2.27 Near Infrared Spectrometry

Near infrared spectrometry is one of spectroscopic methods used to qualitatively and quantitatively evaluate samples from the analysis of data obtained by determining their absorption spectrum of light in the near-infrared range.

The near-infrared range lies between the visible light and infrared light, typically of wavelengths (or wave numbers) between 750 and 2500 nm (13,333 to 4000 cm^{-1}). The absorption of near-infrared light occurs due to harmonic overtones from normal vibration or combination tones in the infrared range from 2500 to 25000 nm (4000 to 400 cm^{-1}), primarily absorption of O-H, N-H, C-H and S-H that involve hydrogen atoms, in particular.

Absorption in the near-infrared range is far weaker than absorption due to normal vibration that occurs in the infrared range. Furthermore, in comparison with visible light, near-infrared light has longer wavelength, which makes it possible for the light to penetrate to a depth of several mm into solid samples including fine particles. This method is often utilized as a nondestructive analysis, as changes occurring with an absorbed light spectrum (transmitted light or reflected light) in this process provide physical and chemical information pertaining to samples.

Near-infrared spectrometry is used as a rapid and nondestructive method of analysis that replaces conventional and established analysis methods. It is necessary to perform a comparison test to evaluate this method against an existing analysis method, to verify that this method is equivalent to such existing analysis method, before using this analysis method as an evaluation test method for quality control.

Applications of this method include qualitative or quantitative evaluation of active ingredients, additives or water contents of drug substances or preparations. Furthermore, near-infrared spectrometry can also be used for the evaluation of physical conditions of substances, such as crystal forms, crystallinity, particle diameters. It is also possible to perform spectrometry on samples that are located in a remote location away from equipment main units, without sampling, by using optical fibers. It can therefore be used as an effective means to perform pharmaceutical manufacturing process control online (or in-line).

1. Equipment

Near-infrared spectrophotometers mainly include a distributed near-infrared spectrophotometer and a Fourier transform near-infrared spectrophotometer.

1.1. Distributed near-infrared spectrophotometer

This equipment is comprised of a light source section, sample section, spectrometry section, photometry section, signal processing section, data processing section, and display-record output section. Halogen lamps, tungsten lamps, light emitting diodes and other such devices that can emit high intensity near-infrared light in a stable manner are used in the light source section. The sample section is comprised of a sample cell and a sample holder. Equipment with an optical fiber section that is comprised of optical fibers and a collimator is equipped with a function for transmitting light to the sample section, which is remotely located away from the spectrophotometer main unit. Quartz is ordinarily used as material for optical fibers.

The spectrometry section is intended to extract light of required wavelength, using dispersive devices and is comprised of slits, mirrors and dispersive devices. The photometry section is comprised of detectors and amplifiers. Sensors include semiconductor detectors, as well as photomultiplier tubes. Detecting methods that use semiconductor detectors generally perform detections with single elements, but there are also occasions where array type detectors that use multiple elements are used. Such detectors are capable of simultaneously detecting multiple wavelengths (or wave numbers). The signal processing section separates signals required for measurements from output signals fed by amplifiers and then outputs such isolated signals. The signal processing methods include analog processing and digital processing.

1.2. Fourier transform near-infrared spectrophotometer

The configuration of the equipment is fundamentally the same as that of the distributed-type equipment described in Section 1.1., except for the spectrophotometry section and the signal processing section.

The spectrophotometry section is comprised of interferometers, sampling signal generators, detectors, amplifiers, A/D conversion devices, etc. The signal processing section is equipped with functions that are required for the spectrometer, as well as a function for translating an acquired interference waveform (interferogram) into an absorption spectrum by Fourier transformation.

2. Measurement method

There are three types of measurement methods that are used with near-infrared spectrometry: transmittance method, diffuse reflectance method and transmittance reflectance method. The selection of measurement methods relies on the shape of samples and applications. For example, the transmittance method or diffuse reflectance method is used for solid samples, including fine particles, and the transmittance method or transmittance reflectance method is used for liquid samples. The measurement mode, etc. of equipment are selected and set.

2.1. Transmittance method

The degree of decay for incident light intensity as the light from a light source passes through a sample, is represented as transmittance rate T (%) or absorbance A with the transmittance method.

