

Development & Validation of Spectroscopy

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ABSTRACT: Organic chemists use spectroscopy as a necessary tool for structure determination. Spectroscopy may be defined as the study of the quantized interaction of electromagnetic radiations with matter. Electromagnetic radiations are produced by the oscillation of electric charge and magnetic field residing on the atom. There are various forms of electromagnetic radiation, e.g. light (visible), ultraviolet, infrared, X-rays, microwaves, radio waves, cosmic rays etc.

Key words: U-v visible spectroscopy, Infrared, FT-IR, Spectroscopy, Determination, Handheld spectroscopy, NIR, Instrumentation, Validation.

I. CHAPTER NO : 1) AIM & OBJECTIVES:-

Development & validation of spectroscopy. Spectroscopy is used in physical and analytical chemistry because atoms and molecules have unique spectra. As a result these spectra can be used to detect identify information about the atom and molecules.

Spectroscopy is used as a tool for studying the structures of atoms and molecules. The large number of wavelengths emitted by these systems makes it possible to investigate their structures in detail, including the electron configurations of group and various excited states.

The main type of spectroscopy: atomic spectroscopy

Atomic spectroscopy :1) Atomic absorption spectroscopy

2) Atomic emission spectroscopy

3) Atomic fluorescence spectroscopy

Spectroscopy is a field of analysis that uses the interaction of light to analyze and detect components within a sample.

II. CHAPTER NO : 2) INTRODUCTION:- Spectroscopy Methods^[1]

: It is the branch of science dealing with the learn about of interplay between Electromagnetic radiation and count number. It is a most find tool available for the locate out about of atomic and molecular structures and is used in the analysis of giant range of samples. Optical spectroscopy includes the region on electromagnetic spectrum between 100 Å and 400 nm.

- U-v spectroscopy
- Infrared spectroscopy
- CD spectroscopy
- FT-IR spectroscopy
- Terahertz pulsed spectroscopy

1) . Ultraviolet-Visible Spectrophotometry^[1]:

UV-Visible spectrophotometry is one of the most regularly employed approach in pharmaceutical analysis. It entails measuring the extent of ultraviolet or visible radiation absorbed by mean of a substance in solution. Instrument which measure the ratio, or function of ratio, of the intensity of two beams of light in the U.V-Visible region are called Ultraviolet-Visible spectrophotometers

UV spectroscopy is kind of absorption spectroscopy in which mild of ultra-violet place (200-400 nm.) is absorbed via the molecule. Absorption of the extremely violet radiations results in the excitation of the electrons from the floor state to higher strength state. The energy of the ultra-violet radiation that are absorbed is equal to the energy difference between the ground state and higher energy states

There are three type of absorbance instruments used to collect UV-vis spectra.

- Single beam spectrometer
- Double beam spectrometer
- Simultaneous spectrometer



Figure 1 2013 ELICO SL 210 DOUBLE BEAM UV-VISIBLE

2) Infrared spectroscopy:- IR is the measurement of the interaction of infrared radiation with matter by absorption, emission, or reflection. It is used to study and identify chemical substances of functional groups in solid, liquid or gaseous forms.

There are mainly two types of infrared spectroscopy:-

- 1) Near infrared spectroscopy
- 2) photospectroscopy

Infrared spectroscopy is an essential method used for the characterization of very complex mixtures. The portion of the infrared area most beneficial for evaluation of organic compounds is that having a wavelength range from 2500 to 16000 nm.^[14]

Infrared spectroscopy exploits the reality that molecules have precise frequencies at which they rotate or vibrate corresponding to discrete energy levels (vibrational modes). These resonant frequencies are determined by way the shape of the molecular plausible energy surfaces, the loads of the atoms and with the aid of the related vibronic coupling. In order to a vibrational mode in a molecule to be infrared active, it have to be related with changes in the everlasting dipole.

