 ml 20 mM glycine solution and 100 ml of 200mM NaOH solution. Calibrate the pH meter with standard buffer solution at room temperature. Take 50 ml of glycine solution in a beaker and add 0.5 ml of NaOH solution and shake well to mix. Note the change in pH. Add subsequent quantities of NaOH with an increment of 0.5 ml each time and note observed pH at regular intervals. Take about 30-35 readings and generate the following observation table:

Volume of NaOH added (ml)	Observed pH
0.0	
1.0	
1.5	
2.0	
2.5	

POSSIBLE QUESTIONS

PART-B (2 Mark Questions)

1. State the Beer-Lambert's Law

2. What is finger-print region in spectroscopy?

3. What is the role of a monochromator in the atomic absorption spectrometer?

4. What is buffer solution?

5. What is chiral shift reagents?

PART-C (6 Mark Questions)

1. i) Draw a dash-wedge structure for (R)-3-methylhexan-3-ol
   ii) Draw a dash-wedge structure for (R)-3-bromo-1,1-dimethylcyclohexane
2. Illustrate the chiral chromatographic techniques
3. Discuss the following
   i. Common ion effect
   ii. pH scale.
4. Write brief note on pH metric titration.
5. Explain the electronic transitions involved in UV spectroscopy?
6. Explain the applications of IR spectroscopy in organic functional groups?
7. Write notes on Atomic emission spectroscopy?
8. Write notes on flame photometry?
9. Explain the statistical test of data (F, Q and T test)
10. Discuss about type of errors

KARPAGAM ACADEMY OF HIGHER EDUCATION
(Deemed to be University)
(Established Under Section 3 of UGC Act 1956)
DEPARTMENT OF CHEMISTRY

UNIT III (Thermal methods of analysis & Electroanalytical methods)
(Multiple Choice Questions)

S.No	Questions	Option a	Option b	Option c	Option d	Answer
1	Which of the abbreviation refered thermogravimetry	TG	DTA	DTG	Th	TG
2	According to thermal methods, when the matter is heated then it undergo -------- changes	physical changes only	chemical changes only	biological change	physical and chemical changes	physical and chemical changes
3	What is the instrument used in the DTG	DTA apparatus	calorimeter	thermobalance	photometer	thermobalance
4	What is the abbreviation for DTA	Differential thermal analysis	Differentiate thermal analysis	Different temperature analysis	Differential thermo analysis	Differential thermal analysis
5	The parameter reflectance is measured by which instrument	DSC	DTG	DRS	DTA	DRS
6	The dynamic reflectance spectroscopy used ------ instruments	calorimeter	spectrophotometer	thermobalance	detector	spectrophotometer
7	The meaning of EC is	Electrodeconductance	Electrical conductivity	Energy concentration	Electroniccurrent	Electrical conductivity
8	The horizontal lines present in the TG curve is called as	Plates	Plateaus	Curve	Peak	Plateaus

9	The instrument of Electrical conductivity is	photometer	spectrophotometer	Electrometer	Thermometer	Electrometer
10	The main reaction occurred in thermal analysis is	oxidation	reduction	decomposition	dehydrogenation	decomposition
11	Thermogravimetery is used to analyse ------ of the samples	size	mass	nature	elements percentage	mass
12	Thermomechanical analysis is used to measure the ----- of the samples	nature	refractive index	magnitude	Volume	Volume
13	The change in dH of the sample according change in ------- of the sample	temperature	volume	size	index	temperature
14	The instrument dilatometer is used to measure the ------ of the sample	length	height	density	mass	length
15	The brief of DSC is	Differential thermal analysis	Differential scanning calorimetry	Differentioalscanning calorimeter	Difficultscannedconductometer	Differential scanning calorimetry
16	The parameter temperature of the sample is measured by	TG	DTA	thermoprocess	thermo titrimetry	thermo titrimetry
17	The isothermal thermogravimetry also called as	Static thermogravimetry	Dynamic thermogravimetry	Differential thermogravimetry	Thermogravimetry	Static thermogravimetry
18	The thermogravimetry is concerned the change in ----- of samples	volume	weight	temperature	density	weight

19	DTA did not measure the ------ change in the samples	temperature	weight	valuble	metals	weight
20	In DTA instruments ----- not change with in the temperature range	heat of reaction	size only	nature	mass	heat of reaction
21	In the gaseous environment, which technique is more sensitive	DTA	TG	DSC	DTG	DTA
22	In DSC, energy necessary to establish a -------- temperature difference between sample and reference	zero	optimum	high	low	zero
23	The sample size which is under DSC analysis	1-7mg	2-3mg	8-9 mg	2-10mg	2-10mg
24	The sensitivity of heat transition in DTA is	1kJ/mole	3kJ/mol	0.5 kJ/mol	4 kJ/mol	0.5 kJ/mol
25	Automatic thermogravimetric analysis----minutes required for entire operation carried out	1 to 5	5	10	12	12
26	In DTA analysis, We can measure the peak corresponds to-------- of the sample	decomposition	combination	reduction	oxidation	decomposition
27	12 minutes for recording the results occur in ----- thermogravimetry	dynamic	static	automatic	mannual	automatic
28	Give the equation for X-ray	$m\lambda = d \sin \theta$	$m\lambda = 2d \sin \theta$	$m\lambda = d \cose \theta$	$m\lambda = d \cos \theta$	$m\lambda = d \sin \theta$

	monochromators?					
29	Which one the following is example for Metal-insoluble metal salt electrode	calomel electrode	standard hydrogen electrode	silver-silver chloride electrode	Gas electrode	calomel electrode
30	The unit of electrical energy is	volts	joules	coulomb	meter	joules
31	In IUPAC conventions, the double vertical line represents	two half cell	cathode half cell	salt bridge	anode half cell	salt bridge
32	Platinum is a	positive electrode	negative electrode	Positve and negative electrode	Inert electrode	Inert electrode
33	If the emf acts in the opposite direction through the cell circuit it is denoted as a	positive	negative	zero	cannot be determined	zero
34	What is the potential of a half cell consisting of zinc electrode in 0.01M $ZnSO_4$ solution 25°c. E°= 0.763 V	0.0591 V	0.6521 V	0.7532 V	0.8221 V	0.0591 V
35	What is R in Nernst equation	rate of the reaction	redox reaction	gas constant	reduction of gas	gas constant
36	What is the free energy change for the reaction $Sn^{4+} + 2e \rightarrow Sn^{2+}$. If its standard reduction potential is +0.15	25.59 kJ	29.52 kJ	28.95 kJ	data inadequate	25.59 kJ
37	The device in which the free energy of a physical or chemical process is converted into electrical energy is called	daniel cell	galvanic cell	laclanche cell	voltaic cell	galvanic cell

38	The electrode in which oxidation occurs is	anode	cathode	Anode and Cathode	Electrolyte	anode
39	The salt bridge is filled with a solution of	potassium chromate	sodium chloride	potassium chloride	zinc chloride	potassium chloride
40	If the electricity produced by the cell is equal to the EMF, the cell is	reversible	irreversible	Sometimes reversible	Sometimes irreversible	irreversible
41	An example for metal-metal ion electrodes is	daniel cell	hydrogen electrode	chlorine electrode	calomel electrode	daniel cell
42	An example for gas electrode is	hydrogen electrode	chlorine electrode	oxygen electrode	hydrogen, Chlorine and oxygen electrode	hydrogen, Chlorine and oxygen electrode
43	calomel is a	potassium chloride	sodium chloride	mercurous chloride	barium chloride	mercurous chloride
44	The wire used in the calomel electrode is made of	platinum	copper	titanium	iron	platinum
45	An example for oxidation-reduction electrode is	calomel electrode	chlorine electrode	quinhydrone electrode	hydrogen electrode	quinhydrone electrode
46	In quinhydrone electrode, the platinum wire is placed in a solution containing	hydroquinone and quinone	only hydroquinone	only quinone	Water	only hydroquinone
47	The tendency of an electrode to lose or gain electrons when contact with its own ions in solution, is called	electrode potential	reduction potential	oxidation potential	Concentration potential	electrode potential
48	The electrode in which reduction occurs is	anode	cathode	Anode and Cathode	Electrolyte	cathode
49	The value of standard	chemical series	potential series	electrochemical	electricity series	electrochemical

	electrode potential arranged in the decreasing order is called			series		series
50	Any two suitable half cells can be combained to form a	daniel cell	electrochemical cell	galvanic cell	leclanche cell	galvanic cell
51	If the electricity produced by the cell is greater than the applied EMF, then the cell is	reversible	irreversible	Sometimes reversible	Sometimes irreversible	reversible
52	In the calomel electrode, the wire used is made of	platinum	copper	titanium	iron	platinum
53	The salt bridge is made of	potassium chromate	sodium chloride	potassium chloride	zinc chloride	potassium chloride
54	An example for metal-insoluble metal salt electrode is	calomel electrode	standard hydrogen electrode	silver-silver chloride electrode	Gas electrode	calomel electrode
55	The voltaic cell Zn-Cu, the standard EMF is	1.20 v	1.15 v	1.25 v	1.10 v	1.10 v
56	Which one is Metal-insoluble metal salt electrode	calomel electrode	standard hydrogen electrode	silver-silver chloride electrode	Gas electrode	calomel electrode
57	The EMF is measured in	volts	coulomb	faraday	joules	volts
58	The electrical energy is measured in	volts	joules	coulomb	meter	joules
59	The EMF generated by an electrochemical cell is given by the symbol	E	E°	V	V°	E
60	The EMF is measured by	voltmeter	galvanometer	potentiometer	ammeter	potentiometer

Separation techniques: Solvent extraction: Classification, principle and efficiency of the technique. Mechanism of extraction: extraction by solvation and chelation. Technique of extraction: batch, continuous and counter current extractions. Qualitative and quantitative aspects of solvent extraction: extraction of metal ions from aqueous solution, extraction of organic species from the aqueous and nonaqueous media. Chromatography: Classification, principle and efficiency of the technique. Mechanism of separation: adsorption, partition & ion exchange. Development of chromatograms: frontal, elution and displacement methods. Qualitative and quantitative aspects of chromatographic methods of analysis: IC, GLC, GPC, TLC and HPLC.

Introduction of Solvent extraction

Analytical chemistry is concerned with the chemical characterization ofmatter and the answer to two important questions: what is it ? (qualitative) andhow much is it ? (quantitative). Chemicals make up everything we use orconsume, and knowledge of chemical composition of many substances isimportant in our daily lives. Analytical chemistry plays an important role innearly all aspects of chemistry, for example agricultural, clinical, environmental,forensic, manufacturing, metallurgical and pharmaceutical chemistry. Thenitrogen content of a fertilizer determines its value. Foods must be analyzed forcontaminants. The air in cities must be analyzed for carbon monoxide. Bloodsugar must be monitored in diabetics (and, in fact most diseases are diagnosedby chemical analysis). The quality of manufactured products often depends onproper chemical proportions, and measurement of the constituents is a necessarypart of quality control.

Solvent extraction technique is a part of analytical chemistry and has beenrecognized as an excellent separation method because of its ease, simplicity,speed, and wide scope. Utilizing apparatus no more complicated than aseparatery funnel, requiring just several minutes, at the most to perform,applicable both to trace and macrolevels of metals, extraction procedures offersmuch to the analytical chemist. A further advantage of the extraction methodover the widely used precipitation method lies in the cleaner separations that canbe achieved by the former. With the later method contamination of precipitatesby coprecipitation phenomena is a decided limitation which is minimized onlywith difficulty, whereas the analog of coprecipitation, i.e., coextraction, is almostunknown in solvent extraction.After world war second, chemists were

engaged in atomic energy research,encountered the problem of separating and purifying almost all the elements, inamounts ranging from very low traces up to the usual micro levels. Among thesolution to these difficult problems were the precipitation method and the ionexchange method. The former was found to be suitable for the treatment ofmaterials in solution at high electrolyte concentrations and the later for thetreatment of the ions in dilute solution or for the separation of chemically similarelements such as lanthanides and transplutonium elements.However, the solvent extraction method proved to be most effective andattractive to separate these metals, because in this method, the separation is almost complete than precipitation method and operation time is very less as compared to ion exchange method. With proper choice of extracting agents, this technique can achieve group separation or selective separation of trace elements with high efficiencies. 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.

Solvent extraction or liquid-liquid extraction by high molecular weight organic amines has become increasingly popular in recent years in studying metal complexes. Extraction by these organic amines combines many of the advantages of both solvent extraction and ion exchange. The main interest of metal extraction by the high molecular weight amines lies in their selectivity towards anionic metal complexes, reversibly formed in an aqueous solution. Hence they are generally referred to as "liquid anion exchangers". The extent of extraction by the organic bases depends on their nature, structure, size, concentration and the nature of the organic solvent used as diluent.

1) The solubility of primary long chain amines in non-polar solvents increases with increasing chain length; secondary amines are generally highly soluble in non-polar solvents and sparingly soluble in highly polar solvents. Tertiary amines are completely miscible with non-polar solvents at room temperature and sparingly soluble in polar solvents.

2) Extractive power of the alkylamines generally increases from primary to

secondary to tertiary to quaternary amines. The trend may be exceptionally changed if there is extraction by bulky amines, where steric factor play an important role.

