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▲〈1856〉 NEAR-INFRARED SPECTROSCOPY—THEORY AND PRACTICE

1. THEORY

Near-infrared (NIR) spectroscopy is a technique with broad and varied applications in pharmaceutical analysis. The NIR spectral region lies between the ultraviolet (UV)-visible and infrared regions, and at one time was considered the forgotten region of the electromagnetic spectrum. It is a branch of vibrational spectroscopy that shares many of the principles that apply to other spectroscopic techniques. The NIR spectral region comprises two sub-ranges (see *Figure 1*) associated with detectors used in instrumentation. The short-wavelength (Herschel) region extends from approximately 780–1100 nm (12,821–9000 cm^{-1}), and the longer wavelengths, which fall between 1100 and 2500 nm (9000–4000 cm^{-1}), compose the traditional NIR spectral region. It is common to express the wavelength (λ) in nanometers (nm) and the frequency (v) in reciprocal centimeters (wavenumber) as acquired by the instrument. Usually, Fourier-transform (FT) spectrometers report the *x*-axis in wavenumber (cm^{-1}), whereas a dispersive, monochromator-based instrument will show the *x*-axis in wavelength (nm).

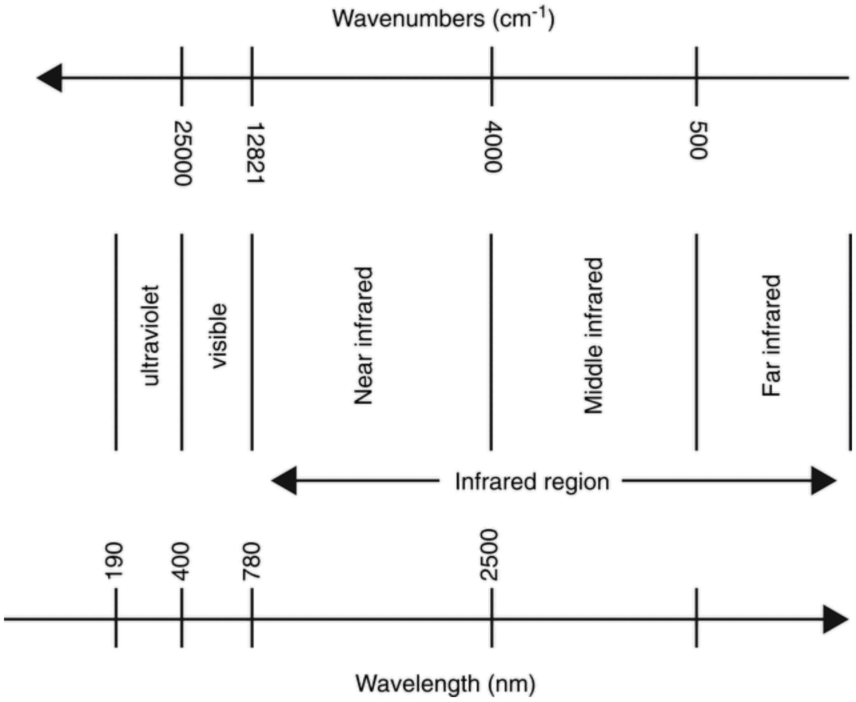

Figure 1. NIR region of the electromagnetic radiation spectrum.

NIR spectra are dominated by C-H, N-H, O-H, and S-H overtone resonances and combinations of fundamental mid-infrared vibrational modes from which they originate. Because molar absorptivities in the NIR range are low, radiation can penetrate several millimeters into materials, including solids where it can be absorbed when the wavelength of the radiation corresponds to a transition between the ground vibrational state of the analyte and either a harmonic of a given vibrational mode (an overtone) or the sum of two or more different modes (a combination band). Fiber-optic technology is readily implemented in the NIR range, which allows real-time, or near real-time, monitoring of processes in environments that might otherwise be inaccessible. As is the case with other spectroscopy measurements, interactions between NIR radiation and matter provide information that can be useful for both qualitative and quantitative assessment of the chemical composition of samples. In addition, qualitative and quantitative characterization of a sample's physical properties can be made because of the sample's influence on NIR spectra.

Measurements can be made directly on samples in situ in addition to applications during standard sampling and testing procedures. Applications of qualitative analysis include identification of raw material, in-process control testing, and finished-product release testing. NIR measurements can be performed off-line, but also at-line, in-line, and on-line for process analytical technology. These applications often involve comparing an NIR spectrum from a sample to a reference spectrum and assessing similarities against acceptance criteria developed and validated for a specific application. In contrast, applications of quantitative analysis involve the development of a predictive relationship between NIR spectral attributes and sample properties. Because of the highly covariant nature of the NIR signal, these applications typically use chemometrics models to quantitatively predict chemical and/or physical properties of the sample on the basis of its spectral attributes.

2. TRANSMISSION, REFLECTION, AND TRANSFLECTION MODES

The most common measurements performed in the NIR spectral range are transmission and reflection spectroscopy. Incident NIR radiation is absorbed or scattered by the sample and is measured as transmittance or reflectance, respectively.

2.1 Transmission Mode

Transmittance (T) is the intensity ratio of the transmitted radiation (I) to the incident radiation (I_0), which represents the decrease in intensity at given wavelengths when radiation is passed through the sample. The sample is placed in the optical beam between the source and the detector. The arrangement is analogous to that in many conventional spectrophotometers, and the result can be presented directly in terms of transmittance and/or absorbance (A). NIR spectra are usually measured in absorbance (A).

$$T = \frac{I}{I_0} \text{ or } T = 10^{-A}$$

I = intensity of transmitted radiation

I_0 = intensity of incident radiation

The measurement of transmittance is dependent on a background transmittance spectrum for its calculation. Examples of background references include air, a polymeric disc, an empty cell, a solvent blank, or in special cases, a reference sample. The method generally applies to gases, liquids (diluted or undiluted), dispersions, solutions, and solids, including tablets and capsules. For transmittance measurements of solids, a suitable sample accessory is to be used. Tablets and capsules can be analyzed using suitably engineered holders, thereby ensuring reproducible measurements. Liquid samples are examined in a cell of suitable path length (typically 0.5–4 mm) that is transparent to NIR radiation, or alternatively by immersion of a fiber-optic probe of a suitable configuration. Gases are usually measured using dedicated long path-length, multiple reflection cells.

