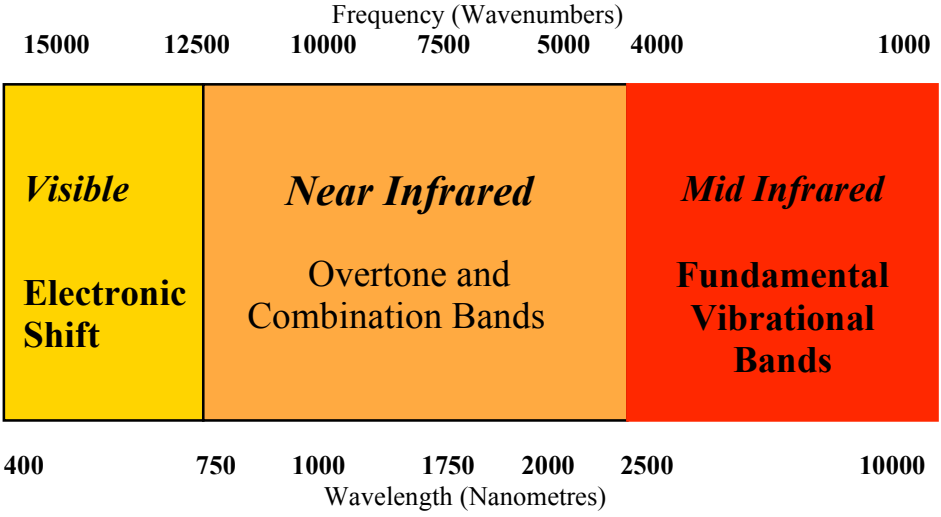
Near Infrared Transmission Spectroscopy in the Food Industry

Introduction:

Near Infrared Spectroscopy is used in many industries including the pharmaceutical, petrochemical, agriculture, cosmetics, chemical and food industries. However in the food industry NIR has an almost universal application. Since food is made mostly from proteins, carbohydrates, fats and water, i.e. >99% by weight, NIR provides a means of measuring these components in almost any food.

NIR Analysis: Simple theory.

Figure 1. shows part of the electromagnetic radiation spectrum from 300nm to 10000nm. It shows the Visible, NIR and Mid IR spectral regions. When light energy interacts with a material, energy is absorbed at resonant frequencies associated with the atomic and molecular interactions of the material. In the Visible region, energy is absorbed when electrons jump from a lower to a higher energy state or orbit. In this region, chromaphores such as metal chelates, absorb visible light. Likewise Atomic Absorption Spectroscopy also uses this phenomenon to measure metals species as they burn in a flame.

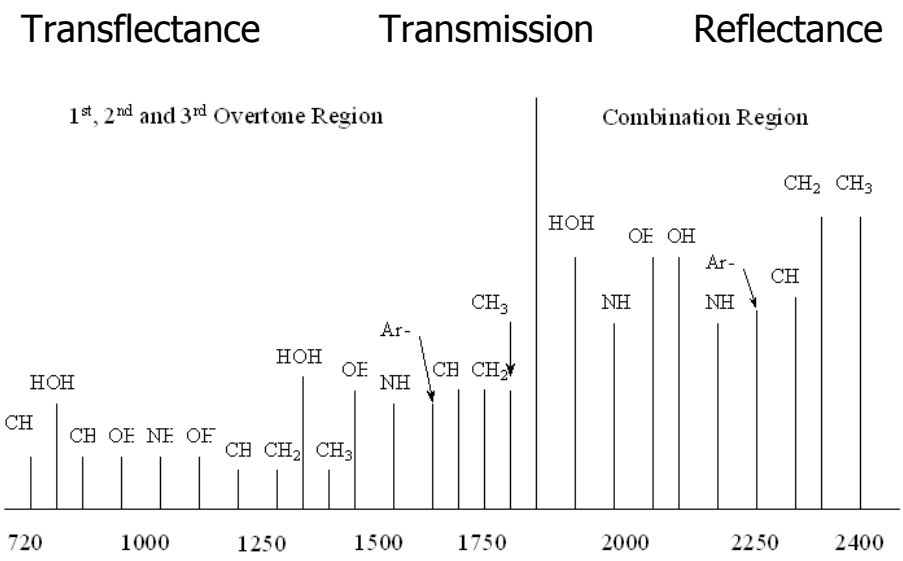


In the Mid Infrared region, i.e. 2500 to 10000nm, energy is absorbed by vibrating molecules at resonant frequencies for each type of vibration, eg, stretching, bending, wagging, twisting etc. In the Mid IR region, all chemical bonds in organic molecules exhibit strong absorption bands. Typically the Mid IR region is suitable for characterizing C-C, C-O, C-N, C-H, O-H, N-H and many other chemical bonds. Sample preparation such as dissolving in a solvent, preparing a nujol mull, drawing film etc, is required in order to get sufficient light through a sample. Mid IR spectroscopy is generally used for non-water bearing materials such petrochemicals, plastics, polymers and chemicals. Mid IR spectroscopy is used mainly for qualitative analysis rather than quantitative analysis.

The NIR spectral region, i.e., 720 to 2500nm, is the Overtone and Combination region of the Mid IR region. NIR spectra contain absorbance bands mainly due to three chemical bonds, i.e., C-H (fats, oil, hydrocarbons), O-H (water, alcohol) and N-H (protein). Other chemical bonds may exhibit overtone bands in the NIR region, however they are generally too weak to be considered for use in analysis of complex mixtures such as foods, agricultural product, pharmaceuticals, toiletries, cosmetics, textiles etc. NIR spectra do not have the resolution of the Mid IR spectra however NIR spectra can generally be collected off or through materials without sample preparation as well as it is suitable for measuring high and low water content materials. Whereas Mid IR is mainly a qualitative technique, NIR is mainly a quantitative technique. NIR provides a very rapid means of measuring multiple components in foods, agricultural products, pharmaceuticals, cosmetics, toiletries, textiles and virtually any organic material or compound.

Three NIR Regions

Figure 2. shows the NIR region from 720 to 2500nm. There are three parts of the NIR spectral region, 1) Reflectance, 2) Transmission and 3) Transflectance.



