JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

A REVIEW ON MODERN SPECTROPHOTOMETERS AND THEIR APPLICATIONS.

RUTUJA RAJENDRA FALKE[1], MR. YOGIRAJ P. MULEY[2], DR. SUNIL S. JAYBHAYE[3], SAURABH M. BONDRE, RAJSHRI SHESHRAO KAMBLE[5].

1. Student of Bachelor in Pharmacy, Dr. Babasaheb Ambedkar Technological University, Raigad, Lonere.
2. Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Chemistry, Dr. Babasaheb Ambedkar Technological University, Raigad, Lonere.
3. Department of Quality Assurance, Faculty of Quality Assurance, Dr. Babasaheb Ambedkar Technological University, Raigad, Lonere
4. Department of Medicinal Chemistry, Faculty of Medicinal Chemistry, Dr. Babasaheb Ambedkar Technological University, Raigad, Lonere
5. Student of Bachelor in Pharmacy, Dr. Babasaheb Ambedkar Technological University, Raigad, Lonere.

1. ABSTRACT

The intensity of light absorbed or transmitted by a sample across a broad range of wavelengths can be measured with modern spectrophotometers, which are sophisticated analytical tools. Modern spectrophotometers use diode array detectors, photodiode arrays, or charge-coupled devices (CCDs) for quick and high-resolution spectral analysis, in contrast to conventional single-beam or filter-based instruments. Real-time monitoring and accurate quantification of chemical, biological, and environmental samples are made possible by these instruments' improved sensitivity, accuracy, and automation. In order to enable complex spectral analysis and laboratory reproducibility, modern spectrophotometers also incorporate digital interfaces, software-controlled wavelength selection, and data processing capabilities. They are essential in modern research and industrial settings because of their wide range of applications, which include pharmaceuticals, environmental monitoring, clinical diagnostics, and material science.

KEYWORDS: Modern spectrophotometer, diode array, CCD, high-resolution spectroscopy, analytical instrumentation.

2. INTRODUCTION

Isaac Newton invented spectroscopy as a science when he used a prism to split light, which was then known as optics. As a result, James Clerk Maxwell's research expanded the study of visible light, or colour, to encompass the entire electromagnetic spectrum. The scientific field that studies how electromagnetic radiation interacts with matter is called spectroscopy. The most significant effect of this interaction is that matter absorbs or releases energy in discrete amounts known as quanta. The practice of spectroscopy involves the experimental determination of radiation frequency (emitted or absorbed) and the inference of energy levels from these measurements.[1]

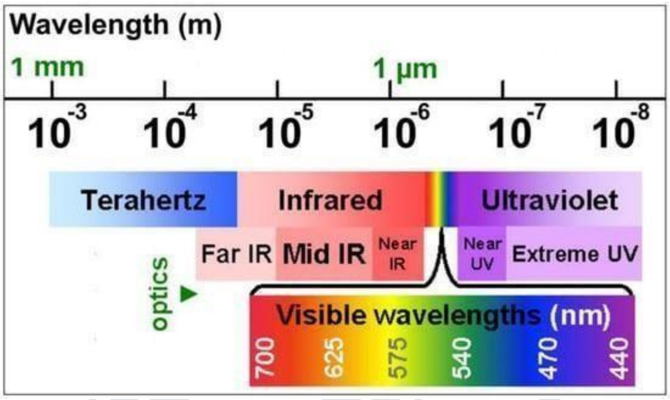

Figure 1: Spectroscopy

Spectroscopy: The measurement and interpretation of Electro Magnetic Radiation [EMR] absorbed and emitted when molecules, atoms, or ions in a sample transition between different energy states is known as spectroscopy.

UV-VIS Spectroscopy: Ultraviolet (UV) spectroscopy is a physical technique of the optical spectroscopy that uses light in the visible, ultraviolet, and near-infrared ranges and it is based on Beer-Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and path length. Thus, for a fixed path length, it can be used to determine the concentration of the absorber in a solution. It is necessary to know how rapidly the absorbance changes with concentration, UV-VIS spectroscopy has been in general use for the last 37 years and over this period it's become the most important analytical instrument in the modern day laboratory.[2]

3. PRINCIPLE

When radiation induces an electronic transition in a molecule's or ion's structure, the molecule or ion will show absorption in the visible or ultraviolet spectrum. As a result, when a sample absorbs light in the visible or ultraviolet spectrum, the electronic state of the molecules within the sample changes. Electrons will be promoted from their ground state orbital to a higher energy orbital, such as an excited state orbital or an anti-bonding orbital, by the energy provided by the light. Three different kinds of ground state orbitals could be at play.[3-4]

1. σ (Bonding) molecular

2.π (Bonding) molecular orbital

3. n (non-Bonding) atomic orbital.

In addition, two types of anti-bonding orbitals may be involved in transition i) σ*

(sigma star) orbital. ii) π* (pi star) orbital.

Since n electrons do not form bonds, there is no such thing as a n anti-bonding orbital. Therefore, the absorption of visible and ultraviolet light can result in the following electronic transitions.

