KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University)

(Established Under Section 3 of UGC Act 1956)

Coimbatore - 641021.

DEPARTMENT OF CHEMISTRY

SUBJECT : INSTRUMENTAL METHODS OF CHEMICAL ANALYSIS
SEMESTER : V
SUBJECT CODE: 16CHU504A CLASS: III - B. Sc – Chemistry

Scope

The course deals with the introduction to spectroscopic methods of analysis like IR, UV-Visible, NMR spectroscopy and mass spectroscopy, Chromatographic separation techniques, elemental analysis, electro analytical techniques, and XRD analysis.

Objectives

This course enables the students to

1. Understand the spectroscopic techniques like IR, UV-Visible, NMR spectroscopy and mass spectroscopy for analytical analysis
2. Understand the Chromatographic separation techniques for analysis
3. Understand the quantitative techniques like elemental analysis, electro analytical techniques, and XRD analysis

Methodology

Blackboard teaching, Powerpoint presentation and group discussion.

UNIT I

Introduction to spectroscopic methods of analysis:

Recap of the spectroscopic methods covered in detail in the core chemistry syllabus: Treatment of analytical data, including error analysis. Classification of analytical methods and the types of instrumental methods. Consideration of electromagnetic radiation.

UNIT II

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).

UNIT III

Separation techniques

Chromatography: Gas chromatography, liquid chromatography, supercritical fluids, Importance of column technology (packing, capillaries), Separation based on increasing number of factors (volatility, solubility, interactions with stationary phase, size, electrical field), Detection: simple vs. specific (gas and liquid), Detection as a means of further analysis (use of tags and coupling to IR and MS), Electrophoresis (plates and capillary) and use with DNA analysis.

Immunoassays and DNA techniques

Mass spectroscopy: Making the gaseous molecule into an ion (electron impact, chemical ionization), Making liquids and solids into ions (electrospray, electrical discharge, laser desorption, fast atom bombardment), Separation of ions on basis of mass to charge ratio, Magnetic, Time of flight, Electric quadrupole. Resolution, time and multiple separations, Detection and interpretation (how this is linked to excitation).

UNIT IV

Elemental analysis:

Mass spectrometry (electrical discharges).

Atomic spectroscopy: Atomic absorption, Atomic emission, and Atomic fluorescence. Excitation and getting sample into gas phase (flames, electrical discharges, plasmas), Wavelength separation and resolution (dependence on technique), Detection of radiation (simultaneous/scanning, signal noise), Interpretation (errors due to molecular and ionic species, matrix effects, other interferences).

UNIT V

NMR spectroscopy: **P**rinciple, Instrumentation, Factors affecting chemical shift, Spincoupling, Applications.

Electroanalytical Methods: Potentiometry & Voltammetry

Radiochemical Methods

X-ray analysis and electron spectroscopy (surface analysis)

Suggested Readings

Text books:

1. Douglas A. Skoog, James Holler, F. & Stanley Crouch. *Principles of Instrumental Analysis*. 6th Edition. (ISBN 0-495-01201-7).

2. Willard, Merritt, Dean & Settle. *Instrumental Methods of Analysis*. 7th ed.

3. P.W. Atkins. *Physical Chemistry*.

Reference Books

1. G.W. Castellan. *Physical Chemistry*.

2. C.N. Banwell. *Fundamentals of Molecular Spectroscopy*.

3. Brian Smith. *Infrared Spectral Interpretations: A Systematic Approach*.

4. W.J. Moore. *Physical Chemistry*.

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LECTURE PLAN

DEPARTMENT OF CHEMISTRY

S. No	Lecture Duration Period	Topics to be Covered	Support Material/Page Nos
		UNIT-I	
1	1	Introduction to spectroscopic methods of analysis	T_1- 158
2	1	Recap of the spectroscopic methods covered in detail in the core chemistry syllabus: Introduction	T_1- 158
3	1	Treatment of analytical data,	T_1-1055
4	1	Error analysis.	T_1-1056
5	1	Classification of analytical methods	T_1- 1074
6	1	The types of instrumental methods.	T_1-14; T_2-02
7	1	Consideration of electromagnetic radiation.	T_1- 160; T_3-500
8	1	Recapitulation and Discussion of Important Questions	
	Total No of Hours Planned for Unit I =08		
		UNIT-II	
1	1	**UNIT II** **Molecular spectroscopy:** *Infrared spectroscopy: In*teractions with molecules: absorption and scattering.	T_3-445,475

2	1	Means of excitation (light sources), separation of spectrum (wavelength dispersion, time resolution),	T_1-489, 493; T_3-479
3	1	detection of the signal (heat, differential detection), Interpretation of spectrum (qualitative, mixtures, resolution),	R_2-112; T_1-509;
4	1	Advantages of Fourier Transform (FTIR). Samples and results expected.	T_1-492; R_2-120;
5	1	Applications: Issues of quality assurance and quality control, Special problems for portable instrumentation and rapid detection.	T_1-503,507; T_3-481
6	1	*UV-Visible/ Near IR* – emission, absorption, fluorescence and photoacoustic, Excitation sources (lasers, time resolution),	T_1-526; T_3-503; T_1-523, T1-489-490
7	1	Wavelength dispersion (gratings, prisms, interference filters, laser, placement of sample relative to dispersion, resolution),	T_1-489, 495,416,524
8	1	Detection of signal (photocells, photomultipliers, diode arrays, sensitivity and S/N),	T_3-481-482, 734,
9	1	Single and Double Beam instruments, Interpretation quantification.	T_1-492,516
10	1	Interpretation- mixtures, absorption vs. fluorescence and the use of time, photoacoustic, fluorescent tags).	T_1-516, 444-446, 523, 446
11	1	Recapitulation and Discussion of Important Questions	
	Total No of Hours Planned for Unit II=11		
		UNIT-III	
1	1	**UNIT III** **Separation techniques** *Chromatography:* Gas chromatography, liquid chromatography, supercritical fluids,	T_1-865, T1-893,935
2	1	Importance of column technology (packing, capillaries),	T_1-880,881
3	1	Separation based on increasing number of factors (volatility, solubility, interactions with stationary phase, size, electrical field),	T_1-850, 894,896
4	1	Detection: simple vs. specific (gas and liquid), Detection as a means of further analysis (use of tags and coupling to IR and MS),	T_1-900, 938,951, T2-572
5	1	Electrophoresis (plates and capillary) and use with DNA analysis.	T_1-845, 947,948
6	1	*Immunoassays and DNA techniques* *Mass spectroscopy:* Making the gaseous molecule into an ion (electron impact, chemical ionization),	T_1-958-960, 607,610,613

7	1	Making liquids and solids into ions (electrospray, electrical discharge, laser desorption, fast atom bombardment),	T_1-615-617, 619
8	1	Separation of ions on basis of mass to charge ratio, Magnetic time of flight, Electric quadrupole.	T_1-624,627
9	1	Resolution, time and multiple separations, Detection and interpretation (how this is linked to excitation).	T_1-622, 641,636,639
10	1	Recapitulation and Discussion of Important Questions	
	Total No of Hours Planned for Unit III=10		
		UNIT-IV	
1	1	**UNIT IV** **Elemental analysis:** Mass spectrometry (electrical discharges). Atomic spectroscopy: Atomic absorption,	T_2-262, 243
2	1	Atomic spectroscopy: Atomic emission, Atomic fluorescence.	T_2-260, 249
3	1	Excitation and getting sample into gas phase (flames, electrical discharges, plasmas),	T_2-250, 260,262,265
4	1	Wavelength separation (dependence on technique),	T_1-270,272
5	1	Detection of radiation (simultaneous/scanning, signal noise)	T_2-277
6	1	Interpretation (errors due to molecular and ionic species,	T_1-332, T_2- 250
7	1	Interpretation-matrix effects, other interferences.	T_1-334
8	1	Recapitulation and Discussion of Important Questions	
	Total No of Hours Planned for Unit IV=08		
		UNIT-V	
1	1	**UNIT V** **NMR spectroscopy**: Introduction and Principle	T_2- 422, 431
2	1	NMR spectroscopy- Instrumentation	T_2-431
3	1	Factors affecting chemical shift, Spin coupling,	T_2-440, 443
4	1	NMR spectroscopy -Applications.	T_2-454-458
5	1	Electroanalytical Methods: Potentiometry, Voltammetry	T_1-724, T2-697
6	1	Radiochemical Methods	T_1-992
7	1	X-ray analysis	T_2-340

8	1	electron spectroscopy (surface analysis)	T_2-386
9	1	Recapitulation and Discussion of important Questions	
10	1	Discussion of Previous ESE Question Papers.	
11	1	Discussion of Previous ESE Question Papers.	
	Total No of Hours Planned for unit V=11		
Total Planned Hours = 48			

Text books:

1. Douglas A. Skoog, James Holler, F. & Stanley Crouch. *Principles of Instrumental Analysis*. 6th Edition. (ISBN 0-495-01201-7).
2. Willard, Merritt, Dean & Settle. *Instrumental Methods of Analysis*. 7th ed.
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3. Brian Smith. *Infrared Spectral Interpretations: A Systematic Approach*.
4. W.J. Moore. *Physical Chemistry*.

UNIT I
Syllabus

Introduction to spectroscopic methods of analysis:

Recap of the spectroscopic methods covered in detail in the core chemistry syllabus: Treatment of analytical data, including error analysis. Classification of analytical methods and the types of instrumental methods. Consideration of electromagnetic radiation.

DATA and ERROR ANALYSIS

Performing the experiment and collecting data is only the beginning of the process of completing an experiment in science. Understanding the results of any given experiment is always the central goal of the experiment. Presenting those results in a clear concise manner completes the experiment. This overview of the complete process is as valid in an instructional laboratory course as in a research environment. You will not have learned any physics if you did not understand the experiment. Presenting the results of your experimental work is fundamentally saying, "This is what I did and this is what I learned." Putting together your presentation of the results should help you clarify the results to yourself. (If your instructor can clearly see what you did and what you learned, you might get a better grade.)

Data analysis should NOT be delayed until all of the data is recorded. Take a low point, a highpoint and maybe a middle point, and do a quick analysis and plot. This will help one avoid the problem of spending an entire class collecting bad data because of a mistake in experimental procedure or an equipment failure.

First and foremost, data analysis means understanding what your results mean. When analyzing your data, try to think through the physical processes which are occurring. Write your train of thought down. Ultimately, the goal is for you to understand physics and the world a bit better. Your understanding of your results probably occurs in stages, with each stage being a refinement and possibly more mathematical than the previous stage.

For example, one might first note that as time increases so does distance. Next a quick graph of distance vs time might verify this understanding but the relationship is NOT linear, i.e. the data does not form a straight line. By further work, one might discover that distance increase linearly with the square of the time. Or sometimes the mathematical relationship may remain hidden.

Relate each successive stage of your understanding and interpretation of your results to the physical principles that are involved. In the above example, one might note that the change in position with time is caused by velocity that is in turn caused by acceleration from the gravitational force. Finally, develop the related mathematics. Equations are nearly meaningly unless they are related to the physical laws. (Remember to identify all the variables and constants in you equations.)

Sometimes, your results will not support and may even contradict the physical explanation suggested by the manual or your instructor. Say so! But of course then a few suggestions as to the reason for this apparent failure of the physical laws, would be in order. Do NOT just say " The equipment was a piece of shot" Try to explain what went wrong or what competing effects have come into play.

One of the reasons that you are encouraged to record everything that is going on as it is going on, is that this information may help explain bad results. For example, partly for fun, you note each time your lab partner sneezes. Later while looking at the data, you discover that each data point that was being collected during a sneeze deviates from the pattern of the rest of the data. This may give you good reason for dropping "bad" data.

The quality of the data, determines to a great extent, what conclusions can be reached from them. If you are looking for a small effect, say a total change of 1 mm, and the uncertainties in your data is 2mm then you really cannot make any solid conclusions. (See the section on error analysis below.)When one considers the quality of a measurement there are two aspects to consider. The first is if one were to repeat the measurement, how close would new results be to the old, i.e., how reproducible is the measurement? Scientists refer to this as the precision of the measurement. Secondly, a measurement is considered “good” if it agrees with the true value. This is known as the accuracy of the measurement. But there is a potential problem in that one needs to know the “true value” to determine the accuracy.

A good measurement must be close to the "true value" and be reproducible. In this experiment, if someone made one measurement of g2 and got 9.79 m/s, it would be an accurate measurement. But if next time they tried they got 4.12 m/s, no one would believe that they were anything but lucky in the first measurement. Similarly, if one group2 got values of 7.31, 7.30, 7.33, and 7.29 m/s their results 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 replicate measurements should be done, however cost and time usually limit the number of replicate measurements possible. Statistics treats each result of a measurement as an item 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 the standard deviation, s. In order to determine the true m and s, the entire population should 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 of several items is done, which constitutes a sample. Estimates of the mean and the standard deviation are calculated and denoted by x and s, respectively. The values of x and s are used to calculate confidence intervals, comparison of precisions and significance of apparent discrepancies. The mean, x, and the standard deviations, of the values $X_1, X_2, \ldots$, Xn obtained from n measurements is given by the equations:

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

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

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

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

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

In some older books the use of the term ‘average’ instead of ‘mean’ (Youden 1994), can be found, but the common term nowadays is ‘mean’. There are different kinds 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 by Equation (2.1). There are several reasons why the arithmetic means and not the other ones are chosen. 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 free units 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 the standard deviation, s. In many cases the variance is not denoted by V, 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 uses n 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 an infinite 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 the Calculation 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 estimate for 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. The standard deviation has the same dimension as that of the measured values, xi often; analysts prefer to use a dimensionless quantity to describe the dispersion of the 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 and instrumentation 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.

The word chemistry used in "analytical chemistry" clearly signifies the analysis of chemical elements and their derived compounds. This branch of science is used in all the fields of sciences. Chemical sciences serve as proof of various advances and evolution of technologies which has led to the growth of high performance instruments. This development of instrumentation has led to increase in more sophisticated and non-destructive method of analysis. Nondestructive techniques plays the most valuable role in forensic science as they can be conducted on very small samples and does not even require extensive sample preparation before the measurement.

With these advances techniques quality and precision requirements can be met efficiently. 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 analysis in various fields of science i.e. pure and applied science. Only a single instrument cannot solve an analytical issue, instead numerous instrumental methods are necessary for efficient analysis to a maximum extent.

Classification of analytical methods:

Analytical Chemistry deals with methods for determining the chemical composition of samples of matter. A qualitative method yields information about the identity of atomic or molecular species or the functional groups in the sample; a quantitative method, in contrast, provides numerical information as to the relative amount of one or more of these components.

Analytical methods are often classified as being either classical or instrumental. This classification is largely historical with classical methods, sometimes called wet-chemical methods, preceding instrumental methods by a century or more.

Classical Methods:

- Separation of analytes by precipitation, extraction, or distillation.

- Qualitative analysis by reaction of analytes with reagents that yielded products that could be recognized by their colors, boiling or melting points, solubilities, optical activities, or refractive indexes.

- Quantitative analysis by gravimetric or by titrimetric techniques.

In the early years of chemistry, most analyses were carried out by separating components of interest in a sample by precipitation, extraction, or distillation. For quantitative analyses, the separated components were then treated with reagents that yielded products that could be recognized by their colors, boiling points or melting points, their solubility in a series of solvents, their odors, their optical activities, or their refractive indexes. For quantitative analyses, the amount of analyte was determined by gravimetric or by titrimetric measurement.

Gravimetric Methods – the mass of the analyte or some compound produced from the analyte was determined.

Titrimetric Methods – the volume or mass of a standard reagent required to react completely with the analyte was measured.

Types of instrumental Methods:

Measurements of physical properties of analytes, such as conductivity, electrode potential, light absorption, or emission, mass to charge ratio, and fluorescence, began to be used for quantitative analysis of a variety of inorganic, organic, and biochemical analyte. Highly efficient chromatographic and electrophoretic techniques began to replace distillation, extraction, and precipitation for the separation of components of complex mixtures prior to their qualitative or quantitative determination. These newer methods for separating and determining chemical species are known collectively as instrumental methods of analysis.

Instrumentation can be divided into two categories: detection and quantitation.

- Measurement of physical properties of analytes - such as conductivity, electrode potential, light absorption or emission, mass-to-charge ratio, and fluorescence-began to be employed for quantitative analysis of inorganic, organic, and biochemical analytes.

- Efficient chromatographic separation techniques are used for the separation of components of complex mixtures.
- Instrumental Methods of analysis (collective name for newer methods for separation and determination of chemical species.)

Instrumentation is necessary to decipher these values. The challenge for the instrumental scientist is to mimic the 5 senses. Substances have physical and chemical fingerprints with unique thresholds. The object is to detect a chemical substance within a matrix and selectively perturb the substance of interest. Signals must be readable (in a voltage or electrical signal).

Types of instrumentation

Signal	Instrumental Methods
Emission of radiation	Emission spectroscopy (X-ray, UV, visible, electron, Auger); fluorescence, phosphorescence, and luminescence (X-ray, UV, and visible)
Absorption of radiation	Spectrophotometry and photometry (X-ray, UV, visible, IR); photoacoustic spectroscopy; nuclear magnetic resonance and electron spin resonance spectroscopy
Scattering of radiation	Turbidimetry; nephelometry; Raman spectroscopy
Refraction of radiation	Refractometry; interferometry
Diffraction of radiation	X-Ray and electron diffraction methods
Rotation of radiation	Polarimetry; optical rotary dispersion; circular dichroism
Electrical potential	Potentiometry; chronopotentiometry
Electrical charge	Coulometry
Electrical current	Polarography; amperometry
Electrical resistance	Conductometry
Mass-to-charge ratio	Mass spectrometry
Rate of reaction	Kinetic methods
Thermal properties	Thermal conductivity and enthalpy
Radioactivity	Activation and isotope dilution methods

Instruments for Analysis

An instrument for chemical analysis converts information about the physical or chemical characteristics of the analyte to information that can be manipulated and interpreted by human.

Detectors: A mechanical, electrical, or chemical device that identifies, records, or indicates a change in one of the variables in the environment, such as pressure, temperature, electrical charge, electromagnetic radiation, nuclear radiation, particulates, or molecules.

Transducers: Devices that convert information in nonelectrical domains to information in electrical domains and the converse. (Photodiodes, photomultipliers)

Sensors: Analytical devices those are capable of monitoring specific chemical species continuously and reversibly.

Instrument Components

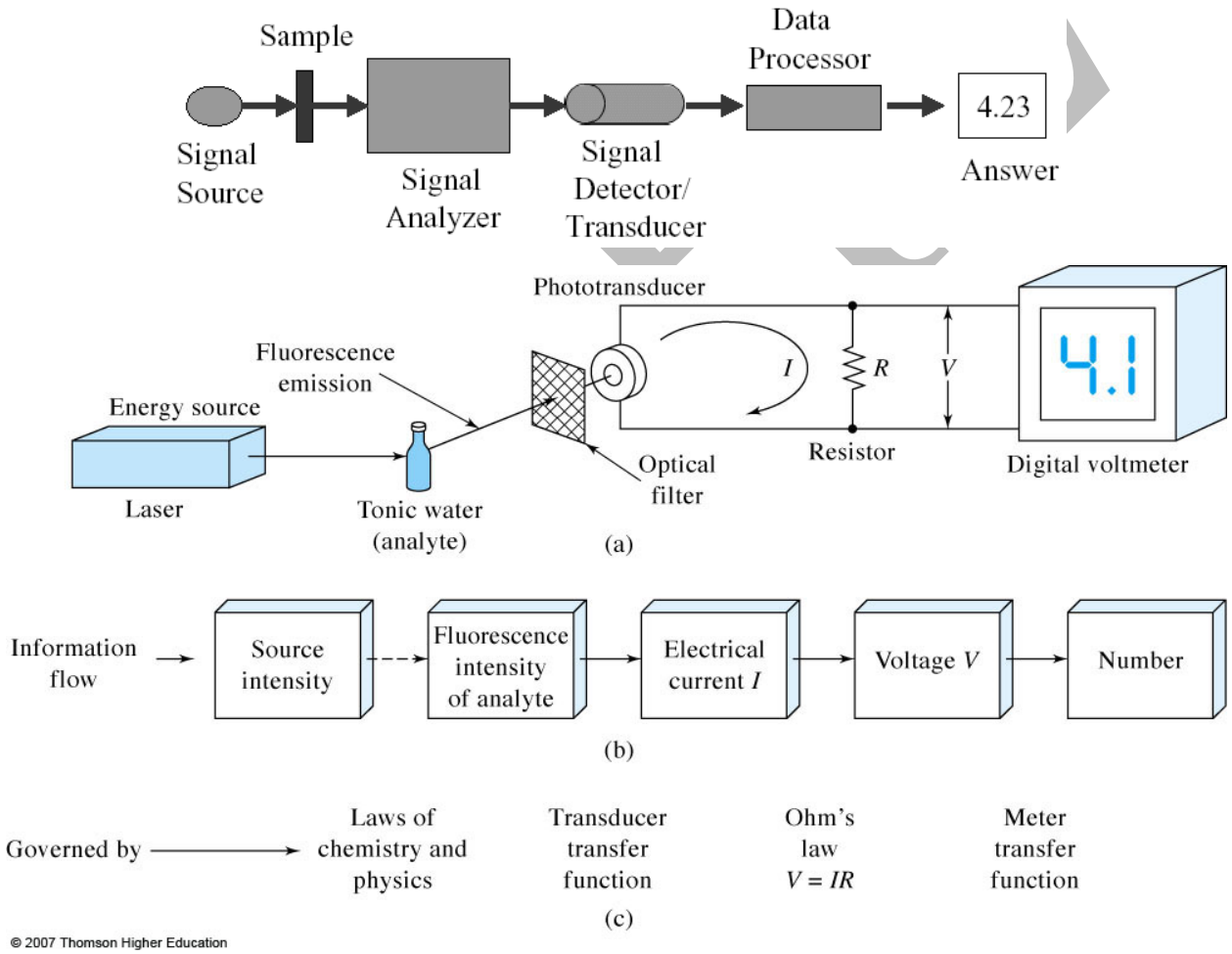

TABLE 1-2 Some Examples of Instrument Components

Instrument	Energy Source (stimulus)	Analytical Information	Information Sorter	Input Transducer	Data Domain of Transduced Information	Signal Processor/ Readout
Photometer	Tungsten lamp	Attenuated light beam	Filter	Photodiode	Electrical current	Amplifier, digitizer, LED display
Atomic emission spectrometer	Inductively coupled plasma	UV or visible radiation	Monochromator	Photomultiplier tube	Electrical current	Amplifier, digitizer, digital display
Coulometer	Direct-current source	Charge required to reduce or oxidize analyte	Cell potential	Electrodes	Time	Amplifier, digital timer
pH meter	Sample/ glass electrode	Hydrogen ion activity	Glass electrode	Glass-calomel electrodes	Electrical voltage	Amplifier, digitizer, digital display
Mass spectrometer	Ion source	Mass-to-charge ratio	Mass analyzer	Electron multiplier	Electrical current	Amplifier, digitizer, computer system
Gas chromatograph with flame ionization	Flame	Ion concentration vs. time	Chromatographic column	Biased electrodes	Electrical current	Electrometer, digitizer, computer system

Selecting an Analytical Method

- Required Accuracy
- Amount of sample
- Concentration range(s) of analyte(s)
- Possible interferences
- Chemical and physical properties of matrix
- Number of samples

Desirable Characteristics for an Analytical Method

- Speed

- Ease and Convenience
- Skill required of operator
- Cost and availability of equipment
- Per-samples cost

Numerical Criteria for Selecting an Analytical Method

- Precision
- Absolute standard deviation
- Relative standard deviation
- Coefficient of variation
- Variance
- Bias
- Absolute systematic error
- Relative systematic error
- Sensitivity
- Calibration
- Analytical
- Detection Limit
- Blank plus three times Std. Dev. of blank
- Concentration Range
- Limit of Quantitation (LOQ)
- Limit of Linearity (LOL)
- Selectivity
- Effects of interferences
- Coefficient of Selectivity

STANDARD DEVIATION:

If the deviations of a measurement were averaged, the result would be zero because of high and low values would cancel each other. Generally one expresses the fluctuation about the

average measurement with by calculating and quoting the standard deviation, F of the n measurements.

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

The standard deviation

The standard deviation of the mean, σ_m, is the standard measure for describing of the precision of a measurement, i.e. how well a number of measurements agree with themselves. Thus:

$$\Delta x = \sigma_m$$

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

Electromagnetic radiation

Electromagnetic radiation, in classical physics, the flow of energy at the universal speed of light through free space or through a material medium in the form of the electric and magnetic fields that make up electromagnetic waves such as radio waves, visible light, and gamma rays. In such a wave, time-varying electric and magnetic fields are mutually linked with each other at right angles and perpendicular to the direction of motion. An electromagnetic wave is characterized by its intensity and the frequency ν of the time variation of the electric and magnetic fields. In terms of the modern quantum theory, electromagnetic radiation is the flow of photons (also called light quanta) through space. Photons are packets of energy hν that always

move with the universal speed of light. The symbol h is Planck's constant, while the value of ν is the same as that of the frequency of the electromagnetic wave of classical theory. Photons having the same energy hν are all alike, and their number density corresponds to the intensity of the radiation. Electromagnetic radiation exhibits a multitude of phenomena as it interacts with charged particles in atoms, molecules, and larger objects of matter. These phenomena as well as the ways in which electromagnetic radiation is created and observed, the manner in which such radiation occurs in nature, and its technological uses depend on its frequency ν. The spectrum of frequencies of electromagnetic radiation extends from very low values over the range of radio waves, television waves, and microwaves to visible light and beyond to the substantially higher values of ultraviolet light, X-rays, and gamma rays. The basic properties and behaviour of electromagnetic radiation are discussed in this article, as are its various forms, including their sources, distinguishing characteristics, and practical applications. The article also traces the development of both the classical and quantum theories of radiation.

General Considerations

Occurrence and importance

Close to 0.01 percent of the mass/energy of the entire universe occurs in the form of electromagnetic radiation. All human life is immersed in it, and modern communications technology and medical services are particularly dependent on one or another of its forms. In fact, all living things on Earth depend on the electromagnetic radiation received from the Sun and on the transformation of solar energy by photosynthesis into plant life or by biosynthesis into zooplankton, the basic step in the food chain in oceans. The eyes of many animals, including those of humans, are adapted to be sensitive to and hence to see the most abundant part of the Sun's electromagnetic radiation-namely, light, which comprises the visible portion of its wide range of frequencies. Green plants also have high sensitivity to the maximum intensity of solar

electromagnetic radiation, which is absorbed by a substance called chlorophyll that is essential for plant growth via photosynthesis. Practically all the fuels that modern society uses—gas, oil, and coal—are stored forms of energy received from the Sun as electromagnetic radiation millions of years ago. Only the energy from nuclear reactors does not originate from the Sun.

The electromagnetic spectrum

The brief account of familiar phenomena given above surveyed electromagnetic radiation from low frequencies of ν (radio waves) to exceedingly high values of ν (gamma rays). Going from the ν values of radio waves to those of visible light is like comparing the thickness of this page with the distance of Earth from the Sun, which represents an increase by a factor of a million billion. Similarly, going from the ν values of visible light to the very much larger ones of gamma rays represents another increase in frequency by a factor of a million billion. This extremely large range of ν values, called the electromagnetic spectrum, is shown in Figure 1, together with the common names used for its various parts, or regions.

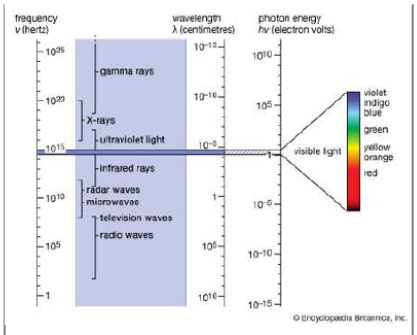

Figure 1: Electromagnetic spectrum. The small visible range (shaded) is shown enlarged at the right.
Encyclopædia Britannica, Inc.

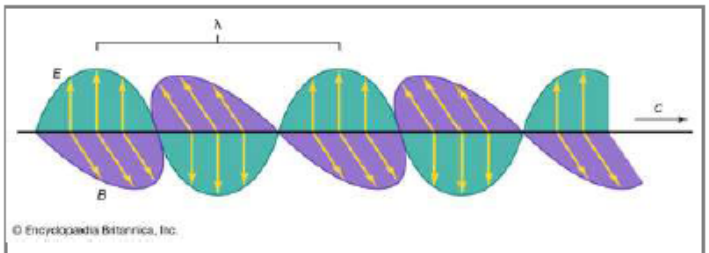

Figure 2: Radiation fields in which vectors {E vector} and {B vector} are perpendicular to each other and to the direction of propagation (see text).
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The number ν is shared by both the classical and the modern interpretation of electromagnetic radiation. In classical language, ν is the frequency of the temporal changes in an electromagnetic wave. The frequency of a wave is related to its speed c and wavelength λ in the following way. If 10 complete waves pass by in one second, one observes 10 wriggles, and one says that the frequency of such a wave is ν = 10 cycles per second (10hertz [Hz]). If the wavelength of the wave is, say, λ = 3 cm, then it is clear that a wave train 30 cm long has passed in that one second to produce the 10 wriggles that were observed. Thus, the speed of the wave is 30 cm per second, and one notes that in general the speed is $c = \lambda\nu$.

The speed of electromagnetic radiation of all kinds is the same universal constant that is defined to be exactly c = 299,792,458 meters per second (186, 282 miles per second). The wavelengths of the classical electromagnetic waves in free space calculated from $c = \lambda\nu$ are also shown on the spectrum in Figure 1, as is the energy hν of modern-day photons. Commonly used as the unit of energy is the electron volt (eV), which is the energy that can be given to an electron

by a one-volt battery. It is clear that the range of wavelengths λ and of photon energies hν is equally as large as the spectrum of ν values. Because the wavelengths and energy quanta hν of electromagnetic radiation of the various parts of the spectrum are so different in magnitude, the sources of the radiations, the interactions with matter, and the detectors employed are correspondingly different. This is why the same electromagnetic radiation is called by different names in various regions of the spectrum. In spite of these obvious differences of scale, all forms of electromagnetic radiation obey certain general rules that are well understood and that allow one to calculate with very high precision their properties and interactions with charged particles in atoms, molecules, and large objects. Electromagnetic radiation is, classically speaking, a wave of electric and magnetic fields propagating at the speed of light c through empty space. In this wave the electric and magnetic fields change their magnitude and direction each second. This rate of change is the frequency ν measured in cycles per second-namely, in hertz. The electric and magnetic fields are always perpendicular to each other and at right angles to the direction of propagation, as shown in Figure 2. There is as much energy carried by the electric component of the wave as by the magnetic component, and the energy is proportional to the square of the field strength.

Generation of electromagnetic radiation

Electromagnetic radiation is produced whenever a charged particle, such as an electron, changes its velocity-i.e., whenever it is accelerated or decelerated. The energy of the electromagnetic radiation thus produced comes from the charged particle and is therefore lost by it. A common example of this phenomenon is the oscillating charge or current in a radio antenna. The antenna of a radio transmitter is part of an electric resonance circuit in which the charge is made to oscillate at a desired frequency. An electromagnetic wave so generated can be received by a similar antenna connected to an oscillating electric circuit in the tuner that is tuned to that same frequency. The electromagnetic wave in turn produces an oscillating motion of charge in

the receiving antenna. In general, one can say that any system which emits electromagnetic radiation of a given frequency can absorb radiation of the same frequency.

Such human-made transmitters and receivers become smaller with decreasing wavelength of the electromagnetic wave and prove impractical in the millimeter range. At even shorter wavelengths down to the wavelengths of X-rays, which are one million times smaller, the oscillating charges arise from moving charges in molecules and atoms. One may classify the generation of electromagnetic radiation into two categories: (1) systems or processes that produce radiation covering a broad continuous spectrum of frequencies and (2) those that emit (and absorb) radiation of discrete frequencies that are characteristic of particular systems. The Sun with its continuous spectrum is an example of the first, while a radio transmitter tuned to one frequency exemplifies the second category.

Properties and behavior
Scattering, reflection, and refraction

If a charged particle interacts with an electromagnetic wave, it experiences a force proportional to the strength of the electric field and thus is forced to change its motion in accordance with the frequency of the electric field wave. In doing so, it becomes a source of electromagnetic radiation of the same frequency, as described in the previous section. The energy for the work done in accelerating the charged particle and emitting this secondary radiation comes from and is lost by the primary wave. This process is called scattering. Since the energy density of the electromagnetic radiation is proportional to the square of the electric field strength and the field strength is caused by acceleration of a charge, the energy radiated by such a charge oscillator increases with the square of the acceleration. On the other hand, the acceleration of an oscillator depends on the frequency of the back-and-forth oscillation. The acceleration increases with the square of the frequency. This leads to the important result that the electromagnetic energy radiated by an oscillator increases very rapidly—namely, with the square

of the square or, as one says, with the fourth power of the frequency. Doubling the frequency thus produces an increase in radiated energy by a factor of 16.This rapid increase in scattering with the frequency of electromagnetic radiation can be seen on any sunny day: it is the reason the sky is blue and the setting Sun is red. The higher frequency blue light from the Sun is scattered much more by the atoms and molecules of Earth's atmosphere than is the lower-frequency red light. Hence, the light of the setting Sun, which passes through a thick layer of atmosphere, has much more red than yellow or blue light, while light scattered from the sky contains much more blue than yellow or red light. The process of scattering, or reradiating part of the electromagnetic wave by a charge oscillator, is fundamental to understanding the interaction of electromagnetic radiation with solid, liquids or any matter that contains a very large number of charges and thus an enormous number of charge oscillators. This also explains why a substance that has charge oscillators of certain frequencies absorbs and emits radiation of those frequencies. When electromagnetic radiation falls on a large collection of individual small charge oscillators, as in a piece of glass or metal or a brick wall, all of these oscillators perform oscillations in unison, following the beat of the electric wave. As a result, all the oscillators emit secondary radiation in unison (or coherently), and the total secondary radiation coming from the solid consists of the sum of all these secondary coherent electromagnetic waves. This sum total yields radiation that is reflected from the surface of the solid and radiation that goes into the solid at a certain angle with respect to the normal of (i.e., a line perpendicular to) the surface. The latter is the refracted radiation that may be attenuated (absorbed) on its way through the solid.

Rayleigh scattering

Rayleigh scattering seen over the ocean.

Rodrigo Nuno Bragança da Cunha

POSSIBLE QUESTIONS
UNIT-I
PART-A (20 MARKS)
(Q.NO 1 TO 20 Online Examination)

PART-B (2 MARKS)

1. Define error analysis
2. Draw the electromagnetic pattern?
3. Write note on standard deviation?
4. What are the types of error?
5. Define precision?

PART-C (6 MARKS)

1. Write about treatment of analytical data,
2. What are Classification of analytical methods
3. The types of instrumental methods.
4. Describe some important characteristic of electromagnetic radiation.
5. write a short note on the following terms a) Error b) Precision c) Standard Deviation

KARPAGAM ACADEMY OF HIGHER EDUCATION

SUBJECT : INSTRUMENTAL METHODS OF CHEMICAL ANALYSIS
SEMESTER : V SUBJECT CODE: 16CHU504A
Class: III-B. Sc Chemistry Batch – (2016-2019)
Unit-I-Multiple choice Questions

Unit-I-Multiple choice Questions

S. No	Question	Option I	Option II	Option III	Option IV	Answer
1	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
2	Branch of statistics which deals with development of particular statistical methods is classified as	industry statistics	economic statistics	applied statistics	mathematical statistics	mathematical statistics
3	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.
4	Quantitative data refers to	graphs and tables	numerical data that could usefully be quantified to help you answer your research	any data you present in your report	statistical analysis	numerical data that could usefully be quantified to help you answer your research question(s) and to meet your

			question(s) and to meet your objectives			objectives
5	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
6	Computers are essential for quantitative data analysis because	increasingly data analysis software contain algorithms that check the data for obvious errors as it is entered	they are so powerful.	they enable easy calculation for those of us not too good with figures	they are fun to use	increasingly data analysis software contains algorithms that check the data for obvious errors as it is entered
7	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
8	Testing the probability of a relationship between variables occurring by chance	chi-squared tests	significance testing	correlation coefficients	multiple regression analysis	significance testing

	alone if there really was no difference in the population from which that sample was drawn is known as					
9	The steady state error due to a ramp input for a type to system is equal to	Zero	infinite	non-zero number	constant	constant
10	Errors that occur during measurement of the quantities are of	2 types	3 types	5 types	4 types	2 types
11	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
12	Error that occurs due to equally affected measurements is called	random error	systematic error	frequent error	precision	systematic error
13	Error that occurs during the measurement of quantities is	random error	systematic error	random and systematic error	random frequent error	random and systematic error
14	Regardless to difference	degree of	statistic error	population	standard error	population mean

	in distribution of sample and population, mean of sampling distribution must be equal to	freedom		mean		
15	In statistical analysis, sample size is considered large if	n > or = 30	n < or = 30	n > or = 50	n < or = 50	n > or = 30
16	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
17	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
18	Standard deviation of a sampling distribution is also classified as	standard error	statistic error	sampling error	probability error	standard error
19	The difference between indicated value and true value of a quantity is	Gross Error	Absolute Error	Dynamic Error	Relative Error	. Absolute Error

20	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
21	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
22	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 conditions	All	All
23	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
24	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 do not depend on the sensitivity of instrument	None of these	The sensitivity of instrument to environmental input as low as possible

25	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
26	Random errors in a measurement system are due to	Environmental changes	Use of uncalibrated instrument	Poor cabling practices	Unpredictable effects	Unpredictable effects
27	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
28	Systematic errors occur due to	overuse of instruments	careful usage of instruments	human sight	instrument quality	over use of instruments
29	Measurement which is close to the true value is	accurate	average	precise	error	accurate
30	Systematic errors can be removed by	buying new instrument	breaking the instrument	dusting the instrument	recalibrating the instrument	recalibrating the instrument
31	A measurement which on repetition gives same or nearly same result is called	accurate measurement	average measurement	precise measurement	estimated measurement	precise measurement
32	The degree of closeness of the measured value of a certain quantity with	Accuracy	Precision	Standard	Sensitivity	Accuracy

	its true value is known as					
33	Relative amounts of elements are discussed in	Testing	quantitative analysis	Qualitative analysis	Physical test	quantitative analysis
34	Which of the following procedures do general analytical procedures not include?	Reasonableness tests	Statistical analysis	Trend analysis	Sequence tests	Statistical analysis
35	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
36	Which of the following is not a data mining method?	Classification and prediction	Data description	Dependency analysis	A priori algorithms	A priori algorithms
37	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	There exist plausible relationships among data that can reasonably be expected to

		among data that can reasonably be expected to continue		among data that is highly accurate	process	continue
38	the following types of analytical procedure, which one uses the most variables?	Trend analysis	Data mining	Ratio analysis	Reasonableness test	Data mining
39	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
40	Which of the following is *not* an example of a 'unit of analysis'?	Validity	significant actors	Words	Themes	significant actors
41	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
42	Which of the following	It is flexible and	It is highly complex	It is only	It is not	It is flexible and is not resource

	is an advantage of content analysis?	is not resource dependent	and requires rigorous training	suitable for the analysis of interview transcripts	suitable for the analysis of business reports	dependent
43	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
44	All of the waves listed below are a part of the electromagnetic spectrum except	sound waves	X rays	gamma rays	radio waves	sound waves
45	When light enters from vacuum in to glass, it's velocity	varies depending on mass of glass	decreases	remains same	increases	decreases
46	Speed of electromagnetic radiation is independent of	wavelength	amplitude	time period	frequency	frequency
47	Speed at which stars and galaxies are moving	blue shift	yellow shift	red shift	orange shift	red shift

	away from us is determined by phenomena of					
48	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 field	along the electric field
49	Speed of electromagnetic waves is same	for all wavelengths	in all media	for all intensities	for all frequencies	for all intensities
50	Electromagnetic waves ware transverse in nature is evident by:	polarization	interference	reflection	diffraction	polarization
51	Electromagnetic waves do not transport:	energy	charge	momentum	information	charge
52	Electromagnetic waves are produced by:	accelerated charged particle	deaccelerated charged particle	charge in uniform motion	none of the above	accelerated charged particle
53	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
54	Which of the following	X-rays	Visible rays	UV-rays	IR-rays	IR-rays

	rays is emitted by a human body?					
55	Who produced the electromagnetic waves first ?	Marconi	Maxwell	J.C. Bose	Hertz	Hertz
56	According to Maxwell, a changing electric field produces	emf	Electric current	magnetic field	radiation pressure	magnetic field
57	Which of the following have zero average value in a plane electromagnetic wave?	Electric energy	Magnetic energy	Electric field	None of these	Electric field
58	Speed of electromagnetic wave is the same	for all wavelengths	in all media	for all intensities	for all frequencies	for all intensities
59	Electromagnetic waves are produced by	a static charge	a moving charge	an accelerating charge	charge less particles	an accelerating charge
60	Systematic errors occur due to	overuse of instruments	careful usage of instruments	human sight	instrument quality	over use of instruments

UNIT II

Syllabus

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. Infrared spectrometer 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 to vibrational 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 the photon, 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 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: Light Source

Getting the colours necessary for optical spectroscopy Amplified pulses from Ti: Sapphire lasers can be readily used for time-resolved spectroscopy experiments. 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 (around 800 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 nm wavelength 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 is comparable 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 right colours for time-resolved spectroscopy.

Dispersion

Light dispersion is the phenomenon of linear optics, but it is important for the further discussion, 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 blue photons (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 arrive earlier, and the blue ones – later. Such pulse is called chirped and resembles the pulses in the cavity of the regenerative amplifier. Dispersion broadening of the ultra short 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 much as possible, optical components of femto second beam lines (like lenses, wave plates or polarisers) are made as thin as possible. This also prevents transporting femto second pulses using optical fibers: in several meters of fiber a femto second pulse will stretch to tens of picoseconds, and different frequency components will scatter in time killing any dreams of good time resolution.

Interpretation of spectrum (qualitative, mixtures, resolution)

Interpretation of the IR spectrum of an unknown compound is an art that requires experience and practice. The more spectra you examine, the easier it will become to recognize the absorption due to an O-H group and to differentiate between that band and one that results from an N-H group. The following table summarizes the positions of the various absorption bands that have been discussed so far. On the basis of these absorptions, it is usually possible to determine the nature of the functional group that is present in the compound whose spectrum is being considered. Many functional groups require the presence of several characteristic absorptions, whereas the absence of a band in a particular region of the spectrum can often be used to eliminate the presence of a particular group.

Infrared spectra of compounds belonging to each of the major functional group classes are provided in the figures in this chapter. Each figure has a summary of the important absorption bands for that functional group. Some of these figures are found on previous pages; others appear on later pages. Table 2 provides a list of the important functional groups and the figure(s) that show IR spectra of typical compounds containing that functional group. Now that all of the important absorption bands have been discussed, this is a good time for you to examine all of these spectra to become more familiar with the combination of bands caused by each functional group.