Nevertheless, resonant frequencies can be in a first method associated the power of the bond, and the mass of the atoms at either end of it. Thus, the frequency of vibrations can be related with a precise bond type.^[2]

Infrared spectrum of a pattern may be received by passing a beam of infrared light through the sample. Examination of the transmitted light exhibits how a top deal energy was absorbed at every wavelength. This can be achieved with a monochromatic beam, which trade in wavelength over time, or via the use of a Fourier transform instrument to measure all wavelengths at as soon as. From this, a transmittance or absorbance spectrum can be produced, displaying at which infrared wavelengths the sample absorbs. Analysis of these absorption characteristics reveals details about the molecular structure of the sample of the sample.^[2]

Infrared spectroscopy is widely used in each research and industry as a simple and reliable method for measurement, high quality manage and dynamic measurement. It is especially used to forensic evaluation in both criminal and civil cases and has been surprisingly successful for purposes in each organic and inorganic chemistry.^[2]

1) Near infrared spectroscopy :-^[2]

statistics of the NIR spectra. While observing the dispersion of the daylight thru a glass prism, William Herschel seen in 1800 that location beneath the area below the red end of the visible spectrum supplied a great heating effect .

The near infrared (NIR) radiation was once observed in 1881, Abney and Festing, using photographic plates, recorded the spectra of organic solvents inside the 1000- 1200 nm wavelength range. Their statement highlighted the importance of the hydrogen bond in the NIR spectrum. [2]

Combining a rock salt prism with a thermopile connected to a galvanometer, Coblenz created the first NIR spectrometer. Even though each substance has

its own fingerprint, Coblenz discovered out that defined characteristic agencies experienced some unique trend in their spectral elements suggesting the opportunity to extract structural information out of the NIR spectra. [2] NIR lookup things to do have been slower than in the mid- infrared (MIR) field. [3]

Consequently, parallel to the improvement of the optics, there was once a need for new tools to extract the relevant facts out of the NIR spectra. Therefore, the recent traits of NIR spectroscopy had been enabled with the aid of advances in micro- computing which allowed performing advanced data evaluation primarily based on Chemometrics.

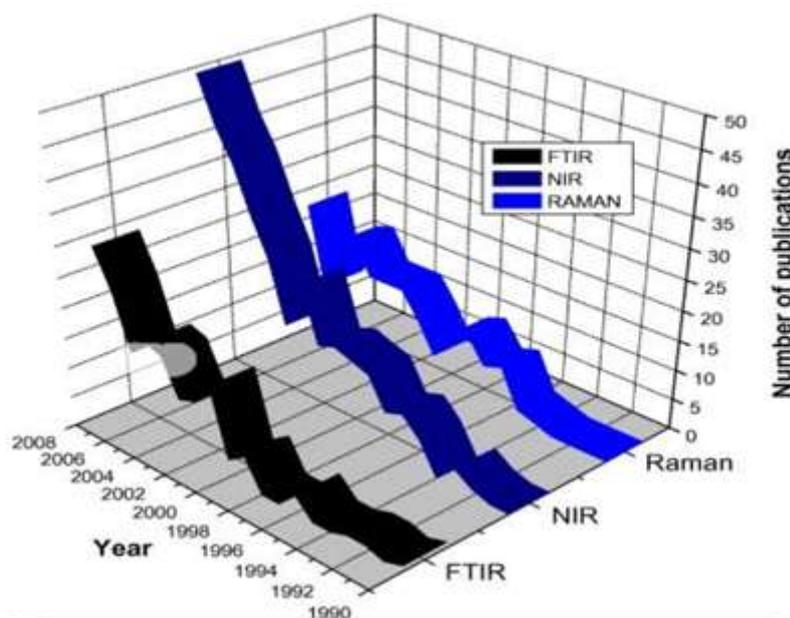
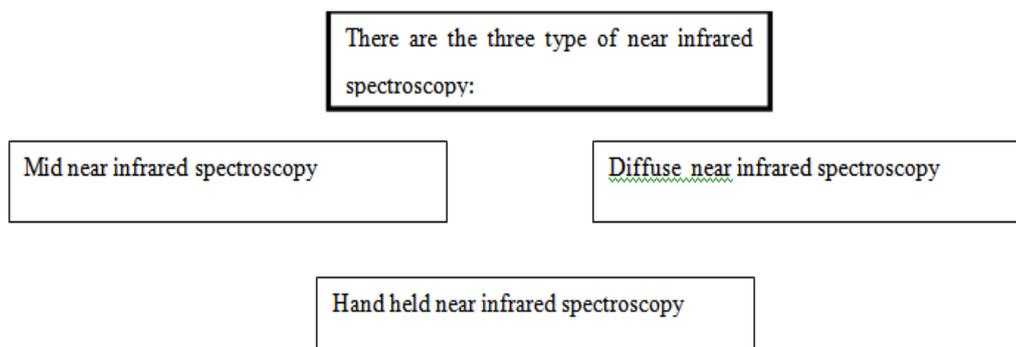