3) Third phase formation :

a) The third phase formation, a second organic one, is more comman in systems where the diluent is an aliphatic hydrocarbon. Aromatic and some derivatives of aliphatic hydrocarbons show the phenomenon usually at high organic phase loading. The splitting of organic phase is less comman, when straight chain alkylamines are used.

c) Formation of third phase is most comman, characteristic of amine sulphate systems. The compatibility increases in the order: sulphate >bisulphate> chloride > nitrate.

d) The formation of the third phase is tempreturedependant. Literature in solvent extraction is reviewed by Freiser every two years in 'Analytical Chemistry'. Morrison and Freiser wrote a comprehensive monograph on solvent extraction in analytical chemistry. The solvent extraction of metal chelate complexes was reviewed by Starry and Zoltov. Marcus and Kertes and De et al, the applications were compiled by Sakine and Hasegawa.

The extraction of many metal ions from various aqueous solutions by high molecular weight amines have been reviewed by Khopkar and Green.

Basic Principles of Solvent Extraction Method:

An extractant, is a substance primarily responsible for the transfer of a solute (here metal) from one phase to the other. The extractant is dissolved in a suitable diluent and together act as a solvent. The diluent is immiscible with other phase which is usually water. The extractant reacts with the solute by solvation/chelation/ion pair formation etc to extract from the aqueous phase. The distribution equilibrium between two phases is governed by Gibbs phase rule, given by

$$P+V=C+2$$

Where,

P = is the number of phases,

V = is the variance or degree of freedom and

C = is the number of components.

In solvent extraction, we have P=2 two phases namely aqueous and organic phase, the component C=1, viz. solute, in solvent and water phase and at constant tempreture and pressure P=1, thus, we therefore have

2+1=1+2 i.e. P+V=C+2

According to Nernst distribution law, If $[X]_1$ is concentration of solute in phase 1 and $[X]_2$ is the concentration of solute in phase 2 at equilibrium

$$K_D = \frac{[X]1}{[X]2}$$

Where K_D is called as the partition coefficient, this partition or distribution coefficient is independent of the total solute concentration in either of the phases. In the above expression for K_D, we have not considered the activity coefficient of the species in the organic as well as in the aqueous phase. We, therefore, use the term distribution ratio (D) to account for the total concentration of species in the two phases.

Distribution Ratio (D)

The distribution of a solute between two immiscible solvents in contact to each other can be described by the distribution ratio "D".

$$D = \frac{[X]_1}{[X]_2}$$

Where [X] represents the stochiometric or formal concentration of a substance X 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;

$$D = \frac{[X]_{org}}{[X]_{aq}}$$

The subscript org and aq refer to the organic and aqueous phases respectively. Distribution ratio 'D' is dimensionless quantity, separation of two solutes by solvent extraction is expressed by the term, separation factor (α), which is related to individual distribution ratios,

$$\alpha = \frac{D_A}{D_B}$$

D_A and D_B are the respective distribution ratios of solute A and B.

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

$$\%\text{Extraction}(E) = \frac{100D}{D + V_{aq}/V_{org}}$$

Where, V represent solvent volume and the other quantities remain as previouslydefined. The percent extraction may be seen to vary with the volume ratio of thetwo phases as well as with D.

Classification of Extraction Systems

The process of metal extraction is based on the formation of neutral metalchelate. All types of chelating agents find useful applications in metal extractionprocedures. Various extraction systems can be classified in several ways. Theclassical one is based on the nature of the extracted species. The present dayclassification is based upon the process of extraction. Thus, based upon theprocess of extraction, extraction systems can be classified into four major classesviz.,

a) Chelate extraction

b) Extraction by solvation

c) Extraction involving ion pair formation

d) Synergic extraction

All the above extractions are based on the fact that neutral or uncharged species

are extracted easily in organic solvents. These are described here briefly :

a) Chelate extraction

In this class, extraction proceeds by the process of formation of chelate orclosed ring structure between the chelating agent and the metal ion to beextracted.e.g.

i) The extraction of Uranium with 8-hydroxyquinoline in chloroform.
ii) The extraction of Iron with cupferron in carbon tetrachloride.

b) Extraction by solvation

In this class, the extraction proceeds by the process of solvation of thespecies which is extracted into organic phase. Oxygenated organic solvents suchas alcohols (C-OH), ketones, ethers and esters show some basicity because of thelone pair of electron on the oxygen atom and can therefore directly solvateprotons and metal ions and bring about their extraction.e.g.

i) The extraction of Uranium with tributyl phosphate from nitric acid

ii) The extraction of Iron(III) with diethyl ether from hydrochloric acid.

c) Extraction involving ion pair formation

The extraction proceeds with the formation of neutral uncharged species which in turn gets extracted in to the organic phase. The best example of this is the extraction of Scandium and Uranium with trioctyl amine from mineral acids. In this case an ion pair is formed between complex of metal ion with high molecular weight amine and anionic species of mineral acids.

d) Synergic extraction

In this case, there is enhancement in the extraction on account of use oftwo extractants.

e.g. the extraction of Uranium with tributylphosphate (TBP) as well as 2-thionyltrifluroacetone (TTA).

Methods of Extraction

Three basic methods of liquid-liquid extraction are generally utilized inthe analytical laboratory.

A) Batch extraction

Batch extraction, the simplest and most commonly used method, consistsof extracting the solute from one immiscible layer in to other by shaking the twolayers until equilibrium is attained, after which the layers are allowed to settlebefore sampling. This is commonly used on the small scale in chemicallaboratories. The most commonly employed apparatus for performing a batchextraction is a separatory funnel. The batch extractions may also be used withadvantage when the distribution ratio is large.

B) Continuous extraction

The second type, continuous extraction, makes use of a continuous flow ofimmiscible solvent through the solution or a continuous countercurrent flow ofboth phases. Continuous extractions are particularly applicable when thedistribution ratio is relatively small. Continuous extraction device operate on thesame general principle, which consist of distilling the extracting solvent from aboiler flask and condencing it and passing it continuously through the solutionbeing extracted. The extracting liquid separates out and flows back into thereceiving flask, where it is again evaporated and recycled while the extractedsolute remains in the receiving flask. When the solvent cannot easily be distilled,a continuous supply of fresh solvent may be added from a reservoir.

C) Countercurrent extractions

Extraction by continuous countercurrent distribution is the third generaltype and is used primarily for fractionation purposes. The separation throughcontinuous countercurrent method is achieved by virtue of the density differencebetween the fluids in contact. In vertical columns, the denser phase enters at thetop and flows downwards while the less dense phase enters from the bottom andflows upwards. The choice of method to be employed will depend primarilyupon the value of the distribution ratio of the solute of interest, as well as on theseparation factors of the interfering materials.

Factors Influencing the Extraction Efficiency

Primary requirement of solvent extraction for separation /removalpurposes is a high distribution ratio of the solute of interest between the twoliquid phases. It is useful to employ a number of different techniques forenhancing the distribution ratio. It depends on the nature of the species beingextracted and extraction system. The attainment of selectivity in an extractionprocedure is also very important. Some of the factors, which affect thedistribution of solute of interest, are given below.

A) Choice of solvent

The most important consideration in the selection of a solvent for use in aparticular extraction procedure is the extractability of the element of interest. Forsubsequent analytical processing a consideration of the solubility of the solute inparticular solvent, the ease of recovery of the solvent or the ease of recovery ofthe solute from the solvent is very important. Thus, the boiling point of thesolvent or the ease of stripping by chemical reagents enters into selection of asolvent when the possibility of a choice existed. Similarly, the degree ofmiscibility of the two phases, the relative specific gravities, viscosities andtendency to form emulsion should be considered. From the point of view ofsafety, the toxicity and the flammability of the solvent must be considered.Use of a suitable solvent for effective separation is very important. Metalchelates and many organic molecules, being essentially covalent compounds donot impose many restrictions on the solvent and the general rules of solubilityare the great use. In ion association systems and particularly in oxonium typeions, the role of solvents is very important. This is due to involvement of solventin the formation of extractable species.

B) Acidity of an aqueous phase

The extractability of metal complexes is greatly influenced by the acidityof an aqueous phase, so it is necessary to assure optimum concentration of H+ions for maximum extraction. In the case of chelate extraction, the chelatingreagent concentration is maintained constant; the distribution of the metal in asystem is a function of pH. For this reason, curves of extractability versus pH atconstant reagent concentration are of great analytical significance. Sometimes itis possible to achieve the desired characteristics of a solvent by employing amixed solvent system.

C) Stripping

Stripping is the removal of the extracted solute from the organic phase forfurther processing or analysis. In many colorimetric procedures and evenradioactive techniques, the concentration of solute is determined directly in theorganic phase. However, where further separation steps are required, it isnecessary to remove the solute from the organic layer to more stable medium.When organic layer is on the steam bath, care should be taken, to avoid loss ofvolatile solute during evaporation. Addition of acid to water before evaporationhelps to break the chelate complexes, thereby causing the metal ion to enter theaqueous phase. In the process of destroying the residual organic matter, hydrochloric acid, nitric acid, perchloric acid or aqua regia is used. The usual procedure is to shake the organic layer with a volume of wateralone or water containing an appropriate concentration of acid, an oxidizing orreducing agent or masking agent. The metal ion is then back extracted in thestripping aqueous phase.The conditions employed depend upon metal ion and the particularextraction system and are such that they promote the reversal of extraction. Purewater or water adjusted to an appropriate pH/ molarity of acid are the morepopular and convenient stripping agents. Washing the organic layer with anoxidizing and reducing agent changes the metal ion to be stripped in anoxidation state in which it is not extracted under the specific conditions.

D) Use of masking agents

In the extraction procedures for metal pairs that are difficult to separate; masking or sequestering agents are introduced to improve the separation factor. Masking agents are themselves metal-complexing agents, which serve to prevent a particular metal from taking part in their usual reaction, and thus to remove their interference without the necessity of actual separation. In solvent extraction, masking agents are used to prevent certain metals from forming extractable complexes and increasing the selectivity of the method. In extraction of metals the application of masking agents, which include cyanide, tartarate, citrate, fluoride, and EDTA. The selection of a particular masking agent largely depends on the acidity of the system and the complex formation constants of the metal with both the masking and the extraction agent.

E) Salting-out agents

The term salting-out agent is applied to those electrolytes whose additiongreatly enhances the extractability of complexes. The function of salting-outagent would be primarily of providing a higher concentration of complex andthus improve the extraction. Water is probably bound as a shell of oriented waterdipoles around the ion and thus becoming unavailable as "free solvent".Addition of salting-out agents decreases the dielectric constant of theaqueous phase, which favors the formation of the ion association complexes.Salting-out agents have been used with great success in separation involving thehalide and thiocynate systems.In addition to enhancement of the extraction of the metal of interest usingsaltingout agents, it is also possible to decrease the extraction of impurities in thesystem. Thus, it is necessary to choose an agent that produces a favorableseparation factor between the element of interest and the impurities. However, itmust be remembered that anomalies sometimes result from specific interactioneffects. Aluminium or calcium salts are strong salting-out agents, whereasammonium salts are much weaker but analytically more convenient.

F) Backwashing

Backwashing is an auxiliary technique used with batch extractions toinfluence quantitative separations of elements. The combined organic phasesfrom several extractions of the original aqueous phase contain practically all thedesired elements and possibly some of the impurities that have been extracted toa much smaller extent. This combined organic phase when shaken with one ormore small portions of a fresh aqueous phase containing the optimum reagent/salting agent concentration, acidity, etc., will result in a redistribution of theimpurities in favor of the aqueous phase since their distribution ratios are low.Under optimum conditions, most of the elements of interest will remain in theorganic layer, since their distribution ratios are high.This technique is analogous in many respects to the re-precipitation stepin a gravimetric recipitation procedure. With the proper conditions, most of theimpurities can be removed by this backwashing operation, with neglisible loss ofthe main component, thereby attaining a selective operation.

F) Variation of oxidation state

The selectivity of an extraction is increased by the modification ofoxidation states of the interfering ions present in solution, in order to prevent theformation of their extractable metal complexes e.g. reduction of Cerium(IV) toCerium(III) prevents extraction of this element from nitrate media, the extractionof Iron(III) from chloride solutions can be prevented by reduction to Iron(II),which is not extractable. Similarly, Antimony(V) may be reduced to thetetravalent state to suppress its extraction. Conversely, it is important in thepreparation of a solution for extraction to adjust the proper valence state of metalion required for the formation of the complex in order to ensure completeextraction of that element. Selectivity can also be achieved by variation of theoxidation state of the co-extracted interfering ions during the stripping operation.

G) Synergic Extraction

Synergism is defined as the combined action of two complexing reagents,which is greater than the sum of the actions of the individual reagents usedalone. An example of the synergic extraction of Ce(III) with picrolonic acid andbenzo-15-crown-5.

H) Use of organic acid media

Organic acid media are having ability of controlling the concentration ofthe complexing ligand, is one of the unique application, the ease of adjustment ofpH and the wide difference in pH at which various metal ions formanionic complexes. The comparative ease of stripping of the complexes from theorganic phase can be achieved by fully exploiting the differences in reactivityof various metals to backwash in the aqueous phase by mineral acid. It is knownthat organic acid media offers better separation of metals possibly due to highstability of metal organic acids complexes.

