Chapter-5

Spectroscopy

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Introduction

Radiation is the broad term for energy released from a source. It is the process that releases energy into the atmosphere as waves or particles. Although it can refer to radiation from electromagnetic waves in general, including radio waves, visible light spectrum, and even gamma waves, it can also refer to sound, heat, or light in more generic terms. The study of electromagnetic radiation, or energy, and how it interacts with or is produced from matter is known as spectroscopy. It is commonly understood to be the branch of physics that studies how atoms and molecules, whether they are in the gas, liquid, or solid phase, absorb, emit, and scatter electromagnetic radiation.The strength of radiation emitted varies with frequency and is quantified in an emission spectrum. We employ and investigate a broad spectrum of spectroscopic methods that yield data on a vast array of distinct materials. Analytical parts of both qualitative and quantitative analysis employ these techniques as well.

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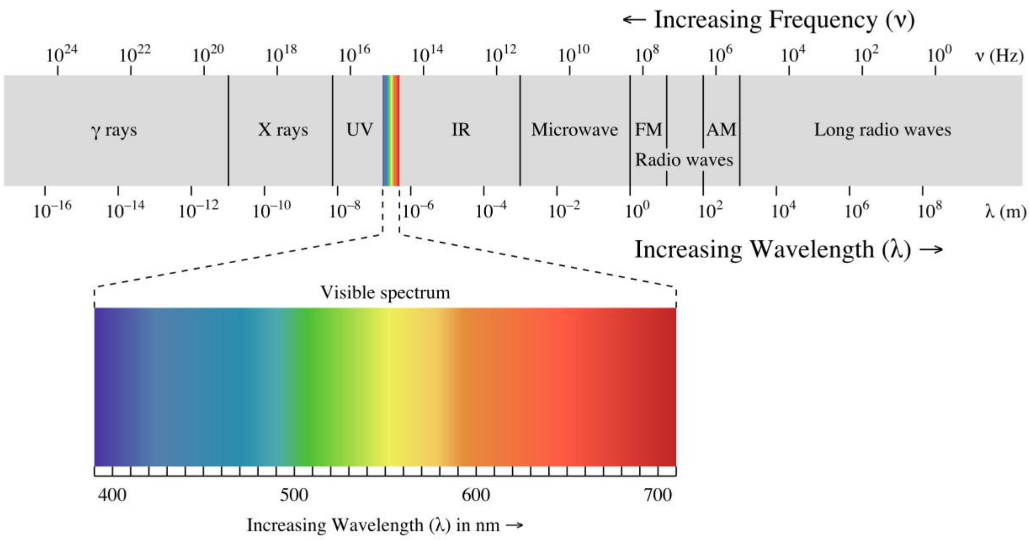
Certain spectroscopic techniques provide information about a material's average or usual qualities, while others are used inside of a microscope and can provide very specific information. The local compositions of materials can be studied using spectroscopy techniques such as electron energy loss spectroscopy in a transmission electron microscope. Cathodoluminescence spectroscopy in a scanning electron microscope can be used to understand the light emission properties of semiconductors and other structures, while spectroscopic methods in a scanning tunneling microscope can reveal the electronic structure of a material. In addition to being sensitive to local order and system dynamics, solid state NMR offers a sensitive probe of material structure. The flow of electrically charged particles through a vacuum or matter, or oscillating electric and magnetic disturbance, is two sources of electromagnetic radiation. The disturbance results from the combined wave moving perpendicular to both the electric and magnetic oscillating fields when they are at right angles to one another.An electric and magnetic disturbance flowing through space at the speed of light (2.998 × 108 m/s) is known as electromagnetic radiation. It moves in radiant energy packets known as photons, or quanta, but has neither mass nor charge.

Photons, which are quantized harmonic waves which travel at the speed of light, are the light energy bundles that are released when an electron is released. Next, this energy is classified into different groups within the electromagnetic spectrum according to its wavelength. These electromagnetic and electric waves have certain properties, such as amplitude, wavelength, and frequency, and they travel perpendicular to one another.

The study of how electromagnetic radiation interacts with matter through absorption, emission, or scattering is known as spectroscopy.



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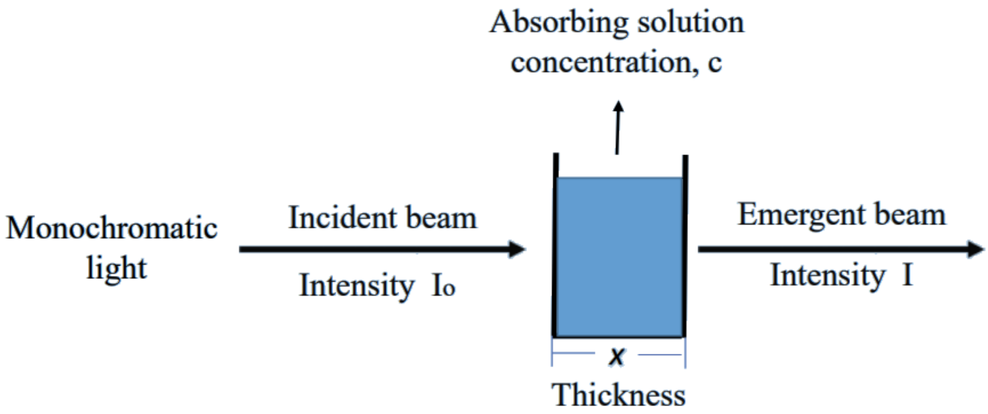
A portion of the incident radiation is absorbed, some is scattered, and some is transmitted when an object is subjected to radiation. Absorption causes an increase in the transmitting material's intensity or the transmitted light's intensity. The amount of incident light that is absorbed depends on the thickness of the absorption medium. A quantifiable relationship was established by Lambert between the decreases in light intensity I that happen when monochromatic light passes through a homogeneous medium of thickness dx.