1) Transflectance: 720 to 1100nm. This section is most suited to transflectance through a thick sample, such as, seeds, slurries, liquids and pastes. The absorption bands are due to 3rd overtones of the fundamental stretch bonds in the Mid IR region.
2) Transmission: 1200 to 1850nm. This section can be used for transmission through liquids and films, as well as diffuse reflectance measurements off samples with high water contents. The absorption bands are due to the 1st and 2nd overtones of the fundamental stretch bonds in the Mid IR region
3) Reflectance: 1850 to 2500nm. This section is predominantly used for making diffuse reflectance measurements off ground or solid materials. The absorption bands are due to combination bands, i.e., C-H stretch and bend combination bands.

The Transflectance region is of particular interest in the analysis of foods because it is suitable for measuring high moisture and high fat content products including meat, dairy products, jams and conserves, dough and batters. The major advantage of working in this region is that longer pathlength sample cells can be used to collect the NIT spectra. Typically a 10-20 mm pathlength can be used. This makes sampling easier and allows viscous and non-homogeneous samples to be scanned without further sample processing.

A major advantage of measuring in Transflectance as compared with Reflectance is that the spectra represent the variation in components throughout the entire sample, not just the surface. In reflectance, the first 1mm contributes as much as 99% of the spectrum. As such uneven distribution of components in the sample, egg, drying at the surface, or separation of a water or oil layer at a glass window, results in reflectance spectra that do not represent the entire sample.

Spectral Collection Modes

Transflectance:
The Transflectance region was first suggested by Norris as a means of measuring whole cereal grains and oil seeds because the NIR light could penetrate through the grains and oil seeds. Actually the Transflectance mechanism is a combination of reflectance and transmission, as the NIR light reflects off the surface of the seeds as it transmits through the sample to the other side.

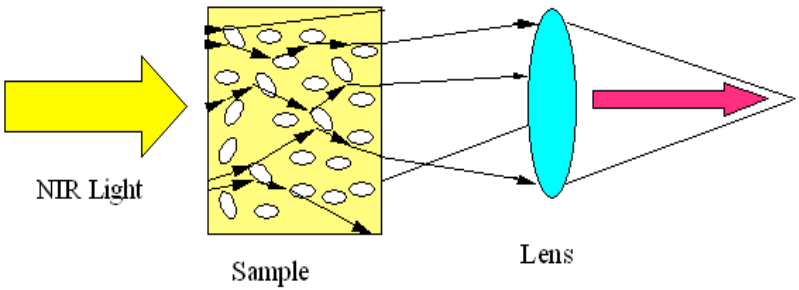


Figure 4. Schematic of Transflectance Optics

Transmission:

Transmission spectroscopy is the most common form of spectroscopy. UV-Visible, Mid IR and Atomic Absorption are major analytical techniques used in the analysis of water, metals, biological and organic materials. The basic principle of transmission spectroscopy is that light passes through a clear or transparent sample and energy is absorbed by the chemical components components. The light is not deflected as it passes through the sample. In the NIR transmission region, 1200 to 1850nm, the same principles apply.

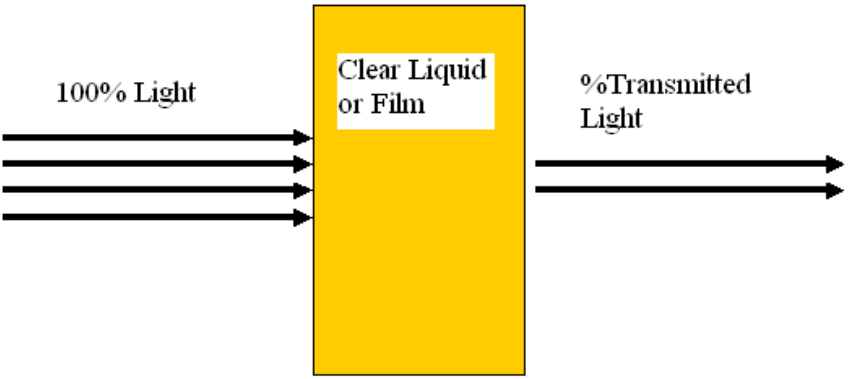


Figure 5. Schematic of Transmission Optics

Reflectance:

In Reflectance spectroscopy, light illuminates sample at 0 degree angle.

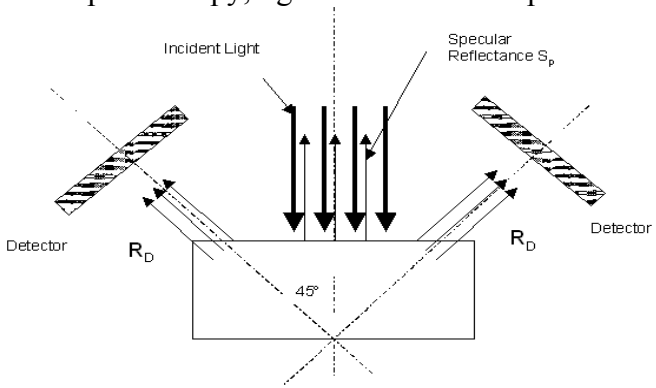


Figure 6. Schematic of Reflectance Optics

Light interacts with material and re-radiates Diffuse Reflected energy back into the plane of illumination. The re-radiated light is detected at 45 degree angle, in order to reduce Specular Reflectance.

Applications of NIT Spectroscopy for Food Products

The following are examples of NIT analyses of food products using the Transflectance region;

Raw and Processed Meats:
NIT spectra of raw and processed meat can be collected using a 10 mm Squeeze Cell as shown in Figure 7. Figure 8. shows the NIT spectra of minced beef, minced ham, pork sausage, salami and bologna. The differences in density of these samples are shown in the significant baseline variances. By converting the spectra with a Multiplicative Scatter Correction (MSC) algorithm, these differences are reduced. Figure 9. shows the MSC converted spectra of these meat samples.

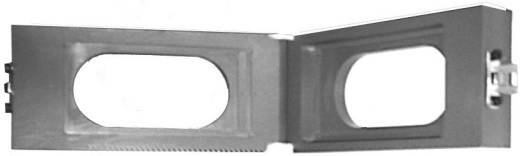


Figure 7. Squeeze Cell used for analyzing meat, cheese and dough.