2.2 Reflection Modes

2.2.1 REFLECTANCE

Reflectance (R) is the ratio of the intensity of light reflected from the sample, I, to that reflected from a background or reference reflective surface, I_R.

It is composed of both the specular (mirror) and the diffuse components (see *Figure 2*).

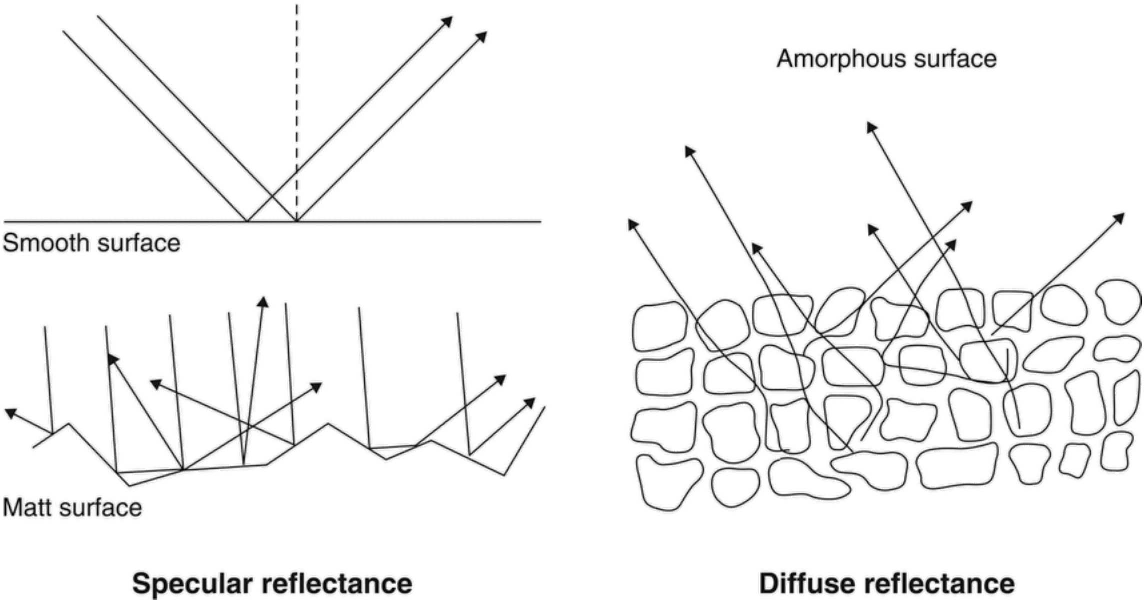

Figure 2. Specular and diffuse reflectances.

The determination of reflectance requires the measurement of a reference reflection spectrum to determine the attenuation of reflected light relative to the unattenuated incident beam. The reflectance spectrum is calculated as the ratio of the single-beam spectrum of the sample to that of the reference material.

$$R = \frac{I}{I_R}$$

I = intensity of light diffusively reflected from the sample

I_R = intensity of light reflected from the background or reference reflective surface

Most reflection measurements in the NIR spectral region are made of scattering samples such as powders and slurries. For such materials, NIR radiation can penetrate a substantial distance into the sample, where it can be absorbed when the wavelength of the radiation corresponds to a transition between the ground vibrational state of the analyte and either a harmonic of a given vibrational mode (an overtone) or the sum of two or more different modes (a combination band). Nonabsorbed radiation is scattered back from the sample to the detector. NIR reflectance spectra typically are accessed by calculating and plotting log (1/R) versus wavelength. Other abscissa data transformations can also be used. This logarithmic form is the pseudo-absorbance of the material and is commonly called absorbance.

2.2.2 DIFFUSE REFLECTION

The diffuse reflection mode gives a measure of reflectance (R) (penetrating the sample surface, interacting with the sample, and passing back through the sample's surface), which is the ratio of the intensity of light reflected from the sample (I) to that reflected from a background or reference reflective surface (I_r), where by using careful instrumental design, the specular component is often excluded. NIR radiation can penetrate a substantial distance into the sample, where it can be absorbed by vibrational combinations and overtone resonances of the analyte species present in the sample. Nonabsorbed radiation is partially reflected back from the sample to the detector.

$$R = \frac{I}{I_r}$$

I = intensity of light diffusively reflected from the sample

I_r = intensity of light reflected from the background or reference reflective surface

This mode generally applies to solids. The sample is examined in a suitable device. For process monitoring, material can be analyzed through a polished glass (e.g., sapphire) window interface, or using an in-line probe. Care must be taken to ensure that the measuring conditions are as reproducible as possible from one sample to another. The reflected radiation of a background reference is scanned to obtain the baseline, and then the reflectance of one or more analytical samples is measured. Common reflectance references include ceramic tiles, thermoplastic resins, and gold. Other suitable materials may be used. In some process-analysis situations, it may be impossible to remove a probe for reference background data collection; in these cases, consider various options including internal referencing; measurement of a background reference using a second detector; and others. Only spectra measured against a background possessing the same optical properties can be compared directly with one another.

