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Near Infra Red Spectroscopy

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1. Introduction

NIR stands for Near Infrared and refers to the region of light immediately adjacent to the visible range, falling between 750 and 3,000 nanometers (nm = nanometers or 1/1000000000 of a meter) in wavelength. Most organic materials have well defined reflectance or transmittance features at these wavelengths. According to the principles of quantum physics, molecules may only assume discrete energy levels. Similar to the vibrating string of a musical instrument, the vibration of a molecule has a fundamental frequency, or wavelength, as well as a series of overtones. For molecules, the fundamental vibrations involve no change in the center of gravity of the molecule. The spectrum shape for any material is the result of these characteristic fundamentals and overtones. Near-infrared spectra are primarily the result of overtones, whereas there are many fundamentals in the mid and far infrared regions. Since the molecular structure of most compounds is very complex, the resulting spectra are actually the result of many overlapping peaks and valleys. Generally speaking, persons performing NIR analysis must then identify and characterize specific features in the spectra by means of statistical methods. Chemometrics software is designed to accomplish this task.

The absorption of NIR radiation by organic molecules is due to overtone and combination bands primarily of O-H, C-H, N-H and C=O groups whose fundamental molecular stretching and bending absorb in the mid-IR region. These overtones are anharmonic, i.e., they do not behave in a simple fashion, making NIR spectra complex and not directly interpretable as in other spectral regions. Below is a graph depicting the prominent absorption bands as they relate to the overtone and combination bands of the fundamental vibrations occurring in the Mid IR region.

To understand the types of measurements possible using NIR light, it is useful to understand several general properties of electromagnetic waves, as well as basics of classical molecular and atomic structure. EM radiation, is in the form of waves, and as such, has all the properties of a wave; including wavelength. Figure 1 graph is a typical wave.

Wavelength is a distance between two points. Wavelength is particularly important to our discussion as it is closely connected to energy. Wavelength and energy are readily convertible from one to the other when speaking of EM waves. *See figure 2 below*

They are related in the following manner

$$E = \frac{hc}{\lambda} \qquad (1)$$

E = energy, *h* = *Planck's* constant (6.626 x 10-27), *c* = *speed* of light (2.998 x 1010dm/s), and l = wavelength.
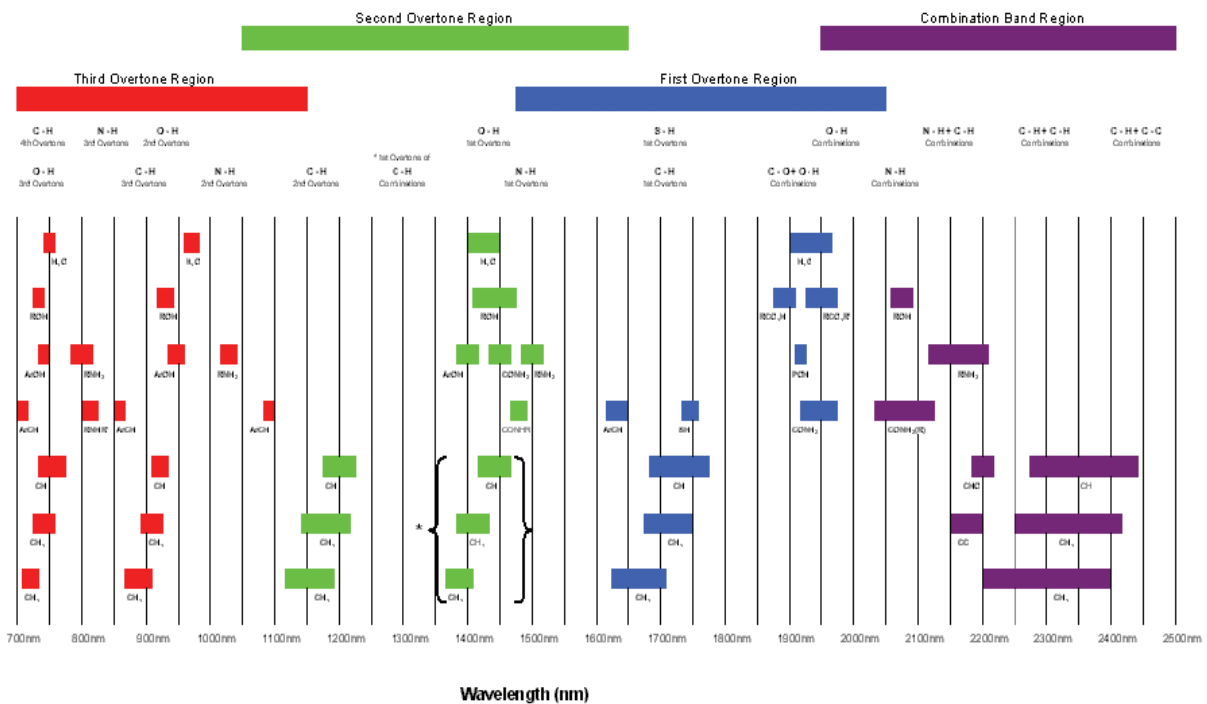

