CHAPTER 2

Theory and Principles of Near Infrared Spectroscopy

2.1. Introduction

The investigation of characteristics of near infrared (NIR) region of the electromagnetic spectrum starts with Herschel in 1800 [Herschel 1800]. In the last ten years, near infrared spectroscopy (NIRS) has become a very popular technique for a wide range of analyses in various fields namely agriculture, pharmaceuticals, industries, process control, food processing, remote imaging spectroscopy and other diverse applications. [Kradiel 2001]. The advantage of this technique is mainly attributed to the rapid and non-destructive analysis of much thicker samples.

Infrared spectroscopy is used to investigate the vibrational properties of a sample. The infrared region of the electromagnetic spectrum (Range: 700 – 10^6 nm) is divided into three regions, near infrared (NIR) in the wavelength range 700 – 2500 nm, mid-infrared (generally referred to as IR) in the range 2500 – 5 \times 10^4 and far infrared in the range 5 \times 10^4 – 10^6 nm. The periodic vibrational motions of atomic nuclei within molecules result in respective molecular spectra. These nuclei move close or apart (stretch) along a straight line vector and they vibrate, rotate, wag, and bend with respect to their centers of gravity. Molecular vibrations give rise to absorption bands generating spectra. When a molecule absorbs infrared radiation, the individual covalent bonds vibrate very similar to the vibrations of a diatomic oscillator [Siesler et al. 2002].

2.2. The diatomic molecule

2.2.1. Ideal harmonic oscillator

For an ideal harmonic oscillator, the potential energy V is given by a quadratic expression:
\[ V = \frac{1}{2} k (r - r_e) \]

Or \[ V = \frac{1}{2} kx^2 \]

where \( k \) is the force constant of the bond, \( x = (r - r_e) \) is the displacement coordinate, \( r \) is the inter-nuclear distance, \( r_e \) is the inter-nuclear distance at equilibrium.

The potential energy versus inter-nuclear distance curve is shown in Fig. 2.1

![Harmonic potential function](image)

Fig.2.1 Harmonic and anharmonic potential functions for a diatomic oscillator.

The frequency at which the ideal harmonic oscillator (atomic or molecular dipole) stretches or bends depends on the bond strength and the masses of the atoms bonded together. When the harmonic oscillator vibrates, the vibrational energy is continuously changing from kinetic to potential and back again. The total energy in the bond is proportional to the frequency of vibration. Considering the elastic properties of a harmonic oscillator, Hooke’s law is applied to explain the properties of the two atoms with a spring-like bond between them.
The natural vibrational frequency for a bond (or any two masses attached to the ends of a spring) is expressed as

\[ \nu = \frac{1}{2 \pi} \sqrt{\frac{k}{\mu}} \]

where \( \mu = \frac{m_1 m_2}{m_1 + m_2} \) is the reduced mass of the system; \( m_1 \) and \( m_2 \) are the masses of the atoms or nuclei.

Note that as masses of atoms increase, the frequency of vibrations decreases. Also note that the force constant \( k \) varies from one bond to another. The force constant for a single bond is approximately ½ times that of a double bond and \( \frac{1}{3} \) times that of a triple bond.

The ideal or simple harmonic oscillator (Normal mode theory) is able to predict, with relative accuracy, the frequency of energy of fundamental absorption bands such as symmetric and asymmetric stretching, bending, scissoring and wagging (detailed descriptions later). However the simple harmonic oscillator cannot predict the overtone band positions because bonds are not true harmonic oscillators. The quantum mechanical effect on a simple harmonic oscillator indicate that the bond between two atoms can not be treated quite as simply as two masses connected by a spring. A quantum mechanical treatment has shown that the vibrational energy between atoms in a molecule is quantized and may have only discrete energy levels. Under suitable conditions, the vibrational energy in a molecules jumps from one level to another.

For the harmonic oscillator (any molecule), the discrete vibrational energy levels are given by

\[ E_{\text{vib}} = (\nu + \frac{1}{2}) \hbar \nu \]
where $h$ is plank's constant, $\nu$ (Greek nu) is the classical vibrational frequency of the bond (defined above) and $\nu$ is the vibrational quantum number which can take only integer values $0, 1, 2, 3, \ldots$

In terms of wave number units ($\text{cm}^{-1}$) the expression for energy levels is

$$G(\nu) = \frac{E_{\text{vib}}}{hc}$$

$$= \frac{\nu}{c} (\nu + \frac{1}{2})$$

i.e. $G(\nu) = \nu (\nu + \frac{1}{2})$

where $\nu$ is the wave number of the vibrational transition.