2.3 Transflection Mode

The term transflection (T^*) is used to describe any double-pass transmission technique. Transflection spectrometry is a hybrid of transmission and reflection wherein a reflector is placed behind the sample so that the optical path through the sample and back to the detector is doubled, compared with a transmission measurement of a sample of the same thickness. Nonabsorbed radiation is reflected back from the sample to the detector. The light may be reflected from a diffuse or specular (mirror) reflector placed behind the sample.

$$T^* = \frac{I}{I_{T^*}}$$

I = intensity of transflected radiation measured with the sample

I_{T^*} = intensity of transflected radiation of the reference material as background

This mode generally applies to liquids and clear plastic materials. This configuration can be adapted to share the same instrument geometry with reflectance and fiber-optic probe systems where the source and the detector are on the same side of the sample. The sample is examined through a cell with a mirror or a suitable diffusive reflector made of either metal or an inert substance (e.g., dried titanium dioxide) not absorbing in the NIR region. Liquids can also be measured using in-line transflectance probes to increase intensity levels for more sensitive applications.

3. FACTORS THAT AFFECT NIR SPECTRA

NIR spectroscopy is advantageous because, as a result of lower molar absorptivities in this region of the electromagnetic spectrum, accurate measurements can often be made quickly without destroying the sample, and with minimal or no sample preparation; this applies whether the samples are solid, semi-solid, liquid, or gas. The NIR spectrum contains information on overtone resonances and combination of fundamental vibrational modes of the sample that can yield both sample and process understanding. Samples may be analyzed directly through packaging or directly in media that is transparent to wavelengths in the NIR spectral region. In addition, by definition NIR spectra are produced using the corresponding NIR radiation, and therefore standard glass/quartz and fiber optics may be used.

The following discussion is not exhaustive, but it includes many of the major factors that affect NIR spectra.

3.1 Environmental Factors

The environmental temperature and humidity must be considered before carrying out measurements. Ideally, ambient conditions will be controlled to meet the operating specifications of the instrument manufacturer. However, tighter controls may not always be practical to the degree necessary for the application, and as such, another justifiable means of accounting for the influence of the environment (e.g., spectral preprocessing) should be utilized, with frequent verification. This is particularly relevant when considering the effect of temperature when using fiber-optic probes.

3.2 Sampling Factors

Depending on the measurement mode, sample preparation and presentation can vary. The following factors should be considered for all sampling techniques:

- Find the best suitable measurement mode for the intended application (transmission, diffuse reflection, or transflection)
- Find the best suitable accessory (e.g., transmission or immersion probes)
- Optimize path length in transmission and transflection modes
- Find a suitable spectroscopic background reference sample
- Show that the background reference sample is reliable over time and that the measurement of the background is reproducible and stable over time
- When measuring moving materials or samples (for process-related measurements), it is important to obtain a representative spectrum (e.g., by adjusting the measuring time or number of scans, by co-adding individual spectra, or by increasing the beam size)
- Ensure that there is not fouling of the sensor, for example, with build-up of material or contamination
- When measuring through packaging material, consider the variability of content and thickness
- Where multiple crystalline forms are present, care must be taken to ensure that the model calibration samples have a distribution of forms relevant to the intended application

3.2.1 SAMPLE PRESENTATION AREA

The sample presentation area or probe end must be clean and free of residue prior to the measurement. Similarly, the in-line or on-line interface to the sample should not have significant product or contamination build-up, which would interfere with the desired measurement.

3.2.2 SAMPLE TEMPERATURE

Sample temperature influences spectra obtained from aqueous solutions and other hydrogen-bonded liquids, and a difference of a few degrees may result in significant spectral changes. Temperature may also affect spectra obtained from less polar liquids, as well as solids that contain solvents and/or water.

3.2.3 MOISTURE AND SOLVENT

Moisture and solvent that are present in the sample material and analytical system may change the spectrum of the sample. Both absorption by moisture and solvent, and their influence on hydrogen bonding of the active pharmaceutical ingredients and excipients, can change the NIR spectrum.

3.2.4 SAMPLE THICKNESS

Sample thickness is a known source of spectral variability and must be understood and/or controlled, particularly for tablet and capsule analysis. The sample thickness in transmission mode is typically controlled by using a fixed optical path length for the sample. In diffuse reflection mode, the sample thickness may be controlled by using samples that are “infinitely thick” relative to the detectable penetration depth of NIR light into a solid material. Here, the term “infinite thickness” implies that the reflection spectrum does not change if the thickness of the sample is increased. For the measurement of compressed powders, an infinite thickness is typically reached after 5 mm of sample depth (e.g., in a filled vial).

3.2.5 SAMPLE OPTICAL PROPERTIES

With solids, both surface and bulk scattering properties of model calibration standards and analytical samples must be taken into account. Surface morphology and refractive index properties affect the scattering properties of solid materials. For powdered materials, particle size and bulk density influence scattering properties and the NIR spectrum. The spectra of physically, chemically, or optically heterogeneous samples may require sample averaging, examination of multiple samples, or spinning the sample to obtain a representative spectrum of the sample. Certain factors, such as differing degree of compaction or particle size in powdered materials and surface finish, can cause significant spectral differences.

3.2.6 SOLID-STATE FORMS

The variations in solid-state forms (polymorphs, hydrates, solvates, and amorphous forms) influence vibrational spectra. Hence, different crystalline forms as well as the amorphous form of a solid may be distinguished from one another on the basis of their NIR spectra. Where multiple crystalline forms are present, care must be taken to ensure that the calibration samples have a distribution of forms relevant to the intended application.

3.2.7 AGE OF SAMPLES

Samples may exhibit changes in their chemical, physical, or optical properties over time. Depending on the storage conditions, solid samples may either absorb or desorb water/solvent, and portions of amorphous materials may crystallize. Materials used for NIR model calibration are representative of future samples and their matrix variables. Hence, care must be taken to ensure that samples for NIR analysis are representative.

