

13.3 Spectroscopy: Electromagnetic Radiation for Identifying and Measuring the Ingredients of Food

H. J. Kersting and H. Zwingelberg

13.3.1 Principles of Spectroscopy

Spectroscopy describes the interactions between electromagnetic radiation (e.g. light) and material.

Besides a knowledge of the laws governing absorption, emission, excitement, resonance etc., the correlation between the concentration of the absorbent substance and the intensity of the light is extremely important (Lambert-Beer Law).

These interactions are recorded and represented in the form of spectra. The information to be gained from these spectra depends on the properties of the substances analyzed and the nature or wavelength range of the radiation acting on them and the different interactions that result. Fig. 79 shows the various sectors of the electromagnetic spectrum.

Spectra can reveal information about the properties of emitters (radiation sources) or of irradiated samples or secondary emitters.

In principle the permeability of a sample to electromagnetic radiation (transmittance) is measured as a function of the wavelength.

The difference between the radiation energy reaching the sample and that leaving the sample is detected in the spectrum. This difference is termed transmittance.

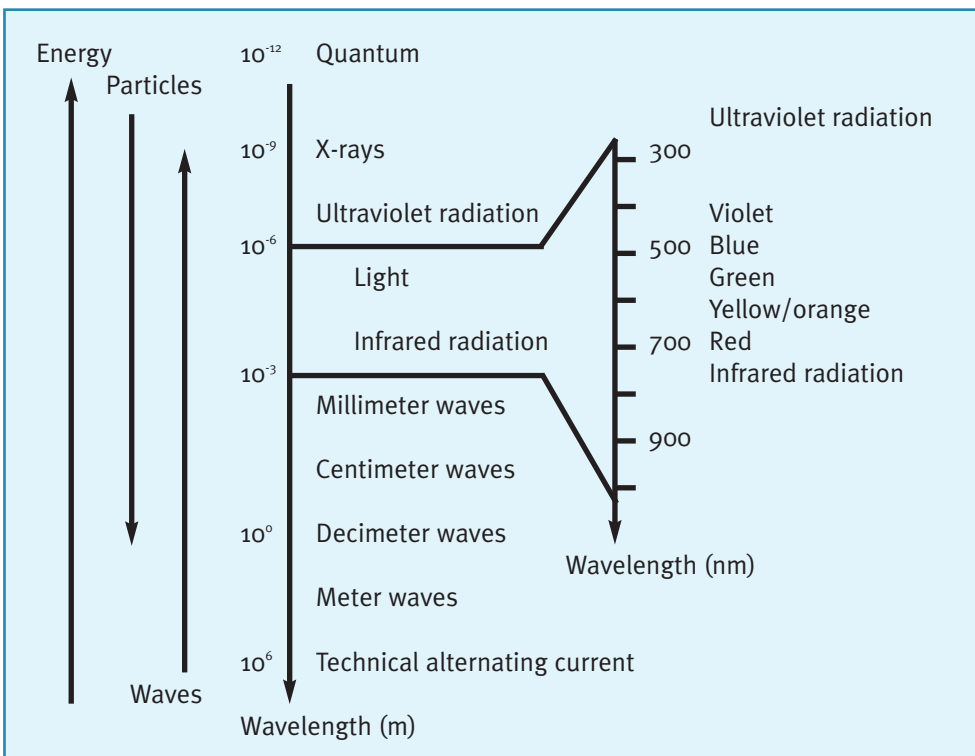


Fig. 79: Electromagnetic spectrum

In process engineering, electromagnetic radiation can be used to differentiate between raw material qualities, to describe the properties of the end product and to control the process itself. On the basis of the interactions between electromagnetic radiation and material a distinction is made between emission, absorption, scatter and reflection spectra.

Emission spectra

occur with substances that are naturally luminous or whose luminosity has been induced. Induced luminosity is caused by the fact that molecules or atoms have been excited optically and thus taken up energy. The energy previously taken up is emitted by the material, mainly in the form of fluorescence and phosphorescence.