Extractants	Metal ion	Diluent	Medium
N-n-octylaniline	Au(III)	Xylene	Sodium malonate pH-10 [32]
Tri-n-octyl amine	Os,Ru(III)	Benzene	HCL [33]
Tri-n-octyl amine	Te	Xylene	NaI,$HClO_4$ [34]

Tridodecyl amine	Th,Pa,Np,U	Benzene	Mixed mineral acids [35]
Tri-n-octyl amine	Np,Ta,Zr	Toluene chloroform	HCl,HF [36]
N-octylaniline	Noble metals	Xylene	Mineral acids [37]
Tridodecyl amine	Pu, U	Benzene	HCl or HBr H_2SO_4[38]
Tridodecyl amine	Cr(IV)	Chloroform	Orthophosporic acid [39]
N-octylaniline	Mo,W,Cu(II), Ni(II),Co,Mn	Toluene	HCl [40]
Alamine336	Zn(II)	Xylene	HCl, HBr [41]
Aliquat336	Pb	Xylene	Chloride [42]
Alamine336, Aliquat336,Amberlite LA-1, Primene JMT	Zn,Cd,Hg,In and Tl	Benzene Chloroform	NH_4SCN [43]
Aliquat336	Am,Eu	Cyclohexane	NaOH [44]
Tricarpyl methyl amine	U,Np	Xylene	HCl,HBr [45]
Tridodecyl amine	Th,Pu	Xylene	HNO_3 [46]
Tri-n-octyl amine	Ga(III),In(III)	Chloroform	Tartaric acid[47]
Tri-n-octyl amine	Al(III)	Chloroform	Tartaric acid Oxalic acid [48]
Aliquat336	Mn,Ni(II),Co ,Cu(II)	Benzene	SCN [49]
Aliquat336	Cd(II)	Benzene	SCN [50]
Aliquat336	Np,Pu,U	Chloroform	HCl [51]
TOA	Zr	Benzene	HCl [52]
Tridodecyl amine trioctyl amine	Np,U	Chloroform	Acetic acid [53]

Tri-n-octyl amine	Ac	Cyclohexane	HCl, HNO_3 [54]
N-octylaniline	Noble metals	-	Mineral acids [55]
Aliquat336	Ac,Eu	-	Alkaline[56]
TOA	Zn(II)	Chloroform CCl_4	HCl, HBr[57]
TOA	V	Benzene	HCl[58]
Tri-n-octyl amine	Fe(III)	Chloroform Toluene CCl_4	HCl[59]

Chromatography

Chromatography (Greek*chroma* "color" and *graphein* "to write") is the collective term for a set of laboratory techniques for the separation of mixtures. The mixture is dissolved in a fluid called the *mobile phase*, which carries it through a structure holding another material called the *stationary phase*. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus changing the separation.

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for more advanced use (and is thus a form of purification). Analytical chromatography is done normally with smaller amounts of material and is for measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive.

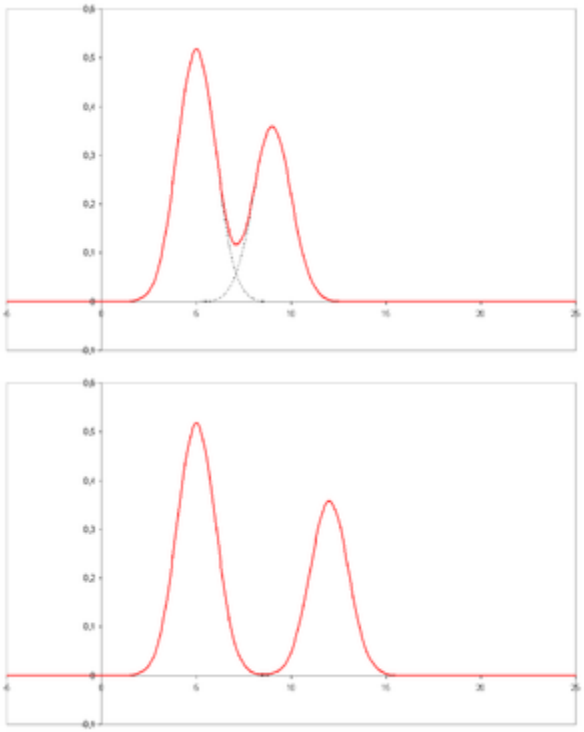
Thin layer chromatography is used to separate components of a plant extract, illustrating the experiment with plant pigments that gave chromatography its name

Chromatography was first employed in Russia by the Italian-born scientist Mikhail Tsvet in 1900. He continued to work with chromatography in the first decade of the 20th century, primarily for the separation of plant pigments such as chlorophyll, carotenes, and xanthophylls. Since these components have different colors (green, orange, and yellow, respectively) they gave the technique its name. New types of chromatography developed during the 1930s and 1940s made the technique useful for many separation processes.

Chromatography technique developed substantially as a result of the work of Archer John Porter Martin and Richard Laurence Millington Synge during the 1940s and 1950s. They established the principles and basic techniques of partition chromatography, and their work encouraged the rapid development of several chromatographic methods: paper chromatography, gas chromatography, and what would become known as high performance liquid chromatography. Since then, the technology has advanced rapidly. Researchers found that the main principles of Tsvet's chromatography could be applied in many different ways, resulting in the different varieties of chromatography described below. Advances are continually improving the technical performance of chromatography, allowing the separation of increasingly similar molecules.

Chromatography terms

- The **analyte** is the substance to be separated during chromatography. It is also normally what is needed from the mixture.
- **Analytical chromatography** is used to determine the existence and possibly also the concentration of analyte(s) in a sample.
- A **bonded phase** is a stationary phase that is covalently bonded to the support particles or to the inside wall of the column tubing.
- A **chromatogram** is the visual output of the chromatograph. In the case of an optimal separation, different peaks or patterns on the chromatogram correspond to different components of the separated mixture.



Plotted on the x-axis is the retention time and plotted on the y-axis a signal (for example obtained by a spectrophotometer, mass spectrometer or a variety of other detectors) corresponding to the response created by the analytes exiting the system. In the case of an optimal system the signal is proportional to the concentration of the specific analyte separated.

- A **chromatograph** is equipment that enables a sophisticated separation, e.g. gas chromatographic or liquid chromatographic separation.
- **Chromatography** is a physical method of separation that distributes components to separate between two phases, one stationary (stationary phase), the other (the mobile phase) moving in a definite direction.
- The **eluate** is the mobile phase leaving the column.
- The **eluent** is the solvent that carries the analyte.
- An **eluotropic series** is a list of solvents ranked according to their eluting power.
- An **immobilized phase** is a stationary phase that is immobilized on the support particles, or on the inner wall of the column tubing.
- The **mobile phase** is the phase that moves in a definite direction. It may be a liquid (LC and Capillary Electrochromatography (CEC)), a gas (GC), or a supercritical fluid (supercritical-fluid chromatography, SFC). The mobile phase consists of the sample being separated/analyzed and the solvent that moves the sample through the column. In the case of HPLC the mobile phase consists of a non-polar solvent(s) such as hexane in normal phase or polar solvents in reverse phase chromatography and the sample being separated. The mobile phase moves through the chromatography column (the stationary phase) where the sample interacts with the stationary phase and is separated.
- **Preparative chromatography** is used to purify sufficient quantities of a substance for further use, rather than analysis.
- The **retention time** is the characteristic time it takes for a particular analyte to pass through the system (from the column inlet to the detector) under set conditions. See also: Kovats' retention index
- The **sample** is the matter analyzed in chromatography. It may consist of a single component or it may be a mixture of components. When the sample is treated in the course of an analysis, the phase or the phases containing the analytes of interest is/are referred to as the sample whereas everything out of interest separated from the sample before or in the course of the analysis is referred to as waste.
- The **solute** refers to the sample components in partition chromatography.

- The **solvent** refers to any substance capable of solubilizing another substance, and especially the liquid mobile phase in liquid chromatography.
- The **stationary phase** is the substance fixed in place for the chromatography procedure. Examples include the silica layer in thin layer chromatography
- The **detector** refers to the instrument used for qualitative and quantitative detection of analytes after separation.

Chromatography is based on the concept of partition coefficient, any solute partitions between two immiscible solvents. When we make one solvent immobile (by adsorption on a solid support matrix) and another mobile it results in most common applications of chromatography. If matrix support is polar (e.g. paper, silica etc.) it is forward phase chromatography, and if it is non-polar (C-18) it is reverse phase.

Techniques by chromatographic bed shape

Column chromatography

Column chromatography is a separation technique in which the stationary bed is within a tube. The particles of the solid stationary phase or the support coated with a liquid stationary phase may fill the whole inside volume of the tube (packed column) or be concentrated on or along the inside tube wall leaving an open, unrestricted path for the mobile phase in the middle part of the tube (open tubular column). Differences in rates of movement through the medium are calculated to different retention times of the sample.

In 1978, W. Clark Still introduced a modified version of column chromatography called **flash column chromatography** (flash). The technique is very similar to the traditional column chromatography, except for that the solvent is driven through the column by applying positive pressure. This allowed most separations to be performed in less than 20 minutes, with improved separations compared to the old method. Modern flash chromatography systems are sold as pre-packed plastic cartridges, and the solvent is pumped through the cartridge. Systems may also be linked with detectors and fraction collectors providing automation. The introduction of gradient pumps resulted in quicker separations and less solvent usage.

In expanded bed adsorption, a fluidized bed is used, rather than a solid phase made by a packed bed. This allows omission of initial clearing steps such as centrifugation and filtration, for culture broths or slurries of broken cells.

Phosphocellulose chromatography utilizes the binding affinity of many DNA-binding proteins for phosphocellulose. The stronger a protein's interaction with DNA, the higher the salt concentration needed to elute that protein.

Planar chromatography

Planar chromatography is a separation technique in which the stationary phase is present as or on a plane. The plane can be a paper, serving as such or impregnated by a substance as the stationary bed (paper chromatography) or a layer of solid particles spread on a support such as a glass plate (thin layer chromatography). Different compounds in the sample mixture travel different distances according to how strongly they interact with the stationary phase as compared to the mobile phase. The specific Retention factor (R_f) of each chemical can be used to aid in the identification of an unknown substance.

Paper chromatography

Paper chromatography is a technique that involves placing a small dot or line of sample solution onto a strip of *chromatography paper*. The paper is placed in a container with a shallow layer of solvent and sealed. As the solvent rises through the paper, it meets the sample mixture, which starts to travel up the paper with the solvent. This paper is made of cellulose, a polar substance, and the compounds within the mixture travel farther if they are non-polar. More polar substances bond with the cellulose paper more quickly, and therefore do not travel as far.

Thin layer chromatography

Thin layer chromatography (TLC) is a widely employed laboratory technique and is similar to paper chromatography. However, instead of using a stationary phase of paper, it involves a stationary phase of a thin layer of adsorbent like silica gel, alumina, or cellulose on a

flat, inert substrate. Compared to paper, it has the advantage of faster runs, better separations, and the choice between different adsorbents. For even better resolution and to allow for quantification, high-performance TLC can be used. An older popular use had been to differentiate chromosomes by observing distance in gel (separation of was a separate step).

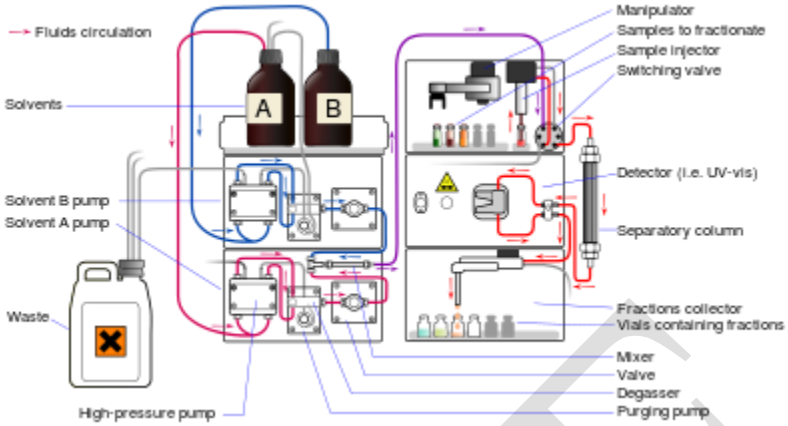
Techniques by physical state of mobile phase

Gas chromatography

Gas chromatography (GC), also sometimes known as gas-liquid chromatography, (GLC), is a separation technique in which the mobile phase is a gas. Gas chromatographic separation is always carried out in a column, which is typically "packed" or "capillary". Packed columns are the routine work horses of gas chromatography, being cheaper and easier to use and often giving adequate performance. Capillary columns generally give far superior resolution and although more expensive are becoming widely used, especially for complex mixtures. Both types of column are made from non-adsorbent and chemically inert materials. Stainless steel and glass are the usual materials for packed columns and quartz or fused silica for capillary columns.

Gas chromatography is based on partition equilibrium of analyte between a solid or viscous liquid stationary phase (often a liquid silicone-based material) and a mobile gas (most often helium). The stationary phase is adhered to the inside of a small-diameter (commonly 0.53 – 0.18mm inside diameter) glass or fused-silica tube (a capillary column) or a solid matrix inside a larger metal tube (a packed column). It is widely used in analytical chemistry; though the high temperatures used in GC make it unsuitable for high molecular weight biopolymers or proteins (heat denatures them), frequently encountered in biochemistry, it is well suited for use in the petrochemical, environmental monitoring and remediation, and industrial chemical fields. It is also used extensively in chemistry research.

Liquid chromatography



Preparative HPLC apparatus

Liquid chromatography (LC) is a separation technique in which the mobile phase is a liquid. It can be carried out either in a column or a plane. Present day liquid chromatography that generally utilizes very small packing particles and a relatively high pressure is referred to as high performance liquid chromatography (HPLC).