4. PRETREATMENT OF NIR SPECTRAL DATA

NIR spectral data to be used in qualitative or quantitative applications often need preprocessing to attenuate environmental or sampling factors, enhance a certain signal, or for other purposes that may include transformation, normalization, or other mathematical treatment. Preprocessing of samples (rows of data) may include mean or median centering, scaling, and other procedures. NIR responses (columns of data) also can be transformed, centered, or scaled. Because of the highly covariant nature of the NIR signal, it is common to remove linear or polynomial trends or apply filtering or smoothing techniques such as derivatives, wavelets, or a Savitzky–Golay filter. Spectral pretreatment techniques may include the numerical calculation of the first- or second-order derivative of the spectrum. Higher-order derivatives are not recommended because of increased spectral noise. For more detail, see *Chemometrics 〈1039〉*.

5. INSTRUMENTATION

5.1 Apparatus

All NIR measurements result from exposing material to incident NIR light radiation and measuring the attenuation of the emerging (transmitted, scattered, or reflected) light. Spectrophotometers for measurement in the NIR region consist of a suitable light source, such as a highly stable quartz–tungsten lamp, a monochromator or interferometer, and a detector. Common monochromators are acousto-optical tunable filters, gratings, or prisms. Traditionally, many NIR instruments have had a single-beam design, although some process instruments use internal referencing and can therefore be dual beam (for example, in diode array instruments). Silicon, lead sulfide, and indium gallium arsenide are examples of detector materials. Examples of sampling devices include conventional cuvette sample holders, fiber-optic probes, transmission dip cells, neutral borosilicate vials, and spinning or traversing sample holders. The choice is made on the basis of the intended application, with particular attention paid to the suitability of the sampling system for the type of sample to be analyzed. Suitable data processing and evaluation units (e.g., software and computer) are usually part of the system.

5.2 Imaging Techniques

NIR imaging is a combination of NIR spectroscopy with digital image processing. A NIR imaging system is basically composed of an illumination source, an imaging optic, a spectral encoder selecting the wavelengths, and a focal plane array. NIR imaging in particular has a huge potential for gaining rapid information about the chemical structure and related physical or biopharmaceutical properties of all types of pharmaceutical dosage forms, thus improving product quality and enhancing production speed.

Within a given material, the distribution of the various compounds can be characterized three-dimensionally by using imaging. Use of NIR imaging allows for the collection of detailed chemical information. NIR microscopy techniques have the following advantages:

- No preparation of the sample required
- Good spatial resolution

- Clear image quality
- Excellent chemical differentiation

It is possible to collect a signal from the entire sample (e.g., a tablet), disperse it into a spectrum using a spectrometer, and detect the spectrum using a multi-channel detector such as a charge couple detector or photo-diode array. In this case, instead of obtaining trivial cumulative information about the spot signal intensity, one can obtain a signal spectrum which can be transformed into detailed information about the chemical composition of the given spot on a sample. The three-dimensional array of data sets, two spatial and one spectral, recorded in such an imaging measurement has become known as a hypercube or data cube. These microscopy systems are capable of determining a spatial resolution down to approximately 1–5 µm.

NIR imaging systems are capable of characterizing the structure and distribution of the active components, and excipients within formulations, dosage forms, and delivery devices, to name a few. For example, chemical mapping for homogeneity testing of solid oral dosage forms, creams, and ointments may be useful in select applications.

5.3 Instrument Calibration Considerations

NIR instrument calibration involves three components: wavelength accuracy and uncertainty (*x*-axis); photometric linearity and response stability (*y*-axis); and photometric noise. *Table 1* provides an overview of performance verification criteria for a wide range of NIR systems.

5.3.1 PHOTOMETRIC NOISE

For any given method, the validation should include an estimation of the photometric noise of the system, as a change in this parameter may affect any future model calibration.

5.3.2 WAVELENGTH ACCURACY AND UNCERTAINTY (*X*-AXIS)

In the case of FT-NIR instruments, primary instrument wavelength-axis calibration is maintained, at least to a first approximation, with an internal helium–neon laser. NIR spectra collected by the conventional grating based spectra from sample and/or reference standard materials can be used to demonstrate an instrument's suitable wavelength-dispersion performance against target specifications. Suitable materials for demonstrating wavelength-accuracy performance include methylene chloride, talc *R*, spectral calibration lamps, polystyrene, mixtures of rare earth oxides, and absorption by water vapor. Certified traceable standards are available from the National Institute of Standards and Technology (NIST) for transmittance measurements (SRM 2035a) and reflectance (SRM 2036) and can be used for wavelength verification. Other suitable standards may also be used. Instrument manufacturers may use polystyrene films (thickness of 0.75–2 mm) as an internal standard for wavelength qualification and control. However, because of the weak asymmetric signals emanating from polystyrene at short wavelengths, this material may not be suitable as a reference standard across the whole NIR range. When choosing an appropriate reference standard, it is important to confirm that the operating parameters (e.g., resolution, band width, and others) are within the limits of the certified material.

The effect of temperature on assignment of wavelength must be taken into account. Typically, standards are measured in environments near 25°C, and the temperature at which certified values were taken will be indicated. The effect of resolution can also have an impact on the measured values. Resolution for FT-NIR spectrometers is typically 8 cm^{-1}, but modern spectrometers are capable of higher resolutions (2 cm^{-1} or 1 cm^{-1}).

Standard certificates will indicate resolution and possibly even peak assessment algorithms. The choice of reference standard for a performance test should always match the resolution and environmental conditions expected for the execution of the test. Additional standards may be necessary, depending on the range of wavelengths or reference standards meeting these conditions.[1] Refer to *Table 1* and *Table 2* for additional information.

5.3.3 PHOTOMETRIC LINEARITY AND RESPONSE STABILITY (*Y*-AXIS)

Calibration of the photometric axis can be critical for successful quantification. Both FT-NIR and dispersive NIR spectrometers should undergo similar instrument calibration procedures. The tolerance of photometric precision acceptable for a given measurement should be assessed during the method development and validation stages.