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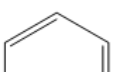
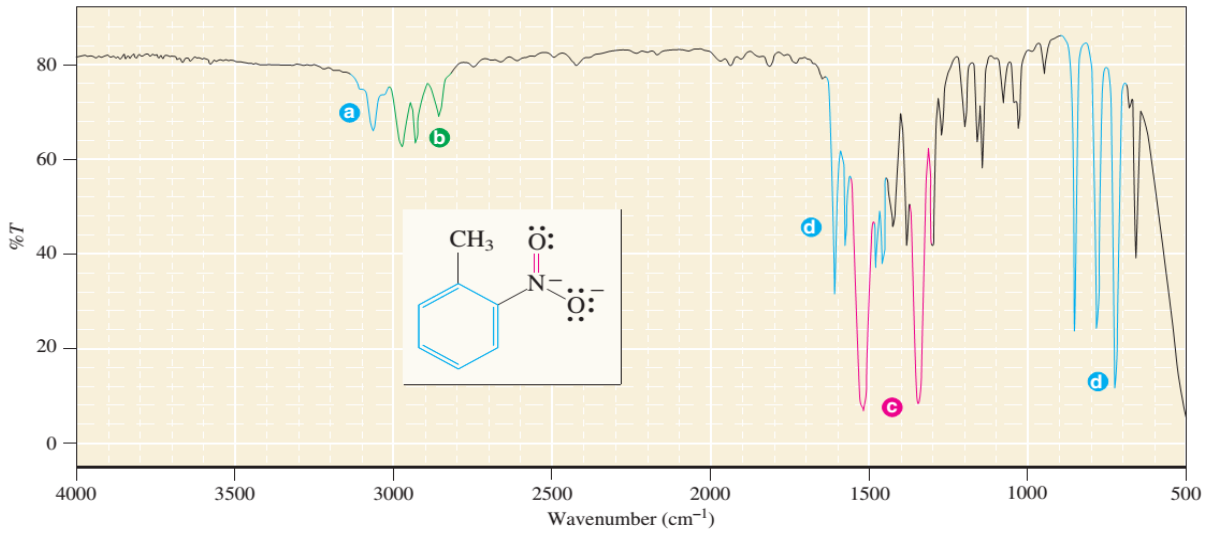
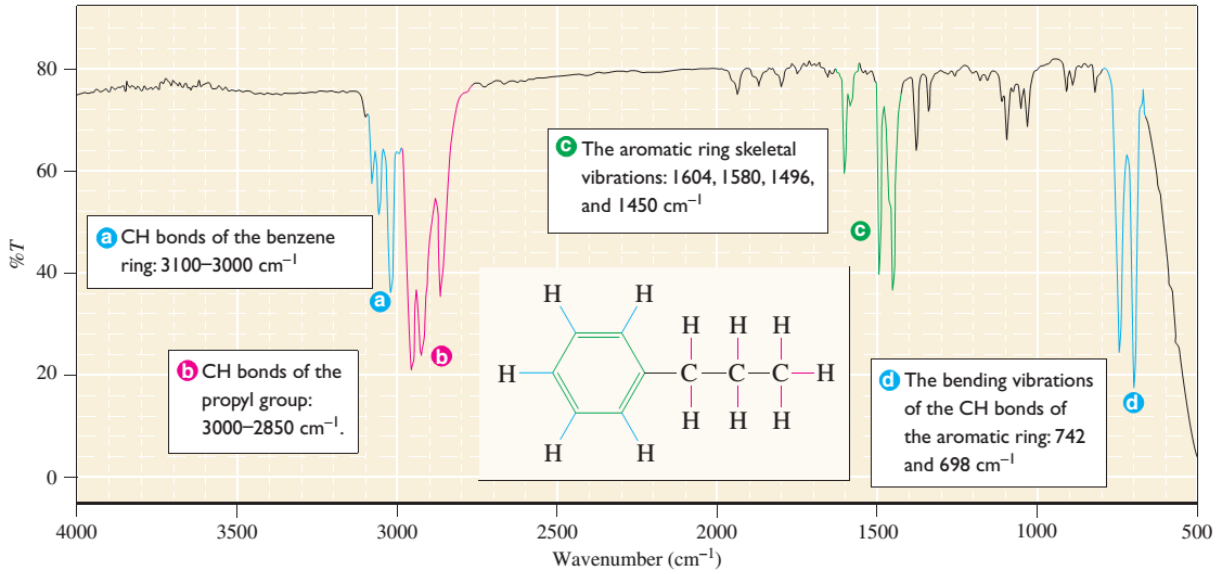
Position (cm^{-1})	Group	Comments
3550–3200	—O—H	Strong intensity, very broad band
3400–3250	—N—H	Weaker intensity and less broad than O—H; NH_2 shows two bands, NH shows one
3300	≡C—H	Sharp, C is *sp* hybridized
3100–3000	=C—H	C is sp^2 hybridized
3000–2850	—C—H	C is sp^3 hybridized; 3000 cm^{-1} is a convenient dividing line between this type of C—H bond and the preceding type
2830–2700	—C(=O)—H	Two bands
2260–2200	—C≡N	Medium intensity
2150–2100	—C≡C—	Weak intensity
1820–1650	—C(=O)—	Strong intensity, exact position depends on substituents; see Table 13.1
1660–1640	C=C	Often weak intensity
1600–1450		Four bands of variable intensity
1550 and 1380	—NO_2	Two strong intensity bands
1300–1000	—C—O—	Strong intensity
900–675		Strong intensity

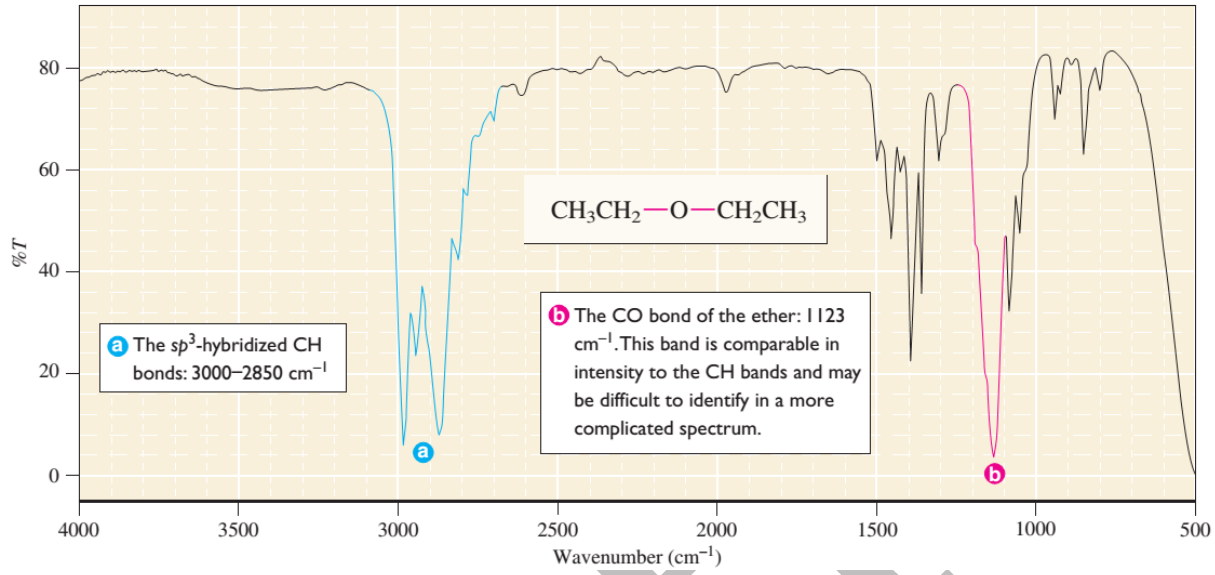


Two types of CH bonds can be detected in this spectrum:
- a sp^2-hybridized CH bonds (3100–3000 cm^{-1})
- b sp^3-hybridized CH bonds (3000–2850 cm^{-1})
- c The absorption bands due to the nitro group: 1523 and 1347 cm^{-1}. They are at lower wavenumbers than usual because the nitro group is conjugated with the benzene ring.
- d The aromatic ring is responsible for the absorptions due to the sp^2 CH stretching vibrations (3100 – 3000 cm^{-1}); the ring skeletal vibrations at 1612, 1577, 1500, and 1461 cm^{-1}; and the CH bending vibrations at 859, 788, and 728 cm^{-1}.

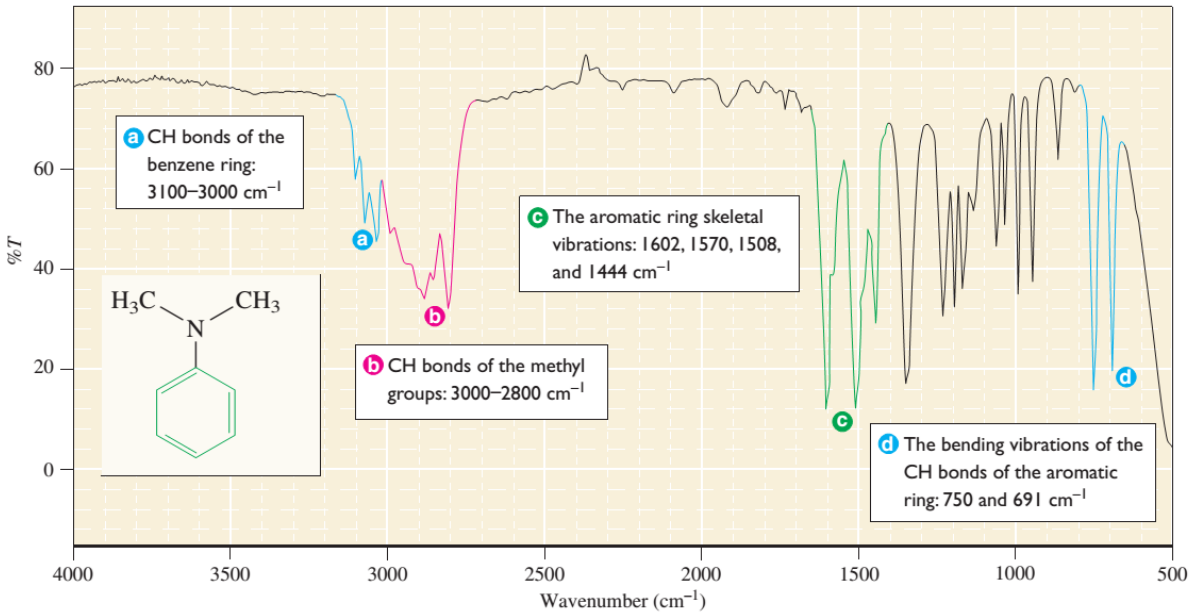
Nitro compounds: Compounds containing nitro groups are identified by the appearance of two strong bands near 1550 and 1380 cm^{-1}. These absorptions appear at lower wavenumbers if the nitro group is conjugated with a benzene ring.



Arenes: Arenes that have hydrogens on the aromatic ring show absorptions in the 3100 and 3000 cm^{-1} region. They also show four absorptions of variable intensity near 1600, 1580, 1500, and 1450 cm^{-1} due to skeletal vibrations of the benzene ring. In addition, most aromatic compounds have at least one strong absorption in the 900 and 675 cm^{-1} region due to bending vibrations of the CH bonds of the aromatic ring. Although care must be used in assigning some of these bands because they occur in the fingerprint region, the presence of all of them provides evidence that the compound contains an aromatic ring.

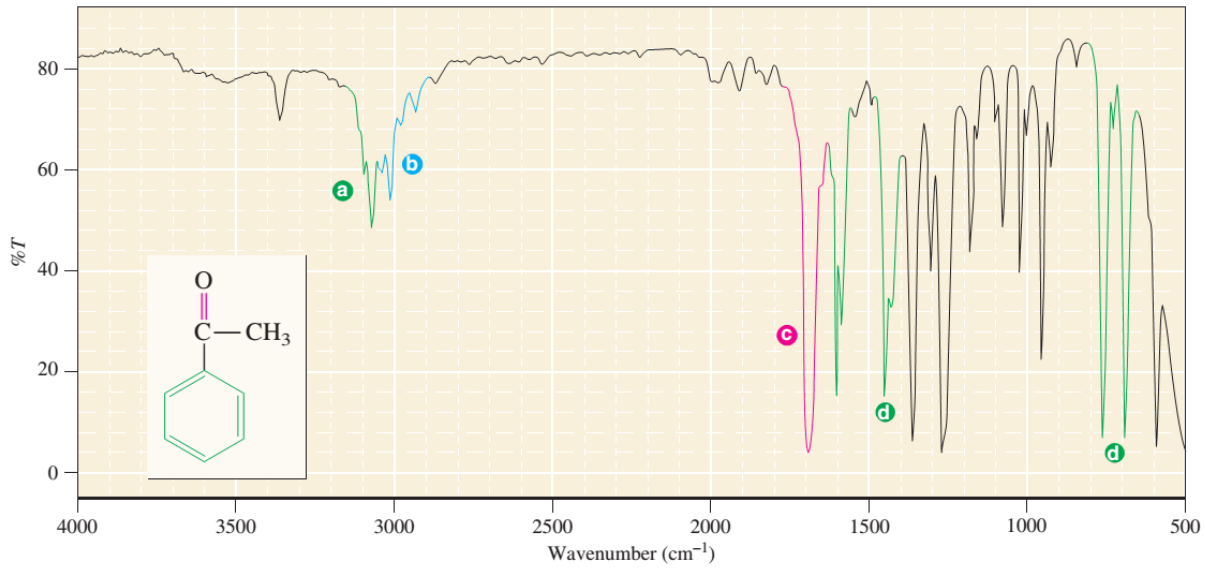


Ethers: Ethers usually have one or more strong bands in the 1300–1000 cm^{-1} region due to the CO bond. As was the case with alcohols, this band is often difficult to identify because it occurs in the fingerprint region with many other absorptions. If oxygen is known to be present from the formula of a compound, the presence of an ether can be inferred by the absence of absorptions due to OH, carbonyl, or other possible oxygen-containing functional groups.



Only the alkyl groups and the aromatic ring can be detected in the spectrum of this tertiary amine. The absorptions due to the aromatic ring skeletal vibrations are stronger than usual, suggesting that the ring is substituted with a polar substituent.

Tertiary amines: Tertiary amines do not have a NH bond, so there is no evidence for the amine group in the 3400–3250 cm^{-1} region. Because the CN bond-stretching vibration is difficult to assign in the fingerprint region, tertiary amines are not readily identified from their IR spectra. Chemical tests are helpful in such cases.



a CH bonds of the benzene ring: 3100–3000 cm^{-1}

b CH bonds of the methyl group: 3000–2850 cm^{-1}

c The carbonyl group: 1685 cm^{-1}. This is the position predicted for a carbonyl group of a ketone that is conjugated to an aromatic ring. 1715 – (20 to 40) = 1695 to 1675 cm^{-1}.

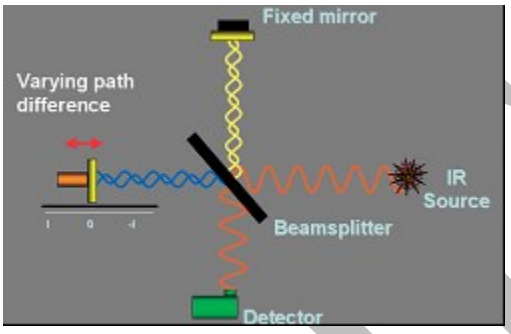
d The aromatic ring vibrations: 1599, 1579, 1449, 760, and 691 cm^{-1}. Note that the band around 1500 cm^{-1} is very weak in this spectrum.

Ketones: The carbonyl of a ketone has an absorption band near 1715 cm^{-1}. This band is shifted to lower wavenumbers if the carbonyl group is conjugated, and it is shifted to higher wavenumbers if the carbonyl group is part of a five-membered ring. Ketones have no other characteristic bands and often can only be distinguished from the other carbonyl-containing functional groups by the absence of the bands required for those other groups. Again, chemical tests can be very useful in confirming the presence of a ketone.

Fourier Transform Instruments (FT-IR)

An FT-IR instrument relies upon interference of various frequencies of light to collect a spectrum. The spectrometer consists of a source, beam splitter, two mirrors, a laser and a detector; the beam splitter and mirrors are collectively called the interferometer. The assembled whole is shown in Figure. The IR light from the source strikes the beam splitter, which produces two beams of roughly the same intensity. One beam strikes a fixed mirror and returns, while the

second strikes a moving mirror. A laser parallels the IR light, and also goes through the interferometer.



FT-IR Advantages

The modern FT-IR spectrometer has three major advantages over a typical dispersive infrared spectrometer. These advantages are the reason FT-IR is now the standard tool, having largely displaced dispersive instruments by the mid-1980.

Multiplex Advantage

As seen from the operations description above, the interferometer does not separate energy into individual frequencies for measurement. Each point in the interferogram contains information from each wavelength of light being measured. Every stroke of the moving mirror equals one scan of the entire infrared spectrum, and individual scans can be combined to allow signal averaging. In the dispersive instrument, every wavelength across the spectrum must be measured individually as the grating scans. This can be a slow process, and typically only one spectral scan of the sample is made in a dispersive instrument. The multiplex advantage means many scans can be completed and averaged on an FT-IR in a shorter time than one scan on most dispersive instruments.

Throughput Advantage

The FT-IR instrument does not limit the amount of light reaching the detector using a slit. The Thermo Scientific FT-IR spectrometers also use the fewest number of mirrors necessary,

which means less reflective losses occur. Overall, these mean more energy reaches the sample and hence the detector in an FT-IR spectrometer than in a dispersive spectrometer. The higher signal leads to an improved signal-to-noise ratio of the FT-IR. Higher signal-to-noise means that the sensitivity of the instrument for small absorptions will be greater, and details in a sample spectrum will be clearer and more distinguishable. The IR analysis of proteins is a good example – this is almost impossible in a classical dispersive instrument, while it is a fairly routine measurement for FT-IR.

The slit in a dispersive instrument becomes even more of a limitation as the spectral resolution desired increases. To see a narrower range, the slit closes down, choking the amount of light passing the instrument, resulting in poor quality spectra for all except ideal samples. Further, high resolution also implies a very slow scan speed, so it can take long times to collect a high resolution dispersive spectrum. To attain ultra-high resolution, the IR also uses an aperture, but the limitation of the light is not nearly so severe, and the multiplex advantage quickly gains back the loss.

Precision Advantage

An FT-IR spectrometer uses a laser to control the velocity of the moving mirror and to time the collection of data points throughout the mirror stroke. This laser is also used as a reference signal within the instrument. The interferogram of the laser is a constant sine-wave, which provides the reference for both precision and accuracy of the infrared spectrometer. Well-designed FT-IR spectrometers rely exclusively on this reference laser, rather than any external reference sample. In this case, spectra collected with an FT-IR spectrometer can be compared with confidence whether they were collected five minutes or five years apart. This capability is not available on a dispersive infrared system, or any system requiring external calibration standards.

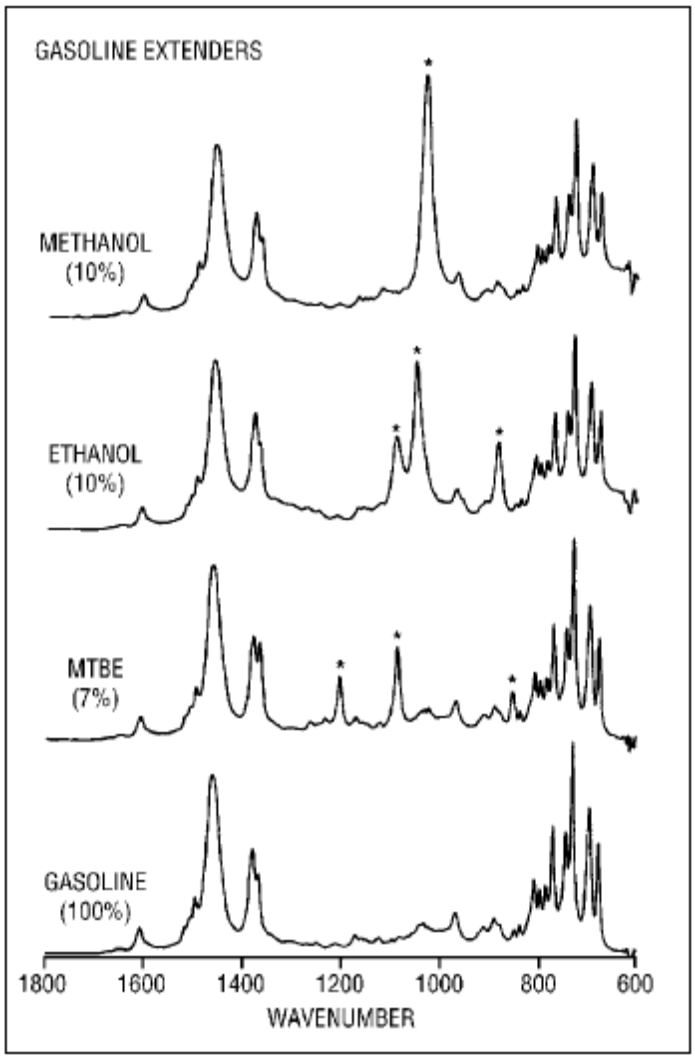
Fourier transforms infrared (FT-IR) spectrometers for use in quality assurance (QA)

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 industrial environments 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 determinations has 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 the full 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 are easily 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.

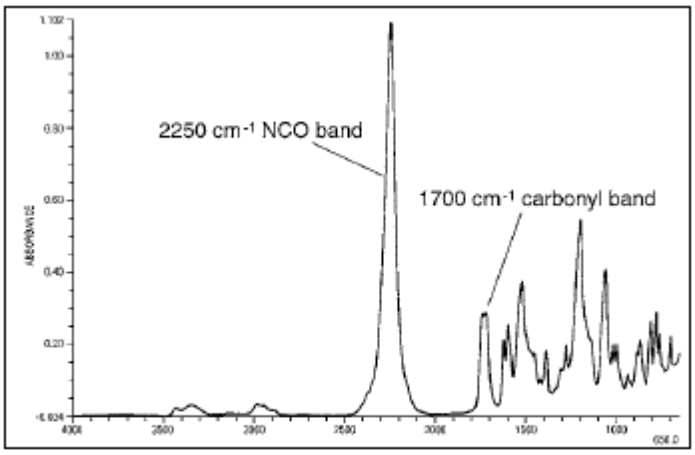


Figure 2: Toluene diisocyanate pre-polymer spectrum obtained on a horizontal ATR accessory. Bands used for quantitative determination are indicated.

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 scatter analysis (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 reference method 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 done using FT-IR.

Fluorescence Excitation and Emission

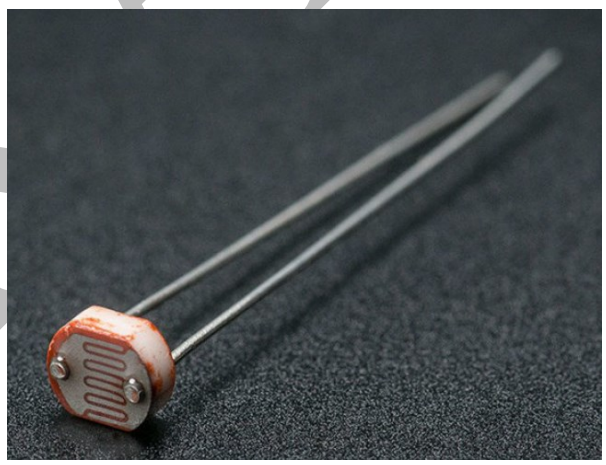
Fluorescence is a member of the ubiquitous luminescence family of processes in which susceptible molecules emit light from electronically excited states created by either a physical (for example, absorption of light), mechanical (friction), or chemical mechanism. Generation of luminescence through excitation of a molecule by ultraviolet or visible light photons is a phenomenon termed photoluminescence, which is formally divided into two categories, fluorescence and phosphorescence, depending upon the electronic configuration of the excited 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 after a 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. Fluorochromes that 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 are divided into two broad classes, termed intrinsic and extrinsic. Intrinsic fluorophores, such as aromatic amino acids, neurotransmitters, porphyrins, and green fluorescent protein, are those that occur naturally. Extrinsic fluorophores are synthetic dyes or modified biochemical's that are added to a specimen to produce fluorescence with specific spectral properties.

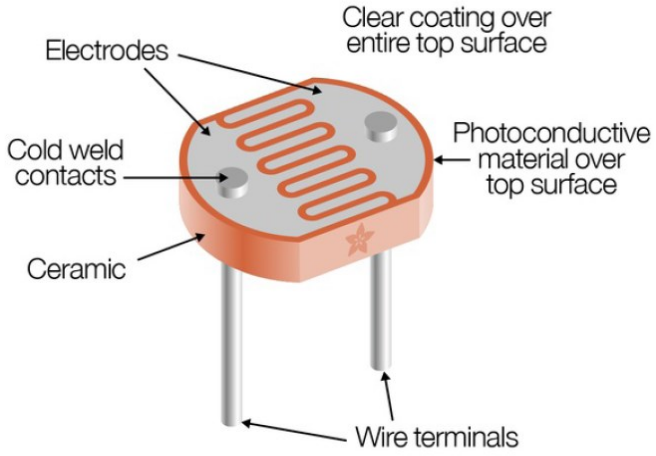
Detection of signal

Photocells

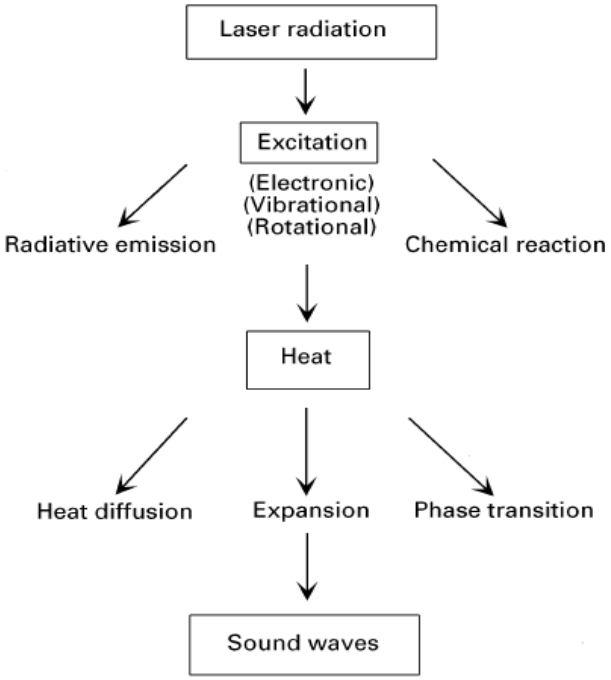
Photocells are sensors that allow you to detect light. They are small, inexpensive, low-power, easy to use and don't wear out. For that reason they often appear in toys, gadgets and appliances. They are often referred to as CdS cells (they are made of Cadmium-Sulfide), light-dependent resistors (LDR),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



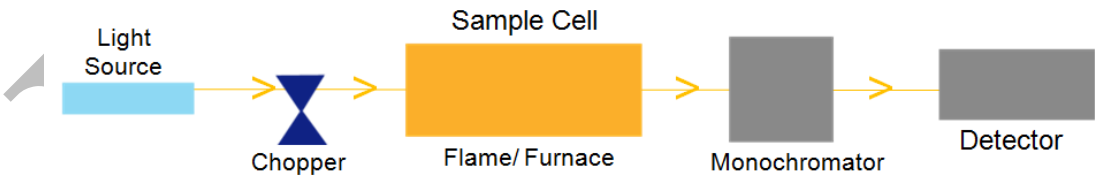
Photocells are basically a resistor that changes its resistive value (in ohms Ω) depending on how much light is shining onto the squiggly face. They are very low cost, easy to get in many sizes and specifications, but are very inaccurate. Each photocell sensor will act a little differently than the other, 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 has the most light hitting it", photocells can be a good choice!



Photoaccoustic



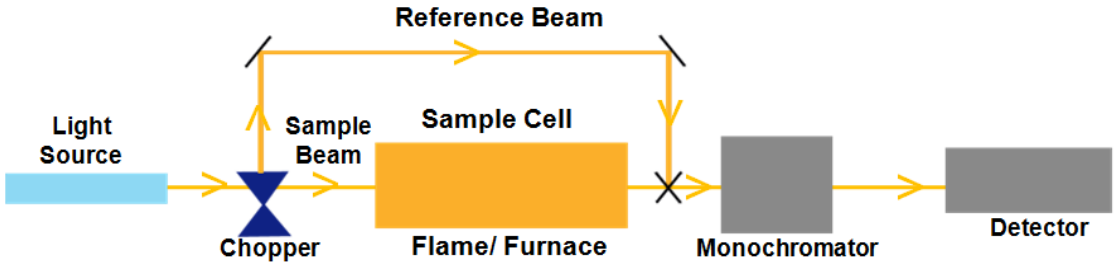
Single Beam spectrometer



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 disk suspended 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. Not shown in the animations that accompany this page is the spectrophotometer's monochromator which selects the analytical wavelength from the source lamp's broad spectrum containing many wavelengths of light. The analytical wavelength is chosen based on the absorbance characteristics of the analyte. Monochromator are instruments whose sole purpose is to allow polychromatic (that is many wavelengths containing) 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. This exiting, well-shaped, narrowly-defined beam now contains a small region of the electromagnetic spectrum. The spread, or band-pass, of the wavelengths depends on the slit settings of the monochromator (usually adjustable) and the quality of the light dispersing element in the monochromator (usually a grating in most modern monochromators). In the instrumental design shown schematically in the animation and below here the source lamp's beam is alternately diverted at right angles by a rotating disk with three 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 though the disk it shines directly into the sample cell. If the

sample is a liquid then this cell contains a cuvette and is made of a transparent material, such as quartz, that does not absorb light in the spectral region of interest. The analyte is dissolved in a solvent held in the cuvette. When the source light is reflected at 90 degrees by the rotating disk instead of striking the sample cuvette it passes through a cuvette in the reference cell which contains ONLY solvent. During the third sequence, when the black sector blocks the source beam, NO light passes through the disk. And as can be seen below, therefore no light arrives at the photo transducer. This part of the cycle is used for the computer to digitize and measure the dark current--the amount of light produced by the photo transducer circuit when no light impinges on the photo transducer. The dark current can be subtracted from the overall light measurements made by the system. After travelling through either the sample cell or reference cell the light that was not absorbed--by far, most of the beam-- is directed onto the photo transducer or light detector. This component converts the arrival of photons into an electrical signal. By the way, the light path through the spectrophotometer need not be in a straight line since 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 beam generate alternating electrical photo transducer signals. A computer, sampling those signals, can now determine the analyte absorption in two ways. Some instruments merely subtract the sample beam signal's digitized light intensity from that of the reference beam. The difference is a measure of the amount of light absorbed by the analyte. Since photo transducers-based system are relatively poor at measuring the absolute difference in two different light intensities especially if that difference is large, light absorbances determined in this manner can contain unacceptable amounts of error. Photo transducer are, however, good at generating signals from light intensities that are close together in intensity; therefore, an alternate means of determining the analyte absorption is used by some instruments: Some spectrophotometer design uses the digitized reference-minus-sample signal difference to activate

a servor motor connected to the computer and a device called an optical wedge. The servor motor slides the optical wedge into the brighter reference beam's path somewhere after the reference cell but before the photo transducer. Remember that since the reference cell does not have any light absorbing analyte, the light exiting the reference cell will always be brighter than that from the sample cell even if the solvent itself absorbs some at the analyte wavelength since both cuvettes contain solvent. The optical wedge is made of a material that absorbs light so that the more the wedge intersects the reference beam the more of that beam will be absorbed by the wedge and the less will be the difference between the sample and reference signals. The wedge is automatically fed into the reference beam until the reference and sample beam signals are of exactly identical intensity as measured by the photo transducer (remember the system is good at this). When the signals are equal the amount of wedge needed to produce this 0 signal difference is a measure of the analyte absorption. Since the computer controls the wedge it converts wedge position to an absorbance reading of the analyte.

Photodiode array

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

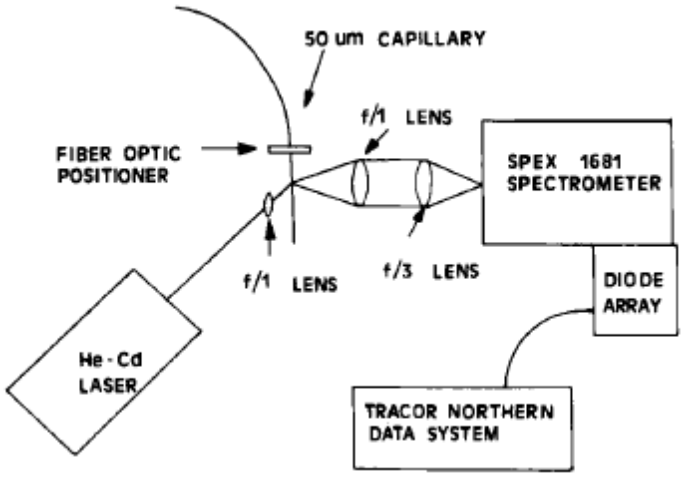
Laser-induced fluorescence detection

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

smaller volume than are possible with arc lamp excitation. The disadvantages are that the wavelengths available from current types of laser sources are rather limited, and that there are possibilities of photo degradation of the analytes caused by the high light intensity.

Fluorometric photodiode array detector

A fluorometric photodiode array detector for CE was developed by Swaile and 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 removing a small section of the capillary polyimide coating near its outlet. Emission was collected at a 90" angle from excitation and collimated by a 4 cm diameter, f / l quartz lens, then focused by a 4 cm diameter, f / 3 quartz lens onto the entrance slits of a spectrometer that dispersed the emission across the diode array. The entrance slit of the spectrometer was set at 1-2 mm, which prevented secular scatter from the sides of the capillary from reaching the photodiode. Detection was accomplished using a photodiode array with 1024 diodes. The diode array was operated at the lowest possible resolution to reduce memory requirement which resulted in a spectral resolution of 4 nm per channel. Calibration data were collected in the histogram mode in order to further reduce memory requirement. Linearity in response over 3 orders of magnitude and detection limits of less than 60fg (10-7 M) were obtained for sodium fluorescein.



Interpretation of Ultraviolet Spectroscopy

Some chromophores and the corresponding transitions

Chromophore an example of compound	Transition	λ_{max} (nm)
H_2O	σ→σ*	183
C-C a C-H, CH_4	σ→σ*	cca 170, 173
C-X, CH_3OH, CH_3NH_2, CH_3I	n→σ*	180-260, 187, 215, 258
C=C, $H_2C=CH_2$	π→π*	160-190, 162
$H_2C=CH-CH=CH_2$	π→π*	217
C=O, H−CH=O	n→π*, π→π*	270, 170-200, 270, 185
$H_2C=CH-CH=O$	n→π*, π→π*	328, 208
C=N	n→σ*, n→π*	190, 300
N=N	n→π*	340
C=S	n→π*	500
NO_2	n→π*	420-450
N=O	n→π*	630-700

Benzene and its derivatives

Compound	λ_{max} (nm)	log ε	λ_{max} (nm)	log ε	λ_{max} (nm)	log ε
benzene	204	3.9	254	2.0	-	-
toluene	207	3.8	261	2.4	-	-
brombenzene	210	3.9	261	2.3	-	-
phenol	211	3.8	270	3.2	-	-
benzaldehyde	250	4.1	280	3.0	320	1.7
acetophenone	246	4.0	280	3.0	320	1.7
benzoic acid	230	4.1	273	3.0	-	-
aniline	230	3.9	280	3.5	-	-
styrene	247	4.0	281	2.0	-	-
cinnamaldehyde	285	4.4	-	-	-	-
cinnamic acid	273	4.3	-	-	-	-
biphenyl	248	4.2	-	-	-	-

Heterocyclic compounds

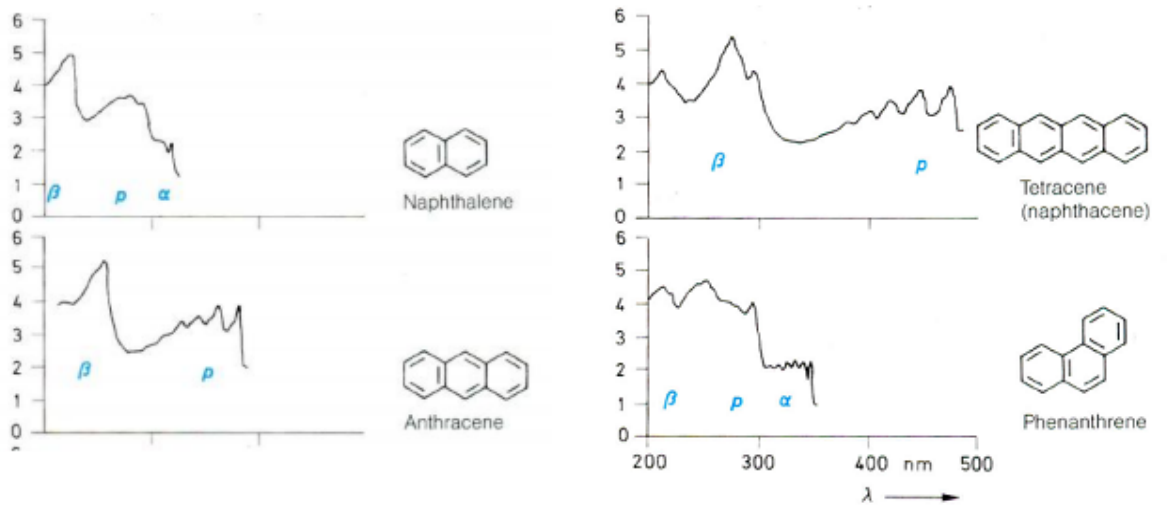
5-Membered

Compound	λ_{max} (nm)	log ε	λ_{max} (nm)	log ε
furan	200	4.0	-	-
2-furaldehyde	227	3.3	272	4.1
2-acetylfuran	225	3.4	269	4.1
pyrrole	210	4.2	240	2.5
2-acetylpyrrole	250	3.6	287	4.2
thiophene	-	-	235	3.7
2-acetylthiophene	260	3.9	285	3.7
thiazole	-	-	240	3.6

6-Membered

Compound	λ_{max} (nm)	log ε	λ_{max} (nm)	log ε	λ_{max} (nm)	log ε
Pyridine	195	-	250	3.3	-	-
2-Picoline	-	-	262	3.4	-	-
Pyrazine	-	-	260	3.7	-	-
Quinoline	227	4.6	275	3.7	313	3.4
Isoquinoline	218	4.9	262	3.6	317	3.5
Pyrimidine	-	-	-	-	343	3.3

Polycyclic aromatic hydrocarbons



POSSIBLE QUESTIONS
UNIT-II
PART-A (20 MARKS)
(Q.NO 1 TO 20 Online Examination)

PART-B (2 MARKS)

1. Define Infra red spectroscopy
2. What is the major requirement for infra red spectroscopy?
3. Why is methanol is good solvent for UV and not for IR determination
4. Write note on FT-IR source of light
5. What type of detectors used in FT-IR and UV spectroscopy?
6. Difference between absorption and emission spectroscopy

PART-C (6 MARKS)

1. How will you distinguish between an aliphatic and aromatic compound in FT-IR
2. Distinguish between the following pairs of compounds with help of infra-red technique
   a) Ethanol and dimethyl ether
   b) Propanal and Propanone
   c) Cis and trans Cinnamic acid
   d) Ethanol from ethyl amine
2. Write short note on finger print region?
3. Write note on advantages of FT-IR?
4. Write note on photocells, photomultipliers, and diode arrays
5. Write about photoaccoustic and fluorescent tags

Unit-II-Multiple choice Questions

S. No	Question	Option I	Option II	Option III	Option IV	Answer
1.	Which one of the following represents the weakest interaction between two species?	Hydrogen bond	Disulfide bond	Ionic bond	Dispersion force	Dispersion force
2.	Which one of the following elements is least likely to participate in a hydrogen bond?	O	F	C	N	S
3.	Which one of the following terms describes a positive and negative charge, which are separated in space within a molecule?	Salt bridge	Polar bond	Dipole	van der Waals interaction	Dipole
4.	Which of the following binding interactions is likely to be the most important initial interaction when a drug enters a binding site?	van der Waals interactions	hydrogen bond	ionic	induced dipole-dipole interactions	ionic
5.	Which of the following	Aromatic ring	Ketone	Alcohol	Alkene	Ketone

	functional groups is most likely to participate in a dipole-dipole interaction?					
6.	The interaction between highly electron deficient hydrogen and highly electronegative atom is called	Covalent bond	ionic bond	dipole-dipole interaction	hydrogen bond	hydrogen bond
7.	When partial positive end of one molecule is attracted weakly to partial negative end then force between them is	Covalent bond	ionic bond	dipole-dipole interaction	hydrogen bond	dipole-dipole interaction
8.	The strength of intermolecular forces from ionic or covalent bond is	weaker	stronger	equal	zero	weaker
9.	How is the wavelength controlled in an FTIR spectrometer?	By a Michelson Interferometer	By a computer	By a laser	By calibration with a standard sample	By a laser
10.	What type of technique is FTIR spectroscopy?	A dispersive technique	An emission technique	An absorbance technique	A UV-Vis technique	An absorbance technique
11.	How is the detector on the Mattson RS/1 FTIR	With water	With liquid nitrogen	With a fan	room temperature detector	room temperature detector

	spectrometer cooled?					
12.	What does the spectrum of Nitrogen(N2) look like?	The same as that of air	It has only p- and r-branches	no dipole change for vibration of N2	The same as that of carbon monoxide	no dipole change for vibration of N2
13.	What are the selection rules for FTIR absorption?	Absorption only occurs for symmetrical molecules	Absorption only occurs with a dipole change	Absorption requires an odd vibrational quantum number	Absorption only occurs with a dipole change and new quantum level	Absorption only occurs with a dipole change and new quantum level
14.	What occurs when the moving mirror in an FTIR spectrometer is the same distance from the beam splitter as the static mirror?	Constructive interference	Destructive interference	Radio interference	The spectrum is measured	Constructive interference
15.	Which infrared technique can measure two spectra at once?	FTIR spectroscopy	FTIR-ATR spectroscopy	Dispersive infrared spectroscopy	FTIR microscopy	Dispersive infrared spectroscopy
16.	In what region of the spectrum does infrared radiation occur?	At the low-energy end	Between the visible and ultraviolet regions	Between the visible and microwave regions	Between the visible and x-ray regions	Between the visible and microwave regions

17.	What occurs when a molecule absorbs infrared radiation?	It warms up	It flies around	It vibrates faster	It emits light	It vibrates faster
18.	What is the Follett advantage of FTIR?	Increased throughput	FTIR is more infrared efficient than dispersive spectroscopy.	All spectral elements measured at the same time	FTIR is a simpler technique, and therefore easier to use	All spectral elements measured at the same time
19.	What is the Jacqui not Advantage?	High throughput.	Errors spread across the spectrum.	Highly accurate measurements.	Another way of saying multiplex advantage	High throughput.
20.	Which technique can measure two spectra at once?	Dispersive spectroscopy.	FTIR spectroscopy	UV spectroscopy	NMR spectroscopy	Dispersive spectroscopy.
21.	What is the Connes advantage?	Higher optical throughput of FTIR instruments	Greater spectral bandwidth	Highly accurate frequencies	High S/N ratio	Highly accurate frequencies
22.	What is the optimum pathlength for polymer samples in transmission mode?	10 micrometres	40 micrometres	30 micrometres	50 micrometres	40 micrometres
23.	What are the units most commonly used to identify bands in FTIR	Wavenumbers(cm-1)	Wavelength(m)	Frequency(Hz)	Wavelength(micrometres)	Wavenumbers(cm-1)

	spectroscopy?					
24.	What are interference fringes caused by?	What are interference fringes caused by?	Gasses in the atmosphere that absorb infrared	Reflection of infrared by the sample.	Impurities in the sample	Reflection of infrared by the sample.
25.	What is the correct way to display spectra?	In absorbance.	In transmission.	Either absorbance or transmission	As raw dat	Either absorbance or transmission
26.	If you measured a spectrum and it was saturated, what could you do to improve the spectrum?	Reduce iris setting OR use a thinner sample	Reduce the iris setting	Scan for a shorter period of time.	Increase iris setting	Reduce iris setting OR use a thinner sample
27.	Which of the following wavelength ranges is associated with UV spectroscopy?	0.8 - 500µm	400 - 100nm	380 - 750nm	0.01 - 10nm	400 - 100nm
28.	Which of the following compounds does not absorb light in the UV/visible spectrum?	Aspirin	Paracetamol	Chloral hydrate	Phenobarbitone	Chloral hydrate
29.	In infrared spectroscopy which frequency range is known as the fingerprint	400 - 1400cm-1	1400 - 900cm-1	900 - 600cm-1	600 - 250cm-1	1400 - 900cm-1

	region?					
30.	In which region of the infrared spectrum would you expect to find a peak characteristic of a triple bond stretch?	4000 - 3000cm-1	2500 -2000cm-1	2000 - 1500cm-1	500 - 750cm-1	2500 - 2000cm-1
31.	Which of the following techniques would be most useful to identify and quantify the presence of a known impurity in a drug substance?	NMR	MS	IR	HPLC	HPLC
32.	In what acoustic frequency range works AE testing usually?	10 Hz - 100 KHz	20 KHz to 1 MHz	20 KHz to 100 KHz	20 KHz to 10 MHz	20 KHz to 1 MHz
33.	What generates the acoustic waves?	the transducer	the crack	microwave	Ultra wave	the crack
34.	How many transducers are used to record the acoustic waves	One and more transducers	Always one transducer	Four transducers	three transducers	One and more transducers
35.	What kind of signal is generated by a crack	shock wave signal	continuous signal	burst signal	UV signal	burst signal
36.	What is a guard sensor	to discriminate from sources	to localize the position of an	to discriminate from sources	scattering emission	to discriminate from sources

		originating outside the area of interest	acoustic emission source	originating inside the area of interest		originating outside the area of interest
37.	What did Hsu-Nielsen invent	an acoustic emission transducer	an aid to simulate an acoustic emission event	a signal filter technique	a signal filter transducer	an aid to simulate an acoustic emission event
38.	The most important region in IR is	15μ-200 μ	2.5 μ-15 μ	0.8 μ-2.5μ	20μ–50μ	2.5 μ-15 μ
39.	Position of C—O stretching band for primary alcohol occur at -----in IR	1050cm-1	1500cm-1	1800cm-1	200cm-1	1050cm-1
40.	The O-H stretching of Phenol exhibit a strong broad band in the range in IR	3600-3200cm-1	1600-1700cm-1	2300-2500cm-1	900-100cm-1	3600-3200cm-1
41.	In IR C—H s stretching vibration occur at the region	1470—1430cm-1	2960-2850cm-1-	1300-1800cm-1	1000-1300cm-1	2960-2850cm-1-
42.	In IR,C=C stretching has the frequency in the region	970—980cm-1	650-610cm-1	1680-1620cm-1	995-985cm-1	1680-1620cm-1
43.	>C=O stretching of	2770-2700 cm-1	1740-1720cm-1	700-970cm-1	3300-3400-1cm	1740-1720cm-1

	aldehydes occur in the region in the IR region.					
44.	The type of H-bonding which give rise to broad lines in IR Technique is ----	Intermolecular	Intramolecular	C-H stretching	C-H bending	Intramolecular
45.	N-H bending vibration for primary amines occurs in the region	700-900cm-1	800-700cm-1	1600-1500cm-1	800-700cm-1	1600-1500cm-1
46.	Which one of the following region in IR is known as functional group region	1300-4000 cm-1	900-1300 cm-1	650-900 cm-1	1000-1300cm-1	1300-4000 cm-1
47.	What will be the C=O stretching band in IR of CH2=CHCOCH3	1700 cm-1	1710 cm-1	1680 cm-1	1780 cm-1	1680 cm-1
48.	Which one of the following statement is false for IR spectroscopy	Conjugation decreases the C=O stretching frequency	higher the frequency if more strained the alicyclic ring containing C=O group	Electron releasing substituents decreases the frequency of C=O band	Electron withdrawing substituents decreases the frequency of C=O band	Electron withdrawing substituents decreases the frequency of C=O band
49.	An alcohol having mol-formula C4H10O gives	1-butanol	2.butanol	2-methyl-2-propanol	isobutanol	2.butanol

	C-O stretching band at1120 cm-1 will be					
50.	The characteristic bond stretching frequency for C-O is about---	104 cm-1	105 cm-1	103 cm-1	102cm-1	103 cm-1
51.	In practical organic chemistry tetramethyl silane is used mainly for--	Making volatile derivatives of alcohols	A spectroscopic standard	A solvent for IR spectra	An antinock in gasolines	A spectroscopic standard
52.	The spectra resulting from changes in vibrational energy levels for the same electronic state fall in which region of the spectrum	microwave	Visible	UV	IR	IR
53.	Which of the following is both IR and microwave active?	Br2	HBr	CS2	CO2	HBr
54.	Which one of the following is microwave inactive?	HCl	HBr	Cl2	NO	Cl2
55.	The rotational constant of a diatomic molecule is	h/4π2I	h2/4π2I	h2/8π2I	h/8π2I	h/8π2I
56.	The degree of degeneracy for a ride rotor in energy	J	2J	2J-1	2J+1	2J+1

	level with quantum number J is--					
57.	From the pure rotation spectrum of HF information can be obtained about	the force constant	The proton spin	The hydrogen bonding	the internuclear distance	the internuclear distance
58.	The characteristic bond stretching frequency for C-O is about---	104 cm-1	105 cm-1	103 cm-1	102cm-1	103 cm-1
59.	Which one of the following statement is false for IR spectroscopy	Conjugation decreases the C=O stretching frequency	higher the frequency if more strained the alicyclic ring containing C=O group	Electron releasing substituents decreases the frequency of C=O band	Electron withdrawing substituents decreases the frequency of C=O band	Electron withdrawing substituents decreases the frequency of C=O band
60.	An alcohol having mol-formula C4H10O gives C-O stretching band at1120 cm-1 will be	1-butanol	2.butanol	2-methyl-2-propanol	isobutanol	2.butanol

UNIT-III
Syllabus

Separation techniques

Chromatography: Gas chromatography, liquid chromatography, supercritical fluids, Importance of column technology (packing, capillaries), Separation based on increasing number of factors (volatility, solubility, interactions with stationary phase, size, electrical field), Detection: simple vs. specific (gas and liquid), Detection as a means of further analysis (use of tags and coupling to IR and MS), Electrophoresis (plates and capillary) and use with DNA analysis.

Immunoassays and DNA techniques

Mass spectroscopy: Making the gaseous molecule into an ion (electron impact, chemical ionization), Making liquids and solids into ions (electrospray, electrical discharge, laser desorption, fast atom bombardment), Separation of ions on basis of mass to charge ratio, Magnetic, Time of flight, Electric quadrupole. Resolution, time and multiple separations, Detection and interpretation (how this is linked to excitation).

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 colours (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.