In HPLC the sample is forced by a liquid at high pressure (the mobile phase) through a column that is packed with a stationary phase composed of irregularly or spherically shaped particles, a porous monolithic layer, or a porous membrane. HPLC is historically divided into two different sub-classes based on the polarity of the mobile and stationary phases. Methods in which the stationary phase is more polar than the mobile phase (e.g., toluene as the mobile phase, silica as the stationary phase) are termed normal phase liquid chromatography (NPLC) and the opposite (e.g., water-methanol mixture as the mobile phase and C18 = octadecylsilyl as the stationary phase) is termed reversed phase liquid chromatography (RPLC).

Specific techniques under this broad heading are listed below.

Affinity chromatography

Affinity chromatography is based on selective non-covalent interaction between an analyte and specific molecules. It is very specific, but not very robust. It is often used in biochemistry in the purification of proteins bound to tags. These fusion proteins are labeled with compounds such as

His-tags, biotin or antigens, which bind to the stationary phase specifically. After purification, some of these tags are usually removed and the pure protein is obtained.

Affinity chromatography often utilizes a biomolecule's affinity for a metal (Zn, Cu, Fe, etc.). Columns are often manually prepared. Traditional affinity columns are used as a preparative step to flush out unwanted biomolecules.

However, HPLC techniques exist that do utilize affinity chromatogaphy properties. Immobilized Metal Affinity Chromatography (IMAC) is useful to separate aforementioned molecules based on the relative affinity for the metal (I.e. Dionex IMAC). Often these columns can be loaded with different metals to create a column with a targeted affinity.

Techniques by separation mechanism

Ion exchange chromatography

Ion exchange chromatography (usually referred to as ion chromatography) uses an ion exchange mechanism to separate analytes based on their respective charges. It is usually performed in columns but can also be useful in planar mode. Ion exchange chromatography uses a charged stationary phase to separate charged compounds including anions, cations, amino acids, peptides, and proteins. In conventional methods the stationary phase is an ion exchange resin that carries charged functional groups that interact with oppositely charged groups of the compound to retain. Ion exchange chromatography is commonly used to purify proteins using FPLC.

Size-exclusion chromatography

Size-exclusion chromatography (SEC) is also known as **gel permeation chromatography** (GPC) or **gel filtration chromatography** and separates molecules according to their size (or more accurately according to their hydrodynamic diameter or hydrodynamic volume). Smaller molecules are able to enter the pores of the media and, therefore, molecules are trapped and removed from the flow of the mobile phase. The average residence time in the pores

depends upon the effective size of the analyte molecules. However, molecules that are larger than the average pore size of the packing are excluded and thus suffer essentially no retention; such species are the first to be eluted. It is generally a low-resolution chromatography technique and thus it is often reserved for the final, "polishing" step of a purification. It is also useful for determining the tertiary structure and quaternary structure of purified proteins, especially since it can be carried out under native solution conditions.

Expanded Bed Adsorption (EBA) Chromatographic Separation

Expanded Bed Adsorption (EBA) Chromatographic Separation captures a target protein from a crude feed stream when it passes through a chromatography column system containing adsorbent beads. With this technique the crude feedstock can be treated directly in the chromatographic column, avoiding the traditional clarification and pre-treatment steps. EBA Chromatographic Separation is highly scalable, from laboratory-based 1 cm diameter columns to large production columns up to 2 meter in diameter. These columns can typically handle feed stock throughput of more than 1,000,000 liter per day with a production capacity of 1000 MT protein per year.

Column chromatography

A chemist in the 1950s using column chromatography. The Erlenmeyer receptacles are on the floor.

Automated fraction collector and sampler for chromatographic techniques

Column chromatography in chemistry is a method used to purify individual chemical compounds from mixtures of compounds. It is often used for preparative applications on scales from micrograms up to kilograms. The main advantage of column chromatography is the relatively low cost and disposability of the stationary phase used in the process. The latter prevents cross-contamination and stationary phase degradation due to recycling.

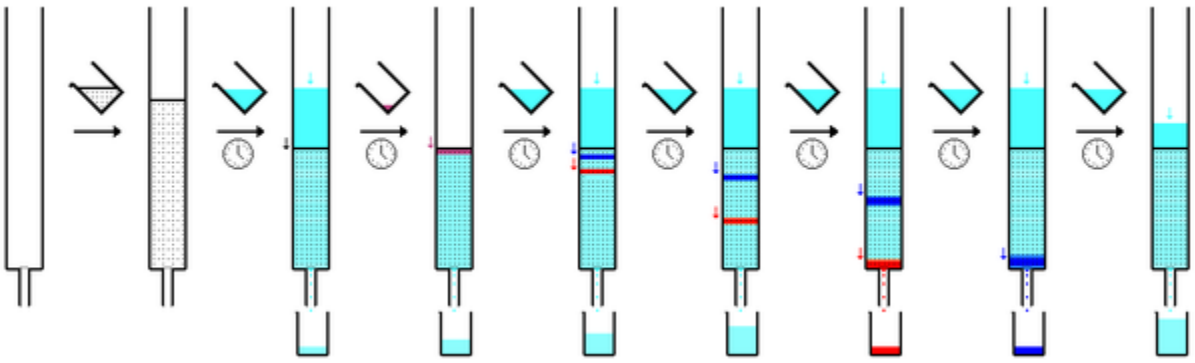
The classical preparative chromatography column is a glass tube with a diameter from 5 mm to 50 mm and a height of 5 cm to 1 m with a tap and some kind of a filter (a glass frit or glass wool plug – to prevent the loss of the stationary phase) at the bottom. Two methods are generally used to prepare a column: the dry method and the wet method.

- For the dry method, the column is first filled with dry stationary phase powder, followed by the addition of mobile phase, which is flushed through the column until it is completely wet, and from this point is never allowed to run dry.

- For the wet method, slurry is prepared of the eluent with the stationary phase powder and then carefully poured into the column. Care must be taken to avoid air bubbles. A solution of the organic material is pipetted on top of the stationary phase. This layer is usually topped with a small layer of sand or with cotton or glass wool to protect the shape of the organic layer from the velocity of newly added eluent. Eluent is slowly passed through the column to advance the organic material. Often a spherical eluent reservoir or an eluent-filled and stoppered separating funnel is put on top of the column.

The individual components are retained by the stationary phase differently and separate from each other while they are running at different speeds through the column with the eluent. At the end of the column they elute one at a time. During the entire chromatography process the eluent is collected in a series of fractions. Fractions can be collected automatically by means of fraction collectors. The productivity of chromatography can be increased by running several columns at a time. In this case multi stream collectors are used. The composition of the eluent flow can be monitored and each fraction is analyzed for dissolved compounds, e.g. by analytical chromatography, UV absorption, or fluorescence. Colored compounds (or fluorescent compounds with the aid of an UV lamp) can be seen through the glass wall as moving bands.

Stationary phase



Column chromatography proceeds by a series of steps.

Photografic sequence of a column chromatography

The *stationary phase* or *adsorbent* in column chromatography is a solid. The most common stationary phase for column chromatography is silica gel, followed by alumina. Cellulose powder has often been used in the past. Also possible are ion exchange chromatography, reversed-phase chromatography (RP), affinity chromatography or expanded bed adsorption (EBA). The stationary phases are usually finely ground powders or gels and/or are microporous for an increased surface; though in EBA a fluidized bed is used. There is an important ratio between the stationary phase weight and the dry weight of the analyte mixture that can be applied onto the column. For silica column chromatography, this ratio lies within 20:1 to 100:1, depending on how close to each other the analyte components are being eluted.

Mobile phase (eluent)

The *mobile phase* or *eluent* is either a pure solvent or a mixture of different solvents. It is chosen so that the retention factor value of the compound of interest is roughly around 0.2 - 0.3 in order to minimize the time and the amount of eluent to run the chromatography. The eluent has also been chosen so that the different compounds can be separated effectively. The eluent is optimized in small scale pretests, often using thin layer chromatography (TLC) with the same stationary phase.

There is an optimum flow rate for each particular separation. A faster flow rate of the eluent minimizes the time required to run a column and thereby minimizes diffusion, resulting in a better separation. However, the maximum flow rate is limited because a finite time is required for the analyte to equilibrate between the stationary phase and mobile phase, see Van Deemter's equation. A simple laboratory column runs by gravity flow. The flow rate of such a column can be increased by extending the fresh eluent filled column above the top of the stationary phase or decreased by the tap controls. Faster flow rates can be achieved by using a pump or by using compressed gas (e.g. air, nitrogen, or argon) to push the solvent through the column (flash column chromatography).

The particle size of the stationary phase is generally finer in flash column chromatography than in gravity column chromatography. For example, one of the most widely used silica gel grades in the former technique is mesh 230 – 400 (40 – 63 µm), while the latter technique typically requires mesh 70 – 230 (63 – 200 µm) silica gel.

A spreadsheet that assists in the successful development of flash columns has been developed. The spreadsheet estimates the retention volume and band volume of analytes, the fraction numbers expected to contain each analyte, and the resolution between adjacent peaks. This information allows users to select optimal parameters for preparative-scale separations before the flash column itself is attempted.

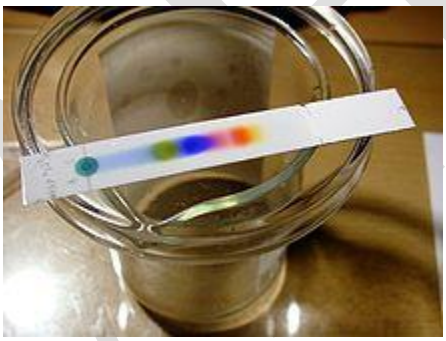
Automated systems

An automated ion chromatography system.

Column chromatography is an extremely time consuming stage in any lab and can quickly become the bottleneck for any process lab. Therefore, several manufacturers like Buchi, Teledyne Isco, have developed automated flash chromatography systems (typically referred to as LPLC, low pressure liquid chromatography, around 350–525 kPa or 50.8–76.1 psi) that minimize human involvement in the purification process. Automated systems will include components normally found on more expensive high performance liquid chromatography (HPLC) systems such as a gradient pump, sample injection ports, a UV detector and a fraction collector to collect the eluent. Typically these automated systems can separate samples from a few milligrams up to an industrial many kilogram scale and offer a much cheaper and quicker solution to doing multiple injections on prep-HPLC systems.

The resolution (or the ability to separate a mixture) on an LPLC system will always be lower compared to HPLC, as the packing material in an HPLC column can be much smaller, typically only 5 micrometre thus increasing stationary phase surface area, increasing surface interactions and giving better separation. However, the use of this small packing media causes the high back pressure and is why it is termed high pressure liquid chromatography. The LPLC columns are typically packed with silica of around 50 micrometres, thus reducing back pressure and resolution, but it also removes the need for expensive high pressure pumps. Manufacturers are now starting to move into higher pressure flash chromatography systems and have termed these as medium pressure liquid chromatography (MPLC) systems which operate above 1 MPa (150 psi).

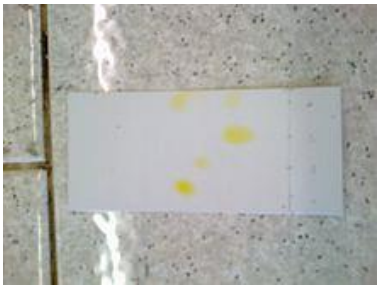
Thin-layer chromatography



Separation of black ink on a TLC plate

Thin-layer chromatography (TLC) is a chromatography technique used to separate non-volatile mixtures. Thin-layer chromatography is performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide, or cellulose. This layer of adsorbent is known as the stationary phase.

After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved.



TLC of three standards (ortho-, meta- and para-isomers) and a sample

Thin-layer chromatography can be used to monitor the progress of a reaction, identify compounds present in a given mixture, and determine the purity of a substance. Specific examples of these applications include: analyzing ceramides and fatty acids, detection of pesticides or insecticides in food and water, analyzing the dye composition of fibers in forensics, assaying the radiochemical purity of radiopharmaceuticals, or identification of medicinal plants and their constituents.

A number of enhancements can be made to the original method to automate the different steps, to increase the resolution achieved with TLC and to allow more accurate quantitative analysis. This method is referred to as HPTLC, or "high-performance TLC".

POSSIBLE QUESTIONS

PART-B (2 Mark Questions)

1. What is meant by the precision?

2. What is molar extension coefficient (ε)?

3. What is the radiation source used in IR spectroscopy?

4. What is meant by thermogravimetry curve.?

5. State the optical purity?

PART-C (6 Mark Questions)

1. Discuss the Methods of Determining Optical Purity.
2. Wirte notes on Enantiomeric excess
3. Explain the principle and applications of TLC?
4. Write notes on High-performance liquid chromatography (HPLC).
5. Calculate the Fundamental modes of vibrational frequency in IR spectrum.
   a. (i) HCl, ii) H_2O iii) CH_4 iv) CO
6. Write notes on finger print region.
7. Illustrate the experimental determination of potentiometric titrations.
8. Explain the electronic transitions involved in UV spectroscopy?
9. Explain the techniques for the quantitative estimation of trace level of metal ions from water samples?
10. Discuss on accuracy and precision.