- **σ to σ***
- **n to σ***
- **n to π***
- **π to π***

Both σ to σ* and n to σ* transitions require a great deal of energy and therefore occur in the far ultraviolet region or weakly in the region 180-240nm. Consequently, saturated groups do not exhibit strong absorption in the ordinary ultraviolet region. Transitions from then to π* and π to π* type occur in molecules with unsaturated centers, they require less energy and occur at longer wavelengths than transitions to σ* anti-bonding orbital. It will be seen presently that the wavelength of maximum absorption and the intensity of absorption are determined by molecular structure. Transitions to π* anti-bonding orbital which occurs in the ultraviolet region for a particular molecule may well take place in the visible region if the molecular structure is modified. Many inorganic compounds in solution also show absorption in the visible region.

These include salts of elements with incomplete inner electron shells whose ions are complexed by hydration. Such absorptions arise from a charge transfer process, where electrons are moved from one part of the system to another by the energy provided by the visible light.[5]

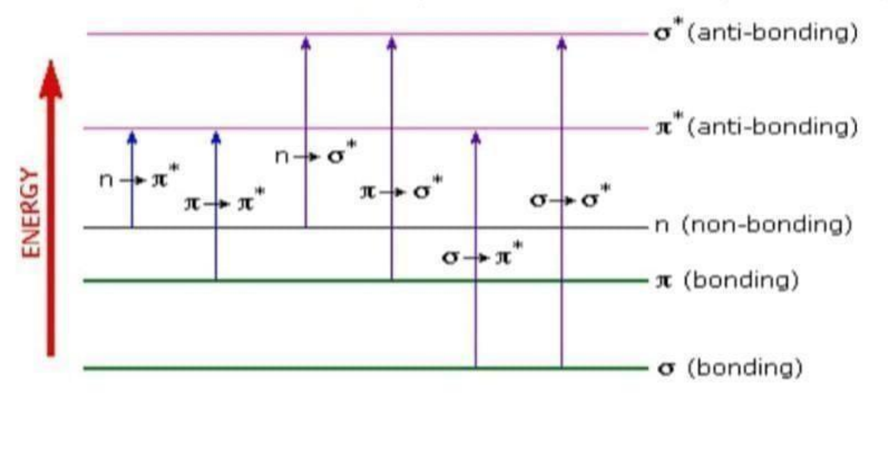

Figure 2: Electron Transition

BEER-LAMBERT LAW

According to this rule, the route length (b) and the concentration of the absorbing species (c) in the solution are directly correlated with the absorbance of a solution (A).

Absorbance A=molar absorptivity constant x cell length x concentration

A = abc. C = A/ab.

where,

A= Absorbance.

A = Molar absorptivity.

B = Path length.

C=Concentration.

ABSORPTION, INTENSITY TRANSMISSION AND UV SPECTRUM

1. **Bathochromic shift:** In this instance, the shift of absorption to a longer wavelength (λ max) is referred to as redshift.

2. **Hypsochromic shift**: In this instance, the shift of absorption to a shorter wavelength (λ max) is also referred to as blue shift.

3. **Hyperchromic transition:** The absorption maximum's intensity (£max) rises.

4. **Hypochromic change:** reduction in the maximum absorption intensity (£max).[6]

Descriptive term	Nature of the shift
Bathochromic shift (Red shift)	Toward longer wavelength
Hypsochromic shift (Blue shift)	Toward shorter wavelength
Hyperchromic effect	Toward higher absorbance
Hypochromic effect	Toward lower absorbance

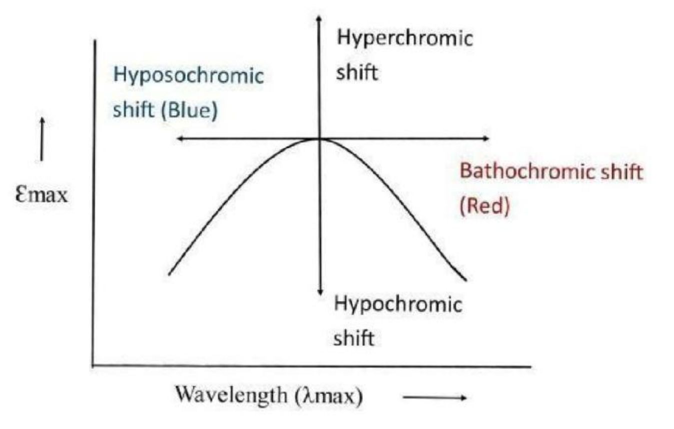

Figure 3: Shifts in absorption position and intensity

4. WHAT IS SPECTROPHOTOMETER ?

One type of spectrometer that measures a sample's transmittance or absorbance as a function of wavelength is called a spectrophotometer. When the sample is exposed to light with a specific intensity and frequency range. A spectrophotometer only measures the intensity of light as a function of wavelength, as opposed to a spectrometer, which is any device that can measure the characteristics of light across a range of wavelengths.