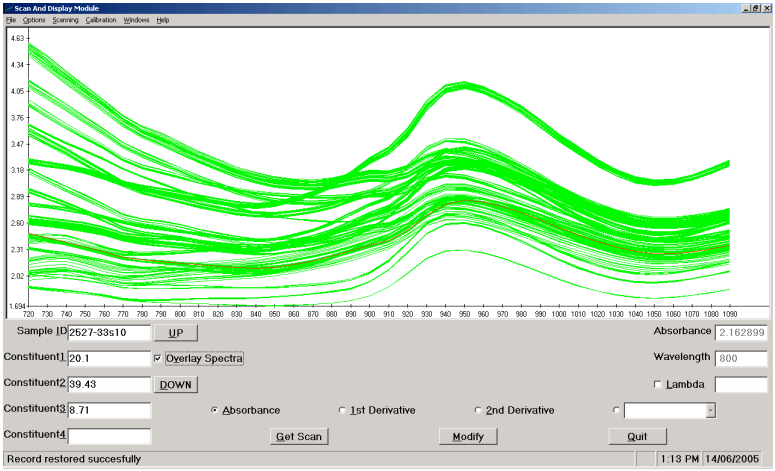


Figure 8. NIT Spectra of various meats, eg, turkey, beef, chicken, pork and sausage mix.

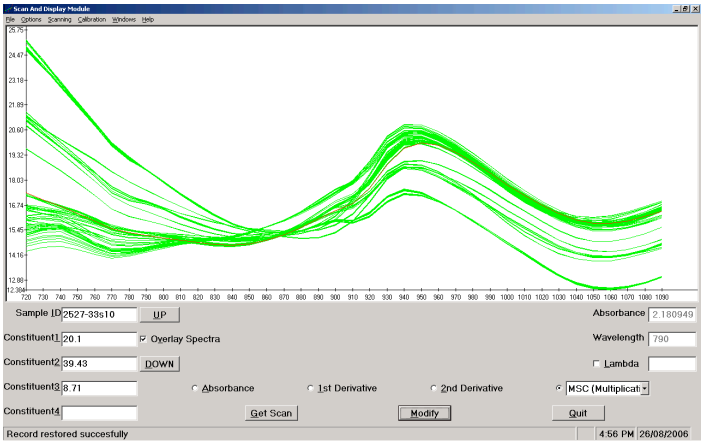


Figure 9. Multiplicative Scatter Corrected Spectra of the meat samples

As an example of quantitative analysis of meat products, figure 10. shows the plot of NIR Fat vs Soxhlet Extracted Fat. A calibration for fat was developed using 79 samples and a further 21 samples were analysed in duplicate to define the accuracy and precision. The accuracy or Standard Error of Prediction (SEP) was calculated as 0.45% fat and the precision or Standard Deviation of Difference (SDD) was calculated as 0.19%. Calibrations for protein, moisture and salt have also been developed for processed meat samples.

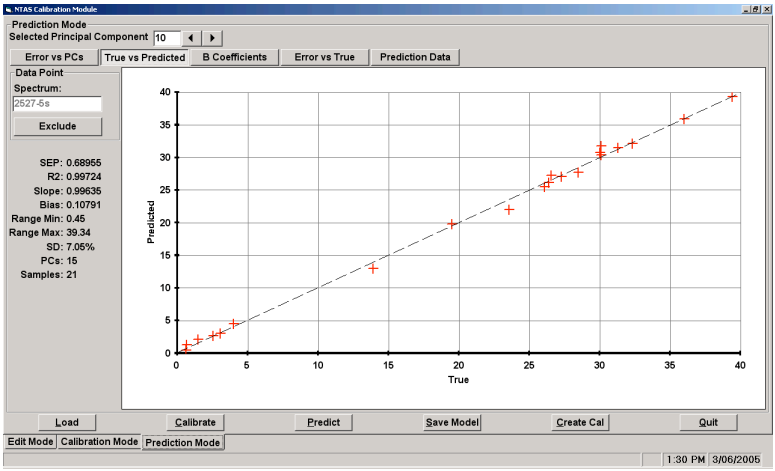


Figure 10. Plot of NIR Fat vs Soxhlet Extracted Fat calibration data.

Dairy Products:

Figure 11. shows the NIT spectra of milk, cheddar cheese, yogurt, milk powder, cream cheese and butter. Except for milk and milk powder, these spectra were also collected using a 10mm pathlength Squeeze Cell. The milk was scanned in a 10mm pathlength glass cuvette and the milk powder was scanned in a 5mm pathlength Powder Cell.

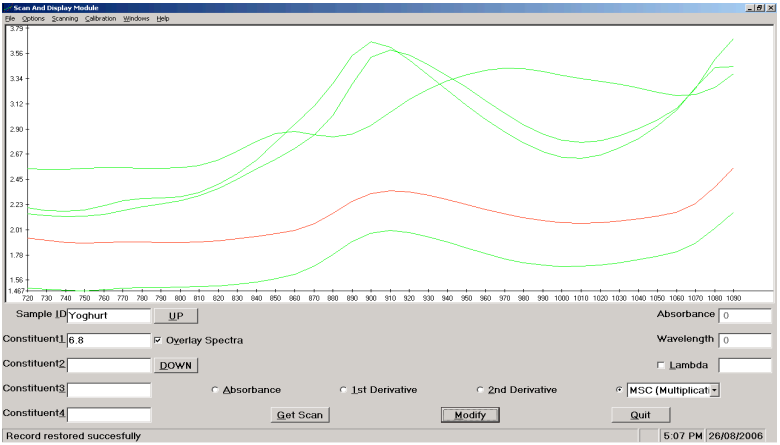


Figure 11. NIT Spectra of Milk, Cheese, Milk Powder, Yogurt, Cream Cheese

As an example of a quantitative analysis of dairy products, figure 12, shows the plots of the analysis of moisture in sliced cheese samples. The SEP's for moisture = 0.55%.

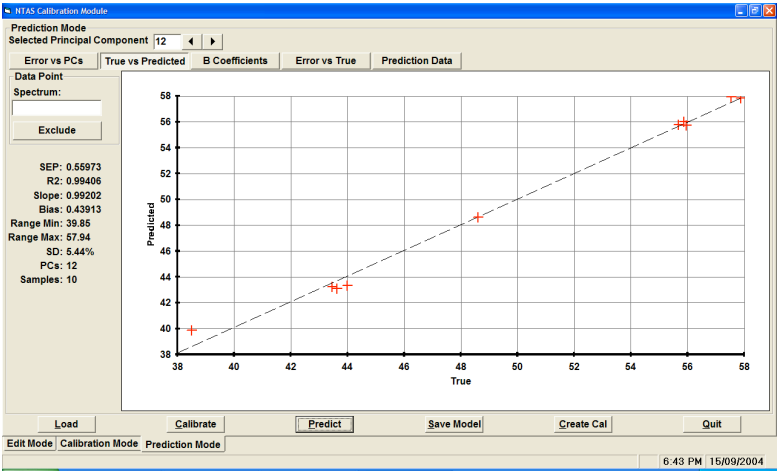


Figure 12. Plot of NIT Fat vs Reference Fat

Baked Goods:

Figure 13. shows the NIT spectra of cookie dough scanned in a 20mm pathlength Squeeze Cell. Fat, moisture, protein and sugar can be measured in dough, however the ability to rapidly measure fat and moisture in the mixing room, means that the dough will spread correctly when the cookies are laid on the hot oven band. If the fat content is too high then the cookies will spread too much, resulting in out of spec product. The accuracy of the fat and moisture measurements has been shown to be 0.13 and 0.28% respectively.