Absorption spectra

Since a body absorbs the type of radiation it emits when excited, spectra can also be viewed from the point of view of absorption. Bodies or materials that absorb all the radiation reaching them are called "black bodies". This expression is in line with the everyday use of language, in which a substance that absorbs visible radiation almost completely is termed "black". Conversely, a body that reflects all radiation is called a "white body". The principle of physics according to which the radiation emitted by a body must equal the radiation absorbed is expressed by Kirchhoff's Law.

Scatter spectra

The characteristic changes in radiation caused by scattering against particles are documented in scatter spectra. In particular the observation and analysis of laser-light scatter spectra in which precise measurements of intensity make it possible to follow the movements of molecules, e.g. in polymers, or Brownian motion, have led to a better understanding of dynamic processes in complex substances and systems.

Reflection spectra

If a powder is irradiated with light, part of the light reaching it is absorbed and a further part is emitted as a diffuse reflection after multiple

scattering by the powder particles and subsequently split up into a spectrum. This is how reflection spectra are produced.

The different kinds of spectra described above can be subdivided into continuous, line, atomic, and band or molecular spectra.

As we have already said, the uptake of electromagnetic radiation can cause the molecules to vibrate. The vibrational spectra of pure substances and mixtures produced in this way are as characteristic as a fingerprint. This is especially true of near-infrared spectra, from which it is possible to determine the identity of substances and the composition of complex mixtures with the aid of computers.

13.3.2 NIR Spectrometry

The NIR method may be regarded as "applied spectroscopy", which means that it falls under the physical analysis procedures (spectral analysis). It is a quick method by which individual organic components of a mixture of substances can be determined both qualitatively and quantitatively on the basis of physical measurements. Identification, receiving and final inspections and in-process analysis – carried out non-destructively in seconds, without any great preparation of the samples – are making this method increasingly important. NIR spectrometry is not a method for trace analysis. The limits of detection and quantitation are in the percent range.

The near-infrared (NIR) region is the wavelength range between 800 nm and 2,500 nm ($12,500\text{ cm}^{-1}$ to $4,000\text{ cm}^{-1}$) of the electromagnetic spectrum (Fig. 79). This range is between the mid-infrared region at longer wavelengths and the visible region at shorter wavelengths.

In the near-infrared region, molecular groups such as -OH, -NH, -CH and -CO are caused to vibrate very strongly.

The -OH vibrations are characteristic of compounds such as alcohols, phenols, acids, keto alcohols and hydroxy esters; the group of -NH vibrations represents both primary and secondary amines and amides. Aromatic

compounds, olefins and paraffins are reflected by the -CH vibrations. The -CO bands occur with carboxylic acids, carboxylic acid esters, ketones, alcohols and also aldehydes.

Tab. 65 gives examples of typical band positions for these molecular groups.

140

Since these groups are typical components of food ingredients such as water, protein, fats and carbohydrates, the NIR method is particularly suitable for characterizing foods.

As we have already mentioned, quantitative information from the NIR technique is based on the Lambert-Beer Law.

$$E = \lg \frac{I_0}{I} = \varepsilon \cdot c \cdot d$$

E = Absorbance

I_0 = Initial intensity of the light ray

I = Intensity of the weakened light ray

ε = Decadic absorption coefficient

c = Concentration of the substance (mol/L)

d = Length of the optical path/thickness (m)

In principle there are two ways of determining the absorption properties of a sample to be analyzed: by detecting the diffuse reflected light (NIR instruments) or by detecting the transmitted light (NIT instruments).

Tab. 65: Wavelengths of typical bands of molecular groups

Group	Wavelength (nm)				
-CH ₃	860-1090	1125-1255	1340-1480	1670-1830	2200-2500
-CH ₂	860-1090	1150-1255	1360-1480	1675-1830	2250-2500
-CH	860-1090	1125-1255	1340-1480	1670-1830	2250-2500
R-CONH ₂	1000-1070	1120-1150	1430-1550	1900-2020	
R-CONHR	1000-1070	1120-1150	1340-1500	1980-2090	2110-2230
R-NH ₂	1000-1070	1460-1600	1950-2050	2060-2210	
R-NHR	1000-1060	1470-1550	1560-1660	2020-2230	
CH ₂ OH	960-1100	1395-1635	1995-2160		
CHOH	940-1100	1390-1630	1980-2170		
CHOR	940-1080	1400-1600	1950-2150		
H ₂ O	1370-1600	1845-2200			
RCOOR	1870-1950	2060-2200			
RCOR	1865-1975	2090-2180			
RSSR	1800-2060				