NIR spectra from samples and/or reference standard materials with known relative transmittance or reflectance can be used to demonstrate a suitable relationship between NIR light attenuation (resulting from absorption) and instrument response. Verification of photometric linearity and verification of photometric noise are not required for instruments using methods to perform simple identifications that do not use the photometric absorbance as part of the model strategy (e.g., simple correlation with absorbing wavelengths). The USP Near IR System Suitability RS is recommended, but other certified traceable standards exist in the market and may be used. For reflectance measurements, commercially available reflectance standards with known reflectance properties are often used.

Spectra obtained from reflection standards are subject to variability because the experimental conditions under which they were factory calibrated differ from those under which they are subsequently put to use. Hence, the reflectance values supplied with a set of calibration standards may not be useful in the attempt to establish an "absolute" calibration for a given instrument. The reproducibility of the photometric scale will be established over the range of standards provided that 1) the standards do not change chemically or physically, 2) the same reference background is also used to obtain the standard values, and 3) the instrument measures each standard under identical conditions (including precise sample positioning). Subsequent measurements on the identical set of standards provide information on long-term stability. Refer to *Table 1* and *Table 2* for additional information.

Photometric linearity is demonstrated by using a set of transmission or reflection standards with known values for percentage transmittance or reflectance. For reflectance measurements, carbon-doped polymer standards are available. It is important to ensure that the absorbance of the materials used is relevant to the intended linear working range of the method. Nonlinear calibration models, which are acceptable, may be used, as long as the user can demonstrate adequate justification for this approach.

Table 1. Control of Bench/Mobile Instrument Performance

Measurement Mode	Reflection	Transflection	Transmission
Verification of wavelength scale (except for filter apparatus)	Measure talc via a suitable medium or by fiber-optic probe. Talc has suitable-for-calibration, characteristic peaks at 948, 1391, 2077, and 2312 nm. Alternatively, other suitable standards may also be used that ensure wavelength accuracy in the region of working methodology. For example, measure an internal polystyrene standard if built in, or measure an NIST standard or other traceable material (e.g., USP Near IR System Suitability RS) and assess 3 peaks across the wavelength range for calibration.	A suspension of 1.2 g of dry titanium dioxide in about 4 mL of methylene chloride is used directly through the cell or using a probe. Titanium dioxide has no absorption in the NIR range. Spectra are recorded with a maximum nominal instrument bandwidth of 10 nm at 2500 nm (16 cm^{-1} at 4000 cm^{-1}). Methylene chloride has characteristic sharp bands at 1155, 1366, 1417, 1690, 1838, 1894, 2068, and 2245 nm. Choose 3 peaks across the wavelength range for calibration. Other suitable standards may also be used, such as TS5 liquid mixed with titanium dioxide or some other reflective medium.	Methylene chloride may be used and has characteristic sharp bands at 1155, 1366, 1417, 1690, 1838, 1894, 2068, and 2245 nm. Choose 3 peaks across the wavelength range for calibration. Other suitable standards may also be used, such as NIST SRM 2065, Polystyrene 65 μm, and TS5 liquid.
	For FT instruments, the calibration of the wavenumber scale may be performed using a narrow, isolated water-vapor line (for example, the line at 7306.74, 7299.45, or 7299.81 cm^{-1}).		
Verification of wavelength repeatability (except for filter apparatus)	The standard deviation of the wavelength is consistent with the specifications of the instrument manufacturer, or otherwise scientifically justified. Verify the wavelength repeatability using a suitable external or internal standard.		
Verification of photometric linearity and response stability[a]	Measure 4 photometric standards across the working method absorbance range. Analyze 4 reference standards, for example, in the range of 10%–90%, including 10%, 20%, 40%, and 80% with respective absorbance values of 1.0, 0.7, 0.4, and 0.1. Evaluate the observed absorbance values against the reference absorbance values (for example, perform a linear regression). Acceptable tolerances are 1.00 ± 0.05 for the slope and 0.00 ± 0.05 for the intercept for the first verification of photometric linearity of an instrument. Subsequent verifications of photometric linearity can use the initial observed absorbance values as the reference values.		
Verification of photometric noise	Determine the photometric noise using a suitable reflectance or transmittance standard (for example, white reflective ceramic tiles or reflective thermoplastic resins [i.e., polytetrafluoroethylene (PTFE)]). Follow the manufacturer's methodology and specifications. Scan the reflectance standard over a suitable wavelength range in accordance with the manufacturer's recommendation and calculate the photometric noise as peak-to-peak noise.		

[a] Burgess C, Hammond J. Wavelength standards for the near-infrared spectral region. *Spectroscopy*. 2007;22(4):40–48.

Table 2. Control of Process Instrument Performance

Measurement Mode	Reflection	Transflection	Transmission
Verification of wavelength scale (except for filter apparatus)	If it is not practically possible to measure a traceable standard material at the point of sample measurement, use internal material such as polystyrene, fiberglass, or solvent and/or water vapor. Alternatively, adopt a second external fiber/probe. For FT instruments, the calibration of the wavenumber scale may be performed using a narrow, isolated water-vapor line (for example, the line at 7306.74, 7299.45, or 7299.81 cm^{-1}) or a narrow line from a certified reference material.		