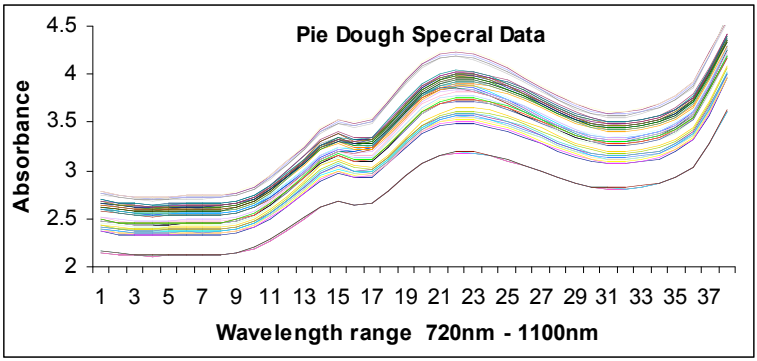


Figure 13. NIT Spectra of Pie Dough

In the baked good industries, NIT can also be used for measuring the incoming flour for protein, moisture and ash, as well as dough stability, dough development and water absorption. Other raw materials used in baking that can be analysed using NIT, include, liquid and powdered eggs, milk and milk powder, shortening, liquid and crystalline sugars and syrups.

A very unique application for NIT spectroscopy is the analysis of whole cookies. Manufacturers can measure the protein, moisture, sugar and fat content of whole cookies. The above spectra were collected by placing a whole cookie into a sample cell and lowering into a light beam. The NIR light passed through the cookies, thus giving a measurement representing the outside and inside of the cookie. Figure 14. shows the NIT spectra through several cookie types.

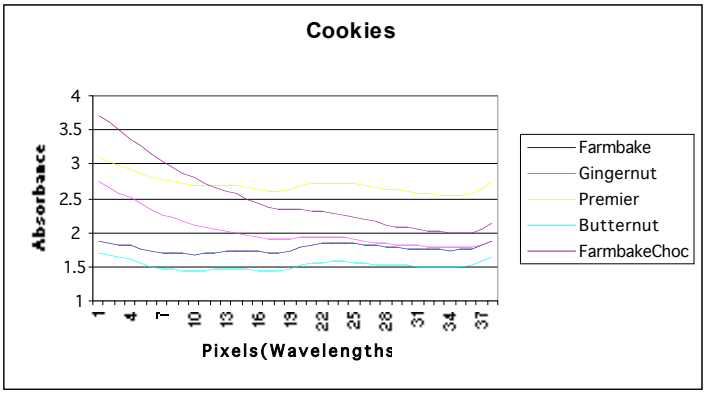


Figure 14. NIT Spectra of Whole Cookies

Confectionery:

Chocolate is the world's most popular confectionery. Figure 15 shows the NIT spectra of a milk chocolate and a dark chocolate using a 5mm pathlength Squeeze Cell. The chocolate samples were softened but not melted. The softened block was compressed between two glass windows and scanned.

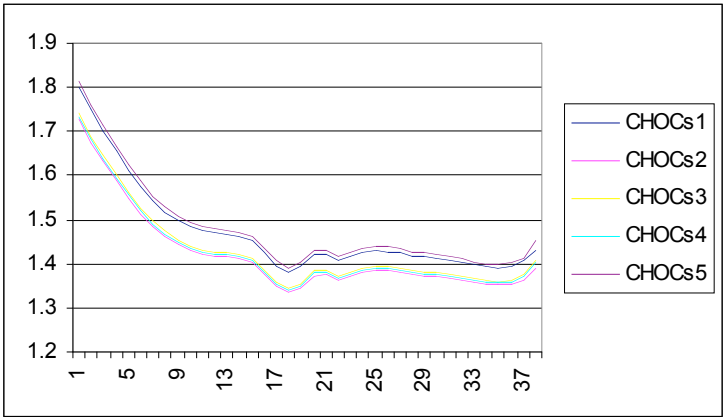


Figure 15 NIT Spectra of Milk and Dark Chocolates

Reflectance spectra of chocolate suffer from the problem of drying and uneven distribution of the fat between the surface and the rest of the sample. Transflectance provides a quick method of measuring fat and moisture throughout the entire chocolate sample.

Figure 16. shows the NIT spectra of several common types of lollies and candies. Sweets such as jubes or jellies, are made of gelatin, i.e., protein, water and sugar. Since these sweets are easily deformed, they can be measured in a 10mm pathlength Squeeze Cell. Nougats and fondants used in many candy bars are a mixture of fat, sugar and emulsifiers. NIT spectra are easily collected and as such provide manufacturers a rapid means of measuring the consistency of the material before it is used in the final bar.

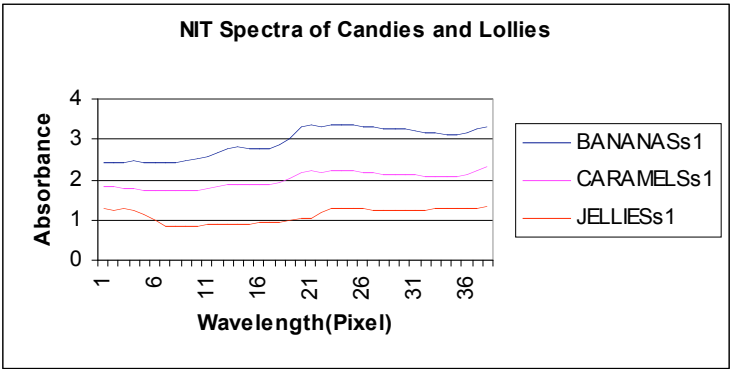


Figure 16. NIT Spectra of Lollies or Candies.

Grains and Oil Seeds:

Figure 17 shows the NIT spectra of wheat, barley, soybeans, corn (maize) and canola seeds.