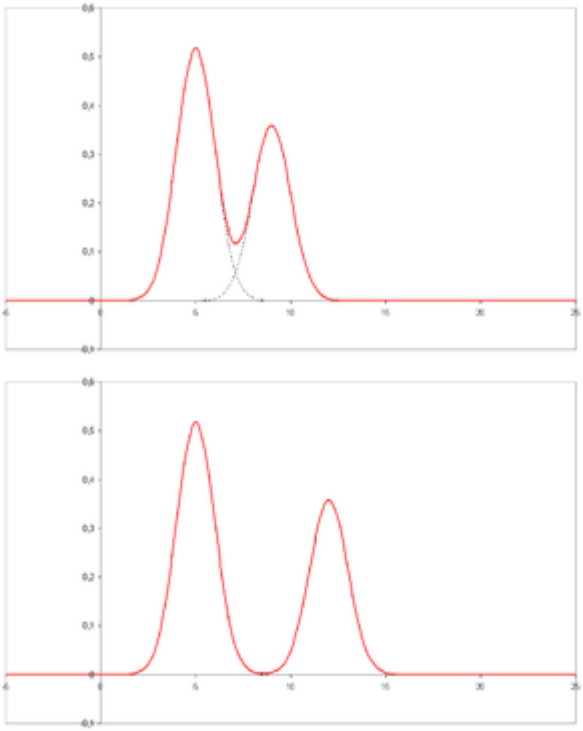
Chromatography technique developed substantially as a result of the work of Archer John Porter Martin and Richard Laurence Millington Synge during the 1940s and 1950s. They established the principles and basic techniques of partition chromatography, and their work encouraged the rapid development of 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.
- **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.



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 physical state of mobile phase

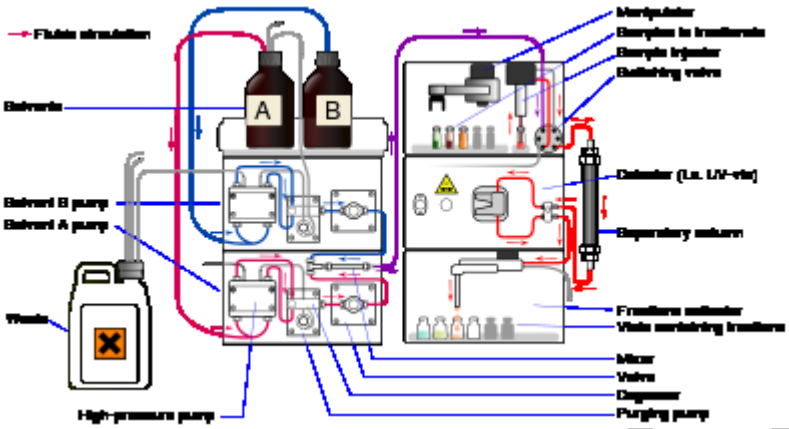
Gas chromatography

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.

Importance of column technology (packing, capillaries)

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).

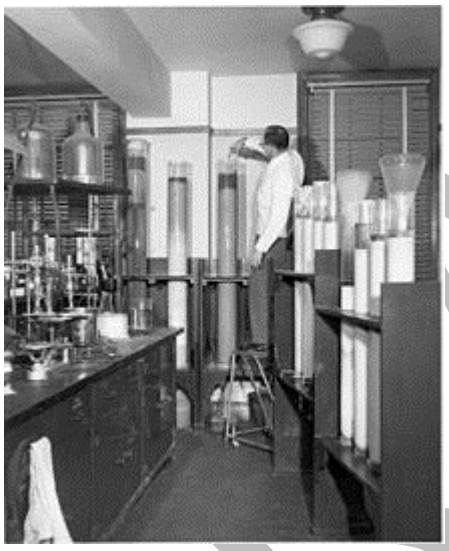
In HPLC the sample is forced by a liquid at high pressure (the mobile phase) through a column that is packed with a stationary phase composed of irregularly or spherically shaped particles, a porous monolithic layer, or a porous membrane. HPLC is historically divided into two different sub-classes based 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).

Separation based on increasing number of factors (volatility, solubility, interactions with stationary phase, size, electrical field)

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.

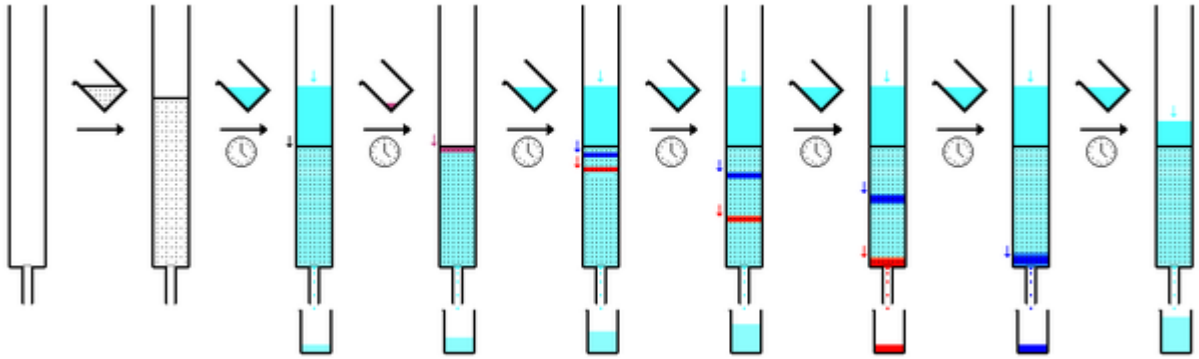
The classical preparative chromatography column is a glass tube with a diameter from 5 mm to 50 mm and a height of 5 cm to 1 m with a tap and some kind of a filter (a glass frit or glass wool plug – to prevent the loss of the stationary phase) at the bottom. Two methods are generally used to prepare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

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.

Mass spectroscopy

The Mass Spectrometer

Making the gaseous molecule into an ion (electron impact, chemical ionization),

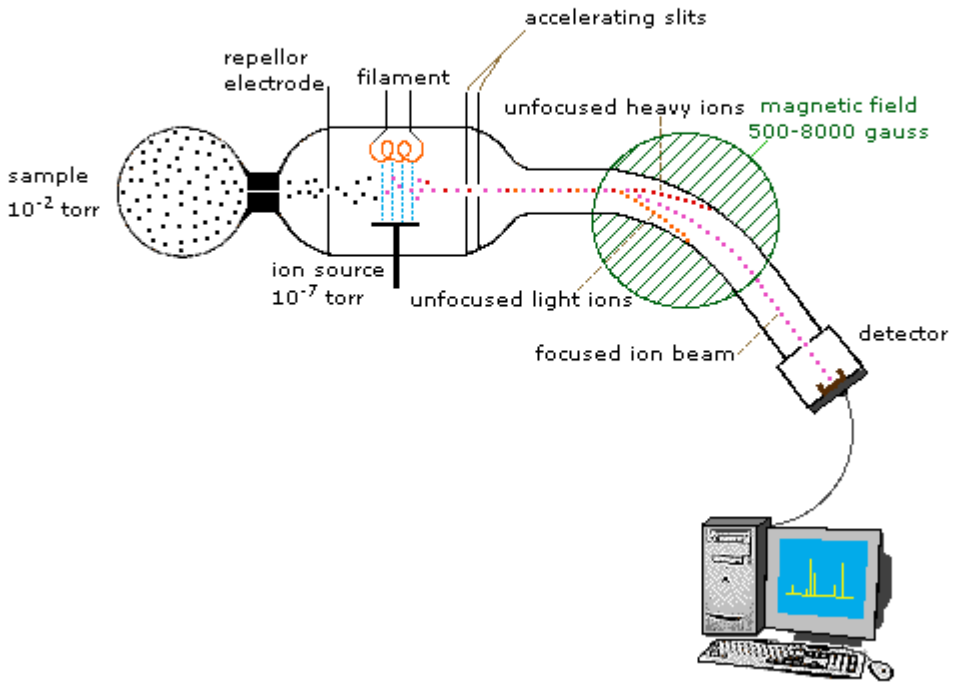
In order to measure the characteristics of individual molecules, a mass spectrometer converts them to ions so that they can be moved about and manipulated by external electric and magnetic fields. The three essential functions of a mass spectrometer, and the associated components, are:

1. A small sample is ionized, usually to cations by loss of an electron. The Ion Source

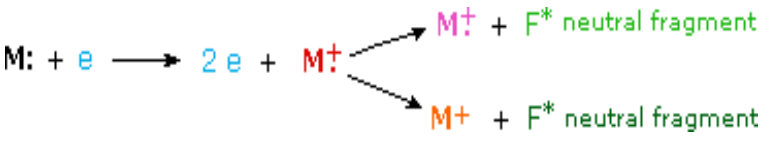
2. The ions are sorted and separated according to their mass and charge. The Mass Analyzer

3. The separated ions are then measured, and the results displayed on a chart. The Detector

Because ions are very reactive and short-lived, their formation and manipulation must be conducted in a vacuum. Atmospheric pressure is around 760 torr (mm of mercury). The pressure under which ions may be handled is roughly 10^{-5} to 10^{-8} torr (less than a billionth of an atmosphere). Each of the three tasks listed above may be accomplished in different ways. In one common procedure, ionization is effected by a high energy beam of electrons, and ion separation is achieved by accelerating and focusing the ions in a beam, which is then bent by an external magnetic field. The ions are then detected electronically and the resulting information is stored and analyzed in a computer. A mass spectrometer operating in this fashion is outlined in the following diagram. The heart of the spectrometer is the ion source. Here molecules of the sample (black dots) are bombarded by electrons (light blue lines) issuing from a heated filament. This is called an **EI** (electron-impact) source. Gases and volatile liquid samples are allowed to leak into the ion source from a reservoir (as shown). Non-volatile solids and liquids may be introduced directly. Cations formed by the electron bombardment (red dots) are pushed away by a charged repeller plate (anions are attracted to it), and accelerated toward other electrodes, having slits through which the ions pass as a beam. Some of these ions fragment into smaller cations and neutral fragments. A perpendicular magnetic field deflects the ion beam in an arc whose radius is inversely proportional to the mass of each ion. Lighter ions are deflected more than heavier ions. By varying the strength of the magnetic field, ions of different mass can be focused progressively on a detector fixed at the end of a curved tube (also under a high vacuum).



When a high energy electron collides with a molecule it often ionizes it by knocking away one of the molecular electrons (either bonding or non-bonding). This leaves behind a **molecular ion** (colored red in the following diagram). Residual energy from the collision may cause the molecular ion to fragment into neutral pieces (colored green) and smaller **fragment ions** (colored pink and orange). The molecular ion is a radical cation, but the fragment ions may either be radical cations (pink) or carbocations (orange), depending on the nature of the neutral fragment. An animated display of this ionization process will appear if you click on the ion source of the mass spectrometer diagram.

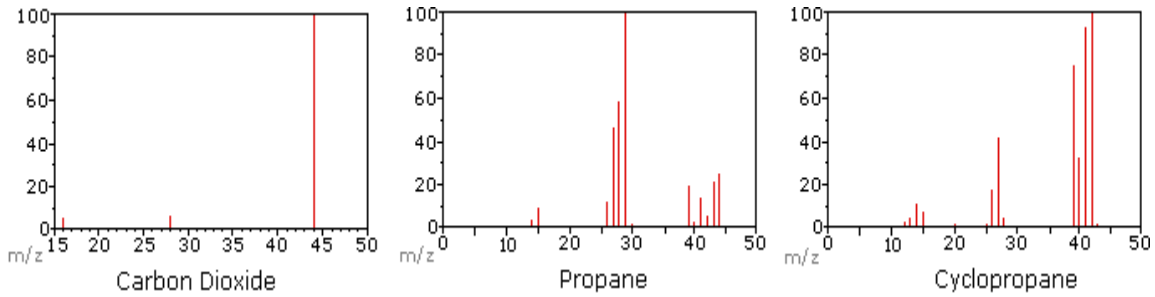


2. The Nature of Mass Spectra

Making liquids and solids into ions (electrospray, electrical discharge, laser desorption, fast atom bombardment)

A mass spectrum will usually be presented as a vertical bar graph, in which each bar represents an ion having a specific mass-to-charge ratio (m/z) and the length of the bar indicates the relative abundance of the ion. The most intense ion is assigned an abundance of 100, and it is referred to as the **base peak**. Most of the ions formed in a mass spectrometer have a single charge, so the m/z value is equivalent to mass itself. Modern mass spectrometers easily distinguish (resolve) ions differing by only a single atomic mass unit (amu), and thus provide completely accurate values for the molecular mass of a compound. The highest-mass ion in a spectrum is normally considered to be the molecular ion, and lower-mass ions are fragments from the molecular ion, assuming the sample is a single pure compound.

The following diagram displays the mass spectra of three simple gaseous compounds, carbon dioxide, propane and cyclopropane. The molecules of these compounds are similar in size, CO_2 and C_3H_8 both have a nominal mass of 44 amu, and C_3H_6 has a mass of 42 amu. The molecular ion is the strongest ion in the spectra of CO_2 and C_3H_6, and it is moderately strong in propane. The unit mass resolution is readily apparent in these spectra (note the separation of ions having m/z=39, 40, 41 and 42 in the cyclopropane spectrum). Even though these compounds are very similar in size, it is a simple matter to identify them from their individual mass spectra. By clicking on each spectrum in turn, a partial fragmentation analysis and peak assignment will be displayed. Even with simple compounds like these, it should be noted that it is rarely possible to explain the origin of all the fragment ions in a spectrum. Also, the structure of most fragment ions is seldom known with certainty.



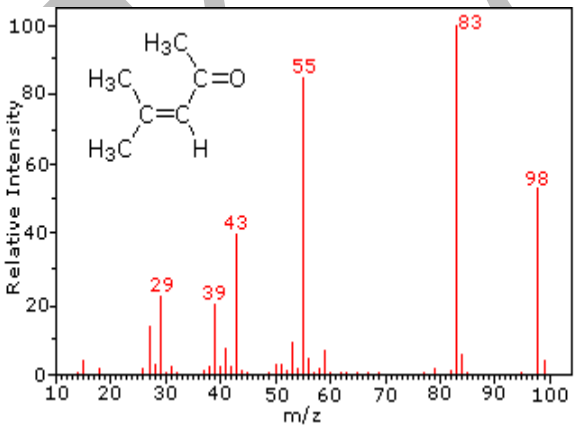
Since a molecule of carbon dioxide is composed of only three atoms, its mass spectrum is very simple. The molecular ion is also the base peak, and the only fragment ions are CO (m/z=28) and O (m/z=16). The molecular ion of propane also has m/z=44, but it is not the most abundant ion in the spectrum. Cleavage of a carbon-carbon bond gives methyl and ethyl fragments, one of which is a carbocation and the other a radical. Both distributions are observed, but the larger ethyl cation (m/z=29) is the most abundant, possibly because its size affords greater charge dispersal. A similar bond cleavage in cyclopropane does not give two fragments, so the molecular ion is stronger than in propane, and is in fact responsible for the the base peak. Loss of a hydrogen atom, either before or after ring opening, produces the stable allyl cation (m/z=41). The third strongest ion in the spectrum has m/z=39 (C_3H_3). Its structure is uncertain, but two possibilities are shown in the diagram. The small m/z=39 ion in propane and the absence of a m/z=29 ion in cyclopropane are particularly significant in distinguishing these hydrocarbons.

Most stable organic compounds have an even number of total electrons, reflecting the fact that electrons occupy atomic and molecular orbitals in pairs. When a single electron is removed from a molecule to give an ion, the total electron count becomes an odd number, and we refer to such ions as **radical cations**. The molecular ion in a mass spectrum is always a radical cation, but the fragment ions may either be even-electron cations or odd-electron radical cations, depending on the neutral fragment lost. The simplest and most common fragmentations are bond cleavages producing a neutral radical (odd number of electrons) and a cation having an even number of electrons. A less common fragmentation, in which an even-electron neutral fragment is lost, produces an odd-electron radical cation fragment ion.

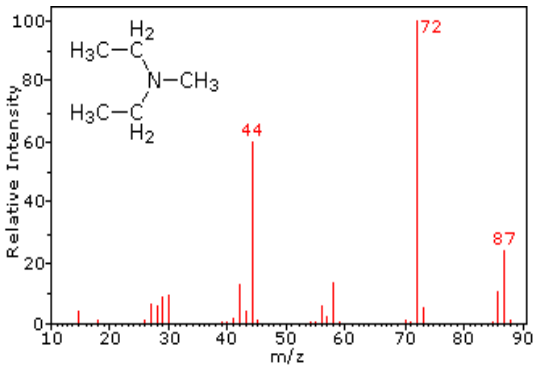
Fragment ions themselves may fragment further. As a rule, odd-electron ions may fragment either to odd or even-electron ions, but even-electron ions fragment only to other even-electron ions. The masses of molecular and fragment ions also reflect the electron count, depending on the number of nitrogen atoms in the species.

Ions with no nitrogen or an even # N atoms	odd-electron ions even-number mass	even-electron ions odd-number mass
Ions having an odd # N atoms	odd-electron ions odd-number mass	even-electron ions even-number mass

This distinction is illustrated nicely by the following two examples. The unsaturated ketone, 4-methyl-3-pentene-2-one, on the left has no nitrogen so the mass of the molecular ion (m/z = 98) is an even number. Most of the fragment ions have odd-numbered masses, and therefore are even-electron cations. Diethylmethylamine, on the other hand, has one nitrogen and its molecular mass (m/z = 87) is an odd number. A majority of the fragment ions have even-numbered masses (ions at m/z = 30, 42, 56 & 58 are not labeled), and are even-electron nitrogen cations. The weak even -electron ions at m/z=15 and 29 are due to methyl and ethyl cations (no nitrogen atoms). The fragmentations leading to the chief fragment ions will be displayed by clicking on the appropriate spectrum. Repeated clicks will cycle the display.



4-methyl-3-pentene-2-one

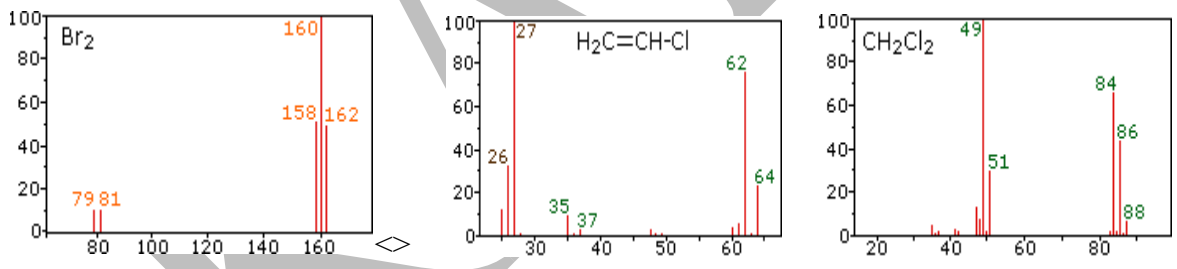


N,N-diethylmethylamine

When non-bonded electron pairs are present in a molecule (e.g. on N or O), fragmentation pathways may sometimes be explained by assuming the missing electron is partially localized on that atom. A few such mechanisms are shown above. Bond cleavage generates a radical and a cation, and both fragments often share these roles, albeit unequally.

Isotopes

Since a mass spectrometer separates and detects ions of slightly different masses, it easily distinguishes different isotopes of a given element. This is manifested most dramatically for compounds containing bromine and chlorine, as illustrated by the following examples. Since molecules of bromine have only two atoms, the spectrum on the left will come as a surprise if a single atomic mass of 80 amu is assumed for Br. The five peaks in this spectrum demonstrate clearly that natural bromine consists of a nearly 50:50 mixture of isotopes having atomic masses of 79 and 81 amu respectively. Thus, the bromine molecule may be composed of two ^{79}Br atoms (mass 158 amu), two ^{81}Br atoms (mass 162 amu) or the more probable combination of ^{79}Br-^{81}Br (mass 160 amu). Fragmentation of Br_2 to a bromine cation then gives rise to equal sized ion peaks at 79 and 81 amu.



bromine **vinyl chloride** **methylene chloride**

The center and right hand spectra show that chlorine is also composed of two isotopes, the more abundant having a mass of 35 amu, and the minor isotope a mass 37 amu. The precise isotopic composition of chlorine and bromine is:

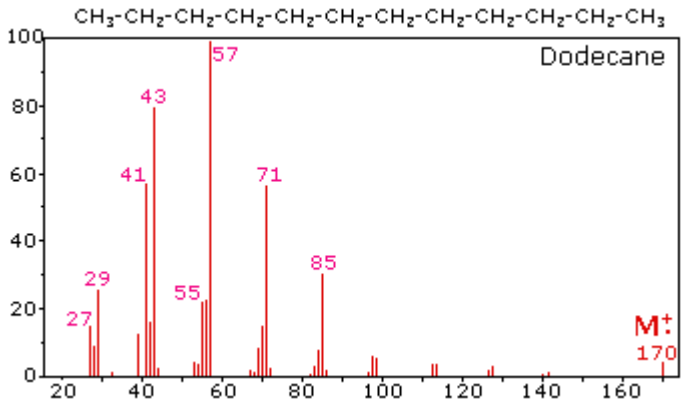
Chlorine: 75.77% ^{35}Cl and 24.23% ^{37}Cl

Bromine: 50.50% ^{79}Br and 49.50% ^{81}Br

The presence of chlorine or bromine in a molecule or ion is easily detected by noticing the intensity ratios of ions differing by 2 amu. In the case of methylene chloride, the molecular ion consists of three peaks at m/z=84, 86 & 88 amu, and their diminishing intensities may be calculated from the natural abundances given above. Loss of a chlorine atom gives two isotopic fragment ions at m/z=49 & 51amu, clearly incorporating a single chlorine atom. Fluorine and iodine, by contrast, are monoisotopic, having masses of 19 amu and 127 amu respectively. It should be noted that the presence of halogen atoms in a molecule or fragment ion does not change the odd-even mass rules given above.

Two other common elements having useful isotope signatures are carbon, ^{13}C is 1.1% natural abundance, and sulfur, ^{33}S and ^{34}S are 0.76% and 4.22% natural abundance respectively. For example, the small m/z=99 amu peak in the spectrum of 4-methyl-3-pentene-2-one (above) is due to the presence of a single ^{13}C atom in the molecular ion. Although less important in this respect, ^{15}N and ^{18}O also make small contributions to higher mass satellites of molecular ions incorporating these elements.

The calculator on the right may be used to calculate the isotope contributions to ion abundances 1 and 2 amu greater than the molecular ion (M). Simply enter an appropriate subscript number to the right of each symbol, leaving those elements not present blank, and press the "Calculate" button. The numbers displayed in the M+1 and M+2 boxes are relative to M being set at 100%.



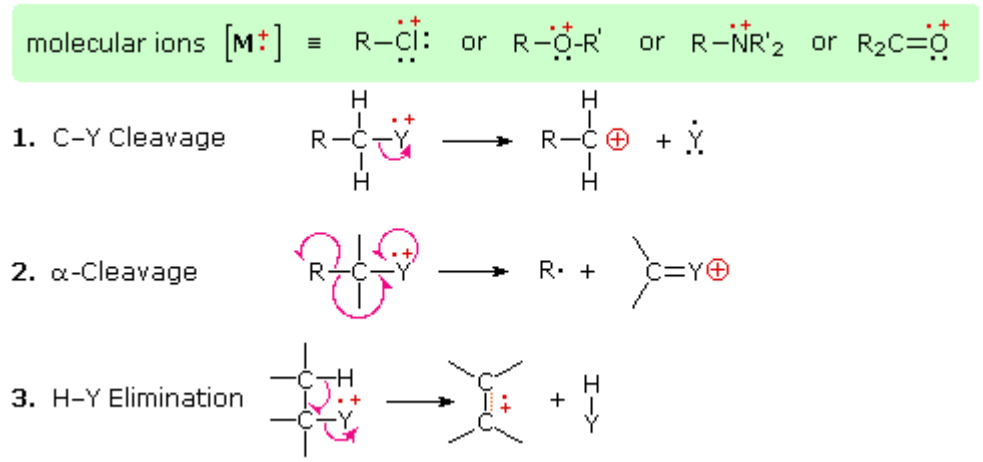
4. Fragmentation Patterns

The fragmentation of molecular ions into an assortment of fragment ions is a mixed blessing. The nature of the fragments often provides a clue to the molecular structure, but if the molecular ion has a lifetime of less than a few microseconds it will not survive long enough to be observed. Without a molecular ion peak as a reference, the difficulty of interpreting a mass spectrum increases markedly. Fortunately, most organic compounds give mass spectra that include a molecular ion, and those that do not often respond successfully to the use of milder ionization conditions. Among simple organic compounds, the most stable molecular ions are those from aromatic rings, other conjugated pi-electron systems and cycloalkanes. Alcohols, ethers and highly branched alkanes generally show the greatest tendency toward fragmentation.

The mass spectrum of dodecane on the right illustrates the behavior of an unbranched alkane. Since there are no heteroatoms in this molecule, there are no non-bonding valence shell electrons. Consequently, the radical cation character of the molecular ion (m/z = 170) is delocalized over all the covalent bonds. Fragmentation of C-C bonds occurs because they are usually weaker than C-H bonds, and this produces a mixture of alkyl radicals and alkyl carbocations. The positive charge commonly resides on the smaller fragment, so we see a homologous series of hexyl (m/z = 85), pentyl (m/z = 71), butyl (m/z = 57), propyl (m/z = 43), ethyl (m/z = 29) and methyl (m/z = 15) cations. These are accompanied by a set of corresponding alkenyl carbocations (e.g. m/z = 55, 41 &27) formed by loss of 2 H. All of the significant fragment ions in this spectrum are even-electron ions. In most alkane spectra the propyl and butyl ions are the most abundant.

The presence of a functional group, particularly one having a heteroatom Y with non-bonding valence electrons (Y = N, O, S, X etc.), can dramatically alter the fragmentation pattern of a compound. This influence is thought to occur because of a "localization" of the radical cation component of the molecular ion on the heteroatom. After all, it is easier to remove (ionize) a

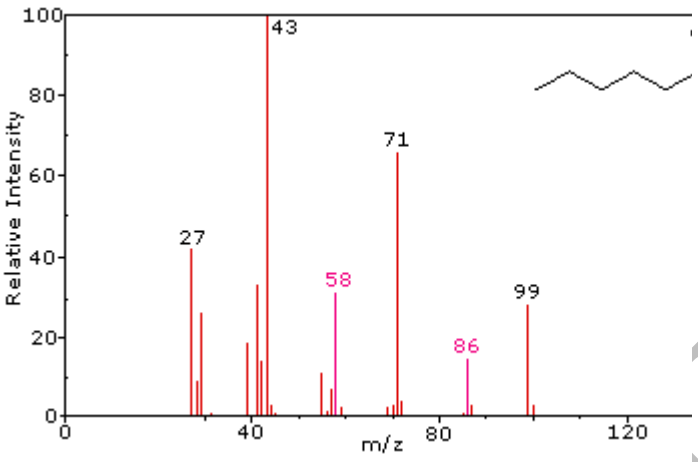
non-bonding electron than one that is part of a covalent bond. By localizing the reactive moiety, certain fragmentation processes will be favored. These are summarized in the following diagram, where the green shaded box at the top displays examples of such "localized" molecular ions. The first two fragmentation paths lead to even-electron ions, and the elimination (path #3) gives an odd-electron ion. Note the use of different curved arrows to show single electron shifts compared with electron pair shifts.



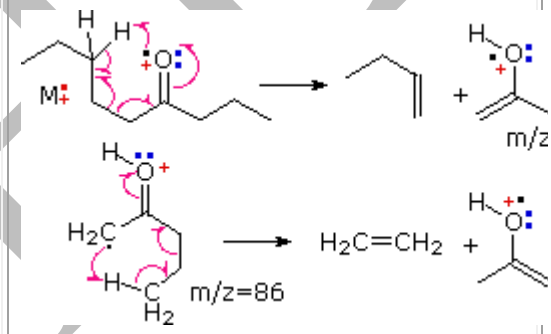
The charge distributions shown above are common, but for each cleavage process the charge may sometimes be carried by the other (neutral) species, and both fragment ions are observed. Of the three cleavage reactions described here, the alpha-cleavage is generally favored for nitrogen, oxygen and sulfur compounds. Indeed, in the previously displayed spectra of 4-methyl-3-pentene-2-one and N,N-diethylmethylamine the major fragment ions come from alpha-cleavages. Further examples of functional group influence on fragmentation are provided by a selection of compounds that may be examined by clicking the left button below. Useful tables of common fragment ions and neutral species may be viewed by clicking the right button.

Rearangement Mechanisms in Fragmentation

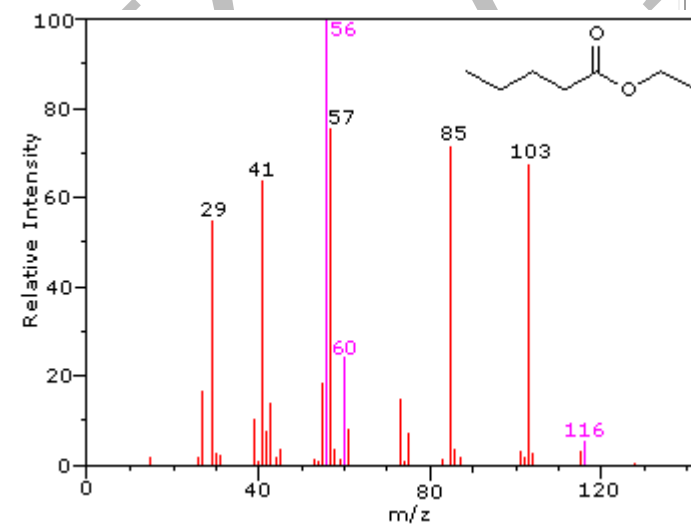
4-nonanone



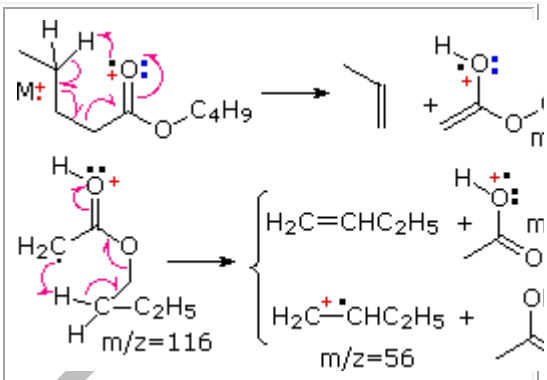
The odd-electron fragment ions at m/z = 86 and 58 are the result of a McLafferty rearrangement, involving the larger alkyl chain, and a subsequent loss of ethene (the "double-McLafferty" rearrangement). Alpha-cleavage leads to the m/z = 99, 71 and 43 ions. The charge is apparently distributed over both fragments.



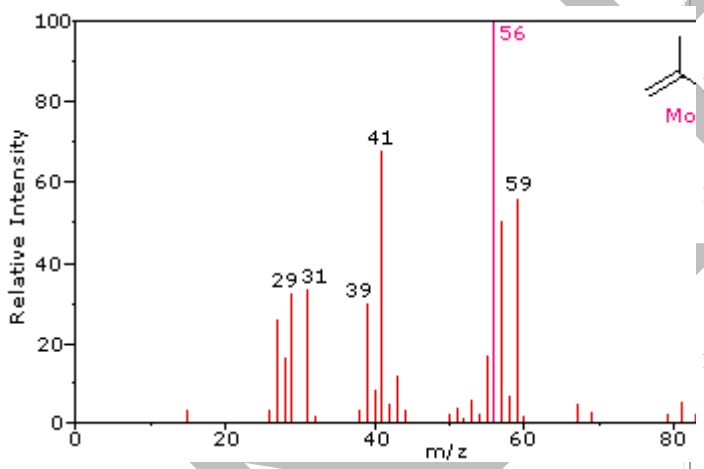
butylpentanoate



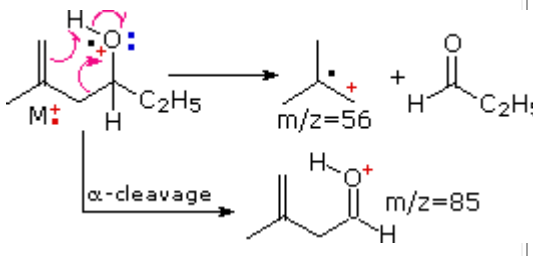
Alpha-cleavage gives ions at m/z=57 & 85 amu. The McLafferty rearrangement on the acid side generates a m/z=116 ion. Subsequent rearrangement on the alcohol side generates m/z=60 and 56 ions. The m/z=103 ion is probably $C_4H_9CO_2H_2^{(+)}$.



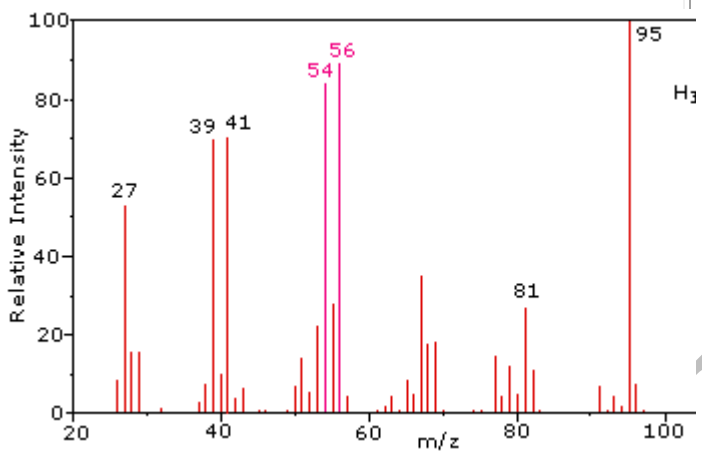
5-methyl-5-hexen-3-ol



The molecular ion (m/z=114 amu) is not observed under electron impact ionization conditions. The highest mass ion (m/z=85) is due to an alpha-cleavage of ethyl; the other alpha-cleavage generates m/z=59. The rearrangement cleavage shown here generates the m/z=56 ion.

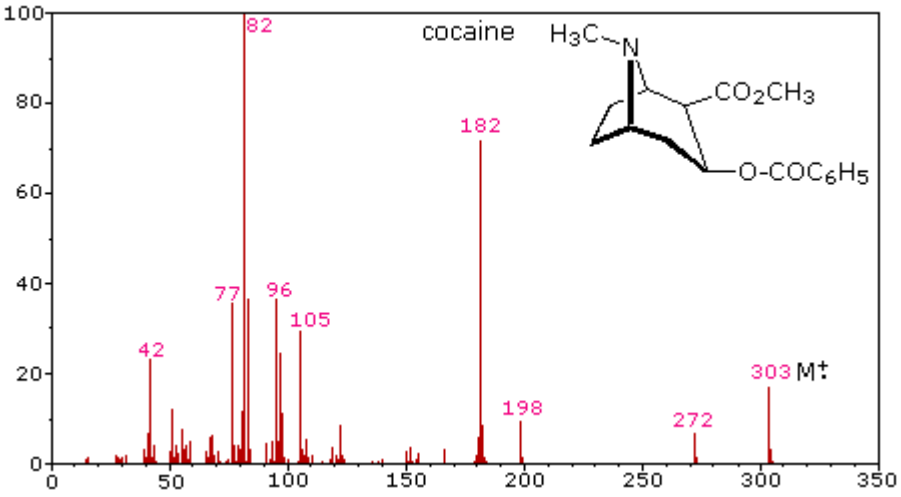


4,4-dimethylcyclohexene



The loss of a methyl radical generates the base peak at m/z=95 amu. The m/z=81 & 67 ions are smaller homologues of this ion (14 mass units less). Cyclohexene compounds undergo a retro-Diel-Alder rearrangement to give diene and alkene fragments. The charge may reside on either fragment, with the larger usually predominating. In this case both ions are relatively strong (m/z=54 & 56).

The complexity of fragmentation patterns has led to mass spectra being used as "fingerprints" for identifying compounds. Environmental pollutants, pesticide residues on food, and controlled substance identification are but a few examples of this application. Extremely small samples of an unknown substance (a microgram or less) are sufficient for such analysis. The following mass spectrum of cocaine demonstrates how a forensic laboratory might determine the nature of an unknown street drug. Even though extensive fragmentation has occurred, many of the more abundant ions (identified by magenta numbers) can be rationalized by the three mechanisms shown above. Plausible assignments may be seen by clicking on the spectrum, and it should be noted that all are even-electron ions. The m/z = 42 ion might be any or all of the following: C_3H_6, C_2H_2O or C_2H_4N. A precise assignment could be made from a high-resolution m/z value .



Odd-electron fragment ions are often formed by characteristic rearrangements in which stable neutral fragments are lost. Mechanisms for some of these rearrangements have been identified by following the course of isotopically labeled molecular ions. A few examples of these rearrangement mechanisms may be seen by clicking the following button.

5. High Resolution Mass Spectrometry

In assigning mass values to atoms and molecules, we have assumed integral values for isotopic masses. However, accurate measurements show that this is not strictly true. Because the strong nuclear forces that bind the components of an atomic nucleus together vary, the actual mass of a given isotope deviates from its nominal integer by a small but characteristic amount (remember $E = mc^2$). Thus, relative to ^{12}C at 12.0000, the isotopic mass of ^{16}O is 15.9949 amu (not 16) and ^{14}N is 14.0031 amu (not 14).

Formula	C_6H_{12}	C_5H_8O	$C_4H_8N_2$
Mass	84.0939	84.0575	84.0688

By designing mass spectrometers that can determine m/z values accurately to four decimal places, it is possible to distinguish different formulas having the same nominal mass. The table on the right illustrates this important feature, and a double-focusing high-resolution mass spectrometer easily distinguishes ions having these compositions. Mass spectrometry therefore not only provides a specific

molecular mass value, but it may also establish the molecular formula of an unknown compound.

Tables of precise mass values for any molecule or ion are available in libraries; however, the mass calculator provided below serves the same purpose. Since a given nominal mass may correspond to several molecular formulas, lists of such possibilities are especially useful when evaluating the spectrum of an unknown compound. Composition tables are available for this purpose, and a particularly useful program for calculating all possible combinations of H, C, N & O that give a specific nominal mass has been written by Jef Rozenski.

POSSIBLE QUESTIONS

UNIT-III

PART-A (20 MARKS)

(Q.NO 1 TO 20 Online Examination)

PART-B (2 Marks)

1. What are the types of chromatography?
2. Write a note on electrophoresis
3. Write a account supercritical fluids
4. What are the solvents used for column chromatography
5. Write a note on stationary phase and mobile phase

PART-C (6 MARKS)

1. Write a detail about Gas chromatography
2. Explain about liquid chromatography
3. What are the importance of Gas and liquid chromatography?
4. Write detail about Mass spectroscopy
5. Write short note on electro spray, electrical discharge, fast atom bombardment
6. What are the terms used in column chromatography? Explain?

Unit-III-Multiple choice Questions

S. No	Question	Option- I	Option- II	Option- III	Option- IV	Answer
1.	Chromatography is a technique used for --------- compounds	separation	identification	measure	analysis	separation
2.	Column chromatography is a type of	partition	adsorption	Absorption	thin layer	adsorption
3.	Primary alhols show M^+----18 peaks corresponding to the loss of	water	H^+ion	OH^-ion	O^{2-}ion	OH^-ion
4.	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
5.	In reverse phase chromatography, the stationary phase is made	non-polar	either non-polar or polar	polar	low polar	non-polar
6.	For primary amines, the base peak is formed at m/e 30 due to $CH_2=N^+$ H2 by	α-cleavage	β- cleavage	γ- cleavage	δ- cleavage	δ- cleavage
7.	The general expression for the appearance of a solute in an effluent is (where V is the elution volume of a substance, V_0 void	$V = V_0 + k_D V_i$	$V = V_0/V_i$	$V = V_0 - k_D V_i$	$V/V_0 = k_D V_i$	$V = V_0 + k_D V_i$

	volume, k_D distribution constant and V_i internal water volume)					
8.	In aliphatic ethers alkyl cation (or)an oxonium ion is formed by	α-cleavage	β-cleavage	γ-cleavage	δ-cleavage	α-cleavage
9.	Which of the following is not used for detection in GC?	Infrared spectroscopy	NMR	Flame ionisation	Electrical conductivity	NMR
10.	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
11.	The GC trace obtained after an experiment is called a	chromatograph	chromatogram	chromatophore	graph	chromatogram
12.	Which of the following detectors give concentration-dependent signals?	Electron-capture detector	non-thermal conductivity	UV detector	Scintillation counter	Electron-capture detector
13.	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
14.	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
15.	Resolution is proportional to the	number of theoretical plates in a column	square root of the number of theoretical plates in a column	square of the number of theoretical plates in a column	cube root of the number of theoretical plates in a column	square root of the number of theoretical plates in a column
16.	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

17.	Which of the statements is correct?	Gas chromatography is notused to analyse gases	Gas chromatography is used to analyse solids	Gas chromatography is not used to analyse gases, solutions	Gas chromatography is not used to analyse solid, gases	Gas chromatography is used to analyse solids
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 do not move	compounds trapped on one column are eluted to another column
19.	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
20.	Which of the following detectors give mass flow-dependent signals?	Electron capture detector	Field ionisation detector	Thermal conductivity detector	Proton capture	Field ionisation detector
21.	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
22.	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 split the sample portions	splitting off some of the sample so that it does not enter the column

23.	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
24.	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
25.	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
26.	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
27.	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
28.	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
29.	Sample retention in the column is measured by	retention time	factor	index	co-efficient	retention time

30.	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
31.	Which of the following are not used as stationary phases in a GC column?	Polysiloxanes	Silica	Cyclodextrins	None are used as stationary phases	Silica
32.	Doubling the column's length increases resolution by a factor of	$(2)^{0.5}$	3	2	4	$(2)^{0.5}$
33.	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
34.	Which of the following gases is unsuitable for use as a GC carrier gas?	Nitrogen	Helium	Oxygen	Hydrogen	Oxygen
35.	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 R_f value for the molecules separated.	use of a mobile and a stationary phase.	use of a mobile and a stationary phase.
36.	In aliphatic acids carboxyl group is directly eliminated by	β-cleavage	α-cleavage	γ-cleavage	δ-cleavage	β-cleavage
37.	Primary amides gives a strong peak at m/e=44 due to	H_2N^-	NH_3	NH_4	$H_2N\text{-}C{=}O^-$	NH_3

38.	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
39.	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.
40.	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.
41.	Acetone is an organic molecule with a semi-structural formula of CH_3COCH_3. 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 components, 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 components, but acetone is not one of them.	the mixture has at least three components, one of which might be acetone.

42.	A mixture of ethanol (C_2H_6O) and butanol ($C_4H_{10}O$) 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.
43.	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.
44.	Which of the following statements about paper and gas chromatography is correct?	The R_f and R_t 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 R_f value in paper chromatography.	A high R_f 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.
45.	The example of bulk property detector used in HPLC is	Refractive index detector	UV detector	fluorescence detector	UV-visible detector	Refractive index detector
46.	In-vitro hydrolysis studies of drugs & kinetic studies of reaction can be performed by	Polarimetry	Refractometry	Potentiometry	Conductometry	Conductometry
47.	The alkenes and aromatic	Refractive index	conductivity detector	Spectrophotometric	Potentiometric	Spectrophotometric

	compounds can be suitably detected using	detector		detector	detector	detector
48.	Formic acid is an example of	protogenic solvent	protophillic solvent	amphiprotic solvent	Aprotic solvent	protogenic solvent
49.	The relationship between concentration, temperature & potential of a solution is given by	Ilkovic equation	Henderson equation	Nernst equation	Hassalbach equation	Nernst equation
50.	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	0.004 to 0.005
51.	Sucrose can be determined after silylation using which chromatographic technique	HPLC	Gel chromatography	Gas liquid chromatography	Paper chromatography	Gas liquid chromatography
52.	R_f is refered as	retention time	retard factor	resistant value	reduced value	retention time
53.	The composition of Silica gel G is	silica gel without binder	silica gel + $CaSO_4$	Silica gel + alumina	silica gel + $MaSO_4$	Silica gel + alumina
54.	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)
55.	Oxygen --------be used as carrier gas in gas chromatography	can	cannot	often	always	can
56.	Snells law is related to	Refractometry	Potentiometry	Non-aqueous	Chromatography	Refractometry

				titrations		
57.	Relative flow (Rf) value ranges from	0 to 1	0 to 2.0	+2 to -2	+1 to -1	0 to 1
58.	In Mass spectroscopy, molecules are bombarded with a beam of energetic	neutrons	positrons	electrons	nucleons	electrons
59.	The intensity of the base peak is taken as	80	100	87	57	100
60.	In Mass spectroscopy, molecules are bombarded with a beam of energetic	neutrons	positrons	electrons	nucleons	electrons

UNIT IV
Syllabus

Elemental analysis:
Mass spectrometry (electrical discharges).
Atomic spectroscopy: Atomic absorption, Atomic emission, and Atomic fluorescence. Excitation and getting sample into gas phase (flames, electrical discharges, plasmas), Wavelength separation and resolution (dependence on technique), Detection of radiation (simultaneous/scanning, signal noise), Interpretation (errors due to molecular and ionic species, matrix effects, other interferences).

Elemental analysis: Mass spectrometry

Atomic Absorption Spectroscopy

Guystav Kirchoff and Robert Bunsen first used atomic absorption spectroscopy-along with atomic emission-in 1859 and 1860 as a means for identify atoms in flames and hot gases. Although atomic emission continued to develop as an analytical technique, progress in atomic absorption languished for almost a century. Modern atomic absorption spectroscopy has its beginnings in 1955 as a result of the independent work of A. C. Walsh and C. T. J. Alkemade.13 Commercial instruments were in place by the early 1960s, and the importance of atomic absorption as an analytical technique was soon evident.

Instrumentation

Atomic absorption spectrophotometers use the same single-beam or double-beam optics described earlier for molecular absorption spectrophotometers (see Figure 10.26 and Figure 10.27). There is, however, an important additional need in atomic absorption spectroscopy—we must covert the analyte into free atoms. In most cases our analyte is in solution form. If our sample is a solid, then we must bring it into solution before the analysis. When analyzing lake sediment for Cu, Zn, and Fe, for example, we bring the analytes into solution as Cu2+, Zn2+, and Fe3+ by extracting them with suitable reagent. For this reason, only the introduction of solution samples is considered in this text. The process of converting an analyte to a free gaseous atom is called atomization. Converting an aqueous analyte into a free atom requires that westrip away the solvent, volatilize the analytes, and, if necessary, dissociate the analyte into free atoms. Desolating an aqueous solution of $CuCl_2$, for example, leaves us with solid particulates of CuCl2. Converting the particulateCuCl2 to gas phases atoms of Cu and Cl requires thermal energy.

$$CuCl_2(aq) \rightarrow CuCl_2\ (s) \rightarrow Cu\ (g) + 2Cl\ (g)$$

There are two common atomization methods: flame atomization and electro thermal atomization, although a few elements are atomized using other methods.