Fig. 1. Graph of near-infrared overtone absorptions, peaks and positions
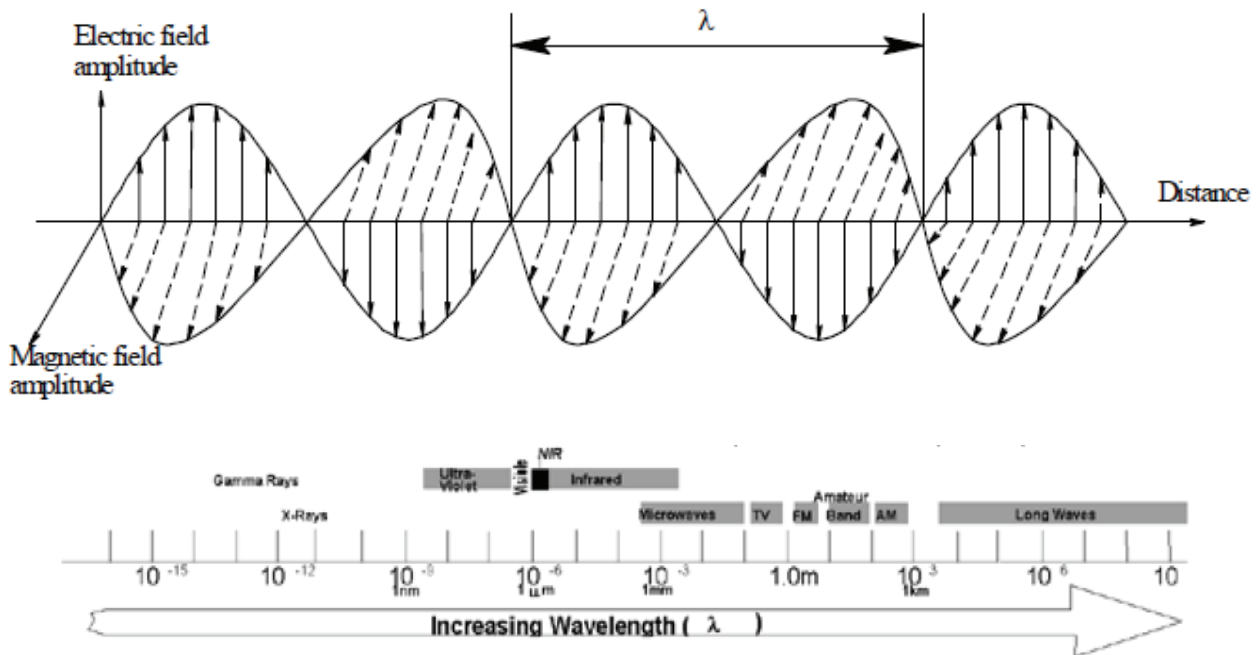