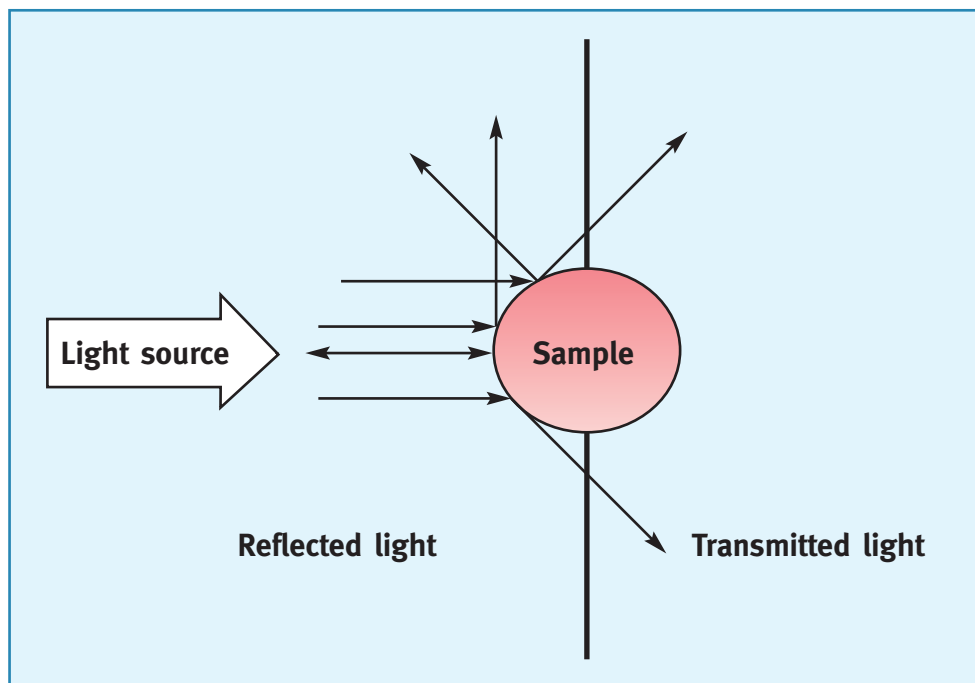


Fig. 8o: Splitting of light into a reflected part and a transmitted part by a solid, opaque sample

The difference between reflection and transmission techniques lies in the physical position of the detector. In the case of the reflection technique the detector receives the light reflected by the sample and is therefore in front of the sample chamber, whereas in the transmission technique the detector is situated behind the sample compartment (Fig. 8o).

In the case of solid, opaque samples it is usually the energy diffusely reflected by the sample that is measured rather than the transmitted energy. This is done by calculating the ratio of the energy reflected by the sample to the total reflection of an internal standard.

$$R = \frac{I_s}{I_o}$$

R = Diffuse reflection
 I_s = Intensity of light reflected by the sample
 I_o = Intensity of light reflected by the internal standard

The values for reflection are shown as a common logarithm of the quotient 1/reflected energy.

Further subdivisions can be made according to the different optical systems. For example, a distinction should be made between single-beam and double-beam systems.

Besides solid-filter systems, which have long been used for standard applications in the food industry, monochromator systems are gaining significance since the greater volume of data generated by them provides more information for characterizing ingredients. In the monochromator, light is split up into its spectrum and a narrow wavelength range is then isolated by means of a slit. In Fourier transform spectrometers a modulator is used instead of the monochromator. This modulator uses one modulating frequency for each wavelength, thus allowing a definite allocation of wavelengths to frequencies. This dispenses with the need for spatial dispersion of the

electromagnetic radiation and allows simultaneous measurement of the information for all wavelengths.

A further advantage of NIR/NIT spectroscopy besides the small amount of work required for sample preparation is doubtless that it permits quick, non-destructive analysis of the materials to be tested. The possibility of online use in routine procedures and the fact that chemicals are not usually required make NIR spectroscopy a user-friendly method.