The spectrophotometer was developed in 1940 by Arnold O. Beckman with assistance from his coworkers at National Technical Laboratories. This would be a remedy for the earlier spectrophotometers that couldn't properly absorb the ultraviolet. It is extensively utilized in labs for a variety of industries, including food and beverage quality control, environmental testing, and pharmaceuticals. Spectrophotometers offer precise and trustworthy data that is necessary for product development, quality control, and research.[7-8]

5. TYPES OF SPECTROPHOTOMETERS

5.1. UV Spectrophotometer

An analytical tool called a UV spectrophotometer is used to determine a solution's transmittance or absorbance in the visible (400–800 nm) and ultraviolet (200–400 nm) portions of the electromagnetic spectrum. According to Beer-Lambert's law, it operates on the premise that some light is absorbed when it travels through a solution and that the quantity of light absorbed is directly proportional to the concentration of the absorbing material. It is frequently used in pharmaceutical analysis for compound identification, purity testing, and quantitative drug determination.[9]

Principle:

It follows Beer–Lambert's law, which states that:

Absorbance (A) = ε × c × l

Where, ε = molar absorptivity, C = concentration,

l = path length of cuvette

A] OLD TECHNOLOGY UVS SPECTROPHOTOMETER

1. Single beam spectrophotometer

The 1940s and 1950s saw the development of the traditional single beam spectrophotometer, most notably the Beckman DU Spectrophotometer (1941), one of the first commercial UV visible spectrophotometers. By enabling quick and precise quantitative analysis of compounds based on their light absorption, it transformed analytical chemistry.[7]

Working principle

To choose a particular wavelength, light from a source is passed through a monochromator in a single-beam spectrophotometer. The sample is then exposed to this light just once. The detector measures the intensity of transmitted light, and the instrument compares it with the initial intensity (I) taken separately using a blank. Beer-Lambert's law uses the drop in light intensity (I) to determine absorbance.

Figure 4: single beam spectrophotometer

Limitations:

- Not suitable for fast or automated measurements
- Requires manual recalibration • Drift in light intensity affects accuracy

Example

1. Beckman DU Spectrophotometer (1941) - first commercial model.
2. Cary 11 and Hilger Uvispek - early laboratory instruments.

B] MODERN TECHNOLOGY UV SPECTROPHOTOMETERS (Advanced Models)

1.Double Beam Spectrophotometer

To get around the drawbacks of the conventional single beam design, the contemporary double beam spectrophotometer was created. The 1950s and 1960s saw the introduction of the first commercial double beam instruments, including the Cary 14 spectrophotometer. Present day instruments are fully digital, microprocessor-controlled, and computer-integrated for high precision.[7]

Working principle:

Beer-Lambert's law, which states that absorbance is directly proportional to concentration, is the basis for how a contemporary double beam spectrophotometer operates. A source of light is divided into two beams, one of which travels through the sample and the other through a blank reference. The device simultaneously measures and compares the intensity of the two beams. The automatic calculation of the absorbance ($A = \log I_o/I$) reduces errors brought on by variations in the light source. Results from this dual-beam system are precise, consistent, and repeatable.

Figure 5: Double Beam Spectrophotometer

Advance Features:

Automatic baseline correction

- Digital readout and data storage
- High-speed scanning and accuracy
- Compact and user-friendly software interface

- Automatic wavelength calibration and temperature control.

Advantages:

- Eliminates drift and light fluctuation errors
- Allows real-time comparison between sample and reference
- Faster, more precise, and automated operation
- Suitable for multi-wavelength and kinetic studies

Applications (Both Old & Modern)

- Quantitative analysis of drugs and pharmaceuticals
- Determination of purity and concentration
- Quality control in industries
- Enzyme kinetics and biochemical assays
- Environmental and food testing

Examples

1. Shimadzu UV-19001
2. Agilent Cary 60 UV-Vis
3. PerkinElmer Lambda 365
4. Thermo Scientific Evolution 350
5. Jasco V-780 UV-Vis-NIR

5.2. IR (Infrared) Spectrophotometer

In analytical tool for examining how molecules absorb infrared radiation (IR) is an IR spectrophotometer. A molecule experiences rotational and vibrational transitions as a result of interactions with infrared radiation. Since each molecule emits a distinct infrared spectrum, IR spectroscopy is frequently used to identify functional groups and describe organic compounds.

Principle

It is predicated on how matter and infrared radiation (4000–400 cm) interact. Certain frequencies of infrared radiation are absorbed by molecules in a sample, leading to vibrational transitions (bond stretching or bending). An IR spectrum, which functions as a molecular fingerprint, is the resultant spectrum (absorbance vs. wavenumber).

A] OLD TECHNOLOGY IR SPECTROPHOTOMETERS

1. Dispersive IR Spectrophotometer

One of the first devices created for infrared spectroscopy was the antiquated dispersive IR spectrophotometer. It disperses infrared radiation into distinct wavelengths using a prism or diffraction grating, which are subsequently scanned across the sample one at a time. These early devices, which were widely used from the 1940s through the 1970s, were big, slow, and mechanical, but they offered the first trustworthy way to identify functional groups and study molecular vibrations. Chemical, pharmaceutical, and industrial analysis relied heavily on old dispersive IR spectrophotometers, which also served as the basis for contemporary FT-IR systems.[8]

Working principle

The idea behind a dispersive infrared spectrophotometer is that molecules absorb particular infrared wavelengths that correspond to their vibrational energy levels. This device first directs the source's infrared radiation into a monochromator, such as a prism or diffraction grating, which splits the light into distinct wavelengths. After that, the sample is exposed to each of these wavelengths individually. A portion of the radiation is absorbed as each wavelength hits the sample, while the remainder travels to the detector. The intensity difference between incident and transmitted infrared radiation is measured by the detector. The device captures this variation in intensity as a function of wavelength and generates an infrared spectrum that displays the sample's distinctive absorption peaks.