Measurement Mode	Reflection	Transflection	Transmission
Verification of wavelength repeatability (except for filter apparatus)	The standard deviation of the wavelength is consistent with the specifications of the instrument manufacturer, or otherwise scientifically justified. Verify the wavelength repeatability using a suitable external or internal standard.		
Verification of photometric linearity and response stability[a]	Measure 4 photometric standards across the working method absorbance range.		
	If photometric reflectance standards cannot be measured at the point of sample measurement, use the manufacturer's internal photometric standards. Process instruments can use internal photometric standards for photometric linearity. Follow the manufacturer's verified tolerances in such cases.	Analyze 4 reference standards to cover the absorbance values over the working absorbance range of the modelled data. Evaluate the observed absorbance values against the reference absorbance values (for example, perform a linear regression). Acceptable tolerances are 1.00 ± 0.05 for the slope and 0.00 ± 0.05 for the intercept for the first verification of photometric linearity of an instrument. Subsequent verifications of photometric linearity can use the initial observed absorbance values as the reference values.	
Verification of photometric noise	Determine the photometric noise using a suitable reflectance or transmittance standard [for example, white reflective ceramic tiles or reflective thermoplastic resins (i.e., PTFE)]. Follow the manufacturer's methodology and specifications.		
	As above, or if not practically possible, use the manufacturer's internal standard for noise testing and specifications.	Scan the transmittance high-flux standard over a suitable wavelength/wavenumber range in accordance with the manufacturer's recommendation and calculate the photometric noise as peak-to-peak noise.	

[a] Burgess C, Hammond J. Wavelength standards for the near-infrared spectral region. *Spectroscopy*. 2007;22(4):40–48.

5.3.4 EXTERNAL INSTRUMENT CALIBRATION

Detailed functional validation employing external reference standards is recommended to demonstrate instrumental suitability of laboratory instruments, even for instruments that possess an internal calibration approach. The use of external reference standards does not obviate the need for internal quality-control procedures; rather, it provides independent documentation of the fitness of the instrument for the specific analysis or purpose. For instruments installed in a process location or in a reactor where positioning of an external standard routinely is not possible, including those instruments that employ an internal calibration approach, the relative performance of an internal versus an external calibration approach should be evaluated periodically. The purpose of this test is to check for changes or drifts in components (e.g., the process lens or fiber-optic probe) that might not be included in the internal calibration method; this could, for example, affect the photometric calibration of the optical system.

5.3.5 MULTI-INSTRUMENT CALIBRATION

There are many approaches for ensuring a transferable calibration across multiple instruments of the same type (same make and model) and different types. This is an area of growing interest because of the globalization of manufacturing and regulatory controls. Today, reference standards have been developed to cover this region, but many deficiencies still exist regarding wavelength uncertainty and photometric response curves. Therefore, verification of compendial procedures, method transfers, and spectrometer platform changes is compromised by the bias involved in differences between instruments. Understanding the risk associated with these variations should be a part of any quality-by-design initiative to use NIR spectroscopic procedures for product control.

The use of appropriate certified reference materials can be helpful in establishing instrumental variance and/or bias. As stated above, the value assignments included on the certification of such materials will contain significant contributions to the overall expanded uncertainty budget from the instrumental factors used in generating these values. For example, the optical geometry, polarization, and other aspects of a reflectance measurement will significantly affect the measured value, and therefore wherever possible these characteristics should be matched to the required measurement configuration. However, by definition, a reference material should have the essential characteristics of appropriate stability and homogeneity to at least allow comparative measurements to be made, albeit they may be biased by known or unknown factors.

6. APPLICATIONS

NIR spectroscopy has a wide variety of applications for chemical, physical, and process analysis.

For chemical analysis, the applications include:

- Identification of active substances, excipients, dosage forms, manufacturing intermediates, chemical absorbance materials, and packaging materials

- Qualification of ingredients, intermediates, and drug products, including batch-to-batch spectral comparison and supplier change assessment
- Quantification of active substances and excipients; determination of chemical values such as hydroxyl value; determination of absolute water content; determination of degree of hydroxylation; and control of solvent content

For physical analysis, the applications include:

- Crystalline form and crystallinity, polymorphism, solvates, and particle size
- Analysis of intact pharmaceutical dosage forms: tablets, capsules, lyophilized products, and implants (e.g., polymeric and microspheres)
- Dissolution behavior, disintegration pattern, and hardness
- Examination of film properties

For process monitoring and process control analysis, the applications include:

- Monitoring of unit operations such as synthesis, crystallization, blending (e.g., powder), pelletization, tableting, capsule filling, drying, granulation, coating (e.g., film), and packaging for the purpose of process control

Measurements in the NIR spectral region are influenced by many chemical and physical factors. The reproducibility and relevance of the results depend on the control of these factors. Usually, measurements are only valid for a defined system.

6.1 Qualitative Analysis: Identification and Characterization

6.1.1 ESTABLISHMENT OF A SPECTRAL REFERENCE LIBRARY

To establish a spectral reference library, record the spectra of a suitable number of representative samples of the substance; the samples should have known, traceable identities. These representative samples should exhibit the variation that is typical for the substance to be analyzed (e.g., variation in solid-state form and particle size). The set of spectra obtained represents the information that can be used for chemical and/or physical identification of the sample to be analyzed.

The collection of spectra in the library may be represented in different ways, which are defined by the mathematical technique used for identification. These may be:

- All individual spectra representing the substance
- A mean spectrum of the measured batches for each chemical substance or physical form
- If necessary, a description of the variability within the substance spectra

The number of substances to be included in the library depends on the specific application. All spectra in the library will have the same spectral range and number of data points; technique of measurement; and data pretreatment. If subgroups (libraries) are created, the above criteria should be applied independently to each group. Origin spectral data for the preparation of the spectral library must be archived. Caution must be exercised when performing any mathematical transformation, as artifacts can be introduced or essential information can be lost. The suitability of the algorithm chosen should be demonstrated by successful method validation, and in all cases, the rationale for the use of transformation must be documented.

6.1.2 NIR REFERENCE SPECTRA

NIR references provide known, stable measurements to which other measurements can be compared; thus, they are used to minimize instrumental and environmental variations that would affect the measurement. Direct comparison of representative spectra of the substance to be examined and a reference substance for qualitative chemical or physical identification purposes may not require use of a reference spectral library, where specificity permits.