Figure2:- Number of publications involving multicomponent analysis by vibrational spectroscopy during the last decade. [3]



- I.
- II. **Mid near infrared spectroscopy**^[4] :- A novel technique, which permits the willpower of fungal infection with *Fusarium graminearum* on corn inside minutes, is presented. The ground sample was sieved and the particle size fraction between >250 µm and 100 µm was used for mid infrared/attenuated total reflection (ATR) measurements.
- III. **Hand held near infrared spectroscopy**^[5] :- While the weight of the majority of Raman and MIR spectrometers is nonetheless in these 1 kg range, the miniaturization of NIR spectrometers has superior down to the ~100 g degree and developments are underway to combine them into cellular phones (Tino et al., 2016). Furthermore, miniaturized NIR systems have currently reached the <1,000 US\$ level. Therefore, solely the acquisition of NIR structures can be taken into consideration for non public use whereas handheld Raman and MIR spectrometers will be confined to industrial, military or homeland security applications and public use by way of first responders, customs or environmental institutions. Due to the fact of the good sized development in the miniaturization of near-infrared spectrometers in combination with a drastic value reduction, advertising professionals predict this type of instrumentation a sizable growth rate. These developments have made hand-held NIR spectroscopy also attractive for everyday life consumer applications of a new, non-expert user community ranging from food testing to the detection of fraud and adulteration in a broad location of materials. Notwithstanding this wide-spread application range of hand-held NIR spectroscopy, the focus of this

conversation will be for plant analytical factor only.

- IV. **Diffused near infrared spectroscopy**^[6] :- Diffuse reflectance spectroscopy offers a nondestructive potential for size of C in soils based totally on the on landscapes is confined via the reflectance spectra of illuminated soil. Each the NIR capacity (400–2500 nm) and MIR (2500–25000 nm) location have been investigated for utility in quantifying soil C.

3) **CD spectroscopy**^[7] :- Circular dichroism (CD) is an optical approach which shortly, simply and with high sensitivity presents statistics on the secondary and tertiary structures of protein and glycoprotein products, and has a position in steadiness and components studies. The spectra tend to have notably negative signal-to-noise and arise from a collection of overlapping huge peaks. Even though broadly used in research and characterization studies of proteins, glycoproteins, nucleic acids and small molecules, and in assessing balance and interactions, there has been little work to advance objective means to discriminate between spectra. Thus CD has no longer been broadly used for best control purposes.

We also recognise that unacceptable alternate in functional properties may occur without significant changes in the CD spectra, in which case different assays to investigate purposeful properties will be required. It may additionally be desirable to achieve information that outline in what way the spectra are different (e.g. to distinguish between thermally- or chemically-induced changes in protein structure), or to quantify these differences.

