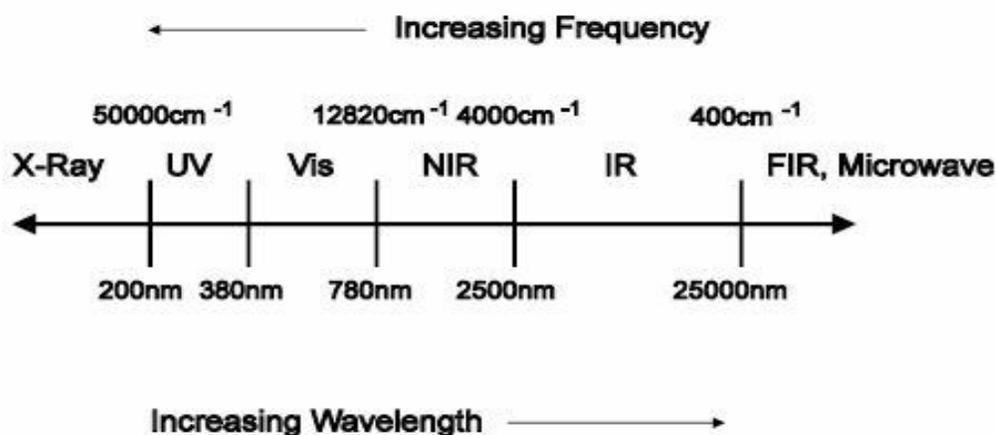


## NEAR IR SPECTROSCOPY

Infra red spectrum is an important record which gives sufficient information about the structure of a compound. In recent years, NIR spectroscopy has become so widespread in process analysis and within pharmaceutical industry for raw material testing, product quality control and process monitoring. Not only in the pharmaceutical industry it has gained wide acceptance in biotechnology, genomics analysis, proteomic analysis, interactomics research, inline textile monitoring, food analysis, plastics, textiles, insect detection, forensic lab application, crime detection, various military applications, and is a major branch of astronomical spectroscopy and so on. NIR absorption bands are typically broad, overlapping and 10–100 times weaker than their corresponding fundamental mid-IR absorption bands. NIR spectroscopy is a vibrational spectroscopic method belongs to the infrared light spectrum which is very close to the visible region (from about 750 to 2500 nm), where the most of organic and some inorganic compounds shows good reflectance or transmission properties. That means they are exhibiting good absorption of light at the NIR region. Figure 1 express the range of electromagnetic radiation.



**Figure 1: Range of Electromagnetic radiation**

**Theory & Principle of near-infrared (NIR) spectroscopy**

The American Society of Testing and Materials (ASTM) defines the NIR region of the electromagnetic spectrum as the wavelength range of 780–2526 nm corresponding to the wave number range 12820–3959  $\text{cm}^{-1}$ . The most prominent absorption bands occurring in the NIR region are related to overtones and combinations of fundamental vibrations of –CH, –NH, –OH (and –SH) functional groups. Combinations arise by interaction of two or more vibrations taking place simultaneously. For a given molecule, a normal mode of vibration corresponds to internal atomic motions in which all atoms move in phase with same frequency but with different amplitude. Additionally to these normal vibrations transitions corresponds to be called overtones. Such transitions are forbidden by the selection rules of quantum mechanics. As a result the molar absorptivity in the near IR region is very small. There are two laws which govern the basics of vibrational spectroscopy, they are Hooke's law and Frank condon principle. Hooke's law states that, for two body harmonic oscillator, the frequency of vibration is

$$\tilde{\nu} \text{ (in } \text{cm}^{-1}\text{)} = \frac{1}{2\pi c} \sqrt{\frac{k(m_1 + m_2)}{m_1 m_2}}$$