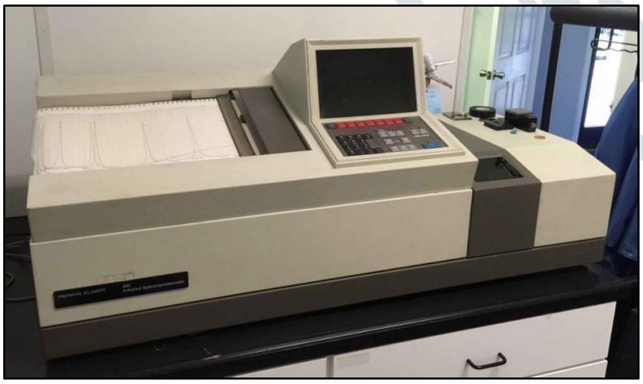

Figure 6: Dispersive IR Spectrophotometer

Limitations:

- Time-consuming (single wavelength scanned at a time)
- Mechanical wear and tear of moving parts
- Poor signal-to-noise ratio
- Frequent calibration needed

Example: Perkin-Elmer 21(Introduced in 1943-one of the first commercial IR instrument)

B] MODERN TECHNOLOGY IR SPECTROPHOTOMETERS

1. FTIR (Fourier Transform Infrared) Spectrophotometer

In 1970s-1980s, Fourier Transform Infrared (FTIR) spectrophotometer is an advanced analytical instrument used to obtain the infrared spectrum of absorption, emission, or transmission of solid, liquid, and gaseous samples. This technique provides rapid, accurate, and highly sensitive spectral data, making FTIR a powerful tool in modern analytical laboratories. In the food industry, forensic analysis, pharmaceuticals, polymer science, environmental monitoring, and research labs, the FTIR spectrophotometer has become indispensable due to its speed, high signal-to-noise ratio, enhanced resolution, and low sample preparation requirements.[8]

Working Principle

The basis of FTIR's operation is interferometry. After passing through a Michelson interferometer, infrared light is divided into two beams, one of which is reflected by a stationary mirror and the other by a moving mirror. All infrared frequencies are present in the interference pattern (interferogram) that is produced when the beams recombine. A Fourier Transform (FT) is then used to process this interferogram and turn it into a traditional infrared spectrum, which displays absorption peaks that match the sample's molecular vibrations.

Figure 7: FTIR Spectrophotometer

Features:

- All wavelengths measured simultaneously (multiplex advantage)
- High sensitivity and resolution
- Very fast scanning (seconds instead of minutes)
- Digital data output with automatic correction

Applications (Old and Modern)

- Identification of organic and inorganic compounds
- Determination of functional groups (C=O, O–H, N–H, etc.)
- Quality control in pharmaceuticals and polymers

- Protein structure and enzyme studies
- Environmental and forensic analysis

Example: Shimadzu IRAffinity-1, PerkinElmer Spectrum Two, Thermo Nicolet iSs

5.3. Flurosence Spectrophotometer:

A device that measures the wavelength and intensity of light emitted by a sample after it has been excited by a particular wavelength of light is called a fluorescence spectrophotometer. A sample is excited by a light source, which causes it to release light with a longer wavelength. The light that is released is then measured by a detector. The light source (such as a xenon lamp), a monochromator or filters, and the detector are essential parts of these devices, which have numerous uses in biochemistry, pharmaceuticals, and environmental science.[10]

Principle

The method relies on a form of photoluminescence called fluorescence. A molecule is excited to a higher electronic state when it absorbs light (excitation) at a specific wavelength. It emits fluorescence, a longer wavelength (lower energy) light, to return to its ground state after a short period of time. The Stokes shift is the difference between the wavelengths of excitation and emission.

A] OLD TECHNOLOGY FLUORESCENCE SPECTROPHOTOMETERS

1. Filter-based Fluorometers (First Generation)

In 1930s-1950s, A device that measures a sample's fluorescence at particular wavelengths is called a filter-based fluorometer. To separate the desired light wavelengths that excite the sample from those released by fluorescence, it uses excitation and emission filters. Fluorescent intensity can be quantitatively measured after the emitted light is detected by a photodetector after passing through an emission filter. These devices are simple, cost-effective, and suitable for routine fluorescence analysis but have limited wavelength flexibility compared to modern spectrofluorometers.[10]

Working principle

An excitation filter chooses the wavelength that excites the fluorescent molecules in the sample from light emitted by a light source. This light is absorbed by the sample, which then releases fluorescent light with a longer wavelength. To identify the precise fluorescence wavelength, the released light is passed through an emission filter concentration.

