

NIRS INSTRUMENTATION

Karl H. Norris
Instrumentation Research Laboratory
Beltsville Agricultural Research Center, ARS, USDA
Beltsville, Maryland 20705

Instrumentation Requirements for Quantitative Analysis by Diffuse Reflectance

Spectroscopy

The near infrared region is generally defined as the wavelength region from 700 to 3000 nm; however, most of the quantitative analyses by reflectance are done in the 1200 to 2500 nm region. Below 1200 nm, the absorption bands are so weak that quantitative measurements by reflectance are difficult and above 2500 nm, the absorption bands are so strong that measurements are difficult. In addition, the signal-to-noise falls off rapidly at the longer wavelengths because the energy from a tungsten lamp falls off rapidly. As a result, the most useful region is the region from 1200 to 2500 nm.

The NIRS method of analysis requires very high wavelength reproducibility, typically within a standard deviation of less than 0.02 nm. The wavelength accuracy requirements within one instrument are not very important, and a tolerance of ± 5 nm is acceptable. However, if calibrations are to be transferred from one instrument to another, corrections must be applied to correct for the wavelength differences.

Diffuse reflectance data in the near infrared region will seldom be less than 1.0% and never greater than 100%. Expressed as $\log (1/R)$, the minimum value will be 0.0 and the maximum approximately 2.0. Therefore, an instrument should have good linearity over the photometric range from 0 to 2.0 in $\log (1/R)$ and a linearity of within 1% over this range is generally considered adequate. The accuracy of the photometric scale within one instrument is not important as long as it is stable. However, between-instrument comparisons require a photometric accuracy of better than 1% or corrections must be

applied. Very small changes in reflectance must be measured in the NIRS method; therefore, photometric noise is a very important parameter. Noise levels of 0.0001 in $\log(1/R)$ can limit the performance of a measurement.

Three different optical geometries are being used in commercial NIRS instruments. These are: illumination at an angle of zero degrees and collection at an angle of 45° as used in instruments manufactured by Pacific Scientific Corporation and Labor-Mim Company, illuminating at an angle of zero degrees and collection of all reflected radiation with an integrating sphere as used by Technicon Industrial Systems and Percon Corporation; and illumination at a small angle and collecting at an angle of zero degrees as used by Dickey-john Corporation. Each of these geometries has advantages and disadvantages, but they all provide adequate reflectance data for quantitative analyses.

Reflectance measurements require a reference standard. The National Bureau of Standards recommends the use of a commercial plastic "Halon" (6) as a reflectance standard for the ultraviolet, visible, and near infrared region. A more rugged standard is desirable for the NIRS method and a special ceramic has been adopted as the working standard. This ceramic has a uniform reflectance across the total near infrared region as shown in Fig. 1. This standard can be cleaned with soap and water or with alcohol to remove contamination. Those instruments which use an integrating sphere use the interior coating of the sphere as the reference. The spheres are coated with a special coating of gold to provide a uniform, high-diffuse reflectance over the near infrared region. Pressed sulfur has been proposed as a reference standard (4), but it has not been widely used in spite of its excellent reflectance properties in the near infrared region.

The reflectance of the reference must remain constant to maintain the calibration of an NIRS instrument. The absolute value of the reflectance is not of great consequence, but if calibrations are to be transferred from one instrument to another, any differences in the references must be taken into consideration. It appears that the ceramic working standard is very stable and does not represent a significant source of error within one instrument, but between-instrument variability occurs because the position of the ceramic and its backing affect the measured reflectance.

Wavelength-Scanning Instruments

Several research groups have developed scanning-monochromator instruments for their use in NIRS research (2,3,5). These have consisted of various modifications of prism-grating spectrophotometers originally manufactured by Cary. The most common modification uses only the monochromator from the Cary and replaces the sample optics with a single-beam diffuse reflectance arrangement of sample illumination at zero degrees, and collection with a bank of four photocells at 45° (Fig. 2). This sample geometry is essentially the same as that used by Pacific Scientific Corporation in several models.

The light source is chopped into an alternating on-off beam, and synchronous detection is used with the photocell signal to reduce the effects from temperature, amplifier drift, and stray radiation. Large-area, lead sulfide detectors are used to measure the radiation reflected from the sample. The signals from the detectors are summed into a high-gain, low-noise amplifier, and the amplified signal is converted to a digital value with different types of signal sorting and analog-to-digital converters. The digital signal, representing the reflected energy, is coupled into a computer for processing and storage of data. A signal representing wavelength is coupled into the

computer in synchronization with the reflectance signal so that a reflectance value is stored for each wavelength point. The wavelength spacing between points is a variable set by the operator to be anywhere from 0.1 nm per point to 10 nm per point with 1.0 to 2.0 nm per point being the most common choice.