At any given position, the reduction in light intensity due to the absorbing medium's thickness is precisely proportional to the increase in light intensity.

$$-\frac{dI}{dx}\alpha\ I \ldots..(1)$$

$$-\frac{dI}{dx} = \alpha\ I \ldots..(2)$$

Where,
dI = the slight decrease in brightness brought on by traveling a short distance dx.
I= the intensity of monochromatic light priorentering the medium.
-dI/dx = the rate of strength drop with thickness dx
a = absorption coefficient.
Integrating equation (2), we get;

$$-lnI = ax + C \ldots..(3)$$

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Substituting values, x=0, I = Io and C= −lnIo in above equation, we get,

$$ln\frac{I}{I_0} = -ax \dots (4)$$

$$log\frac{I}{I_0} = -\frac{\propto}{2.0303x} \dots . (5)$$

$$log\frac{I}{I_0} = -a'x$$

Where,
a' = (a / 2.303) = the extinction coefficient and
ln/Io = the medium's absorbance.
A = absorbance.

The concentration C of the solution and its intensity I (light intensity) both affect how quickly incident light is absorbed when it passes through a solution of a certain thickness, according to the Beer-Lambert Law. The Beer-Lambert law states that this is;

$$-\frac{dI}{dx} \propto C$$

Combining two laws; we get;

$$-\frac{dI}{dx} \propto I \times C$$

$$-\frac{dI}{dx} = b \times I \times C$$

b is known as the molar absorption coefficient when the concentration, **c**, is stated in mol/L.

Lambert's law equation can also be written as;

$$log\frac{I}{I_0} = \frac{-b}{2.303} \times c \times x$$

$$log\frac{I}{I_0} = -\in \times c \times x$$

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This is the commonly known as Beer-lambert Law equation.
Where ∈ = (-*b*/2.303) is called the molar extinction coefficient which is expressed in L/mol/cm.
Both the type of absorbing solute and the input light's wavelength affect the molar extinction coefficient, or∈.

Applications of Beer-Lambert Law

Beer-Lambert law has various applications:

1. **Drug Analysis:** Biological and dosimetric materials, as well as pharmaceuticals, are analyzed using Beer-Lambert Law. The molecular content of a pill or bilirubin in blood plasma samples can be ascertained using this method. With electromagnetic spectroscopy, we use electromagnetic radiation (UV rays) to scan the tablet in order to determine both its quantitative (drug presence) and qualitative (drug concentration) properties.

2. **Spectroscopy:** In spectroscopy, the absorption of light at a specific wavelength is used to calculate the concentration of a material in a solution. This technique can be applied to environmental science, biochemistry, pharmacology, and other fields to determine the concentration of a compound in a solution by measuring the absorption of light at a specific wavelength.

3. **Chemical Analysis:** One can determine a substance's concentration in a solution by using chemical analysis. The Beer-Lambert Law is used in analytical techniques such as UV-Visible Spectroscopy, Chromatography, and Capillary Electrophoresis to determine an ingredient's concentration in a solution by measuring the absorption of light at a specific wavelength. The same method can be used to determine the amount of bilirubin that blood plasma samples are absorbing.

4. **Quality Assurance:** The beer-lambert law can be used to determine the concentration of particular ingredients in a variety of products, including food, drinks, and medications.

5. **Applications in Chemistry:** Beer's Law is used to gauge polymer deterioration in Chemistry.

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Limitations of Beer-Lambert Law

- The relationship between absorbance and concentration in the Beer Lambert Law requires linearity over a wide range of concentrations. However, the relationship could not be linear at extremely high or low concentrations.
- For the law to be applicable, the material must be uniformly and consistently distributed throughout the solution. This law is predicated on the idea that the light source being used for analysis is monochromatic (having only one wavelength). Practically the majority of light sources generate light at various wavelengths, which cause measurement mistakes.
- The law presupposes that the temperature of the solution will stay constant during the measurement. However, temperature changes can affect both the substance's absorbance and the measurement's accuracy.
- The legislation states that the solution can't contain any extra ingredients that can taint the measurement. In actuality, though, different materials could absorb light with the same wavelength as the material, leading to inaccurate measurements.
- Light travels through materials at a set distance in accordance with the law. However, there are times when factors like the shape of the container or the presence of bubbles or particles in the solution might alter the route length.
- Because the analyte's molecules are closer together and have stronger intramolecular and electrostatic interactions, the law will produce false results at high concentrations. In addition to changing the molar absorptivity of the solution, high concentrations also change its refractive index, which causes departures from the Beer-Lambert equation.

Spectroscopy is a broad field with many sub-disciplines and a large number of techniques. While there are numerous varieties of spectroscopy, nuclear magnetic resonance, atomic, Raman, visible, and ultraviolet spectroscopy, colourimetry, infrared, and infrared spectroscopy are the most often utilized forms for analysis. A few of these techniques are covered in further depth below

Colorimetry

The science of measuring colour appearance and intensity using a colourimeter, an instrument for making such measurements, is known as colourimetry.It is a common analytical technique in which the intensities of colour channels are measured for certain wavelengths and colour is quantified physically.