This method is applied for taking measurements of samples that are liquids and solutions. Quartz glass cells and flow cells are used, with the layer length of 1-5 mm along. Furthermore, this method can also be applied for taking measurements of samples that are solids including fine particles. It is also known as the diffuse transmittance method. Selecting appropriate layer length is critical for this method, since the transmitted light intensity varies depending on grain sizes and surface condition of samples.

2.2. Diffuse reflectance method

The ratio of reflection light intensity I emitted from a sample in a wide reflectance range and control reflection light intensity I_r emitted from the surface of a control substance is expressed as reflectance R (%) with the diffuse reflectance method. The near-infrared light penetrates to a depth of several mm into solid samples including fine particles. In that process, transmission, refraction, reflection and dispersion are repeated, and diffusion takes place, but a portion of the diffused light is emitted again from the surface of the sample and captured by a detector. The spectrum for the diffuse reflectance absorbance (A_r) can ordinarily be obtained by plotting logarithm of inverse numbers for reflectance against wavelengths (or wave numbers).

This method is applied to solid samples including fine particles, and requires a diffuse reflector such as a probe.

2.3. Transmittance reflectance method

The transmittance reflectance method is a combination of the transmittance method and reflectance method. A mirror is used to re-reflect the light that has passed through a sample in order to measure transmittance reflectance rate, T^* (%). The light path must be twice the thickness of the sample. On the other hand, the light reflected by a mirror and being introduced into a detector is used as the control light. When this method is applied to suspended samples, however, a metal plate or a ceramic reflector with a rough surface that causes diffuse reflectance is used instead of a mirror.

This is a method that is applied to solid samples, including fine particles, as well as liquids and suspended samples. The thickness of a sample must be adjusted when applying this method to a solid sample. Ordinarily adjustment is made by setting absorbance to 0.1 – 2 (transmittance of 79 – 1%), which provides the best linearity and SN ratio of a detector. A cell with appropriate layer length, according to the grain size of the fine particle, must be selected when applying the method to a fine particle sample.

3. Factors that affect spectra

Following items must be considered as factors that can affect spectra when applying near-infrared spectrometry, particularly when conducting quantitative analysis.

(i) Measurement conditions: A significant change (wavelength shift, for example) can occur when the temperature varies by a several degrees (℃). Care must be taken, particularly when a sample contains water. Also, water or residual solvent contents of a sample, as well as water (humidity) in the environment where in measurements are taken, can significantly affect absorption bands of the near-infrared range.

The thickness of a sample is a factor for spectral changes and therefore needs to be controlled at a certain thickness. Furthermore, since the condition of sample fill can potentially affect spectra when taking measurements of samples that are solids or fine particles, care must be taken with filling samples in a cell, to ensure that a certain amount is filled through a specific procedure.

Samples can potentially undergo chemical, physical or optical property changes, due to passing of time or storage after sampling. In creating calibration curves, therefore, it is necessary to pay attention that the samples for calibration curves must be prepared with adequate considerations for reducing the time to be measured, such as the measurement is carried out offline in a laboratory or online in manufacturing process (or inline).

(ii) Characteristics of samples: When a sample is physically, chemically or optically uneven, relatively large beam size must be used, multiple samples must be used, measurements must be taken at multiple points on the same samples, or a sample must be pulverized to ensure averaging of the sample. Grain size, fill condition, as well as roughness of surfaces can also affect the spectra of fine particle samples. Since variations in crystal structures (crystal polymorphism) can also affect spectra, in cases where multiple crystal forms exist, care must be taken to ensure that even standard samples for the calibration curve method have diversified distributions similar to that of samples to be analyzed.

4. Control of equipment performance

4.1. Accuracy of wavelengths (or wave numbers)

The accuracy of wavelengths (or wave numbers) of equipment is derived from the deviation of suitable substances for which peak absorption wavelengths (or wave numbers) have been defined, such as polystyrene, mixture of rare earth oxides (dysprosium, holmium and erbium; 1:1:1) or steam, from the figures indicated on the equipment. Tolerance figures in the vicinity of 3 peaks are ordinarily set in the following manner, though appropriate tolerance figures can be set depending on the intended purpose:

1200 ± 1 nm (8300 ± 8 cm^{-1})
1600 ± 1 nm (6250 ± 4 cm^{-1})
2000 ± 1.5 nm (5000 ± 4 cm^{-1})

Since the location of absorption peaks vary, depending on the substance used as reference, absorption peaks of wavelengths (or wave numbers) that are closest to the above 3 peaks are selected for suitability evaluation. A mixture of rare

earth oxides, for instance, would indicate characteristic absorption peaks at 1261 nm (7930 cm^{-1}), 1681 nm (5949 cm^{-1}) and 1971 nm (5074 cm^{-1}).