In operation, the spectrum for the reference standard is measured and stored in the computer. The spectrum for the sample is then measured and the corrected sample reflectance spectrum is computed. The reflectance spectrum for each sample may be recorded on disk or magnetic tape for future analysis. The most common analyses make use of the $\log (1/R)$ where R is the reflectance; therefore, the data are often converted to $\log (1/R)$ before being stored on disk or tape.

The Cary monochromator has variable slits so the spectral resolution can be varied. The low energy output of the monochromator limits the measurements to relatively wide pass bands, typically 5 to 10 nm or slit widths from 1.5 to 3.0 mm. The Cary monochromator provides excellent stray light rejection and excellent wavelength precision. It may be operated at scanning speeds as high as 10 nm per second so that the whole spectrum from 1000 to 2600 nm can be scanned in less than 3 minutes.

Two U.S. firms have produced and marketed scanning monochromator instruments specifically for rapid quantitative NIRS analyses. The Model 6100 grating monochromator instrument was introduced by Neotec, now a subdivision of Pacific Scientific Corporation, in 1978 and has been marketed under several different model numbers with the major distinction being the computer used with each of the models. This instrument uses a large, high-efficiency grating to achieve high energy output and low noise with a rapid scan (1). The spectral region from 1100 to 2500 nm is scanned at five scans per second and multiple scans are averaged to minimize the noise.

Spectral data may be collected at either 1.0 or 2.0 nm per wavelength point with 2.0 being the more common. The instrument uses the sample geometry of Fig. 2 and operates in the single-beam mode. A reference sample of ceramic is scanned and the spectral data are stored in the computer so that when a sample is scanned, the corrected reflectance of the sample is obtained. From 20 to 100 scans are averaged for both the reference and the sample, but since the scans are at five scans per second, a low-noise, corrected-reflectance spectrum of a sample can be obtained in 20 seconds. The reflectance data are normally converted to $\log(1/R)$ before storage on the disk. The instrument is operated at a fixed-slit width providing a nominal bandpass of 10 run. An accessory is available for diffuse transmittance measurements of solutions and slurries. The instrument can also be modified to operate in the 600 to 1100 run region using silicon detectors to replace the lead sulfide detectors.

The 6100 monochromator was originally marketed with a Data General computer and a floppy disk. This has now been changed to a North Star Computer, incorporating both a hard disk and a floppy disk, and includes a full line of operating software for collecting, plotting, and analyzing the spectral data. The software includes a selection of several regression procedures for calibration. The software also includes diagnostic routines to test the performance of the spectrophotometer.

The 6100 monochromator has also been marketed with a Digital Equipment Company computer, and it is this version that was adopted for use by the USDA's Forage Network. These models are typically equipped with 3 removable hard disk and dual floppies for storage of data and programs. The operating software for these units was developed as part of the Forage Network and the backup support for this software comes from the Network. This software, which will be described in a later section, provides full capabilities for collecting and analyzing the spectral data as well as routines for testing instrument performance.

The Model 500 grating monochromator instrument was introduced by Technicon Industrial Systems in 1982. Ibis instrument also uses a large, high-efficiency grating to achieve high energy output, but otherwise the design is quite different. The Model 500 operates in a double-beam mode using an integrating sphere (Fig. 3) to collect the reflected energy as well as to provide the dual-beam capability. The wavelength drive is by a stepping motor, which provides the flexibility of continuous scanning from 1100 to 2500 nm or rapid jumping to specific wavelengths or wavelength regions. The instrument requires 90 seconds for a complete scan at 2 nm per wavelength point from 1100 to 2500 nm. During this scan, the reference and the sample are both measured, and the computer reads out the corrected reflectance or $\log(1/R)$ as desired. The monochromator slits are configured to provide a bandpass of 10 nm across the spectrum.

The Model 500 is normally equipped with a Hewlett Packard computer and two floppy disk drives, but a hard disk is available. Software is provided for collecting and processing the spectral data, and several choices of regression procedures are included for calibration. The software also includes diagnostic routines for testing instrument performance.

The Model 500 monochromator has also been marketed with a Digital Equipment Company computer, but in this configuration, the software support is from the Forage Network.