4) **FI –IR spectroscopy**^[8] :- Evidence of plant root biomass and manufacturing in peatlands at the stage of species or plant purposeful kind is wanted type (PFT) for defining ecosystem functioning and

predicting its future development. However, such source are local due to methodological difficulties and the toil some ness of deductive roots from peat. We developed Fourier transform infrared (FTIR) spectroscopy based calibration models for quantifying the mass proportions of several common peatland species, and alternatively, the PFTs that these species represented, in composite root samples. Near infrared and FTIR spectroscopy are non-hazardous bodily methods.

The spectrum of light absorbed by as amplein the near-infrared (1100–2500 nm) or infrared (4000–400 cm⁻¹) region gives a chemical signature of the sample, providing information about the presence, character and abundance of chemical bonds or functional groups.

5) Terahertz pulsed spectroscopy ^[9]:- Terahertz pulsed spectroscopy (TPS) and terahertz pulsed imaging (TPI) are two novel techniques for the bodily characterization of pharmaceutical drug materials and closing solid dosage forms, utilizing spectral statistics in the a long way infrared location of the electromagnetic spectrum. TPS can be used to characterize crystalline properties of drugs and excipients. unique polymorphic type of a drug can be easily exceptional and quantified.

Over the past few years terahertz technological know how has come to be a new device for the physical characterization of solid materials (Beard etal 2002). Radiation in the far-infrared location of the electromagnetic spectrum, so-called terahertz radiation (60 GHz–4THz=2–130cm⁻¹, Figure 3), is used to find out about prescribed drug materials.

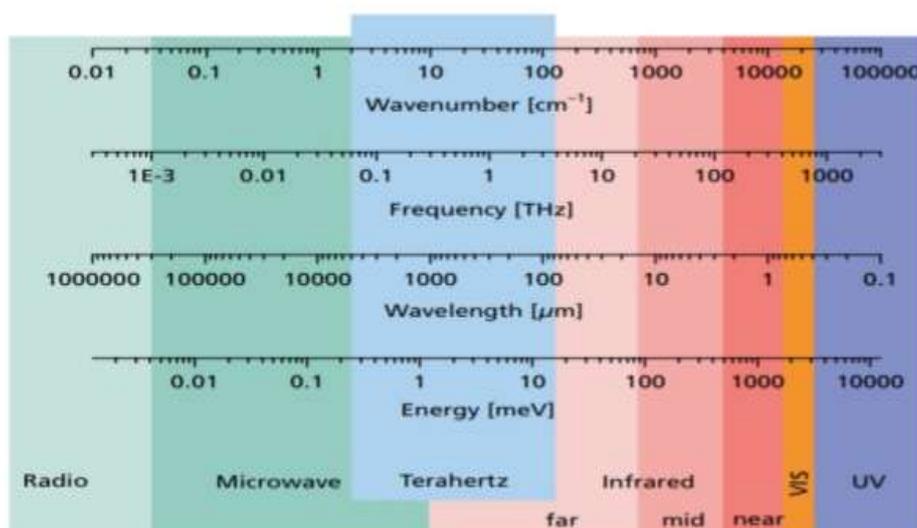


Figure 2 The electromagnetic spectrum showing terahertz radiation in relation to adjacent spectral regimes. ^[9]

Both imaging and spectroscopic measurements have been developed. Using science it is possible to directly access and exploit structural information of condensed matter at unparalleled speed. The energies of terahertz radiation are reduce than most interior vibrations of the molecules and the predominant mode of absorption of terahertz radiation is due to intermolecular vibrations.

As a substitute than probing intramolecular vibrations, terahertz spectra represent statistics on translations and liberations of molecules (Chantry 1971). It is an fantastic technique for characterizing the crystalline

properties of solid materials, as the phonon lattice modes are probed directly.

TPS in ATR mode is an interesting device for polymorph screening, as solely milligrams of sample material are crucial for analysis.