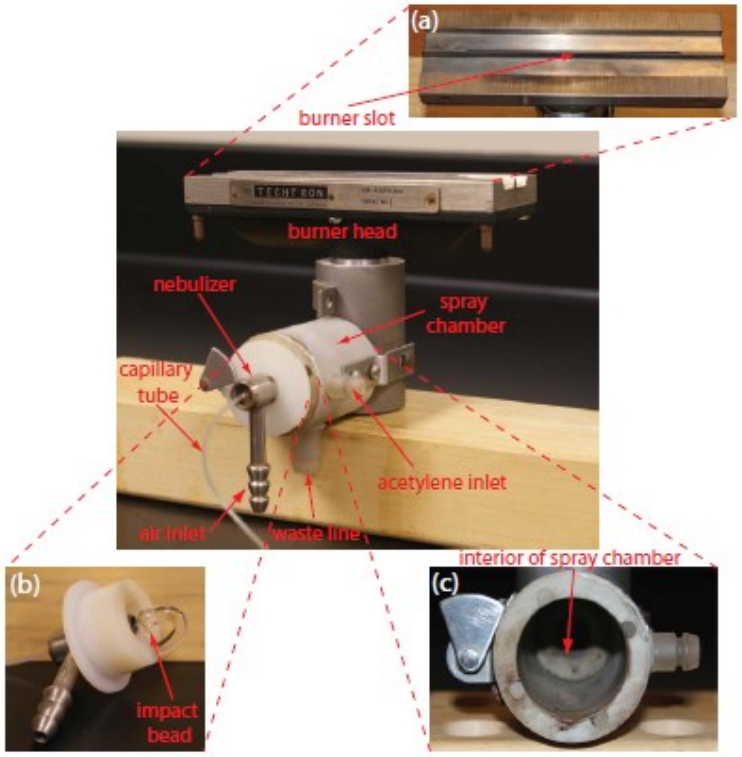


Figure 10.42 Flame atomization assembly with expanded views of (a) the burner head showing the burner slot where the flame is located; (b) the nebulizer's impact bead; and (c) the interior of the spray chamber. Although the unit shown here is from an older instrument, the basic components of a modern flame AA spectrometer are the same.

FLAME ATOMIZER

Figure 10.42 shows a typical flame atomization assembly with close-up views of several key components. In the unit shown here, the aqueous sample is drawn into the assembly by passing a high-pressure stream of compressed air past the end of a capillary tube immersed in the sample. When the sample exits the nebulizer it strikes a glass impact bead, converting it into a fine aerosol mist within the spray chamber. The aerosol mist is swept through the spray chamber

by the combustion gases-compressed air and acetylene in this case-to the burner head where the flame's thermal energy desolates the aerosol mist to a dry aerosol of small, solid particles. The flame's thermal energy then volatilizes the particles, producing a vapor consisting of molecular species, ionic species, and free atoms.

Burner

The slot burner in Figure 10.42a provides a long optical path length and a stable flame. Because absorbance increases linearly with the path length, a long path length provides greater sensitivity. A stable flame minimizes uncertainty due to fluctuations in the flame. The burner is mounted on an adjustable stage that allows the entire assembly to move horizontally and vertically. Horizontal adjustments ensure that the flame is aligned with the instrument's optical path. Vertical adjustments adjust the height within the flame from which absorbance is monitored. This is important because two competing processes affect the concentration of free atoms in the flame. The more time the analyte spends in the flame the greater the atomization efficiency; thus, the production of free atoms increases with height. On the other hand, a longer residence time allows more opportunity for the free atoms to combine with oxygen to form a molecular oxide. For an easily oxidized metal, such as Cr, the concentration of free atoms is greatest just above the burner head. For metals, such as Ag, which are difficult to oxidize, the concentration of free atoms increases steadily with height (Figure 10.43). Other atoms show concentration profiles that maximize at a characteristic height Flame. The flame's temperature, which affects the efficiency of atomization, depends on the fuel-oxidant mixture, several examples of which are listed in Table 10.9. Of these, the air-acetylene and the nitrous oxide–acetylene flames are the most popular. Normally the fuel and oxidant are mixed in an approximately stoichiometric ratio; however, a fuel-rich mixture may be necessary for easily oxidized analytes. Figure 10.44 shows a cross-section through the flame, looking down the source radiation's optical path. The primary combustion zone is usually rich in gas combustion

products that emit radiation, limiting is usefulness for atomic absorption. The inter zonal region generally is rich in free atoms and provides the best location for measuring atomic absorption. The hottest part of the flame is typically 2-3 cm above the primary combustion zone. As atoms approach the flame's secondary combustion zone, the decrease in temperature allows for formation of stable molecular species. Sample Introduction. The most common means for introducing samples into a flame atomizer is a continuous aspiration in which the sample flows through the burner while we monitor the absorbance. Continuous aspiration is sample intensive, typically requiring from 2-5 mL of sample. Flame micro sampling allows us to introduce a discrete sample of fixed volume, and is useful when we have a limited amount of sample or when the sample's matrix is incompatible with the flame atomizer. For example, continuously aspirating a sample that has a high concentration of dissolved solids-sea water, for example, comes to mind-may build-up a solid deposition the burner head that obstructs the flame and that lowers the absorbance. Flame micro sampling is accomplished using a micro pipet to place

Table 10.9 Fuels and Oxidants Used for Flame Combustion

fuel	oxidant	temperature range (°C)
natural gas	air	1700–1900
hydrogen	air	2000–2100
acetylene	air	2100–2400
acetylene	nitrous oxide	2600–2800
acetylene	oxygen	3050–3150

50–250 µL of sample in a Teflon funnel connected to the nebulizer or by dipping the nebulizer tubing into the sample for a short time. Dip sampling is usually accomplished with an

automatic sampler. The signal for flame micro sampling is a transitory peak whose height or area is proportional to the amount of analyte that is injected. Advantages and Disadvantages of Flame Atomization. The principal advantage of flame atomization is the reproducibility with which the sample is introduced into the spectrophotometer. A significant disadvantage to flame atomizers is that the efficiency of atomization may be quite poor. There are two reasons for poor atomization efficiency. First, the majority of the aerosol droplets produced during nebulization are too large to be carried to the flame by the combustion gases. Consequently, as much as 95% of the sample never reaches the flame. A second reason for poor atomization efficiency is that the large volume of combustion gases significantly dilutes the sample. Together, these contributions to the efficiency of atomization reduce sensitivity because the analyte's concentration in the flame may be factor of 2.5 × 10–6 less than that in solution.

ELECTROTHERMAL ATOMIZERS

A significant improvement in sensitivity is achieved by using the resistive heating of a graphite tube in place of a flame. A typical electro thermal atomizer, also known as a graphite furnace, consists of a cylindrical graphite tube approximately 1-3 cm in length and 3-8 mm in diameter. As shown in Figure 10.45, the graphite tube is housed in an sealed assembly that has optically transparent windows at each end. A continuous stream of an inert gas is passed through the furnace, protecting the graphite tube from oxidation and removing the gaseous products produced during atomization. Power supply is used to pass a current through the graphite tube, resulting in resistive heating. Samples of between 5-50 μL are injected into the graphite tube through small hole at the top of the tube. Atomization is achieved in three stages. In the first stage the sample is dried to a solid residue using a current that raises the temperature of the graphite tube to about 110 ^{o}C. In the second stage, which is called asking, the temperature is

increased to between 350–1200 ^{o}C. At these temperatures any organic material in the sample is converted to CO_2 and H_2O, and volatile inorganic materials are vaporized. These gases are removed by the inert gas flow. In the final stage the sample is atomized by rapidly increasing the temperature to between 2000–3000 ^{o}C. The result is a transient absorbance peak whose height or area is proportional to the absolute amount of analyte injected into the graphite tube. Together, the three stages take approximately 45–90s, with most of this time used for drying and asking the sample. Electro thermal atomization provides a significant improvement in sensitivity by trapping the gaseous analyte in the small volume within the graphite tube. The analyte's concentration in the resulting vapor phase maybe as much as 1000× greater than in a flame atomization. This improvement in sensitivity-and the resulting improvement in detection limits-is offset by a significant decrease in precision. Atomization efficiency is strongly influenced by the sample's contact with the graphite tube, which is difficult to control reproducibly.

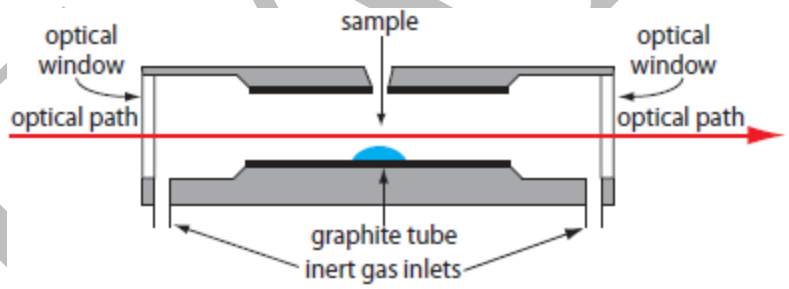


Diagram showing a cross section of an electro thermal analyzer

MISCELLANEOUS ATOMIZATION METHODS

A few elements may be atomized by a chemical reaction that produces a volatile product. Elements such as As, Se, Sb, Bi, Ge, Sn, Te, and Pb, for example, form volatile hydrides when

reacted with $NaBH_4$ in acid. An inert gas carries the volatile hydrides to either a flame or to a heated quartz observation tube situated in the optical path. Mercury is determined by the cold-vapor method in which it is reduced to elemental mercury with SnCl2. The volatile Hg is carried by an inert gas to an unheated observation tube situated in the instrument's optical path.

Quantitative Applications

Atomic absorption is widely used for the analysis of trace metals in a variety of sample matrices. Using Zn as an example, atomic absorption methods have been developed for its determination in samples as diverse as water and wastewater, air, blood, urine, muscle tissue, hair, milk, breakfast cereals, shampoos, alloys, industrial plating baths, gasoline, oil, sediments, and rocks. Developing a quantitative atomic absorption method requires several considerations, including choosing a method of atomization, selecting the wavelength and slit width, preparing the sample for analysis, minimizing spectral and chemical interferences, and selecting a method of standardization. Each of these topics is considered in this section.

DEVELOPING A QUANTITATIVE METHOD

Flame or Electro thermal Atomization:

The most important factor in choosing a method of atomization is the analyte's concentration. Because of its greater sensitivity, it takes less analyte to achieve a given absorbance when using electro thermal atomization. Table 10.10, which compares the amount of analyte needed to achieve an absorbance of 0.20 when using flame atomization and electro thermal atomization, is useful when selecting an atomization method. For example, flame atomization is the method of choice if our samples contain 1-10 mg Zn^{2+}/L, but electro thermal atomization is the best choice for samples containing 1-10 μg Zn^{2+}/L. Selecting the Wavelength and Slit Width. The source for atomic absorption is a hollow cathode lamp consisting of a

cathode and anode enclosed within a glass tube filled with a low pressure of Ne or Ar (Figure 10.46). Applying a potential across the electrodes ionizes the filler gas.

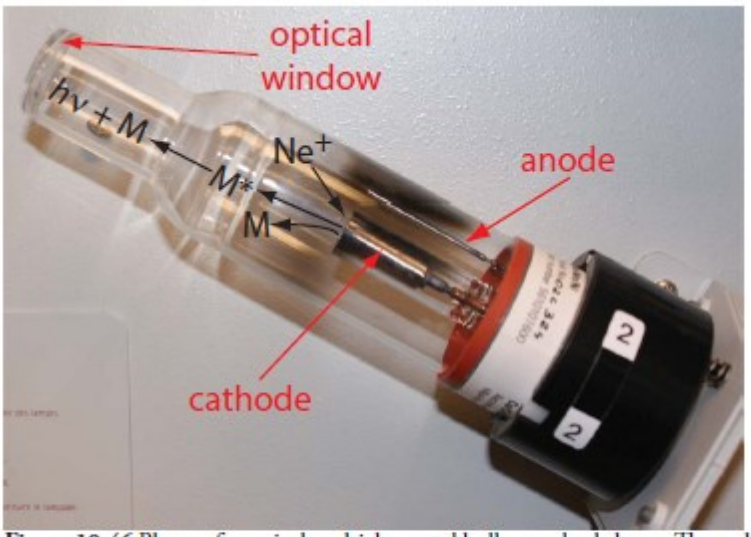


Figure 10.46 Photo of a typical multielemental hollow cathode lamp. The cathode in this lamp is fashioned from an alloy containing Co, Cr, Cu, Fe, Mn, and Ni, and is surrounded by a glass shield to isolate it from the anode. The lamp is filled with Ne gas. Also shown is the process leading to atomic emission. See the text for an explanation.

The positively charged gas ions collide with the negatively charged cathode, sputtering atoms from the cathode's surface. Some of the sputtered atoms are in the excited state and emit radiation characteristic of the metal(s) from which the cathode was manufactured. By fashioning the cathode from the metallic analyte, a hollow cathode lamp provides emission lines that correspond to the analyte's absorption spectrum. Each element in a hollow cathode lamp provides several atomic emission lines that we can use for atomic absorption. Usually the wavelength that provides the best sensitivity is the one we choose to use, although a less sensitive wavelength may be more appropriate for a larger concentration of analyte. For the Cr

hollow cathode lamp in Table 10.11, for example, the best sensitivity is obtained using a wavelength of 357.9 nm. Another consideration is the intensity of the emission line. If several emission lines meet our need for sensitivity, we may wish to use the emission line with the largest relative P0 because there is less uncertainty in measuring P0 and PT. When analyzing samples containing ≈10 mg Cr/L, for example, the first three wavelengths in Table 10.11 provide an appropriate sensitivity. The wavelengths of 425.5 nm and 429.0 nm, however, have a greater P0 and will provide less uncertainty in the measured absorbance. The emission spectrum from a hollow cathode lamp includes, besides emission lines for the analyte, additional emission lines for impurities present in the metallic cathode and from the filler gas. These additional linesare a source of stray radiation that leads to an instrumental deviation from Beer's law. The monochromator's slit width is set as wide as possible, improving the throughput of radiation, while, at the same time, being narrow enough to eliminate the stray radiation. Preparing the Sample. Flame and electro thermal atomization require that the sample be in solution. Solid samples are brought into solution by dissolving in an appropriate solvent. If the sample is not soluble it may be digested, either on a hot-plate or by microwave, using HNO3, H2SO4, orHClO4. Alternatively, we can extract the analyte using a Soxhlet extractor. Liquid samples may be analyzed directly or extracted if the matrix is incompatible with the method of atomization. A serum sample, for instance, is difficult to aspirate when using flame atomization and may produce an unacceptably high background absorbance when using electrothermal atomization. A liquid–liquid extraction using an organic solvent and a chelating agent is frequently used to concentrate analytes. Dilute solutions of Cd2+,Co2+, Cu2+, Fe3+, Pb2+, Ni2+, and Zn2+, for example, can be concentrated by extracting with a solution of ammonium pyrrolidine dithiocarbamate in methyl isobutyl ketone. Minimizing Spectral Interference. A spectral interference occurs when an analyte's absorption line overlaps with an interferent's absorption line or band. Because they are so narrow, the overlap of two atomic absorption lines

is seldom a problem. On the other hand, a molecule's broad absorption band or the scattering of source radiation is a potentially serious spectral interference. An important consideration when using a flame as an atomization source is its effect on the measured absorbance. Among the products of combustion are molecular species that exhibit broad absorption bands and particulates that scatter radiation from the source. If we fail to compensate for this spectral interference, then the intensity of transmitted radiation decreases. The result is an apparent increase in the sample's absorbance. Fortunately, absorption and scattering of radiation by the flame are corrected by analyzing a blank. Spectral interferences also occur when components of the sample's matrix other than the analyte react to form molecular species, such as oxides and hydroxides. The resulting absorption and scattering constitutes the sample's background and may present a significant problem, particularly at wavelengths below 300 nm where the scattering of radiation becomes more important. If we know the composition of the sample's matrix, then we can prepare our samples using an identical matrix. In this case the background absorption is the same for both the samples and standards. Alternatively, if the background is due to a known matrix component, then we can add that component in excess to all samples and standards so that the contribution of the naturally occurring interferent is insignificant. Finally, much interference due to the sample's matrix can be eliminated by increasing the atomization temperature. For example, by switching to a higher temperature flame it may be possible to prevent the formation of interfering oxides and hydroxides. If the identity of the matrix interference is unknown, or if it is not possible to adjust the flame or furnace conditions to eliminate the interference, then we must find another method to compensate for the background interference. Several methods have been developed to compensate for matrix interferences, and most atomic absorption spectrophotometers include one or more of these methods. One of the most common methods for background correction is to use a continuum source, such as a D2 lamp.

Table 10.11 Atomic Emission Lines for a Cr Hollow Cathode Lamp			
wavelength (nm)	slit width (nm)	mg Cr/L giving $A = 0.20$	P_0 (relative)
357.9	0.2	2.5	40
425.4	0.2	12	85
429.0	0.5	20	100
520.5	0.2	1500	15
520.8	0.2	500	20

Because a D2 lamp is a continuum source, absorbance of its radiation by the analyte's narrow absorption line is negligible. Only the background, therefore, absorbs radiation from the D2 lamp. Both the analyte and the background, on the other hand, absorb the hollow cathode's radiation. Subtracting the absorbance for the D2lamp from that for the hollow cathode lamp gives a corrected absorbance that compensates for the background interference. Although this method of background correction may be quite effective, it does assume that the background absorbance is constant over the range of wavelengths passed by the monochromator. If this is not true, subtracting the two absorbances may underestimate or overestimate the background.

Minimizing Chemical Interferences

The quantitative analysis of some elements is complicated by chemical interferences occurring during atomization. The two most common chemical interferences are the formation of nonvolatile compounds containing the analyte and ionization of the analyte. One example of the formation of nonvolatile compounds is the effect of PO43– or Al3+ on the flame atomic absorption analysis of Ca2+. In one study, for example, adding 100 ppm Al3+ to a solution of 5 ppm Ca2+ decreased the calcium ion's absorbance from 0.50 to 0.14, while adding 500 ppmPO43– to a similar solution of Ca2+ decreased the absorbance from 0.50 to0.38. These interferences were attributed to the formation of nonvolatile particles of Ca3(PO4)2 and an Al–Ca–O oxide.16When using flame atomization, we can minimize the formation of nonvolatile compounds by increasing the flame's temperature, either by changing the fuel-to-oxidant ratio or

by switching to a different combination of fuel and oxidant. Another approach is to add a releasing agent or a protecting agent to the samples. A releasing agent is a species that reacts with the interferent, releasing the analyte during atomization. AddingSr2+ or La3+ to solutions of Ca2+, for example, minimizes the effect ofPO43– and Al3+ by reacting in place of the analyte. Thus, adding 2000 ppmSrCl2 to the Ca2+/PO43– and Ca2+/Al3+ mixtures described in the previous paragraph increased the absorbance to 0.48. A protecting agent reacts with the analyte to form a stable volatile complex. Adding 1% w/w EDTA to the Ca2+/PO43– solution described in the previous paragraph increased the absorbance to 0.52.Ionization interferences occur when thermal energy from the flame or the electrothermal atomizer is sufficient to ionize the analyte M(g)ÉM (g) e + + − 10.24where M is the analyte. Because the absorption spectra for M and M+ are different, the position of the equilibrium in reaction 10.24 affects absorbance at wavelengths where M absorbs. To limit ionization we add a high concentration of an ionization suppressor, which is simply a species that ionizes more easily than the analyte. If the concentration of the ionization suppressor is sufficient, then the increased concentration of electrons in the flame pushes reaction 10.24 to the left, preventing the analyte's ionization. Potassium and cesium are frequently used as an ionization suppress or because of their low ionization energy. Standardizing the Method. Because Beer's law also applies to atomic absorption, we might expect atomic absorption calibration curves to be linear. In practice, however, most atomic absorption calibration curves are nonlinear, or linear for only a limited range of concentrations. Nonlinearity in atomic absorption is a consequence of instrumental limitations, including stray radiation from the hollow cathode lamp and the variation in molar absorptivity across the absorption line. Accurate quantitative work, therefore, often requires a suitable means for computing the calibration curve from a set of standards. When possible, a quantitative analysis is best conducted using external standards. Unfortunately, matrix interferences are a frequent problem, particularly when using electrothermal atomization. For

this reason the method of standard additions is often used. One limitation to this method of standardization, however, is the requirement that there be a linear relationship between absorbance and concentration.

Evaluation of Atomic Absorption Spectroscopy

SCALE OF OPERATION

Atomic absorption spectroscopy is ideally suited for the analysis of trace and ultra trace analytes, particularly when using electro thermal atomization. For minor and major analyte, sample can be diluted before the analysis. Most analyses use a macro or a meso sample. The small volume requirement for electro thermal atomization or flame micro sampling, however, makes practical the analysis micro and ultra micro samples.

ACCURACY

If spectral and chemical interferences are minimized, an accuracy of 0.5–5%is routinely attainable. When the calibration curve is nonlinear, accuracy may be improved by using a pair of standards whose absorbance's closely bracket the sample's absorbance and assuming that the change in absorbance is linear over this limited concentration range. Determinate errors for electro thermal atomization are often greater than that obtained with flame atomization due to more serious matrix interferences.

PRECISION

For absorbance values greater than 0.1–0.2, the relative standard deviation for atomic absorption is 0.3–1% for flame atomization and 1–5% for electro thermal atomization. The principle limitation is the variation in the concentration of free analyte atoms resulting from variations in the rate of aspiration, nebulization, and atomization when using a flame atomizer, and the consistency of injecting samples when using electro thermal atomization.

SENSITIVITY

The sensitivity of a flame atomic absorption analysis is influenced strongly by the flame's composition and by the position in the flame from which we monitor the absorbance. Normally the sensitivity of an analysis is optimized by aspirating a standard solution of the analyte and adjusting operating conditions, such as the fuel-to-oxidant ratio, the nebulizer flow rate, and the height of the burner, to give the greatest absorbance. With electro thermal atomization, sensitivity is influenced by the drying and asking stages that precede atomization. The temperature and time used for each stage must be optimized for each type of sample. Sensitivity is also influenced by the sample's matrix. We have already noted, for example, that sensitivity can be decreased by chemical interferences. An increase in sensitivity may be realized by adding a low molecular weight alcohol, ester, or ketone to the solution, or by using an organic solvent.

SELECTIVITY

Due to the narrow width of absorption lines, atomic absorption provides excellent selectivity. Atomic absorption can be used for the analysis of over60 elements at concentrations at or below the level of μg/L.

TIME, COST, AND EQUIPMENT

The analysis time when using flame atomization is short, with sample through puts of 250-350 determinations per hour when using a fully automated system. Electro thermal atomization requires substantially more time per analysis, with maximum sample throughputs of 20-30 determinations per hour. The cost of a new instrument ranges from between $10,000–$50,000 for flame atomization, and from $18,000–$70,000 for electro thermal atomization. The more expensive instruments in each price range include double-beam optics, automatic samplers, and can be programmed for multi elemental analysis by allowing the wavelength and hollow cathode lamp to be changed automatically.

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.18 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 1964.10G.1 Atomic Emission Spectra Atomic emission occurs when a valence electron in a higher energy atomic orbital returns to a lower energy atomic orbital. Figure 10.57 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. The intensity of an atomic emission line, I.e., is proportional to the number of atoms, N*, populating the excited state, Ie = kN * 10.30where k is a constant accounting for the efficiency of the transition. If a system of atoms is in thermal equilibrium, the population of excited state is related to the total concentration of atoms, N, by the Boltzmann distribution. For many elements at temperatures of less than 5000 K the Boltzmann distribution is approximated as where Gi and g0 are statistical factors that account for the number of equivalent energy levels for the excited state and the ground state, Ei is the energy of the excited state relative to a ground state energy, E0, of 0, k is Boltzmann's constant (1.3807 × 10–23 J/K), and T is the temperature in kelvin. From equation 10.31 we expect that excited states with lower energies have larger populations and more intense emission lines. We also expect emission intensity to increase with temperature. Where Gi and g0 are statistical factors that account for the number of equivalent energy levels for the excited state and the ground state, Ei is the energy of the excited state relative to a ground state energy, E0, of 0, k is Boltzmann's constant (1.3807 × 10–23 J/K), and T is the temperature in kelvin. From equation 10.31 we expect that excited states

with lower energies have larger populations and more intense emission lines. We also expect emission intensity to increase with temperature.

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 multielement 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 10.42). The burner head consists of single or multiple slots, ora 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.

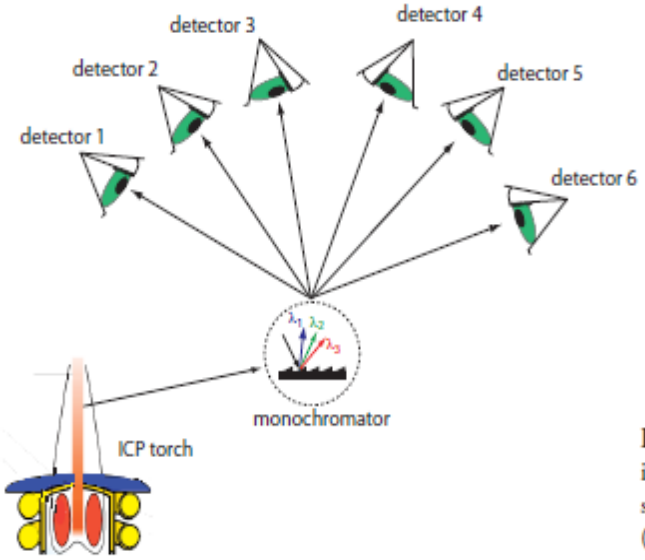


Figure 10.59 Schematic diagram of a multichannel atomic emission spectrometer for the simultaneous analysis of several elements. The ICP torch is modified from Xvlun (commons.wikipedia.org). Instruments may contain as many as 48–60 detectors.

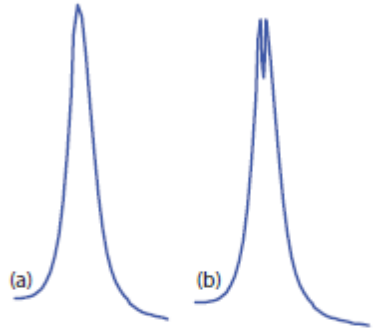


Figure 10.61 Atomic emission lines for (a) a low concentration of analyte, and (b) a high concentration of analyte showing the effect of self-absorption.

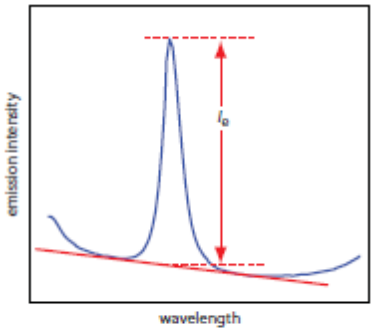


Figure 10.60 Method for correcting an analyte's emission for the flame's background emission.

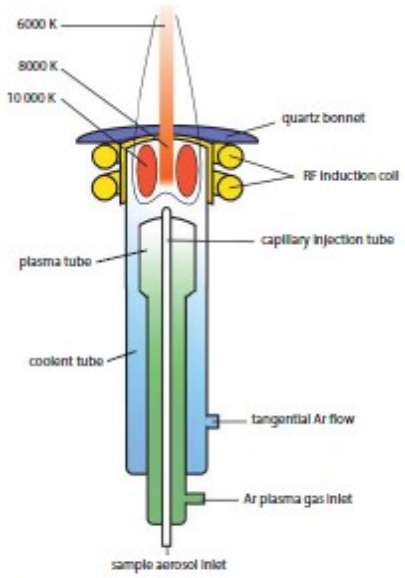


Figure 10.58 Schematic diagram of an inductively coupled plasma torch. Source: modified from Xvlun (commons.wikipedia.org).

Table 10.14 Detection Limits for Atomic Emission[a]

element	detection limit in μg/mL flame emission	ICP
Ag	2	0.2
Al	3	0.2
As	2000	2
Ca	0.1	0.0001
Cd	300	0.07
Co	5	0.1
Cr	1	0.08
Fe	10	0.09
Hg	150	1
K	0.01	30
Li	0.001	0.02
Mg	1	0.003
Mn	1	0.01
Na	0.01	0.1
Ni	10	0.2
Pb	0.2	1
Pt	2000	0.9
Sn	100	3
Zn	1000	0.1

[a] Source: Parsons, M. L.; Major, S.; Forster, A. R.; App. Spectrosc. 1983, 37, 411–418.

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 10.58. 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.

MULTIELEMENTAL ANALYSIS

Atomic emission spectroscopy is ideally suited for multielement 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 multielement 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 10.59).

Quantitative Applications

Atomic emission is widely used for the analysis of trace metals in a variety of sample matrices. The development of a quantitative atomic emission method requires several considerations, including choosing a source for atomization and excitation, selecting a wavelength and slit width, preparing the sample for analysis, minimizing spectral and chemical interferences, and selecting a method of standardization.

CHOICE OF ATOMIZATION AND EXCITATION SOURCE

Except for the alkali metals, detection limits when using an ICP are significantly better than those obtained with flame emission (Table 10.14). Plasmas also are subject to fewer spectral and chemical interferences. For these reasons a plasma emission source is usually the better choice.

SELECTING THE WAVELENGTH AND SLIT WIDTH

The choice of wavelength is dictated by the need for sensitivity and the need to avoid interferences from the emission lines of other constituents in the sample. Because an analyte's atomic emission spectrum has an abundance of emission lines—particularly when using a high temperature plasma source—it is inevitable that there will be some overlap between emission lines. For example, an analysis for Ni using the atomic emission line at349.30 nm is complicated by the atomic emission line for Fe at 349.06 nm. Narrower slit widths provide better resolution, but at the cost of less radiation reaching the detector. The easiest approach to selecting a wavelength is to record the sample's emission spectrum and look for an emission line that provides an intense signal and is resolved from other emission lines.

PREPARING THE SAMPLE

Flame and plasma sources are best suited for samples in solution and liquid form. Although a solid sample can be analyzed by directly inserting it into the flame or plasma, they usually are first brought into solution by digestion or extraction.

MINIMIZING SPECTRAL INTERFERENCES

The most important spectral interference is broad, background emission from the flame or plasma and emission bands from molecular species. This background emission is particularly severe for flames because the temperatures insufficient to break down refractory compounds, such as oxides and hydroxides. Background corrections for flame emission are made by scanning over the emission line and drawing a baseline (Figure 10.60). Because a plasma's temperature is much higher, a background interference due to molecular emission is less of a problem. Although emission from the plasma's core is strong, it is insignificant at a height of 10–30 mm above the core where measurements normally are made.

MINIMIZING CHEMICAL INTERFERENCES

Flame emission is subject to the same types of chemical interferences as atomic absorption. These interferences are minimized by adjusting the flame's composition and adding protecting agents, releasing agents, or ionization suppressors. An additional chemical interference results from self-absorption. Because the flame's temperature is greatest at its center, the concentration of analyte atoms in an excited state is greater at the flame's center than at its outer edges. If an excited state atom in the flame's center emits a photon while returning to its ground state, then a ground state atom in the cooler, outer regions of the flame may absorb the photon, decreasing the emission intensity. For higher concentrations of analyte self-absorption may invert the center of the emission band (Figure 10.61). Chemical interferences with plasma sources generally are not significant because the plasma's higher temperature limits the formation of nonvolatile species. For example, PO_4^{3-} is a significant interferent when analyzing samples for Ca^{2+} by flame emission but has a negligible effect when using a plasma source. In addition, the high concentration of electrons from the ionization of argon minimizes ionization interferences.

STANDARDIZING THE METHOD

From equation 10.30 we know that emission intensity is proportional to the population of the analyte's excited state, N*. If the flame or plasma is in thermal equilibrium, then the excited state population is proportional to the analyte's total population, N, through the Boltzmann distribution (equation 10.31). A calibration curve for flame emission is usually linear over two to three orders of magnitude, with ionization limiting linearity when the analyte's concentrations is small and self-absorption limiting linearity for higher concentrations of analyte. When using a plasma, which suffers from fewer chemical interferences, the calibration curve often is linear over four to five orders of magnitude and is not affected significantly by changes in the matrix of the standards. Emission intensity may be affected significantly by many parameters, including the temperature of the excitation source and the efficiency of atomization. An increase in temperature of 10 K, for example, produces 4% increase in the fraction of Na atoms occupying the 3p excited state. This is potentially significant uncertainty that may limit the use of external standards. The method of internal standards can be used when variations in source parameters are difficult to control. To compensate for changes in the temperature of the excitation source, the internal standard is selected so that its emission line is close to the analyte's emission line. In addition, the internal standard should be subject to the same chemical interferences to compensate for changes in atomization efficiency. To accurately compensate for these errors the analyte and internal standard emission lines must be monitored simultaneously.

Evaluation of Atomic Emission Spectroscopy

SCALE OF OPERATION

The scale of operations for atomic emission is ideal for the direct analysis of trace and ultrarace analytes in macro and meso samples. With appropriate dilutions, atomic emission also can be applied to major and minor analytes.

ACCURACY

When spectral and chemical interferences are insignificant, atomic emissions capable of producing quantitative results with accuracies of between1–5%. Accuracy frequently is limited by chemical interferences. Because the higher temperature of a plasma source gives rise to more emission lines, the accuracy of using plasma emission often is limited by stray radiation from overlapping emission lines.

PRECISION

For samples and standards in which the analyte's concentration exceeds the detection limit by at least a factor of 50, the relative standard deviation for both flame and plasma emission is about 1–5%. Perhaps the most important factor affecting precision is the stability of the flame's or the plasma's temperature. For example, in a 2500 K flame a temperature fluctuation of ±2.5 K gives a relative standard deviation of 1% in emission intensity. Significant improvements in precision may be realized when using internal standards.

SENSITIVITY

Sensitivity is strongly influenced by the temperature of the excitation source and the composition of the sample matrix. Sensitivity is optimized by aspirating standard solution of analyte and maximizing the emission by adjusting the flame's composition and the height from which we monitor the emission. Chemical interferences, when present, decrease the sensitivity of the analysis. The sensitivity of plasma emission is less affected by the sample matrix. In some cases, a calibration curve prepared using standards in a matrix of distilled water can be used for samples with more complex matrices.

SELECTIVITY

The selectivity of atomic emission is similar to that of atomic absorption. Atomic emission has the further advantage of rapid sequential or simultaneous analysis.

TIME, COST, AND EQUIPMENT

Sample throughput with atomic emission is very rapid when using automated systems capable of multielement analysis. For example, sampling rates of 3000 determinations per hour have been achieved using a multichannel, and 300 determinations per hour with a sequential ICP. Flame emission is often accomplished using an atomic absorption spectrometer, which typically costs between $10,000–$50,000. Sequential ICP's range in price from $55,000–$150,000, while an ICP capable of simultaneous multielement analysis costs between $80,000–$200,000. Combinatorics' that are capable of both sequential and simultaneous analysis range in price from $150,000–$300,000. The cost of Ar, which is consumed insignificant quantities, cannot be overlooked when considering the expense of operating an ICP.

Emission Spectroscopy

An analyte in an excited state possesses an energy, E2, that is greater than its energy when it is in a lower energy state, E1. When the analyte returns to its lower energy state—a process we call relaxation—the excess energy, ΔEΔE = E −E 2 1must be released. Figure 10.4 shows a simplified picture of this process. The amount of time the analyte spends in the excited state—its lifetime—is short, typically 10–5–10–9 s for electronic excited states and 10–15 for vibrational excited states. Relaxation of an analyte's excited-state, A*, occurs through several mechanisms, including collisions with other species in the sample, by photochemical reactions, and by the emission of photons. In the first process, which is called vibrational relaxation, or nonradiative relaxation, the excess energy is released as heat. A*→ A +heat Relaxation by a photochemical reaction may involve a decomposition reaction A*→ X + Y or a reaction between A* and another species A *+Z→ X + Y In both cases the excess energy is used up in the chemical reaction or released as heat. In the third mechanism, the excess energy is released as a photon of electromagnetic radiation. A*→ A +hν. The release of a photon following thermal excitation is called emission and that following the absorption of a photon is called photoluminescence. In chemiluminescence and bioluminescence, excitation results from a

chemical or biochemical reaction, respectively. Spectroscopic methods based on photoluminescence are the subject of the next section and atomic emissions covered in Section 10G.Photoluminescence Spectroscopy

Photoluminescence is divided into two categories: fluorescence and phosphorescence. A pair of electrons occupying the same electronic ground state have opposite spins and are said to be in a singlet spin state (Figure 10.47a). When an analyte absorbs an ultraviolet or visible photon, one of its valence electrons moves from the ground state to an excited state with a conservation of the electron's spin (Figure 10.47b). Emission of a photon from the singlet excited state to the singlet ground state or between any two energy levels with the same spin is called fluorescence. The probability of fluorescence is very high and the average lifetime of an electron in the excited state is only 10–5–10–8 s. Fluorescence, therefore, decays rapidly once the source of excitation is removed. In some cases, an electron in a singlet excited state is transformed to a triplet excited state (Figure 10.47c) in which its spin is no longer paired with the ground state. Emission between a triplet excited state and a singlet ground state or between any two energy levels that differ in their respective spin states–is called phosphorescence. Because the average lifetime for phosphorescence ranges from 10–4–104 s, phosphorescence may continue for some time after removing the excitation source. The use of molecular fluorescence for qualitative analysis and semiquantitative analysis can be traced to the early to mid-1800s, with more accurate quantitative methods appearing in the 1920s. Instrumentation for fluorescence spectroscopy using a filter or a monochromator for wavelength selection appeared in, respectively, the 1930s and 1950s.

Although the discovery of phosphorescence preceded that of fluorescence by almost200 years, qualitative and quantitative applications of molecular phosphorescence did not receive much attention until after the development of fluorescence instrumentation.10F.1 Fluorescence and

Phosphorescence Spectra To appreciate the origin of fluorescence and phosphorescence we must consider what happens to a molecule following the absorption of a photon. Let's assume that the molecule initially occupies the lowest vibrational energy level of its electronic ground state, which is a singlet state labeled S0 in Figure 10.48. Absorption of a photon excites the molecule to one of several vibrational energy levels in the first excited electronic state, S1, or the second electronic excited state, S2, both of which are singlet states. Relaxation to the ground state occurs by a number of mechanisms, some involving the emission of photons and others occurring without emitting photons. These relaxation mechanisms are shown in Figure 10.48. The most likely relaxation pathway is the one with the shortest lifetime for the excited state. radiation less deactivation When a molecule relaxes without emitting a photon we call the process radiation less deactivation. One example of radiation less deactivation is vibrational relaxation, in which a molecule in an excited vibrational energy level loses energy by moving to a lower vibrational energy level in the same electronic state. Vibrational relaxation is very rapid, with an average lifetime of <10–12 s. Because vibrational relaxation is so efficient, a molecule in one of its excited state's higher vibrational energy levels quickly returns to the excited state's lowest vibrational energy level. Another form of radiation less deactivation is an internal conversion in which a molecule in the ground vibrational level of an excited state passes directly into a higher vibrational energy level of a lower energy electronic state of the same spin state. By a combination of internal conversions and vibrational relaxations, a molecule in an excited electronic state may return to the ground electronic state without emitting a photon. A related form of radiation less deactivation is an external conversion in which excess energy is transferred to the solvent or to another component of the sample's matrix. A final form of radiation less deactivation is an intersystem crossing in which a molecule in the ground vibrational energy level of an excited electronic state passes into a higher vibrational energy level of a lower energy electronic state with a different spin state. For example, an intersystem crossing is shown in

Figure 10.48 between a singlet excited state, S1, and a triplet excited state, T1. RELAXATION BY FLUORESCENCE. Fluorescence occurs when a molecule in an excited state's lowest vibrational energy level returns to a lower energy electronic state by emitting a photon. Because molecules return to their ground state by the fastest mechanism, fluorescence is observed only if it is a more efficient means of relaxation than a combination of internal conversions and vibrational relaxations. A quantitative expression of fluorescence efficiency is the fluorescent quantum yield which is the fraction of excited state molecules returning to the ground state by fluorescence. Fluorescent quantum yields range from 1, when every molecule in an excited state undergoes fluorescence, to0 when fluorescence does not occur. The intensity of fluorescence, If, is proportional to the amount of radiation absorbed by the sample, P0 – PT, and the fluorescent quantum yield

$$I_f = k\Phi_f(P_0 - P_T) \qquad \frac{P_T}{P_0} = 10^{-\varepsilon bC} \qquad I_f = k\Phi_f P_0(1 - 10^{-\varepsilon bC}) \qquad I_f = 2.303k\Phi_f \varepsilon bCP_0 = k'P_0$$

where k is a constant accounting for the efficiency of collecting and detecting the fluorescent emission. From Beer's law we know that When εbC < 0.01, which often is the case when concentration is small, equation 10.27 simplifies to where k′ is a collection of constants. The intensity of fluorescent emission, therefore, increases with an increase in the quantum efficiency, the source's incident power, and the molar absorptivity and the concentration of the fluorescing species. Fluorescence is generally observed when the molecule's lowest energy absorption is a π é π* transition, although some n é π* transitions show weak fluorescence. Most unsubstituted, nonheterocyclic aromatic compounds have favorable fluorescence quantum yields, although substitutions on the aromatic ring can significantly affect Φf. For example, the presence of an electron-withdrawing group, such as –NO2, decreases Φf, while adding an electron-donating

group, such as –OH, increases Φf. Fluorescence also increases for aromatic ring systems and for aromatic molecules with rigid planar structures. Figure 10.49 shows the fluorescence of quinine under a UV lamp. A molecule's fluorescent quantum yield is also influenced by external variables, such as temperature and solvent. Increasing the temperature generally decreases Φf because more frequent collisions between the molecule and the solvent increases external conversion. A decrease in the solvent's viscosity decreases Φf for similar reasons. For an analyte with acidic or basic functional groups, a change in pH may change the analyte's structure and its fluorescent properties. As shown in Figure 10.48, fluorescence may return the molecule to any of several vibrational energy levels in the ground electronic state. Fluorescence, therefore, occurs over a range of wavelengths. Because the change in energy for fluorescent emission is generally less than that for absorption, a molecule's fluorescence spectrum is shifted to higher wavelengths than its absorption spectrum. RELAXATION BY PHOSPHORESCENCEA molecule in a triplet electronic excited state's lowest vibrational energy level normally relaxes to the ground state by an intersystem crossing to a singlet state or by an external conversion. Phosphorescence occurs when the molecule relaxes by emitting a photon. As shown in Figure 10.48, phosphorescence occurs over a range of wavelengths, all of which are at lower energies than the molecule's absorption band. The intensity of phosphorescence, is given by an equation similar to equation 10.28 for fluorescence

$$I_p = 2.303k\Phi_p \varepsilon bCP_o = k'P_o$$

where Φp is the phosphorescent quantum yield. Phosphorescence is most favorable for molecules with n é π* transitions, which have a higher probability for an intersystem crossing thanπ é π* transitions. For example, phosphorescence is observed with aromatic molecules containing carbonyl groups or heteroatoms. Aromatic compounds containing halide atoms also have a higher efficiency for phosphorescence. In general, an increase in phosphorescence

corresponds to a decrease in fluorescence. Because the average lifetime for phosphorescence is very long, ranging from 10–4–104 s, the phosphorescent quantum yield is usually quite small.

An improvement in Φp is realized by decreasing the efficiency of external conversion. This may be accomplished in several ways, including lowering the temperature, using a more viscous solvent, depositing the sample on a solid substrate, or trapping the molecule in solution. Figure 10.50 shows an example of phosphorescence.

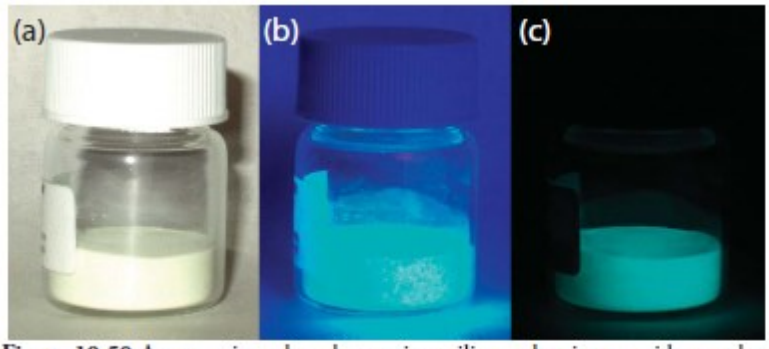


Figure 10.50 An europium doped strontium silicate-aluminum oxide powder under (a) natural light, (b) a long-wave UV lamp, and (c) in total darkness. The photo taken in total darkness shows the phosphorescent emission. Source: modified from Splarka (commons.wikipedia.org).

EXCITATION VERSUS EMISSION SPECTRA

Photoluminescence spectra are recorded by measuring the intensity of emitted radiation as a function of either the excitation wavelength or the emission wavelength. An excitation spectrum is obtained by monitoring emission at a fixed wavelength while varying the excitation wavelength. When corrected for variations in the source's intensity and the detector's response, a sample's excitation spectrum is nearly identical to its absorbance spectrum. The excitation spectrum provides a convenient means for selecting the best excitation wavelength for a quantitative or qualitative analysis. In an emission spectrum a fixed wavelength is used to excite the sample and the intensity of emitted radiation is monitored as function of wavelength. Although a molecule has only a single excitation spectrum, it has two emission spectra, one for

fluorescence and one for phosphorescence. Figure 10.51 shows the UV absorption spectrum and the UV fluorescence emission spectrum for tyrosine.

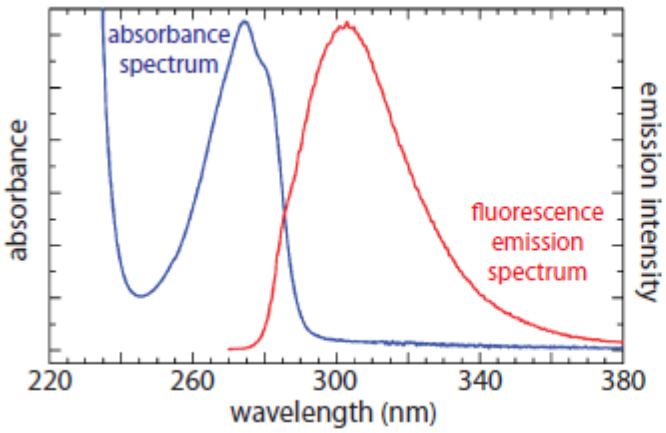


Figure 10.51 Absorbance spectrum and fluorescence emission spectrum for tyrosine in a pH 7, 0.1 M phosphate buffer. The emission spectrum uses an excitation wavelength of 260 nm. Source: modified from Mark Somoza (commons.wikipedia.org).