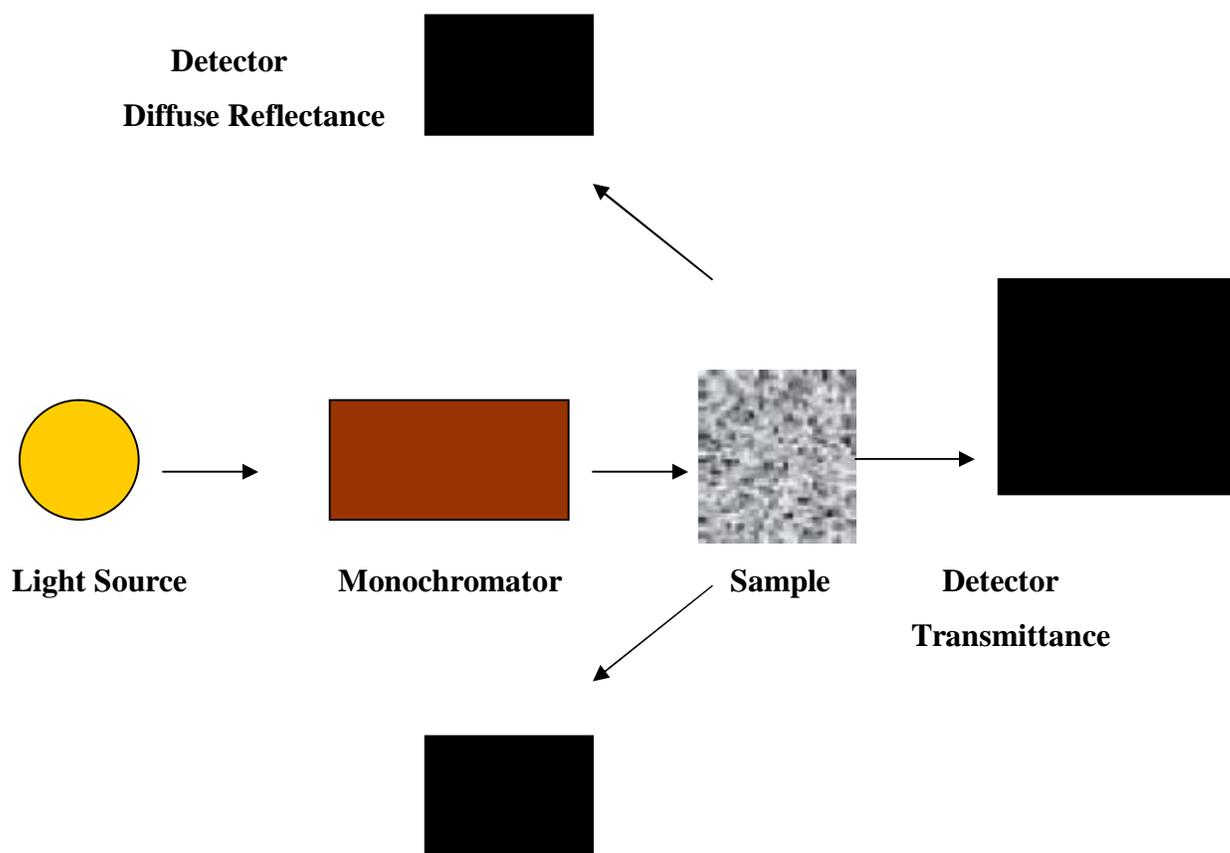
Where, C = speed of light, K = force constant ( $5 \times 10^5$  dynes/cm).

Hooke's law can be used to calculate the fundamental vibrations for diatomic molecules in IR. Transition from the ground state to the first excited state absorbs light strongly in IR region and give rise to intense bands called the fundamental bands. Transition from the ground state to the second excited state with the absorption of NIR give rise to weak bands called 1st overtone in NIR. Transition from the ground state to the third excited state with the absorption of NIR give rise to weak bands called 2<sup>nd</sup> overtone in NIR. Like wise 3rd and 4th overtone bands will occur based on the transition to the fourth and fifth excited state with the absorption of NIR.