Fig. 2.2 shows the vibrational energy levels corresponding to different values of $\nu$. The various energy levels are represented as horizontal lines. These levels are equally spaced.

Fig. 2.2 Vibrational energy levels of the harmonic oscillator
The selection rules that state which transitions between levels are allowed can be deduced from the expression for the transition moment given by

\[ \int \psi^{\ast}_{\nu'} \varepsilon \psi_{\nu''} \text{d} \tau \]

where \( \varepsilon \) is the dipole moment, \( \psi^{\ast}_{\nu'} \) and \( \psi_{\nu''} \) are the wave functions for the \( \nu' \) and \( \nu'' \) states (\( \psi^{\ast}_{\nu} \) is the complex conjugate of \( \psi_{\nu} \)).

The dipole moment \( \varepsilon \) is expressed as a linear function of \( x \) for small displacements about the equilibrium position.

\[ \varepsilon = \varepsilon_0 + \left( \frac{d\varepsilon}{dx} \right)_e x \]

where \( \varepsilon_0 \) is the dipole moment at the equilibrium internuclear distance.

The selection rule states that, in the quantum mechanical harmonic oscillator (dipole involving two atoms), a molecule may only absorb (or emit) electromagnetic radiation of energy equal to the spacing between the two levels. The transitions can only occur from one level to the next higher (or lower) level. i.e. Vibrational quantum number can only change by one unit. Hence transitions are allowed only if the value of the transition moment is non zero.

\[ \left( \frac{d\varepsilon}{dx} \right)_e \neq 0 \]

i.e. \( \Delta \nu = \pm 1 \)

Most molecules prefer to stay at their lowest energy state (ground state for which \( \nu = 0 \)). If a molecule absorbs radiation energy, it changes to an exited state and creates an absorption spectrum. The allowed transition \( \nu = 0 \rightarrow \nu = 1 \) is called fundamental transition. This transition is responsible for most of the infrared absorption to which a
spectroscopist is of much interested. The other allowed transition \( \nu = 1 \rightarrow \nu = 2 \), \( \nu = 2 \rightarrow \nu = 3 \) etc. originate from vibrationally excited levels \( (\nu > 0) \). The corresponding bands which are much weaker than the fundamental absorption are called ‘hot bands’. These excited levels are having a relatively low population. When temperature increases, this population will increase and thus the intensity of the bands also increase. For ideal or simple harmonic oscillator, the transitions of hot bands have same frequency as that of the fundamental transition (see Fig. 2.2)

Experimental observations give evidence that molecules are not ideal oscillators. When two atoms become closer, the columbic repulsion between their nuclei causes a rapid increase in the potential energy which the harmonic approach predicts. When the interatomic distance comes closer to the distance at which dissociation (or breakage) of bond takes place, the level of potential energy becomes stabilized. Thus the real molecules approach more the behavior of an anharmonic oscillator (see Fig. 2.1)

### 2.2.2. The anharmonic oscillator

In the anharmonic oscillator, it is possible to observe fundamental bands \( (\Delta \nu = \pm 1) \) and bands corresponding to transition states \( (\Delta \nu = \pm 2, \pm 3, \ldots) \) called overtones. The first consequence due to anharmonicities is that the vibrational energy levels are not equally spaced and hence the hot bands do not have exactly the same frequency as the fundamental band.

The second consequence is that overtone transitions such as \( \nu = 0 \rightarrow \nu = 2, \nu = 0 \rightarrow \nu = 3 \) etc are allowed.

Fig. 2.3 shows the energy level diagram and the associated transitions of harmonic and anharmonic oscillators.
The deviation from harmonic behavior can be expressed by two effects namely mechanical anharmonicities and electrical anharmonicities. The potential energy expression for an anharmonic oscillator contains higher order terms compared to that of an ideal harmonic oscillator.

$$V = \frac{1}{2}kx^2 + k^1x^3 + \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots (k^1 \ll k)$$

The effect of higher order terms gives rise to mechanical anharmonicities. A solution to the above expression (obtained by applying the expression in Schrodinger equation) yields an energy level in cm$^{-1}$ given by

$$G(v) = \frac{E_{\text{sub}}}{hc}$$

$$= \sqrt{v(v + \frac{1}{2})} - x_v \sqrt{v(v + \frac{1}{2})^2}$$

Fig. 2.3 Energy level diagram and associated transitions of the harmonic and anharmonic oscillator.
Here $x_e$ is the anharmonicity constant. It is because of the second term that, unlike the harmonic oscillator, energy levels are no longer equally spaced.