Fig. 2. Electromagnetic spectrum

It is the energy or wavelength that gives a wave its particular properties, and it is the amount of energy an EM wave carries (its wavelength) that determines whether or not a wave (radiation) is harmful. Waves with different wavelengths (energies) act differently. Wavelengths with certain energies will produce the effects associated with an x-ray to microwaves. The general properties of waves of certain energies allow us to classify them across the full EM spectrum. Another property of light is the manner in which energy is transferred from itself to whatever it may encounter. Light, as well as being a wave, consists of photons. Photons have properties of both waves and particles. For this discussion, we will think of photons as the "carriers" and "transferers" of energy. Now that we have discussed light and its properties, it is appropriate to talk about matter. Matter is defined as anything which has mass and takes up space. Matter (pen, paper, ink) is made up of atoms. Atoms are made up of smaller constituents known as neutrons, protons, and electrons. Protons are charged electrically positive, neutrons have no charge, and electrons are negatively charged. This means that protons and electrons are attracted to one another in a similar manner as are magnets of differing polarities. This also means that protons are repelled by other protons, and electrons are repelled by other electrons. These small particles can be arranged in many different ways. The simplest model is shown in *Figure 3*.
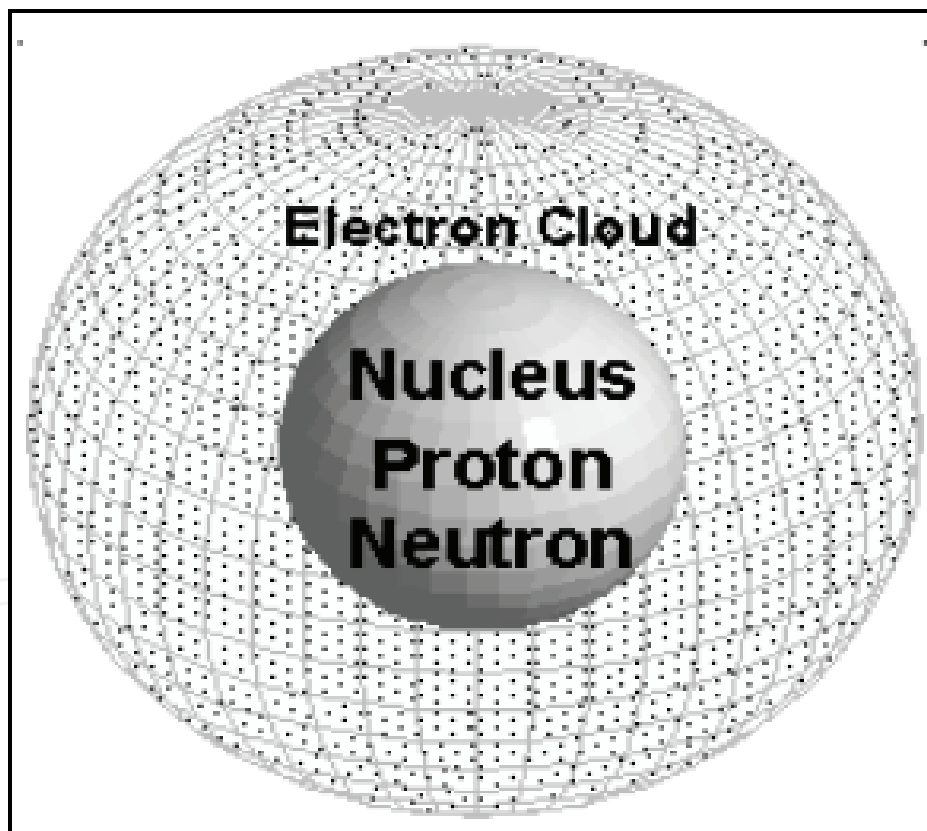

Fig. 3. Hydrogen atom

The center area, where the neutrons and protons are located, is referred to as the nucleus. Around the nucleus is the space in which the electrons reside and is knows as an orbital. Orbitals are distinct areas where an electron can exist. Orbitals also have distinct energies with which they are associated. Continuing addition of protons, neutrons, and electrons would produce atoms in the numeric sequence listed in the periodic chart of the elements.

Molecules are a group of atoms which have combined together to form a chemical compound. Molecules are simply substances made of several atoms of similar or different elements. Chemicals made of different types of atoms may have completely different properties than the properties exhibited by the individual atoms of which they are made. The interactions of protons and electrons help to hold the molecules together by producing bonds between the different atoms. Different arrangements of different numbers and kinds of atoms produce different properties and characteristics.

With NIR we will only deal with organic molecules (generally water, H_2O, is an exception). This will limit the types of molecules we will observe with NIR, since organic molecules are classified as molecules that contain carbon. Every living thing on earth is made up of thousands upon thousands of different organic molecules. Generally speaking, the interactions of EM waves with matter will simply involve the transfer of energy. The type of interaction we will observe and use is absorption of EM radiation by molecules. Actually, only a small portion of the molecule is involved in the absorption process—the electrons. As stated before, we know electrons exist in orbitals around the nuclei of atoms. Orbitals are also energy levels and if the electron is orbiting about, at a particular distance from the nucleus and with a particular speed, it will have a particular energy. Because of quantum mechanics, scientists now know that electrons can exist only in specified energy states; in other words, specific orbitals. Electrons cannot exist in between energy states (orbitals). This means electrons can only absorb discrete amounts (packages) of energy as the next orbital is a specific amount of energy away. *Figures 4a* and *4b* illustrate the process of light being absorbed by an electron.

The photon is absorbed by an electron causing the electron to jump up to a higher energy level. Electrons in differing original orbits will absorb different amounts of energy. Remember that energy and wavelength are closely related (see *Equation 1*) so if electrons absorb differing energies, this also translates into different wavelengths.