6.1.3 DATA EVALUATION

Direct comparison is made between the representative spectrum of the substance to be examined and the individual or mean reference spectra of all substances in the database on the basis of their mathematical correlation or other suitable algorithms. A set of known reference mean spectra and the variability around this mean can be used with an algorithm for classification; alternatively, this can be achieved visually by overlaying spectral data if specificity is inherent. Different calibration techniques are available, such as principal component analysis, cluster analysis, etc.

6.1.4 VALIDATION OF THE MODEL

Chemical or physical identification methods using direct spectral comparison must be validated in accordance with identification method validation procedures. The validation parameters for qualitative methods are robustness and specificity.

6.1.5 RELATIVE COMPARISON OF SPECTRA

A model calibration is not required when comparing a set of spectra for limit analysis purposes, such as determining the maximum or minimum absorbance at which an analyte absorbs. Also, in-process control of a drying operation may use a qualitative approach around a specific absorbing wavelength. Appropriate spectral ranges and pretreatments (if used) must be shown to be fit for purpose.

6.1.6 SPECIFICITY

The relative discriminatory power and selectivity of a limit test must be demonstrated. The extent of specificity testing is dependent on the application and the risks being controlled. Variations in matrix concentrations within the operating range of the method must not affect the measurement.

6.2 Trend Analysis

6.2.1 RELATIVE COMPARISON OF SPECTRA

NIR-based models can also be used to assess process trajectories. An example application is the use of NIR-based models to determine the endpoint of blending operation. Typically, a reference trajectory is defined based on a pool of batches that are deemed to present nominal variability; then each new batch trajectory gets compared with the reference trajectory and associated tolerances.

A model calibration is not necessarily required when comparing a set of spectra for trend analysis purposes, for example, using the moving-block approach to estimate statistical parameters such as mean, median, and standard deviation. For example, blend-uniformity monitoring using NIR spectroscopy has adopted such data analysis approaches. Appropriate spectral ranges and algorithms must be used for trend analyses.

6.2.2 SPECIFICITY

The relative discriminatory power and selectivity for trend analysis must be demonstrated. The extent of specificity testing is dependent on the application and the risks being controlled. Variations in matrix concentrations within the operating range of the method must not affect the trend analysis.

6.3 Quantitative Analysis

6.3.1 SPECTRAL REFERENCE LIBRARY FOR A CALIBRATION MODEL

Calibration is the process of constructing a mathematical model to relate the response from an analytical instrument to the properties of the samples. Any calibration model that can be defined clearly in a mathematical expression and gives suitable results can be used. Record the spectra of a suitable number of representative samples with known or future-established values of the attribute of interest (for example, content of water) throughout the range to be measured. The number of samples for calibration will depend on the complexity of the sample matrix and interferences (e.g., temperature, particle size, and others). It is encouraged to take a risk management approach to identify critical interferences that may need to be assessed. All samples must give quantitative results within a calibration interval as defined by the intended purpose of the method. Multiple linear regression, principal component regression (PCR), and partial least squares regression (PLS) are commonly used. For PLS or PCR calibrations, the regression coefficients and/or the loadings should be plotted, and the regions of large coefficients or loadings should be compared with the spectrum of the analyte. Predicted residual error sum of squares plots or similar plots are useful for facilitating the optimization of the number of PCR or PLS factors. For more information, see ISO 12099.

6.3.2 PRETREATMENT OF DATA

Wavelength selection, or exclusion of certain wavelength ranges, may enhance the efficiency of calibration models. Wavelength compression (wavelength averaging) techniques, multiplicative signal correction (MSC), standard normal variate transformation (SNV), and derivatives may be applied to the data.

6.3.3 MODEL VALIDATION PARAMETERS

Analytical performance characteristics to be considered for demonstrating the validation of NIR methods are similar to those required for any analytical procedure. Specific acceptance criteria for each validation parameter must be consistent with the intended use of the method. Validation parameters for quantitative methods are accuracy, linearity over the operational range, precision (repeatability and intermediate precision), robustness, and specificity.

6.3.4 ONGOING MODEL EVALUATION

NIR models validated for use are subjected to ongoing performance evaluation and monitoring of validation parameters. If discrepancies are found, corrective action is necessary.

7. PROCEDURE VALIDATION

Validation of NIR methods will follow the same protocols described in *Validation of Compendial Procedures* ⟨1225⟩ in terms of accuracy, precision, and other suitable parameters. It may be necessary to determine QL for methods of detection and quantification of an impurity or polymorphic form.

Detector linearity must be confirmed over the range of possible signal levels. Method precision must also encompass sample position. The sample presentation is a critical factor for both solids and liquids, and must be either tightly controlled or accounted for in the calibration model. Sample-position sensitivity can often be minimized by appropriate sample preparation or sample holder geometry, but will vary from instrument to instrument on the basis of excitation and optical configuration. In addition, many suitable chemometric algorithms for data pretreatment and calibration are available. Selection of an algorithm should be based on sound scientific judgment and suitability for the intended application.

7.1 Ongoing Method Evaluation

Validated NIR methods should be subject to ongoing performance evaluation, which may include monitoring accuracy, precision, and other suitable method parameters. If performance is unacceptable, corrective action is necessary. This involves conducting an investigation to identify the cause of change in method performance, and may indicate that the NIR method is not suitable for continued use. Improving the NIR method to meet measurement suitability criteria may require additional method development and documentation of validation experiments demonstrating that the improved method is suitable for the intended application. The extent of revalidation that is required depends on the cause of change in method performance and the nature of corrective action needed to establish suitable method performance. Appropriate change controls should be implemented to document ongoing method improvement activities.