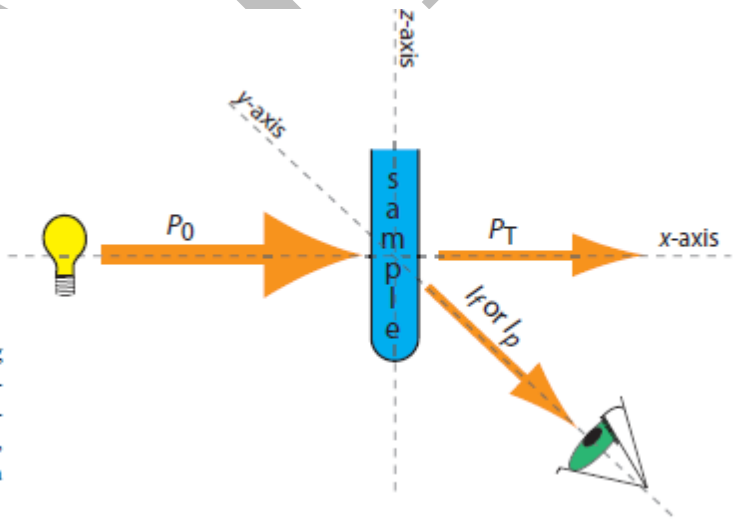


Figure 10.52 Schematic diagram showing the orientation of the source and the detector when measuring fluorescence and phosphorescence. Contrast this to Figure 10.21, which shows the orientation for absorption spectroscopy.

Instrumentation

The basic instrumental needs for monitoring fluorescence and phosphorescence—a source of radiation, a means of selecting a narrow band of radiation, and a detector—are the

same as those for absorption spectroscopy. The unique demands of both techniques, however, require some modifications to the instrument designs seen earlier in Figure 10.25 (filter photometer), Figure 10.26 (single-beam spectrophotometer), Figure 10.27(double-beam spectrophotometer), and Figure 10.28 (diode array spectrometer). The most important difference is the detector cannot be placed directly across from the source. Figure 10.52 shows why this is the case. If we place the detector along the source's axis it will receive both the transmitted source radiation, PT, and the fluorescent, If, or phosphorescent, radiation. Instead, we rotate the director and place it at 90o to the source. INSTRUMENTS FOR MEASURING FLUORESCENCE. Figure shows the basic design of an instrument for measuring fluorescence, which includes two wavelength selectors, one for selecting an excitation wavelength from the source and one for selecting the emission wavelength from the sample. In a fluorimeter the excitation and emission wavelengths are selected using absorption or interference filters. The excitation source for a fluorimeter is usually a low-pressure Hg vapor lamp that provides intense emission lines distributed throughout the ultraviolet and visible region (254, 312, 365, 405, 436, 546, 577, 691, and 773nm). When a monochromator is used to select the excitation and emission wavelengths, the instrument is called a Spectro fluorimeter. With a monochromator the excitation source is usually high-pressure Xe arc lamp, which has a continuous emission spectrum. Either instrumental design is appropriate for quantitative work, although only a spectrofluorimetric can be used to record an excitation or emission spectrum. The sample cells for molecular fluorescence are similar to those for molecular absorption. Remote sensing with fiber optic probes also can be adapted for use with either a fluorimeter or Spectro fluorimeter. An analyte that is fluorescent can be monitored directly. For analytes that are not fluorescent, a suitable fluorescent probe molecule can be incorporated into the tip of the fiber optic probe. The analyte's reaction with the probe molecule leads to an increase or decrease in fluorescence.

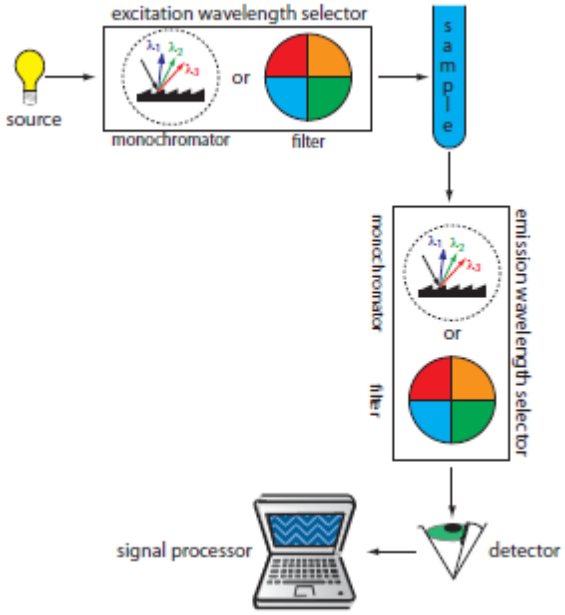


Figure 10.53 Schematic diagram for measuring fluorescence showing the placement of the wavelength selectors for excitation and emission. When a filter is used the instrument is called a fluorimeter, and when a monochromator is used the instrument is called a spectrofluorimeter.

INSTRUMENTS FOR MEASURING PHOSPHORESCENCE

Instrumentation for molecular phosphorescence must discriminate between phosphorescence and fluorescence. Because the lifetime for fluorescence is shorter than that for phosphorescence, discrimination is easily achieved by incorporating a delay between exciting the sample and measuring phosphorescent emission. Figure 10.54 shows how two out-of-phase choppers can be used to block emission from reaching the detector when the sample is being excited, and to prevent source radiation from reaching the sample while we are measuring the phosphorescent emission. Because phosphorescence is such a slow process, we must prevent the excited state from relaxing by external conversion. Traditionally, this has been accomplished by dissolving the sample in a suitable organic solvent, usually a mixture of ethanol, is open Tane,

and diethyl ether. The resulting solution is frozen at liquid-N2 temperatures, forming an optically clear solid. The solid matrix minimizes external conversion due to collisions between the analyte and the solvent. External conversion also is minimized by immobilizing the sample on a solid substrate, making possible room temperature measurements. One approach is to place a drop of the solution containing the analyte on a small disc of filter paper. After drying the sample under a heat lamp, the sample is placed in the Spectro fluorimeter for analysis. Other solid surfaces that have been used include silica gel, alumina, sodium acetate, and sucrose. This approach is particularly useful for the analysis of thin layer chromatography plates.

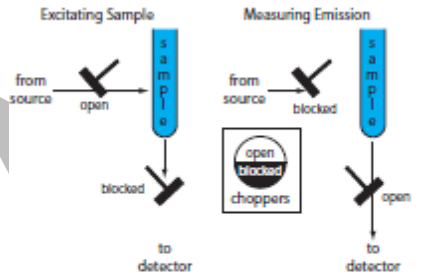


Figure 10.54 Schematic diagram showing how choppers are used to prevent fluorescent emission from interfering with the measurement of phosphorescent emission.

Interpretation

Inductively coupled plasma-optical emission spectrometry (ICP-OES) is an attractive technique that has led many analysts to ask whether it is wiser to buy an ICP-OES or to stay with their trusted atomic absorption technique (AAS) (1).More recently, a new and more expensive technique, inductively coupled plasma-mass spectrometry(ICP-MS), has been introduced as a routine tool (2).ICP-MS offers initially, albeit at higher cost, the advantages of ICP-OES and the

detection limit advantages of graphite furnace-atomic absorption spectrometry (GF-AAS). Unlike the famous prediction by Fassel, "…that AAS would be dead by year 2000….", low cost flame AAS will always have a future for the small lab with simple needs.

GF-AAS interferences

Spectral

There are only a few spectral interferences in GF-AAS when deuterium background correction is used, e.g. effect of Fe on Se, at 196.0 nm but these are interferences can be eliminated by the use of Zeeman GF-AAS.

Background

For many matrices careful programming of the ash stage is required to minimize the background signal during the atomization. The use of chemical modifier scan be helpful in increasing the allowable ash temperature. For example, a Ni chemical modifier for Se determinations allows ash temperatures of up to1000 ºC before Se loss. The use of Zeeman background correction can give an improvement in accuracy compared with D2 arc background correction in many GF-AAS applications.

Vapor phase interferences

These can be caused by the atomization of the analyte into a cooler gas environment. These interferences have been minimized in recent years by isothermal tube design, and use of platforms to delay the atomization of the analyte, whereby the sample is atomized into a hot inert gas environment.

Matrix effects

Matrix effects are exhibited by varying retention of the analyte on the graphite tube depending on the sample type. The dry and ash stages can have a dramatic effect on the shape of the transient peak. The use of matrix modifiers (e.g. $PdCl_2$) and hot injection can be quite effective in minimizing these effects and the use of peak area measurement can be advantageous in some cases.

Flame AAS interferences

Chemical

Due to the low temperature of the air/acetylene flame (2,200 ºC), there are many chemical interferences examples are PO4 on Ca and the effects of precious metals on other precious metals. The use of "releasing agents" can overcome these interferences, e.g. Lanthanum Chloride for the Ca in phosphate solutions and Uranium Oxide or Lanthanum Oxide for precious metals. The list is very long so methodology involves much work on accuracy before routine analysis can be accepted. For many, these interferences are well documented, but work with reference materials to ascertain the accuracy is often useful.

Matrix effects

Flame AAS, like ICP-MS and ICP-OES, uses a nebulizer and spray, so it has similar interferences such as viscosity differences between samples and calibration standards. Matrix matching is often mandatory (due to direct aspiration of the sample) and the method of standard additions is often used, especially because an internal standard is not possible on modern AA spectrophotometers. Spray chamber" adaptation" effects are less in Flame AAS probably due to the large droplet size and volume of aerosol in the spray chamber.

Background effects

For most applications the flame creates a different spectral background for different samples when compared with blank and standards. It is for this reason most elements are determined with the use of background correction, this involves the use of aD2 continuum source.

Ease of use

For routine analyses, ICP-OES has matured in automation to the point where relatively unskilled personnel can use methods created by the ICP-OES specialist, similar in ease of use as flame AAS. Until recently, ICP-MS was still the domain of the specialist chemist making fine adjustments before performing routine analysis. The trend to simplicity has been evident since 1993 and will continue in the future. One of the reasons for this is full computer control of parameters stored within a method. Another reason is the use of a multitasking graphical user interface, to show the operator several indicators of data integrity on the same screen. The use of such software also has a very positive effect on method development time, in the hitherto complex subject of ICP-MS. GF-AAS, although relatively simple for routine analysis, requires considerable skill in setting up the methods.

Total dissolved solids (TDS)

Recent ICP-OES spectrometers have been able to analyze routinely up to 10% TDS and even up to30% for simple salt solutions. Although the analysis of 0.5% TDS for ICP-MS may be possible for a limited time-scale, most chemists are happier with0.2% maximum TDS. This should be borne in mind when the original sample is a solid. The ultimate detection limit for some elements in ICP-MS may not be so impressive when expressed in the solid, compared with ICP-OES. Flame AAS can usually cope with up to 5% TDS although this figure is reduced to about 1% for N2O/C2H2 flame work. GFAA Scan cope with extremely high levels of dissolved solids.

Linear dynamic range (LDR)

ICP-MS can have a LDR in excess of 105. Various methods for extending the linear range up to 107include de-sensitizing one of the ion lenses, use of detector analog mode, or use of a separate faraday cup as a second detector. These should be used with caution, however, as high matrix component concentrations may cause problems best solved by dilution, and/or have different curve characteristics for the extended range. For this reason, and because of the problems with high levels of dissolved solids, ICP-MS should be mainly the domain of trace/ultra-trace analysis. The exception is when using isotope dilution. With the isotope dilution technique, very good results have been obtained with high concentrations. Flame AAS has a LDR of approximately 103, so constant dilutions for the various elements may be required. It is for this reason, over-range dilution using an auto-diluter is very important for much flame AAS work. GF-AAS has a very limited LDR of102 -103. It can be used for higher concentrations less sensitive line is available and selected. Trace to major element analysis may be performed by ICP-OES because of its excellent 106 LDR. ICP-OES is ideal for analysis up to and including percentage levels using radial viewing. For this reason ICP-OES, in addition to ICP-MS or GF-AAS, is often needed to fulfill laboratory requirements.

Precision

The short-term (in-run) precision of ICP-MS is generally1-3%. This is improved routinely by use of multiple internal standards. The longer term precision (over a period of hours) is still <5% RSD. The use of isotope dilution can give results of very high precision and accuracy, although the cost can be prohibitive for routine analysis, due to the cost of the standards. ICP-OES has generally in-run precision of 0.3-2%RSD and again less than 3% RSD over several hours (for some spectrometers <1% for 4 hours) (3). GFAAS, however, will generally have short-term precision of 0.5-5% RSD. Long-term precision is a function of the

number of graphite tube firings, rather than time. In-run precision of flame AAS is excellent0.1-1%, however, long term precision is poor especially if nitrous oxide/acetylene flame is used. Constant manual "de-coking" of the burner may be required by the operator.

Sample throughput

The ICP-MS has an incredible capacity to analyze a vast number of samples for trace elements. Typical analysis time is less than 5 minutes/sample for the whole suite of required trace elements. For some applications this may only take a couple of minutes. Consulting laboratories find the sample through put a major advantage. While the speed of ICP-OES will depend on whether simultaneous or sequential instruments are used; generally this can vary from 2 to 6 minutes. Simultaneous ICP-OES can be faster, typically 2minutes/sample, but sometimes its accuracy can be compromised by spectral interferences present with some types of samples (e.g. rocks). As the detection limit can be better on a sequential ICP spectrometer, the integration times are typically shorter and therefore for a limited number of elements maybe faster than simultaneous ICP. Sometimes there is a need for speed due to the limited sample volume available (e.g. 2 mL). In this case the latest micro-concentric nebulizers have given analysts the power of similar LODs with only100 µL/minute sample consumption. The speed of GF-AAS is typically 3-4 minutes per element per sample (assuming 2 replicates).Automated overnight runs can be performed, and this will improve throughput of samples. Total sample through put for trace element analysis can be a major factor in favor of ICP-MS in the busy laboratory. The following examples (expressed as solution concentrations), will give a guide:1. One to three elements/sample, at sub/low-ppb concentration will generally be better by GF-AAS, assuming the elements of interest can be determined by this technique.2. Five to twenty elements/sample at

10's of ppb to percent levels will generally be better by ICP-OES.3. Six or more elements/sample at sub ppb and ppb concentrations will generally be better by ICP-MS, if the number of samples to be analyzed is high.4. One to four elements for ppm to percent levels, flame AAS may be attractive, depending on the element and sample load. Table 3 (page 10) presents detection limit comparison data for a number of elements for ICP-MS, ICPOES, Flame AAS and GFAAS.

Unattended operation

ICP-MS, ICP-OES and GF-AAS can all operate unattended overnight because of the modern automated designs and the safety inherent in the use of inert argon gas in these techniques. For highest productivity, overnight operation is mandatory. For reasons of safety, it is not possible to leave a flame AAS left unattended for any period of time.

Cost of ownership

The running cost of ICP-MS is more than ICP-OES because several components have a limited lifetime and have to be replaced. These include the turbo molecular pumps, the sampler and skimmer cones and the detector. The torch and nebulizer have similar lifetimes for both ICP-OES and ICP-MS techniques. If ICP-OES is chosen instead of ICP-MS the laboratory may require GF-AAS as well if sub-ppb levels are required in the samples. The cost of graphite tubes for the GF-AAS has to be taken into account. In all three techniques the cost of argon isa significant budget item, with the ICP techniques requiring more than GF-AAS. Flame AAS will always be a low running cost item because the hollow cathode lamps and the occasional replacement of the nebulizer are the main consumable costs.

Capital cost

This is always a difficult subject to quantify because it will depend on the amount of automation, the accessories and the supplier. In very approximate terms, you can estimate that an

ICPOES will cost twice as much as a GF-AAS and 2-3times less than ICP-MS. It should be noted, however, that the accessories could distort these figures considerably. Another cost that needs to be taken into account is that ultra-trace analysis requires a clean laboratory and ultra-pure chemicals. These are not in expensive items. Flame AAS is a low cost investment. If only a few elements are required, for a limited number of samples, at ppm levels or above and refractory elements are not required, then the extra cost of other techniques may be difficult to justify. However, as the number of elements and samples increase, or the use of nitrous oxide flame for refractory elements become a requirement, the position shifts more to ICP-OES.

POSSIBLE QUESTIONS
UNIT-IV
PART-A (20 MARKS)
(Q.NO 1 TO 20 Online Examination)

PART-B (2 Marks)

1. Write a note on Atomic absorption

2. What is meant by Excitation?

3. Write a note on Atomic fluorescence

4. What are the detectors used in Atomic absorption emission, and fluorescence?

5. How will get the sample solid into liquid Mass spectrometry?

Part-C (6 Marks)

1. What are the difference between Atomic absorption and Atomic Emission?

2. Write detail about atomic fluorescence spectroscopy

3. Write details about flames, electrical discharges, plasmas?

4. How will detect the sample using Atomic absorption and emission spectroscopy?

5. What are the sources used in Atomic absorption, Atomic emission, and Atomic fluorescence? Give explanation?

Unit-IV-Multiple choice Questions

S. No	Question	Option- I	Option- II	Option- III	Option- IV	Answer
1.	Atomic spectra are an example of	line spectra	continuous spectra	band spectra	both a and b	line spectra
2.	Electron in the atom are held in the atom due to	coulombs force	nuclear force	atomic force	both a and b	coulombs force
3.	For an electron to be confined to a nucleus, its speed relative to the speed of light would have to be	equal	less	greater	equal to infinity	greater
4.	Absorbed wavelengths in atomic absorption spectrum appear as	dark background	dark lines	light background	light lines	dark lines
5.	Lines which appear in absorption and emission spectrum are	same	different	very different	far apart	same
6.	Background in atomic absorption spectrum is	bright	dark	brown	purple	bright
7.	The ionization energies are influenced by shielding effect of	outer electrons	inner electrons	middle electrons	protons	inner electrons
8.	Azimuthal quantum number is represented by	m	n	l	p	l
9.	In which period the decrease of	second	third	first	fourth	second

	atomic radii is very prominent					
10.	London dispersion forces were explained in	1978	1980	1987	1930	1930
11.	The number of electrons in valence shell of first element of period is	two	one	three	four	one
12.	The number of lobes in the fifth d-orbital are	four	eight	two	three	two
13.	Line spectrum is actually characteristic of	iquids	gases	atom	plasma	atom
14.	The modern method for separation of isotopes is	laser separation	chromatography	ionization	X-ray	laser separation
15.	The lines which appear in absorption and emission spectrum are	same	different	very different	far apart	same
16.	In Atomic Absorption Spectroscopy, which of the following is the generally used radiation source?	Tungsten lamp	Xenon mercury arc lamp	Hydrogen or deuterium discharge lamp	Hollow cathode lamp	Hollow cathode lamp
17.	n Atomic Absorption Spectroscopy, with what material is the cathode in Hollow cathode lamp constructed?	Tungsten	Quartz	Element to be investigated	Aluminium	Element to be investigated

18.	How can the intensity of radiation be increased in Hollow cathode lamp?	Addition of non-conductive protective shield of mica	Addition of nitrogen to neon or argon in the lamp	Increasing the pressure of the filling gas	Changing the metal of the anode	Addition of non-conductive protective shield of mica
19.	Which of the following is the function of the chopper in Atomic Absorption Spectroscopy?	To split the beam into two	To break the steady light into pulsating light	To filter unwanted components	To reduce the sample into atomic state	To break the steady light into pulsating light
20.	Which of the following is the function of Flame or Emission system in Atomic Absorption Spectroscopy?	To split the beam into two	To break the steady light into pulsating light	To filter unwanted components	To reduce the sample into atomic state	To reduce the sample into atomic state
21.	Which of the following is not a component of emission system in Flame photometer?	Burner	Atomiser	Fuel gases and their regulation	Chopper	Chopper
22.	Which of the following is the function of atomiser in the emission system of Atomic Absorption Spectroscopy?	To split the beam into two	To break the steady light into pulsating light	To break large mass of liquid into small drops	To reduce the sample into atomic state	To break large mass of liquid into small drops
23.	Which of the following is not a fuel used in flame photometry	Acetylene	Propane	Hydrogen	Camphor oil	Camphor oil
24.	Which of the following is not the requirement of a good flame in flame photometer?	Liquid sample must be evaporated to	Solid residue must decompose to form atoms	Atoms must be produced such that they have the	Atoms must be produced such that they	Atoms must be produced such that they are in

		form solid residue		ability to get excited to higher states	are in stable state	stable state
25.	At what pressure should the gases in the sealed tube be maintained in the Hollow cathode lamp?	1 to 5 torr	20 to 30 torr	40 to 50 torr	50 to 55 torr	1 to 5 torr
26.	An example of an absorption spectrum is the spectrum of	A mercury vapour lamp	Atomic hydrogen	Sodium vapour	The sun	A mercury vapour lamp
27.	Spectroscopy measures the change in behaviour of a molecule when it is exposed to which of the following?	A centrifugal force	Electromagnetic radiation	An electrical charge	Acidic conditions	Electromagnetic radiation
28.	Which of the following types of spectroscopy can tell us the most about the carbon framework of an organic compound?	UV-visible spectroscopy	Infra-red spectroscopy	NMR spectroscopy	Mass spectrometry	NMR spectroscopy
29.	In ionization chamber vapors are bombarded with fast moving	protons	electrons	neutrons	antineutron	electrons
30.	In mass spectrometer M/E is plotted on	x axis	y axis	z axis	x and y axis	x axis
31.	The correct order for the basic features of a mass spectrometer is...	acceleration, deflection, detection,	ionisation, acceleration, deflection,	acceleration, ionisation, deflection,	acceleration, deflection, ionisation,	ionisation, acceleration, deflection,

		ionisation	detection	detection	detection	detection
32.	Which one of the following statements about ionisation in a mass spectrometer is incorrect?	gaseous atoms are ionised by bombarding them with high energy electrons	atoms are ionised so they can be accelerated	atoms are ionised so they can be deflected	it doesn't matter how much energy you use to ionise the atoms	it doesn't matter how much energy you use to ionise the atoms
33.	The path of ions after deflection depends on...	only the mass of the ion	only the charge on the ion	both the charge and the mass of the ion	neither the charge nor the mass of the ion	both the charge and the mass of the ion
34.	Which of the following is not a use for mass spectrometry?	calculating the isotopic abundance in elements	investigating the elemental composition of planets	confirming the presence of O-H and C=O in organic compounds	calculating the molecular mass of organic compounds	confirming the presence of O-H and C=O in organic compounds
35.	Which one of the following pieces of information cannot be obtained from an infra-red spectrum?	the molecular mass	the presence of C=O bonds	the presence of O-H bonds	the identity of a compound through comparison with other spectra	the molecular mass
36.	The region of an infra-red spectrum where many absorptions take place is known	thumbprint region	handprint region	footprint region	fingerprint region	fingerprint region

	as the...					
37.	To work out the molecular mass of an organic molecule you would look at its...	infrared spectrum	proton nmr spectrum	mass spectrum	boiling point	mass spectrum
38.	Which one of the following methods would be best for finding the identity of an organic compound?	finding the m/z value of the molecular ion in its mass spectrum	its proton nmr spectrum	measuring its melting point	comparing its infrared spectrum with known examples	comparing its infrared spectrum with known examples
39.	When crystalline solids' properties are changed with respect to direction the phenomenon is called	spectroscopy	anisotropy	spectrometry	Xray	anisotropy
40.	The analysis on the Kennedy assassination bullets were performed by the technique of:	x-ray diffraction	inductively coupled plasma emission spectrometry.	atomic absorption spetrophotometer.	neutron activation analysis.	
41.	Inductively coupled plasma emission spectrometry has been successfully applied in the identification and characterization of:	glass fragments and mutilated bullets	paint chips and soil comparisons.	glass fragments and paint chips.	mutilated bullets and paint chips.	glass fragments and mutilated bullets.
42.	Which technique has been in use for chemical analysis since 1955, but has not found wide	Atomic absorption spectrophotometer	X-ray diffraction	Inductively coupled plasma emission	Neutron activation analysis	Atomic absorption spectrophotometer

	application for solving forensic problems?			spectrometry		
43.	The major advantage to this technique is that it provides a nondestructive method for identifying and quantifying trace elements.	X-ray diffraction	Neutron activation analysis	Atomic absorption spectrophotometer	Inductively coupled plasma emission spectrometry	Neutron activation analysis
44.	In atomic absorption spectroscopy, radiation is used to cause electrons to move to an excited state. When these electrons return to the ground state ...	no radiation is emitted because this is absorption spectroscopy	light is emitted with a very different wavelength to the light absorbed	radiation is emitted that interferes with the study of the light absorbed by the sample	radiation is emitted, but this radiation does not interfere with the analysis because the original radiation was in a pulsed form.	radiation is emitted, but this radiation does not interfere with the analysis because the original radiation was in a pulsed form.
45.	Which alternative lists techniques usually applied to the analysis of organic molecules?	High performance liquid chromatography and gravimetric analysis using precipitation reactions.	Infrared spectroscopy and nuclear magnetic resonance spectroscopy	Emission spectroscopy and gas liquid chromatography	UV spectroscopy and atomic absorption spectroscopy	Infrared spectroscopy and nuclear magnetic resonance spectroscopy.

46.	The presence of helium in the Sun was first detected by scientists using a knowledge of ...	nuclear magnetic resonance (NMR) spectroscopy	emission spectroscopy	mass spectroscopy	infrared spectroscopy	emission spectroscopy
47.	Which of the following analyses is qualitative only?	Use of gravimetric analysis to determine the molar mass of an organic compound	Analysis of the blood alcohol content of a motorist.	Measurement of the level of a banned steroid in the urine of an athlete	Detection of strontium ions in polluted water	Detection of strontium ions in polluted water
48.	An electronic transition takes place so rapidly that a vibrating molecule does not change its internuclear distance appreciably during the transition. This principle is known as	Paulis principle	Heisenberg principle	Franck condon principle.	Uncertinity principle	Franck condon principle.
49.	Which among the following is the laws of Photochemistry	Hooks law	Beer-Lambertz law	Charls law	Avagdros law.	Beer-Lambertz law
50.	Reciprocal of transmittance is called	Absorbance	Opacity	Incidence	Molar exinction coefficient	Opacity
51.	The quantity Ecl is known as	Absorbance	Opacity	Transmittance	absorbtivity	Absorbance

52.	If water used as solvent in UV absorption studies, ------ nm to be added with parent value.	-4	4	8	-8	-8
53.	Cycles/sec is unit for	Wavelength	frequency	wave number	energy	energy
54.	The arrangement of all type of electromagnetic radiation in the order of -------- wavelength is called electromagnetic spectrum.	increasing	decreasing	same	approximate	increasing
55.	Greater value of Molar extinction coefficient indicates that the probability of transition------	less	more	nil	zero	more
56.	Molar extinction coefficient value less than ------- is called forbidden transition	1	10	100	1000	100
57.	According to spin conservation rule, which of the following is allowed transition	singlet to singlet	singlet to triplet	triplet to singlet	none	singlet to singlet
58.	The time taken for an electronic transition is very small compared to time taken for one vibration. This principle is known as	Paulis principle	Frank-condon principle	predissociation	Hunds principle	Frank-condon principle
59.	In predissociation, some	Higher	lower	zero	without	lower

	molecules dissociation occurs at ------ energy					
60.	One nm is equal to	10A°	0.1 A°	10-9 cm	10-8 cm	10A°

UNIT V
Syllabus

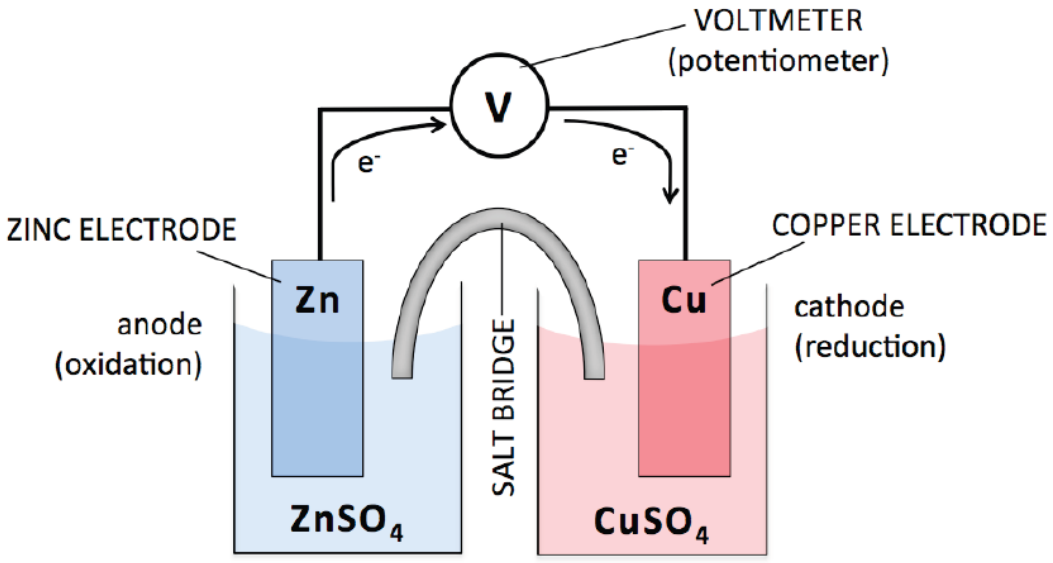
NMR spectroscopy: **P**rinciple, Instrumentation, Factors affecting chemical shift, Spincoupling, Applications.
Electroanalytical Methods: Potentiometry & Voltammetry

Radiochemical Methods

X-ray analysis and electron spectroscopy (surface analysis)

Electrochemical cell

Potentiometry is one type of electrochemical analysis methods. Electrochemistry is a part of chemistry, which determines electrochemical properties of substances. An electrical circuit is required for measuring current (unit: ampere, A) and potential (also voltage, unit: volt (V)) created by movement of charged particles. Galvanic cell (electrochemical cell, Fig. 1) serves as an example of such system.



. A galvanic electrochemical cell.

Electrochemical cell consists of two solutions connected by a salt bridge and electrodes to form electrical circuit. Sample cell on figure 1 consists of solutions of ZnSO4 and CuSO4. Metallic Zn and Cu electrodes are immersed in respective solutions. Electrodes have contacts firstly through wires connected to the voltmeter and secondly through solutions and a salt bridge, forming an

electric circuit. Salt bridge consists of a tube filled with saturated salt solution (e.g. KCl solution). The ends of the tube are capped with porous frits that prevent solutions from mixing, but permit movement of ions.

Three distinct charge transfer processes are described for the system in Fig 1:

1. Electrons move in electrodes and wires from zinc electrode to copper electrode.

2. Ions move in solutions:

a. In solution on the left, zinc ions move away from the electrode and sulfate ions move towards it.

b. In solution on the right, copper ions move towards the electrode and negatively charged ions (sulfate) away from it.

c. In salt bridge positive ions move right and negative ions left.

3. On the surfaces of electrodes electrons are transferred to ions or vice versa:

Zinc electrode dissolves:

b. Metallic copper is deposited on the electrode surface:

$$Zn \rightarrow Zn^{2+} + 2e^-$$

$$Cu^{2+} + 2e^- \rightarrow Cu$$

Three processes mentioned above are important parts of a closed electrical circuit making the flow of electrical current possible. Potential on an electrode depends on the ions present in the solution and their concentration. These way electrochemical cells can be used to determine ions and their concentration in solution. The dependence of potential between electrodes from concentration of ions is expressed by Nernst equation (Eq. 1).

$$E = E_0 - \frac{RT}{nF} \ln a$$

E - Electrode potential,

E_0 - Standard potential of the electrode,

R - Universal gas constant (8.314 J/(K*mol)),

F - Faraday constant (96485 C/mol),

T - Temperature in kelvins,

n - Charge of the ion or number of electrons participating in the reaction,

a - Activity of the ions.

Activity of the ions is a function of concentration. For solutions with concentrations lower than about 0.1 mol/l, activity can be approximated to concentration. Thus a logarithmic dependence exists between potential and the activity (concentration) of ions in solution.

Potentiometry

Potential is measured under the conditions of no current flow The measured potential is proportional to the concentration of some component of the analyte The potential that develops in the electrochemical cell is the result of the free energy change that would occur if the chemical phenomena were to proceed until the equilibrium condition has been satisfied.

Potentiometry is based on the measurement of the potential of an electrode system (e.g. electrochemical cell). Potentiometric measurement system consists of two electrodes called reference and indicator electrode, potentiometer and a solution of analyte (figure 2). Reference electrode is an electrode with potential which is a) independent of concentration of analyte (or other) ions in solution; b) independent of temperature. Potential of an indicator electrode depends mainly on the concentration of the analyte ions (in this case hydrogen ions). Potentiometric measurements enable selective detection of ions in presence of multitude of other substances. In case of figure 2, the potential of the indicator electrode is sensitive to hydrogen ions. In a system

like this, the potential is measured in reference to a calomel electrode, e.g. calomel electrode functions as the reference electrode.

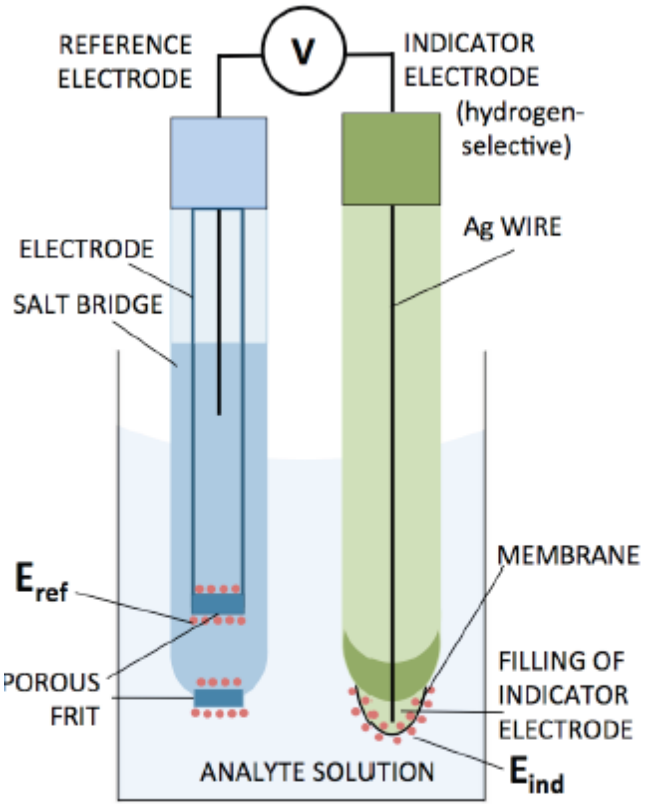


Figure 2. Potentiometric measurement system (for pH measurement)

Voltammetry

Introduction

Historically, the branch of electrochemistry we now call voltammetry developed from the discovery of polarography in 1922 by the Czech chemist Jaroslav Heyrovsky, for which he

received the 1959 Nobel Prize in chemistry. The early voltammetry methods experienced a number of difficulties, making them less than ideal for routine analytical use. However, in the 1960s and 1970s significant advances were made in all areas of voltammetry (theory, methodology, and instrumentation), which enhanced the sensitivity and expanded the repertoire of analytical methods. The coincidence of these advances with the advent of low-cost operational amplifiers also facilitated the rapid commercial development of relatively inexpensive instrumentation.

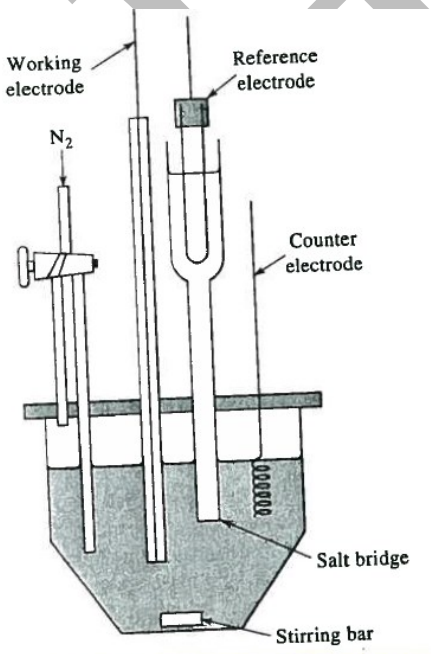
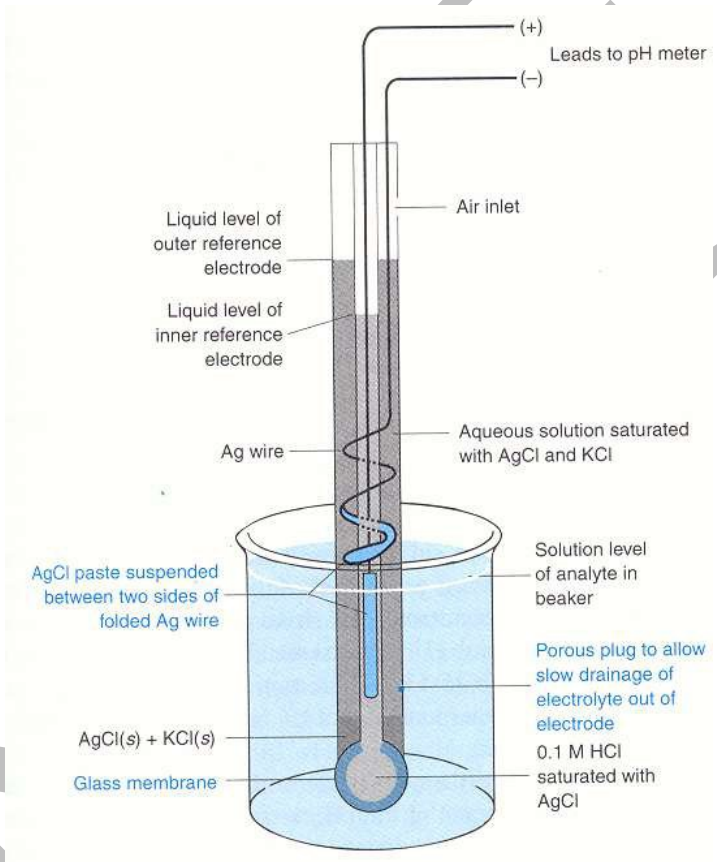


FIGURE 25-8 A three-electrode cell for hydrodynamic voltammetry.

Instrumentation

The basic components of a modern electro analytical system for voltammetry are a potentiostat, computer, and the electrochemical cell (Fig. 37.1). In some cases the potentiostat and computer are bundled into one package, whereas in other systems the computer and the A/D and D/A converters and microcontroller are separate, and the potentiostat can operate independently.



The Electrodes and Cell

A typical electrochemical cell consists of the sample dissolved in a solvent, an ionic electrolyte, and three (or sometimes two) electrodes. Cells (that is, sample holders) come in a variety of sizes, shapes, and materials. The type used depends on the amount and type of sample,

the technique, and the analytical data to be obtained. The material of the cell (glass, Teflon, polyethylene) is selected to minimize reaction with the sample. In most cases the reference electrode should be as close as possible to the working electrode; in some cases, to avoid contamination, it may be necessary to place the reference electrode in a separate compartment. The unique requirements for each of the voltammetric techniques are described under the individual techniques. Reference Electrodes The reference electrode should provide a reversible half-reaction with Nernstian behavior, be constant over time, and be easy to assemble and maintain. The most commonly used reference electrodes for aqueous solutions are the calomel electrode, with potential determined by the reaction $Hg_2Cl_2(s) + 2e^- = 2Hg(l) + 2Cl^-$ and the silver/silver chloride electrode (Ag/AgCl), with potential determined by the reaction $AgCl(s) + e^- = Ag(s) + Cl^-$. Table 37.1 shows the potentials of the commonly used calomel electrodes, along with those of some other reference electrodes. These electrodes are commercially available in a variety of sizes and shapes. Counter Electrodes In most voltammetric techniques the analytical reactions at the electrode surfaces occur over very short time periods and rarely produce any appreciable changes in bulk concentrations of R or O. Thus, isolation of the counter electrode from the sample is not normally necessary. Most often the counter electrode consists of a thin Pt wire, although Au and sometimes graphite have also been used. Working Electrodes The working electrodes are of various geometries and materials, ranging from small Hg drops to flat Pt disks. Mercury is useful because it displays a wide negative potential range (because it is difficult to reduce hydrogen ion or water at the mercury surface), its surface is readily regenerated by producing a new drop or film, and many metal ions can be reversibly reduced into it. Other commonly used electrode materials are gold, platinum, and glassy carbon.

Polarography

Even though polarography could be considered just another variation of technique within vol-tammetry, it differs from other voltammetric methods both because of its unique place in the history of electrochemistry and in respect to its unique working electrode, the dropping mercury electrode (DME). The DME consists of a glass capillary through which mercury flows under gravity to form a succession of mercury drops. Each new drop provides a clean surface at which the redox process takes place, giving rise to a current increase with increasing area as the drop grows, and then falling when the drop falls. Figure 37.3 shows a polarogram for a 1 M solution of HCl that is 5 mM in Cd^{2+}. The effect of drop growth and dislodging can be clearly seen. The potential when the current attains half the value of the plateau current is called the half-wave potential and is specific to the analyte's matrix. The plateau current is proportional to the concentration of analyte. For example, Fig. 37.4 shows a differential pulse polarogram for the acetyl derivative of chlordiazepoxide. In this case the peak height is proportional to the analyte concentration. The current for the polarographic plateau can be predicted by the Ilkovic equation:

$$i_d = 708nD^{1/2}m^{2/3}t^{1/6}c^0$$

where m is the rate of flow of the Hg through the capillary, t is the drop time, and c0 is the bulk analyte concentration. Even though polarography with the DME is the best technique for some analytical determinations, it has several limitations. Mercury is oxidized at potentials more positive than +0.2 V versus SCE, which makes it impossible to analyze for any analytes in the positive region of potential. Another limitation is the residual current that results from charging of the large capacitance of the electrode surface. By manipulating the potential and synchronizing potential pulses with current sampling, the same basic experiment can be made to yield a more useful result.

Cyclic Voltammetry

Cyclic voltammetry (CV) has become an important and widely used electroanalytical technique in many areas of chemistry. It is rarely used for quantitative determinations, but it is widely used for the study of redox processes, for understanding reaction intermediates, and for obtaining stability of reaction products. This technique is based on varying the applied potential at a working electrode in both forward and reverse directions (at some scan rate) while monitoring the current. For example, the initial scan could be in the negative direction to the switching potential. At that point the scan would be reversed and run in the positive direction. Depending on the analysis, one full cycle, a partial cycle, or a series of cycles can be performed. The response obtained from a CV can be very simple, as shown in Fig. 37.5 for the reversible redox system:

$$Fe(CN)_6^{-3} + e^- = Fe(CN)_6^{-4}$$

in which the complexed Fe (III) is reduced to Fe (II). The important parameters in a cyclic voltammogram are the peak potentials (Epc, Epa) and peak currents (ipc , ipa) of the cathodic and anodic peaks, respectively. If the electron transfer process is fast compared with other processes (such as diffusion), the reaction is said to be electrochemically reversible, and the peak separation is

$$\Delta E_p = |E_{pa} - E_{pc}| = 2.303\ RT/nF$$

Thus, for a reversible redox reaction at 25 °C with n electrons DEp should be 0.0592/n V or about 60 mV for one electron. In practice this value is difficult to attain because of such factors as cell resistance. Irreversibility due to a slow electron transfer rate results in DEp > 0.0592/n V, greater, say, than 70 mV for a one-electron reaction. The formal reduction potential (Eo) for a reversible couple is given by

$$E^o = \frac{E_{pc} + E_{pa}}{2}$$

For a reversible reaction, the concentration is related to peak current by the Randles–Sevcik expression (at 25 °C):

$$i_p = 2.686 \times 10^5 n^{3/2} A c^0 D^{1/2} \nu^{1/2}$$

where ip is the peak current in amps, A is the electrode area (cm2), D is the diffusion coefficient (cm2 s–1), c0 is the concentration in mol cm–3, and n is the scan rate in V s–1. Cyclic voltammetry is carried out in quiescent solution to ensure diffusion control. A three-electrode arrangement is used. Mercury film electrodes are used because of their good negative potential range. Other working electrodes include glassy carbon, platinum, gold, graphite, and carbon paste.

Pulse Methods

In order to increase speed and sensitivity, many forms of potential modulation (other than just a simple staircase ramp) have been tried over the years. Three of these pulse techniques, shown in Fig. 37.6, are widely used.

Normal Pulse Voltammetry (NPV)

This technique uses a series of potential pulses of increasing amplitude. The current measurement is made near the end of each pulse, which allows time for the charging current to decay. It is usually carried out in an unstirred solution at either DME (called normal pulse polarography) or solid electrodes. The potential is pulsed from an initial potential Ei. The duration of the pulse, t, is usually 1 to 100 msec and the interval between pulses typically 0.1 to 5

sec. The resulting voltammogram displays the sampled current on the vertical axis and the potential to which the pulse is stepped on the horizontal axis.

Differential Pulse Voltammetry (DPV)

This technique is comparable to normal pulse voltammetry in that the potential is also scanned with a series of pulses. However, it differs from NPV because each potential pulse is fixed, of small amplitude (10 to 100 mV), and is superimposed on a slowly changing base potential. Current is measured at two points for each pulse, the first point (1) just before the application of the pulse and the second (2) at the end of the pulse. These sampling points are selected to allow for the decay of the nonfaradaic (charging) current. The difference between current measurements at these points for each pulse is determined and plotted against the base potential.

Square-Wave Voltammetry (SWV)

The excitation signal in SWV consists of a symmetrical square-wave pulse of amplitude Esw superimposed on a staircase waveform of step height DE, where the forward pulse of the square wave coincides with the staircase step. The net current, inet, is obtained by taking the difference between the forward and reverse currents (ifor – irev) and is centered on the redox potential. The peak height is directly proportional to the concentration of the electro active species and direct detection limits as low as 10–8M are possible. Square-wave voltammetry has several advantages. Among these are its excellent sensitivity and the rejection of background currents. Another is the speed (for example, its ability to scan the voltage range over one drop during polarography with the DME). This speed, coupled with computer control and signal averaging, allows for experiments to be performed repetitively and increases the signal to- noise ratio. Applications of square-wave voltammetry include the study of electrode kinetics with regard to preceding, following, or catalytic homogeneous chemical reactions, determination of some species at trace levels, and its use with electrochemical detection in HPLC.

Radiochemical Methods

Radiochemical analysis is concerned with the determination of the radioactivity associated with the elements in a sample. Such measurements may subsequently be used to compute masses or concentrations, but the activity measurement is the primary one. The radiochemist must be familiar with the chemistry of many elements, including the rarer ones, and must know something of the physics of radioactive decay and radiation detection.

Radiochemical soiling materials

Radiochemical model soiling consists of the bulk soiling material, labelling radioisotope and solvent. The model soiling used should contain typical components of the environment of the evaluated materials. The natural soiling materials of buildings may contain inorganic particles, organic particles and oil components (Pesonen-Leinonen, 2003). In radiochemical studies, an inorganic compound chromium oxide (Cr_2O_3) has often been used as a model of natural inorganic soiling materials, whereas an organic compound chromium acetyl acetonate ($Cr(C_5H_7O_2)_3$) has used to represent natural organic soiling materials (Table 2). Triglyceride (triolein, $C_{57}H_{104}O_6$) has been used as a model of natural oils and sebum.