A scanning monochromator instrument has also been developed in Hungary by Labor-Min, but has not been marketed in the United States. This instrument operates in a single-beam mode using the geometry of Fig. 2. It scans the spectral region from 1200 to 2400 nm in 60 seconds and has a nominal bandpass of 15 nm. It uses a small grating monochromator and does not achieve the same signal-to-noise as the larger grating instruments. The instrument incorporates

a dedicated microcomputer to collect and process the reflectance data. Software is provided for limited data analysis.

L.T. Industries has announced a rapid scanning monochromator instrument for the near infrared region, but details of the instrument have not been made available.

Tilting-Filter Instruments

The transmitted pass band of an interference filter moves to shorter wavelengths as the filter is tilted away from perpendicular to the incident source. The effective wavelength of a filter may be shifted as much as 10% without serious distortion of the pass band. Therefore, it is possible to scan a limited wavelength region by tilting an interference filter. One of the original commercial NIRS instruments used this principle with three interference filters in a paddle wheel to scan the spectral region from 1800 to 2310 nm. The principle is still used by Pacific Scientific Corporation in all of their filter-type NIRS instruments. Models using this principle are: Models 31, 41, 51, 101, 102, 4250, and 7000. They use the optical geometry of Fig. 2 for reflectance measurements, and incorporate various levels of computer capability to collect and process the reflectance data.

These tilting-filter instruments are generally marketed as calibrated instruments with calibration constants stored in the instruments to give digital readout of from two to five constituents for each of several products. The calibration constants can be changed by the user to adjust to new products or new constituents. Some of these instruments can also be used in a transmittance mode, and by changing filters, it is possible to change the effective wavelength region. Silicon detectors replace the lead sulfide detectors for operation in the 600 to 1100 nm region.

These instruments may be used as fixed-wavelength instruments or as limited-range scanning spectrophotometers. As scanning spectrophotometers, they offer the capability of first or second derivative treatment of data, and they are most often used in this mode.

The instruments all incorporate a ceramic reference standard which is measured between each sample, and the computer provides the output as $\log (1/R)$.

Fixed-Filter Instruments

Fixed-filter NIRS instruments have been developed by four firms. The first model was developed by Dickey-john Corporation and was marketed as the 2.5A Grain Analysis Computer by Technicon Industrial Systems. It is a six-filter instrument with wavelengths chosen to measure oil, moisture, and protein. The wavelengths, 1680, 1940, 2100, 2180, 2230, and 2310 nm, were specified by the Instrumentation Research Laboratory of USDA to coincide with absorption bands for water at 1940 nm, carbohydrates at 2100 nm, protein at 2180 nm, and oil at 2310 nm. The wavelengths of 1680 and 2230 were chosen as reference wavelengths of minimum absorption for the constituents. The six, narrow-band, interference filters are mounted in a wheel so that the sample is successively illuminated for brief times with radiation from each of the filters. The optical geometry provides for illumination of the sample at a small angle and collection of the reflected energy at zero angle. A single, lead-sulfide detector is used to measure the radiation from the sample. The detector incorporates a thermoelectric element for cooling the detector to enhance the signal to noise. The 2.5A used an analog computer to sort the reflectance values and compute the constituent values. This unit has since been upgraded with a digital computer to GAC III Models 640, 650, 660, and 800. The new units are marketed directly by Dickey-john Corporation. The

newer units use the same six wavelengths originally chosen with options of adding up to four additional wavelengths. The Dickey-john units have an additional feature of rotating the sample during measurement to minimize sample variability. All of these instruments operate in the single-beam mode with a ceramic reference standard being measured between each sample.

Fixed-filter instruments for NIRS have also been developed by Technicon Industrial Systems under the Model numbers of 300 and 400. These instruments use the integrating sphere geometry of Fig. 3 and operate in a double-beam mode with the interior of the sphere being the reference standard. These units use the same six wavelengths that were originally chosen to measure oil, moisture, and protein, but the 400 offers up to 19 wavelengths. The standard choice for the 19 are: 1445, 1680, 1722, 1734, 1759, 1778, 1818, 1940, 1982, 2100, 2139, 2180, 2190, 2208, 2230, 2270, 2310, 2336, and 2348 nm. These instruments incorporate a microcomputer to process the data into constituent analyses. A liquid sample compartment is available for the Technicon 400 and 500 instruments. This sample compartment has been used for milk and dairy products as well as other liquid samples. With the liquid sample cell, a thin sample of the liquid covers a ceramic disk, and the radiation is transmitted through the liquid reflected by the ceramic and transmitted back through the liquid into the integrating sphere for measurement. This mode of operation is referred to as the "Transflectance Mode."