Various techniques of sample analysis such as whole grain measurement, cell measurement and measurement by means of glass-fibre probes in containers of various kinds have contributed to an increased use of this method. By using light-conducting fibre-optic probes it is possible to examine the sample at a considerable distance from the measuring instrument. For example, analyses of transparent and opaque solids can be carried out with fibre lengths of 1 m to 50 m. This means that the sample no longer has to be conveyed to the site of the equipment (e.g. the laboratory); the measuring cell comes to the sample – a great advantage in many sectors of routine analysis.

143

Moreover, the method permits "in-line" measurement for continuous characterization of materials and process control.

The choice of the instrument and the measuring technique to be used depends on the frame of reference and the planned application (Tab. 66).

Examples of the uses of such techniques are to be found in the publications of Zwingelberg, *et al.* (1987), and Bolling and Gerstenkorn (1987). These studies show how the NIR method can be used to determine quality parameters of products made from wheat and wheat flour.

The information derived from the NIR measurements depends to a very large extent on the parameters chosen for sample preparation and on the measuring parameters. To ensure reproducibility of the measurements and the results derived from them it is essential to maintain corresponding conditions (similar conditions for preparation and measurement).

The evaluation of scatter spectra makes it possible to assess the hardness of grain and the lightness of its colour. However, when determining quality parameters a distinction has to be made between values that can be determined directly and those that must be determined indirectly. The protein, water and starch content of a sample and also its fat content are variables that can be ascertained directly from NIR data, since their spectroscopic signals are attributable to defined molecular vibrations (vibrations of the amide and hydroxyl group).

Tab. 66: Suitable measuring techniques for various applications and types of sample

Nature of the sample	Application	Measuring technique
Clear or slightly light-dispersing liquids	Carbohydrate solutions	Transmission
	Doughs	
Opaque liquids, suspensions, foams	Liquids	Transmission
	Suspensions	Reflection
	Foams	
Powder, granular solids	Flours, grains	Reflection
		Transmission

Values determined indirectly include the sedimentation rate, damage to the starch, water absorption and baked volume. These are derived from the IR vibration spectra by calculation.

Specific uses of NIR transmission spectroscopy for ascertaining the quality of individual grains in respect of moisture and protein content and grain hardness are the subject of various publications.

As we have already said, one of the great advantages of the NIR applications is their speed. Constituents that contribute to the value of a product can be determined simultaneously within a few minutes and called up in sequence if the spectrometer is suitably calibrated.

NIR Methods and Procedures

The choice of the sample preparation method (whole grain or powder sample) is of central importance for the conduct of NIR analyses. For both measuring procedures the homogeneous condition of the material is the most important parameter. If the material is comminuted, the absorption behaviour of the sample depends on the degree of grinding. The light from the source is altered by the sample and then recorded by detectors; a computer then converts it to a figure that shows the amount of the constituent present.

The routine use of this method does, however, require extensive calibration for the specific constituents or parameters that are to be determined. The ratio of reflection to transmission is determined by measuring samples with known levels of the constituents concerned, and this ratio is stored as a calibration constant. Comprehensive calibration requires a large number of samples varying as widely as possible; the amount of the desired constituent must be determined in each sample by reference analysis.

The influence of variability is taken into account by the fact that spectra determined over a long period are stored in special

reference libraries. These are used to establish mean spectra that provide information on possible deviations.

For standard applications with ground products (water, protein, starch and fat content), filter instruments have been used successfully for years. Their construction makes them especially suitable for the production sector.

However, the filter instruments only cover those wavelength ranges which have been set in order to determine the constituents to which they relate. Besides the filter instruments there are also scanning instruments, which are used chiefly to analyze complex substances. Since these scanning instruments use the whole NIR wavelength range for calibration they generate a larger volume of data and thus enable much more information on the samples to be collected and evaluated.

Many aspects of quality assurance can be covered quickly and at low cost with the very accurate FT-NIR spectrometers.

FT-NIR and AOTS (acusto-optical-tunable scanning) systems are further developments that make systematic use of the possibilities offered by modern microelectronics in the fields of signal generation, control engineering and data processing.