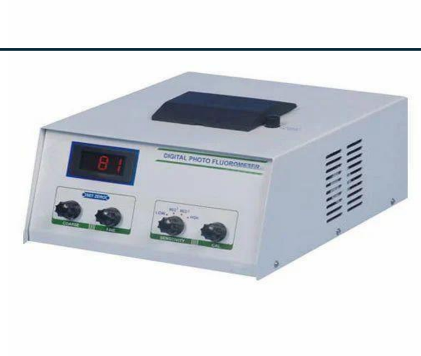

Figure 8: Filter-based Fluorometers

Limitations:

- Filters cannot provide narrow wavelength selection → poor resolution
- Interference from scattered light and background noise
- Manual data recording (no digital storage)
- Slow operation and less reproducible results

Example: Early Turner Fluorometer (developed around 1950s)

B] MODERN TECHNOLOGY FLUORESCENCE SPECTROPHOTOMETERS

1. Spectrofluorometer (Monochromator-Based, Advanced Type)

In1970s–present, A spectrofluorometer is used to measure the spectrum and intensity of fluorescence from a sample across a range of excitation and emission wavelengths. In contrast to filter-based fluorometers, it selects precise wavelengths using monochromators, enabling flexible and high-resolution measurements. For the sensitive and quantitative detection of fluorescent molecules, spectrofluorometers are extensively utilized in material science, biochemistry, and environmental analysis.[11]

Working principle

A wide range of light is emitted by a light source, which is typically a xenon or mercury lamp. To excite the fluorescent molecules in the sample, an excitation monochromator chooses a certain wavelength. The desired emission wavelength is isolated by passing the light through an emission monochromator. The intensity of the light is measured using a photodetector, also known as a photomultiplier tube or photodiode.

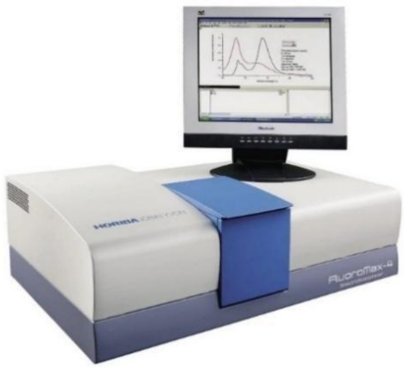

Figure 9: Spectroflurometer

Modern Enhancements:

- Dual monochromator design for high resolution
- Digital signal processing
- Software-based spectral correction and data analysis
- Fluorescence lifetime and anisotropy measurements

Applications (Old and Modern)

- Quantitative estimation of fluorescent drugs and biomolecules
- Detection of impurities or trace contaminants
- Enzyme kinetics and biochemical assays
- DNA/RNA and protein fluorescence studies
- Environmental and clinical analysis

Examples

Shimadzu RF-6000, Agilent Cary Eclipse, PerkinElmer LS-55

5.4. Atomic Absorption Spectrophotometer (AAS)

The concentration of metal elements in a sample can be ascertained analytically using an Atomic Absorption Spectrophotometer (AAS). This quantitative method is frequently used for trace metal analysis (e.g., Fe, Cu, Zn, Pb, Na, K, Ca) in the food, chemical, pharmaceutical, and environmental sectors. AAS is utilized in pharmacology, biophysics, archeology, and toxicology research. It can identify more than 70 distinct elements in liquids or directly in solid samples using electrothermal vaporization.[12]

Principle

Atomic absorption spectroscopy is based on the idea that free atoms in a gaseous state absorb light. Metal ion-containing samples absorb light energy proportional to their concentration when they are atomized (converted to free atoms) and exposed to light of a particular wavelength (emitted by a hollow cathode lamp).

Beer–Lambert's Law: $A = \varepsilon \times c \times l$ where A = absorbance, ε = molar absorptivity, c = concentration, l = path length. Specific and sensitive quantitative analysis is made possible by the unique wavelengths that each element absorbs.

A] OLD TECHNOLOGY ATOMIC ABSORPTION SPECTROPHOTOMETERS

1. Single-Beam AAS (Classical or Traditional Type)

In1950s, Alan Walsh (1953, CSIRO, Australia) An analytical tool called a single-beam Atomic Absorption Spectrophotometer (AAS) measures the amount of light absorbed by free atoms to ascertain the concentration of metal ions. The hollow-cathode lamp's light beam only goes through the atomizer once in this design before arriving at the detector. By comparing the light intensity before and after the sample, the device makes it possible to quantify elements at trace levels. Because of its simplicity, affordability, and ease of use, single-beam AAS is frequently utilized in clinical laboratories, metallurgy, food quality control, pharmaceutical testing, and environmental analysis. The method is based on the idea that light with a particular wavelength is absorbed by ground-state atoms, and that the quantity of light absorbed is proportional to the number of atoms in the sample.[13]

Working Principle

The single-beam AAS operates on the premise that light of a particular wavelength is absorbed by ground-state atoms, and that the quantity of light absorbed is proportional tothe analyte metal concentration in the sample. The atomizer, monochromator, and detector all receive light from a hollow cathode lamp (HCL) once, without being divided into two beams. In order to transform the sample solution into free ground-state atoms, it is aspirated into a flame or graphite furnace. The lamp's emission of radiation with their characteristic wavelength is absorbed by these atoms. The detector measures the absorbance, or drop in light intensity. This absorbance is directly proportional to the element's concentration in the sample, as per Beer's Lambert's Law.

Figure 10: Single beam AAS

Limitations:

- Low sensitivity for trace elements.
- High noise and signal drift.
- Manual operation and frequent recalibration.
- No automation or computer data storage.
- Only flame atomization possible (no graphite furnace).