Molecules' atoms are built of electrons, protons, and neutrons in different configurations. Similarly, the electrons, protons, and neutrons in water have different characteristics than those in protein. This also means varying substances absorb different wavelengths of light. This type of absorption is considered an *electronic* absorption. The absorptions in the NIR are slightly more complicated though they still involve the absorption of energy (light) by electrons. Remember molecules consist of atoms bonded together. Bonds are produced by atoms sharing and/or giving up electrons to another atom. These bonds actually act similar to little springs (see *Figure 4c*). As an electron moves about the atom(s), the bonded atom is drawn or repulsed from the atom to which it is bonded, creating a vibrating motion. Whenever something moves consistently (vibrates) in time in this manner, it is said to have a frequency (*n*=frequency*)*. The frequency is the number of times the atom vibrates in a second. The absorptions occurring in the NIR region will therefore be considered *vibrational* absorptions. These possible absorptions are also quantum mechanical in nature; only discrete energy amounts can be absorbed. These levels can be roughly calculated using *Equation 2*

$$En = \left(n + \frac{1}{2} \right) \frac{h}{2\pi} \sqrt{\frac{k}{\mu}} \tag{2}$$

Where *En* = the molecule vibrational energy, *n* = (0,1,2,3 ...), *h* = Plank's constant, *k* = the force = the reduced mass.
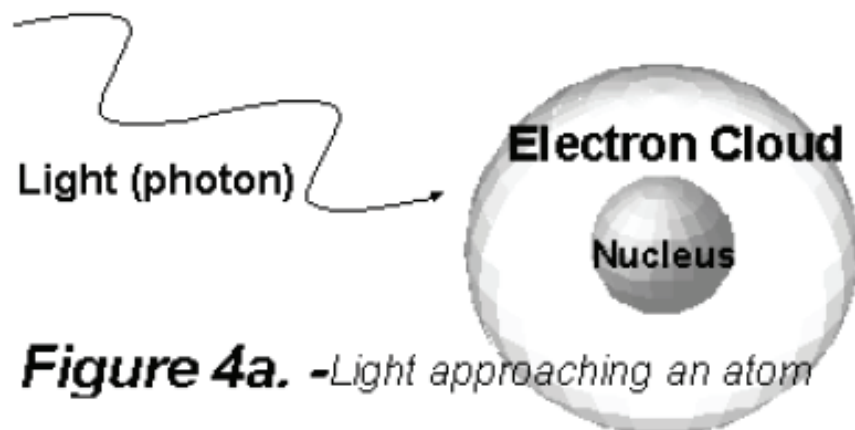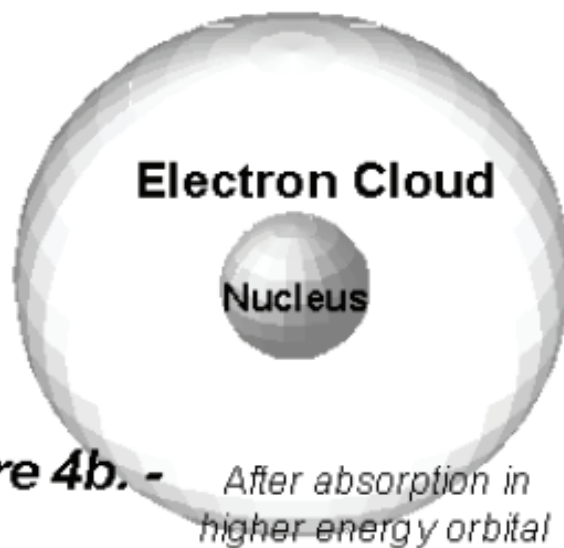
Figure 4a. -Light approaching an atom

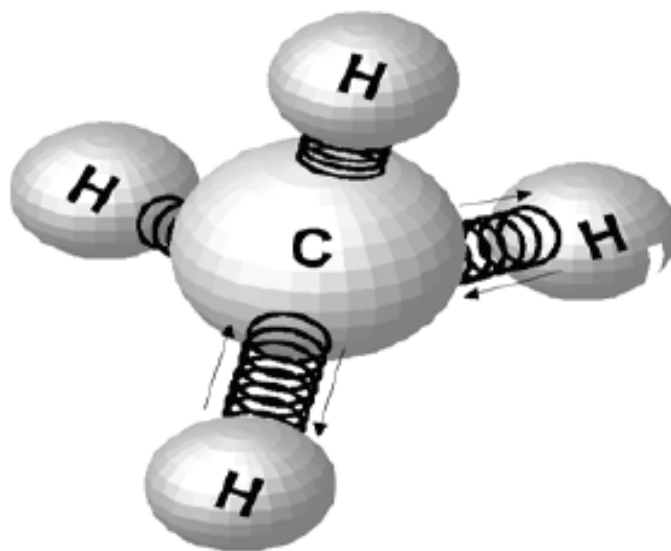Figure 4b. - After absorption in higher energy orbital