Franck Condon Principle: When a molecule vibrates the probability of finding of given atom at a certain point is inversely proportional to its velocity. Therefore the atoms in a vibrating molecule spend most of their time in configuration in which the kinetic energy is low, that is the configuration in which potential energy is nearly identical with total energy or at intersection of the vibrational energy level with the potential energy surface of the molecule. Thus the photon is most likely to be absorbed when the nuclei are stationary or moving slowly. The excitation resulting from the absorption of photon can not be transferred immediately to the nuclei. The nuclei will therefore tend to continue moving slowly after absorption. The nuclear configuration also tends to be close to intersection of the vibrational energy level with the potential energy surface of the molecule. Therefore transitions tend to take place between vibrational levels in which nuclear configurations are same in both states. Thus these small variations give rise to anharmonicity which causes the combination and overtone bands. The spectrum can be measured based on the sample nature either in transmission or reflection. Transparent materials are usually measured in transmittance, solids and turbid, semi solid solutions measured in diffuse reflection, transfection or transmission. In the 1100–2500 nm region, the amount of scattering makes the path length so great that transmittance through 1 cm of most samples, such as wheat meal, flour or milk powder, is negligible. This situation is called diffuse reflectance because most of the incident radiation is reflected. Hybrid mode of transmittance and reflectance called transflectance, where the radiation is transmitted through the sample reflected from a ceramic tile beneath the sample and then transmitted back through the sample before finally reaching the detector.

### Instrumentation

A NIR spectrometer is generally composed of a light source, a monochromator, a sample holder or a sample presentation interface, and a detector, allowing for transmittance or reflectance measurements (Fig. 2).



**Fig. 2. Basic NIR spectrometer configurations.**

**Source:** The light source is usually a tungsten halogen lamp, which produce heat up to 1100k. These lamps emit visible radiation along with considerable NIR radiation. So filters are needed in order to eliminate visible radiation which helps in prevention of unnecessary sample heating. The advantages of this source are cheap in cost and are readily available and these lamps can not increase their energy out put as the voltage of source is increased.

Most of the applications requires only selected wavelength. LEDs emit radiation at specific wavelength, but it is relatively broad energy emitted. LEDs combined with filters are using as wavelength selector and these are very efficient, cheap and ideal for portable, so low cost instruments can be produced without having moving parts.

### **Grating:**

Gratings comprises metal or glass engraved surface with many fine parallel lines. When the light beam strikes the surface it divides into various wavelengths by diffraction. In interferometer wavelength selector, the light beam is split into two beams with a beam splitter. The two separated beams strikes the fixed and moving mirrors respectively and they are reflected back to beam splitter. They are then recombined and exit the interferometer in the direction of sample. Based on the material ( KBr, CaF<sub>2</sub> or quartz) used for the beam splitter interferometer operate at specific wavelength.

### **Sample Holder**

Sample holder Cells made up of quartz or glass to perform transmission with liquids. Depending on the design of the instrument the cells are of varied sizes and designs. Some instruments use round sample cups for dry solid and grained samples. Transport cells are designed for analyzing the bulk samples. Round cups are often rotated in order to scan maximum amount of sample and to eliminate the lack of sample homogeneity. Transport cells move vertically where the scanning beam remains stationary.

### **Detector**

Detector The choice of detectors depends on Wavelength range, Spectrometer design characteristics, detector characteristics such as photosensitivity (responsivity), noise equivalent power (NEP), detectivity. Photosensitivity measures the voltage output per unit of incident radiant at a particular wavelength when noise is not considerable. NEP

measures the quantity of light when the signal to noise ratio is 1. Detectivity is a parameter used to compare the performance of different detectors. Best detector should possess the higher in the signal of detectivity. Detectivity is the signal to noise ratio at particular electrical frequency and in a 1HZ bandwidth when 1 watt of radiant on a  $1\text{cm}^2$  active area detector.