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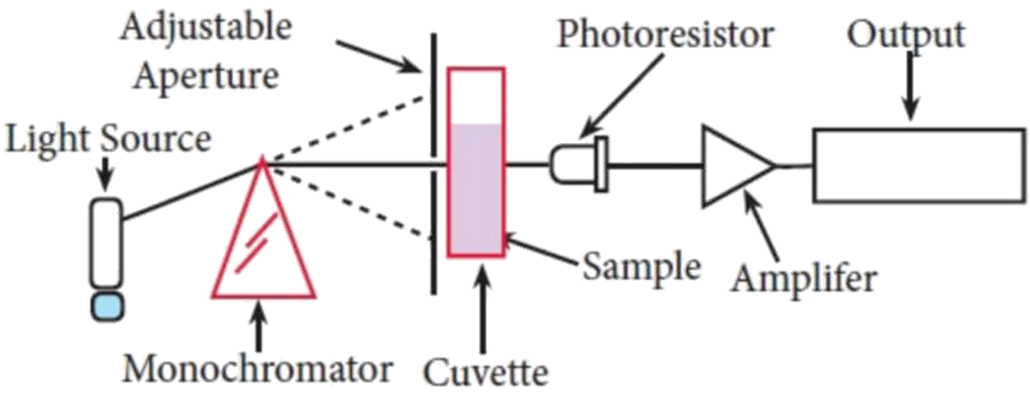
Principle

A material needs to be coloured or possess the ability to create chromogens by the addition of reagents that will absorb light in a way that allows the intensity of the colour to be measured. According to Beer and Lambert's law, which states that the amount of light transmitted through a coloured solution decreases exponentially with an increase in the concentration of coloured solution and an increase in the path length of the cuvette or thickness of the coloured solution, the intensity of the colour is proportionate to the concentration of coloured compound.



Colorimeter

Instrumentation

1. **Light Source:** Energy from the light source should be intense enough to span the whole visible spectrum, which is between 380 and 780 nm. Tungsten lamps are frequently employed as light sources for measurements in the near-infrared and visible spectrums. UV measurements using halogen deuterium is appropriate (200-900 nm).

2. **Slit:** It lets a light beam through, reducing stray or unwanted light.

3. **Condensing Lens:** After passing through slit cut on it, a condensing lens produces a parallel beam of light.

4. **Monochromator:** It allows only monochromatic light to pass through while blocking polychromatic light, which absorbs undesirable light wavelengths. There are three different kinds of these: glass, grating, and prism.

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5. **Prism:** When light travels through different media, it makes the process easier.

6. **Glass:** It selectively transmits light in certain ranges of wavelengths.

7. **Gratings:** Graphite, the material used to make them, divides light into many wavelengths.

8. **Cuvette (Sample Cell):** The colourful sample solution in the cuvette is illuminated by the monochromatic light coming from the filter. Their fixed diameter is 1cm, and their sizes range from square and rectangle to circular. Based on the materials they are composed of, these can be divided into three categories: glass, quartz, and plastic cuvettes.

    - Glass cuvettes are inexpensive and can absorb light with a wavelength of 340 nm.
    - Both UV and visible light entry is facilitated by quartz cuvettes.
    - Plastic cuvettes last less time, are more affordable, and are more prone to scratches.

9. **Photocell (Photodetector):** These photosensitive devices use an electrical energy conversion process to measure light intensity.

10. **Galvanometer:** Through the use of a galvanometer, the electrical signal produced in a photocell is identified and quantified. It shows the percentage of transmission as well as the optical density (OD).

Applications

- It's used in the printing industry to assess the quality of printing paper and ink.
- The food and food processing industries use these.
- It is widely employed in medical facilities and labs to ascertain the biochemical makeup of various samples, including blood, urine, serum, plasma, and cerebral spinal fluid.
- The paint and textile industries use it.
- Using a colourimeter, diamond merchants also assess the aesthetic qualities of precious stones.
- The device is also used in cosmetology to gauge how much UV protection skin care products offer.
- They serve as a filter to remove impurities such as cyanide, iron, fluorine, chlorine, molybdenum, etc. and to assess the quality of the water.

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- In order to provide users with the best possible viewing experience, they are used to assess the colour contrast and brightness of screens on computers, mobile devices, and televisions.
- A colourimeter is also used in the pharmaceutical industry to identify subpar products and drugs.
- A colourimeter is used to examine blood samples in order to ascertain the amount of haemoglobin present.

Spectrophotometry

Spectrophotometry is the measurement of intensity over the complete electromagnetic spectrum at various wavelengths. A colourimeter evaluates a sample's intensity of light absorption or transmission at a single wavelength, whereas a spectrophotometer examines the sample's quantity of light absorption or transmission at many wavelengths.

Applications

- Chemical analysis and research in the biotechnology and pharmaceutical sectors.
- Examining how light is absorbed by organic, inorganic, and biological materials.
- Pigment and dye analysis.
- Examining the environmental gas concentrations and air pollution.
- Examination of DNA and RNA specimens.
- Quantitative examination of metal ions in water samples.
- The manufacture of electronic components with quality monitoring.

The electromagnetic spectrum's ultraviolet (UV) and visible sections correlate to changes in an atom's or molecule's electron energy level. Hence, by examining the electronic structure of molecules in a sample, UV/Vis spectroscopy can be used to identify the compounds that are present. When it comes to recognizing peptide bonds, specific amino acid sidechains, prosthetic groups, and coenzymes, UV/Vis spectroscopy is especially helpful.

Infrared (IR) Spectroscopy

Measurement of the absorption, emission, or reflection of infrared radiation by matter is known as infrared spectroscopy (also known as vibrational spectroscopy).

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Since the energy of photons in the infrared portion of the electromagnetic spectrum are characteristic of molecular vibrations, infrared spectroscopy (IR spectroscopy) is still the major method used to study the vibrational and rotational modes of molecules.

IR spectrometers are commonly used to quantify the relative absorption of various frequencies in the infrared spectrum by a given substance.