KARPAGAM ACADEMY OF HIGHER EDUCATION
(Deemed to be University)
(Established Under Section 3 of UGC Act 1956)
DEPARTMENT OF CHEMISTRY

UNIT IV (Separation Techniques-Chromatography)

(Multiple Choice Questions)

S.No	Questions	Option a	Option b	Option c	Option d	Answer
1	A combination of paper chromatography and electrophoresis involves	Absorption	electrical mobility of the ionic species	ionization	adsorption chromatography	electrical mobility of the ionic species
2	A mixture of ethanol (C2H6O) and butanol (C4H10O) is approximately 90% ethanol and 10% butanol. The mixture is passed through a gas liquid chromatogram. The printout obtained is likely to show that, compared to butanol, the ethanol has ...	a shorter retention time and a smaller area under the peak.	a shorter retention time and a larger area under the peak.	a longer retention time and a larger area under the peak.	a smaller retardation factor and a larger area under the peak.	a shorter retention time and a larger area under the peak.
3	A new youth drink contains sugar, salt, alcohol and vitamin C. A gas chromatogram could be used to determine the ...	alcohol content only.	alcohol, sugar and vitamin C content only.	concentration of all ingredients in the drink.	alcohol and sugar content only.	alcohol content only.
4	A retention gap is placed between the injector and the front of the column to	retain contaminants and prevent them from reaching the column	retain the sample and release it gradually to the column	prevent backflush of the injected solution	release random to the column	retain contaminants and prevent them from reaching the column
5	A student sets up a paper chromatogram and places a spot	26	8	18	15	15

	of green food dye on the origin. After six minutes the solvent has moved 12 cm and a blue spot has advanced 9 cm. After fourteen minutes the solvent has advanced a further 8 cm. How many cm from the origin is the blue spot likely to be?					
6	Acetone is an organic molecule with a semi-structural formula of CH3COCH3. A student runs a sample of acetone through a gas chromatogram at 50°C. The acetone produces a peak after 4.2 minutes. The student then injects a mixture of unknown organic substances into the same column at the same temperature. There are peaks after 3.1, 4.2 and 7.4 minutes. From this information, it can be concluded that ...	the mixture has three componenets, one of which must be acetone.	the mixture has at least three components, one of which might be acetone.	the mixture has at least three components, one of which must be acetone.	the mixture has three componenets, but acetone is not one of them.	the mixture has at least three components, one of which might be acetone.
7	characteristic feature of any form of chromatography is the ...	use of molecules that are soluble in water.	. use of an inert carrier gas.	. calculation of an Rf value for the molecules separated.	use of a mobile and a stationary phase.	use of a mobile and a stationary phase.
8	Chromatography is a technique used for --------- compounds	separation	identification	measure	analysis	separation
9	Column bleeding occurs when	elution of the analyte is extended over time	the column is cracked and stationary phase leaks out	traces of the stationary phase are eluted	the column breaks during installation and causes personal injury	traces of the stationary phase are eluted
10	Column chromatography is atype of	partition	adsorption	Absorption	thin layer	adsorption

11	Derivatisation of a sample is carried out to	reduce polarity of the analytes	decrease the detector response	decrease volatility of the analytes	irreducible polarity	reduce polarity of the analytes
12	Doubling the column's length increases resolution by a factor of	(2)0.5	3	2	4	(2)0.5
13	Electrostatic attraction is a function present in ------ chromatography	column	paper	ion exchange	gas	ion exchange
14	Formic acid is an example of	protogenic solvent	protophillic solvent	amphiprotic solvent	Aprotic solvent	protogenic solvent
15	Headspace analysis is carried out in order to	analyse volatile compounds from solid or liquid samples	determine the psychological state of the tutor	analyse the column contents ahead of the sample	determine non-volatiles	analyse volatile compounds from solid or liquid samples
16	Helium is generally preferred as carrier gas over nitrogen and hydrogen because	it is inert	it has a high viscosity	it not doubles up as a party gas for balloons and funny voices	it is reactive	it is inert
17	High performance liquid chromatography (HPLC) cannot be used to ...	separate types of organic pesticides.	determine the mercury content of a fish sample.	identify the various pigments from a leaf extract.	determine the caffeine content of coffee samples.	determine the mercury content of a fish sample.
18	In column switching chromatography	compounds trapped on one column are eluted to another column	one column is removed and replaced by another	the flow to the column is switched on and off repeatedly	compounds does not move	compounds trapped on one column are eluted to another column
19	In gas chromatography, the basis for separation of the components of the volatile material is the difference in	partition coefficients	conductivity	molecular weight	elements percentage	partition coefficients
20	In refractometric analysis, if temperature is increased by 1 °C then refractive index decreases by	0.001 to 0.002	0.002 to 0.003	0.003 to 0.004	0.004 to 0.005	Nernst equation

21	In reverse phase chromatography, the stationary phase is made	non-polar	either non-polar or polar	polar	low polar	non-polar
22	In-vitro hydrolysis studies of drugs & kinetic studies of reaction can be performed by	Polarimetry	Refractometry	Potentiometry	Conductometry	Conductometry
23	Ion exchange chromatography is based on the	electrostatic attraction	electrical mobility of ionic species	adsorption chromatography	partition chromatography	electrostatic attraction
24	Oxygen --------be used as carrier gas in gas chromatography	can	cannot	often	always	can
25	Relative flow (Rf) value ranges from	0 to 1	0 to 2.0	+2 to -2	+1 to -1	0 to 1
26	Resolution is proportional to the …….. Of the theoretical plates in a column	number	square root	square	cube root	square root
27	Rf is refered as	retention time	retard factor	resistant value	reduced value	retention time
28	Sample injection is considered successful if	all of the sample in the injector has been added to the column	the sample is concentrated at the start of the column	the sample is spread evenly along the column	the sample is homogenously spread along the column	the sample is concentrated at the start of the column
29	Sample retention in the column is measured by	retention time	factor	index	co-efficient	retention time
30	Snells law is related to	Refractometry	Potentiometry	Non-aqueous titrations	Chromatography	Refractometry
31	Split injection is carried out by	splitting the sample into smaller portions to inject sequentially	splitting the sample into smaller portions to inject at the same time through parallel ports	splitting off some of the sample so that it does not enter the column	It does not splitting the sample portions	splitting off some of the sample so that it does not enter the column
32	Sucrose can be determined after silylation using which	HPLC	Gel chromatography	Gas liquid chromatography	Paper chromatography	Gas liquid chromatography

	chromatographic technique					
33	The alkenes and aromatic compounds can be suitably detected using	Refractive index detector	conductivity detector	Spectrophotometric detector	Potentiometric detector	Spectrophotometric detector
34	The basis of the technique of chromatography for separating components of a mixture is ...	the absorption of infrared radiation by the components.	the interaction of the components with both stationary and mobile phases.	the differing movement of particles of different mass in an electric field.	the deflection of charged particles in a magnetic field.	the interaction of the components with both stationary and mobile phases.
35	The column is heated to	prevent analyte condensation within the column	control elution of the same analytes	irreduce band broadening to get sharper peaks	control elution of the different analytes	control elution of the different analytes
36	The composition of Silica gel G is	silica gel without binder	silica gel + CaSO4	Silica gel + alumina	silica gel + MaSO4	Silica gel + alumina
37	The example of bulk property detector used in HPLC is	Refractive index detector	UV detector	fluorescence detector	UV-visible detector	Refractive index detector
38	The formula for resolution (R) between peaks in gas chromatography is (where d = distance between peak 1 and 2; W1 and W2 are width of peak 1 and 2, respectively)	2d / (W1+W2)	d / (W1+W2)	2d / (W1-W2)	d / (W1-W2)	2d / (W1+W2)
39	The GC trace obtained after an experiment is called a	chromatograph	chromatogram	chromatophore	graph	chromatogram
40	The general expression for the appearance of a solute in an effluent is (where V is the elution volume of a substance , V0 void volume, kD distribution constant and Vi internal water volume)	V = V0 + kDVi	V = V0/Vi	V = V0 - kDVi	V/V0 = kDVi	V = V0 + kDVi
41	The mechanism present in the ion exchange chromatography	reversible	irreversible	ionisation	bromination	reversible

42	The relationship between concentration, temperature & potential of a solution is given by	Ilkovic equation	Henderson equation	Nernst equation	Hassalbach equation	Nernst equation
43	Theoretical plates are used to	estimate the efficiency of a column	determine the thickness of the stationary phase	measure the distribution of the analyte between mobile and stationary phases	Estimate the compounds	estimate the efficiency of a column
44	Thin layer chromatography can be used to distinguish between different amino acids. If a particular amino acid has low solubility in the mobile phase used, then the amino acid ...	will have a low Rf value.	will spend more time dissolved in the mobile phase than attached to the stationary phase.	must have a high molecular mass.	will move at a speed close to that of the solvent.	will have a low Rf value.
45	Thin layer chromatography is	ionization	partition chromatography	electrical mobility of ionic species	adsorption chromatography	adsorption chromatography
46	TLC means	thinlayer chromatography	thicklayer chromatography	thermolinear chromatography	therotical layer chromatography	thinlayer chromatography
47	What are the benefits of decreasing the column internal diameter?	Increased sample capacity	Increased resolution	Reduced risk of column overloading	non-Reduced risk of column overloading	Increased resolution
48	What does the retention factor, k', describe?	The distribution of an analyte between the stationary and the mobile phase	The migration rate of an analyte through a column	The velocity of the mobile phase	The velocity of the stationary phase	The migration rate of an analyte through a column
49	What does the selectivity factor describe?	The proportional difference in widths of two chromatographic peaks	The maximum number of different species which a column can separate simultaneously	The relative separation achieved between two species	does not separate the species	The relative separation achieved between two species

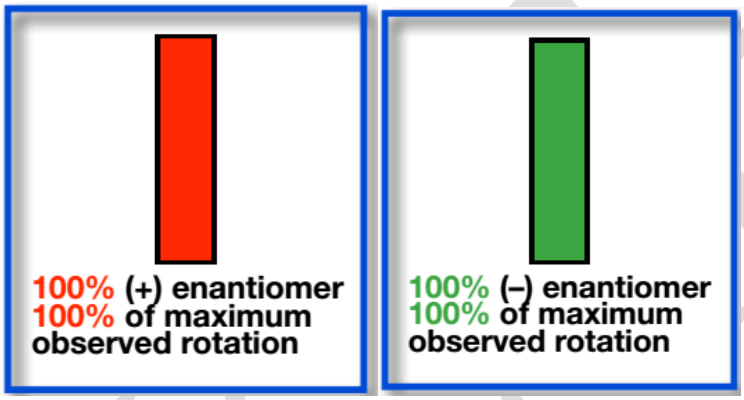
50	What is the typical internal diameter of fused silica capillary columns?	0.2-0.3 mm	0.3-0.5mm	0.5-1.0 mm	1.0-2.0 mm	0.2-0.3 mm
51	What useful information can be found from a Van Deemter plot?	The selectivity factor	Optimum mobile phase flow rate	Optimum column temperature	Optimum column length	Optimum mobile phase flow rate
52	Which of the following are not used as stationary phases in a GC column?	Polysiloxanes	Silica	Cyclodextrins	None are used as stationary phases	Silica
53	Which of the following detectors give concentration-dependent signals?	Electron-capture detector	non thermal conductivity	UV detector	Scintillation counter	Electron-capture detector
54	Which of the following detectors give mass flow-dependent signals?	Electron capture detector	Field ionisation detector	Thermal conductivity detector	Proton capture	Field ionisation detector
55	Which of the following gases is unsuitable for use as a GC carrier gas?	Nitrogen	Helium	Oxygen	Hydrogen	Oxygen
56	Which of the following is not used for detection in GC?	Infrared spectroscopy	NMR	Flame ionisation	Electrical conductivity	NMR
57	Which of the following is the most suitable gas to use as a carrier gas in a gas chromatogram?	Helium	Oxygen	Methane	Carbon dioxide	Helium
58	Which of the following statements about paper and gas chromatography is correct?	The Rf and Rt values of a substance are determined solely by the interaction of the substance with the stationary phase.	A substance with a long retention time in gas chromatography is likely to have a high Rf value in paper chromatography .	A high Rf value is indicative of a substance that adsorbs strongly onto the stationary phase.	A long retention time in gas chromatography is indicative of a substance with a strong adsorption on to the stationary phase.	A long retention time in gas chromatography is indicative of a substance with a strong adsorption on to the stationary phase.
59	Which of the statements is correct?	Gas chromatography	Gas chromatography	Gas chromatography is	Gas chromatography	Gas chromatography is

		is notused to analyse gases	is used to analyse solids	not used to analyse gases, solutions	is not used to analyse solid , gases	used to analyse solids
60	Which of these effects result from slow injection of a large sample volume?	Increased resolution	Decreased resolution	Non-linear detector response	Constant resolution	Decreased resolution

Separation and analysis: Measurement of optical rotation, calculation of Enantiomeric excess (ee)/ diastereomeric excess (de) ratios and determination of enantiomeric composition using NMR, Chiral solvents and chiral shift reagents. Chiral chromatographic techniques using chiral columns (GC and HPLC).Role of computers in instrumental methods of analysis.