**Table 1: Detectors used in NIR spectroscopy**

Detector	Wavelength Range	Region	Responsivity/Detectivity	Remark
PbS	1100-2500 400-2600 1100-4500	NIR UV-NIR NIR-MIR	Intermediate/Intermediate	PbS sandwiched with silicon photodiodes are often used for VIS-NIR
PbSe	1100-5000	NIR-MIR	Fast/High	The detector must be cooled with liquid nitrogen
InGa As	700-1700	NIR NIR Raman	Fast/ Very High	Linear arrays High sensitivity, dynamic range, signal to noise performance and stability FT-NIR Diode arrays

				spectrometer
InSb/In As	1000-5500	NIR IR NIR-MIR	Fast/ Very High	High quality detector, Detector Photodiodes
CCD	800-2200	NIR	Fast/ High	High performance detector, Applied in cameras, Diode arrays spectrometer

Detectors using in NIR spectrometers are Lead sulphide detectors (PbS), Lead selenide detectors (PbSe), Silicon detectors, Indium antimonide detectors, InGaAs, InSb, Common Charged Coupled Devices (CCD). Silicon detectors are fast, low noise, small and highly sensitive from the visible region to 1100 nm. PbS detectors are slower, but very popular since they are sensitive from 1100 to 2500 nm and provide good signal-to-noise properties. The most expensive InGaAs detector combines the speed and size characteristics of the silicon detector with the wavelength range of the PbS detector

### **Types of instrument used in NIR Spectroscopy**

Modern NIR instruments are classified in terms of the technology employed for wavelength selection. These are Filter based instruments, LED based instruments, AOTF based instruments, Dispersive optics-based instrument, Fourier-transform based instruments.

#### **Filter based instruments**

These instruments employing filters as wavelength selectors are commercially available for dedicated applications. For example, an instrument for determination of the quality

parameters of gasoline (Zeltex Inc.) employs interference (Fabri-Perrot) filters and LED (Light Emitting Diode) sources in the NIR region. Variable (scanning) interference filters based on the variation of the dielectric layer thickness by using piezoelectric drivers have recently been used.

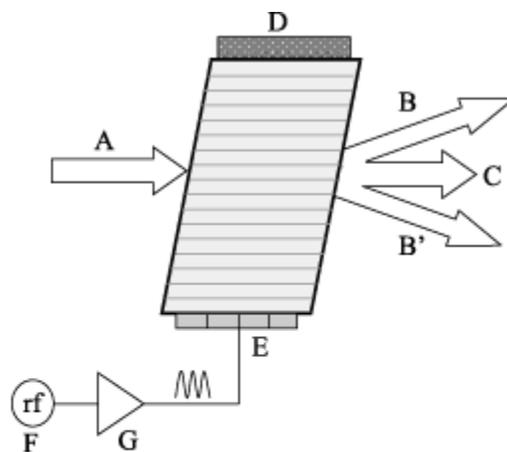
### **LED based instruments**

Recently a tendency to reduce the price and size of the instruments, aiming for use in the field, has taken advantage of the use of Light Emitting Diodes (LED). These devices can produce NIR radiation with a band width of about 30 - 50 nm, centred in any wavelength of the spectral region. The instruments can employ a set of LEDs as sources of narrow bands of near infrared radiation or use them to produce a polychromatic, highly stable source whose radiation is dispersed by using common monochromator devices such as those based on gratings or filter optics.

### **AOTF based instruments**

The instruments based on Acousto-Optical Tunable Filters (AOTF) are modern scan spectrophotometers employing a technology that allows constructing instruments with no moving parts, capable of reaching very high scan speeds over a broad range of the NIR spectral region. Scan speed is fast and up to 2000 wavelengths can be selected per second. The scan speed is usually limited by the detector response time.

The AOTF operating in a non-collinear configuration (acoustic wave and radiation beam propagating at almost perpendicular angles), is a device made of a birefringent crystal of TeO<sub>2</sub>, cut in a special angle. Other materials can be used but the characteristics of TeO<sub>2</sub> are suitable for the NIR regions and has been chosen by all instrument manufacturers.