A fixed-filter instrument has also been developed in Germany by Percon. This instrument is very similar to the Technicon filter instruments using the same integrating sphere geometry and double-beam mode. The major difference is the use of a flow-thru sample compartment rather than a separately packed sample cup.

Instrument Error Sources

The major source of noise in NIRS measurements is from the sample itself: sampling errors, sample stability, non-uniformity errors, sample-packing errors, errors in the laboratory analyses, or errors resulting from attempting to use NIRS for constituents having no near-infrared signal to measure. This is true because the instrument manufacturers have done an excellent job of reducing the instrument noise to a minimum. The noise contribution of the instrument can be separated into long-term noise or drift and short-term noise. The major contributors to long-term drift are changes in ambient temperature and degradation of components. The instruments are designed to operate in a fairly constant-temperature environment and changes of 10 to 20 degrees will cause changes in the constituent readings. Component degradation may be as simple as accumulation of dust on the reference standard or on windows, lenses, or other optical components, or the degradation may be from mechanical wear on moving parts as well as from deterioration of electronic components. Such changes can produce major errors or complete malfunction. Instruments operating in the double-beam mode are less subject to some of these errors because they affect both the reference and the sample, but errors resulting from dust on the sample window and errors from wavelength changes are not cancelled by the double-beam optics.

Wavelength instability is the main source of short-term noise in wavelength-scanning instruments. Detector and input amplifiers represent the next most common source of short-term noise for instruments which are functioning properly. Interference from noise outside the instrument can be a problem. These include brush-type motors, heater switches, and similar electrical devices which generate electrical pulses. Such pulses can be carried on the electrical lines into the instrument or they can be transmitted as radio-frequency pulses through the air.

Single-beam instruments require very stable light sources. Therefore, the lamp and lamp power supply can be a source of noise. Dual-beam instruments must switch beams from reference to sample very fast. Ibis switching action can be a source of noise in such instruments.

Other sources of noise include stray light, photometric linearity, and surface reflectance. All of these introduce nonlinearity into the measurement, but since the reflectance signal is not linear with constituent content, any nonlinearity effects tend to be masked. These nonlinearity errors become a more serious problem for multiple-instrument applications where it is desirable to transfer calibrations from one instrument to another.

Stray light is of two forms: it may be from room light reaching the detectors because of inadequate shielding or it may be from leakage of light through the monochromator without being blocked by the dispersion element (gratings or filters). Room light is seldom a source of difficulty if the instrument is operated properly (sample drawer closed, etc.). The stray light in a well-designed grating monochromator is typically less than 0.1%. This would introduce an error of 10% in measuring the magnitude of an isolated absorption band giving a $\log (1/R)$ value of 2.0. However, isolated absorption bands do not occur in forage samples, and the presence of the other absorbers reduces the stray-light effect. The maximum $\log (1/R)$ value for dry forage samples is less than 1.0, and at this level, the stray-light of 0.1% causes an error of less than 1%. High-moisture samples such as fresh silage will have $\log (1/R)$ values exceeding 2.0 and, for these samples, stray light becomes a more important source of error.

Well-designed filter instruments should also have less than 0.1% stray light, but an instrument with poor or damaged filters could have very high stray light. The combination of the spectral response of the lead sulfide

detector and the spectral emission of the tungsten lamp results in a peak energy response in the 1300 to 1600 nm region. Therefore, measurements below 800 nm and above 2600 nm are more subject to stray light, because the available energy at these wavelengths is very low.