Fig. 4. (a) Light approaching an atom. (b) After absorption in higher energy orbital (c) Vibrating methan molecul

N is considered a quantum number and can be constant and take on only whole integer values. A transition where *n=1* is known as a fundamental absorption. These fundamental absorptions are about 100 times less energetic in the NIR region and less energetic means longer wavelength. When *n* is greater than 1, the transition is known as an overtone. By looking at *Equation 2*, it is evident that as *n* increases, the energy to be absorbed also increases. This in turn indicates that shorter wavelengths will need to be absorbed. These absorptions generally occur in the NIR region. *Equation 2* predicts fairly well the absorptions of two atoms bonded together (called diatomic molecules), but does not take into account all of the surrounding effects for polyatomic (many atom) molecules, such as overlapping absorption bands or hydrogen bonding. Organic molecules exist in energy states that absorb NIR wavelengths (energies). Metals, such as silver, lead, and most inorganics, cannot absorb NIR light because they have electrons incapable of absorbing NIR wavelengths, therefore there is no interaction to measure. Generally, only organic molecules can absorb wavelengths in the NIR region. It is actually the energy state of a molecule which allows us to perform a measurement with NIR.

Now imagine a sample made up of many, many electrons, protons and neutrons. These particles are arranged into atoms, and further into molecules. The sample can be made of different types of molecules, meaning there can be water molecules, protein molecules and so on. When they take on these arrangements, they also take on different properties such as the ability to absorb different wavelengths of light, therefore, quite a few different energies might be absorbed. When a measurement is performed on this sample, what the instrument is measuring is the number of photons which undergo the absorption process for a particular wavelength. The number of photons absorbed is proportional to the amount of particular type of molecule present in the sample. This statement is more or less *Beer's Law* which states that "absorption is proportional to concentration." In principle, that is what is occurring and is the basis for an NIR measurement.

Bouguer-Lambert-Beer Law (BLB Law, 'Beer's Law)[1]

$$\log(1 / Transmittance_\lambda) = \alpha_\lambda lc$$

where α_λ is the molar absorption coefficient, *l* is the path length, and *c* is the analyte concentration. This equation is called the BLB Law and the quantity $\log(1 / Transmittance_\lambda)$ is called 'absorbance'. Absorbance is a unitless quantity, however, the term absorbance units (AU) is often used to indicate this type of measurement. BLB is valid only for transmittance measurements and much has been written on the mathematics and physics of this law. There is no rigorous derivation of a similar law that relates reflectance to analyte concentration (see *Log(1/Reflectance)*). Absorbance cannot be measured directly since there is no way to directly count the number of photons as they disappear one-by-one. Therefore, what is being measured is actually transmittance.

1.1 Chemometric models[2]

The single step in NIR analysis requiring the most planning preparation is the assembly of the samples, often called the training set to be used for the development of calibrations. A crucial step in achieving success is ensuring the samples have been analyzed as accurately and precisely as conventional techniques allow. These analyses are termed reference analyses. In order for any NIR analyzer to make quantitative measurements or qualitative discriminations, the controlling computer must have access to one or more chemometrics

models which represent the type of material being tested. The model is a mathematical construct developed using samples of the same product or class of products. The controlling computer applies the model(s) to the target spectrum and returns a model result. A chemometrics model is developed by collecting spectral readings from a group of samples that display (a) the maximum variability of the characteristic of interest, and (b) non-correlating or random variability in all other characteristics. The same samples are submitted for independent testing to measure the characteristic of interest by a standard analytical method. The spectral data and independent test data are then analyzed using commercially available chemometrics software. The statistical processes used in quantitative spectral analysis include multiple linear regression, classical least squares, inverse least squares, and principal component regression. The statistical processes used in qualitative spectral analysis include K-nearest neighbors, SIMCA and others.