Example

Early Perkin-Elmer 303 or Unicam SP90A instruments

B] MODERN TECHNOLOGY ATOMIC ABSORPTION SPECTROPHOTOMETERS **1. Double-Beam and Automated AAS (Modern Type)**

In1980s–Present, it is analytical tool for the quantitative identification of metals and trace elements in a variety of samples is a Double Beam Atomic Absorption Spectrophotometer (AAS)[12]. The double-beam system divides the light from the source into two distinct paths, one of which travels through the sample and the other through a reference pathway, in contrast to a single-beam AAS. This configuration improves accuracy, stability, and reproducibility by enabling the instrument to continuously adjust for variations in lamp intensity, drift in detector response, and background noise.[13-14]

Working principle

A hollow cathode lamp's light is split into two beams for a double-beam AAS, one of which passes through the sample and the other serves as a reference. When the sample atoms are transformed into free atoms in the furnace or flame, they absorb particular wavelengths. In order to determine absorbance, the detector compares the intensity of the sample beam with that of the reference beam. This dual-path system provides more accurate and stable metal analysis by automatically correcting for lamp fluctuations.

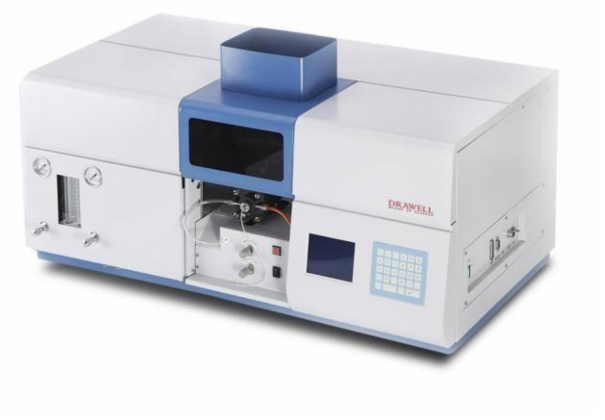

Figure 11: Double beam AAS

Features of Modern AAS:

- Automatic wavelength and slit-width selection.
- Fast scanning and digital output.
- Can analyze multiple elements sequentially.
- High sensitivity (ppb–ng/mL detection).
- Compact and software-controlled.
- Background correction and safety interlocks.

Applications (Both Old & Modern)

- Determination of metals in pharmaceutical and biological samples
- Water and soil analysis for trace metals
- Food and beverage industry (nutrient and contamination check)
- Clinical and toxicological studies
- Mining, metallurgy, and environmental pollution control

Example

1. PerkinElmer PiaAAcle series
2. ShimadzuAA-7000
3. Agilent 280FS

5.5. Nuclear Magnetic Resonance (NMR) spectrophotometer

It is analytical tool for figuring out the molecular structure of both organic and inorganic substances is a Nuclear Magnetic Resonance (NMR) spectrophotometer.

It operates on the premise that some nuclei absorb energy at particular frequencies when exposed to radiofrequency (RF) radiation and behave like tiny magnets when placed in a strong magnetic field. The absorption pattern reveals details about the environment, bonding, and molecular structure of atoms, particularly carbon (^{13}C) and hydrogen (^{1}H).[15]

Principle

The behavior of nuclei with non-zero spin (such as ^{1}H, ^{13}C, ^{19}F, and ^{31}P) in a magnetic field is the foundation of NMR. Such nuclei align with or against an external magnetic field (B_0) when exposed to it.

These energy levels change when exposed to radiofrequency (RF) radiation.

A] OLD TECHNOLOGY NMR SPECTROMETERS (Classical Systems)

1. Continuous Wave (CW) NMR Spectrophotometer

In 1950s–1970s, Felix Bloch and Edward Purcell (1946, Nobel Prize in 1952), Originally created in the early 1950s, old Nuclear Magnetic Resonance (NMR) spectrophotometers were groundbreaking devices used to investigate the magnetic characteristics of atomic nuclei in both organic and inorganic compounds. The continuouswave (CW) methods used by these early NMR systems involved gradually varying the magnetic field while maintaining a constant radiofrequency. The instrument required large electromagnets, vacuum-tube electronics, and manual tuning, but it provided the first reliable method to determine chemical structure, molecular environment, and purity.[15]

Working Principle

Either the radiofrequency (v) or the magnetic field (B_0) is continuously changed in CW NMR until resonance for a particular nucleus is reached. The NMR spectrum is created by recording each absorption point individually after the nucleus absorbs RF energy when it reaches its resonance condition.

Figure 12: Continues wave NMR spectrophotometer

Limitations:

- Slow analysis — took several hours per spectrum.
- Poor signal-to-noise ratio.
- Manual operation (no automation).
- Low resolution and limited nuclei detection (mostly ^{1}H only).
- No Fourier Transform (FT) or computer interface.

