History and Theory of Near-Infrared Spectroscopic Analysis (NIRS)

Eilert, A.J.
Blue Sun Scientific

Abstract

The use of near infrared spectroscopy (NIRS) as an analytical technique has evolved greatly since it was first demonstrated to be a viable method of obtaining molecular compositional information from the "scanning" of a sample. Potential applications of the technology envisioned by early pioneers in the field have been realized or exceeded time after time, and it has now become a very broadly applied means of instrumental analysis. It is worth recalling the historical progression of the technology and the fact that the earliest applications were developed to meet the challenges of agricultural product analysis. In particular, forage analysis is one of the areas where the advantages and possibilities made development of systems dedicated to overcoming the challenges well worth the effort, and helped usher in innovations that could be more widely used in other areas. Various attributes of the technology combine or individually lend themselves to development of systems that can be routinely used for rapid multiconsitiuent analysis, with either limited or no sample preparation. When developed to their ultimate potential, such systems can be utilized with little-to-no understanding of the underlying principles. In many cases a user can simply "scan" a sample using the NIRS "black box" and obtain results on a computer screen or in a printout in less time than it would take to write them down in a notebook. However, an understanding of how NIRS works, and the inherent theoretical capabilities and limitations of what it can do, will give the user a deeper appreciation for the technology and a basis for ensuring that it is operating properly and being optimally utilized.

Introduction

Near-infrared spectroscopy is perhaps the most significant breakthrough in the last half-century for the routine quantitative analysis of a wide range of constituents in organic products. It provides a rapid method for making routine analyses of many different constituents in parallel, with minimal sample preparation. The early history of NIRS is largely a story of dedicated, clever people making insightful connections across disciplines to solve practical problems. However, it really begins with an appreciation of a spectral region consisting of "uninterestingly" weak/broad/overlapping overtone and combination molecular absorption bands that no "right-minded" spectroscopist could be expected to envision putting to practical use, particularly when the unambiguous band assignments for sample identification could be achieved by just looking at the distinct primary absorption bands in the mid-infrared spectrum (Burns and Margoshes 1992). It is also a story of the underlying principles of diffuse reflection and transmission and the physics of light scattering, and an understanding of the inherent advantages that near-infrared radiation could provide for material sampling. Finally it is a story of technology, particularly the emerging technologies of electronics, electro-optics and computer science in the 1960s and 1970s era, coupled with various mathematical modelling schemes that would ultimately be classified under the name "chemometrics". All the pieces were first put together for the purpose of quantitative NIRS by Karl Norris at USDA in Beltsville MD. His successful experimentation prompted the birth of a powerful new quantitative analytical technique that can also be considered the entirely new analytical concept of using models based on historical spectral observations of known composition samples to "predict" composition of future samples. Once the principle of quantitative analysis using the near-infrared region was understood to be possible, and its potential for rapid, multi-consitituent analysis of agricultural products was recognized, it was destined to be made into a practical technique in the intervening decade through the work of numerous other pioneers. For a full appreciation of the technology and its development, some basic understanding of the theory of the physical processes that are being put to use is necessary. This includes how the radiation (light) in the near-infrared region interacts with a sample, how that is being "sensed" by the spectrometer in the process of generating an absorbance spectrum, and some of the different approaches that can be employed in near-infrared instruments for obtaining the spectrum. Another important aspect related to the practical usage of NIRS is the theory and methodologies involved in spectrum evaluation (using chemometrics) to obtain analytical results. This is an area of development tha is particularly relevant when it comes to the usage of NIRS for forage analysis.