Terahertz spectroscopy addresses the inter-molecular structure and tends to incorporate physical data alternatively than chemical, as can be observed by using NMR and IR. TPS instruments currently cover the wave range between 2–133cm⁻¹, but very lately terahertz units have been developed which emit at frequencies up to 180cm⁻¹.

Even though it may want to be proven that, for a rigid dimer molecular structure such as

carbamazepine, the phonon modes are restricted to the wavenumber range between 10–140cm⁻¹ (Day et al 2006), this would possibly now not be actual for bendy flexible polymorphic forms.

Most intramolecular vibrations are expected at wavenumbers above 200cm⁻¹.

For this reason it would be suitable to extend the spectral bandwidth of the instruments to include dimension of at least some of the intramolecular vibrations.

The polymorphic purity of the sample material used to be analysed with the aid of temperature-dependent TPS measurements.

The extra advantages of terahertz spectroscopy were apparent. TPS can be used to learn about very speedy solid-state tactics without influencing the experimental conditions. Good quality spectra can be acquired in much less than a second. Terahertz spectroscopy can furnish complementary information to assist interpret thermal analysis data, such as differential scanning calorimetry and thermo gravimetric analysis.

III. CHAPTER NO: 3) FORMULATION ASPECT AND METHOD OF PREPARATION:-

✓ UV –VISIBLE SPECTROSCOPY^[1]

Method Development :-

Basic criteria for new method development of drug analysis :-

- The drug or drug combination may not be official in any pharmacopoeias.
- A proper analytical procedure for the drug may not be available in the literature due to patent regulations; Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients.
- Analytical methods for the quantization of the drug in biological fluids may not be available, Analytical methods for a drug in combination with other drugs may not be available the existing analytical procedures may require expensive reagents and solvents.
- It may also involve cumbersome extraction and separation procedures and these may not be reliable.

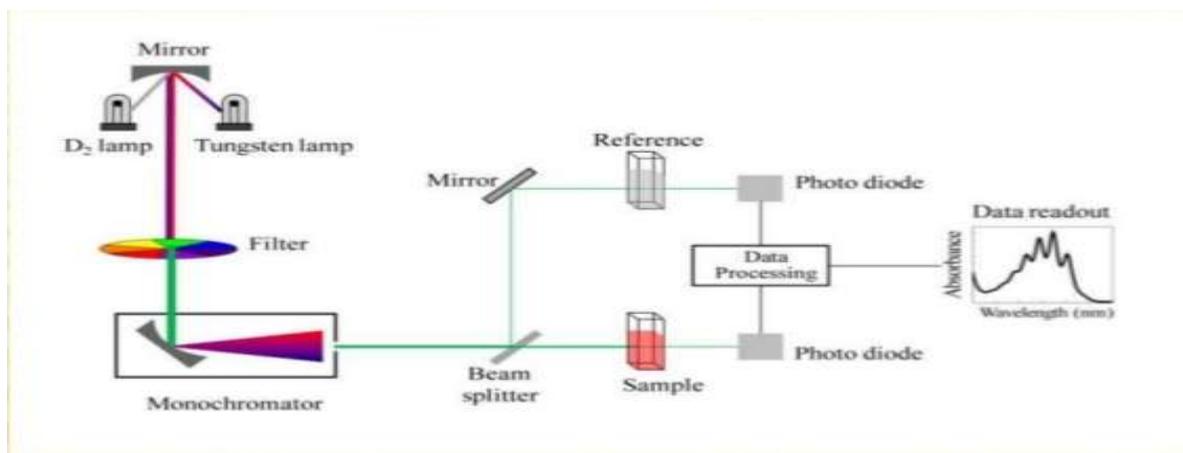


FIG 3:- UV VISIBLE INSTRUMENTATION^[1]

The fundamental law that governs the quantitative spectrophotometric analysis is the Beer -Lambert law.