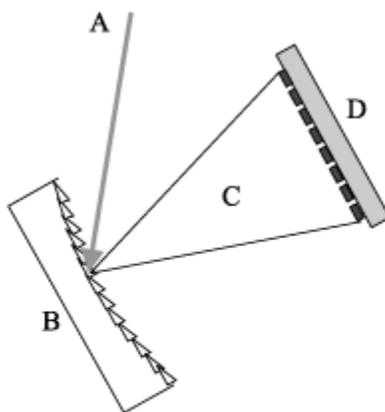
**Figure 3.** A Monochromator based on an Acousto-Optic Tunable Filter. A, incident polychromatic radiation; B and B', monochromatic beams (same wavelength); C, remaining polychromatic radiation; D, acousto absorber; E, piezoelectric transducer; F, generator of radio-frequency signal, and G, radio frequency amplifier.

A piezoelectric material (usually  $\text{LiNiO}_4$ ) is attached to one end of the crystal which, under excitation from an external radio frequency signal (rf), produces a mechanical (acoustic) wave which propagates through the crystal. The acoustic wave produces a periodic variation of the refractive index of the crystal in a frequency determined by the Rf signal, in the range of 50 to 120 MHz. The interaction of the electromagnetic wave and the acoustic wave causes the crystal to refract selectively a narrow wavelength band. The birefringence of the  $\text{TeO}_2$  crystal leads to the production of two monochromatic beams whose angular separation is about  $7^\circ$ . Both or only one diffracted beam can be used by NIR instruments. Dual beam (with a reference beam produced by splitting one of the beams) or single beam instruments can be found. Alternatively, the two monochromatic beams can be employed for the construction of a dual beam instrument with an optimised use of the radiation throughput.

The non-moving parts concept of the AOTF-based NIR spectrophotometers impart to them some unbeatable qualifying characteristics for use in the field or on the factory floor, aiming at in-line monitoring. The wavelength precision is about  $\pm 0.05$  nm and the resolution is dependent on the wavelength, with typical values in the range 5 to 15 nm for the wavelength in the range 1000 to 2500 nm.

### Dispersive optics-based instruments

Dispersive instruments based on diffraction gratings were employed in the early days of NIR spectroscopy and were responsible for the research initially developed to consolidate NIR spectroscopy as an analytical tool. This technology has continued through the 90's and is still being used, with many manufacturers offering instruments with performance suitable for many practical applications.



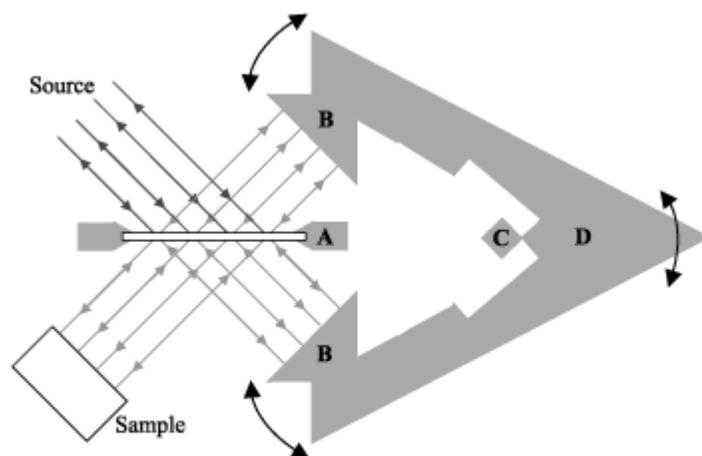
**Figure 4.** Dispersive optics based on concave grating and sensor array employed in modern non-moving-parts spectrophotometers. A, incident polychromatic beam; B, concave grating; C, dispersed radiation, and D array of sensors.