Optical purity and Enantiomeric excess

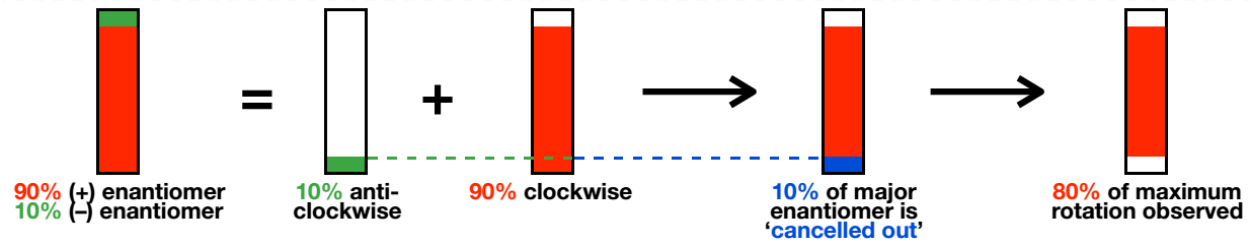
Molecules with chirality centers cause the rotation of plane polarised light and are said to be "optical active" (hence the term optical isomers).
Enantiomeric molecules rotate the plane in opposite directions but with the same magnitude.
This provides a means of measuring the "**optical purity**" or "**enantiomeric excess** (ee)" of a sample of a mixture of enantiomers.
These two terms mean the same thing : How much more of one enantiomer is there than the other ?



Specific rotation is a physical property like boiling point and can be looked up in references. It is defined according to the following equation based on the experimental measurements:

$$\text{Specific rotation } [\alpha]_D = \alpha_{obs} / c\, l$$

where "α_{obs}" is the experimentally observed rotation, "c" is the concentration in g/ml and "*l*" is the pathlength of the cell used expressed in dm (10 cm).
The most important factor is that two enantiomers will have the ***same magnitude*** specific rotations but in ***opposite directions***.



For example: (S)-bromobutane has a specific rotation of +23.1^o, therefore, (R)-bromobutane has a specific rotation of -23.1^o

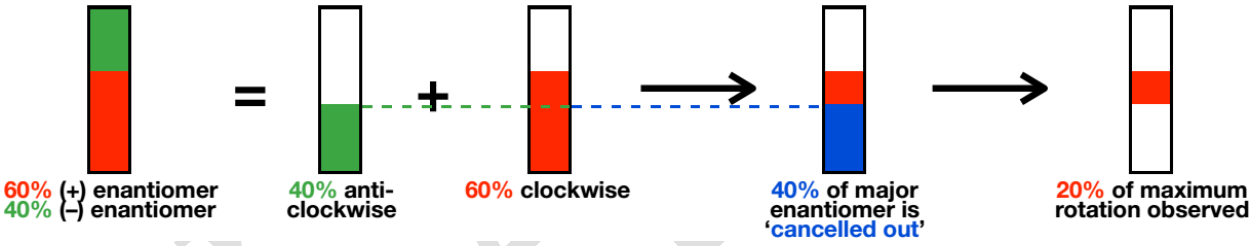
As a consequnce of this, a 50:50 mixture of the two enantiomers will not rotate plane polarised light because the effects of the two enantiomers cancel each other out, molecule for molecule. This type of mixture is called a ***racemate*** or a ***racemic mixture***. The specific rotation of a racemic mixture is zero.

The optical purity of a mixture of enantiomers is given by:

% Optical purity of sample = 100 * (specific rotation of sample) / (specific rotation of a pure enantiomer)

Based on the above example data for the bromobutanes:

Optical purity of a racemic mixture = 100 * (0^o) / (+23.1^o) = 0% *i.e. there is no one enantiomer present in excess.*



Another way to express optical purity is as the "enantiomeric excess" or ee:

ee % = 100 * ([R] - [S]) / ([R] + [S])

where [-] indicates the concentration of the species. Repeating the above example (to show the identity) in a racemic mixture [R] = [S], therefore,

ee% = 100 * 0 / ([R] + [S]) = 0%

or, if we use the % as concentrations, then [R] = [S] = 50 % we get,

ee% = 100 * (50 - 50)/ (50 + 50) = 0 %

So, what about for a pure enantiomer, say 100% R ?

Optical purity % = 100 * (-23.1^o / -23.1^o) = 100 %, or, ee% = 100 * (100% - 0%) / (100% + 0%) = 100%

So, finally, what about another mixture of the 2-bromobutanes of measured specific rotation = -9.2^o ?
Well the fact that the sign is negative tells us that in this case the R enantiomer is the dominant one.

Optical purity % = 100 * (- 9.2^o / - 23.1^o) = 40 % *ie there is a 40% excess of R over S.*

This corresponds to a mixture of 70% R and 30% S. How do you get this quickly ?

Well, if there is a 40% excess of R, then the 60% leftover must be equal amounts of both R and S *ie*. 30% of each. So the total amount of R is 30% + 40% excess = 70%.

You should also be able to work out the specific rotation of a mixture given the % composition. Try it out for the above example.

As a further example for the same system, what would the optical purity be is the measured specific rotation were +18.4^o ? Now the enantiomer in excess must be the S.

Optical purity% = 100 * (+18.4^o / +23.1^o) = 80% corresponding to a 90% S : 10% R mixture.

How do we measure enantiomeric excess?
• Problem - all the physical properties of enantiomers are identical (in an achiral environment) except rotation of plane polarised light
• Solution - the interaction of a chiral molecule with other chiral compounds is different depending on the enantiomer used...
• Imagine you have a mixture of left and right-handed gloves and you are asked to separate them...suddenly there is a power cut, and you are left in a darkened room. How would you do it? Use just one hand and try the gloves on...

Chiral Chromatography

Resolution - the separation of enantiomers from either a racemic mixture or

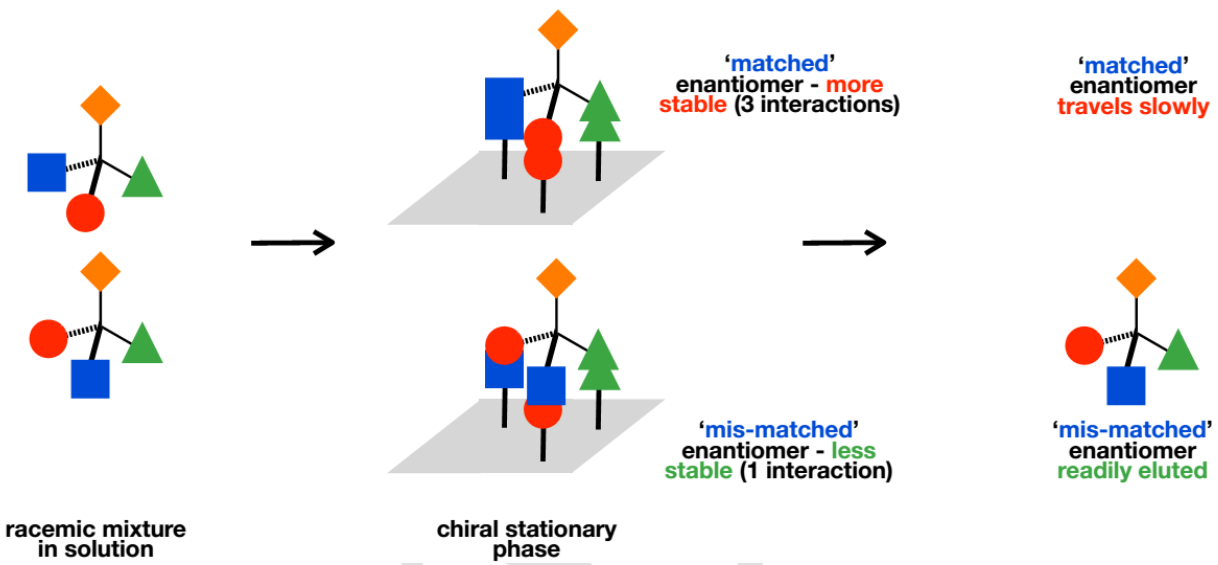
enantiomerically enriched mixture

• Chiral chromatography - Normally HPLC or GC

• A racemic solution is passed over a chiral stationary phase

• Compound has rapid and reversible diastereotopic interaction with stationary phase

• Hopefully, each complex has a different stability allowing separation

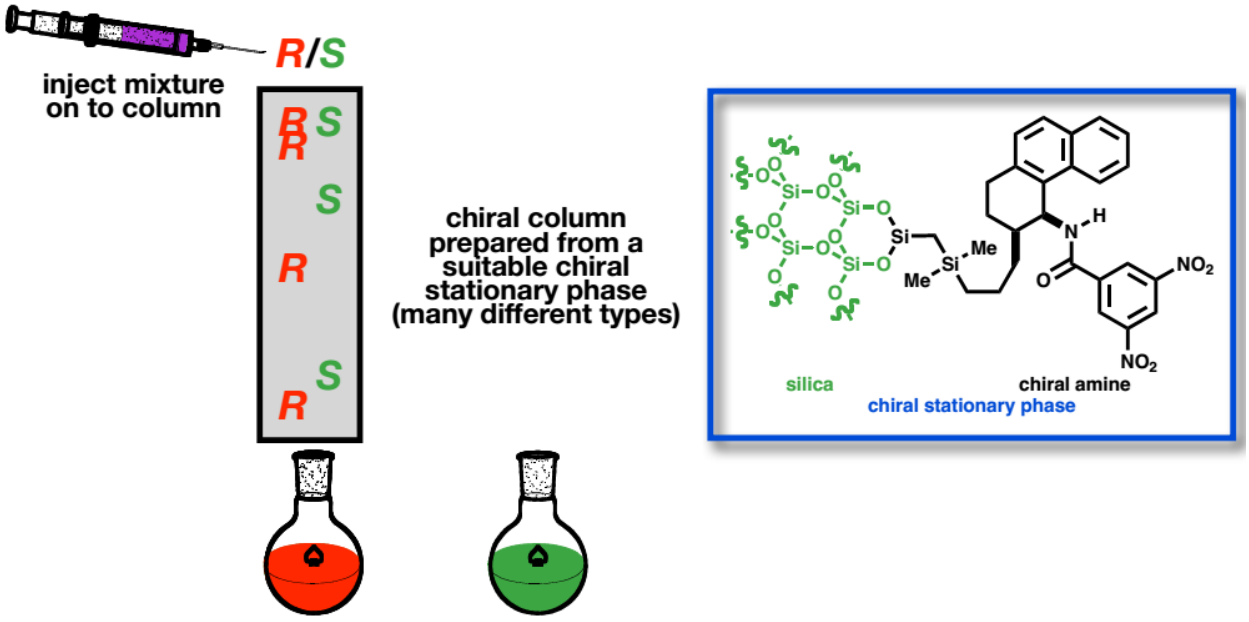


Measurements of ee by HPLC or GC are quick and accurate (±0.05%)

• Chiral stationary phase may only work for limited types of compounds

• Columns are expensive (>£1000)

• Need both enantiomers to set-up an accurate method



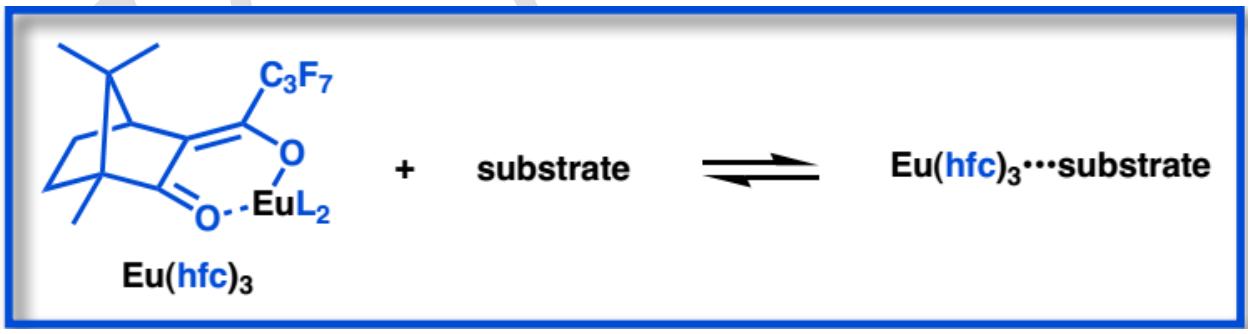
NMR spectroscopy: chiral shift reagents

Chiral paramagnetic lanthanide complexes can bind reversibly to certain chiralmolecules via the metal centre

• Process faster than nmr timescale and normally observe a downfield shift (higherppm)

• Two diastereomeric complexes are formed on coordination; these may have different

nmr signals



Chiral derivatising agents

A racemic mixture of enantiomers can be converted to a mixture of

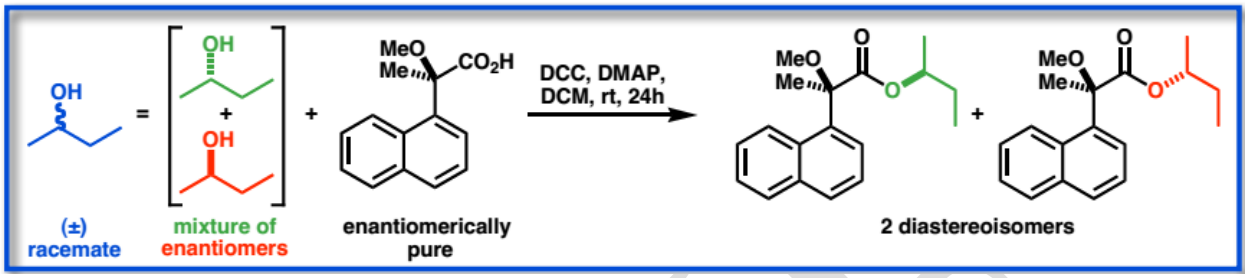
diastereoisomers by covalently attaching a second, enantiomerically pure unit