The study of how a molecule interacts with infrared light is known as infrared spectroscopy. It addresses the absorption of electromagnetic spectrum radiation in the infrared range. Vibrational-rotational spectroscopy, another name for infrared spectroscopy, is derived from the fact that molecules absorb infrared light, which modifies their vibrational and rotational energy levels.

Principle

Let us look at the example of a diatomic molecule, AB, to better understand the IRspectrum principle.It is made up of two atoms, A and B, along with the electrons that go with them. The molecule may undergo the following modifications as a result of being excited from a lower energy level to a higher energy level as it absorbs energy.

- Electrons can move from one orbit to another due to changes in energy.
- Modification in the molecule AB's overall rotational energy.
- Variation in the atomic A and B's vibrational energy with respect to one another within the molecule.

The wavelength range of the infrared area that is most helpful for analysing organic molecules is between 2,500 and 16,000 nm. This region of the infrared spectrum has photon energy between 1 and 15 kcal/mole, which are too small to excite electrons but could excite covalently bound atoms and groups vibrationally.

Apart from the straightforward and seamless rotation of groups around single bonds, molecules undergo an extensive range of vibrational movements, which are indicative of the individual atoms that make them up.

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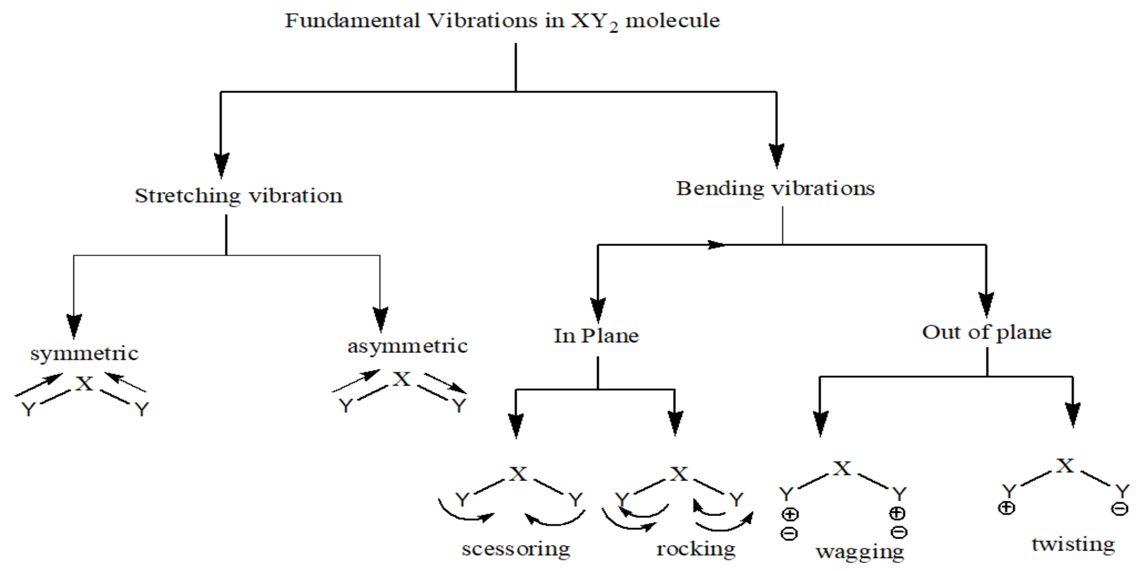

We go over fundamental vibrations below:

1. **Stretching Vibration:** The bond angle stays constant when the distance between two atoms increases and decreases.Varieties of vibrations that stretch. When there is a symmetric stretching vibration, both atoms are squeezed or stretched in the same direction.

Asymmetric Stretching Vibration: In this type of vibration, one atom experiences compression while the other experiences stretching, and vice versa.

2. **Bending Vibrations:** The bond angle shifts while the distance between two atoms stays the same. Either in-plane or out-of-plane vibrations are possible.

Vibrations in Plane Bending

- **Scissoring:** the two atoms travel in the same direction towards one another as a scissor

- **Rocking:** both the atoms move in same direction, either in left side or right side.

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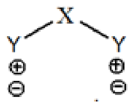
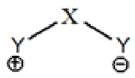
Bending Vibrations Outside of the Plane

- **Wagging:** both atoms move up and down in relation to the centre atom.

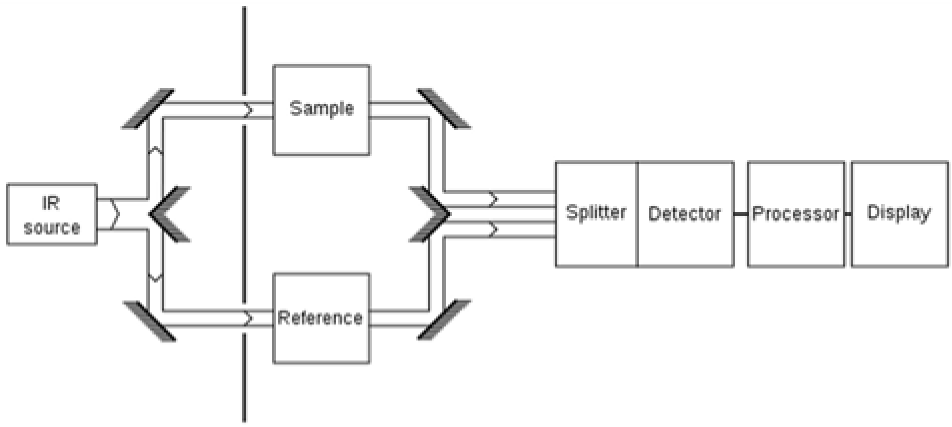


- **Twisting:** one atom move up and other atom move down with respect to central atom.