The first commercial Brazilian-made NIR spectrophotometer, manufactured by Femto Ltda., was launched in 1999 and is based on a monochromator employing a moving diffraction grating and Czerny-Turner optics. Its performance suffices for many transmittance measurement-based analytical methods employed in the control of sugar and alcohol production, a very important Brazilian industrial sector. The instruments based on grating monochromators present the advantage of a relatively low cost when compared with other scanning instruments employing modern technologies. The main disadvantages of dispersive instruments are the slow scan speed and a lack of wavelength precision, which deteriorates for long term operation due to mechanically driven mechanism fatigue. This can be a negative regarding maintenance of multivariate models. Also, the presence of moving parts limits the use of dispersive instruments in the field

and in more aggressive environments. On the other hand, recent evolution in sensor production technology gives dispersive optics a longer life. That is because it is now possible to construct linear arrays of PbS and InGaAs sensors containing up to 256 independent elements. Placed in the focal plane of plane or concave grating optics, the sensor array allows to scan an entire spectra in a few milliseconds.

### Fourier-transform based instruments

Spectrophotometers based on the use of interferometers and Fourier transform to recover the intensities of individual wavelengths in the NIR region are, undoubtedly, the instruments combining most of the best characteristics in terms of wavelength precision and accuracy, high signal-to-noise ratio and scan speed (although slower than AOTF based instruments). These instruments add to the previously mentioned Fellgett gain the Jaquinot gain, which is not attainable with dispersive Hadamard instruments. The Jaquinot gain arises from the high radiation throughput achieved for a Fourier instrument, due the fact that it does not employ entrance or exit slits to limit the radiation intensity reaching the detector. Typical wavelength accuracy is better than 0.05 nm and the resolution can achieve values below 1 nm in the NIR region, at cost of decreasing the scan speed.



**Figure 5.** "Wishbone" interferometric system employed in modern NIR spectrophotometers based on Fourier Transform. A, beam splitter; B, corner cubic mirrors; C, anchor, and D, "wishbone".

These advantages turn the NIR spectrophotometer based on interferometric measurement and Fourier transform into an unbeatable research instrument. On the other hand, the spectrophotometer is not as robust as an AOTF-based instrument which is assembled without any moving parts. The robustness of the Fourier spectrophotometer has been improved by using a "wishbone" type of interferometer, such as the one found in the Bomem instruments. This type of interferometer employs half cubic mirrors to compensate for beam travelling distance changes arising from non uniform mirror movement during data acquisition. The result is a Fourier-based spectrophotometer that can tolerate some environmental vibrations without losing wavelength precision and photometric reproducibility. The price of a Fourier based instrument is comparable with the AOTF-based spectrophotometer and, therefore, both are considered expensive relative to other options.

### **Pharmaceutical applications**

NIR spectroscopy combined with multivariate data analysis opens many interesting perspectives in pharmaceutical analysis, both qualitatively and quantitatively.

- **Identification and qualification of raw materials and intermediates**

The chemical identity of a active ingredients and excipients are usually confirmed by NIR techniques with a spectral library approach.

- **Analysis of intact dosage forms**

The nondestructive and multivariate nature of NIR techniques opens new perspectives in the pharmaceutical analysis of intact dosage forms, including chemical, physical and related biopharmaceutical aspects. Fast and nondestructive identification of active ingredients and excipients in whole tablets, even through the blister packaging, is certainly a domain of NIR spectroscopy.

- **Analysis of polymorphs**

NIR spectroscopy for the quantitative determination of polymorphs in a formulation matrix. Most polymorphs exhibit spectral differences in the mid-IR, since NIR spectra arise from overtones and combinations of mid-IR absorbances, NIR was also reasoned to be suitable for the analysis of polymorphs.

- **Determination of Adulteration of Essential Oils**

Near-infrared (NIR) technologies using for the assessment of essential oil components and in the identification of individual essential oils. NIR can be used to discriminate between the ravintsara(adultrant) and ravensara(authentic) essential oils.

- **Protein quantification within lipid implants**

Lipid implants have been proposed as promising sustained release devices for the parenteral application of pharmaceutical proteins. Near infrared spectroscopy (NIRS) as tool for drug quantification within controlled release matrix systems based on poly-(lactic-co-glycolic) acid (PLGA).

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