In recent years IR spectroscopy has become an important tool, with applications that range from routine identity and purity testing through qualitative and quantitative analysis of reaction mixtures to the identification of unknown compounds. One reason for this development is the enhanced use of IR technology in conjunction with microelectronics by the manufacturers and the systematic practical implementation of the theoretical benefits of these IR methods. A further reason is the flood of new regulations and the demands they make on quality control and quality assurance, which has speeded up the use of IR spectroscopy.

The introduction of the ISO 9000 series of standards has resulted in a tremendous increase

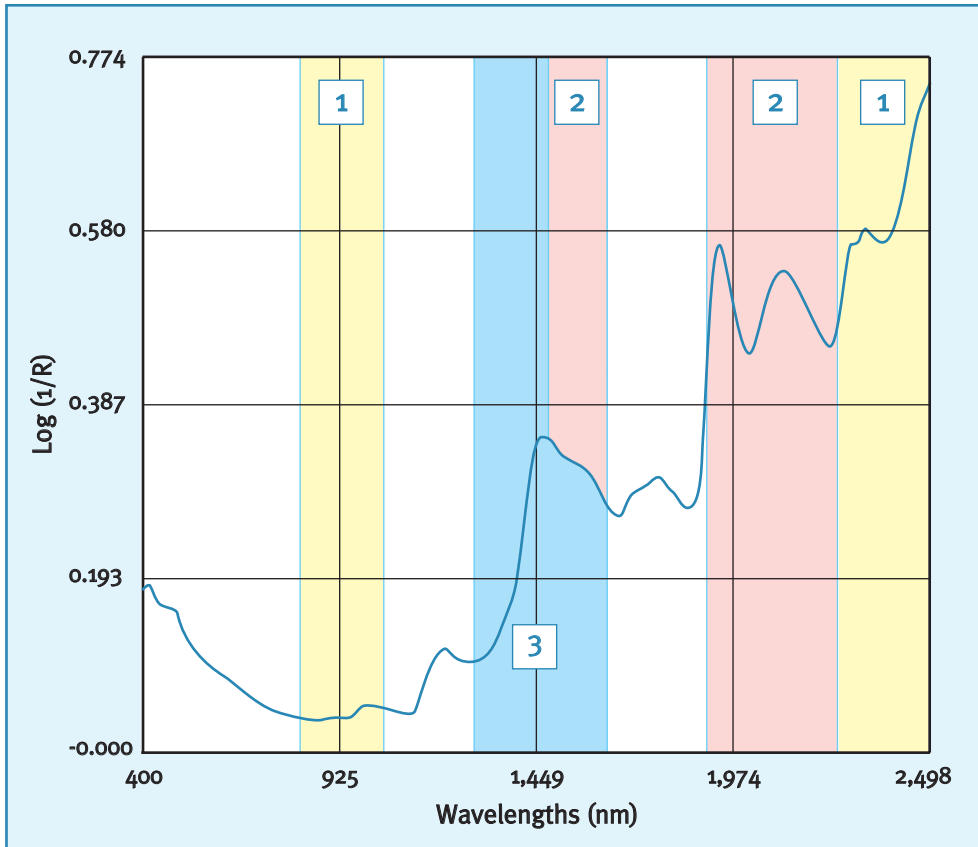


Fig. 81: NIR spectrum (reflection) of a Type 550 flour

in the analytical work accompanying the production process. This applies both to the speed of analysis and the number of analyses to be performed. In particular near-infrared spectroscopy (NIR) has gained importance in this sector.

The future will doubtless see an increase in the importance of networks and questions of the administration of near-infrared networks under practical conditions in the field of spectral analysis.

Fig. 81 shows an NIR spectrum of a Type 550 flour measured by the reflection method. In this spectrum the various group vibrations are allocated to the relevant wavelength range as an example.

The NIR bands occurring are seen to correlate with the functional molecule groups. The $-\text{CH}$, $-\text{CH}_2$ and $-\text{CH}_3$ vibrations occur in region 1; region 2 represents the vibrations of the $\text{R}-\text{CONH}_2$, $\text{R}-\text{CONHR}$, $\text{R}-\text{CNH}_2$ and $\text{R}-\text{CNHR}$ groups; region 3 shows the $-\text{OH}$ vibrations.

When evaluating the spectra it should be taken into account that various spectral regions often overlap, making a definite allocation of of wavelengths to molecular groups virtually impossible. In the above example the regions 2 and 3 overlap in the wavelength range around 1,450 nm.