History of NIRS

The region of the electromagnetic spectrum referred to as near-infrared starts where the red end of the visible spectrum stops and extends to the wavelength at which the mid-infrared region starts. By this definition, in wavelength terms, it starts at about 700 nm and ends at 2500 nm. The existence of what we refer to as near-infrared ("light" beyond the red portion of the visible spectrum) was first discovered by Sir William Hershel in 1800 (Hershel 1800). Karl Norris is widely regarded as the father of modern NIRS technology, although studies of the near infrared region and utilization of it for spectroscopic analysis were being considered by scientists prior to Norris, such as Wilbur Kaye in the 1950s and Kermit Whetsel in the 1960s. However, in addition to sharing their understanding of the potential of the region, he also understood how computer technology could enable realization of its potential via computerized calibration and prediction, and was first to demonstrate use of the region for practical quantitative NIRS instrumental analysis. Commercial NIRS instruments started to become available in the mid-1970s that were designed to measure absorbances in the NIR region and generate analytical results, based on the "Norris strategy". The earliest of these used multiple bandpass filters, with filter wavelengths selected according to the spectroscopic regions where constituents of interest were known to provide the strongest unambiguous indications of their presence and best potential for quantitative determination. Starting with major constituents such as moisture, protein, and fat, analytical success could be achieved with measurements at only two or three specifically-selected wavelengths for each constituent. As is still true today, determination of the optimal wavelengths for analyzing a particular constituent in the presence of other interfering components (e.g. overlapping absorption bands) and sample characteristics (e.g. particle size, packing density, temperature) that affect the spectrum is largely an empirical science. If full-spectrum data on a set of known composition samples are available, computer algorithms can generate models that can identify a small number of wavelengths whose linear comination of absorbances can generate "predicted" concentrations optimally-correlated to the actual constituent concentrations of interest. The simplest of these modelling algorithms is known as multiple linear regression, and that is what was used. Different combinations of wavelengths could be tried on a computer, and a winning (high correlation) combination could be identified and used in commercial instrumentation. In the 1970s, starting with Neotec's GQA Model 1 in 1972 (Williams et al. 2019) were being tested and later adopted for practical routine use for wheat protein analysis. These early instruments were essentially dedicated application-specific devices with embedded calibrations and were accuracy-adjusted by analog means. The GQA used a tilting-filter approach, which later provided for optimization by changing "pulse points" corresponding to the degree of tilt of a filter. This particular scheme was a proven success for specific applications, but was not to be continued as a design approach beyond the 1980s. Another early multi-filter instrument design, with six turret wheel mounted filters, was produced by the DICKEY-john company, that was named GAC (for "grain analysis computer"). They became associated with Technicon in 1974, who made their own improvements to the design, which was given the name "InfraAlyzer". DICKEY-john also improved on their original design and marketed an instrument that was named "InstaLab". By 1978 both Technicon and DICKEY-john were commercially producing NIR instruments, and the technology was beinning to be adopted on an even more widespread level.

Based on how incident sample interrogation energy is selected, commercial NIRS instruments that are in common use today can be broadly classified into four basic categories: Filter, Monochromator, Selective diode (e.g. diode array), and Fourier-transform (FT-NIR). The turret-wheel filter instrument design (utilizing up to 20 filters) was remarkably successful for a wide range of applications, particularly in the grain, food, feed, and textile industries, and it continues to be used for certain applications today (Wetzel et al. 2002). On the other hand, dispersion type (grating) monochromator instruments were responsible for producing the bulk of the data used for early NIRS application research, but not so much for routine analysis applications. However, as instrument development progressed through the 1980s into the 1990s, commercial instruments utilizing this technology, such as those manufactured by Perstorp / NIR Systems (later acquired by Foss) and Technicon (later acquired by Bran+Luebbe), were being increasingly used for routine analysis work. These instruments were inherently more flexible with regard to both range of applications and for optimization of calibration model accuracy. A key advantage of acquiring continuous wavelength full spectrum data was that it made possible spectrum preprocessing techniques to minimize particle size and compaction scattering variability and enabled transformation of a spectrum into its derivatives. By the mid 1990s, dispersion type monochromator instruments were being used more than any other technique in laboratory settings (McClure 1994). A crucial partner to this growth was the personal computer revolution that occurred during the same time period. A computer was an essential data acquisition and evaluation component if one wanted to take full advantage of the capabilities that a full-spectra-range scanning instrument had to offer. Early commercially available grating monochromator systems came with more obscure personal computers, such