An infrared spectrum, which is a plot of recorded infrared intensity vs wavelength (or frequency) of light, is the primary measurement acquired in infrared spectroscopy.

Instrumentation of Infrared (IR) Spectroscopy



IR Spectrophotometer, Schematic Diagram

The following are the components that make up the IR spectrometer:

- Source of radiation
- Substance sampling and sample cells
- Separators
- Indicators
- Take note.

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IR Radiation Sources

A source of radiant energy that consistently emits infrared radiations that span the required wavelength and are strong enough to be detected is needed for infrared equipment.

Various Sources of IR Radiations are as Follows

- Nernst glower
- Incandescent lamp
- Mercury arc
- Tungsten lamp
- Glober source
- Nichrome wire

Sample Cells and Sampling of Substances

Characterization of solid, liquid, or gas samples can be done by IR spectroscopy.

1. **Solid:** Solid samples can be prepared using a variety of methods, including the pressed pellet technique, solid run in solution, solid films, mull process, etc. In order to prevent interference with absorbance, KBr is used as a carrier for the IR spectrum sample since it is optically transparent to light in the IR measurement range. In the wave number range of 4000–400 cm^{-1}, KBr exhibits 100% transmittance.

2. **Liquid:** Alkali halide liquid sample cells can be used to store samples. It is not possible to utilise aqueous solvents since they will dissolve alkali halides. You can only use organic solvents, such as chloroform.

3. **Gas:** Gas sampling is comparable to liquid sampling.

Monochromators

Because the sample will only absorb a limited range of IR radiation frequencies, it is helpful to choose the required IR radiation frequencies.

Various types of monochromators used are prism, gratings and filters.

- One can make prisms out of calcium iodide, sodium chloride, or potassium bromide.

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- Alkali halides are used to make diffraction gratings, and lithium fluoride is used to make filters.

Detectors

The intensity of the infrared radiation that the sample has not absorbed is measured by a detector. It is important to position and build the detector so that it can pick up even low-frequency signals. Thermal detectors are therefore frequently used in infrared spectrometry. Detectors used after that

- Bolometers
- Thermocouple
- Thermistors
- Golay cell
- Photoconducting cell
- Semiconductivity cell
- Pyroelectric detectors

Working of IR-Spectrophotometer

It is well known that compounds with both single and double bond functional groups are always in a state of vibration. We call this waving and stretching. They only absorb a certain range of infrared radiation; the remaining, undesirable frequencies are transmitted out of the compound unabated. The detector picks up this transmitted radiation, and a graph is made.

For instance, if a molecule exhibits stretching and wagging at 2Hz and 4Hz, respectively, and is exposed to radiation at a frequency of 1Hz, the sample will transmit 100% of the radiation without experiencing any absorption because there is no stretching or wagging at this frequency.

Now, if the sample is exposed to 2Hz infrared radiation, it will coincide with the molecule's stretching frequency. The radiation will be absorbed by the sample, resulting in a significant decline in transmittance, which will also be seen on the graph.Now, if the sample is exposed to 3Hz infrared light, it will exhibit 100% transmittance once more since no group in the sample will stretch or wag at this frequency. As the radiation reaches 4Hz, the transmittance will once more sharply decrease in time to align with the compound's 4Hz wagging frequency. We continue this method till we get our finished graph.

The fingerprint region and the functional group/absorption region are the two regions that make up the graph produced by IR spectroscopy. While the

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fingerprint area aids in the identification of other small groups connected to the chemical in addition to functional groups, the functional group region aids in the identification of specific functional groups.

Applications

In research as well as industry, infrared spectroscopy is a straightforward and trustworthy method that is utilized extensively in both organic and inorganic chemistry.

- It has been of great significance to scientific researchers in many fields such as:
- Nanoscale semiconductor analysis and
- Protein characterization
- Analysis of gaseous, liquid or solid samples
- Space exploration.
- Identification of compounds
- Quantitative analysis
- An understanding of the functional groups and molecular composition can be derived from the infrared spectra.
- IR spectroscopy provides a valuable instrument for analyzing the structures of proteins and peptides, especially in relation to conformation and reaction research.
- To understand how molecules interact with one another infrared spectroscopy can also be used to gauge the degree of polymerization during the production of polymers.

Table 1: Characteristic IR Absorptions of Some Functional Groups

Functional Group		Absorption (cm^{-1})	Intensity
Alkane	C–H	2850–2960	Medium
Alkene	=C–H	3020–3100	Medium
	C=C	1640–1680	Medium
Alkyne	≡C–H	3300	Strong
	C≡C	2100–2260	Medium
Alkyl halide	C–Cl	600–800	Strong
	C–Br	500–600	Strong
Alcohol	O–H	3400–3650	Strong, broad
	C–O	1050–1150	Strong
Arene	C–H	3030	Weak

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Aromatic ring		1660–2000	Weak
		1450–1600	Medium
Amine	N–H	3300–3500	Medium
	C–N	1030–1230	Medium
Carbonyl compound	C═OC═O	1670–1780	Strong
	Aldehyde	1730	Strong
	Ketone	1715	Strong
	Ester	1735	Strong
	Amide	1690	Strong
	Carboxylic acid	1710	Strong
Carboxylic acid	O–H	2500–3100	Strong, broad
Nitrile	C≡NC≡N	2210–2260	Medium
Nitro	NO_2	1540	Strong

Information regarding the existence or absence of particular functional groupings is best provided by IR. When comparing samples, infrared radiation (IR) can produce a molecular fingerprint. It can be claimed that two pure samples are the same substance if they have the same infrared spectrum.