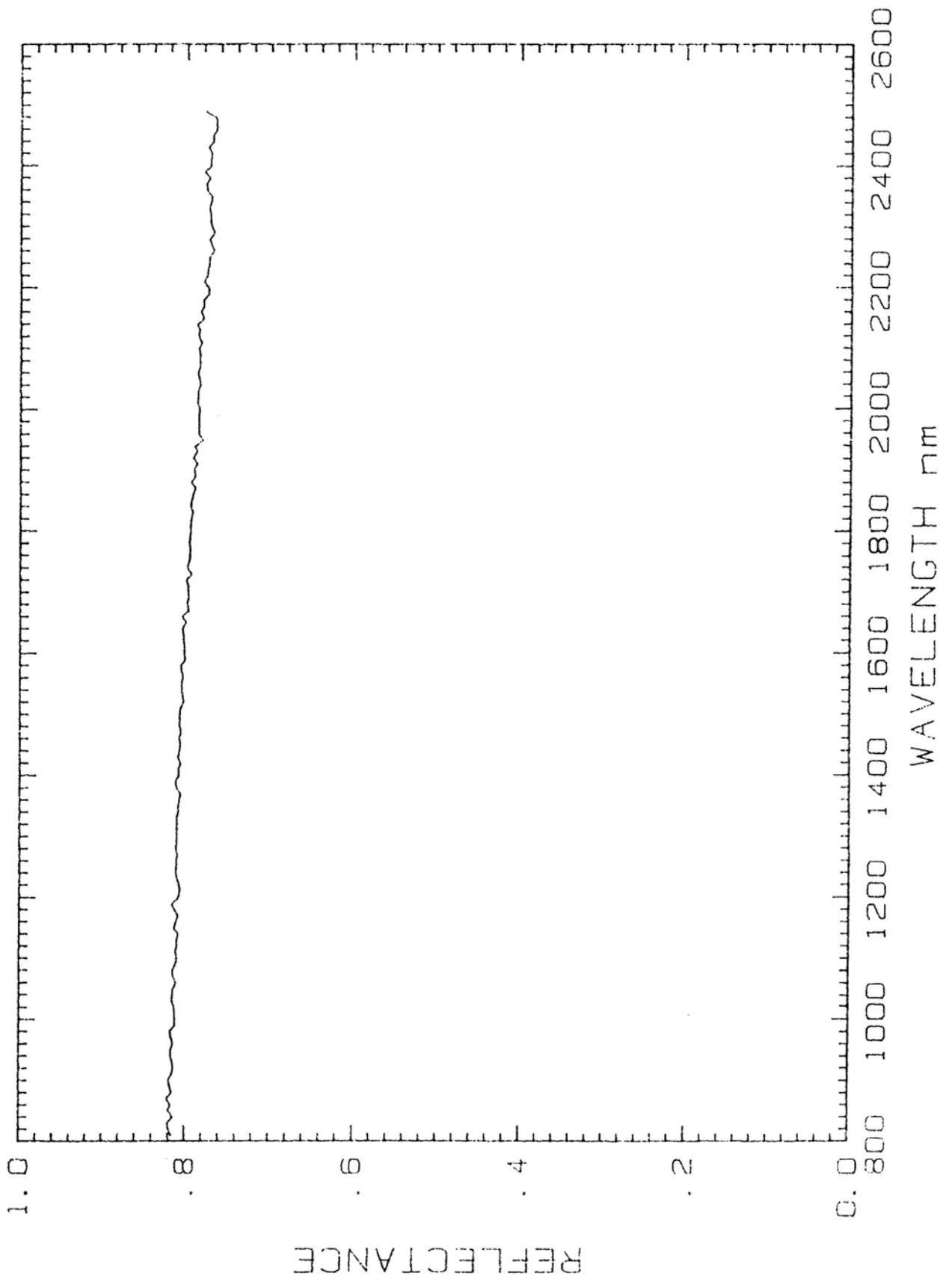
Logarithmic amplifiers were used in the first instruments. These represented a source of photometric nonlinearity, but they are no longer used and photometric linearity is not a serious source of error on newer instruments. The nonlinearity generated from the surface reflectance signal represents a serious error for samples having high $\log(1/R)$ values. It is difficult to keep this reflectance signal below 0.3% which represents an error of 3% for a sample having a $\log(1/R)$ of 1.0 and 33% for a sample having a $\log(1/R)$ of 2.0. This limits useful diffuse reflectance measurements to wavelengths at which the $\log(1/R)$ values are less than 2.0. Corrections for the surface reflectance can be included in the data collection to minimize this error, but such corrections are based on an average surface reflectance and are not specific to the sample being measured. Therefore, it has not been possible to adequately correct for the surface reflectance.

References

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Figure Captions

1. Absolute reflectance spectrum for a ceramic reference standard. Data provided by National Bureau of Standards.
2. Optical geometry for diffuse reflectance measurements using zero illumination and forty-five degree collection with large area photocells.
3. Optical geometry for dual-beam diffuse reflectance measurements using an integrating sphere to collect the reflectance.



MONOCHROMATIC
LIGHT