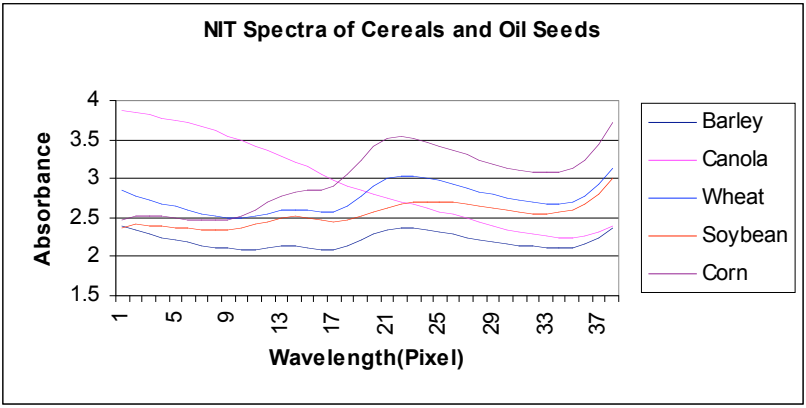


Figure 17. NIT Spectra of Cereal Grains and Oil Seeds

It is difficult to measure whole seeds in the Transmission and Reflectance regions because of the amount of light absorbed by the sample. However in the Transflectance region, the NIT spectra can be collected using an elongated sample, cell with pathlengths from 8 to 28mm, depending on the size of the seeds or grains. This makes sampling very easy and lends itself to on line measurements.

Beverages:
Wine, Beer, Spirits and even alcoholic mixer drinks, can be analysed using NIT for alcohol. Figure 18 shows the NIT spectra of several alcoholic beverages.

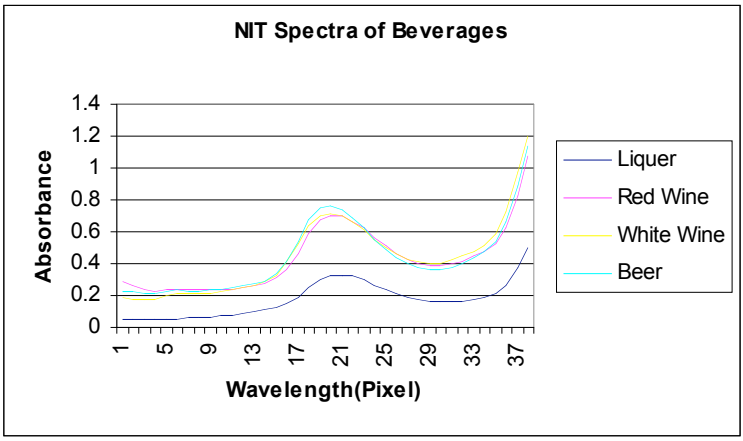


Figure 18. NIT Spectra of Beverages

Figure 19 shows the linearity and accuracy of measuring alcohol in beer.

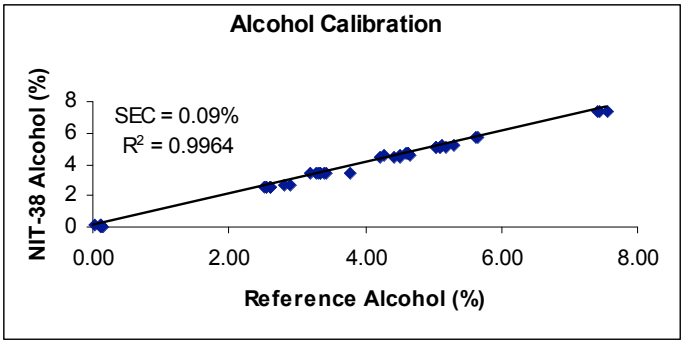


Figure 19. Plot of NIT Alcohol vs Reference Alcohol

Other non-alcoholic beverage scan also be analysed by NIT, such as soy milk, fruit juice and yakult. Figure 20. shows the plot of NIT Protein and Reference Protein in Soy Milk. The SEC is 0.04%.

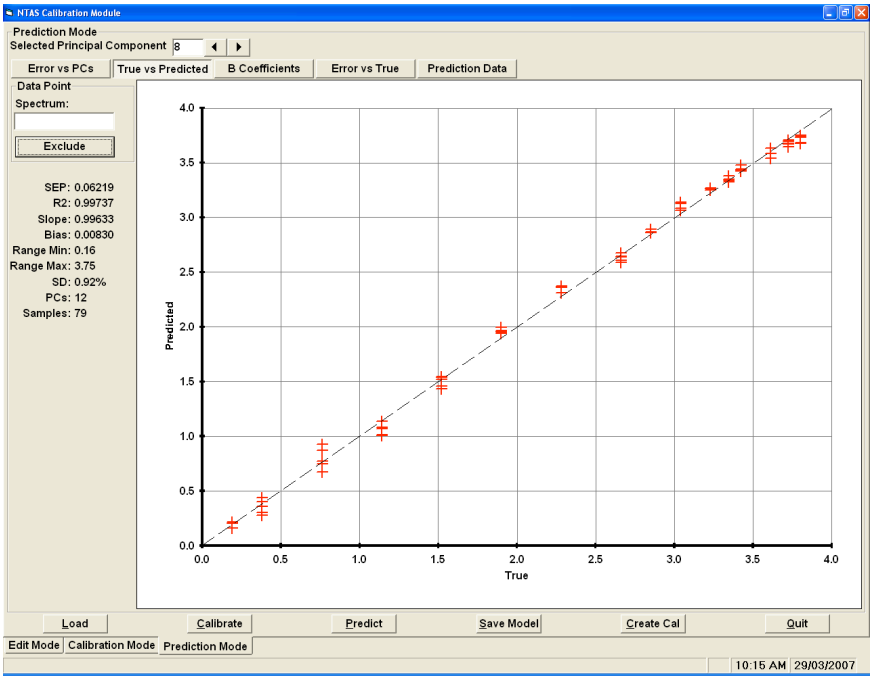


Figure 20. Plot of Protein Results in Soy Milk.

Where as Refractometers are a low cost instrument for measuring sugars, NIT is capable of measuring sugar, protein, fat and solids in the same sample.