Example: Varian A-60 (introduced in early 1960s – first commercial NMR)

B] MODERN TECHNOLOGY NMR SPECTROMETERS (Advanced Systems)

1. Fourier Transform NMR (FT-NMR)

In 1970s – revolutionized NMR technology, Fourier Transform A brief, strong radiofrequency pulse that excites all nuclear frequencies at once is applied to the sample in the Nuclear Magnetic Resonance (FT-NMR) technique, a contemporary NMR method. A Fourier Transform is used to transform the resulting time-domain signal (free induction decay, or FID) into a frequency-domain spectrum. This approach is the norm in contemporary structural chemistry since it is quicker, more sensitive, and more accurate than earlier continuous-wave NMR.[16-17]

Working Principle:

In FT-NMR, the sample is excited with a brief radiofrequency pulse that contains all frequencies at once, as opposed to scanning one frequency at a time. In response, a free induction decay (FID) signal is generated by the nuclei and captured in the time domain. This data is transformed into a frequency-domain spectrum using a mathematical operation called a Fourier Transform.

Figure 13: Fourier Transform NMR

Features:

- High magnetic field (400–1200 MHz) → better resolution.
- Fast data acquisition (seconds).
- Can perform multinuclear analysis (^{1}H, ^{13}C, ^{19}F, ^{31}P, etc.).
- Digital control and data storage.
- 2D and 3D NMR techniques (COSY, HSQC, NOESY, etc.).
- Software for structure elucidation and simulation.

Applications (Old and Modern NMR)

- Determination of molecular structure and conformation.
- Identification of unknown organic compounds.
- Quantitative analysis (purity, drug content).
- Protein, peptide, and nucleic acid studies.
- Quality control in polymers, food, and biochemistry

Example: Bruker Avance Neo, JEOL ECZ-R, Agilent 500 MHz, Varian Inova

5.6. Reman Spectrophotometer

An analytical tool for examining molecular rotations, vibrations, and other low frequency modes in a sample is a Raman spectrophotometer, also known as a Raman spectrometer. It is extensively utilized in chemical research, forensics, materials science, and pharmaceuticals and offers details on molecular structure, chemical bonding, and functional groups.

Principle

The Raman Effect, which Sir C.V. Raman discovered in 1928 and was given the Nobel Prize in 1930, is the foundation of Raman spectroscopy.

The majority of monochromatic light, typically from a laser, is elastically scattered (Rayleigh scattering) when it interacts with a molecule, but a tiny percentage is inelastically scattered (Raman scattering).

The vibrational energy levels of the molecule are represented by the energy difference between the incident and scattered light.[18]

Raman Shift ($\Delta\nu$) = $\nu_0 - \nu_s$ where ν_0 = incident light frequency, ν_s = scattered light frequency.

The Raman spectrum plots intensity vs. Raman shift (cm^{-1}).

A] OLD TECHNOLOGY RAMAN SPECTROPHOTOMETERS

1. Classical (Dispersive) Raman Spectrometer

Period: 1930s–1970s

Discovered: Sir C.V. Raman and K.S. Krishnan (1928)

Working principle

Monochromatic light interacts with a sample, a tiny portion of it is inelastically scattered, resulting in energy shifts that correspond to molecular vibrations. This process is the basis for a traditional Raman spectrophotometer. To produce a Raman spectrum that offers molecular structural information, the scattered light is filtered to eliminate Rayleigh scattering, dispersed by a monochromator, and detected (traditionally on photographic plates or PMTs).

Figure 14: Classical reman Spectrophotometer

Limitations:

- Low sensitivity and poor resolution.
- Weak light sources — required high sample concentration.
- Interference from fluorescence.
- Bulky setup and manual operation.
- Difficult to record spectra quickly.

Example: Early Hilger spectrometers using mercury lamps or arc lamps as light sources.

B] MODERN TECHNOLOGY RAMAN SPECTROPHOTOMETERS

1. Laser-Based Raman Spectrometer

Introduced: Late 1960s–Present

An advanced tool for studying molecular vibrations using Raman scattering is a laser based Raman spectrophotometer. It uses a monochromatic laser as the excitation source, producing intense, stable, and highly collimated light, in contrast to traditional setups that used lamps. Inelastically scattered photons (Raman scattering) are created when the laser light interacts with the sample. These photons are filtered to eliminate Rayleigh scattered light before being distributed via a monochromator. In comparison to classical systems, modern detectors that capture the Raman spectrum, like charge-coupled devices (CCDs), offer better spectral resolution, faster acquisition, and higher sensitivity. This configuration enables quick and accurate material characterization, chemical composition, and molecular structure identification.[18]

Working principle

The sample is exposed to a monochromatic laser in a laser-based Raman spectrophotometer. A tiny percentage of light is Raman scattered, with energy shifts corresponding to molecular vibrations, while the majority is elastically scattered (Rayleigh scattering). To create a Raman spectrum that reveals molecular structure and composition, the Raman-scattered light is filtered to eliminate Rayleigh light, dispersed by a monochromator, and detected by a sensitive detector (CCD or PMT).