Beer's law:-

It states that the intensity of a beam of parallel monochromatic radiation decreases exponentially with the number of absorbing molecules. In other words, absorbance is proportional to the concentration

Lambert's law:-

It states that the intensity of a beam of parallel monochromatic radiation decreases exponentially as it passes through a medium of homogeneous thickness. A combination of these two laws yields the Beer-Lambert law.

Beer-Lambert law

When beam of light is passed through a transparent cell containing a solution of an absorbing substance, reduction of the intensity of light may occur. Mathematically, Beer Lambert law is expressed as ,

$$A = a b c$$

Where, A=absorbance or optical density, a=absorptivity or extinction coefficient, b=path length of radiation through sample (cm), c=concentration of solute in solution. Both b and a are constant so a is directly proportional to the concentration c.

When c is in gm/100 ml, then the constant is called A (1%, 1 cm)

$$A = A \frac{1\%}{1 \text{ CM}} bc$$

✓ . **Infrared spectroscopy** :- infrared spectroscopy is measurement of the interaction of infrared radiation with matter by absorption ,emission or reflection.

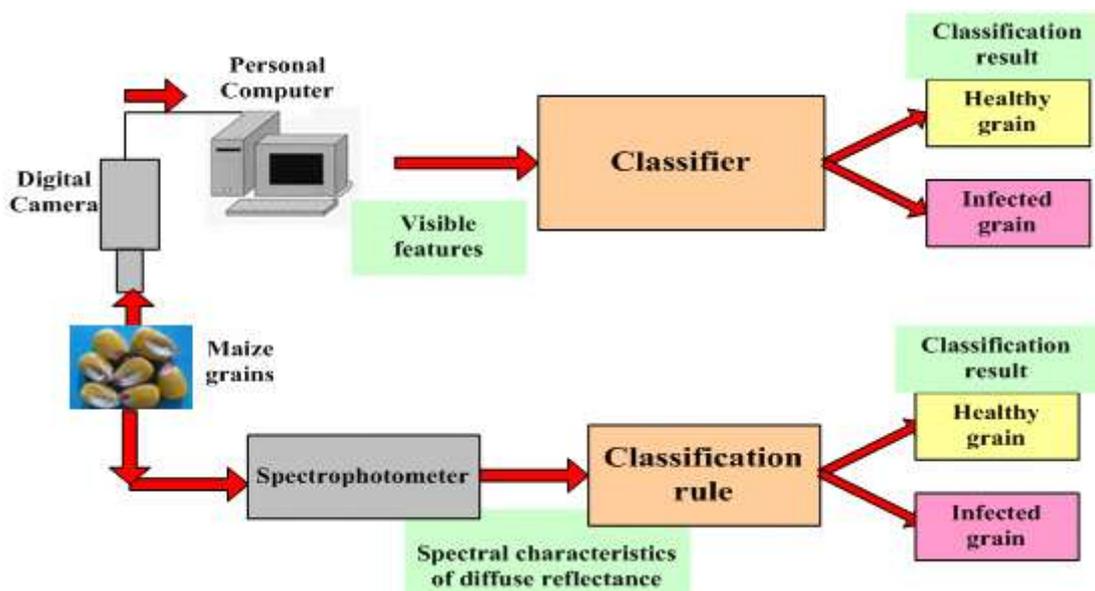


FIG 4:- INFRAD SPECTROSCOPY METHOD OF WORKING

✓ **CD spectroscopy** :- is an absorption spectroscopy method based on the different absorption of left & right circularly polarized light. optically active chiral molecules will preferentially absorb one direction of the circularly polarized light.