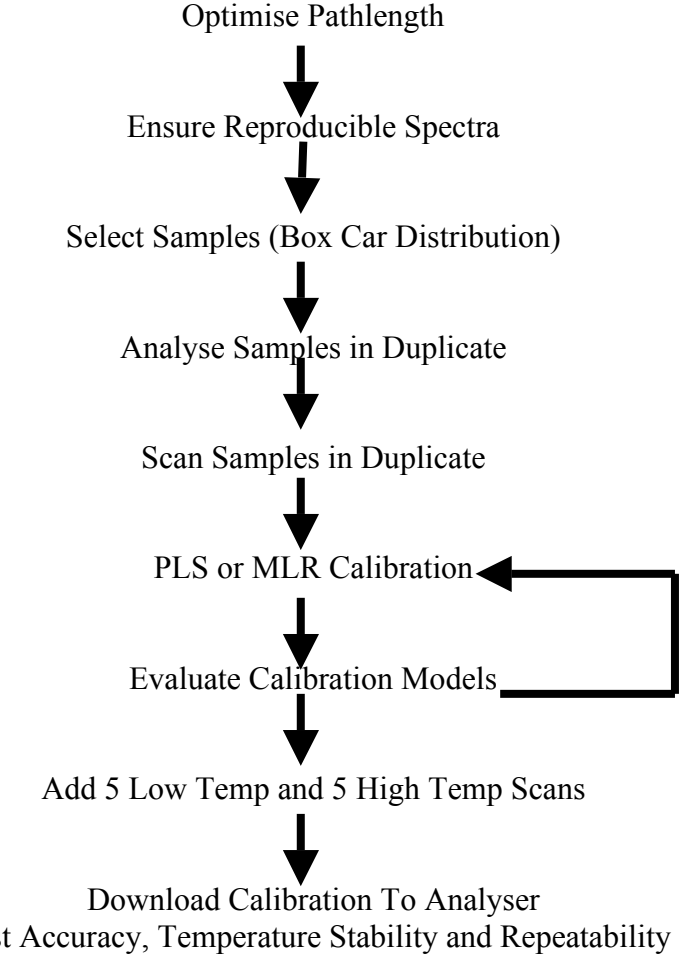
Calibrating NIT Analyser

Introduction

NIR spectroscopy is a secondary or correlative technique i.e. the spectral data collected is correlated through statistical means to some reference (laboratory) data. Regression Analysis techniques are used to develop calibration models, which are subsequently downloaded into a NIR analyser for use in predicting unknown samples.

The following steps presented in the flow chart, outline the procedure in developing a NIT calibration:

Flow Chart for Calibration Development



Calibration Techniques

The concentration of a chemical component is proportional to the amount of light absorbed at a frequency specific to that component. This relationship can be expressed as follows;

Concentration = Conc Factor x Absorbance

Traditional spectroscopy methods such as UV-Visible, use Simple Linear Regression (SLR), sometimes known as Univariate Regression, to estimate the Concentration Factor, CF, which relates the absorbance of a chemical species at a known wavelength.

SLR estimates the equation of the line:

$$\%Y = b_1 x + b_0$$

Where: Y is the concentration of the constituent of interest
X is the absorbance at a specific wavelength
b_1 is the Calibration Factor or Slope
b_0 is the Intercept or Offset

By plotting the absorbance versus concentration, a straight line calibration curve is produced. The slope of the line is the Concentration Factor.

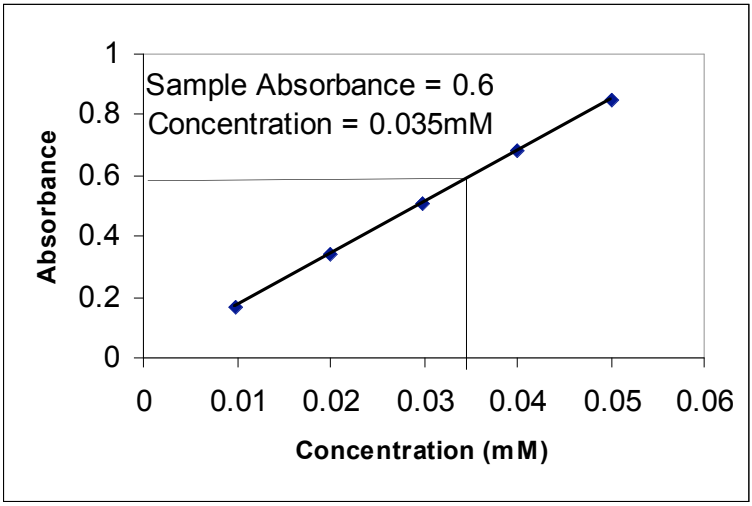


Figure 21. Plot of Absorbance vs Concentration.

Multiple Linear Regression (MLR)

In NIR Spectroscopy the spectral bands are commonly overlapping and exhibit some form of baseline correction due to scatter As such, NIR spectra do not follow the same linear relationship between absorbance and concentration as seen in UV and Visible spectroscopy. Multiple Linear Regression (MLR) techniques are require to develop multi term calibration models to compensate for overlapping bands, interferences, matrix effects and scatter.

A simple example of MLR is Bi-Modal calibration, where one component is being interfered with by another, eg, tryptophan in the presence of Tyrosine. A simple two-component calibration can be developed. The model would take the following general form.

$$Y=b_0+b_1X_1+b_2X_2$$

Where: b_0=y-intercept
b_1 and b_2=partial regression coefficients
x_n are the absorbances at each wavelength

MLR would be used to estimate the values of b0, b1 and b2.

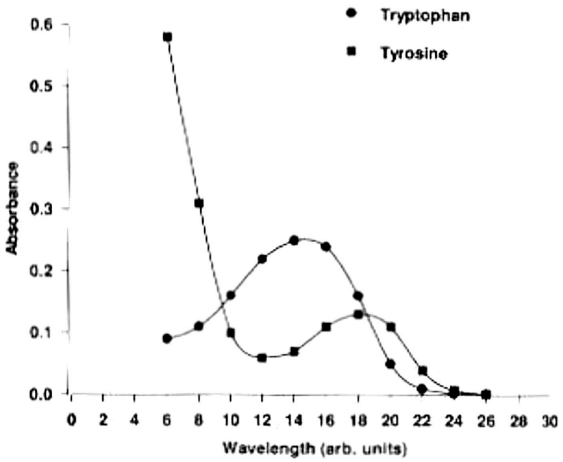


Figure 22. Spectra of Tryptophan and Tyrosine

When the sample matrix is complex, ie, a solid sample with multiple components and inconsistent particle size or distribution, then the MLR model becomes more sophisticated and can include up to 20 terms. For example;

$$Y=b_0+b_1X_1+b_2X_2+b_3X_3 \ldots\ldots +b_{20}X_{20}$$

There are two methods for performing MLR

- Stepwise Forward
- Stepwise Backward

Stepwise Backward

- Develop a model using all wavelengths
- Reduce the model, one wavelength at a time until the Standard error of Calibration (SEC) blows out.
- Used in original filter based instruments (Technicon, Dickey-John Perten).
- Can easily overfit data set resulting in poor prediction and poor reproducibility.