When a sufficient number of samples have been collected and properly analyzed, a mathematical model is constructed that describes the relationship between specific spectral features and the sample characteristic of interest. Thereafter, a chemist or technician may quickly measure that same characteristic in a new target sample by applying the chemometrics model to the spectrum of the target sample. Essentially a calibration is interpreting the information coming from the instrument. If the instrument is taught (calibrated) properly, it will predict the correct amount of parameter in our sample. Once calibrations are obtained, they are entered into the NIR spectrophotometer. Following the scanning of unknowns, requiring a few seconds per sample, numerous constituents or parameters of interest are simultaneously predicted. In this mode, NIR is a rapid, cost-effective, non-destructive, accurate and efficient analytical method.

1.2 Advantages

The biggest advantage of NIR over Mid-IR and Far-IR is little or no sample preparation, and near real-time analysis. Unlike most conventional analytical methods, NIRS is rapid, non-destructive, does not use chemicals, or generate chemical wastes requiring disposal, simultaneously determines numerous constituents or parameters, and can be transported to nearly any environment, or true portable for field work. NIR instrumentation is simple to operate by non-chemists, and operates without fume hoods, drains, or other installations. NIR is not a stand-alone technology. Its accuracy is dependent upon the accuracy of the reference method used for training, however, the data from the NIR method has better reproducibility than the primary method.

Another advantage of NIR over Mid-IR and Far-IR is 'thermal' noise. All internal electronic components are a source of thermal noise in the Mid-IR and Far-IR. However, internal sources of IR are either insignificant to NIR detectors or can be made insignificant by minor shielding.

With NIR analysis most of the useful features in a spectrum consist of overtones, or combinations of overtones, which are more subtle than the fundamentals found in Mid-IR and Far-IR spectra. However, recent developments in off-the-shelf chemometrics software and powerful PC's have made NIR analysis the practical choice for most applications. Because the absorbances in the NIR region are lower than in neighboring regions and generally obey the Beer/Lambert Law, i.e., absorbance increases linearly with concentration, it is possible to analyze bulk samples without the need for dilution or other elaborate sample preparation. Thus, the results provided by NIR are typically more representative than that provided by other analytical means.

1.3 Disadvantage

NIR is not a stand-alone technology. Separate calibrations are required for each constituent or parameter and a portion of unknown samples must periodically be analyzed by the reference method to ensure that calibrations remain reliable. It may be necessary to update calibrations several times during the initial phases of use to incorporate "outlying" samples, until the calibration is acceptable. Despite the intuitive disadvantage of broad and overlapping absorption bands, sophisticated chemometric techniques can extract meaningful information from the complex NIR spectra.

2. Making an NIR analyzer work for you

2.1 Abstract [3]

In recent years, NIR analysis has steadily grown in popularity because of its ability to quickly provide qualitative and quantitative information on many products, especially raw materials. To determine if NIR spectroscopy is a reasonable alternative to more traditional methods, many factors must be considered. These factors include sample characteristics, experiment configuration, and data analysis.

2.2 Sample consideration

The chemical constituents and physical phenomena of interest should have direct or indirect absorbance in the NIR region. Virtually all organic compounds do, particularly those with functional groups like hydroxyl, carboxyl, amine and carbon-hydrogen. A good reference for researching near infrared spectra is *The Atlas of Near Infrared Spectra*, Bio-Rad Sadtler Division, Philadelphia, Pennsylvania. For calibration samples, the amount of analyte in the sample set should be above the detection limit and have sufficient variability. Some analytes, e.g. water, are detectable at the ppm level. For most analytes, the nominal detection limit is 1% or above. The analytical chemist must have an accurate independent method for measurement of the properties and must know the level of error in the reference methods. Errors in NIR prediction most often arise from errors in the reference methods, instability of the NIR spectrometer, and/or inappropriate choice of the calibration model method. The samples used in the development of calibration sets must be representative. All the variations in the future unknown samples should be covered in the "training" calibration sets—for example, sample composition and particle size, homogeneity, and temperature variation at the working environment. As a rule of thumb, the more samples you have for the training set, the more reliable the calibration model.