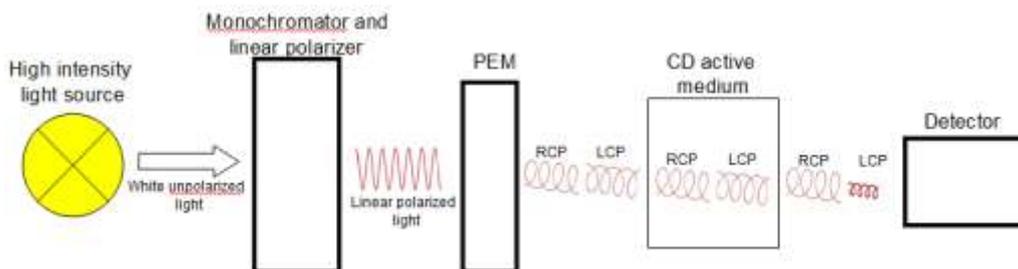


FIG 5:- CD SPECTROSCOPY METHOD OF WORKING

- ✓ **FT-IR spectroscopy:-** is used to identify organic ,polymeric &in some cases, inorganic materials. The FTIR analysis method used infrared light to scan test sample and observe chemical properties.
- ✓ **Terahertz pulsed spectroscopy :-** the propagate a THz pulse through a semiconductor

sample and measure the transmitted and reflected field as function of time.there,one collect information of semiconductor excitation dynamics completely in time domain, which is the general principle of the terahertz time domain spectroscopy.

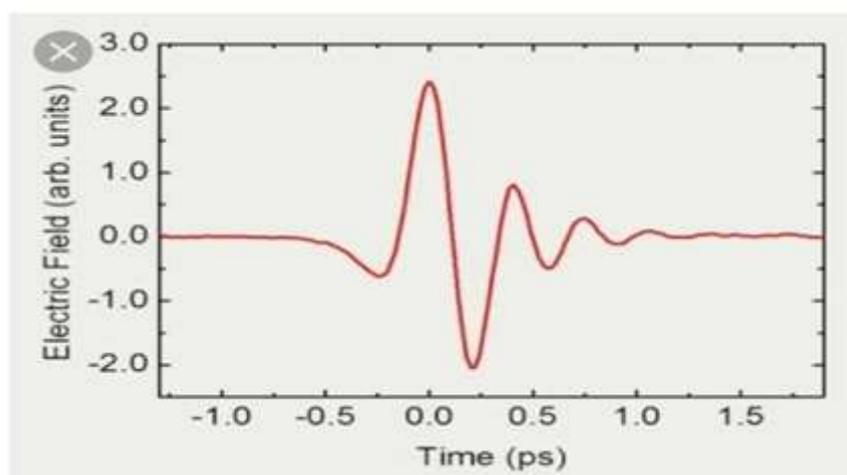


FIG 6:- TERAHERTZ PULSED SPECTROSCOPY WAVELENGTH^[9]