as the HP-1000 A600, but the trend to IBM (or IBM compatible) computers soon became the norm, and this facilitated a range of chemometric software offerings. Instrument manufacturers had their own offerings such as NSAS (near-infrared spectral analysis software) and IDAS (InfraAlyzer data analysis software), but third party providers were also on the scene providing software with some key advanced features. Examples include Unscrambler from CAMO, SpectraCalc (later GRAMS) from Galactic Industries, and most notably for the NIRSC forage analysis community, ISI from InfraSoft International. In 1975-1976, a group of workers under the leadership of Dr. John Shenk recognized the potential of NIRS for determination of quality in forages, and on-farm testing of the technology for that purpose was done. Shenk, in conjunction with others, including Mark Westerhaus, would subsequently develop the first NIRS network, and Westerhaus would then go on to develop the ISI software to support it. The DOS compatible ISI software, later WinISI, incorporated many of Dr. Shenk's ideas about how a network of standardized instruments could be supported (along with some initial provisions for support of different instrument platforms). It was destined to become the world's most widely used NIRS software (Williams et al. 2019).

From the 1990s to today, hardware and software would undergo further development beyond what can be covered here. Diode array and FT-NIR would first come into routine use in the1990s and are available today. This historical discussion has been limited to diffuse reflectance instrumentation/applications. Diffuse transmission mode near-infrared is of secondary importance for forage analysis, but is quite important and influencial in other areas, such as with whole grain analysis. With regard to utilization of NIRS instruments, it is worth noting that historically, the very first were used by scientists, but not necessarily spectroscopists. Later instruments, particularly those with full spectral coverage, were used by scientists with training in spectroscopy. At the end of the 20th century, commercial instruments of various types were commonly being used by personnel with little to no training in NIRS (Norris 1999). The trend has certainly continued steadily in that direction since then, and this constitutes a fulfilment of the promise of widespread routine application of the technology.

Theory of NIRS

It is the physical and optical characteristics of a given material that dictate the most appropriate approach for presenting a sample of it to the spectrometer (Eilert et al. 2002). This theoretical discussion will concentrate on diffuse reflectance NIRS. Diffuse reflectance measurements require instrumentation that is specifically designed to collect the scattered light that impinges upon, interacts with, and is diffusely reflected from the surface of a sample. The ratio of the intensity detected at a given wavelength reflecting from the sample to that reflected from an "ideal" diffuse reflector is referred to as the reflectance (R). Unlike the expected close-to-ideal scattering of the reflectance standard, diffuse reflection from real samples is never quite ideal, and analytical repeatability and accuracy will be impacted somewhat by this. Figure 1 illustrates the difference.
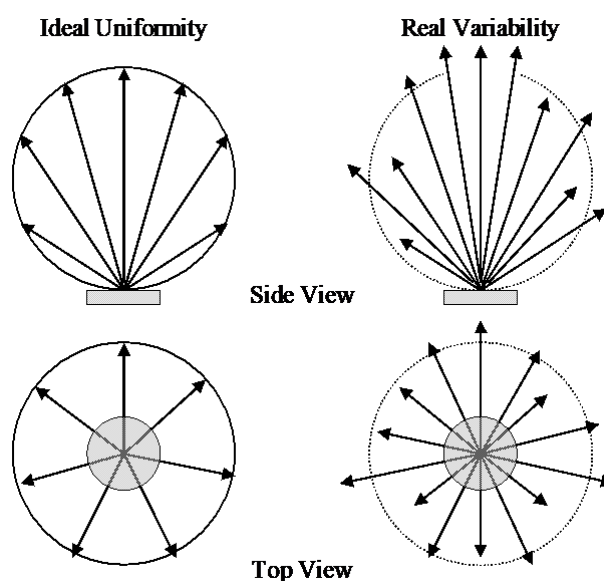

Figure 1. Ideal diffuse reflectance profile compared to what might be obtained when interrogating a real sample.