2.3 Experiment configuration

When using an NIR analyzer, instrument characteristics such as sensitivity, resolution, and signal-to-noise ratio parameters need to be evaluated. The quality of these values is a function of the light source stability, optics throughput, dispersion/filter element accuracy, and detector sensitivity in the instrument. The choice of accessories is application dependent. For liquid samples, transmission and transflectance modes are commonly employed using fiber optic probes or cuvettes. The optimum path length is sample.
dependent, usually ranging from 0.1 to 1 cm. The advantage of using a fiber-optic probe is that sample preparation is significantly reduced, and noninvasive or nondestructive

measurements are possible. For solid samples, diffuse-reflectance spectra collected by a reflectance probe will provide information for analytes. Diffuse reflectance should be measured without interference from specular reflectance. The setup configuration, such as the angle of incident light and the distance of light illumination/collection ought to be consistent throughout all the measurements, including those taken in developing the calibration set and for predicting the future unknowns. For solid samples, the sample should be rotated and measurements done on different spots of the sample to average out surface effects and sampling error. A group of spectra may be averaged to increase the signal-to-noise ratio. Random noise is reduced by the factor square root of the number of spectra averaged. For ASD's NIR spectrometer, it takes 0.1 seconds to acquire one spectrum. Therefore, a 10 second measurement reduces the random noise by a factor of 10.

2.4 Data analysis

NIR spectroscopy is an extremely rapid method of measurement, capable of performing an analysis in under a minute. The time-consuming part of NIR work is the data analysis phase, where chemists try to find the correlation between near-infrared spectral characteristics and the property, or properties, of interest as measured by more traditional methods. There are several commercially available software packages for accomplishing this task. Data analysis involves the following steps.

Data preprocessing

When the spectral data plots are presented, first determine if there is any baseline drift or slope in the spectra, which often occurs in diffuse-reflectance measurements. If necessary, baseline subtraction, first derivative and second derivative transformations may be performed to reduce these effects. There is a trade-off though as each successive degree of derivative taken introduces additional noise into the spectral data.

Outlier detection

An outlier is a data point that falls well outside the main population. Outliers result from lab measurement errors, samples from different categories, and instrument error. It is important to check for, and remove, outliers in both the training set and the set of unknowns on which calibration testing will occur (see "validation" and "prediction").

Building a good calibration model

This is one of the most important steps in NIR analysis. Developing a calibration model involves calculating the regression equation based on the NIR spectra and the known analyte information. The model is then used to predict the future unknowns. Multiple Linear Regression (MLR), Principal Component Regression (PCR) and Partial Least Squares (PLS) are commonly used linear calibration methods, along with Locally Weighted Regression (LWR) for nonlinear models. In developing a calibration model, several parameters are evaluated: factors, loadings, and scores. When choosing the number of factors, one should try to avoid under-fitting, i.e. too few factors, and over-fitting, i.e. too many factors. If an insufficient number of factors are chosen, the prediction is not reliable because useful information has been omitted. If too many factors are chosen, however, more uncertainty is included in the calibration set which results in errors in prediction. Scores are used to check the sample homogeneity and possible clusters, while loadings are used to interpret how the variables are weighted in principal component space.

Approximate [4] Wavelengths of Some Common Functional Groups Functional Group	Wave-length (nm)	Functional Group	Wave-length (nm)
C-H second overtone	1143	O-H stretch/C-O stretch second overtone combination	1820
C=O stretch fourth overtone	1160	C-Cl stretch sixth overtone	1860
C-H second overtone	1170	C=O stretch second overtone	1900
C-H second overtone	1195	O-H stretch first overtone	1908
C-H second overtone	1215	C=O stretch second overtone	1920
C-H second overtone	1225	O-H stretch/HOH deformation combination	1930
C-H combination	1360	O-H bend second overtone	1940
C-H combination	1395	C=O stretch second overtone	1950
O-H first overtone	1410	O-H stretch/O-H bend combination	1960
C-H combination	1415	Asym N-H stretch/N-H in-plane bend; C-N stretch combination	1980
C-H combination	1417	N-H stretch/N-H bend combination	1990
O-H first overtone	1420	C=O stretch second overtone	2030
C-H combination	1440	N-H/N-H in-plane bend; C-N stretch or N-H/C-N stretch; N-H in-plane bend or combination	2050
C-H combination	1446	Sym N-H stretch/C=O stretch combination	2060
O-H stretch first overtone	1450	N-H bend second overtone or N-H bend/N-H stretch combination	2060
C=O stretch third overtone	1450	N-H deformation overtone	2070
Sym N-H stretch first overtone	1460	O-H combination	2070
N-H stretch first overtone	1471	C-H combination	2090
N-H stretch first overtone	1483	O-H bend/C-O stretch combination	2100
N-H stretch first overtone	1490	Asym C-O-O stretch third overtone	2100
O-H stretch first overtone	1490	C-H stretch/C=O stretch combination or sym C-H deformation	2140
Sym N-H stretch first overtone	1490	Asym C-H stretch/C-H deformation combination	2170
N-H stretch first overtone	1492	N-H bend second overtone or C-H stretch/C=O stretch combination, or C=O stretch C-N stretch; N-H in-plane bend. Combination	2180
N-H stretch first overtone	1500	C-H stretch/C=O stretch combination	2200
N-H stretch first overtone	1510	O-H stretch/C-O stretch combination	2270
N-H stretch first overtone	1520	C-H stretch/CH2 deformation	2280
N-H stretch first overtone	1530	C-H bend second overtone	2300
O-H stretch first overtone	1540	C-H bend second overtone	2310
N-H stretch first overtone	1570	C-H stretch/CH2 deformation combination	2322
C-H stretch first overtone	1620	C-H stretch/CH2 deformation combination	2330
C-H stretch first overtone	1685	C-H stretch/C-H deformation	2335
C-H stretch first overtone	1695	CH2 bend second overtone	2352
C-H stretch first overtone	1705	C-H stretch/C-C stretch combination	2380
C-H stretch first overtone	1725	C-H combination	2470
S-H stretch first overtone	1740	Sym C-N-C stretch overtone	2470