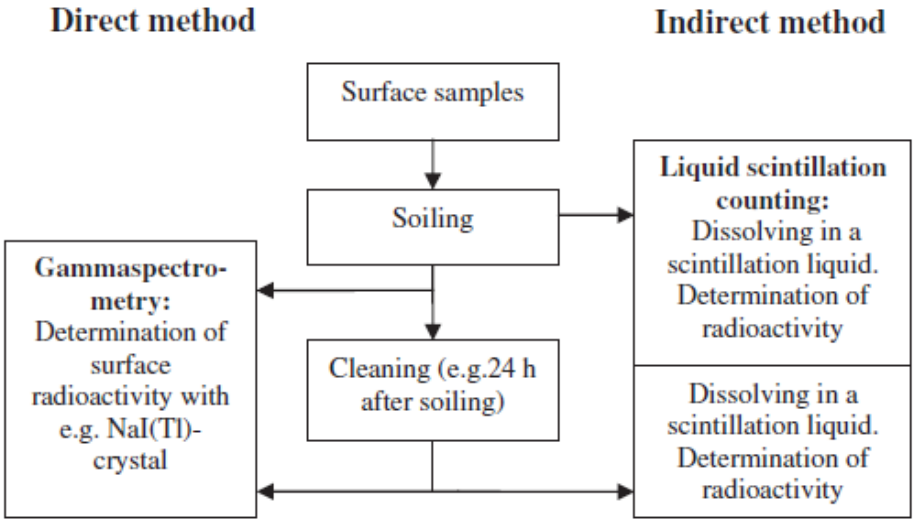


Fig. 1. Flow diagram of determination of the cleanability of radiochemical soiling materials with direct and indirect determination methods.

Radiochemical methods

Radiochemical methods tend to be labor intensive and generate liquid waste due to the chemical separations that are involved. Furthermore, detection limits tend to be high for long-lived isotopes using radiochemical methods.

Radioactive Isotopes:

Radioactive isotopes have unstable nuclei that spontaneously change to form more stable nuclei. As a result, either new isotopes or new elements are produced. In this process of nuclear change, three kinds of rays, called alpha, beta, and gamma rays, are emitted from the nuclei of radioactive isotopes. Alpha rays are a stream of positively charged helium ions (He^{2+}), which consist of two protons and two neutrons. Beta rays are a stream of electrons formed as neutrons

change into protons. The electrons are ejected from the nuclei, and protons remain in the nuclei. Gamma rays are a form of electromagnetic radiation released from nuclei as they lose energy.

All isotopes of an element have the same atomic number, and their chemical behavior is very similar. For example, tritium can substitute for hydrogen, and either 125 iodine or 131 iodine can substitute for 126 iodine in chemical reactions.

Clinicians and researchers commonly use radioactive isotopes because sensitive measuring devices can detect the radioactive rays emitted from isotopes, even when they are present in very small amounts. Several procedures that are used to determine the concentration of substances such as hormones depend on the incorporation of small amounts of radioactive isotopes such as hormones depend on the incorporation of small amounts of radioactive isotopes such as 125 iodine into the substances being measured. Disorders of the thyroid gland, the adrenal gland, and the reproductive organs can be more accurately diagnosed using these procedures.

Radioactive isotopes are also used to treat cancer. Some of the particles released from isotopes have a very high energy content and can penetrate and destroy tissues. Thus radioactive isotopes can be used to destroy rapidly growing tumors, which are more sensitive to radiation than healthy cells. Radiation can also be used to sterilize materials that cannot be exposed to high temperatures (for example, some fabric and plastic items used during surgical procedures). In addition, radioactive emissions provide a convenient and safe method of sterilizing food and other items.

Radioactive isotopes are given as liquids, either in capsules that are swallowed or as a drink, or by injection into a vein (an intravenous injection). The most common form of radioisotope treatment is radioactive iodine. Used to treat tumors of the thyroid gland, it is given as an odorless and colorless drink. The same safety precautions are taken with this type of treatment as for radioactive sources.

Any radioactive-iodine that is not absorbed by the thyroid will be passed from the body in sweat and urine. You should drink plenty of fluids during your treatment as this helps to flush the iodine out of the body. The amount of radiation in your body will be checked regularly and as soon as it falls to a safe level, after about four to seven days, you will be able to go home. You may need to take some special precautions for a short time after going home - particularly with regard to young children and pregnant women. The hospital staff will explain these to you.

Radioactive iodine does not usually cause side effects, but you may feel very tired for a few weeks after having this treatment. Radioisotope treatment can also be given if certain types of cancer have spread to the bones (secondary cancer in the bone). A radioisotope is injected into a vein, for which you can attend as an out-patient. Before you go home you will be given some simple advice to follow, as your urine and blood will be slightly radioactive for a few days. This type of radiotherapy treatment does not usually cause any side effects, apart from tiredness for a few weeks.

The presence of both natural and artificial radioactive isotopes has made possible the development of analytical methods that are both sensitive and specific. Radiochemical methods are characterized by good accuracy and their ability to be adapted to a wide number of applications. Another advantage to this method is that they minimize or even eliminate the need for separations that are required in other analytical methods.

A numerical (or "absolute") age is a specific number of years, like 150 million years ago. A relative age simply states whether one rock formation is older or younger than another formation. The Geologic Time Scale was originally laid out using relative dating principles. Numerical dating takes advantage of the "clocks in rocks" - radioactive isotopes ("parents") that spontaneously decay to form new isotopes ("daughters") while releasing energy. For example, decay of the parent isotope Rb-87 (Rubidium) produces a stable daughter isotope, Sr-87

(Strontium), while releasing a beta particle (an electron from the nucleus). ("87" is the atomic mass number = protons + neutrons.

Numerical ages have been added to the Geologic Time Scale since the advent of radioactive age-dating techniques.

Many minerals contain radioactive isotopes. In theory, the age of any of these minerals can be determined by:

1) Counting the number of daughter isotopes in the mineral, and

2) Using the known decay rate to calculate the length of time required to produce that number of daughters.

It illustrates how the amount of a radioactive parent isotope decreases with time. This amount is a percentage of the original parent amount. Time is expressed in half-lives. Experiment by dragging on the graph. For example, when 42% of the parent still remains, 1.23 Half-Lives of time has passed.

Parent Decay and Daughter Growth Curves:

The half-life of U-235 decaying to Pb-207 is 713 million years. Note that this half-life can be obtained from the graph at the point where the decay and growth curves cross.

Radiocarbon Dating:

Willard F. Libby and a team of scientists at the University of Chicago developed the radiocarbon dating method in the 1940's. It subsequently evolved into the most powerful method of dating late Pleistocene and Holocene artifacts and geologic events up to about 50,000 years in age. The radiocarbon method is applied in many different scientific fields, including archeology, geology, oceanography, hydrology, atmospheric science, and paleoclimatology. For his leadership, Libby received the Nobel Prize in Chemistry in 1960.h

There are three types of radiochemical methods:

1. Activation analysis. The activity is induced in one or more elements of the sample by irradiation with suitable radiation or particles. Most commonly thermal neutrons from a nuclear reactor source is used.

2. Tracer method. Radioactivity is introduced into the sample by adding a measured amount of a radioactive species.

3. Isotope dilution. This method is the most important class of radiochemical quantitative method. In this method, a weighed quantity of radioactively tagged analyte having a known activity is added to a measured amount of sample. The mixture is then mixed to homogeneity and then a fraction of the compound of interest is isolated and purified. The analysis is based upon the activity of the isolated fraction.

All atoms, except hydrogen, is made up of a collection of protons and neutrons. The chemical properties of an atom are determined by its atomic number, Z, the number of protons. The sum of neutrons and protons is the mass number, A. The nuclei of isotopes of an element contain the same number of protons, but have different numbers of neutrons. Stable isotopes are those that decay spontaneously. Radioactive isotopes (radionuclides), undergo spontaneous disintegration, which ultimately leads to stable isotopes. Radioactive decay of isotopes occurs with the emission of electromagnetic radiation in the form of x-rays or gamma ray. Accompanying this emission is the formation of electrons, positrons and the helium nucleus or fusion in which a nucleus breaks up into smaller nuclei.

Some of the most chemically important types of radiation from radioactive decay are listed in the table. Four types of these types - alpha particles, beta particles, gamma-ray photons and X-ray photons can be detected and counted.

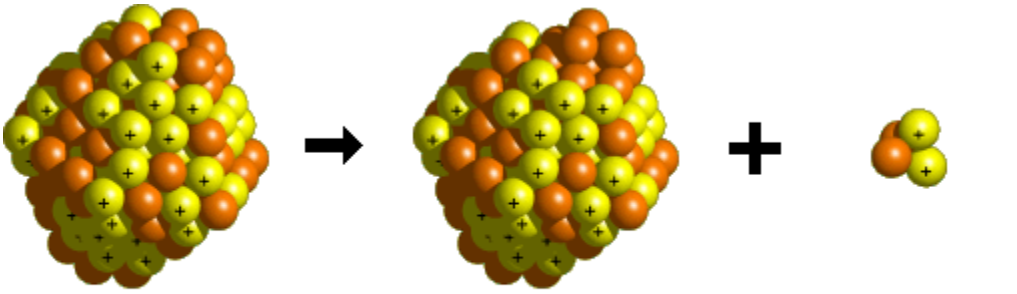
Alpha Decay:

Alpha decay is a common radioactive process encountered with heavier isotopes. The alpha particle is a helium nucleus having a mass of 4 and a charge of +2. Isotopes with mass numbers less than about 150 (Z 60) seldom yield alpha particles. Alpha particles progressively lose their energy as a result of collisions as they pass through matter and are ultimately converted into helium atoms through capture of two electrons from their surroundings. Since alpha particles have high mass and charge, alpha particles have a low penetrating power. Because of this, alpha particles are relatively ineffective for producing artificial isotopes. An example of alpha decay is shown by the equation below:

$$^{238}_{92}U \longrightarrow {}^{234}_{90}Th + {}^{4}_{2}He$$

Here, uranium-238 is converted to thorium-234 a daughter nuclide having an atomic number that is two less than the parent. Alpha particles from a particular decay process are either monoenergetic or are distributed among relatively few discrete energies. As shown above, the decay process proceeds by two distinct pathways. The first, which accounts for 77% of the decays, produces an alpha particle with an energy of 4.196MeV. The second pathway produces an alpha particle having an energy of 4.149MeV.

Alpha Decay



Beta Decay:

Beta decay is a radioactive process in which, the atomic number changes but the mass number stays the same. There are types of β decay are encountered: negatron formation, positron formation and electron capture. Example of these three processes are:

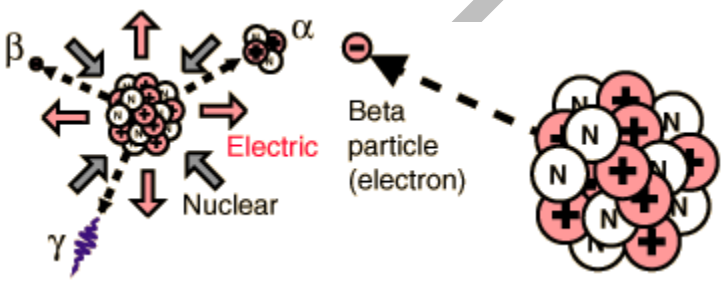
$$^{14}_{6}C \rightarrow ^{14}_{7}N + \beta^{-} + \bar{\nu} \qquad \text{negatron formation}$$

$$^{65}_{30}Zn \rightarrow ^{65}_{29}Cu + \beta^{+} + \nu \qquad \text{positron formation}$$

$$^{48}_{24}Cr + {}^{0}_{1}e \rightarrow ^{48}_{23}V + X - rays \qquad \text{electron capture}$$

Here $\bar{\nu}$ and ν in the first two equations represent an antineutrino and a neutrino particle of no significance in analytical chemistry. The third equation illustrates a beta-decay process called electron capture. Negatrons (β^-) are electrons that form when one of the neutrons in the nucleus is converted to a proton. A positron (β^+), with the mass of the electrons forms when the number of protons in the nucleus is decreased by one. In contrast to alpha emission, beta emission is characterized by the production of a continuous spectrum of energies ranging from nearly zero to

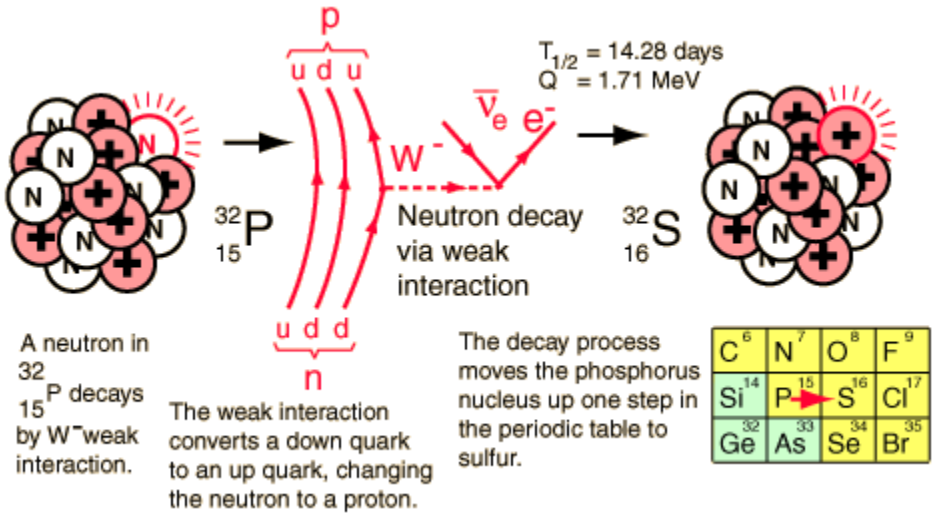
some maximum that is characteristic for each decay process. The beta particle is not nearly as effective as the alpha particle in producing ion pairs in matter because of its small mass (about 1/7000 that of an alpha particle). The penetrating power of beta particles is substantially higher.



Beta Radioactivity

Beta particles are just electrons from the nucleus, the term "beta particle" being an historical term used in the early description of radioactivity. The high-energy electrons have greater range of penetration than alpha particles, but still much less than gamma rays. The radiation hazard from betas is greatest if they are ingested. Beta emission is accompanied by the emission of an electron antineutrino, which shares the momentum, and energy of the decay.

The emission of the electron's antiparticle, the positron, is also called beta decay. Beta decay can be seen as the decay of one of the neutrons to a proton via the weak interaction. The use of a weak interaction Feynman diagram can clarify the process.



Gamma-Ray Emission:

Gamma rays are produced by nuclear relaxations. Gamma-ray emission is the result of a nucleus in an excited state returning to the ground state in one or more quantized steps with the release of monoenergetic gamma rays. Gamma rays, except for their source, are indistinguishable from X-rays of the same energy. The gamma-ray emission spectrum is characteristic for each nucleus and is thus useful for identifying radioisotopes. Gamma radiation is highly penetrating. Gamma rays in interacting with matter lose energy by three mechanisms; the one that predominates depends upon the energy of the gamma-ray photon. With low-energy gamma radiation, the photoelectric effect predominates. In this interaction, the gamma-ray photon disappears after rejecting an electron from an atomic orbital of the target atom. The photon energy is totally consumed in overcoming the binding energy of the electron and in overcoming the binding energy of the electron and in imparting kinetic energy to the ejected

electron. Relatively energetic gamma interacts via the Compton effect. In this, an electron is also ejected from an atom but acquires only a part of the photon energy. The photon now with diminished energy, recoiled from the electron and goes on to further Compton or photoelectric interactions. If the gamma-ray photon possesses sufficiently high energy (at least 1.02 MeV), pair production can occur. In this scenario the photon is totally absorbed in creating a positron and an electron in the field surrounding a nucleus.

Paul Villard, a French physicist, is credited with discovering gamma rays. Most sources put this in 1900, although I've seen a few sources use 1898. Villard recognized them as different from X-rays (discovered in 1896 by Roentgen) because the gamma rays had a much greater penetrating depth. It wasn't until 1914 that Rutherford showed that they were a form of light with a much shorter wavelength than X-rays.

Gamma rays are more dangerous than radio waves. This is due to the fact that light can be thought of as particles (photons) as well as electromagnetic waves. A radio photon doesn't have much energy and doesn't travel through matter well (that's why you don't pick up radio well in a tunnel). A gamma-ray photon has enough energy to damage atoms in your body and make them radioactive, and gamma rays can easily penetrate into your body. It's like the difference between getting hit by sand or a bullet. It takes a lot of sand to do any damage, but only one bullet.

X-Ray Emission:

X-Ray emission is formed from electronic transitions in which outer electrons fill the vacancies created by the nuclear process. One of the processes is electron capture. A second process which may lead to X-rays is internal conversion, a type of nuclear process that is an alternative to gamma-ray emission. In this process, an electromagnetic interaction between the excited nucleus and an extranuclear electron results in the ejection of an orbital electron with a

kinetic energy equal to the difference between the energy of the electron. The emission of this so-called internal conversion electron leaves a vacancy in the K, L or higher orbitals. X-rays are emitted as the orbital is filled by an electronic transition.

On the earth, we generate the energetic particles in analytical instruments such as the scanning electron microscope (called an electron microprobe) and measure the energies of the X-rays emitted from the sample. The general concept: electrons are knocked out of occupied levels by energetic X-rays, electrons, or ions (either protons - H^+ - or alpha particles - He^{++}), electrons make transitions from filled to empty states and emit an X-ray. The energy of the emitted characteristic X-ray identifies the atom.

Radioactive Decay Rates:

Radioactive decay is a completely random process. Although no predictions can be made concerning the lifetime of an individual nucleus, the behavior of a large ensemble of like nuclei can be described by the first order rate expression.

$$-\frac{dN}{dt} = \lambda N$$

where N is the number of radioactive nuclei of a particular kind in the sample at time t and λ is the decay constant for the particular radioisotope. Over this interval this expression can be rearranged to

$$N = N_0 e^{-kt}$$

The half-life, $t_{½}$ is

$$t_{½} = \frac{\ln 2}{\lambda} \approx \frac{0.693}{\lambda}$$

The half-lives of radioactive species range from small fractions of a second to many billions of years. The activity A or a radionuclide is defined by its disintegration rate.

$$A = -\frac{dN}{dt} = \lambda N$$

Activity is given in units of s^{-1}. The becquerel (Bq) corresponds to 1 decay per second (1 Bq = 1 s^{-1}). An older, but still widely used unit of activity is the Curie (Ci), which was originally defined as the activity of 1 g of radium-226. One curie is equal to 3.70×10^{10} Bq. In analytical radiochemistry, activities of analytes range from a nanocurie to less to a few microcuries. In the laboratory, solution activities are seldom measured because detection efficiencies are seldom 100%. Instead, the counting rate, R is employed, where

$$R = cA$$

Substituting this into the activity expression yields

$$R = c\lambda N$$

Here, c is constant called the detection coefficient, which depends upon the nature of the detector, the efficiency of counting disintegrations, and the geometric arrangement of sample and detector. The decay law given then can be written as

$$R = R_0 e^{-kt}$$

Radioactivity is measured by means of a detector that produces a pulse of electricity for each atom undergoing decay. Quantitative information about decay rates is obtained by counting these pulses for a specific period. Shown below is a table with decay data obtained by successive one-minute counts.

Minutes	Counts	Minutes	Counts
1	180	7	168
2	187	8	170

3	166	9	173
4	173	10	132
5	170	11	154
6	164	12	167

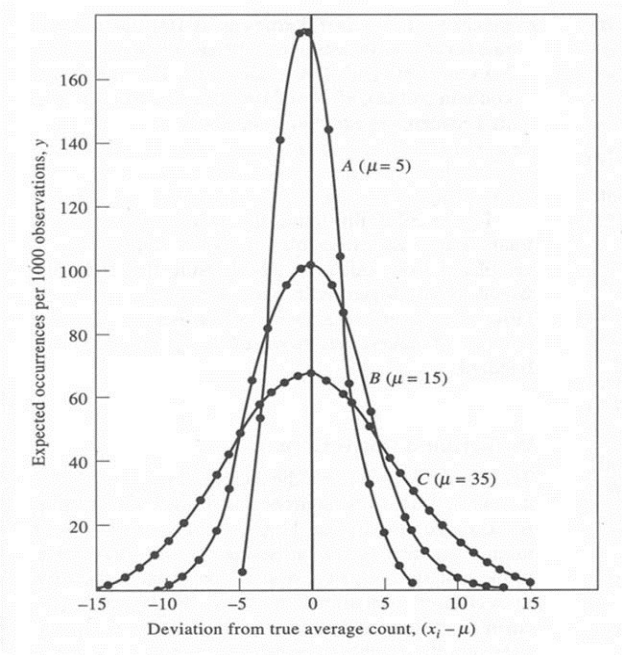
Total counts = 2004

Average counts/min = 167

As discussed earlier, radioactive decay is random. In order to accurately describe radioactive behavior, it is necessary to assume a Poisson distribution, which is given by the equation

$$y = \frac{\mu^{x_i}}{x_i!} e^{-\mu}$$

where y is the frequency of occurrence of a given count x_i and μ is the mean for a large set of counting data. The data plotted in the figure below, shows the deviation (x_i - μ) from the true average count that would be expected if 1000 replicate observations were made on the same sample. Curve A gives the distribution for a substance for which the true average count μ for a selected period is 5. Curve B and C correspond to samples having the true means of 15 and 35. The absolution deviation becomes greater with increases in μ, but the relative deviations become smaller. Note that for the two smaller number of counts, the distribution is not symmetric around the average. This lack of symmetry is a consequence of the fact that a negative count is impossible, while a finite probability exists that a given count will exceed the average by severalfold.



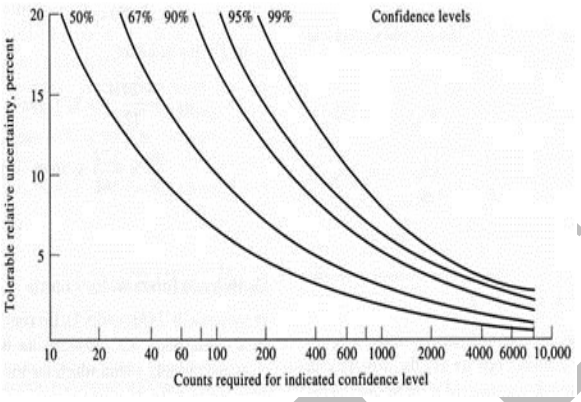
The confidence interval for a measurement was defined as the limits around a measured quantity within the true mean can be expected to fall with a stated probability. When the measured standard deviation is believed to be a good approximation of the true standard deviation ($s \rightarrow \sigma$), the confidence limit CL is

$$\text{CL for } \mu = \bar{x} \pm z\sigma$$

For counting rates, this equation takes the following form

$$\text{CL for } R = R \pm z\sigma_R$$

where z is dependent upon the desired level of confidence. The relative uncertainties in counting are shown below.



The counts recorded in a radiochemical analysis include a contribution from sources other than the sample. Background activity can be traced to the existence of minute quantities of radon isotopes in the atmosphere, to the materials used in construction of the laboratory, to accidental contamination within the laboratory, to cosmic radiation, and to the release of radioactive materials into the Earth's atmosphere. In order to obtain a true assay, then, it is necessary to correct the total count for background. The counting period required to establish the background correction frequently differs from that for the sample, as a result, it is more convenient to employ counting rates. The standard deviation of the corrected counting rate is

$$\sigma_{R_C} = \sqrt{\frac{R_x}{t_x} + \frac{R_b}{t_b}}$$

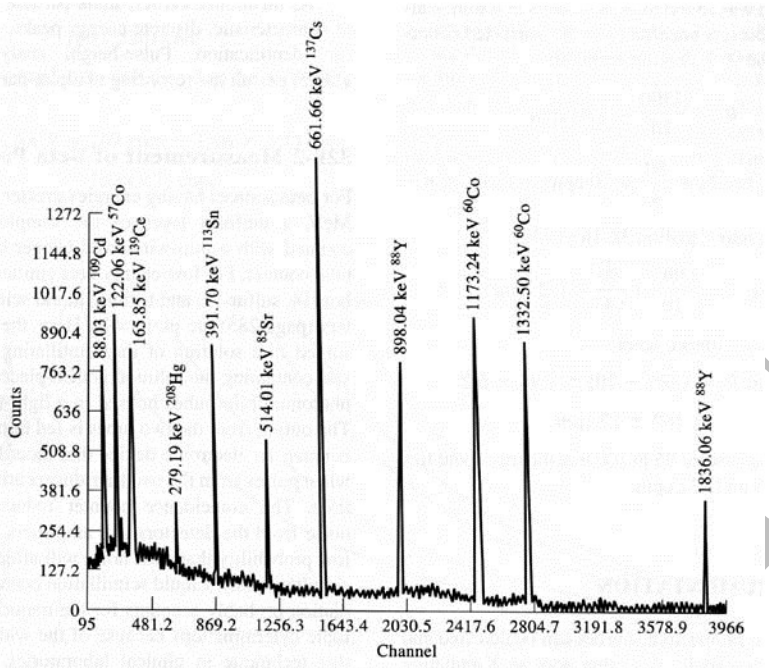
where R_c is the corrected counting rate and R_x and R_b are the rates for the sample and the background, respectively.

Instrumentation:

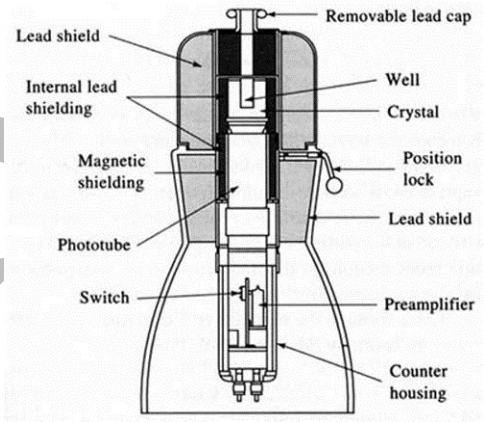
Radiation from radioactive sources can be detected and measured in essentially the same way as X-radiation. Gas-filled transducers, scintillation counters and semiconductor detectors are all sensitive to alpha and beta particles and gamma rays because absorption of these particles

induces ionization or photoelectrons, which can in turn produce thousands of ion pairs. Alpha-emitting samples are generally counted as thin deposits prepared by electrodeposition or by distillation and condensation. These deposits are then sealed with think windows and counted in windowless gas-flow proportional counters or ionization chambers. Liquid scintillation counting is becoming more important for counting alpha emitters because of the ease of sample preparation and the higher sensitivity for alpha-particle detection.

For beta sources having energies greater than about 0.2 MeV, a uniform layer of the sample is counted with a thin-windowed Geiger or proportional tube counter. For low-energy beta emitter, such as carbon-14, sulfur-35 and tritium, liquid scintillation counters are preferable. In a liquid scintillation counter a vial containing the solution is placed between two photomultiplier tubes housed in a light-tight container. The output from the two transducers is fed into a coincidence counter, an electronic device that only records a count only when pules from the two transducers arrive at same. The coincidence counter reduces the background noise from the detector and amplifiers because of the low probability that such noise will affect both systems simultaneously. Gamma radiation is detected and measured by the methods used for the detection for X-radiation. Interference from alpha- and beta- radiation is readily avoided by filtering the radiation with a thin film piece of aluminum or Mylar. Gamma-ray spectrometer is similar to the pulse-height analyzer. The figure below shows a gamma-ray reference spectrum obtained with a 4000 channel analyzer. The characteristic peaks for the various elements are superimposed on the continuum that arises primarily from the Compton effect.



The schematic below shows a well-type scintillation counter that is used for gamma-ray counting.



Isotope Dilution Method:

Isotope dilution methods predate activation procedure and are still being extensively applied to problems in all branches of chemistry. Stable and radioactive isotopes are employed in the isotope dilution technique. The isotope dilution method is more convenient because of the ease with which the concentration of the isotope can be determined.

Principles of the Isotope Dilution Procedure:

Isotope dilution methods require the preparation of a quantity of the analyte in a radioactive form. A known weight of the isotopically labeled species is then mixed with the sample to be analyzed. After treating to assure homogeneity between the active and nonactive species, a part of the analyte is isolated chemically in the form of a purified compound. By counting a weighed portion of this product, the extent of dilution of the active material can be calculated and related to the amount of nonactive substance in the original sample. The following equation relates the activity of the isolated and purified mixture of analyte and tracer to the original amount of the analyte,

$$W_x = \frac{R_t}{R_m} W_m - W_t$$

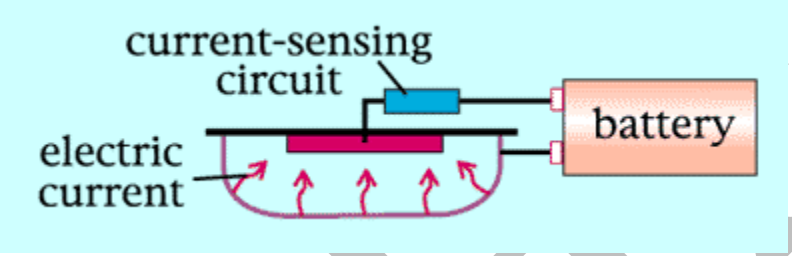
where, W_x is the grams of tracer having a counting rate of R_t (cpm), W_m is the grams of isolated and purified mixture of active and inactive species with a counting rate of R_m, and W_t is the weight of the tracer. The isotopic dilution technique has been used for the determination of about 30 elements in a variety of matrix materials.

Modern Uses of Radioactive Isotopes:

Smoke Detectors and Americium-241

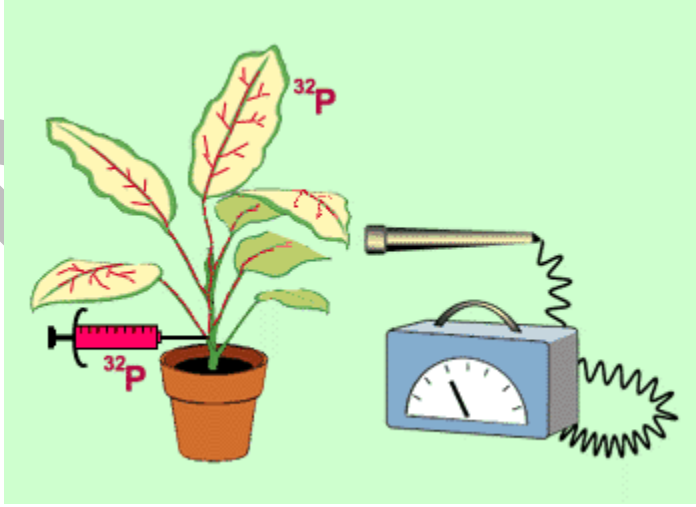
Chances are that a great number of homes have had one or more of these devices installed as an early warning system in case of fire. What most consumers don't know is that many of these

units contain a small amount of americium-241. By utilizing the radioactive properties of this material, smoke from a fire can be detected at a very early stage. This early warning capability has saved many lives. In fact, studies have shown that 80% of fire injuries and 80% of fire fatalities occur in homes without smoke detectors.



Agricultural Applications - radioactive tracers

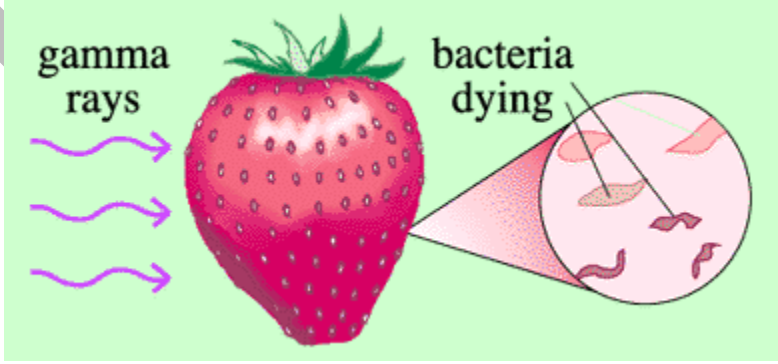
Radioisotopes can be used to help understand chemical and biological processes in plants. This is true for two reasons: 1) radioisotopes are chemically identical with other isotopes of the same element and will be substituted in chemical reactions and 2) radioactive forms of the element can be easily detected with a Geiger counter or other such device.



A solution of phosphate, containing radioactive phosphorus-32, is injected into the root system of a plant. Since phosphorus-32 behaves identically to that of phosphorus-31, the more common and non-radioactive form of the element, the plant uses it in the same way. A Geiger counter is then used to detect the movement of the radioactive phosphorus-32 throughout the plant. This information helps scientists understand the detailed mechanism of how plants utilized phosphorus to grow and reproduce.

Food Irradiation

Food irradiation is a method of treating food in order to make it safer to eat and have a longer shelf life. This process is not very different from other treatments such as pesticide application, canning, freezing and drying. The end result is that the growth of disease-causing microorganisms or those that cause spoilage are slowed or are eliminated altogether. This makes food safer and also keeps it fresh longer. Food irradiated by exposing it to the gamma rays of a radioisotope -- one that is widely used is cobalt-60. The energy from the gamma ray passing through the food is enough to destroy many disease-causing bacteria as well as those that cause food to spoil, but is not strong enough to change the quality, flavor or texture of the food. It is important to keep in mind that the food never comes in contact with the radioisotope and is never at risk of becoming radioactive.



The FDA has approved the irradiation of several food categories, but irradiation is most widely used on spices, herbs and dehydrated vegetables. Since these food items are grown in or on the ground it is clear to see that they are at risk for being exposed to naturally occurring pests such as bacteria, molds, fungi, insects, and rodents. It is impossible to harvest and package these items without having some contamination from these naturally occurring pathogens. Irradiation of this material can help to ensure that the final product you purchase is pest free. Some meats are irradiated. Pork, for example, is irradiated to control the trichina parasite that resides in the muscle tissue of some pigs.

Poultry is irradiated to eliminate the chance of foodborne illness due to bacterial contamination. Irradiation of certain foods also has additional benefits. Since the energy passing through the food can disrupt cellular processes (this is the mechanism for destroying microorganisms) it also can halt the cellular processes that lead to the sprouting or ripening of foods. Potatoes and onions are irradiated to retard their sprouting. Fruits and vegetables are irradiated to slow down the ripening process. In this way, delicate fruits won't reach their peak ripeness before they arrive at the supermarket.

Food irradiation sounds like a wonderful use of nuclear chemistry principles. Although this process is routinely used in Europe, Canada, and Mexico, the United States has been a little more hesitant to adopt food irradiation. This is due mainly to the public's perceived fear and limited understanding of nuclear science. For example, although the FDA has approved the irradiation of poultry, the industry hesitates to adopt the process because they are afraid of a negative response from consumers. With recent public education, however; many people are learning to appreciate and value its usefulness.

Archaeological Dating

Significant progress has been made in this field of study since the discovery of radioactivity and its properties. One application is **carbon-14** dating. Recalling that all biologic organisms contain a given concentration of carbon-14, we can use this information to help solve questions about when the organism died. It works like this: when an organism dies it has a specific ratio by mass of carbon-14 to carbon-12 incorporated in the cells of its body. (The same ratio as in the atmosphere.) At the moment of death, no new carbon-14 containing molecules are metabolized; therefore the ratio is at a maximum. After death, the carbon-14 to carbon-12 ratio begins to decrease because carbon-14 is decaying away at a constant and predictable rate. Remembering that the half-life of carbon-14 is 5700 years, then after 5700 years half as much carbon-14 remains within the organism.

Medical Uses

Bone imaging is an extremely important use of radioactive properties. Supposed a runner is experiencing severe pain in both shins. The doctor decides to check to see if either tibia has a stress fracture. The runner is given an injection containing 99Tcm. This radioisotope is a gamma ray producer with a half-life of 6 hours.

After a several hour wait, the patient undergoes bone imaging. At this point, any area of the body that is undergoing unusually high bone growth will show up as a stronger image on the screen. Therefore if the runner has a stress fracture, it will show up on the bone-imaging scan.
This technique is also good for arthritic patients, bone abnormalities and various other diagnostics.

Surface Analysis - Techniques Available

Properties and reactivity of the surface will depend on:

bonding geometry of molecules to the surface

physical topography

chemical composition

chemical structure

atomic structure

electronic state

Brief History of the Development of X-ray Fluorescence and Powder Diffraction Methods

X-ray photons are a form of electromagnetic radiation produced following the ejection of an inner orbital electron and subsequent transition of atomic orbital electrons from states of high to low energy. When a monochromatic beam of X-ray photons falls onto a given specimen three basic phenomena may result, namely absorption, scatter or fluorescence. The coherently scattered photons may undergo subsequent interference leading in turn to the generation of diffraction maxima. These three basic phenomena form the bases of three important X-ray methods: the absorption technique, which is the basis of radiographic analysis; the scattering effect, which is the basis of X-ray diffraction; and the fluorescence effect, which is the basis of XRF spectrometry.

X-rays were discovered by Wilhelm Roentgen in 1895.1/and the property of the atomic number dependence of the absorption of X-ray photons was quickly established and applied for medical diagnostic purposes. Following the discovery of the diffraction of X-rays by Max von Laue in 1913, two major fields of materials analysis have developed. XRF spectrometry uses either the diffracting power of a single crystal to isolate narrow wavelength bands, or a proportional detector to isolate narrow energy bands, from the polychromatic beam characteristic radiation excited in the sample. The first of these methods is called wavelength-dispersive spectrometry and the second, energy-dispersive spectrometry.

Because of the known relationship between emission wavelength and atomic number, isolation of individual characteristic lines allows the unique identification of an element to be made and elemental concentrations can be estimated from characteristic line intensities. Thus, this technique is a means of materials characterization in terms of chemical composition. X-ray spectrometric techniques provided important information for the theoretical physicist in the first half of this century and since the early 1950s they have found an increasing use in the field of materials characterization. While most of the early work in X-ray spectrometry was carried out using electron excitation,.2/ today most stand-alone X-ray spectrometers use X-ray excitation sources rather than electron excitation. XRF spectrometry typically uses a polychromatic beam of short wavelength X-radiation to excite longer wavelength characteristic lines from the sample to be analyzed.

Modern X-ray spectrometers use either the diffracting power of a single crystal to isolate narrow wavelength bands, or a proportional detector to isolate narrow energy bands, from the polychromatic radiation (including characteristic radiation) excited in the sample.3/ The second field of materials analysis involves characterization by means of atomic arrangement in the crystal lattice. XRD uses single or multiphase specimens comprising a random orientation of small crystallites, each of the order of 1–50 mm in diameter. Each crystallite in turn is made up of a regular, ordered array of atoms. An ordered arrangement of atoms (the crystal lattice) contains planes of high atomic density which in turn means planes of high electron density. A monochromatic beam of X-ray photons will be scattered by these atomic electrons and if the scattered photons interfere with each other, diffraction maxima may occur. In general, one diffracted line will occur for each unique set of planes in the lattice. A diffraction pattern is typically in the form of a graph of diffraction angle (or interplanar spacing) against diffracted line intensity. The pattern is thus made up of a series of superimposed diffractograms, one foreach unique phase in the specimen. Each of these unique patterns can act as an empirical

''fingerprint'' for the identification of the various phases, using pattern recognition techniques based on a file of standard single-phase patterns. Quantitative phase analysis is also possible, albeit with some difficulty because of various experimental another problem, not the least of which is of the large number of diffraction lines occurring from multiphase materials.

Role of X-ray Methods in the Modern Analytical Laboratory

Wavelength-dispersive spectrometers have been commercially available since the early 1950s and there are probably about 20 000 units in use in the world today. Energy-dispersive spectrometer systems became available in the early 1970s, and there are probably about8000 stand-alone spectrometers in use, with perhaps slightly more than this number attached to scanning electron microscopes. XRF analysis finds a wide range of application since it allows the quantitation of all elements in the periodic table from F (atomic number 9) upwards.4/ Newer developments allow the determination of the ultralow atomic number elements including B, C, O and N. Accuracies of a few tenths of one percentage possible for most of the atomic number range, and elements are detectable in many cases to the low parts per million level. Excellent data treatment software is available allowing the rapid application of quantitative and semi-quantitative procedures.

X-ray powder diffractometers have been available in their modern form since the late 1940s, although camera systems were available back to the 1920s. There are probably of the order of 30 000 powder diffractometers in use – about half of these being automated to some degree. XRD is applicable to any ordered (crystalline)material and although much less accurate or sensitive than

the fluorescence method, is almost unique in its ability to differentiate phases. Quantitative phase analysis is possible, but the accuracy of a determination is often compromised by preferred orientation of the specimen. The fluorescence and diffraction techniques are to a large extent complementary, since one allows accurate quantitation of elements to be made and the other

allows qualitative and semiquantitative estimations to be made of the way in which the matrix elements are combined to make up the phases in the specimen. Thus, a combination of the two techniques will often allow the accurate determination of the material balance of a sample.

PROPERTIES OF X-RADIATION

Continuous and Characteristic Radiation

When a high-energy electron beam is incident upon a specimen, one of the products of the interaction is an emission of a broad-wavelength band of radiation called continuum, also referred to as white radiation or bremsstrahlung. This white radiation is produced as the impinging high-energy electrons are decelerated by the atomic electrons of the elements making up the specimen. The intensity/wavelength distribution of this radiation is typified by a minimum wavelength limn which is inversely proportional to the maximum accelerating potential V of the electrons. The intensity distribution of the continuum

reaches a maximum at a wavelength 1.5 to 2 times greater than limn. Increasing the accelerating potential causes the intensity distribution of the continuum to shift towards shorter wavelengths. Most commercially available spectrometers utilize a sealed X-ray tube as an excitation source, and these tubes typically employ a heated tungsten filament as a source of electrons, and a layer of pure metal, such as chromium, rhodium or tungsten, as the anode. The broad band of white radiation produced by this type of tube is ideal for the excitation of the characteristic lines from a wide range of atomic numbers. In general, the higher the atomic number of the anode material, the more intense the beam of radiation produced by the tube. X-ray diffractometers typically employ a more focused filament assembly (12 0:05mm) and run at a somewhat lower loading than the XRF tube. A key factoring the design of an X-ray tube for powder diffraction is the specific loading (watts per mm2) of the tube.

X-ray Spectra

In addition to electron interactions leading to the production of white radiation, there are also electron interactions which produce characteristic radiation. If a high-energy particle, such as an electron, strikes a bound atomic electron, and the energy of the particle is greater than the binding energy of the atomic electron, it is possible that the atomic electron will be ejected from its atomic position, departing from the atom with a kinetic energy (E f) equivalent to the difference between the energy E of the initial particle and the binding energy f of the atomic electron. Where the exciting particles are X-ray photons, the ejected electron is called a photoelectron and the interaction between primary X-ray photons and atomic electrons is called the photoelectric effect. As long as the vacancy in the shell exists, the atom is in an unstable state and can regain stability by transference off an electron from one of the outer orbitals to fill the vacancy. The energy difference between the initial and final states of the transferred electron may be given off in the form of an X-ray photon. Since all emitted X-ray photons have energies proportional to the differences in the energy states of atomic electrons, the lines from a given element will be characteristic of that element. The relationship (Equation 1) between the wavelength of a characteristic X-ray photon and the atomic number Z of the excited element was first established by Moseley.

$$\frac{1}{\lambda} = K(Z - \sigma)^2 \qquad \lambda(\text{Å}) = \frac{12.4}{E(\text{keV})}$$

in which K is a constant that takes on different values foreach spectral series. s is the shielding constant that has a value of just less than unity. The wavelength of the X-ray photon is inversely related to the energy E of the photon according to Equation.

Not all vacancies result in the production of characteristic X-ray photons since there is a competing internal rearrangement process known as the Auger effect. 6/The ratio of the number of vacancies resulting in the production of characteristic X-ray photons to the total number of vacancies created in the excitation process is called the fluorescent yield. Fluorescent yield values are several orders of magnitude less for the very low atomic numbers. In practice this means that if, for example, one was to compare the intensities obtained from pure barium (Z D 56) and pure aluminum (Z D 13), all other things being equal, pure barium would give about 50 times more counts than would pure aluminum. The L-fluorescent yield for a given atomic number is always less by about a factor of three than the corresponding K-fluorescent yield, where K and L refer to the first and second electron shells. An excited atom can revert to its original ground state by transferring an electron from an outer atomic level to fill the vacancy in the inner shell. An X-ray photon is emitted from the atom as part of this de-excitation step, the emitted photon having an energy equal to the energy difference between the initial and final states of the transferred electron. The selection rules for the production of normal (diagram) lines require that the principal quantum number n must change by at least one, the angular quantum number ` must change by 1, and the J quantum number (the total momentum 'J' of an electron is given by the vector sum of ` C s where s is the spin quantum number) must change by 0 or 1. In effect this means that for the K series only p! s transitions are allowed, yielding two lines for each principal level change. Vacancies in the L level follow similar rules and give rise to L series lines. There are more of the L lines since p!s, s! p and d!p transitions are all allowed within the selection rules. Transition groups may now be constructed, based on the appropriate number of transition levels. Figure 1 shows plots of the reciprocal of the square root of the wavelength, as a function of atomic number, for the K, L and M series. As indicated by Moseley's law such plots should be linear. A scale directly in wavelength is also shown, to indicate the range of wavelengths over which a given series occurs. In practice, the number of lines observed from a

given element will depend upon the atomic number of the element, the excitation conditions and the wavelength range of the spectrometer employed.

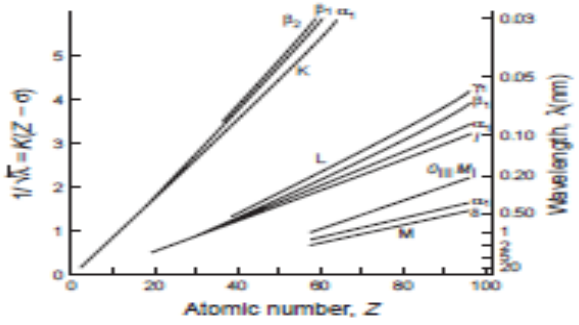


Figure 1 Moseley diagrams for the K, L and M series. (Reproduced from Jenkins, *X-ray Fluorescence Spectrometry*, 2nd edition, Wiley-Interscience, 1999. With permission from John Wiley & Sons.)

Generally, commercial spectrometers cover the K series, the L series and the M series, corresponding to transitions to K, L and M levels respectively. There are many more lines in the higher series and for a detailed list of all of the reported wavelengths the reader is referred to the work of Bearden. 7/ In X-ray spectrometry most of the analytical work is carried out using either the K or the L series wavelengths. While most of the observed fluorescent lines are normal, certain lines may also occur in X-ray spectra that do not at first sight fit the basic selection rules. These lines are called forbidden lines and are shown in the center portion of Figure 1. Forbidden lines typically arise from outer orbital levels where there is no sharp energy distinction between orbitals. A third type of line may also occur – called satellite lines – which arise from dual ionizations. Neither forbidden transitions nor satellite lines have great analytical significance; they may cause some confusion in qualitative interpretation of spectra and may even be misinterpreted as coming from trace elements. Most commercially available X-ray spectrometers have a range from about 0.2 to20A° (60–0.6 keV, 1A° D 10□10 m D 10□1 nm) which will

allow measurement of the K series from F (Z D 9) to Lu (Z D 71), and for the L series from Mn (Z D 25) to U (Z D 92). Other line series can occur from the M and N levels but these have little use in analytical X-ray spectrometry.