Averaged subscans with automated rotation/transport of the sample during the scan is a common way to help reduce the effect of non-uniformity in the scattering from a sample surface. In addition, some materials may be satisfactorally scanned as received, while others need further "conditioning", such as by grinding.

Sometimes the choice of a sample preparation step such as grinding or drying a sample can be considered a matter of how precise/accurate the results need to be. But it can achieve more than make the scattering more efficient or uniform. For instance, grinding a whole grain sample will both improve the homogeneity and expose the interior portions of the individual grains or seeds that may otherwise not "seen" in doing the scan.

Once the reflectance readings are obtained at each wavelength by the spectrometer, they are converted to absorbance (A) using the relationship A = log[1/R], or A = - log[R]. The reason for converting to absorbance is that the Beer-Lambert law, though not strictly valid for diffuse reflectance, indicates that absorbance will be proportional to changes in concentration of an absorbing constituent. This linear relationship is important when it comes to developing calibration models. Absorbances in the near-infrared region are relatively weak in comparison to the mid-IR, where the fundamental molecular vibrational modes are excited. It turns out that this is a big advantage, because the sample does NOT completely attenuate the incident intensity, and there is a sufficient fraction left to measure, so that degrees of change can be quantified. Very small changes in absorbance can be significant, so the instrumentation must be designed to be stable and provide exceptional signal-to-noise performance. Of the constituents that may be present in a natural product, moisture is the one that is most ubiquitous. It turns out that moisture is a quite strong absorber of near-infrared energy. This is good from the standpoint of measuring it, but it can present challenges when moisture levels get too high and attenuate signal that is needed to achieve accuracy for other constituents. Drying is an effective strategy used in the forage industry to deal with this. NIRS is most effective for analyzing constituents with N-H, C-H, and O-H molecular bonds, which exist in all of the primary constituents of organic materials, such as protein, oil, fiber, starch, sugars, and cellulose in forages. This is because strong bonds between relatively light atoms represent the strongest-absorbing molecular vibrational overtones seen in that region. It is also quite effective at analyzing other constituents of interest in forages that are associated with organic composition, and can be useful for analyzing concentrations of some non-organic constituents, such as ash and some minerals, via secondary correlations.

A quality near-infrared spectrum is one with low photometric noise and a high degree of wavelength accuracy, covering wavelengths where each and every one of the analytes of interest absorb. It should also be one taken from an appropriately prepared and representative sample. A sufficiently large collection of such spectra, obtained by scanning samples of accurately-known composition (e.g. reference lab analysis results) for the constituents of interest, representing the compositional variation of a product, can be used to assemble a calibration database. These data can then be used to produce computer-generated models that can "predict" composition of future samples that are scanned in the same manner. Chemometric software is used to produce such prediction models, outputting analytical equations (or some other form of prediction scheme) that is customized for each represented constituent. These are intended to convert newly-acquired spectra to concentration values for future scanned samples. Of the many mathematical techniques that are available to do this, the one that is used most often is partial least squares (PLS) regression.

The complete NIRS "system" is one comprised of a spectrometer with computer/software plus analytical equations and a specific sampling technique with associated hardware as needed. The job of the system is to "predict" results that would have been achieved if a given sample were submitted to the lab(s) responsible for reference data used in the calibration process. It is important to insure that the model remains valid and the "system" is otherwise continuing to work as it should over time. Note that NIRS results are usually more precise than a lab, so ability to accurately predict lab results can be most affected by lab precision. Periodic validation should be done to determine reference lab agreement, comparing predicted results to those obtained for the same samples from the lab, with updates/changes made to improve performance as needed.

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