• The advantage of this over the previous methods is there is normally larger signal

separation in nmr

• There is no reversibility

• Diastereoisomers can often be separated by normal, achiral chromatography

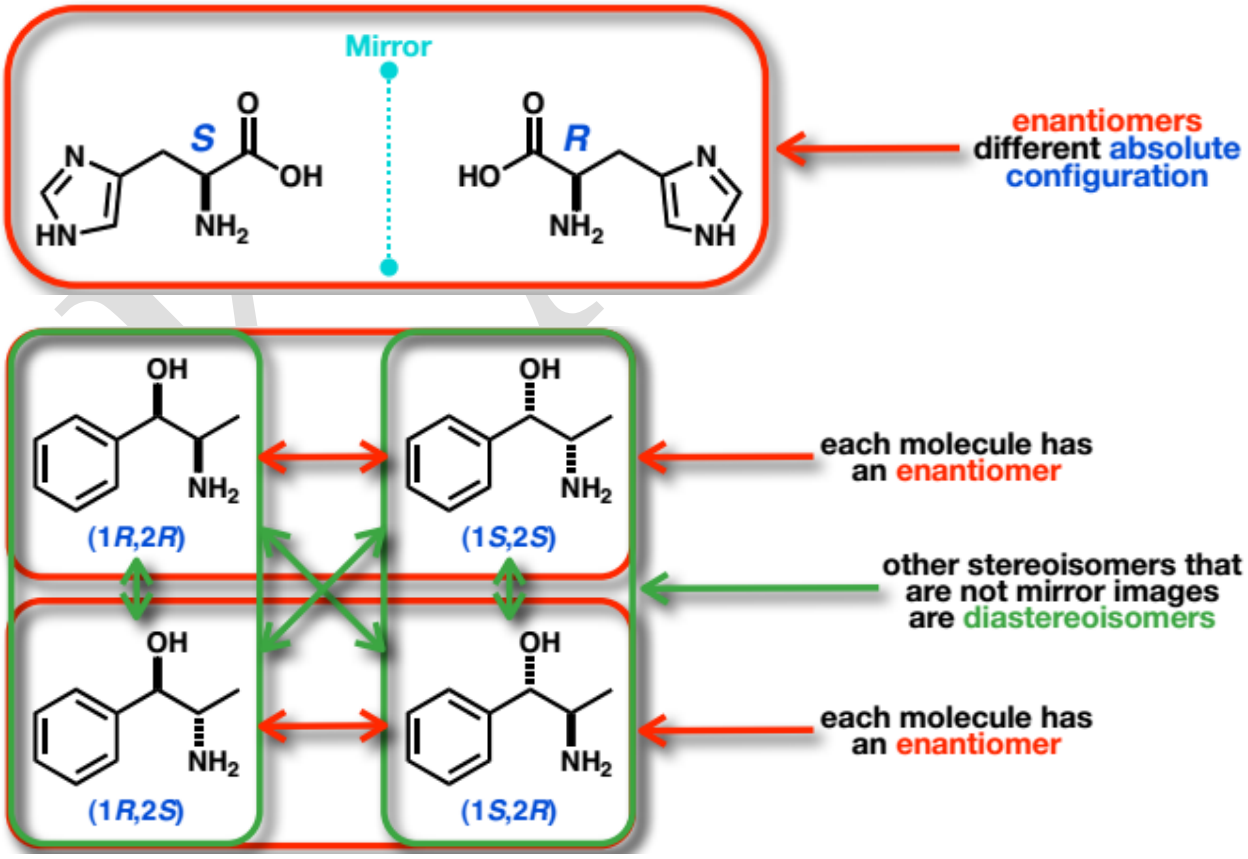


• To understand why diastereoisomers are useful we need to do some more revision.

• A molecule with one stereogeniccentre exists as two stereoisomers or enantiomers

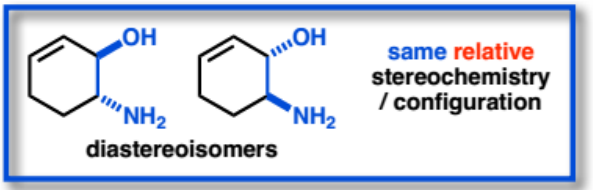
• The two enantiomers differ by their absolute configuration

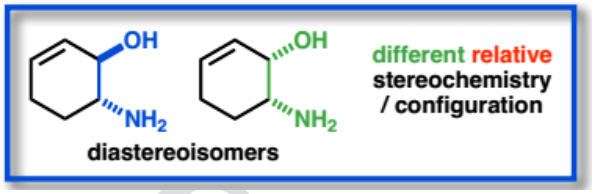
• A molecule with two stereogeniccentres can exist as four stereoisomers



- A molecule can have one enantiomer but any number of diastereoisomers

Diastereoisomers





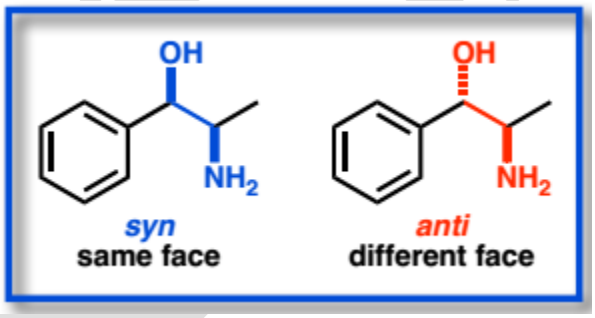
- Diastereoisomers can have the same relative stereochemistry

• The stereoisomers above differ only by their absolute stereochemistry

• Or they can have different relative stereochemistry

• Relative stereochemistry - defines configuration with respect to any other stereogenic element within the molecule but does NOT differentiate between enantiomers

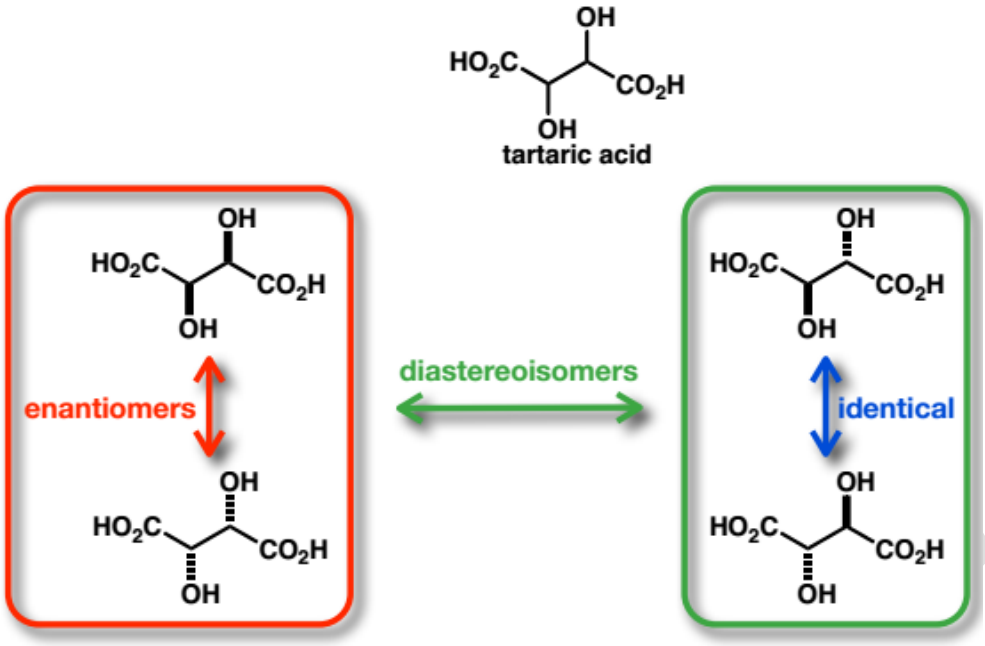
• In simple systems the two different relative stereochemistries are defined as below



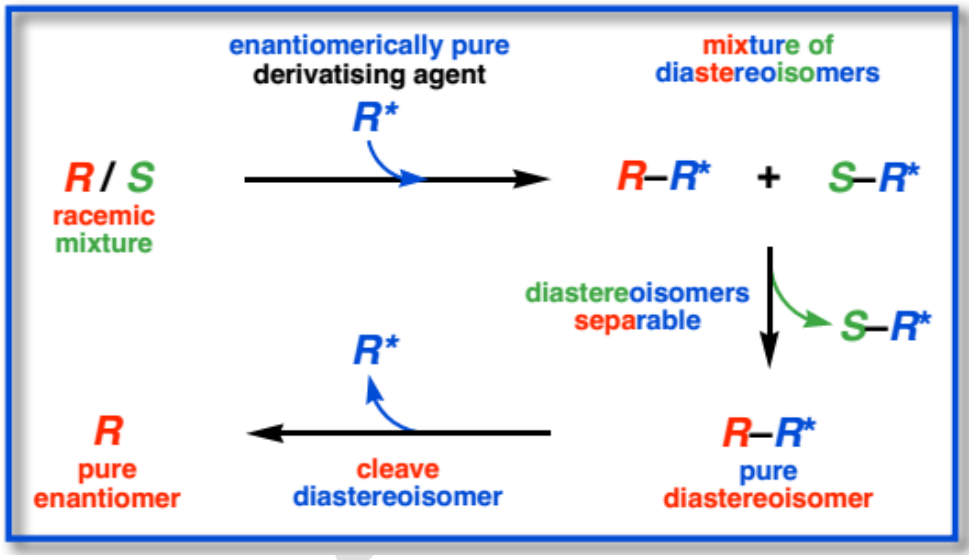
The terms erthyro&threo - depending on the convention used, these can mean two either relative stereochemistry so I will not use them!

Meso compounds

- Tartaric acid has 2 stereogeniccentres. But does it have 4 diastereoisomers?
- 2 diastereoisomers with different relative stereochemistry
- 2 mirror images with different relative stereochemistry
- 1 is an enantiomer
- The other is identical / same compound
- Simple rotation shows that the two mirror images are superimposable



Chiral derivatising agents II



This difference allows chiral derivatising agents to resolve enantiomers

Be enantiomerically pure (or it is pointless)

• Coupling reaction of both enantiomers must reach 100% (if you are measuring ee)

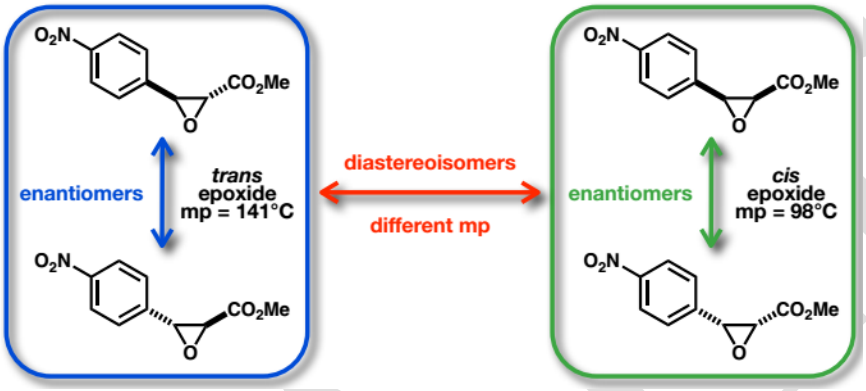
• Coupling conditions should not racemisestereogeniccentre

• Enantiomers must contain point of attachment

• Above list probably influenced depending whether you are measuring %ee or preparatively separating enantiomers

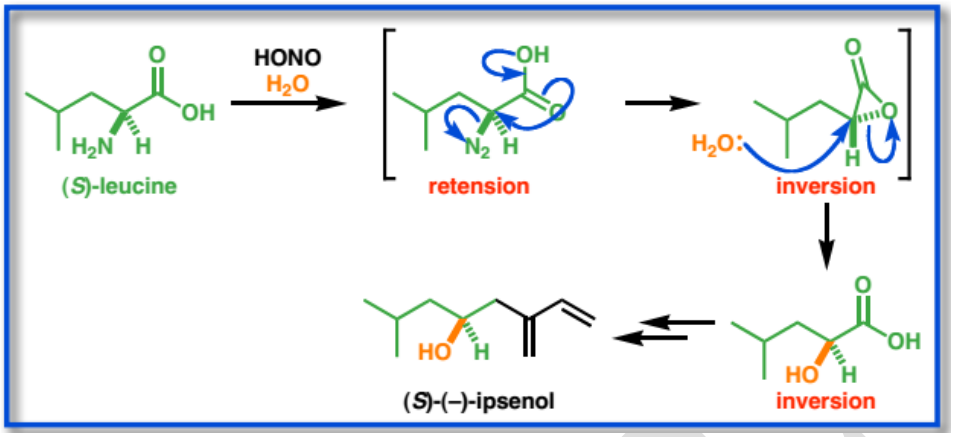
Enantiomers vs. diastereoisomers

- Two enantiomers have identical physical properties in an achiral environment
- Two diastereoisomers have different physical properties



Stereoselective synthesis

- The term 'asymmetric synthesis' should be used with caution. As we shall see, a number of important chiral compounds are symmetric!!
- As such this course will primarily focus on diastereoselective or enantioselective synthesis or the synthesis of chiral molecules
- Chiral compounds can be prepared in a number of ways