Absorption

When a beam of X-ray photons falls onto an absorber, a number of different processes may occur. The more important of these are illustrated in Figure 2. In this example, a monochromatic beam of radiation of wavelengthl0 and intensity I0, is incident on an absorber of thickness and density. The fate of each individual X-ray photon is governed by the following processes. Absorption occurs where only a certain fraction (I=I0)of the radiation may pass through the absorber. Where

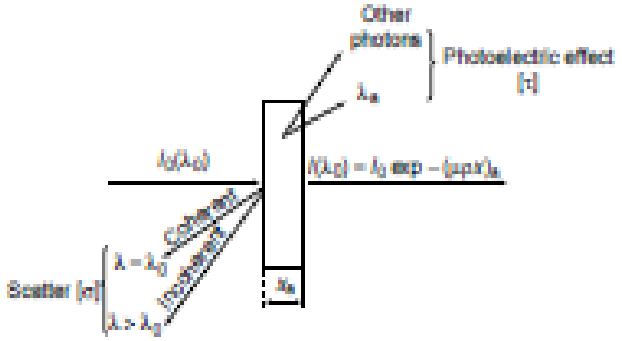


Figure 2 Interaction of X-ray photons with matter.

this happens the wavelength of the transmitted beam isunchanged and the intensity of the transmitted beam I.l0/ is given by Equation (3):

$$I(\lambda_0) = I_0 \exp -(\mu_a \rho_a x_a) \qquad \mu = f(\tau) + f(\sigma)$$

where ma is the mass attenuation coefficient of absorber a for the wavelength l0. It will be apparent from the above that a number of photons equal to (I0/ I) have been lost in the absorption process, most of this loss being due to the photoelectric effect. Photoelectric absorption, usually designated t, will occur at each of the energy levels of the atom. Thus, the total photoelectric absorption will be determined by the sum of each individual absorption within a specific shell. The value of the mass attenuation m referred to in Equation (3) is a function of both the photoelectric absorption and the scatter, Equation (4): However, f(t) is usually large in comparison with f(s). Because the photoelectric absorption is made up of absorption in the various atomic levels, it is an atomic number- dependent function. A plot of m against l

contains a number of discontinuities called absorption edges, at wavelengths corresponding to the binding energies of the electrons in the various subshells. The absorption discontinuities are a major source of nonlinearity between X-ray intensity and composition in both XRF and XRD.2.4 Scattering Scatter, s, will occur when an X-ray photon collides with one of the electrons of the absorbing element. Where this collision is elastic, i.e. when no energy is lost in the collision process, the scatter is said to be coherent (Rayleigh) scatter. Since no energy change is involved,

the coherently scattered radiation will retain exactly the same wavelength as the incident beam. As will be shown later, X-ray diffraction is a special case of coherent scatter, where the scattered photons interfere with each other. It can also happen that the scattered photon gives up a

small part of its energy during the collision, especially where the electron with which the photon collides is only loosely bound. In this instance the scatter is said to be incoherent (Compton scatter) and the wavelength of the incoherently scattered photon will be greater than l0.

Role of Crystal Structure in X-ray Scattering and Diffraction

All substances are built up of individual atoms and nearly all substances have some degree of order of periodicity in the arrangement of these atoms. A crystal can be defined as a homogeneous, anisotropic body having the natural shape of a polyhedron. In practical terms, whether a substance is homogeneous or not can only be defined by the means that are available for measuring the crystallinity. In general, the shorter the wavelength, the smaller the crystalline region that is able to be recognized. Even noncrystalline materials have a degree of order and each will give some sort of a diffraction pattern. For example, glassy materials and liquids will give diffraction patterns of sorts, generally in the form of one or more broad diffuse peaks or halos. In XRD one is generally dealing exclusively with crystalline materials. The diffraction pattern can, moreover, be used to determine the degree of crystallinity, that is the dimensions of the crystalline regions in otherwise amorphous substances. A crystalline substance has a definite form which is retained no matter what the physical size of the crystal. A certain type of crystal can thus be defined in terms of specific physical characteristics which determine its shape. For example, a sodium chloride crystal has cubic symmetry and by definition the angles between all of the principal faces should be 90°. In fact there are other crystal classes which also have angles

between faces of 90°, but the cube is unique in that the lengths of the sides are also equal. If our sodium chloride crystal were reduced in size until the smallest repeat unit was found (the so-called unit cell) it would indeed be found that all sides were equal. Hence it is apparent that a crystal type can be defined in terms of the lengths of the sides of its unit cell and the angles

between the faces. Since every ordered material is made p of a unique arrangement and number of atoms, every ordered material will give a diffraction pattern which is, to all intents and purposes, also unique. Figure 3 illustrates in a simplified manner the diffraction patterns that would be obtained from (a) a single

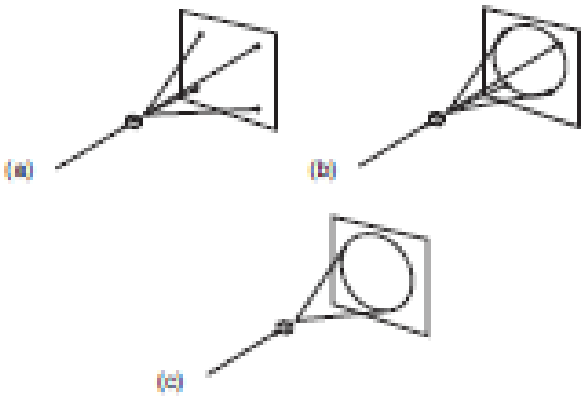


Figure 3 Diffraction patterns from (a) single crystal; (b) a random powder; and (c) an oriented powder. (Reproduced from Jenkins, Snyder, *Introduction to Powder Diffractometry*, Wiley-Interscience, 1996. With permission from John Wiley & Sons.)

crystal, (b) an oriented powder and (c) a random powder. In each instance the specimen is placed between a monochromatic beam of radiation and a piece of photographic film. In the case of the single crystal, because only certain crystal planes are in the position to diffract radiation onto the film, the pattern will appear as a series of spots on the film, the position of the spots being dependent upon the structure and orientation of the crystal. Changing the position of the crystal will bring other planes into diffracting position and the pattern of spots will change. In the case of a random powder, whatever the orientation of the specimen there is always a sufficient number of crystallites to diffract from the appropriate d-spacing(s) (see section 2.6). In the case of an oriented powder, however, there are more crystallites in certain orientations and less in others. Thus the pattern obtained is somewhere between the single-crystal and the random-powder patterns.

Interference and Diffraction Effects

As illustrated in Figure 4(a), a crystal lattice consists of a regular arrangement of atoms, with layers of high atomic density existing throughout the crystal structure. Planes of high atomic density means planes of high electron density. Since scattering occurs between impinging X-ray photons and the loosely bound outer orbital atomic electrons, when a monochromatic beam of radiation falls onto the high atomic density layers scattering will occur. In order to satisfy the requirement for constructive interference, it is necessary that the scattered waves originating from the individual atoms, the scattering points, be in phase with

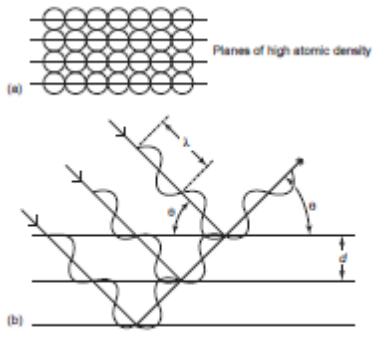


Figure 4 Scatter and diffraction from an ordered arrangement of atoms. (Reproduced from Jenkins, *X-ray Fluorescence Spectrometry*, 2nd edition, Wiley-Interscience, 1999. With permission from John Wiley & Sons.)

one another. The geometric conditions for this condition to occur are illustrated in Figure 4(b). Here, a series of parallel rays strike a set of crystal planes at an angle q and are scattered as previously described. Reinforcement will occur when the difference in the path lengths of the two interfering waves is equal to a whole number of wavelengths. This path length difference is 2d sin q where d is the inter-planar spacing; hence the overall condition (Equation 5) for

$$n\lambda = 2d\sin\theta$$

reinforcement is that:

where n is an integer. Equation (5) is a statement of Bragg's law. Diffraction experiments are generally made at a fixed wavelength; thus, a measure of the diffraction angles will allow the associated d-spacings to be calculated. Bragg's law is also important in wavelength dispersive spectrometry since by using a crystal of fixed 2d, each unique wavelength will be diffracted at a unique diffraction angle. Thus, measuring the diffraction angle 2q allows the determination of the wavelength (inter-planar spacing) if the d-spacing of the crystal is known. Since there is a simple relationship between wavelength and atomic number, one can establish the atomic number(s) of the element(s) from which the wavelengths were emitted.

POSSIBLE QUESTIONS
UNIT-V
PART-A (20 MARKS)
(Q.NO 1 TO 20 Online Examination)

PART-B (2 marks)

1. Define Chemical shift?
2. What is Spin coupling
3. what is the principle used in potentiometry and voltammetry
4. What is the principal used in NMR Spectroscopy?
5. Write a note on TMS?

PART-C (6 Marks)

1. Write the Principle and instrumentation about NMR spectroscopy
2. What are Factors affecting chemical shift
3. Write about Applications of NMR spectroscopy.
4. write details about Electro analytical Methods of Potentiometry and Voltammetry
5. what are the Radiochemical Methods? Explain any two methods detail
6. Describe about X-ray analysis and electron spectroscopy ?

Unit-V-Multiple choice Questions

S. No	Question	Option I	Option II	Option III	Option IV	Answer
1.	What is precessional motion	top moves around the vertical	top moves away the vertical	top moves away from the vertical	none of the above	top moves around the vertical
2.	The gyromagnetic ratio is	2 pi M/Hi	2C pi M/hI	hI/2 pi M	hI/ 2C pi M.	2 pi M/Hi
3.	What is flipping	energy transfer within the molecule	energy transfer outside the molecule	Energy transfer from one energy	none of the above	energy transfer within the molecule
4.	Acetone gives	one signal NMR	two signal NMR	three signal NMR	four signal NMR	one signal NMR
5.	Methyl cyclo propane gives	one signal NMR	two signal NMR	three signal NMR	four signal NMR	three signal NMR
6.	For non-viscous system NMR gives	splitting signal	sharp signal	broad signal	none of the above	sharp signal
7.	The relationship between delta value in ppm and frequency in hertz is fundamental	$\delta x = v_x - v_{TMS/V0}$	$\delta x = v_{TMS} - v_{x/V0}$	$v_0 / v_x - v_{TMS}$	$v_0 / v_{TMS} - v_x$	$\delta x = v_x - v_{TMS/V0}$
8.	What is the rule of spectral multiplicity	I(n+1)	n(I+1)	n+1	n-1	n+1

9.	Which compound gives AMX spectrum	poly styrene compound	poly ehylene compound	vinyl compound	polyvinyl compound	vinyl compound
10.	What is karplus equation	Ó between 0° and 90° Jvic = $8.5COS^2$ Ó-0.28	Ó between 0° and 90° Jvic = $9.5COS^2$ Ó-0.28	Ó between 0° and 180° Jvic = $8.5COS^2$ Ó-0.28	None of the above	Ó between 0° and 90° Jvic = $8.5COS^2$ Ó-0.28
11.	What is double resonance	At a time use of two radio frequency sources	alternate use of two radio frequency source	simultaneous use of two radio frequency source	combined use of two radio frequency source	At a time use of two radio frequency sources
12.	What is NOE signal	nucleous oriented energy level	nucleous over effect	nuclear over hauser effect	nucleous opposite energy level	nuclear over hauser effect
13.	How many double bonds presents in norbornene	single	double	triple	multiplet	double
14.	How many nitrogen's presents in imidazole	1	2	3	5	2
15.	What is SPI	spin permittive intense peak	selective population inversion	selective possible interaction	spin pulse energy intensive peak.	selective population inversion

16.	(i)Magnetically equivalent protons are chemically equivalent (ii)each signal corresponds to a set of equivalent protons	(i) and (ii) are true	(i) true	(ii) true	(i) and (ii) false	(i) and (ii) are true
17.	Propanal gives	1 NMR signal	2 NMR signal	3 NMR signal	4 NMR signal	2 NMR signal
18.	The DMSO is a	highly polar solvent	highly non-polar solvent	weakly polar solvent	weakly non-polar solvent	highly polar solvent
19.	NMR spectrum obtained in	microwave region	radio frequency region	IR region	none of the above	radio frequency region
20.	For NMR, (downfield due to deshielding (upfield due to shielding	both are true	a true b false	b true a false	both are false	both are true
21.	What is Geminal coupling protons attached as the same carbon having different chemical environment protons attached as the same carbon having same chemical environment	a and b are true	a and d are false	b and a false	both are false	a and b are true

22.	I=0	NMR signals observed	NMR signals not observed	half NMR signals	none of the above	NMR signals not observed
23.	How many gem methyl group presents in □-pinene	one	two	three	zero	two
24.	For NMR the aromatic character relates to cyclic delocalization of	(4n+2) □ electrons	(2n+4) □ electrons	(4n+□) 2 electrons	(2n+□) 4 electrons	(4n+2) □ electrons
25.	What is magnetic equivalence	chemical shift equivalence	coupling equivalence	chemical equivalence	magnetic equivalence	chemical equivalence
26.	What is accidental equivalence	chemical shift of 2 proton are equal	environment of 2 protons are equal	coupling constants are equal	magnetic field of 2 protons are equal	chemical shift of 2 proton are equal
27.	For NMR the number of orientation's is	(2m+I)	(2I+1)	(2n+1)	(2n+1)	(2I+1)
28.	Oppose orientation most stable and aligned orientation least stable most stable least stale	both are true	a true b false	both are false	a false b true	both are true

29.	How many methyl group present I methanol	2	3	4	0	3
30.	Which is used as a lanthanide shift reagent	Europium	TMS	6-methyl quinoline	combination of europium and TMS	6-methyl quinoline
31.	What is the natural abundance of ^{13C} NMR	1%	0.1%	1.1%	11%	1.1%
32.	What is the chemical shift range of ^{1}H spectrum	4ppm	40 ppm	80 ppm	none	80 ppm
33.	What is the chemical shift range of	4ppm	40 ppm	80 ppm	120 ppm	4ppm
34.	What is “CI DNP”	Chemically induced dynamic nuclear polymerization	Chemically induced binuclear polarisation	Chemically induced dynamic number polarisation	magnetically induced	Chemically induced dynamic nuclear polymerization
35.	What is MRI	magnetic radio frequency imagines	magnetic resolution imagines	magnetic resonance imagine	none	magnetic resonance imagine
36.	What is “COSY”	2D NMR-Shift correlation spectra	2D NMR-Shift corrected spectra	NMR shift corrected spectra	NMR shift correction spectra	2D NMR-Shift correlation spectra

37.	The energy of free spin is related to give their environment is called as	spin-spin relaxation	spin-spin coupling	spin-lattice relaxation	spin-lattice coupling	spin-lattice relaxation
38.	How many number of hydrogen present in annulene	12H	14H	16 H	18 H	18 H
39.	What is shielding	spin opposes to magnetic field	spin aligns to magnetic field	spin lateral to magnetic field	spin oppose lateral to magnetic field	spin aligns to magnetic field
40.	What is anisotropic effect	for protons attached to C=C in alkenes	for protons attached to C=C in alkynes	for protons attached to cyclo hexanes	for protons attached to para hexanes	for protons attached to C=C in alkenes
41.	The resultant spin of $^{1}_{1}H$ is	0-100 nm	1	1/2	3/2	1/2
42.	The nucleus which gives NMR spectrum is	$^{16}_{8}O$	$^{10}_{5}B$	^{12}O	$^{4}_{2}He$	$^{10}_{5}B$
43.	The $^{1}_{1}H$ spectrum $CH_2(Cl)CH(Cl)OCH_3$ would show	a 3-proton singlet, I proton triplet and 2 proton doublets	a 3-proton doublet 1 proton triplet and 2 proton singlets	a 3-proton triplet 1 proton doublet 2 proton doublet	a3 proton singlet 1 proton singlet 2 proton doublet	a 3-proton singlet, I proton triplet and 2 proton doublets

44.	The chemical shift of $^{1}_{1}H$ on the δ scale is 2.56. The value on ϒ scale is	12.56	7.44	2.56	1.44	7.44
45.	The bond length on NO is 0.116 nm. Its rotational constant is	5.0^{30} x 10^{10} sec	1.23x 10^{10} sec	5.030x10^{10} sec^{-1}	1.23x 10^{10} sec-1	5.030x10^{10} sec^{-1}
46.	Among the following which does not give NMR spectra	$^{16}_{8}O$	$^{10}_{5}B$	$^{15}_{7}N$	$^{1}_{1}H$	$^{16}_{8}O$
47.	The NMR spectroscopy is based on the magnetic moment by the spinning of a	Atom	Charged nucleus	Electron	neutrons	Charged nucleus
48.	In the PMR spectrum $FCH_2 CHF_2$ would give	4 signals	3 signals	2 signals	1 signals	2 signals
49.	In an NMR spectrum number of protons of each kind in a molecule is indicated by the	number of signals	Intensity of signals	Splitting of a signals	Charged nucleus	Intensity of signals

50.	In a low-resolution NMR spectrum of ethanol, the area under the peak corresponding to OH, CH_2 and CH_3 protons respectively will be in the ratio	3:2:1	1:3:2	3:1:2	1:2:3	3:2:1
51.	What is the spin number of the following nuclei ^{11}B, ^{35}Cl, ^{79}Br, ^{81}Br?	3/2	1/2	1	5/2	3/2
52.	The spin angular momentum of the nucleus, H is----	root of 1/2x h/2π	root of 1/2x11/2	root of 1/2x1 1/2xh/2π	root of 1/2	root of 1/2x1 1/2xh/2π
53.	The Bohr magneton is a unit of and it is nearly times the nuclear magneton	Magnetic field 1850	Magnetic moment,1850	Magnetic field 1/1850	Magnetic moment, 1/1850	Magnetic moment,1850
54.	The proton magnetic resonance is studied in---	Radiofrequency	microwave region	IR region	Visible region	Radiofrequency

55.	Under high resolution, ethanol containing acid impurity gives PMR signals and the hydroxyl proton appears as a	3 triplet	3.singlet	2 singlet	3 quintet	3 triplet
56.	The chemical shift of a proton on the δ scale is 4. The value of the τ scale is--	14	6	2.5	4	6
57.	The fine structure and intensity ratios expected in the proton NMR spectrum of NH_4^+ ion (for ^{14}N, I=1) are	singlet	Doublet 1:1	Triplet 1:1:1	triplet1:2:1	Triplet 1:1:1
58.	In the proton NMR spectrum of toluene, the resonance due to CH_3, group is expected to	δ 0.5	δ 1.25	δ2.5	δ 3.5	δ2.5
59.	How many NMR signals are found in cis dimethyl cyclopropane and trans dimethyl cyclopropane?	2,3	3,4	1,2	8,10	3,4

60.	In NMR spectroscopy the value of coupling constant (5)	Changes with field strength	Changes with temperature	Changes with solvent	Is independent to field strength, temperature and solvent	Is independent to field strength, temperature and solvent

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KARPAGAM ACADEMY OF HIGHER EDUCATION

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

(For the candidates admitted from 2016 onwards)

B.sc Degree Examination

III B.Sc. Chemistry

Internal Exam-I

INSTRUMENTAL METHODS OF CHEMICAL ANALYSIS

Date: **Maximum: 50 marks**

Time: 2 hrs.

Part A

Answer all the questions (20 x 1 = 20 marks)

1. Measurement which is close to the true value is

a) Accurate b) average c) precise d) error

2. Errors that occur during measurement of the quantities are of

a) 2 typesb) 3 types c) 5 types d) 4 types

3. The precision of an analytical measurement

a) Is described by calculating percent relative error.

b) Is related to the width of the population distribution of the measurement.

c) Is guaranteed to be high if the measurement also has high accuracy.

d) Is described by calculating random error.

4. Electromagnetic waves are produced by

a) Deaccelerated charged particle b) charge in uniform motion c) magnet

d) accelerated charged particle

5. Quantitative data refers to

a) Graphs and tables b) numerical data that could usefully be quantified to help you answer your research questions and to meet your objectives c) any data you present in your report d) statistical analysis

6. Error that occurs during the measurement of quantities is

a) Random error b) systematic error c) random and systematic error d) frequent error

7. Analysis based on study of price fluctuations, production of commodities and deposits in

a) Sample series analysis b) time series analysis c) numerical analysis d) experimental analysis

8. The steady state error due to a ramp input for a type 0 system is equal to

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

9. Standard deviation of a sampling distribution is also classified as

a) Standard error b) statistic error c) sampling error d) probability error

10. The reliability of an instrument refers to

a) The measurement of changes due to temperature variation b) The degree to which repeatability continues to remain within specified limits c) The life of an instrument

d) The extent to which the characteristics remain later

11. Random errors in a measurement system are due to

a) Environmental changes b) Use of uncalibrated instrument c) Poor cabling practices d) Unpredictable effects

12. Systematic errors can be removed by

a) Buying new instrument b) breaking the instrument c) dusting the instrument

d) Recalibrating the instrument

13. Relative amounts of elements are discussed in

a) Testing b) quantitative analysis c) Qualitative analysis d) Physical test

14. Among the following types of analytical procedure, which one uses the most variables?

a) Trend analysis b) Data mining c) Ratio analysis d) Reasonableness test

15. Electromagnetic waves do not transport

a) Energy b) charge c) momentum d) information

16. the most important region in IR is

a) 15μ-200 μ b) 2.5 μ-15 μ c) 0.8 μ-2.5μ d) 20μ-50μ

17. The energy of first overtone is

a) ΔEvib= 2hυ b) ΔEvib= hυ+3\2 c) ΔEvib= 3hυ d) ΔEvib= hυ\2

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

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

19. 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}

20. Which of the following is both IR and microwave active?

a) Br_2 b) CS_2 c) HBr d) CO_2

PART-B (3 x 2 = 6 MARKS)

21. Write short notes on precision?

22. What are different sources of errors?

23. Why is methanol a good solvent for UV and not for IR determination?

PART-C (3 x 8 = 24 MARKS)

24. a. Explain the various instrumental methods of analysis.

OR

b. Describe some impotent characteristics of electromagnetic radiation.

25. a. What is error? Explain the types and their significance.

OR

b. Explain the following analytical methods.

(i) Classical method (ii) instrumental method

26. a. Explain the instrumentation of FT-IR spectroscopy.

OR

b. Describe some characteristic absorption bands with their probable region and intensity for the following functional groups:

(i) Ketones (ii) amides (iii) acids (iv) esters

[16CHU504A] **Reg. No----------**

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B.sc Degree Examination

III B.Sc. Chemistry

Internal Exam-I

INSTRUMENTAL METHODS OF CHEMICAL ANALYSIS

Date: **Maximum: 50 marks**

Time: 2 hrs.

Part A

Answer all the questions (20 x 1 = 20 marks)

1. a) Accurate

2. a) 2 types

3. b) Is related to the width of the population distribution of the measurement

4. d) accelerated charged particle

5. b) numerical data that could usefully be quantified to help you answer your research questions and to meet your objectives

6. c) random and systematic error

7. b) time series analysis

8. d) constant

9. a) Standard error

10. b) The degree to which repeatability continues to remain within specified limits

11. d) Unpredictable effects

12. d) Recalibrating the instrument

13. b) quantitative analysis

14. b) Data mining

15. b) charge

16. b) 2.5 μ-15 μ

17. a) $\Delta E_{vib} = 2h\upsilon$

18. b) 1740-1720cm-1

19. c) 1600-1500cm-1

20. c) HBr

PART-B (3 x 2 = 6 MARKS)

21. Write short notes on precision?

Precision refers to the closeness of two or more measurements to each other. Using the example above, if you weigh a given substance five times, and get 3.2 kg each time, then your measurement is very precise. Precision is independent of accuracy. You can be very precise but inaccurate,

More commonly, it is a description of systematic errors, a measure of statistical bias; as these cause a difference between a result and a "true" value, ISO calls this trueness.

Alternatively, ISO defines accuracy as describing a combination of both types of observational error above (random and systematic), so high accuracy requires both high precision and high trueness.

22. What are different sources of errors?

Types of Errors in Measurement System

Systematic Errors.

Random Errors.

1) Gross Errors. Gross errors are caused by mistake in using instruments or meters, calculating measurement and recording data results.

2) Blunders.

3) Measurement Error.

Systematic Errors.

Instrumental Errors.

Environmental Errors.

23. Why is methanol a good solvent for UV and not for IR determination?

The analytical UV range is around 190–400nm and the cut-off wavelength of ethanol is 205nm, which is almost at the lower limit. So there is no significant interference of ethanol in UV spectroscopy.

the basic criteria for a molecule to have IR absorption is that it should have a dipole moment. Basically, all covalently bonded molecules have IR absorption. Ethanol is an aliphatic alcohol with sigma bonds, so it has IR absorption.

PART-C (3 x 8 = 24 MARKS)

24. a. Explain the various instrumental methods of analysis.

Classification of analytical methods:

Analytical Chemistry deals with methods for determining the chemical composition of samples of matter. A qualitative method yields information about the identity of atomic or molecular species or the functional groups in the sample; a quantitative method, in contrast, provides numerical information as to the relative amount of one or more of these components.

Analytical methods are often classified as being either classical or instrumental. This classification is largely historical with classical methods, sometimes called wet-chemical methods, preceding instrumental methods by a century or more.

Classical Methods:

- Separation of analytes by precipitation, extraction, or distillation.
- Qualitative analysis by reaction of analytes with reagents that yielded products that could be recognized by their colors, boiling or melting points, solubilities, optical activities, or refractive indexes.
- Quantitative analysis by gravimetric or by titrimetric techniques.

In the early years of chemistry, most analyses were carried out by separating components of interest in a sample by precipitation, extraction, or distillation. For quantitative analyses, the separated components were then treated with reagents that yielded products that could be recognized by their colors, boiling points or melting points, their solubility in a series of solvents, their odors, their optical activities, or their refractive indexes. For quantitative analyses, the amount of analyte was determined by gravimetric or by titrimetric measurement.

Gravimetric Methods – the mass of the analyte or some compound produced from the analyte was determined.

Titrimetric Methods – the volume or mass of a standard reagent required to react completely with the analyte was measured.

Types of instrumental Methods:

Measurements of physical properties of analytes, such as conductivity, electrode potential, light absorption, or emission, mass to charge ratio, and fluorescence, began to be used for quantitative analysis of a variety of inorganic, organic, and biochemical analyte. Highly efficient chromatographic and electrophoretic techniques began to replace distillation, extraction, and precipitation for the separation of components of complex mixtures prior to their qualitative or quantitative determination. These newer methods for separating and determining chemical species are known collectively as instrumental methods of analysis.

Instrumentation can be divided into two categories: detection and quantitation.

- Measurement of physical properties of analytes - such as conductivity, electrode potential, light absorption or emission, mass-to-charge ratio, and fluorescence-began to be employed for quantitative analysis of inorganic, organic, and biochemical analytes.
- Efficient chromatographic separation techniques are used for the separation of components of complex mixtures.
- Instrumental Methods of analysis (collective name for newer methods for separation and determination of chemical species.)

Instrumentation is necessary to decipher these values. The challenge for the instrumental scientist is to mimic the 5 senses. Substances have physical and chemical fingerprints with unique thresholds. The object is to detect a chemical substance within a matrix and selectively perturb the substance of interest. Signals must be readable (in a voltage or electrical signal).

Types of instrumentation

Signal	**Instrumental Methods**
Emission of radiation	Emission spectroscopy (X-ray, UV, visible, electron, Auger); fluorescence, phosphorescence, and luminescence (X-ray, UV, and visible)

Absorption of radiation	Spectrophotometry and photometry (X-ray, UV, visible, IR); photoacoustic spectroscopy; nuclear magnetic resonance and electron spin resonance spectroscopy
Scattering of radiation	Turbidimetry; nephelometry; Raman spectroscopy
Refraction of radiation	Refractometry; interferometry
Diffraction of radiation	X-Ray and electron diffraction methods
Rotation of radiation	Polarimetry; optical rotary dispersion; circular dichroism
Electrical potential	Potentiometry; chronopotentiometry
Electrical charge	Coulometry
Electrical current	Polarography; amperometry
Electrical resistance	Conductometry
Mass-to-charge ratio	Mass spectrometry
Rate of reaction	Kinetic methods
Thermal properties	Thermal conductivity and enthalpy
Radioactivity	Activation and isotope dilution methods

Instruments for Analysis

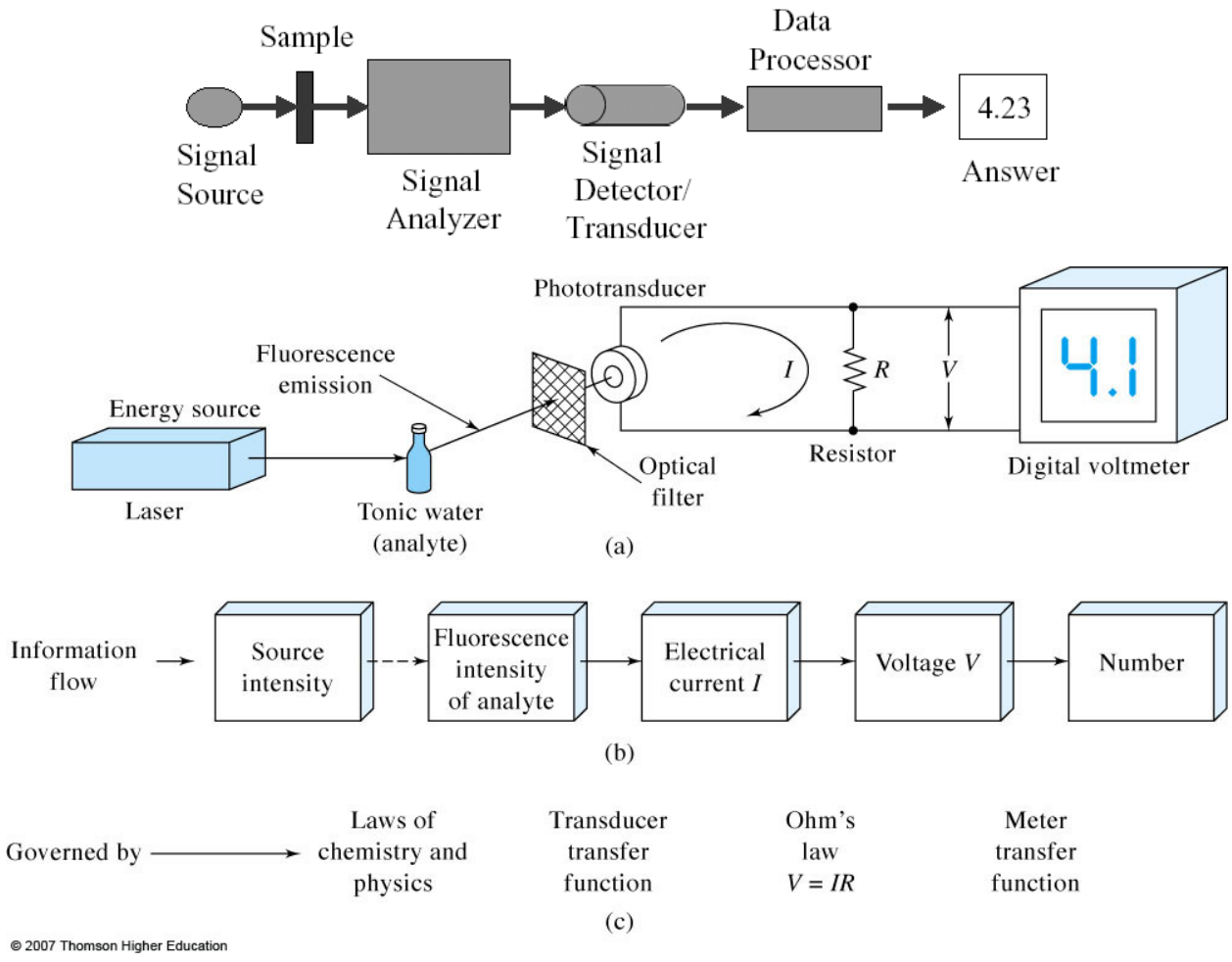
An instrument for chemical analysis converts information about the physical or chemical characteristics of the analyte to information that can be manipulated and interpreted by human.

Detectors: A mechanical, electrical, or chemical device that identifies, records, or indicates a change in one of the variables in the environment, such as pressure, temperature, electrical charge, electromagnetic radiation, nuclear radiation, particulates, or molecules.

Transducers: Devices that convert information in nonelectrical domains to information in electrical domains and the converse. (Photodiodes, photomultipliers)

Sensors: Analytical devices those are capable of monitoring specific chemical species continuously and reversibly.

Instrument Components



OR

b. Describe some important characteristics of electromagnetic radiation.

Light Rays, Geometric Optics is often a convenient way to look at EM wave propagation. The 'rays' are straight normal to local wave fronts, with the following properties. Reflection: Equal angles of incidence and reflection as measured from a normal to the surface. This principle allows mirrors to focus light. Refraction (dispersion): Generally light rays are bent as they pass through the boundary between two media, or through a medium with a

temperature or pressure gradient. Dispersion results when the bending (or index of refraction) depends on wavelength.

Particle Aspect of Electromagnetic Radiation.

In addition to its wave nature, electromagnetic radiation also seems to come in discrete bundles of energy called ‘photons.’ The energy of a photon depends on the frequency of the radiation via Planck’s law: $\varepsilon = h\nu = hc/\lambda$.

This relation connects the wave-particle dual characteristics of EM radiation. The particle aspect is most relevant at high energies, or very low light levels, i.e., where there are few photons.

Monochromatic flux - is easier to define! Integrate intensity in solid angle over a hemisphere. Thus, flux is the energy in frequency range (ν, $\nu+\Delta\nu$) flowing through a unit area per unit time, and into ‘any’ direction (of the hemisphere).

25. a. What is error? Explain the types and their significance.

An 'error' is a deviation from accuracy or correctness. A 'mistake' is an error caused by a fault: the fault being misjudgment, carelessness, or forgetfulness. Now, say that I run a stop sign because I was in a hurry, and wasn't concentrating, and the police stop me, that is a mistake.

There are three types of error: syntax errors, logical errors and run-time errors. (Logical errors are also called semantic errors).

Performing the experiment and collecting data is only the beginning of the process of completing an experiment in science. Understanding the results of any given experiment is always the central goal of the experiment. Presenting those results in a clear concise manner completes the experiment. This overview of the complete process is as valid in an instructional laboratory course as in a research environment. You will not have learned any physics if you did not understand the experiment. Presenting the results of your experimental work is fundamentally saying, "This is what I did and this is what I learned." Putting together your presentation of the results should help you clarify the results to yourself. (If your instructor can clearly see what you did and what you learned, you might get a better grade.)

Data analysis should NOT be delayed until all of the data is recorded. Take a low point, a highpoint and maybe a middle point, and do a quick analysis and plot. This will help one avoid the problem

of spending an entire class collecting bad data because of a mistake in experimental procedure or an equipment failure.

First and foremost, data analysis means understanding what your results mean. When analyzing your data, try to think through the physical processes which are occurring. Write your train of thought down. Ultimately, the goal is for you to understand physics and the world a bit better. Your understanding of your results probably occurs in stages, with each stage being a refinement and possibly more mathematical than the previous stage.

For example, one might first note that as time increases so does distance. Next a quick graph of distance vs time might verify this understanding but the relationship is NOT linear, i.e. the data does not form a straight line. By further work, one might discover that distance increase linearly with the square of the time. Or sometimes the mathematical relationship may remain hidden.

OR

b. Explain the following analytical methods.

(i) Classical method (ii) instrumental method

The majority of the classical analytical methods rely on chemical reactions to perform an analysis. In contrast, instrumental methods typically depend on the measurement of a physical property of the analyte.

Classical qualitative analysis is performed by adding one or a series of chemical reagents to the analyte. By observing the chemical reactions and their products, one can deduce the identity of the analyte. The added reagents are chosen so that they selectively react with one or a single class of chemical compounds to form a distinctive reaction product. Normally the reaction product is a precipitate or a gas, or it is coloured. Take for example copper (II), which reacts with ammonia to form a copper-ammonia complex that is characteristically deep blue. Similarly, dissolved lead (II) reacts with solutions containing chromate to form a yellow lead chromate precipitate. Negative ions (anions) as well as positive ions (cations) can be qualitatively analyzed using the same approach. The reaction between carbonates and strong acids to form bubbles of carbon dioxide gas is a typical example.

Prior to the qualitative analysis of any given compound, the analyte generally has been identified as either organic or inorganic. Consequently, qualitative analysis is divided into organic and inorganic categories. Organic compounds consist of carbon compounds, whereas inorganic

compounds primarily contain elements other than carbon. Sugar ($C_{12}H_{22}O_{11}$) is an example of an organic compound, while table salt (NaCl) is inorganic.

The instrumental methods of chemical analysis are divided into categories according to the property of the analyte that is to be measured. Many of the methods can be used for both qualitative and quantitative analysis. The major categories of instrumental methods are the spectral, electroanalytical, and separatory.

Spectral methods

Spectral methods measure the electromagnetic radiation that is absorbed, scattered, or emitted by the analyte. Because the types of radiation that can be monitored are multitudinous and the manner in which the radiation is measured can significantly vary from one method to another, the spectral methods constitute the largest category of instrumental methods. (See spectroscopy for a more detailed treatment of this subject.)

Absorptiometry
Ultraviolet-visible spectrophotometry
Nuclear magnetic resonance
Microwave absorptiometry
Thermal analysis
X-ray absorption
Scattered radiation
Electron spectroscopy
Radiochemical methods
Electroanalysis

26. a. Explain the instrumentation of FT-IR spectroscopy.

FT-IR stands for Fourier Transform Infra-Red, the preferred method of infrared spectroscopy.

- In infrared spectroscopy, IR radiation is passed through a sample, some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample.

- Like a fingerprint no two unique molecular structures produce the same infrared spectrum.
- This makes infrared spectroscopy useful for several types of analysis.
- This system is based on the Michelson- Morley experiment used to measure the influence of earth rotation on the speed of light.

INTRUMENTATION He-Ne gas laser Movable mirror Beam splitter Sample chamber Fixed mirror Interferometer Detector

Principle of FTIR Spectroscopy Lambert-Beer´s law • FTIR spectra can provide quantitative information • Lambert-Beer´s law correlates physical properties and chemical composition: – The concentration of a sample can be estimated by: A = ε.c.d – Where: • ε is the molar absorption coefficient • c is the sample concentration • d is the sample thickness

Practical FTIR applications in packaging

1. Polymer processing – curing

2. Plasma etching

3. Identification of materials: – polymer dielectrics – inorganic thin films – contamination – unknown compounds

4. Analysis of formulations

6. Drying and curing polymers

Drying of photo-sensitive materials is critical – Impacts photo response Optimizing curing: – Determine optimum intermediate curing in multi-layer applications • Curing level kept low for layer to promote inter-layer bonding. • Curing level high enough to withstand sputtering thermal load. – Checking on consistency of curing level. – Determining curing level and completion. – Checking on the effects of novel curing methods • Microwave

7. Optimizing curing profile – Ramping speed. – Monitoring effects of background curing atmosphere.

•Drying photo resist materials. • Drying polyimide - Identification of material condensing on walls of a poorly ventilated drying oven.

8. Drying Polyimide

9. Monitoring product after curing 1. Curing atmosphere: – Evaluation of thermo-oxidative and thermal stability. – Stability check of cured polymers to environment. • Post curing oxidation in air. – Troubleshooting curing oven problems. 2. Moisture absorption. 3. Evaluation of oxygen or

moisture barrier capabilities. 4. Detection of molecular impurities or additives present in amounts of 1% and in some cases as low as 0.01%.

10. Plasma Etching 1. Detection of etching endpoint – contact via holes – polymer – oxide 2. Detection of etching problems – Residual Fluorine on polymer surface – Polymer or metal oxidation – Polymer degradation: Identification of bonds damaged by plasma chemistry 3. Cleaning of via holes – Very thin films are not detectable in an optical microscope – Over-etching and under-etching control – Detection and identification of residues (e.g. ash)

11. Identification of contamination 1. Chemical contamination of parts in processing – e.g. Permeation or absorption of chemicals in a polymer. 2. Contamination of parts induced by handling, processing, shipping etc. 3. Aging of vacuum roughing lubricants – Deterioration of plasma pump oil. • Acidification, oxidation or fluorination. 4. Vacuum chamber contamination.

12. Identifying Contamination

13. Identification of Materials and Chemicals 1. Identification of compounds – Matching spectrum of unknown compound with reference spectrum (fingerprinting). 2. Identification of functional groups in unknown substances. Ex. Ketones, Aldehydes, Carboxylic Acids Etc. 3. Identification of reaction components and kinetic studies of reactions.

14. 4. Identification of molecular orientation in polymer films – Need polarized IR set-up. 5. Identification of polymers, plastics, and resins. 6. Analysis of formulations – Wet etchants – Cleaning solutions – Solvents.

15. Specific groups Alcohols Show a strong, broad band for the O-H stretch from 3200-3400 cm -1 1-butanol

16. Primary Amines Shows the –N-H stretch for NH2 as a doublet between 3200-3500 cm-1 2-aminopentane

17. Spectra of Thin Inorganic Films Monitoring of oxidation of an Aluminium film.

18. Other FTIR Applications Opaque or cloudy samples. Energy limiting accessories such as diffuse reflectance or FT-IR microscopes. High resolution experiments (as high as 0.001 cm-1 resolution). Trace analysis of raw materials or finished products. Depth profiling and microscopic mapping of samples. Kinetics reactions on the microsecond time-scale. Analysis of chromatographic and thermo gravimetric sample fractions.

19. FTIR limitations 1. Molecule must be active in the IR region. (When exposed to IR radiation, a minimum of one vibrational motion must alter the net dipole moment of the molecule in order

for absorption to be observed.) 2. Minimal elemental information is given for most samples. 3. Material under test must have some transparency in the spectral region of interest. 4. Accuracy greater than 1% obtainable when analysis is done under favorable conditions.

20. Comparison of FT-IR & IR Dispersive IR Fourier transform IR 1. There are many moving parts, resulting in mechanical slippage. 1. Only the mirror moves during the experiment. 2. Calibration against reference spectra is required to measure frequency. 2. Use of laser provides high frequency accuracy (to 0.01 cm-1). 3. Stray light causes spurious readings. 3. Stray radiations do not affect the detector. 4. To improve resolution only a small amount of IR beam is allowed to pass. 4. A much larger beam may be used at all time. Data collection is easier.

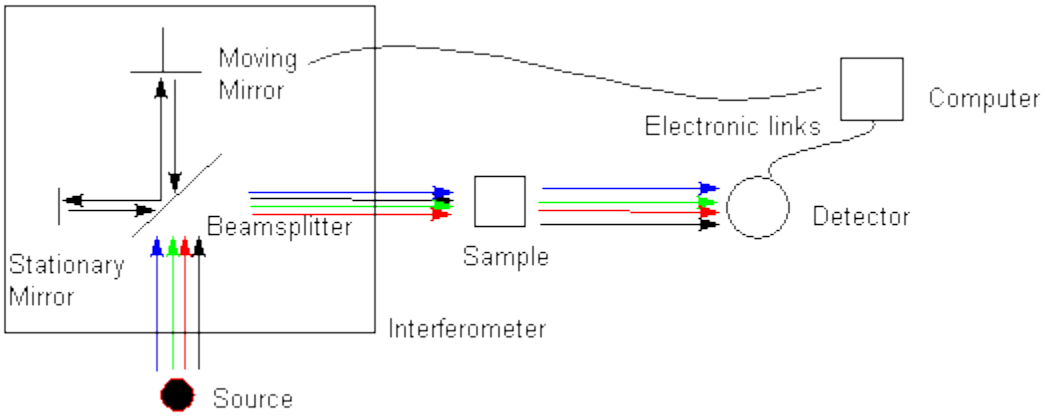
21. Dispersive IR Fourier transform IR 5. Only radiation of a narrow frequency range falls on the detector at one time. 5. All frequency of radiation falls on the detector simultaneously. 6. Slow scan speed. 6. Rapid scan speed.

22. Advantages Fellgett's (multiplex) Advantage- FT-IR collects all resolution elements with a complete scan of the interferometer. Successive scans of the FT-IR instrument are co added and averaged to enhance the signal-to-noise (S/N ratio) of the spectrum. Connes Advantage – □ An FT-IR uses a He-Ne laser as an internal wavelength standard. The infrared wavelengths are calculated using the laser wavelength, itself a very precise and repeatable 'standard'. □ Wavelength assignment for the FT-IR spectrum is very repeatable and reproducible and data can be compared to digital libraries for identification purposes.

23. Advantages Jacquinot Advantage- FT-IR uses a combination of circular apertures and interferometer travel to define resolution. To improve signal-to-noise ratio, one simply collects more scans.

24. Conclusion Advantages– FTIR is a simple and sensitive analytical tool. – Provide fast data acquisition tool. – Simple to operate – Most useful analytical tool • To determine the composition of organic materials • To identify IR transparent or semi-transparent inorganic films • Provides quantitative determination of compounds in mixtures

25. Disadvantages– Interpretation of the data requires some experience. – No useful detailed database available for the semiconductor processes. – Carbon di-oxide & Water Sensitive.



OR

b. Describe some characteristic absorption bands with their probable region and intensity for the following functional groups:

(i) Ketones (ii) amides (iii) acids (iv) esters

(i) Ketones

The carbonyl stretching vibration band C=O of saturated aliphatic ketones appears at 1715 cm-1. Conjugation of the carbonyl group with carbon-carbon double bonds or phenyl groups, as in alpha, beta-unsaturated aldehydes and benzaldehyde, shifts this band to lower wavenumbers, 1685-1666 cm-1.

C=O stretch:

aliphatic ketones 1715 cm-1

alpha, beta-unsaturated ketones 1685-1666 cm-1

The spectrum of 2-butanone is shown below. This is a saturated ketone, and the C=O band appears at 1715. Note the C–H stretches (around 2991) of alkyl groups. It's usually not necessary to mark any of the bands in the fingerprint region (less than 1500 cm-1).

(ii) amides

	Amide		
C=O	stretch	1640-1690	strong

N-H	stretch	3100-3500	unsubstituted have two bands
N-H	bending	1550-1640	

(iii) Acids

Carboxylic acids show a strong, wide band for the O–H stretch. Unlike the O–H stretch band observed in alcohols, the carboxylic acid O–H stretch appears as a very broad band in the region 3300-2500 cm-1, centered at about 3000 cm-1. This is in the same region as the C–H stretching bands of both alkyl and aromatic groups. Thus a carboxylic acid shows a somewhat "messy" absorption pattern in the region 3300-2500 cm-1, with the broad O–H band superimposed on the sharp C–H stretching bands. The reason that the O–H stretch band of carboxylic acids is so broad is becase carboxylic acids usually exist as hydrogen-bonded dimers.

The carbonyl stretch C=O of a carboxylic acid appears as an intense band from 1760-1690 cm-1. The exact position of this broad band depends on whether the carboxylic acid is saturated or unsaturated, dimerized, or has internal hydrogen bonding. See also:

carbonyl stretching frequencies

The C–O stretch appears in the region 1320-1210 cm-1, and the O–H bend is in the region 1440-1395 cm-1 and 950-910 cm-1, although the 1440-1395 band may not be distinguishable from C–H bending bands in the same region.