Turbidimetry

The study of the optical characteristics that lead to light scattering through water is known as turbidity analysis. That's one of the factors that determines how much water's clarity is lost due to the presence of suspended solid particles. The turbidity level increases with the concentration of particles in the water. The turbidity level of the water increases with the concentration of particles in it. Thus,Turbidimetry is the measurement of the reduction in transmitted light intensity caused by the presence of non-transparent particles in the sample,
Or The measurement of the intensity loss of transmitted light, caused by the scattering effect of suspended particles in the sample is Turbidimetry.

Nephelometry

Another method for detecting the scattered light from nontransparent particles suspended in a solution is nephelometry; however, the way the two techniques measure the scattered radiation is different. While the quantity of light that the particles scatter is measured in nephelometry at an angle (typically 90 0} to the incident beam), the amount of radiation scattered by the particles is measured in turbidimetry at an angle of 180 0 to the incident beam.

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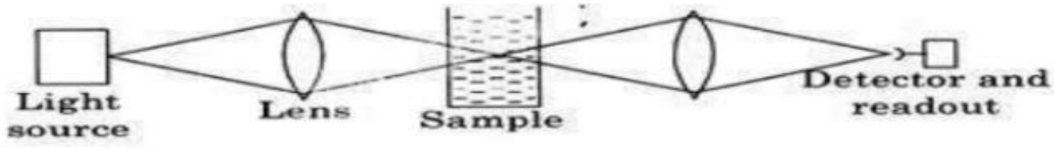
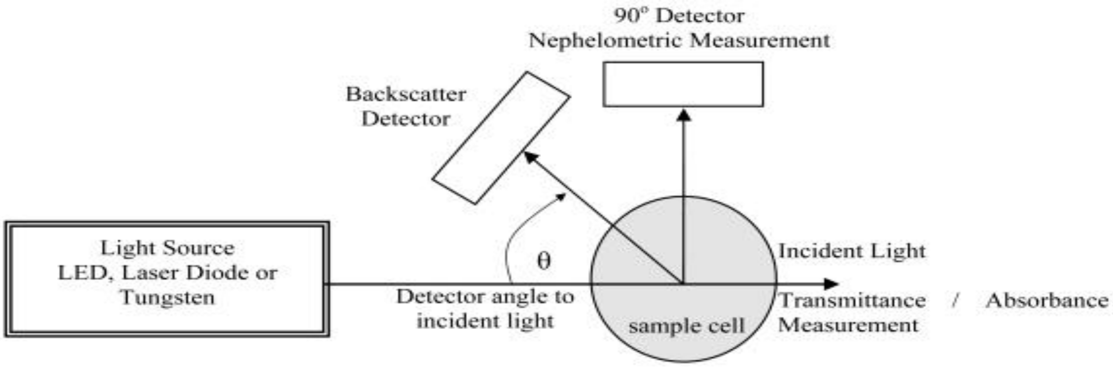


Turbidimetry



Nephelometry

Principle

The scattering or absorption of light by solid or colloidal particles suspended in solution is the foundation of the turbidimetry principle. A portion of the incident radiant energy is wasted by absorption, reflection, and reaction when light passes through the suspension, while the remaining portion is transferred. As a result, the concentration of dispersed phase depends on the following factors when measuring the intensity of transmitted light:

- Number of particles
- Size of particles

$$log\frac{I_0}{I_t}\text{=K'lc.}$$

I_t=Transmitted intensity
C=Concentration of absorbing particles in the solution
I= Thickness of absorbing of solution.

For turbidimetric measurements the transmitted intensity I can be determined from the equation.

This equation is known as the basic equation of turbidimetry.
It is similar to Bouguer-Lambert-Beer equation.

Where,
I_o=Incident intensity

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Applications

Turbidimetry can be applied to transparent solid or gaseous liquid samples.

1. **Pollution of the Air and Water:** Gaseous concentrations of dust and smoke are measured in the air, while turbidity is recorded in the water.

2. **Inorganic Analysis:** Sulfate ($BaSO_4$), chloride (AgCl), fluoride (CaF_2), cyanide (AgCN), calcium (Oxalate), carbonate ($BaCO_3$), and zinc (ferrocanide) are all determined.

3. **Organic Analysis:** Food and drink analyses for clarity, fruit juices, and alcohol benzene

4. **Biochemical Analysis**

   - Growth curve, complete blood count (CBC), quantitative examination of vitamins, antibiotics, and amino acids.
   - Utilized to ascertain the quantity of cells and antigen concentration in the mixture.
   - The turbidity changes that such activity produces in the media allow for the monitoring of cell and bacterial growth.
   - Turbidimetric assays use the production of immunological complexes between antibodies and antigens to assess serum protein concentrations that are not detectable using traditional clinical chemistry techniques.

5. In the water supply and wastewater management industries, turbidity values can act as a simple and convenient alternative measure of the concentration of suspended solids, sulphate ions (which precipitate as $BaSO_4$ in acidic media (HCl) with barium chloride), and other particulate material.

6. In the food manufacturing industry, it is frequently used to monitor the quality of product and efficiency of the treatment process, especially in the dairy and brewing industries.

Atomic Absorption Spectroscopy

Introduction

Approximately 75% of the chemical elements on Earth are metals. Metals can be pollutants or desirable in a substance in certain situations. As a result, determining the metal content is essential for numerous applications. A highly sensitive elemental analysis technique, atomic absorption spectrometry (AAS)

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enables the identification of metals at the picogram level in a wide range of samples. It has been applied to thousands of applications with a broad range of sample types. As a result, AAS is an analytical method for figuring out how much metal atoms or ions are present in a sample.