The Morse function (an empirical relation) as illustrated in Fig. 2.1 is used for the anharmonic potential function

$$V = D_e \left( 1 - e^{-\beta x} \right)^2$$

where $D_e$ is the dissociation energy at the equilibrium position (minimum of the parabolic curve) and $\beta$ is a constant. $D_e$ is given by $D_e = \frac{\varphi}{4x_e}$

The dipole moment expression can be written as

$$\mathcal{E} = \mathcal{E}_0 + \left( \frac{d\mathcal{E}}{dx} \right)_{x_e} x + \frac{1}{2} \left( \frac{d^2\mathcal{E}}{dx^2} \right)_{x_e} x^2 + \ldots$$

The effect of higher order terms in this expression gives rise to the electrical anharmonicities which is responsible for the appearance of overtones corresponding to transitions between energy levels for which $\Delta v = \pm 2, \pm 3, \ldots$

As can be seen in Fig. 2.3, the frequencies of the overtone absorptions are not exactly 2, 3… times the frequency of the fundamental absorption. The frequency of the ‘hot bands’ is less than that of the fundamental transition. At this point, an investigator may conclude that it is the anharmonicity which determines the occurrence of the NIR absorption bands. The intensities of the overtones also depend on anharmonicities.

### 2.3 The polyatomic molecules

The polyatomic molecules containing $N$ atoms will have $(3N - 6)$ vibrational types of motion $(3N - 6)$ degrees of freedom. If the molecule is linear, there are $3N - 5$ types of motion. The number of vibrational types of motion gives the number of fundamental
vibrational frequencies (normal modes). As long as the bonds do not break and the vibrations have amplitudes of about 10 – 15% of the average distance between atoms, the vibrations are considered as harmonic. Any harmonic (vibrational motion of the molecule) is considered to be the superposition of (3N – 6) simple harmonic motions and carries the term ‘normal vibration’. In the harmonic oscillator approximation, only the fundamental modes are allowed.

In practice, the vibrations in polyatomic molecules tend to be anharmonic i.e. vibrations about the equilibrium position are non-symmetric. This anharmonicity introduces overtones and combinations of the fundamental bands in the NIR region also (The fundamental bands are more common in the mid IR region; 2500 – 5 × 10^4 nm). However they will be much weaker than the fundamental bands since the anharmonicities are in general, slight. When dealing with organic compounds like oils, the most prominent NIR bands are those related to –CH, –NH, –OH and –SH functional groups.

2.4. Overtones and Combination bands in the NIR region.

Near infrared (780 – 2500 nm) spectral features arise from the molecular absorptions of the overtones and combination bands that originate fundamental vibrational bands generally founds in the mid- infrared region. The fundamental vibrations are caused by an energy transfer from the ground level to the first vibrational level. ‘Overtones’ are vibrations that are due to an energy transfer from the ground level to the second and higher levels [Osborne et al.1993]. They require a higher energy than the fundamental vibrations and hence occur at smaller wavelengths i.e. in the NIR region. Compared to the first overtones, higher order overtones are less intense and less likely to occur. ‘Combination bands’ arise as summation fundamental bands, their intensity decrease with the increase in the summation frequency.
Most of the absorption bands in the NIR region result from the harmonics and overtones of X – H fundamental stretching and bending modes of vibration.

2.5 Types of molecular vibrations.

In the near infrared region, the molecular vibrations consist of overtone bands and combination bands due to stretching and bending vibrations. ‘Stretching vibrations’ are either symmetric or asymmetric; bending vibrations are either in plane (scissoring and rocking) or out-of-plane (wagging and twisting). Stretching vibrations occur at higher frequencies (lower wavelengths) than bending vibrations. From the highest to lowest frequency, the vibrational modes occur as stretching and bending (scissoring, wagging, twisting and rocking).

The most-often observed bands in the NIR region consist of the combination bands and first, second or third overtones of C – H, O – H and N – H fundamentals. Generally, the shift in frequency and the broadening of bands are due to the formation of hydrogen bonding. The frequency shifts related to hydrogen bonding have a greater relation on overtones and combination bands than on their corresponding fundamentals. The normal types of molecular vibrations active within the NIR region are illustrated in the following sections.