O–H stretch from 3300-2500 cm-1
C=O stretch from 1760-1690 cm-1
C–O stretch from 1320-1210 cm-1
O–H bend from 1440-1395 and 950-910 cm-1

The spectrum of hexanoic acid is shown below. Note the broad peak due to O–H stretch superimposed on the sharp band due to C–H stretch. Note the C=O stretch (1721), C–O stretch (1296), O–H bends (1419, 948), and C–O stretch (1296).

(iv) Esters

The carbonyl stretch C=O of aliphatic esters appears from 1750-1735 cm-1; that of α, β-unsaturated esters appears from 1730-1715 cm-1. See also:

carbonyl stretching frequencies

The C–O stretches appear as two or more bands in the region 1300-1000 cm-1.

C=O stretch

aliphatic from 1750-1735 cm-1

α, β-unsaturated from 1730-1715 cm-1

C–O stretch from 1300-1000 cm-1

The spectra of ethyl acetate and ethyl benzoate are shown below. Note that the C=O stretch of ethyl acetate (1752) is at a higher wavelength than that of the α, β-unsaturated ester ethyl benzoate (1726). Also note the C–O stretches in the region 1300-1000 cm-1.

No. of copies : 50

Reg. No.....

[16CHU504A]

KARPAGAM ACADEMY OF HIGHER EDUCATION
COIMBATORE-21
(For the candidates admitted from 2016 & onwards)
B. Sc DEGREE EXAMINATION-September-2018
III-B.Sc., CHEMISTRY
Internal Exam-II
INSTRUMENTAL METHODS OF CHEMICAL ANALYSIS

DATE: 11/9/18 AN
TIME: 2Hrs

SUBJECT CODE: 16CHU504A
TOTAL: 50 MARKS

PART-A ANSWER ALL THE QUESTIONS (20 x 1 = 20 Marks)

1. Which one of the following represents the weakest interaction between two species?
a) Hydrogen bond b) Disulfide bond c) Ionic bond d) Dispersion force

2. Which one of the following terms describes a positive and negative charge, which are separated in space within a molecule?
a) Salt bridge b) Polar bond c) Dipole d) van der Waals interaction

3. Which of the following functional groups is most likely to participate in a dipole-dipole interaction?
a) Aromatic ring b) Ketone c) Alcohol d) Alkene

4. Headspace analysis is carried out in order to
a) Analyse volatile compounds from solid or liquid samples
b) Determine the psychological state of the tutor
c) Analyse the column contents ahead of the sample
d) Determine non-volatiles

5. Resolution is proportional to the
a) Number of theoretical plates in a column
b) Square root of the number of theoretical plates in a column
c) Square of the number of theoretical plates in a column
d) Cube root of the number of theoretical plates in a column

6. Sample retention in the column is measured by
a) Retention time b) factor c) index d) co-efficient

7. Column chromatography is a type of
a) Partition b) adsorption c) Absorption d) thin layer

8. Chromatography is a technique used for --------- compounds
a) Separation b) identification c) measure d) analysis

9. In reverse phase chromatography, the stationary phase is made
a) Non-polar b) either non-polar or polar c) polar d) low polar

10. Split injection is carried out by
a) Splitting the sample into smaller portions to inject sequentially
b) Splitting the sample into smaller portions to inject at the same time through parallel ports
c) Splitting off some of the sample so that it does not enter the column
d) It does not split the sample portions

11. The column is heated to
a) Preventanalyte condensation within the column
b) Control elution of the same analytes
c) irreduce band broadening to get sharper peaks
d) Control elution of the different analytes

12. The composition of Silica gel G is
a) Silica gel without binder b) silica gel + $CaSO_4$
c) Silica gel + alumina d) silica gel + $MaSO_4$

13. Which of the following gases is unsuitable for use as a GC carrier gas?
a) Nitrogen b) Helium c) Oxygen d) Hydrogen

14. Formic acid is an example of
a) Protogenic solvent b) protophillic solvent
c) Amphiprotic solvent d) Aprotic solvent

15. Sucrose can be determined after silylation using which chromatographic technique
a) HPLC b) Gel chromatography
c) Gas liquid chromatography d) Paper chromatography

16. Snells law is related to
a) Refractometry b) Potentiometry
c) Non-aqueous titrations d) Chromatography

17. The spectra resulting from changes in vibrational energy levels for the same electronic state fall in which region of the spectrum
a) Microwave b) Visible c) UV d) IR

18. The rotational constant (B) of a diatomicmolecule is
a) h/4π2I b) h2/4π2I c) h/8π2I d) h2/8π2I

19. In practical organic chemistry tetramethylsilane is used mainly for--
a) Making volatile derivatives of alcohols b) A spectroscopic standard
c) A solvent for IR spectra d)anantinock in gasolines

20. Which of the following compounds does not absorb light in the UV/visible spectrum?
a) Aspirin b) Paracetamol c) Chloral hydrate d) Phenobarbitone

PART-B ANSWER ALL THE QUESTIONS **(3 x 2 = 6 MARKS)**

21. Write note on FT-IR source of light?

22. Define Base Peak in Mass Spectrometry?

23. What are the solvents used for column chromatography?

PART-C ANSWER ALL THE QUESTIONS **(3 x 8 = 24 MARKS)**

24. a. Write a note on single beam and double beam spectrophotometer?

OR

b. Write note on photocells, photomultipliers and diode arrays?

25. a. Illustrate a gas Chromatography instrument: Describe the principal components?

OR

b. Name four different types of detectors used in column Chromatography?

26. a. Write note on advantages of FT-IR?

OR

b. What are the differences between packed column Chromatography and capillary Column Chromatography?

Reg. No…….. **[16CHU504A]**

KARPAGAM ACADEMY OF HIGHER EDUCATION

COIMBATORE-21

(For the candidates admitted from 2016 & onwards)

B. Sc DEGREE EXAMINATION-November-2018

III-B.Sc., CHEMISTRY

Internal Exam-II

INSTRUMENTAL METHODS OF CHEMICAL ANALYSIS

DATE: **SUBJECT CODE: 16CHU504A**

TIME: 2 Hrs **TOTAL: 50 MARKS**

PART-A ANSWER ALL THE QUESTIONS (20 x 1 = 20 Marks)

1. d) Dispersion force
2. c) Dipole
3. b) Ketone
4. a) Analyse volatile compounds from solid or liquid samples
5. b) Square root of the number of theoretical plates in a column
6. a) Retention time
7. b) adsorption
8. a) Separation
9. a) Non-polar
10. c) Splitting off some of the sample so that it does not enter the column
11. d) Control elution of the different analytes
13. c) Oxygen
14. a) Protogenic solvent
15. c) Gas liquid chromatography
16. a) Refractometry

17. d) IR

18. c) h/8π2I

19. b) A spectroscopic standard

20. c) Chloral hydrate

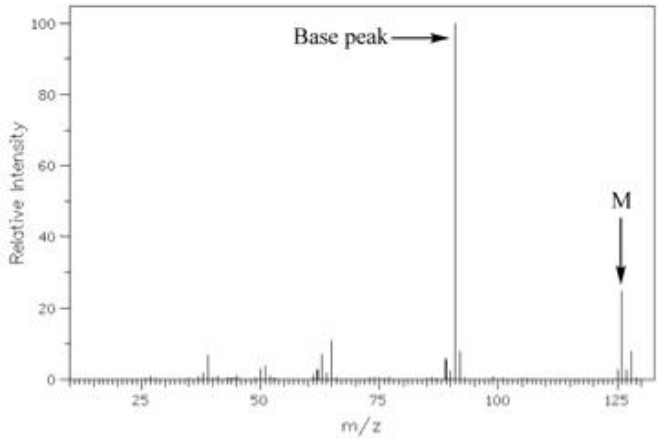
PART-B ANSWER ALL THE QUESTIONS (3 x 2 = 6 MARKS)

21. Write note on FT-IR source of light?

Instruments for measuring infrared absorption all require a source of continuous infrared radiation and a sensitive infrared transducer, or detector. Infrared sources consist of an inert solid that is electrically heated to a temperature between 1,500 and 2,200 K. The heated material will then emit infra red radiation. FT-IR Scanners as part of the modular solution to FT-IR spectroscopy. We offer a QTH version (80009) and SiC version (80007). Modular and interchangeable infrared sources, SiC emitter or QTH IR sources Includes power supply, source, and cables, Compatible with Oriel MIR8035™ FT-IR Scanners

22. Define Base peak in mass Spectroscopy?

Base peak: The most intense (tallest) peak in a mass spectrum, due to the ion with the greatest relative abundance (relative intensity; height of peak along the spectrum's y-axis). Not to be confused with molecular ion: base peaks are not always molecular ions, and molecular ions are not always base peaks.



23. What are the solvents used for column chromatography?

Hydrocarbons: pentane, petroleum ether, hexanes

Ether and dichloromethane: (very similar polarity)

Ethyl acetate: The most common two-component solvent systems (listed from the least polar to the most polar):

Ether/Petroleum Ether, Ether/Hexane, Ether/Pentane: Choice of hydrocarbon component depends upon availability and requirements for boiling range. Pentane is expensive and low-boiling, petroleum ether can be low-boiling, hexane is readily available.

Ethyl Acetate/Hexane: The standard, good for ordinary compounds and best for difficult separations.

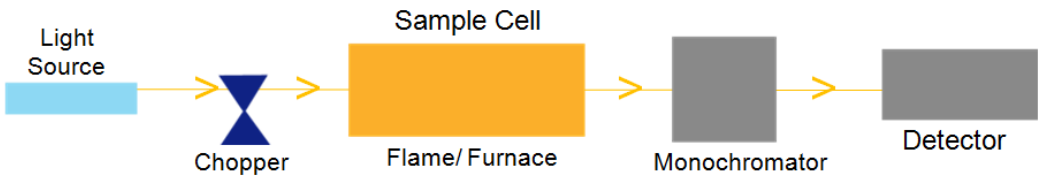
Methanol/Dichloromethane: For polar compounds.

10 percent Ammonia in Methanol Solution/Dichloromethane: Sometimes moves stubborn amines off the baseline.

PART-C ANSWER ALL THE QUESTIONS (3 x 8 = 24 MARKS)

24. a. Write a note on single beam and double beam spectrophotometer?

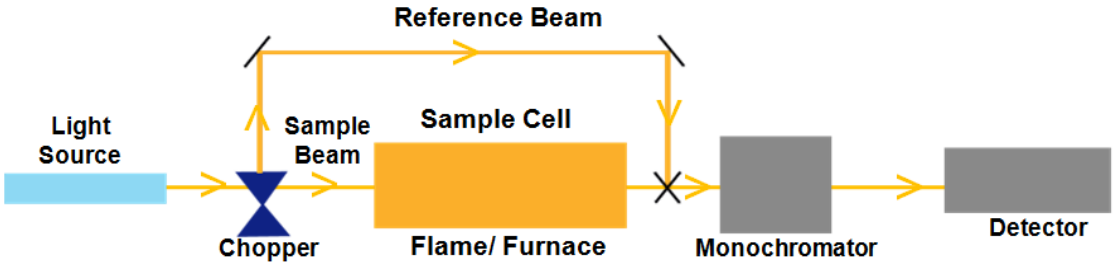
Single Beam spectrometer



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 disk suspended 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. Not shown in the animations that accompany this page is the spectrophotometer's monochromator which selects the analytical wavelength from the source lamp's broad spectrum containing many wavelengths of light. The analytical wavelength is chosen based on the absorbance characteristics of the analyte. Monochromator are instruments whose sole purpose is to allow polychromatic (that is many wavelengths containing) 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. This exiting, well-shaped, narrowly-defined beam now contains a small region of the electromagnetic spectrum. The spread, or band-pass, of the wavelengths depends on the slit settings of the monochromatic (usually adjustable) and the quality of the light dispersing element in the monochromatic (usually a grating in most modern monochromators). In the instrumental design shown schematically in the animation and below here the source lamp's beam is alternately diverted at right angles by a rotating disk with three 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 though the disk it shines directly into the sample cell. If the sample is a liquid then this cell contains a cuvette and is made of a transparent material, such as quartz, that does not absorb light in the spectral region of interest. The analyte is dissolved in a solvent held in the cuvette. When the source light is reflected at 90 degrees by the rotating disk instead of striking the sample cuvette it passes through a cuvette in the reference cell which contains ONLY solvent. During the third sequence, when the black sector blocks the source beam, NO light passes through the disk. And as can be seen below, therefore no light arrives at the photo transducer. This part of the cycle is used for the computer to

digitize and measure the dark current--the amount of light produced by the photo transducer circuit when no light impinges on the photo transducer. The dark current can be subtracted from the overall light measurements made by the system. After travelling through either the sample cell or reference cell the light that was not absorbed--by far, most of the beam-- is directed onto the photo transducer or light detector. This component converts the arrival of photons into an electrical signal. By the way, the light path through the spectrophotometer need not be in a straight line since 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 beam generate alternating electrical photo transducer signals. A computer, sampling those signals, can now determine the analyte absorption in two ways. Some instruments merely subtract the sample beam signal's digitized light intensity from that of the reference beam. The difference is a measure of the amount of light absorbed by the analyte. Since photo transducers-based system are relatively poor at measuring the absolute difference in two different light intensities especially if that difference is large, light absorbances determined in this manner can contain unacceptable amounts of error. Photo transducer are, however, good at generating signals from light intensities that are close together in intensity; therefore, an alternate means of determining the analyte absorption is used by some instruments: Some spectrophotometer design uses the digitized reference-minus-sample signal difference to activate a servor motor connected to the computer and a device called an optical wedge. The servor motor slides the optical wedge into the brighter reference beam's path somewhere after the reference cell but before the photo transducer. Remember that since the reference cell does not have any light absorbing analyte, the light exiting the reference cell will always be brighter than that from the sample cell even if the solvent itself absorbs some at the analyte wavelength since both cuvettes contain solvent. The optical wedge is made of a material that absorbs light so that the more the wedge intersects the reference beam the more of that beam will be absorbed by the wedge and the less will be the difference between the sample and reference signals. The wedge is automatically fed into the reference beam until the reference and sample beam signals are of exactly identical intensity as measured by the photo transducer (remember the system is good at this). When the signals are equal the amount of wedge needed to produce this 0 signal difference is a measure of the analyte absorption. Since the computer controls the wedge it converts wedge position to an absorbance reading of the analyte.

OR

b. Write note on photocells, photomultipliers and diode arrays?

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 photo resistors. 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 has the most light hitting it", photocells can be a good choice.

Photodiode array

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

Photomultiplier tubes (photomultipliers or PMTs for short), members of the class of vacuum tubes, and more specifically vacuum phototubes, are extremely sensitive detectors of light in the ultraviolet, visible, and near-infrared ranges of the electromagnetic spectrum

25. a. Illustrate a gas Chromatography instrument: Describe the principal components?

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.
Importance of column technology (packing, capillaries)

Liquid chromatography

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 = octadecyl silyl as the stationary phase) is termed reversed phase liquid chromatography (RPLC).

OR

b. Name four different types of detectors used in column Chromatography?

Charged aerosol detector (CAD)
Flame ionization detector (FID)
Aerosol-based detector (NQA)
Flame photometric detector (FPD)
Atomic-emission detector (AED)
Nitrogen Phosphorus Detector (NPD)
Evaporative light scattering detector (ELSD)
Mass spectrometer (MS)
Electrolytic Conductivity detector (ELCD)

The Charged Aerosol Detector (CAD) is a universal detector used in conjunction with high-performance liquid chromatography (HPLC) and ultra high-performance liquid chromatography (UHPLC) to measure the amount of chemicals in a sample by creating charged aerosol particles which are detected using an electrometer. A flame ionization detector (FID) is a scientific instrument that measures analyte in a gas stream. It is frequently used as a detector in gas chromatography. The most commonly used detectors are the flame ionization detector (FID) and the thermal conductivity detector (TCD). Both are sensitive to a wide range of components, and both work over a wide range of concentrations. The nitrogen–phosphorus detector (NPD) is also known as thermionic specific detector (TSD) is a detector commonly used with gas chromatography, in which thermal energy is used to ionize an analyte. An evaporative light scattering detector (ELSD) is a detector used in conjunction with high-performance liquid chromatography (HPLC). Mass spectrometry (MS) is an analytical technique that ionizes chemical species and sorts the ions based on their mass-to-charge ratio.

26. a. Write note on advantages of FT-IR?

FT-IR Advantages

The modern FT-IR spectrometer has three major advantages over a typical dispersive infrared spectrometer. These advantages are the reason FT-IR is now the standard tool, having largely displaced dispersive instruments by the mid-1980's. Multiplex Advantage As seen from the operations description above, the interferometer does not separate energy into individual frequencies for measurement. Each point in the interferogram contains information from each wavelength of light being measured. Every stroke of the moving mirror equals one scan of the entire infrared spectrum, and individual scans can be combined to allow signal averaging. In the dispersive instrument, every wavelength across the spectrum must be measured individually as the grating scans. This can be a slow process, and typically only one spectral scan of the sample is made in a dispersive instrument. The multiplex advantage means many scans can be completed and averaged on an FT-IR in a shorter time than one scan on most dispersive instruments.

Throughput Advantage

The FT-IR instrument does not limit the amount of light reaching the detector using a slit. The Thermo ScientificFT-IR spectrometers also use the fewest number of mirrorsnecessary, which means less reflective losses occur.Overall, these mean more energy reaches the sample and hence the detector in an FT-IR spectrometer than in adispersive spectrometer. The higher signal leads to animproved signal-to-noise ratio of the FT-IR. Highersignal-to-noise means that the sensitivity of the instrumentfor small absorptions will be greater, and details in asample spectrum will be clearer and more distinguishable.The IR analysis of proteins is a good example – this is almost impossible in a classical dispersive instrument,while it is a fairly routine measurement for FT-IR.The slit in a dispersive instrument becomes even more of alimitation as the spectral resolution desired increases. Tosee a narrower range, the slit closes down, choking theamount of light passing the instrument, resulting in poorquality spectra for all except ideal samples. Further, highresolution also implies a very slow scan speed, so it cantake long times to collect a high resolution dispersivespectrum. To attain ultra-high resolution, the IR also usesan aperture, but the limitation of the light is not nearly sosevere, and the multiplex advantage quickly gains back the loss.

Precision Advantage

An FT-IR spectrometer uses a laser to control the velocity of the moving mirror and to time the collection of data points throughout the mirror stroke. This laser is also used as a reference signal within the instrument. The interferogram of the laser is a constant sine-wave, which provides the reference for both precision and accuracy of the infrared spectrometer. Well-designed FT-IR spectro meters rely exclusively on this reference laser, rather than any external reference sample. In this case, spectra collected with an FT-IR spectrometer can be compared with confidence whether they were collected five minutes or five years apart. This capability is not available on a dispersive infrared system, or any system requiring external calibration standards.

OR

b. What are the differences between packed column Chromatography and capillary Column Chromatography?

The main difference between packed column and capillary column is that, in a packed column, the stationary phase is packed into the cavity of the column whereas, in a capillary column, the stationary phase coats the inner surface of the cavity of the column. Furthermore, we mainly use packed columns in liquid-liquid extractions and capillary columns in gas chromatography.

Packed column and capillary column are two types of columns used as the stationary phase during chromatographic extractions. Stationary phase is the fixed phase of the chromatography through which the mobile phase carries the components of the mixture.

Packed column gas chromatography (GC) involves the use of a larger column (usually made out of a tube of metal in excess of several mm in diameter) that is filled by tiny grains of a solid or solid/high-melting liquid mixture that acts as the solid or liquid (stationary) phase in the chromatography. The gas phase interacts with those solid particles and separation of a mixture of compounds can occur due to the differences in volatility vs adsorption on the stationary phase of each component in the mixture.

Capillary GC is similar in principal to the above method, except that the tube one uses as the column is usually less than 1 mm in inner diameter. The capillary column is usually made of fused silica or stainless steel that has been very thinly coated with a substance on the inside of the tube. This adsorptive substance is the solid phase in the chromatography, rather than the grains of solid that are present in a packed column method.

Gas chromatography (GC) is an analytical method for the separation and identification of components that are gaseous or vaporized without decomposition. Hereby the sample is added into a stream of carrier gas (mobile phase) via an injector and separated on the column into the individual components at a stationary phase. The components of the sample are analyzed in the detector after separation on the column.

In gas chromatography the separation of compounds takes places due to interactions between the volatile analytes and the stationary phase. The stationary phase can consist of a liquid film (partition chromatography) or a solid one (adsorption chromatography). Different partition equilibria or adsorption forces ultimately enable the separation of analytes.

No. of topics : 50

16CHU504A] Reg. No----------

KARPAGAM ACADEMY OF HIGHER EDUCATION

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

(For the candidates admitted from 2016 onwards)

B.sc Degree Examination

III B.Sc. Chemistry

Internal Exam-III

INSTRUMENTAL METHODS OF CHEMICAL ANALYSIS

Date: 9.10.2018(AN) Maximum: 50 marks

Time: 2 hrs.

PART A ANSWER ALL THE QUESTIONS (20 x 1 = 20 marks)

1. What is NOE signal
a) Nucleolus oriented energy level b) nucleolus over effect
c) Nuclear over Hauser effect d) nucleolus opposite energy level

2. The DMSO is a
a) Highly polar solvent b) highly non-polar solvent
c) Weakly polar solvent d) weakly non-polar solvent

3. NMR spectrum obtained in
a) Microwave region b) radio frequency region
c) IR region d) UV region

4. What is anisotropic effect
a) for protons attached to C=C in alkenes
b) for protons attached to C=C in alkynes
c) for protons attached to cyclo hexanes
d) for protons attached to para hexanes

5. Absorbed wavelengths in atomic absorption spectrum appear as
a) dark background b) dark lines c) light background d) light lines

6. Background in atomic absorption spectrum is
a) bright b) dark c) brown d) purple

7. What is the rule of spectral multiplicity?
a) I(n+1) b) n(I+1) C) n+1 d) n-1

8. How many gem methyl group presents in μ-pinene?
a) one b) two c) three d) zero

9. What is double resonance?
a) At a time use of two radio frequency sources
b) Alternate use of two radio frequency source
c) Simultaneous use of two radio frequency source
d) Combined use of two radio frequency source

10. For non-viscous system NMR gives
a) Splitting signal b) sharp signal c) broad signal d) no Signal

11. The nucleus which gives NMR spectrum is
a) $^{16}_{8}O$ b) $^{10}_{5}B$ c) $^{12}_{6}O$ d) $^{4}_{2}He$

12. The NMR spectroscopy is based on the magnetic moment by the spinning of
a) Charged nucleus b) Electron c) neutrons d) proton

13. How many NMR signals are found in cis dimethyl cyclopropane and trans dimethyl cyclopropane?
a) 2,3 b) 3,4 c) 1,2 d) 8,10

14. What is flipping?
a) Energy transfer within the molecule b) energy transfer outside the molecule
c) Energy transfer from one energy d) energy transfer another molecule

15. The major advantage to this technique is that it provides a nondestructive method for identifying and quantifying trace elements.
a) X-ray diffraction b) Atomic absorption spectrophotometer
c) Neutron activation analysis d) Inductively coupled plasma emission spectrometry

16. The presence of helium in the Sun was first detected by scientists using a knowledge of ...
a) Nuclear magnetic resonance spectroscopy b) emission spectroscopy
c) Mass spectroscopy d) infrared spectroscopy

17. The proton magnetic resonance is studied in---
a) Radiofrequency b) microwave region c) IR region d) Visible region

18. Acetone gives
a) one signal NMR b) two signal NMR c) three signal NMR d) four signal NMR

19. In Atomic Absorption Spectroscopy, which of the following is the generally used radiation source?

a) Tungsten lamp b) Xenon mercury arc lamp
c) Hydrogen or deuterium discharge lamp d) Hollow cathode lamp

20. In ionization chamber vapors are bombarded with fast moving

a) protons b) electrons c) neutrons d) antineutron

PART-B **(3 x 2 = 6 MARKS)**

ANSWER ALL THE QUESTIONS

21. What are the detectors used in Atomic absorption spectroscopy?
22. Why TMS is used as a reference standard in NMR spectroscopy?
23. Define Chemical shift?

PART-C **(3 x 8 = 24 MARKS)**

ANSWER ALL THE QUESTIONS

24. a. What are the sources used in Atomic absorption, Atomic emission, and Atomic fluorescence? Give explanation?

OR

b. What are the advantages of atomic absorption spectroscopy over flame emission spectroscopy?

25. a. Describe with examples the various factors which affect the magnitude of the chemical shift in NMR spectra.

OR

b. How will you determine particle size by X-ray absorption method?

26. a. What are the advantages of a voltammetry titration?

OR

b. Explain in detail about NMR instrumentation?

16CHU504A] **Reg. No----------**

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B.sc Degree Examination

III B.Sc. Chemistry

Internal Exam-III

INSTRUMENTAL METHODS OF CHEMICAL ANALYSIS

Date: **Maximum: 50 marks**

Time: 2 hrs.

PART A **ANSWER ALL THE QUESTIONS** **(20 x 1 = 20 marks)**

1. c) Nuclear over Hauser effect
2. a) highly polar solvent
3. b) radio frequency region
4. a) for protons attached to C=C in alkenes
5. b) dark lines
6. a) bright
7. C) n+1
8. b) two
9. a) At a time use of two radio frequency sources
10. b) sharp signal
11. b) 105B
12. a) Charged nucleus
13. b) 3,4
14. a) Energy transfer within the molecule
15. b) Neutron activation analysis
16. b) emission spectroscopy

17. a) Radiofrequency

18. a) one signal NMR

19. d) Hollow cathode lamp

20. b) electrons

PART-B (3 x 2 = 6 MARKS)

21. What are the detectors used in Atomic absorption spectroscopy?

Photodiode

PN Junction

Photodiode Array

Photomultiplier Tube

22. Why TMS is used as a reference standard in NMR spectroscopy?

- Its high volatility, **TMS** can easily be evaporated, which is convenient for recovery of samples analyzed by **NMR spectroscopy**.
- the chemical shifts are largely temperature and concentration independent
- it offered a single-phase method for referencing (That is, it was an internal standard. Most common methods at the time used an external reference, such as a coaxial insert; a method which is still acceptably used today)
- The TMS peak lies outside the 'usual spectral region and is readily identifiable'
- It is largely inert, and is not likely to react with most samples
- It can be used for almost amy solvent (except H2O, D2O)

23. Define Chemical shift?

The chemical shift in absolute terms is defined by the frequency of the resonance expressed with reference to a standard compound which is defined to be at 0 ppm. The scale is made more manageable by expressing it in parts per million (ppm) and is indepedent of the spectrometer frequency. The applied frequency increases from left to right, thus the left side of the plot is the low field, downfield or deshielded side and the right side of the plot is the high field, upfield or shielded side (see the figure below). The concept of shielding will be explained shortly.

PART-C (3 x 8 = 24 MARKS)

24. a. What are the sources used in Atomic absorption, Atomic emission, and Atomic fluorescence? Give explanation?

Atomic Spectroscopy

Atomic spectroscopy is used for the qualitative and quantitative determination of perhaps 70 elements. Sensitivities of atomic methods lie typically in the parts-per-million to parts-per-billion range. Additional virtues of these methods are speed, convenience, unusually high selectivity, and moderate costs.

The efficiency and reproducibility of the atomization step in a large measure determine the method's sensitivity, precision, and accuracy, so atomization is by far the most critical step in atomic spectroscopy.

In AAS and AFS there are two factors involved. These are:

- The intensity of light source
- The probability of transition.

Sources of Atomic Spectra:

Without chemical bonding, there can be no vibrational or rotational energy states and transitions. 'I'herefore, atomic emission absorption, and fluorescence spectra are made up of a limited number of narrow peaks, or lines.

Flame Atomization:

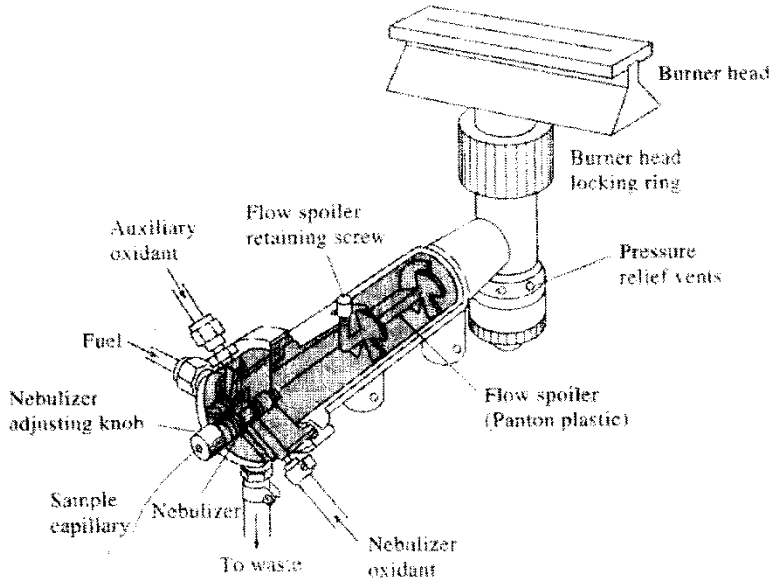
In a flame atomizer, a solution of the sample is nebulized by the flow of gaseous oxidant, mixed with a gaseous fuel, and carried into a flame where atomization occurs.

The first step is de-solvation, in which the solvent is evaporated to produce a finely divided solid *molecular* aerosol. As a result de-solvation of these molecules then leads to an atomic gas. Some of the atoms ionize and give cations and electrons. Undoubtedly, other molecules and atoms are also produced in the flame as a result of interactions of the fuel with the oxidant and with various species in the sample. As shown in the diagram above, we see that a fraction of the molecules, atoms, and ions are also excited by the heat of the flame, thus giving atomic, ionic, and molecular emission spectra.

Flame Atomizers:

A flame atomizer of a pneumatic nebulizer, which converts the sample solution into a mist, or aerosol, that is then fed into a burner, A common type of nebulizer is the concentric tube type. In which the liquid sample is sucked through a capillary tube by -a high-pressure stream of a gas flowing around the tip of the tube. This process of liquid transport is called aspiration. The high velocity gas breaks the liquid into the fine droplets of various sizes, which are then carried into the flame. Cross-flow nebulizers are also employed in which the high-pressure gas flows across a capillary tip at right angles. Often in this type of nebulizer, the liquid is pumped through the capillary. In most atomizers, the high-pressure gas is the oxidant, with the aerosol containing oxidant being mixed subsequently with the fuel.

On this figure we see a typical commercial laminar flow burner that employs a concentric tube nebulizer.



A laminar flow burner. (courtesy of Perkin-Elmer Corporation.)

The aerosol is mixed with fuel and flows past a series of baffles that remove all but the finest droplets. As a result of the baffles, the a-majority of the sample collects in the bottom of the mixing chamber, where it is drained to a waste container. The aerosol, oxidant, alit fuel are then directed into a slotted burner, which provides a flame that is usually 5 or 10 cm in length.

Laminar flow burners provide a relatively quiet flame and a long path length. These properties tend to enhance sensitivity and reproducibility. The mixing chamber in this type of burner contains

a potentially explosive mixture, which can be ignited by flashback if the flow rates are not sufficient.

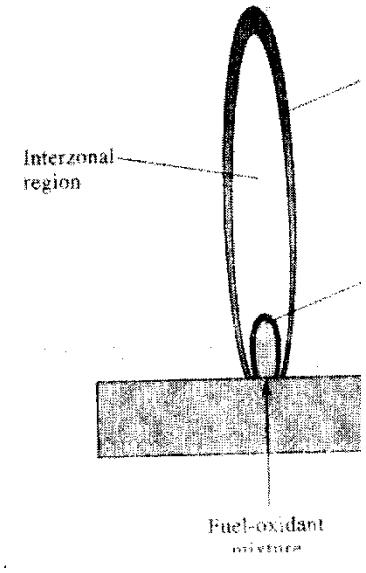
Types of flames:

Table below lists the common fuels and oxidants employed in flame spectroscopy and the approximate range of temperature realized with each of these mixtures. These temperatures in the range of 1700-2400^{o}C were obtained with the various fuels when air serves as the oxidant.

Fuel	Oxidant	Temperature, ^{0}C	Maximum burning velocity(cm s^{-1})
Natural gas	Air	1700-1900	39-43
Natural gas	Oxygen	2700-2800	370-390
Hydrogen	Air	2000-2100	300-440
Hydrogen	Oxygen	2550-2700	900-1400
Acetylene	Air	2100-2400	158-266
Acetylene	Oxygen	3050-3150	1100-2480
Acetylene	Nitrous oxide	2600-2800	285

The burning velocities listed in the last column are of considerable importance because flames are stable in certain ranges of gas flow rates only.

If the gas flow rate does not exceed the burning velocity, the flame propagates itself back into the burner, giving flashback. As the flow rate increases, the flame rises until it reaches a point above the burner where the flow velocity and the burning velocity are equal. This region is where the flame is stable.



Flame Structure:

In the figure, we see all the important regions of a flame. It includes the primary combustion zone, the interzonal region, and the secondary combustion zone. The appearance and relative size of these regions vary considerably with the fuel-to-oxidant ratio as well as with the type of fuel and oxidant. The primary combustion zone in a hydrocarbon flame is recognizable by its blue luminescence arising from the band spectra of C2 and CH, and other radicals. Thermal equilibrium is ordinarily not reached in this region, and it is seldom used for flame spectroscopy.

Furthermore, the interzonal area, -which is narrow in stoichiometric hydrocarbon flames, may reach several centimeters in height in fuel rich acetylene/oxygen acetylene/nitrous oxide sources. The zone is rich in free atoms and it is the most widely used part of the flame.

OR

b. What are the advantages of atomic absorption spectroscopy over flame emission spectroscopy?

Atomic Absorption Spectrometry (AAS) is an analytical technique that measures the concentration of an element by measuring the amount of light (intensity of light) that is absorbed - at a characteristic wavelength - when it passes through a cloud of atoms of this element.

As the number of atoms in the light path increases, the amount of light absorbed increases in a predictable way.

The main advantages of AAS are given below:

High sample throughput

Easy to use

High precision

Inexpensive technique

The main disadvantages of AAS are as follows:

only solutions can be analyzed

less sensitivity compared to graphite furnace

relatively large sample quantities are required (1-3 ml)

problems with refractory elements

Graphite furnace is by far the most advanced and widely used high sensitivity sampling technique for atomic absorption.

The main advantages of graphite furnace (GFAAS) over AAS can be summarized as follows:

Slurries and solid samples can be analyzed in addition to samples in solution

It shows greater sensitivity than AAS

Smaller quantities of sample are required (normally 5-60 μL)

The atomization process is more efficient comparing to AAS

The main disadvantages of graphite furnace are given:

It is an expensive technique

Low sample throughput

It requires experienced operators.

Disadvantages of Flame Atomic Absorption Spectroscopy

• only solutions can be analyzed

• relatively large sample quantities required (1 –2 mL)

• less sensitivity (compared to graphite furnace)

• problems with refractory elements Advantages

• inexpensive (equipment, day-to-day running)

• high sample throughput

• easy to use

• high precision

Advantages Over Flame Atomic Absorption Spectroscopy

• Solutions, slurries and solid samples can be analyzed.

• Much more efficient atomization

• Greater sensitivity

• Smaller quantities of sample (typically 5 –50 µl)

25. a. Describe with examples the various factors which affect the magnitude of the chemical shift in NMR spectra.

Factors affecting chemical shift

1. Inductive effect
2. Vanderwaals deshielding
3. Anisotropic effect
4. Hydrogen bonding

INDUCTIVE EFFECT

- Greater the electronegativity.
- Greater is the deshielding.
- Delta value will be more.

VANDERWAALS DESHIELDING

The Electron cloud of the bulky group will tend to repel,the electron cloud (in overcrowded molecules) surrounding the proton , & the proton is deshielded .

ANISOTROPIC EFFECT

- The opposite of isotropy which means uniformity in all directions.
- So, anisotropy is non-uniformity.

Now for different compounds this anisotropy is different as different distribution of electrons around nuclei. Anisotropy effect of Alkene, Alkyne, Benzene , ketone/Aldehyde

ALKENES: protons adjacent to alkene (C=C-H) are deshielded by anisotropy effect,so, chemical shift will be induced.

Anisotropy refers to the property of the molecule where a part of the molecule opposes the applied field and the other part reinforces the applied field. Chemical shifts are dependent on the

orientation of neighbouring bonds in particular the π bonds. Examples of nucleus showing chemical shifts due to π bonds are aromatics, alkenes and alkynes. Such anisotropic shifts are useful in characterizing the presence of aromatics or other conjugated structures in molecules.

ALKYNES:

All groups in a molecule having πelectrons generate secondary anisotropic field. In alkynes the system will shields the proton,thus causing decrease in chemical shift of alkynes. Also the proton next to the alkynes carbon experience the anisotropic effect towards shielded side.

HYDROGEN BONDING

Downfield shift depends upon the strength of H-bonding. Intra-molecular H-bonding doesn't show any shifting absorption. Hydrogen bonding results from the presence of electronegative atoms in neighbourhood of protons .The resulting deshielding leads to higher values of chemical shifts. This confirms the presence of hydrogen bonding in the molecules.

Electronegativity

Electronegative atoms present in molecules tend to draw the electron density towards themselves and deshield the nucleus. An increase in electronegativity of the surrounding groups will result in decrease of the electron density and lead to an increase in chemical shift value due to the shielding of the nucleus.

Chemical shifts of NMR active protons and other nuclei serve to provide a wealth of structural information on molecules.

OR

b. How will you determine particle size by X-ray absorption method?

Despite the emergence of other particle sizing methods over the past several decades, X-ray sedimentation remains the method of choice for many ceramic manufacturers. In X-ray sedimentation, a narrow, collimated beam of X-rays is used to directly measure the particle concentration in the liquid medium.

An exact method of determining particle size by sedimentation was developed in the 1850s by George Gabriel Stokes, who derived an equation (known as Stokes' law) describing the settling rates of spheres in a fluid as a function of size. Since early in the 20th century, it has been known that the attenuation of X-radiation is proportional to the mass of the absorber. In the mid-'60s, Olivier and Hickin,1 working in a Freeport Kaolin laboratory, combined these two principles and developed an instrument that uses sedimentation to determine particle size and X-ray absorption

to measure the time-dependent change in a mass concentration of solids settling from suspension. The technology was acquired by Micromeritics Instrument Corp. and introduced as a commercial instrument* in 1967. Implementation of the X-ray sedimentation technique for particle sizing has evolved considerably over the years.

Since the introduction of the X-ray sedimentation instrument, a number of other types of analytical instruments have been developed based on different techniques of sizing and quantity determination. Scientists and technologists newly entering the field of particle technology often wonder why X-ray sedimentation continues to be the method of choice in a number of applications. The reasons for remaining with this technique vary from user to user, but two reasons seem to prevail: first, the direct way in which size and quantity are determined, and second, the ability of the user to understand how variations from the theoretical model affect reported results. Because of these advantages, X-ray sedimentation continues to be widely used throughout the ceramic and related industries for accurate particle size analysis. *The SediGraph

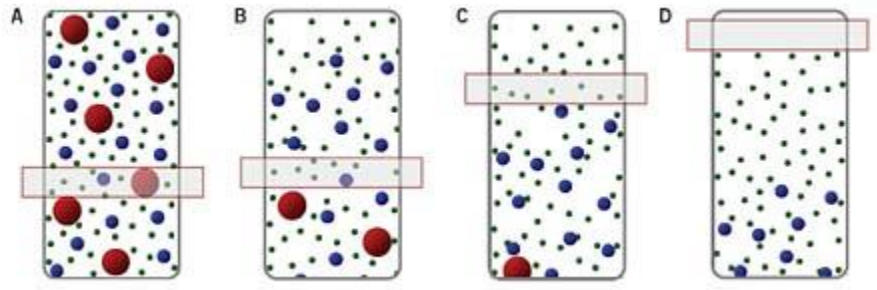
Determining Size and Relative Mass

The X-ray sedimentation method is based on two well-established and well-understood physical phenomena-gravitational sedimentation and low energy X-ray absorption.

Stokes' law describes the gravitational sedimentation of spherical particles as a function of particle diameter. The law states simply that the terminal settling velocity of a spherical particle in a fluid medium is directly proportional to the square of the diameter of the particle. The Stokes settling model applies rigorously, provided that laminar flow around the particle is maintained as the particle settles and displaces the liquid. This condition can be confirmed by calculating the Reynolds number of the largest particle in the suspension-a Reynolds number less than 0.3 indicates sufficient laminar flow around the particle to satisfy the theoretical model within acceptable tolerance. Smaller particles will have a smaller Reynolds number value, thus also satisfying the conditions.

Classifying particles by settling velocity is equivalent to classifying them by particle size. The settling velocity is determined by measuring the time required for particles to fall a known distance. The simplest case, at least from the point of appreciating the technique, is to imagine introducing all particles simultaneously at the same level at the top of the settling cell. The particles will

separate by settling velocity as they fall. At any instant, a specific range of velocities exists within a defined vertical column of liquid below the level of introduction. If all particles have the same density, then they will also be separated by size.



26. a. What are the advantages of a voltammetry titration?

The dropping mercury electrode (DME) is a working electrode made of mercury and used in polarography. Experiments run with mercury electrodes are referred to as forms of polarography even if the experiments are identical or very similar to a corresponding voltammetry experiment which uses solid working electrodes. Like other working electrodes these electrodes are used in electrochemical studies using three electrode systems when investigating reaction mechanisms related to redox chemistry among other chemical phenomena. continuously, and the actual current value is measured as the dependent variable. The opposite, i.e., amperometry, is also possible but not common. The shape of the curves depends on the speed of potential variation (nature of driving force) and on whether the solution is stirred or quiescent (mass transfer). Most experiments control the potential (volts) of an electrode in contact with the analyte while measuring the resulting current (amperes).

Voltammetry experiments investigate the half-cellreactivity of an analyte. Voltammetry is the study of current as a function of applied potential. These curves I = f(E) are called voltammograms. The potential is varied arbitrarily either step by step or

Voltammetric sensors

A number of voltammetric systems are produced commercially for the determination of specific species that are of interest in industry and research. These devices are sometimes called electrodes

but are, in fact, complete voltammetric cells and are better referred to as sensors. These sensors can be employed for the analysis of various organic and inorganic analytes in various matrices

The oxygen electrode

The determination of dissolved oxygen in a variety of aqueous environments, such as sea water, blood, sewage, effluents from chemical plants, and soils is of tremendous importance to industry, biomedical and environmental research, and clinical medicine. One of the most common and convenient methods for making such measurements is with the Clark oxygen sensor, which was patented by L.C. Clark, Jr. in 1956.

OR

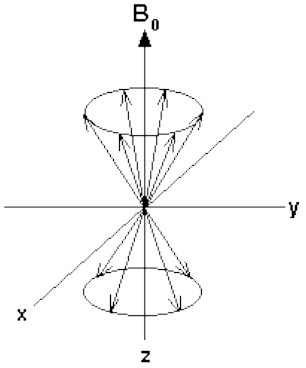
b. Explain in detail about NMR instrumentation?

Introduction

There are two general types of NMR instrument; continuous wave and Fourier transform. Early experiments were conducted with continuous wave (C.W.) instruments, and in 1970 the first Fourier transform (F.T.) instruments became available. This type now dominates the market.

Continuous wave NMR instruments

Continuous wave NMR spectrometers are similar in principle to optical spectrometers. The sample is held in a strong magnetic field, and the frequency of the source is slowly scanned (in some instruments, the source frequency is held constant, and the field is scanned).



Fourier transform NMR instruments

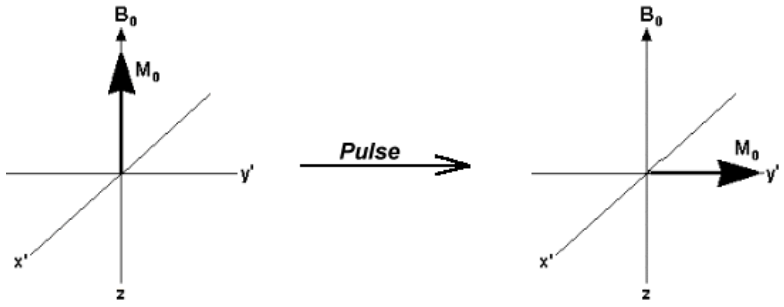
The magnitude of the energy changes involved in NMR spectroscopy are small. This means that sensitivity is a major limitation. One way to increase sensitivity would be to record many spectra, and then add them together; because noise is random, it adds as the square root of the

number of spectra recorded. For example, if one hundred spectra of a compound were recorded and summed, then the noise would increase by a factor of ten, but the signal would increase in magnitude by a factor of one hundred - giving a large increase in sensitivity. However, if this is done using a continuous wave instrument, the time needed to collect the spectra is very large (one scan takes two to eight minutes).

In FT-NMR, all frequencies in a spectrum are irradiated simultaneously with a radio frequency pulse. Following the pulse, the nuclei return to thermal equilibrium. A time domain emission signal is recorded by the instrument as the nuclei relax. A frequency domain spectrum is obtained by Fourier transformation.

The pulse

If a signal of frequency, F, is turned on and then off again very rapidly, then the result is an output consisting of many frequencies centred about F with a bandwidth of 1/t, where t is the duration of the pulse. This means that radiation is produced of all frequencies in the range $F \pm 1/t$. If t is very small, then a large range of frequencies will be produced simultaneously, and all target nuclei in a sample will be excited.



The effect of applying the pulse

To understand the effect of the radio frequency pulse, consider the processing nuclei;

As can be seen, there are more nuclei aligned with the field than against it. This means that there is a resultant magnetization vector aligned with the field. Now, imagine that the laboratory itself is spinning at the Lamor frequency (the frequency of precession). To an observer in the

laboratory, the nuclei would appear to be stationary, i.e. not precessing. Because more nuclei are aligned with the field than against, the magnetization vector is aligned with the field. The idea of spinning the laboratory is called the "rotating frame of reference". Using the rotating frame of reference, the magnetic behaviour of the system can be shown like this;

A pulse of radio frequency radiation is applied along the x' axis. The magnetic field of this radiation is given the symbol B1. In the rotating frame of reference, B1 and M0 are stationary, and at right angles. The pulse causes the bulk magnetization vector, M0 , to rotate clockwise about the x' axis. The extent of the rotation is determined by the duration of the pulse. In many FT-NMR experiments, the duration of the pulse is chosen so that the magnetization vector rotates by 90°.

The detector is aligned along the y' axis. If we return to a static frame of reference (i.e. stop spinning the laboratory at the Lamor frequency) the net magnetic moment will be spinning around the y axis at the Lamor frequency. This motion constitutes a radio-frequency signal which can be detected. When the pulse ends, the nuclei relax and return to their equilibrium positions, and the signal decays. This decaying signal contains the sum of the frequencies from all the target nuclei. The signal cannot be recorded directly, because its frequency is too high. It is mixed with a lower frequency signal to produce an interferogram of low frequency. This interferogram is digitised, and is called the Free Induction Decay, (FID). Fourier transformation of the FID yields a frequency domain spectrum.