Revalidation of a qualitative model may be necessary as a result of the following:

- Changes in instrument hardware
- Addition of a new material to the spectral reference library
- Changes in the physical properties of the material
- Changes in the source of material supply
- Identification of previously unknown critical attribute(s) of material(s)

Revalidation of a quantitative model may be necessary as a result of the following:

- Changes in the composition of the test sample or finished product
- Changes in the manufacturing process
- Changes in the sources or grades of raw materials
- Changes in the reference analytical method
- Major changes in instrument hardware

7.2 Method Transfer

Controls and measures for demonstrating the suitability of NIR method performance following method transfer are similar to those required for any analytical procedure. Any exceptions to general principles for conducting method transfer for NIR methods should be justified on a case-by-case basis. The transfer of an NIR method is often performed by using an NIR calibration model on a second instrument that is similar to the primary instrument used to develop and validate the method. When a calibration model is transferred to another instrument, procedures and criteria must be applied to demonstrate that the calibration model meets suitable measurement criteria on the second instrument. The selection of an appropriate calibration-model transfer procedure should be based on sound scientific judgment.

USP REFERENCE STANDARDS ⟨11⟩

USP Near IR System Suitability RS

GLOSSARY

[Note—This section refers to the technique of NIR in general. Please see *Analytical Instrument Qualification* ⟨1058⟩ and ⟨1039⟩ for additional information.]

Absorbance: Absorbance (A) is represented by the equation:

$$A = -\log T = \log (1/T)$$

where T is the transmittance of the sample. Absorbance is also frequently given as:

$$A = \log (1/R)$$

where R is the reflectance of the sample.

Background spectrum: This is used for generating a sample spectrum with minimal contributions from instrument response; it is also referred to as a “reference spectrum” or “background reference”. The ratio of the sample spectrum to the background spectrum produces a transmittance or reflectance spectrum dominated by NIR spectral response associated with the sample. In reflection measurements, a highly reflective, diffuse standard reference material is used for the measurement of the background spectrum. For transmission measurement, the background spectrum may be measured with no sample present in the spectrometer or using a cell with the solvent blank or a cell filled with appropriate reference material.

Diffuse reflectance: Ratio of the spectrum of radiated light (penetrating the sample surface, interacting with the sample, and passing back through the sample’s surface) reaching the detector to the background spectrum. This is the component of the overall reflectance that produces the absorption spectrum of the sample.

Fiber-optic probe: This consists of two components: optical fibers that may vary in length and number, and a terminus, which contains specially designed optics for examination of the sample matrix.

Installation qualification: The documented collection of activities necessary to establish that an instrument is delivered as designed and specified and is properly installed in the selected environment, which is suitable for the instrument’s intended purpose.

Instrument bandwidth or resolution: A measure of the ability of a spectrometer to separate radiation of similar wavelengths.

Operational qualification: The process of demonstrating and documenting that an instrument performs according to specifications and that it can perform the intended task. This process is required following any significant change, such as instrument installation, relocation, or major repair.

Overall reflectance: The sum of diffuse and specular reflectance.

Performance qualification: The process of using one or more well-characterized and stable reference materials to verify consistent instrument performance. Performance qualification may employ the same or different standards for different performance characteristics.

Photometric linearity: Also referred to as “photometric verification”, it is the process of verifying the response of the photometric scale of an instrument.

Pseudo-absorbance: A, is represented by the equation:

$$A = -\log R = \log (1/R)$$

where R is the diffuse reflectance of the sample.

Reference spectrum: See *Background spectrum*.

Reflectance: Is described by the equation:

$$R = \frac{I}{I_R}$$

in which I is the intensity of radiation reflected from the surface of the sample and I_R is the intensity of radiation reflected from a background reference material and its incorporated losses due to solvent absorption, refraction, and scattering.

Root-mean-square (RMS): Calculated using the equation:

$$\mathrm{RMS} = \sqrt{\sum_{i=1}^{N} \frac{\left(\hat{A} - A_i\right)^2}{N}}$$

where $\hat{A}$ is the mean absorbance over the spectral segment; A_i is the absorbance for each data point; and N is the number of points per segment.

Specular (surface) reflectance: The reflectance from the front surface of the sample, where the angle of reflection matches the angle of incidence (mirror effect).

Standard error of calibration (SEC): A measure of the capability of a model to fit reference data. SEC is the standard deviation of the residuals obtained from comparing the known values for each of the calibration samples to the values that are calculated from the calibration. SEC should not be used as an assessment tool for the expected method accuracy (trueness and precision of prediction) of the predicted value of future samples. The method accuracy should generally be verified by calculating the *Standard error of prediction (SEP)* using an independent validation set of samples. An accepted method is to mark a part of the calibration set as the validation set. This set is not fully independent but can be used as an alternative for the determination of the accuracy.

Standard error of the laboratory (SEL): A calculation based on repeated readings of one or more samples to estimate the precision and/or accuracy of the reference laboratory method, depending on how the data were collected.

Standard error of prediction (SEP): A measure of model accuracy of an analytical method based on applying a given calibration model to the spectral data from a set of samples different from, but similar to, those used to calculate the calibration model. SEP is the standard deviation of the residuals obtained from comparing the values from the reference laboratory to those from the method under test for the specified samples. SEP provides a measure of the model accuracy to be expected when one measures future samples.

Surface reflectance: Also known as "specular reflection", it is that portion of the radiation not interacting with the sample but simply reflecting back from the sample surface layer (i.e., the sample–air interface).

Transflection: A transmittance measurement technique in which the radiation traverses the sample twice. The second time occurs after the radiation is reflected from a surface behind the sample.

Transmittance: Represented by the equation:

$$T = \frac{I}{I_0} \text{ or } T = 10^{-A}$$

where I is the intensity of the radiation transmitted through the sample; I_0 is the intensity of the radiant energy incident on the sample ; and A is the absorbance.▲ (USP 1-May-2020)

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[1] Burgess C, Hammond J. Wavelength standards for the near-infrared spectral region. *Spectroscopy*. 2007;22(4):40–48.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
<1856> NEAR-INFRARED SPECTROSCOPY - THEORY AND PRACTICE	Yang Liu Manager, Product Quality and Analytical Methods	GCCA2020 General Chapters - Chemical Analysis 2020

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