Stepwise Forward

- Select the first wavelength with the highest correlation.
- Regression looks for next wavelength, which will increase the correlation and reduce the error.
- Process is continued until the addition of the next wavelength has no effect or starts to reduce the correlation and increase the error.
- Best results from derivative spectra (removes baseline effects and spectral data are not intercorrelated).

This technique, when used with derivative spectra, usually requires two or three terms in the calibration equation. This reduces the tendency to overfit the data and results in more reliable predictions. (Note, in order to use derivative spectra, a full scanning spectrophotometer is required).

Principal Components Regression

In Principal Components Regression (PCR), the objective is to isolate Principal Components (PC's) (or vectors of maximum variation) of the spectral data and then use these as terms in a calibration equation. Rather than using a single wavelength to characterise a peak, PCR estimate the shape of the peak or factors affecting the spectra and use these as x terms (spectral reconstruction).

Partial Least Squares (PLS) Calibrations

PLS regression is very similar to PCR, however in PLS the constituent data is used in the computation of the PC's. Generally PLS is used in preference to PCR in NIR spectroscopy, as PCR tends to incorporate too much redundant information. PLS models also averages the spectra and improve the signal to noise ratio.

PLS is defined as a global technique, i.e. the entire spectrum is used to develop a calibration and not just some highly correlated wavelengths. An example of a PLS regression profile is shown in figure 23.

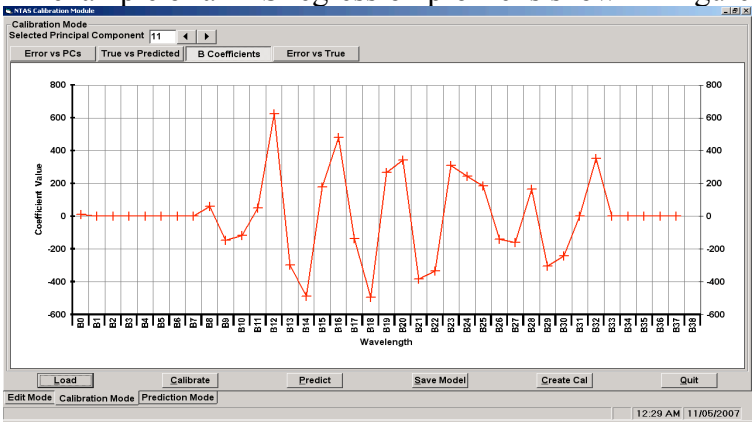


Figure 23. Plot of B Coefficients of a PLS Model.

The plot represents the weightings or loadings, which are placed on each part of the spectrum. By adding the weighted wavelength readings and adding the regression offset b_0, a value of the constituent is obtained using the following equation:

$$\%Y = b_0 + b_1\lambda_1 + b_2\lambda_2 + \ldots\ldots\ldots + b_{38}\lambda_{38}$$

Qualitative Analysis

Discriminant Analysis is a qualitative tool used to group (or classify) spectra into pre-defined classes. The first step of the process is to perform a Principal Components Analysis (PCA) and plot the most significant PC's in order to identify any clustering of spectra (known as Cluster Analysis).

Cluster Analysis is commonly called an unsupervised analysis, as the number of clusters is not known and must be determined by visual inspection of the data. Clusters are usually found using two methods:

- Correlation techniques- Samples are grouped based on how they correlate to each other.

- Distance measures- Samples are grouped based on how close they are to each other.

One distance of measure is known as the Mahalanobis distance, and is commonly used in statistical methods to define closeness of objects. The Mahalanobis Distance can be visualised in the following diagram. Each wavelength reading, ie. absorbance value in the spectrum, is considered an independent axis with a value equivalent to the absorbance value. The diagram only shows three axes, however in NIR the number of vectors is generally hundreds. The resultant vector, ie, the sum of the three vectors produces a unique vector that represents the entire spectrum.

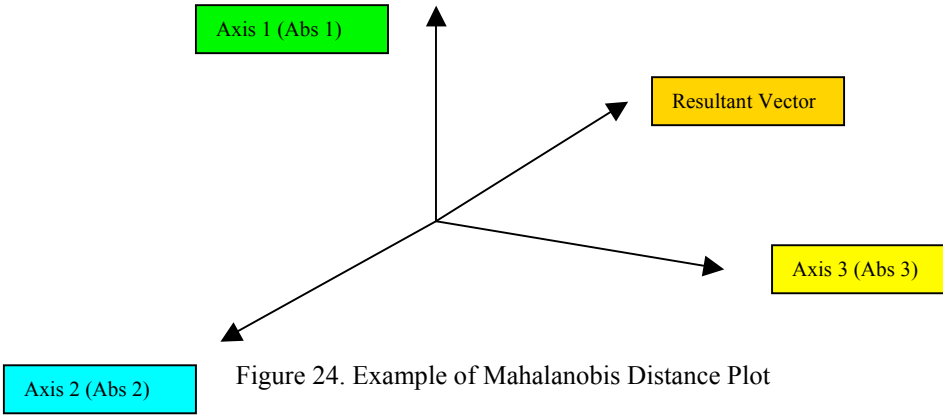


Figure 24. Example of Mahalanobis Distance Plot

Spectra that are similar will have a Resultant Vector with the same angle and length. Normally spectra of even the same sample vary due to packing density, particle size and purity. As such the resultant vectors of spectra from many samples of the same material form an ellipse in space. This is the Cluster that defines the variation in spectra of the specific material. By building a library of spectra for each material, Discriminant Analysis can be used to identify unknown spectra against the library. The closer the resultant vector of the unknown sample is to the Cluster, then the better the fit. The Mahalanobis Distance is the parameter that is calculated to determine how close the unknown spectrum is to each Cluster in the library. If the Mahalanobis Distance is "0", then the spectrum matches perfectly. As such, the smallest Mahalanobis Distance is used to pick which Cluster the unknown spectrum fits best.