Principle

The phenomenon known as atomic absorption is the measurement of the decrease in optical radiation strength that occurs after the light passes through a cell that contains gaseous atoms.

Light is absorbed by all atoms and ions at particular, distinct wavelengths. Only the atoms or ions of that specific metal will absorb light when it is exposed to light at its characteristic wavelength in a sample containing that specific metal. The concentration of the absorbing atoms or ions is directly proportional to the amount of light absorbed at this wavelength.

Instrumentation

The light source, atomization system, monochromator and detecting system are the four primary parts of a standard atomic absorption spectrometer. (Figure 1).

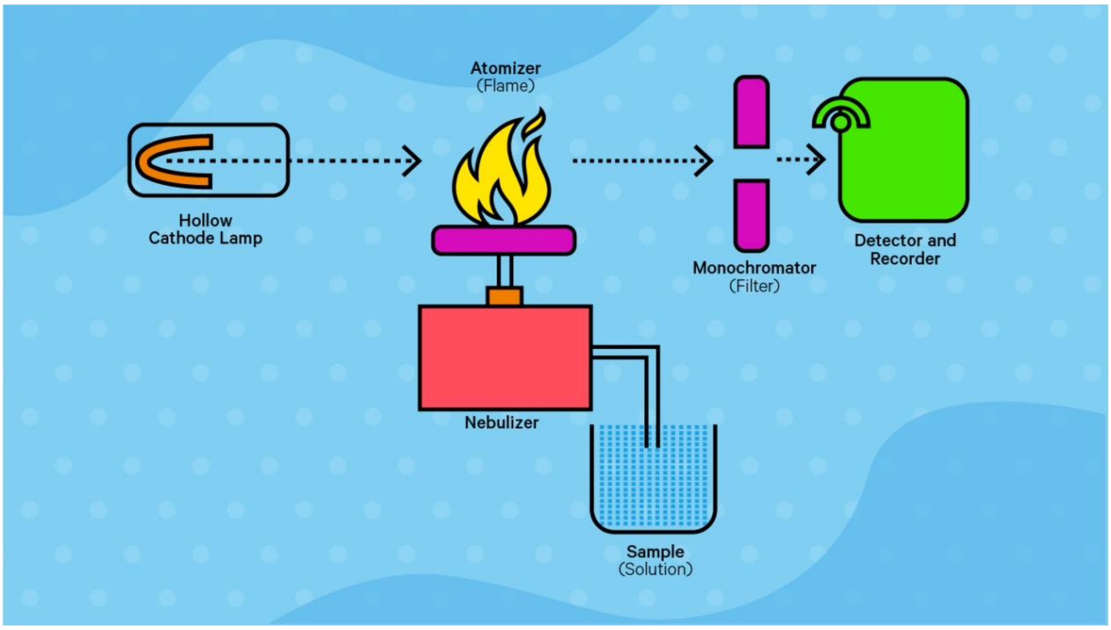

Figure 1: Schematic Diagram of a Typical Atomic Absorption Spectrometer

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Method

Step 1: Sample Preparation: To establish a liquid solution that guarantees the analytes are present as free atoms and ready for absorption, the sample is dissolved in an appropriate solvent (acids, water).

Additional processes like grinding and digestion can be needed for solid materials like ores or minerals in order to release the analytes and break down the matrix.

Step 2: Atomization: After the solution is ready, it is nebulized into a fine mist and put into a graphite furnace or an air-acetylene or nitrous oxide-acetylene mix high-temperature flame.

The analyte atoms' electrons are excited by the flame's extreme heat, which raises their energy levels. The sample and detector are separated by a monochromator to lessen background interference.

Graphite furnaces are typically used for the analysis of solid samples because they allow for controlled electrical heating of the sample rather than direct flame heating.

Step 3: Absorption: The energy difference between the excited and ground states of the analyte atoms is represented by the wavelength of light that is simultaneously emitted by a hollow cathode lamp that contains the same element as the analyte.

The excited analyte atoms absorb part of the photons that are emitted as the light passes through the atomized sample, which causes them to revert to their ground state. Because of this absorption, the light at that particular wavelength is less intense.

Step 4: Measurement and Analysis: A detector that measures the intensity of the light beam and converts it to absorption data measures the light intensity before and after it passes through the sample.

According to the Beer-Lambert equation, the intensity difference is directly proportional to the analyte concentration in the sample:

$A = \varepsilon cl$, where,
A is the absorbance measured.
ε is the molar absorptivity (a constant specific to the element and wavelength).
c is the concentration of the analyte.

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l is the path length of the light through the sample.

Step 5: Calibration and Quantification: The equipment is calibrated using standard solutions that contain known quantities of the element in order to ascertain the actual concentration of the analyte.

The concentration of the analyte in the original sample can be determined by comparing the measured absorbance of the sample to the calibration curve.

Applications

Applications for atomic absorption spectroscopy (AAS) are numerous.They fall under the general categories of biological analysis, environmental and marine analysis, and geological analysis because of their distinctiveness.

1. **Biological Analysis:** Biological samples includes both human tissue samples and food samples.

    - The amount of different amounts of metals and other electrolytes in human tissue samples can be measured using AAS. Analyses can be performed on some trace elements, such as arsenic, mercury, and lead, which are dangerous at specific concentrations.Salt and potassium electrolyte measurements in plasma are also performed using AAS. This measurement is significant because readings that deviate from the usual range may indicate a number of different illnesses.
    - To help customers assess whether they are consuming enough, food samples are examined to measure the quantities of minerals and trace elements.
    - Samples are examined further to identify any heavy metals that might be dangerous for consumers.

2. **Environmental and Marine Analysis:** Water analysis is commonly referred to in environmental and marine analysis.The leaching of lead and zinc from tin-lead solder, which seals the joints of copper pipes, into water is an illustration of water analysis.