2.5.1 Stretching vibrations.

‘Stretching vibration’ is a continuous change in the interatomic distance along the axis (bond length) between two atoms. Figures 2.4 (a) through 2.4. (h) demonstrate symmetric and asymmetric stretching vibrations in CH containing molecules.
Fig. 2.4 (a) and (b). Methyl (−CH₃) symmetric stretching (all atoms move along an identical external / internal direction).

Fig. 2.4 (c) and (d) Methyl (−CH₃) asymmetric stretching (all atoms move in opposite directions relative to the carbon atom).

Fig. 2.4 (e) and (f). Methylene (−CH₂) symmetric stretching (The two hydrogen atoms move away simultaneously from the carbon atom in the outward motion and approach it simultaneously in the inward motion).
Fig. 2.4 (g) and (h). Methylene (−CH₂) asymmetric stretching (when one of the hydrogen atoms moves away from the carbon atom, the other approaches it)

2.5.2 Bending vibrations

‘Bending vibrations’ is a change in the bond angle between the atoms. Fig. 2.5 (a) through 2.5 (f) demonstrate the molecular bending vibrations.

Fig. 2.5 (a) and (b). Methyl (−CH₃) symmetric bending (The bond angles are tightened simultaneously in the inward movement and are increased simultaneously in the outward movement).
Fig. 2.5 (c) and (d). Methyl (–CH$_3$) symmetric bending (one of the three bond angles is tightened (in the outward motion) while the other two are increased and vice versa in the return motion)

Fig. 2.5 (e) and (f). Methylene (–CH$_2$) symmetric bending (The two hydrogen atoms approach each other or recede from each other simultaneously)

2.6. Assignments of NIR bands and their interpretation to spectra.

NIR spectra need to be interpreted so as to be used for qualitative and quantitative analyses. The NIR spectral region is dominated by absorption related to X – H functional groups. These absorptions originate from the overtones of fundamental stretching bands or combination bands involving stretching and bending modes of vibrations of such groups. The main absorption bands of NIR spectra (bands of interest for chemical analysis in this study) are given in Table 2.1. A detailed description about the specific molecular bonds
most active in the NIR and their interpretation to the spectra can be obtained from literatures [Workman 1996; Hourant *et al.*2000].

**Table 2.1: Assignment of major NIR absorption bands.**

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Absorption band</th>
<th>Molecule</th>
<th>Vibrational mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>1090 – 1180</td>
<td>– CH₂ (methylene)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1100 – 1200</td>
<td>– CH₃ (methyl)</td>
<td>2ⁿᵈ overtone</td>
<td></td>
</tr>
<tr>
<td>1150 – 1260</td>
<td>– CH= CH⁻ (ethynl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1350 – 1430</td>
<td>– CH₂</td>
<td></td>
<td>Combination</td>
</tr>
<tr>
<td>1360 – 1420</td>
<td>– CH₃</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1390 – 1450</td>
<td>– OH</td>
<td>1ˢᵗ overtone</td>
<td></td>
</tr>
<tr>
<td></td>
<td>– CH₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1650 – 1850</td>
<td>– CH₃</td>
<td>1ˢᵗ overtone</td>
<td></td>
</tr>
<tr>
<td></td>
<td>– CH = CH⁻</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1880 – 1930</td>
<td>– OH</td>
<td></td>
<td>Combination</td>
</tr>
<tr>
<td>2010 – 2020</td>
<td>– CH = CH⁻</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2100 – 2200</td>
<td>– CH = CH⁻</td>
<td></td>
<td>Combination</td>
</tr>
<tr>
<td>2240 – 2360</td>
<td>– CH₃</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2290 – 2470</td>
<td>– CH₂</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The absorption bands of NIR contain both chemical and physical information of all the sample components. Therefore the knowledge of NIR absorption wavelength bands for each fundamental group is necessary to be able to locate the corresponding bands in the NIR region.


The type of molecular vibration determines the frequency at which NIR energy is absorbed. The intensity of absorption at any specific wavelength or wave number is determined by its absorptivity and the concentration of the molecules (number of molecules) encountered within the beam path of the spectrophotometer.