The absorption peak of steam at 1368.6 nm (7306.7 cm^{-1}) can be used with a Fourier transformation type spectrophotometer, as its wave number resolution ability is high.

Other substances can also be used as the reference, so long as their adequacy for the purpose can be verified.

4.2. Spectroscopic linearity

Appropriate standard plates, such as plate-shaped polymer impregnated with varying concentrations of carbon (Carbon-doped polymer standards), can be used to evaluate spectroscopic linearity. In order to verify linearity, however, standard plates with no less than 4 levels of concentration within the reflectance of 10 – 90% must be used. When measurements are expected to be taken with absorbance of not less than 1.0, it is necessary to add standard plates with the reflectance of either 2% or 5% or both.

In order to plot absorbance of such standard plates at locations in the vicinity of wavelengths 1200 nm (8300 cm^{-1}), 1600 nm (6250 cm^{-1}) and 2000 nm (5000 cm^{-1}) against absorbance at each wavelength (or wave number) assigned to each standard plate, ensure that the gradient of linearity and ordinate intercept obtained are ordinarily within the ranges of 1.00 ± 0.05 and 0.00 ± 0.05, respectively. Depending on the intended purpose, appropriate tolerance figures can be set.

5. Application of qualitative or quantitative analysis

Ordinarily, chemometrics methods are used for analyzing a near-infrared absorption spectrum. Conventional spectrometric methods, such as a calibration curve method, may be used as a method whenever applicable. Chemometrics ordinarily involve the quantification of chemical data, as well as numerical and statistical procedures for computerization of information. Various types of multivariate analysis are used as chemometrics for near infrared spectrometry, and are selected according to the intended purpose. Characteristics of near-infrared absorption spectrum must be emphasized and effects of complexities of spectra, as well as overlay of absorption bands must be reduced by performing mathematical preprocesses, such as primary or secondary spectral differentiation processes or normalizations, which becomes one of vital procedures in establishing analysis methods that use methodologies of chemometrics.

In near-infrared spectroscopy, sustaining and managing performance of an analysis method, once established, are critical. Continuous and systematic maintenance and inspection work are required. Furthermore, it is necessary to pay attention to whether or not appropriate evaluation procedures are available to deal with change controls or implementation of re-validation on changes made in manufacturing processes or raw materials, as well as changes arising from replacement of major components in equipment.

5.1. Qualitative analysis

Qualitative analysis is performed for each substance to be analyzed after preparing a reference library that includes inter-lot variations within the tolerance range and establishing an analysis method using chemometrics methodology. The identity of substances can be confirmed by comparison with a standard spectrum or by methods using validated chemometrics software. Also, substances can be identified by their absorbance bands.

Furthermore, multivariate analysis includes direct analysis methods that consider wavelengths (or wave numbers) and absorption as variables, such as wavelength correlation method, residual sum of squares, range sum of squares, along with factor analysis method, cluster analysis method, discriminant analysis method and SIMCA (Soft independent modeling of class analogy) that are applied after processing such as principal component analysis.

It is also possible to consider the overall near-infrared absorption spectrum as a single pattern and to identify parameters obtained by applying multivariate analysis methods or characteristic wavelength (or wave number) peaks of components to be analyzed as indices for monitoring, for the purpose of manufacturing process control for drug substances or preparations.

5.2. Quantitative analysis

Quantitative analysis ordinarily uses spectra of sample groups and analysis values obtained through the existing and established analysis methods, to obtain quantitative models with methodologies of chemometrics. These are used to calculate concentrations of individual ingredients and material values of samples being measured, using conversion formulas. Chemometrics methodologies for obtaining quantitative models include multiple regression analysis method and PLS (Partial least squares) regression analysis method.

In cases where the composition of a sample is simple, concentrations of ingredients in the sample to be analyzed can be calculated by plotting a calibration curve using the absorbance of a specific wavelength (or wave number) or the correlating relationship between the parameters and concentration, using samples for preparation of calibration curves with known concentrations (calibration curve method).