Only when further regions of the spectrum are considered is it possible to allocate them with certainty.

13.3.3 References

- Alava JM, Millar SJ and Salmon SE, 2001. The determination of wheat breadmaking performance and bread dough mixing time by NIR spectroscopy for high speed mixers. *J. Cereal Sci.* 33(1):71-81.
- Anon., 1996. NIT-Analysengerät. Mühle Mischfuttertechnik 133(24):398.
- Bolling H and Gerstenkorn P, 1987. Möglichkeiten und Grenzen beim Einsatz der NIR-Messtechnik für Züchtung, Erfassung und Verarbeitung von Getreide. In: Bericht über die 13. Getreide-Tagung, p 23.
- Deblasi RA, Cope M, Elwell C, Safoue F and Ferrari M, 1993. Noninvasive Measurement of Human Forearm Oxygen Consumption by Near Infrared Spectroscopy. *Eur. J. Appl. Physiol. Occupational Physiol.* 67(1):20.
- Delwiche SR and Massie DR, 1996. Classification of wheat by visible and near-infrared reflectance from single kernels. *Cereal Chem.* 73(3):309.
- Delwiche SR, Mckenzie SR and Webb BD, 1996. Quality characteristics in rice by near-infrared reflectance analysis of whole-grain milled samples. *Cereal Chem.* 73(2):257-263.
- Delwiche SR, 1995. Single Wheat Kernel Analysis by NIR-Transmittance; Protein Content. *Cereal Chem.* 72(1):11-16.
- Delwiche SR, Chen YR and Hruschka WR, 1995. Differentiation of Hard Red Wheat by NIR Analysis of Bulk Samples. *Cereal Chem.* 72(3):243.
- Delwiche SR, Pitt RE and Norris KH, 1991. Examination of Starch-Water and Cellulose-Water Interactions With Near Infrared (NIR) Diffuse Reflectance Spectroscopy. *Starch/Stärke* 43(3):85.
- Felder R, 1996. NIR-On-Line Messung an Mehl und Gries. *Getreide Mehl Brot* 50(3):147.
- Gerold J, 2002. NIR-Messtechnik zur Qualitätssicherung und Prozeßoptimierung bei der Getreideannahme. *Getreide Mehl Brot* 1(1):15-17.
- Halasz A, Hassa A, Toth A and Varadi M, 1997. NIR techniques in the yeast identification. *Z. Lebensm. Unters. Forsch.* 204:72-74.
- Kohlus R, Zürner I, Bottlinger M and Jansen HD, 1994: Neuronale Netzwerke zum Auswerten von NIR-Spektren (Teil 1). *ZFL, Int. Z. Lebensm.-Technol.-Verfahrenstech.* 45 (3):55.
- Mika V, Bartko A, Nerusil P and Smital F, 1997. The use of NIR method for evaluation of the rape quality. *Rostl Vyroba* 43(6):283-286.
- Millar S, 2000. Mixed fractions. *European Baker* (5/6):18-23.
- Oelichmann J, 2002. Was macht die NIR-Spektroskopie so attraktiv? *GIT-Labor-Fachzeitschrift* (8):906-907.
- Conzen P, 1998. Methodvalidierung in der quantitativen NIR-Spektroskopie. *GIT Labor-Fachzeitschrift* 22:97.
- Wehrle K, Seibel W, Gerstenkorn P and Kuhn M, 1996. Schnellmethoden zur qualitativen Beurteilung von Durumweizen. 1. Mitteilung: NIR-Untersuchungen. *Getreide Mehl Brot* 50(3):181.
- Wesley IJ, Larsen N, Osborne BG and Skerritt JH, 1998. Non-invasive Monitoring of Dough Mixing by NIR. *J. Cereal Sci.* 27:61-69.
- Zwingelberg H, Bolling H and Gerstenkorn G, 1987. Zur Möglichkeit des erweiterten Einsatzes der NIR-Spektroskopie bei der Erfassung von Weizen. Sonderdruck aus Bericht über 38. Müllereitagung.
- Zwingelberg H, 1982. Infrarotspektroskopie. Bedeutung und Messung der Kornhärte. *Getreide Mehl Brot* 36:16.