**Beer – Lambert’s law:**

The law states that the absorbance (A) of an analyte at a particular wavelength \( \lambda \) is proportional to

1. The absorptivity (\( \varepsilon \)) of a specific type of molecular vibration.
2. The concentration (c) of the molecules in the measurement beam and
3. The path length (l) of the sample holder with in the measurement beam

i.e. \( A = \varepsilon cl \);

When the NIR light of initial energy interacts with the sample, absorbance is given by

\[
A = -\log_{10}\left(\frac{I}{I_0}\right)
\]

where \( I \) and \( I_0 \) represent the attenuated energy detected (measured) after sample interaction and the initial energy incident to the sample respectively
For near infrared spectroscopy, several species (n) absorb light at the same wavelength (\( \lambda \)). The light absorption is said to be additive.

\[
A = \sum_{a=1}^{n} \varepsilon_a / C_a
\]

The product \( \varepsilon_a l = S_a \), the molar absorptivity. Absorption spectra of number of samples over a number of wavelengths can be arranged in a matrix \( X \) (I samples x J wavelengths) with one spectrum in each row:

\[
X = \sum_{a=1}^{n} C_a S_a^T = CS^T
\]

(The superscript \( T \) indicates ‘transpose’ of a matrix) Each column in the matrix \( C \) (I \( \times \) n) corresponds to a concentration profile. Each column in the matrix \( S \) (J \( \times \) n) corresponds to a spectral profile.

Modern spectrophotometers make use of the assumptions for spectroscopic measurement and generally display the NIR data as transmittance (T) or reflectance (R) or absorbance (\( A \)) along Y-axis and wavelengths along X-axis. (In our work, absorbance mode is preferred because of the direct proportionality between absorbance and concentration).

### 2.8 NIR equipment

The conventional instrumentation used for the visible or infrared regions can also be employed for the NIR region, depending on purpose or environment. Interference – filter spectroscopy, multichannel Fourier transform spectroscopy and acousto – optic tunable filter spectroscopy are particularly well – known as suitable for NIR spectroscopic measurements. The grating spectroscopy, which is most generally used for the regions ranging from ultraviolet to far infrared, is also used as a standard method for the near
infrared region. Unlike an interference filter, a grating can obtain the full range of the NIR spectrum with high resolution (0.1 nm to 1 nm). Considering that ease of operation is required for field use and in–process NIR measurement, grating spectroscopy with an acousto optical element and multichannel Fourier transform spectroscopy with an array detector would have future potential.

In the present study, the NIR absorption measurement of the samples is carried out using a Cary 5000 UV-VIS-NIR spectrophotometer with a wavelength range of 175 – 3300 nm and a resolution of 0.01nm. The main components are (i) 150 w deuterium lamp as a light source (ii) double sided grating monochromator (iii) a sample chamber (iv) a PbS or InGaAs smart technology detector. Fig. 2.6 depicts the features of the carry 5000 spectrophotometer.
Fig 2.6 Diagram showing the various components of Cary 5000 and their features.
The spectrometer measures the amount of optical absorption in material sample as a function of wavelength. The absorption spectrum in the double beam spectrometer is measured as the ratio of light intensity for the sample and the reference used [Operational manual of Cary – 5000 2010]. The optical absorption at a particular wavelength \( \lambda \) is measured in terms of optical absorbance \( A \) or optical density given by Beer – Lambert’s law.

The absorbance can be obtained as

\[
A(\lambda) = -\log \left( \frac{I(\lambda)}{I_0(\lambda)} \right) = \frac{\mu \lambda}{2.303}
\]

where \( I = \) the intensity of the light transmitted by the sample.

\( I_0 = \) intensity of the incident monochromatic light

\( \mu = \) absorption coefficient

\( t = \) thickness of the sample

\( \lambda = \) particular wavelength (nm) of light

### 2.9 Applications of NIR spectroscopy

Now a day near infrared spectroscopy, with the advancement of simpler and cheaper instrumentation, minimal or no sample preparation requirement, easy and fast data collection, and easy sample handling together with the possibility of avoiding the use of hazardous and expensive chemical solvents that are used in traditional chromatographic methods, is emerging as a potential tool for rapid and non destructive quantitative as well as qualitative analysis.

NIRS has been widely recognized as an analytical technique in different areas including agriculture [Batten 1998], food science [Osborne et al.1993], chemistry
Donahue et al. 1988], environmental science [Espinoza et al. 1999], pharmacy [Mac Donald & Prebble 1993], and medicine [Hall & Pollard 1989] etc.

Limitations of NIR spectra concerning complexity of spectra, influence by physical parameters such as temperature, moisture, particle size of samples, positioning of the sample etc can be overcome with the development of the information extraction tool − chemometrics and multivariate algorithms − and the advancement in computational softwares.