Discrimination Analysis is used for two tasks:

- Identification of Unknown Materials
    - Requires library of known materials
    - Matches unknown material to a class defined by the training set
    - Displays top five matches along with the statistical relevance
- Quantification of Materials or Sameness

  Comparison of known material to a library sample to measure sameness. The concept of Sameness may be a strange term, but the idea is simple. If a manufacturer buys 10 tonnes of a chemical additive, they want to know if it is the same as the last batch or at least as same as the batches which have proven to be acceptable.

Since the NIR spectrum of a material contains information about the chemical composition, as well as the physical characteristics, eg. Particle size and size distribution, compaction, crystallinity etc, then Discriminant Analysis provides a means of inspecting the whole material rather than making a QC assessment on just a couple of chemical tests.

Routine NIR Analysis

Once a calibration model or models have been developed, they are downloaded into the NIR analyser for use in analysing every day samples.

The fundamental assumption in performing MLR or PLS regressions to develop calibration models, is that the calibration set represents all the future variations in the samples to be tested. It also assumes that the environmental conditions are the same for the calibration and the unknown samples. Since it is impossible to include all future sample variations and environmental variations, it is common to add more sample spectra into the calibration set. As such it is important to put aside around 5% of the everyday samples for analysis using the laboratory methods. By comparing the results for these samples against the NIR results, an estimate of the accuracy and stability of the NIR method can be obtained. Plotting control charts of the error between the laboratory and the NIR methods is very useful as it shows whether the errors are random or systematic.

An alternative strategy is to use a test sample to check the stability of the NIR analyser on a daily basis. The test sample is analysed each day and if the difference between the tested value and the NIR result is greater repeatability statistic, then a bias adjustment can be made to the calibration. Figure 25. shows an example of calculating a Bias adjustment.

Sample	Cropscan	Ref	Diff
1	9.5	10	0.5
2	11	11.6	0.6
3	12.2	12.6	0.4
4	13.4	14	0.6
5	14.2	14.5	0.3
	Bias = Average		0.5

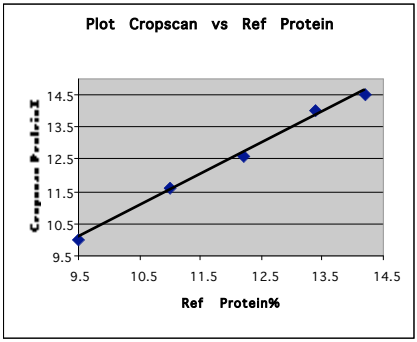


Figure 25. Example of Bias Adjustment

Periodically NIR calibrations may require more than a Bias adjustment. A Slope and Bias adjustment can be necessary when the low readings are too high and the high readings are too low and the middle readings are about right. Figure 26. shows an example of where a Slope and Bias adjustment to the calibration are required.

Sample	Cropscan	Ref	Diff
1	10.5	10	-0.5
2	11.8	11.6	-0.2
3	12.6	12.6	0
4	13.2	13.8	0.6
5	13.8	14.5	0.7
	Bias = Average		0.1

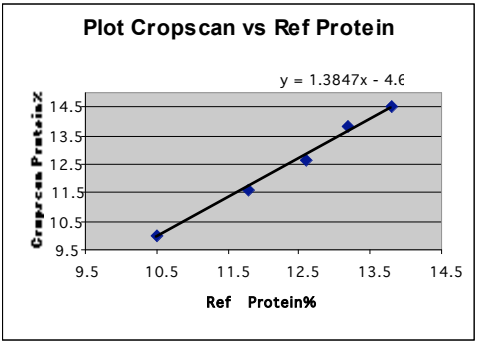


Figure 26. Example of Slope and Bias Adjustment

The Slope and Intercept of the line of fit between the NIR results and the reference results can be used to adjust the calibration model so that the analyser measures all samples correctly.

Temperature Effects

NIR analysers are excellent thermometers. As such the sample temperature and the instrument temperature can affect NIR results.

To correct for the effects of temperature, one of the following procedures can be used;

1. Add high and low temperature sample and instrument spectra into the calibration.
2. Include a temperature reading into the calibration model.
3. Measure the sample and instrument temperature and adjust the results using a linear calculation.

1) Cross Temperature Stabilisation:

By scanning 5 samples at both high and low temperatures with the instrument at room temperature and then scanning the same five samples at room temperature but with the instrument at high and low temperatures, the calibration can be stabilised against temperature effects. Add these scans to the calibration set and recalibrate. The additional of the temperature samples will force the software to choose wavelengths that are stabilised against temperature changes. This method is the best for use with grains, powders or solid materials

2) Calibrate Against Temperature:

In this method, an extra variable is added into the calibration set, i.e., sample temperature. The calibration procedure then includes temperature into the calibration model. This method requires a thermo couple or thermistor reading placed in the sample, to be read by the instrument. This is difficult to implement in laboratory type analysers but more suited to on line analysers.

3) Linear Temperature Correction:

Over narrow temperature ranges, i.e., +/- 10C, the effects of temperature on the predicted results are linear. As such, by measuring the sample temperature, the analyser's computer can make a linear correction. For example, for measuring alcohol in wine the temperature coefficient is approximately -.05% per degree. The predicted result can be corrected by using the simple equation:

%Alcohol (temp corrected) = %Alcohol + (20C - Sample Temp)*-.05

This method is ideal for liquids since a thermistor can be placed inside the measuring cell and a quick and reliable temperature reading obtained.

Note that all three methods are corrections. As such there is always an error introduced from measuring temperature. The best way to reduce errors due to temperature changes is to maintain the sample and instrument temperatures as close to the temperature used to develop the calibration models.

Sample Preparation:

Sample presentation is a major source of errors in NIT spectroscopy. The first issue is that the calibration models can be tuned to a very narrow variation in sample packing, grinding, mincing etc. As such, a model may work well at the time of calibration but fail as soon as the sample presentation changes. Just like in the case of temperature, you can add sample presentation variation into the calibration and thereby force the software to choose wavelengths that are less influenced by packing, grinding etc. Alternatively design the sample preparation and presentation procedure to ensure consistency.

Weight of the sample is a very important factor in NIT spectroscopy. The amount of light that is absorbed by a sample is proportional to the mass concentration of the components. However most NIT methods do not weigh the sample but simply fill the sample cup. This assumes that the density of the material is always the same and that the operator fills the cup up the same every time. A better method is to weight the sample cup before and after loading and then adjust the predicted results based on mass. By connecting a balance to a NIT analyser and reading the weight of the sample, recording this into the analyser's computer board, then analysing the sample followed by a mass correction to the predicted result will provide far more accurate and reproducible results.