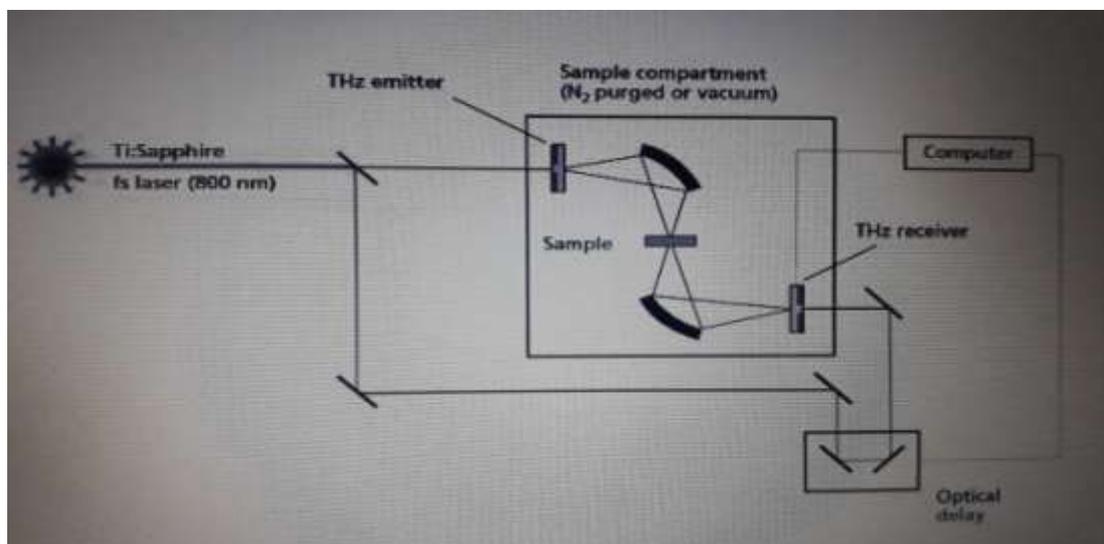


FIG 7:- TERAHERTZ PULSED SPECTROSCOPY METHOD OF WORKING^[9]

IV. CHAPTER NO: 4) EVALUATION PARAMETERS:-

- ✓ **UV –VISIBLE SPECTROSCOPY^[1] :-** Method Validations Analytical Method Validation can be defined as (ICH) “Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its

predetermined specifications and quality characteristics”.

1. Specificity
2. Linearity
3. Accuracy
4. Precision
5. Detection Limit (LOD)
6. Quantitation Limit (LOQ)
7. Range

Table 1 :ACCEPTANCE CRITERIA FOR THE DIFFERENT CHARACTERISTICS OF VALIDATION BY ICH^[1]

❖ S	Characteristics	Acceptance Criteria
u	Linearity	$R^2 \geq 0.99$, similar response ratios
m	Precision-System	RSD<2%
m	Precision-Method	RSD<2%
a	Accuracy	FDA 98-102%, EPA 50-150%
r	Specificity	No interference
y	Detection Limit	>2 times base line
o	Quantitative Limit	Signal-to-Noise=10:1
f	Range	Concentration where data can be reliably detected
v		
a		
l		
i		

Validation parameters :-

- λ range
- Regression Equation ($y=mx+c$)
- Measured wavelength
- Linearity range
- Intercept
- Correlation coefficient (R2)
- Limit of Detection (LOD)
- Limit of Quantitation (LOQ)
- Accuracy (Mean % Recovery)
- Precision (%RSD)

Infraredspectroscopy:-

Identification method external validation The external validation of the identification method was carried out on full scale API bags processed in SMB's manufacturing area.

V. CHAPTER NO: 5) SUMMERY, CONCLUSION AND FUTURE SCOPE:-

Summary:- A general term to describe analytical methods based on absorbance, chemiluminescence, emission, or fluorescence; i.e. an optical method mainly based on the interaction between electromagnetic radiation and matter. The process of resolving electromagnetic radiation (e.g. light, ultraviolet, infrared, microwave, etc.) into its component wavelengths to produce spectra, plotting the function of radiant intensity versus wavelength or frequency. Electromagnetic spectra used in Spectroscopy: It encompasses a wide range of wavelength (1fm - 10m) and frequencies and energies, in which the visible region extends only from about 400nm to 700nm; the most common spectroscopic methods based on electromagnetic radiation are:

Table 2 :SUMMERY OF SPECTROSCOPY

Type of spectroscopy	Wavelength Range [λ . m]
Gamma-ray emission	$0.05-15 \cdot E^{-9}$
X-Ray absorption	0.001-10
Ultraviolet visible absorption	$180-780 \cdot E^{-9}$
Infrared absorption and Raman scattering	$0.78-300 \cdot E^{-6}$
Nuclear magnetic resonance	0.6-10

CONCLUSION :-

Using spectroscopy, we can get information on kind of source, radial velocity, composition, (including ions and molecules) and temperature, from position and intensities of lines.

Spectroscopy is the study of the interaction between matter and electromagnetic radiation as a function of the wavelength or frequency of the radiation. Important applications arise from biomedical spectroscopy in the areas of tissue analysis and medical imaging.

❖ Future scope :-

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