3. **Geological Analysis:** Environmental studies and mineral reserves are both included in geological analyses.Gold and other precious metals are recovered from abandoned mine piles with great attention in mining. The amount of gold can be measured with the use of AAS to ascertain whether extraction would be economical.

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4. **In Pharmaceuticals:** An AAS can be used to make sure that an antibiotic is devoid of catalysts such as palladium or platinum before employing it.

Flame Spectroscopy

Introduction

The spectrum technique of flame photometry, sometimes referred to as flame atomic emission spectroscopy or FAES, is used to determine the concentration of particular chemical elements in a sample. The process includes spraying a sample solution over a hot flame to excite the sample.

Principle

This process is predicated on the idea that some materials absorb energy and change to higher energy states when heated to high temperatures. Photons, or light at particular wavelengths, are released when these excited atoms return to their ground state. The concentration of the element of interest can be ascertained by measuring the intensity of this emitted light.

1. **Flame Source:** In flame photometers, a mixture of air and a hydrocarbon gas, like acetylene or propane, powers a flame that serves as the excitation source.

2. **Systemfor Introducing Sample:** A nebulizer or aspirator is used to deliver a small aerosol or mist into the flame in order to evaluate a sample. Proper atomization guarantees uniform dispersion of the material in the flame, which is necessary for dependable analysis.

3. **Monochromator:** An optical component called a monochromator separates out certain light wavelengths released by excited atoms. Finding emission lines connected to the constituent of interest requires this stage.

4. **Photodetector:** A photodetector is a device that is used to select the intensity of the emitted light at the chosen wavelength.

Working

Preparation of Samples

In flame photometry, sample preparation is essential to guaranteeing accurate measurements. These are the main procedures in sample preparation:

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1. **Sample Selection:** Select the relevant sample solution that includes the relevant ingredient. Making ensuring the solution works with flame photometry analysis is crucial.

2. **Dilution and Standardization:** The element may need to be diluted to bring it within the linear range of the instrument if its concentration in the sample is too high. For calibration reasons, standard solutions with established concentrations are also made.

3. **Filtering:** Use the proper filters to remove any particles or contaminants from the sample solution.

4. **pH Adjustment:** To guarantee optimum performance during analysis, some samples might need to have their pH adjusted.

5. **Conditions of Flame and Optimization:** An essential component of flame photometry study is the flame conditions. The following elements are taken into account when maximizing the flame:

6. **Both Fuel and Oxidant Requirements:** Modify the proportions of oxidant (air or oxygen) and fuel (propane or natural gas) to establish a steady flame at the right temperature for effective atomization.
7. **Flame Stability:** Make sure the flame is constant and doesn't flicker or fluctuate too much because this can interfere with the measurements' accuracy and precision.

8. **Burner Design:** Select the right burner design in accordance with the analysis's requirements. Variable burners, like a premix or slot burner, have different benefits with regard to stability and sensitivity.

9. **Sample Flow Rate:** To guarantee effective atomization and minimum sample consumption, optimize the sample flow rate.

Steps Involved in Operation of Flame Spectroscopy

1. **Sample Atomization**

   - The process begins with the liquid sample being introduced into the flame photometer.
   - The liquid sample is then turned into a fine aerosol or mist using a nebulizer or aspirator to ensure uniform distribution and effective vaporization.

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- The atomization step is crucial because it enables the thermal excitation of the sample's atoms.

2. **Flame Ignition**

- Carefully combining an oxidizing gas, such as air or oxygen, with a combustible fuel gas, such as propane or acetylene creates a flame source.
- The flame acts as an excitation source, supplying the energy required to raise the electrons of the sample's atoms to higher energy levels.
- This mixture is ignited to produce a high-temperature, continuous flame.

3. **Atom Excitation**

- The atoms in the sample absorb energy at these high temperatures, causing some of their electrons to transition to higher energy states.
- Thermal excitation happens as the fine droplets of the sample enter the high-temperature flame.

4. **Wavelength Selection**

- This wavelength selection improves the specificity of the analysis by lowering interference from other sources of light.
- A monochromator is used to separate the appropriate wavelength of light associated with the constituent of interest.
- Only the relevant emission lines pass through the monochromator, which functions as an optical filter.

5. **Emission of Light**

- When the excited atoms return to their ground state, they release the excess energy as photons, or light, because they are unstable at higher energy levels and will quickly return to that state.
- The wavelengths of the light released precisely correspond to the electronic transitions of the element under study.

6. **Photodetection**

- A photodetector receives the released light, which is now at the correct wavelength.
- This light's intensity is measured by the photodetector, which then turns it into an electrical signal.

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7. Data Analysis and Concentration Determination

- The electronics of the device process the electrical signal from the photodetector.
- There exists a relationship between the element concentration in the sample and the intensity of light produced.
- The gadget compares the signal to known-value calibration standards in order to calculate the element concentration of the sample.

8. Display and Reporting

- The concentration results are usually recorded for further analysis or displayed on the device's screen.
- Finding the element's presence and concentration in the sample can be done with the use of these results.

Applications of Flame Photometer

Applications for flame photometry can be found in several domains:

1. **Clinical Chemistry:** Assessing electrolytes in blood or urine samples, such as potassium and sodium.

2. **Environmental Analysis:** Alkali metal content in plant, water, and soil samples is determined.

   Quantification of trace components in pharmaceutical formulations is known as pharmaceutical analysis.

3. **Food and Beverage Industry:** Mineral content analysis for food and drink quality assurance.

4. **Geological Studies:** Elements in geological samples are measured in order to explore and study minerals.