POSSIBLE QUESTIONS

PART-A **(1 Mark Questions)**

1. The number of configurational isomers of molecules having (n) different chiral carbons is
   a) 2n b) **2^n** c) 2^{n-1} d) 2^{n+1}
2. For a molecule with two like chiral carbon atoms, the number of optically inactive form is
   a) **1** b) 2 c) 3 d) 4
3. What is the relationship between *trans*-2-butene and *cis*-2-butene?
   a) unrelated compounds b) **constitutional isomers**
   c) enantiomers d) diastereomers
4. What is the relationship between 1-butene and cis-2-butene?
   a) unrelated compounds b) constitutional isomers
   c) enantiomers d) **diastereomers**
5. Example for a weak electrolyte
   a) NaOH b) NH_4OH c) KCl d)NaCl
6. Molar conductance decreses with increase in concentration is not due to fall the degree of ionisation but to fall in mobilities of ions due to greater
   a) interionic effect b) wien effect c) viscous effect d) **interionic effect**
7. Central ion drag in a concentrated or weak electrolytic solution is due to
   a) **assymmetry effect** b) symmetry effect c) viscous effect d) interionic effect
8. Slow down the ion by counter current in the same way as counter current in a stream slow down a swimmer this effect is known as
   a)**electrophoretic effect** b) viscous effect c)Interionic effect d) wein effect
9. One electron- volt of energy is equivalent to a photon with a wave length of about
   a) 300 A° b) 30 A° c) 3000A° **d) 12000A°**
10. To get parallel band in IR for CO_2,the oscillating dipole moment is ------- to the molecular Axis is

a) parallel b)perpendicular c)angular d)linear

11.Water has the electronic transition

a) n-π* b)π- π* c)α- α* **d) n-α***

12. Bathochromic shift is also called

a) blue shift **b) red shift** c) yellow shift d) orange shift

>C=O stretching of aldehydes occur in the region in the IR region.

a)2770-2700 cm-1 **b)1740-1720cm-1** c)700-970cm-1 d)3300-3400-1cm

13.The wavelength dependence of

a) **optical activity** b) optical rotation c) rotation d) optical dispersion

14. A measurement which on repetition gives same or nearly same result is called

a) accurate measurement b) average measurement
c) **precise measurement** d) estimated measurement

15.The steady state error due to a ramp input for a type to system is equal to

a) Zero b) infinite c) non-zero number d) **constant**

16. Electrostatic attraction is a function present in

a)Column chromatography b) Paper chromatography
c) **ion exchange chromatography** d) gas chromatography

17. In-vitro hydrolysis studies of drugs & kinetic studies of reaction can be performed by

a) Polarimetry b) Refractometry c) Potentiometry d) **Conductometry**

18. What is the instrument used in the DTG

a)DTA apparatus b) calorimeter c) **thermo balance** d) photometer

19. The radioactivity of the sample is detected by using

a)**ETA** b) EC c) EGD d) DTG

20. The instrument of Electrical conductivity is

a)Photometer b) spectrophotometer c) **Electrometer** d) Thermometer

PART-B (2 Mark Questions)

21. What is Random sampling?
22. Define the term chromophore?
23. What is Hooke's law?
24. What is neutralization reaction?
25. What is keto-enol tautomers?

PART-C (6 Mark Questions)

1. Describe the instrumentation of thermogravimetry?
2. Explain the characteristics of TGA curves for $CaC_2O_4.H_2O$
3. Explain the instrumentation of UV spectroscopy?
4. Explain the various applications of UV spectroscopy.
5. Explain the applications of IR spectroscopy in organic compounds?
6. Explain briefly conductometric titrations
7. Discuss on optical purity and enantiomeric excess (ee)
8. Explain the stereoisomerism in tartaric acid
9. Explain with example column chromatography. What are its advantages?
10. Write notes on the principle and applications of GC?

KARPAGAM ACADEMY OF HIGHER EDUCATION
(Deemed to be University)
(Established Under Section 3 of UGC Act 1956)
DEPARTMENT OF CHEMISTRY

UNIT V (Separation and analysis)

(Multiple Choice Questions)

S.No	Questions	Option a	Option b	Option c	Option d	Answer
1	------ type of molecules have the CD and ORD	Chiral only	optical only	symmetrical	chiral,optical	chiral,optical
2	1 Part of the ammonia concentration in 160 millian parts of ammonia detected by adding	indicator	Nessler's reagent	buffer solution	acidic solution	Nessler's reagent
3	A blue iris in an eye because of	Rayleigh scattering	Compton effect	Rayleigh absorption	Tyndall scattering	Tyndall scattering
4	What is the relationship between 1-butene and *cis*-2-butene?	unrelated compounds	constitutional isomers	enantiomers	diastereomers	constitutional isomers
5	What is the relationship between trans-2-butene and cis-2-butene?	unrelated compounds	constitutional isomers	enantiomers	diastereomers	diastereomers
6	Which of the following molecules exists as a pair of enantiomers?	2-Bromopropane	1-Bromo-3-methylbutane	2-Cyclohexen-1-ol	*cis*-1,2-Dichlorocyclobutane	2-Cyclohexen-1-ol
7	Which of the following diols exists as a pair of enantiomers?	cis-1,3-Cyclohexanediol	trans-1.3-Cyclohexanediol	cis-1,4-Cyclohexanediol	trans-1,4-Cyclohexanediol	*trans*-1.3-Cyclohexanediol
8	Which of the following is capable of existing as a	2-methylpropane	2-methylpentane	3-methylpentane	3-methylhexane	3-methylhexane

	pair of enantiomers?					
9	Conventional spectrophotometers are designed to determine the difference between ----------	absorbances of a sample	adsorbances of a sample	size of sample	reflection of sample	absorbances of a sample
10	Electric field and magnetic field oscillates only in one plane ----- occur	linearly polarized light	circularly polarized light	square polarized light	randomly polarized light	circularly polarized light
11	Electric field and magnetic field oscillates perpendicular to the plane ----- occur	linearly polarized light	circularly polarized light	square polarized light	randomly polarized light	linearly polarized light
12	How many types are in the circular resolvers?	1	2	3	4	3
13	In axial haloketone rule, when the +ve cotton effect occurred, the α-halogen present in the ----- side of the view	right	left	axial	equtorial	right
14	In axial haloketone rule, when the -ve cotton effect occurred, the α-halogen present in the ----- side of the view	right	axial	left	equtorial	left
15	In CE optical rotation decreases with wavelength increases is occur	+ve CE	-ve CE	±ve CE	no CE	-ve CE
16	In CE optical rotation increases with wavelength decrease is occur	+ve CE	-ve CE	±ve CE	no CE	-ve CE

17	Which of the 7 isomers of dichlorocyclohexane possess a plane of symmetry?	cis-1,2; cis-1,3;	1,1; cis-1,2; cis-1,3;	cis-1,2; cis-1,3; cis-1,4;	1,1; cis-1,2; cis-1,3; cis-1,4; trans-1,4	1,1; *cis*-1,2; *cis*-1,3; *cis*-1,4; *trans*-1,4
18	Which of the following has a plane of symmetry?	cis-1,2-Dimethylcyclohexane	trans-1,3-Dimethylcyclohexane	1,3-Dimethylcyclohexene	1,4-Dimethylcyclohexene	*cis*-1,2-Dimethylcyclohexane
19	In the 2,6th position of the cyclohexanonen has ----- to the CE effect	small contribution	high contribution	medium contribution	optimum contribution	small contribution
20	In the 4th position of the cyclohexanonen has ------ CE effect	less	no	high	very high	no
21	In the octant rule consider which sector	front sector	side plane	side sector	rear sector	front sector
22	In the octant rule consideration the carbonyl group of the cyclohexanone is ----- of the chair position	tail	axial	equtorial	head	head
23	In the rayleigh scattering , the indensity of light depends on the ----th power of freguency	3	2	4	5	2
24	Ketones substituted with a halogen atom at the------	α-carbon	β-carbon	ϒ-carbon	4-th carbon	α-carbon
25	Magnetic optical rotation is known as	Tyndall effect	Compton effect	cotton effect	Faraday effect	Faraday effect
26	Nephelometry and turbidimetry only differ in the ----- of scattered radiation	measuring	solublity of particles	Absorption	reflection	measuring
27	Nephelometry is similar	conductometry	colorimetry	polarimetry	flurometry	flurometry

	to the					
28	Nephlometry method applied for ----- concentration of collidal particles suspention	low	high	very low	minute	low
29	ORD and CD are known as ------- properties	chiral	achiral	optical	chiroptical	chiroptical
30	ORD curves are affected by	chromophoric bands	chromophoric absorption	chromophoric adsorption	chromophoric reflection	chromophoric bands
31	The light is -------- while the shorter wavelength	transmissed	scattered	emitted	reflected	scattered
32	Which of the following physical properties differ for each of a pair of enantiomers?	solubility in ethanol	direction of rotation of plane-polarized light	boiling point and melting point	index of refraction	direction of rotation of plane-polarized light
33	An optically active compound is composed of 75% of the (R) enantiomer and 25% of the (S) enantiomer. The enantiomeric excess (ee) is equal to	87.5%.	75%.	50%.	37.5%.	50%.
34	If a sample of 2-butanol has an enantiomeric excess of 60% of l-2-butanol, how much of each isomer is present?	60% levorotatory and 40% dextrorotatory	80% levorotatory and 20% dextrorotatory	70% levorotatory and 30% dextrorotatory	66% levorotatory and 34% dextrorotatory	80% levorotatory and 20% dextrorotatory
35	Which of the following statements is TRUE?	To be diastereomers, a pair of molecules must have 2 or more chiral centers.	To be diastereomers, a pair of molecules must have at least 1 chiral center.	To be diastereomers, a pair of molecules must be stereoisomers.	To be diastereomers, a pair of molecules must be a racemate	To be diastereomers, a pair of molecules must be a racemate
36	The cotton effect was discovered by	compton	amine cotton	roberg	john dolton	amine cotton

37	The degree of red in a sunset varies depending on ------	light	weather	scattering particle	cotton effect	weather
38	The effect of CE associates with ----- transition	n-π* transition	n-n transition	n-σ transition	σ-σ transition	n-π* transition
39	The Faraday cell,which consists of a glass or silica rod surrounded by a coil carrying ----- alternating current	12-HZ	14-HZ	16-HZ	60-HZ	60-HZ
40	The french physicst discover the ------- Effect	compton	tyndall	cotton	rayleigh	cotton
41	The indices of refraction of an optical medium for the left and right hand circularly polarized components may not be the same ,and will be designated	nL	nR	nL,nR	nL - nR	nL,nR
42	The majority of CD measurements are for the purpose of what?	Structure determination	identify plane of symmetry	identify the phase effect	identify sample density	Structure determination
43	The octant rule s applied for steroids because of its ----	structure	chemical charge	rigid property	reactivity	rigid property
44	The pockels modulator , a high potential is applied across a plate of crystalline--------?	KH3PO4	KH2PO4	KHPO4	KH2PO3	KH2PO4
45	The servoamplifier responds only to the ------ frequency	12-HZ	14-HZ	16-HZ	17-HZ	12-HZ
46	The size and density of	Rayleigh effect	Compton effect	cotton effect	Tyndall effect	Tyndall effect

	the particles determined by -------					
47	The wavelength dependence of --------	optical activity	optical rotation	rotation	optical dispersion	optical activity
48	Turbidimetry is similar to the	conductometry	colorimetry	polarimetry	flurometry	colorimetry
49	Turbidimetry method applied for ----- concentration of collidal particles suspention	low	high	very low	minute	high
50	Tyndall effect also known as-------	collidal scatterin	Tyndall scattering	α-emission	α-radiation	Tyndall scattering
51	Tyndall effect applied to light scattering for macroscopic particles ---------- takes place	scattering	reflection	transmission	absorption	reflection
52	Tyndall effect in ------ glass,appears the blue colour with orange shining	transparent	opaque	quartz	opalescent	opalescent
53	Tyndall scattering is mathematically analysable in terms of----- theory	langmuir	rayleigh	mie	binomial	mie
54	What are the second steps of circular polarisation	The beam of radiation must not plane polarized	The polarized beam must be passed through a device	The beam of radiation should not be plane polarized	The polarized beam may not be passed through a device	The polarized beam must be passed through a device
55	What is the relationship between (1R,2S)-dibromocyclohexane and (1S,2R)-dibromocyclohexane?	identical	enantiomers	diastereomers	constitutional isomers	enantiomers
56	What is the relationship	identical	enantiomers	diastereomers	constitutional	identical

	between (1R,2S)-1-bromo-2-methylcyclohexane and (1S,2R)-1-bromo-2-methylcyclohexane?				isomers	
57	Which of the following may be separated by ordinary physical methods?	a pair of identical molecules	a pair of enantiomers	a pair of diastereomers	a pair of identical atoms	a pair of diastereomers
58	Which of the following may be separated by ordinary physical methods?	(R)-3-bromo-1-butene and (S)-3-bromo-1-butene	cis-2-bromo-2-butene and trans-2-bromo-2-butene	(2R,3S)-1,2-dibromobutane and (2S,3R)-1,2-dibromobutane	(R)-2-bromobutane and (S)-2-bromobutane	cis-2-bromo-2-butene and trans-2-bromo-2-butene
59	Which source is used for occuring monochromatic radiation	white light	UV light	IR light	gaseous	white light
60	Why Axial substitution often occurred in the compound because	dipole-dipole intraction	dipole-dipole repulsion	dipole-dipole combination	steric effect	dipole-dipole repulsion