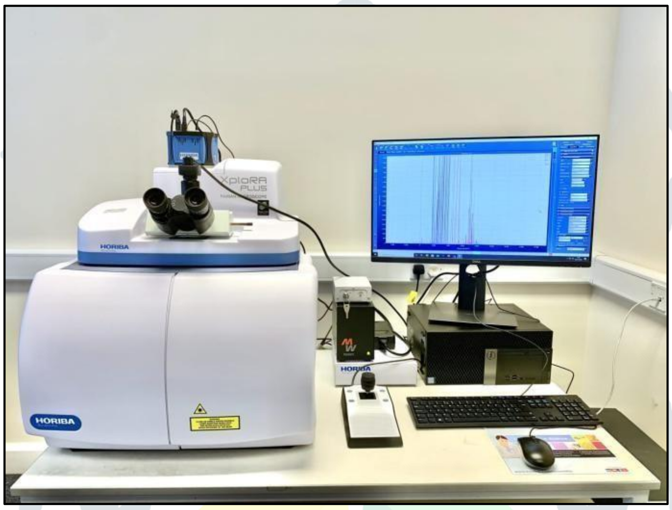

Figure 15: Laser reman spectrophotometer

Features:

- Laser excitation → strong and stable Raman signals.
- High sensitivity and rapid data acquisition.
- Compact and automated.
- Compatible with solids, liquids, and gases.

Applications (Old and Modern Raman Spectrophotometer)

- Identification of molecules and functional groups
- Structural and crystallinity studies
- Pharmaceutical analysis (polymorphs, impurities, formulation)
- Material science (graphene, carbon nanotubes, polymers)
- Forensic and environmental analysis

Examples: Renishaw in Via, Lab RAM HR Evolution

Model Make

SR.NO	Type	Example
1.	UV-Vis spectrophotometer	Shimadzu UV-1800, thermos scientific GENESYS 50, Perkin Elme lambda 35
2.	Infrared (IR)/ FTIR spectrophotometer	Bruker Alpha II, Tharmo Nicolet is10
3.	Fluorometer	Jasco FP-8300, Horiba Floro max, PerkinElmer LS 55
4.	Atomic absorption spectrophotometer (AAS)	Shimadzu AA-7000, PerkinElmer PinAAcle 900
5.	NMR spectrophotometer	Bruker Avance III,
6.	Raman spectrophotometer	Renishaw in Via, Horiba lab RAM HR

6. CONCLUSION

With their increased automation, stability, and compactness, modern spectrophotometers mark a substantial advancement over their classical predecessors. While improved software interfaces make data acquisition and analysis more efficient and intuitive, advancements in optics and detector technology have significantly increased sensitivity and dynamic range. These devices are essential in industries ranging from environmental monitoring to pharmaceuticals because they maintain the fundamental benefits of spectrophotometry, which include speed, non-destructive measurement, and wide applicability. But there are still issues: accuracy can be impacted by stray light interference, the requirement for meticulous calibration, and constraints when working with intricate sample matrices. Notwithstanding these limitations, the ongoing trend toward integration, miniaturization, and more intelligent data processing guarantees that contemporary spectrophotometers are not only more accessible but also more potent analytical instruments. They will probably become even more adaptable, dependable, and accessible as technology develops for a variety of industrial and scientific uses.

7. REFERENCE

1 Chatwal GR, Sharma A. Instrumental Methods of Analysis. 5th ed. Himalaya Publishing House; 2002:2.566-2.587

2 Gandhimathi R, Vijay Raj S, Jyothirmaie MP. Analytical process of drugs by ultraviolet (UV) spectroscopy – a review. Dept of Pharmaceutical Analysis, Sree Vidyanikethan College of Pharmacy, Tirupathi, Andhra Pradesh, India72-78

3 Sharma YR. Elementary Organic Spectroscopy: Ultraviolet and Visible Spectroscopy. 1st ed. S. Chand & Company Ltd; 2004:9-60

4 Basic UV-Vis Theory, Concepts and Applications. pp. 7-10

5 Skoog DA, Holler FJ, Nieman DA. Introduction to UV spectroscopy. In: Principles of Instrumental Analysis. 5th ed. Cole Publications; 2004; 300-305

6 Kisner H, Brown W, Kavarnos G. Multiple analytical frequency and standards for least squares analysis of serum lipids. Anal Chem. 1983;55,1703.

7 Sharma BK. Instrumental Methods of Chemical Analysis. Goel Publishing; 2020.

8 Griffiths PR, de Haseth A. Fourier Transform Infrared Spectrometry. 2nd ed.; 2007.

9 Beckman AO. U.S. Patent No. 2,058,761. 1936

10 Lakowicz JR. Principles of Fluorescence Spectroscopy. 3rd ed.; 2006

11 Shimadzu RF-6000 and Agilent Cary Eclipse Instrument Manuals

12 Walsh A. The application of atomic absorption spectra to chemical analysis. Spectrochim Acta. 1955;7, 108-117.

13 Welz B, Sperling M. Atomic Absorption Spectrometry. Wiley-VCH; 1999.

14 Willard H, Merritt L, Dean J, Settle F. Instrumental Methods of Analysis. 7th ed.; 1986.

15 Emsley JW, Feeney J, Sutcliffe LH. High Resolution Nuclear Magnetic Resonance Spectroscopy. 1965.

16 Ernst RR, Bodenhausen G, Wokaun A. Principles of Nuclear Magnetic Resonance. 1987 17 Bloch F, Hansen WW, Packard M. Nuclear induction experiment. Phys Rev. 1946;69.

18 Long DA. The Raman Effect: A Unified Treatment of the Theory of Raman Scattering by Molecules. Wiley; 2002.