Table 1.

Validation

The validity of the model must be tested. This is usually done by splitting the sample set into two sets; one set for calibration and the other for validation. If there are not enough samples, "leave-one-out" cross validation can be performed. This means leaving one sample out, using the rest of the samples to build a calibration model and then using the model to predict the one left out. The advantage of doing cross validation is that unlike calibration with a full data set, the sample being predicted is not included in the calibration model. Thus, the model can be tested independently.

2.5 Prediction

Finally, the calibration can be used to predict future unknowns, assuming the unknowns are in the same sample population as those used in the calibration set. Whether the unknown is an outlier needs to be tested.

2.6 Summary

Applying an NIR analyzer to a particular application requires the development of a reliable calibration model. The most important steps involve a thorough consideration of experimental design and multivariate calibration. Once this is established, one can enjoy the advantages of the NIR analysis. The speed of the analysis will save time and avoid mistakes instantaneously. The speed advantage is so valuable to engineers involved with on-line process monitoring that instruments are routinely installed in or near the process line with feedback loops. With an NIR analyzer such as QualitySpec® Pro spectrometer, samples can be non-invasively analyzed on-the-spot, dramatically reducing costly and time consuming laboratory analysis as well as preventing unnecessary product waste and/or downtime. The low absorptivity in the NIR region allows measurements to be taken on raw materials, in process and finshed product without elaborate sample preparation. In the food, agricultural, pharmaceutical, polymer, cosmetics, environmental, textile, and medical fields, NIR analysis serves a wide range of applications, with still many unknown applications waiting to be discovered. With the maturity of this technique, more and more people will use NIR analysis for convenience and flexibility.

2.7 Wavenumber and wavelength

$$Y = 10^7 / X$$

where
Y = the number of nanometers (nm)
X = the number of wavenumbers (cm^{-1})

$$Y = 10^7 / 28571 = 350$$

Resolution in cm^{-1}, ($R_{cm^{-1}}$) is dependent upon wavelength position.
So, Resolution in nanometers, (R_{nm}) is calculated as follows:

$$
\begin{aligned}
R_{nm} &= +/- [Y - Y'] \\
&= +/- \{[10^7 / X] - [10^7 / X']\} \\
&= +/- 10^7 * \{[1 / X] - [1 / X']\} \\
&= +/- 10^7 * \{[1 / X] - [1 / (X - R_{cm^{-1}})]\}
\end{aligned}
$$

3. References

[1] Burns D. A. and E. W. Ciurczak (Eds.), Handbook of Near-Infrared Analysis, (Volume 13 in Practical Spectroscopy Series), Marcel Dekker, Inc., New York, 1992

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[4] Burns D. A. and E. W. Ciurczak (Eds.), Handbook of Near-Infrared Analysis, (Volume 13 in Practical Spectroscopy Series), Marcel Dekker, Inc., New York, 1992, pgs 393-395.

Wide Spectra of Quality Control

Edited by Dr. Isin Akyar

Quality control is a standard which certainly has become a style of living. With the improvement of technology every day, we meet new and complicated devices and methods in different fields. Quality control explains the directed use of testing to measure the achievement of a specific standard. It is the process, procedures and authority used to accept or reject all components, drug product containers, closures, in-process materials, packaging material, labeling and drug products, and the authority to review production records to assure that no errors have occurred.The quality which is supposed to be achieved is not a concept which can be controlled by easy, numerical or other means, but it is the control over the intrinsic quality of a test facility and its studies. The aim of this book is to share useful and practical knowledge about quality control in several fields with the people who want to improve their knowledge.

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