Near Infrared Spectroscopy

What is it?

Near Infrared Spectroscopy (NIRS) is an analytical technique based on the absorption of infrared radiations by organic matter. This absorption is linked to the chemical composition; thus this latter can be estimated by the simple measurement of light absorption by the sample.

This measurement is done with a spectrometer, either in “transmission” mode (light going through a thin sample) or in “reflection” mode (light reflected by a thick sample).

NIRS requires a calibration phase based on reference measurements performed in the laboratory by “classical” methods (chemical composition, nutritional value, etc.) and the establishment of mathematical models which allow to relate the infrared spectrum to the results of these measurements.

NIRS has a wide range of applications in the industry (chemicals, pharmaceutical, agro-industries). At Animal Feed Laboratory of Cirad it is used to estimate the chemical composition of animal feeds, forages, animal products (meat), faecal samples (digestibility studies).

This technique has several advantages:

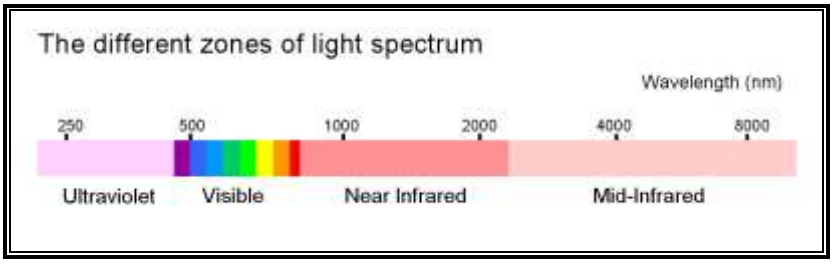
- ***It is rapid***: The spectrum of a sample can be obtained within a few minutes, allowing the immediate prediction of composition. This speed has to be compared with the duration of a classical analysis in the laboratory, which sometimes requires several days.
- ***It is non-destructive***: The sample is recovered intact after the analysis. This property is particularly important for samples available in low quantity or samples which have to be kept for further analysis or other purposes.
- ***It does not require large sample quantity***: Generally 4 to 5 grams are sufficient! In some conditions, spectra can be done on extremely low quantities: less than 1 gram samples, and even on a single seed (which is useful in plant genetic studies). The principal limit to the reduction of the quantities is to ensure that the sample is representative of the matter to be analysed (feed, forage, etc.).
- ***It is cheap***: Apart from the initial investment (cost of the spectrometer) and the building (or purchase) of the calibration equation for every product, the marginal cost of analysis for a sample is extremely low. The only cost is the “maintenance” of calibrations, which consists of the laboratory analysis of samples from time to time in order to ensure that the calibration remains valid through time and well adapted to new samples. Less than 10% control analyses are generally performed, and even less when the calibration is stable.

However it is important to know that:

- NIRS can generally not be used to evaluate minerals (as Ca, P) since it is based on the absorption of light by organic molecules. Nevertheless, total minerals (ashes) can be predicted as this information is equivalent to total organic matter.
- In most cases trace elements cannot be estimated by NIRS because their spectral signal is too weak compared to that of major compounds.

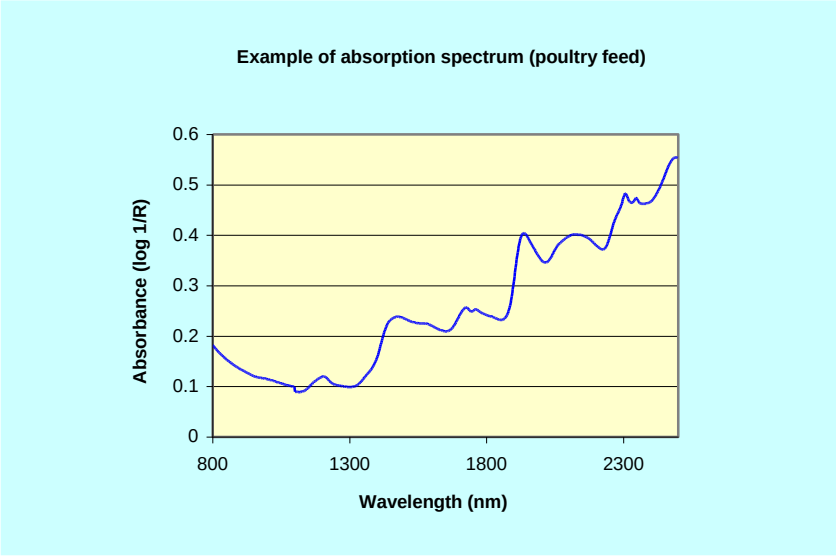
Principle

“Infrared” is the name given to the radiation corresponding to wavelengths directly above those of visible light. By convention “Near Infrared” corresponds to wavelengths between 800 and 2500 nm.



The absorption of radiations by samples depends on the composition of organic matter. Indeed, the chemical bounds can absorb the energy of light photons corresponding to particular frequencies. It is this property of chemical bonds of organic matter that is used by Near Infrared Spectroscopy to establish a link between the absorption of light and chemical composition (or other properties) of the sample.

In order to perform this analysis, the sample is lighted with a range of light wavelengths (or frequencies). The series of data represented by the absorption of light at each of these wavelengths is called the “spectrum” of the sample. This spectrum can include several hundreds of wavelengths for which light absorption has been measured.



The spectrum is characteristic of a sample since it gathers the information (quantity and characteristics) of every organic component (proteins, fat and fatty acids, fibre, etc.). Two samples with the same spectrum would be strictly similar.

This wealth of information constitutes at the same time the advantage and the difficulty of NIRS analysis: a lot of information is present in a spectrum, but it is completely mixed! To overcome this problem, complex statistical methods have to be applied in order to relate chemical analyses to their fingerprint in spectra: this is the calibration phase. Every calibration equation is specific of a particular chemical parameter in a particular material: this work has thus to be done on each new raw material.

How to read a calibration?

A calibration is generally a linear regression between the characteristics of samples (*e.g.* content of chemical compounds) and the infrared information presented as spectrum, the values of absorbance at various wavelengths being a series of variables. The interpretation of results therefore uses the criteria (R^2, etc) classically used to evaluate the quality of a linear regression equation

The knowledge of the principal criteria of model characterization allows to evaluate quickly the quality of the calibrations presented in a report or a publication. The example below (calibration of composition parameters in a forage) presents the synthetic table used to describe a calibration. Three groups of complementary information are presented: *i)* description of the population of samples on which the calibration was established, *ii)* quality of the regression and *iii)* indicators of validation, *i.e.* the precision which can be expected when the calibration will be used in practice.

	Population			Calibration			Validation		
Parameter	N	Mean	Std	Terms	SEC	R^2	SECV	SEP	RPD
O.M.	236	93.0	1.54	11	0.25	0.97	0.34	0.40	4.5
C. Prot.	226	12.0	4.92	9	0.32	1.00	0.41	0.43	12.0
NDF	237	62.8	7.83	8	1.12	0.98	1.29	1.45	6.1
ADF	236	33.5	5.60	9	0.61	0.99	0.77	0.85	7.3
ADL	237	5.5	1.84	8	0.34	0.97	0.42	0.80	4.3

Characterization of the population on which the model was built

Number, mean and standard deviation of the samples used in the development of calibration. It is generally considered that a calibration has to be built on a minimum number of 60-100 samples depending on the nature of the materials. However, in certain conditions a low number of samples (40-60) can allow a « feasibility study » for a given product.
To obtain robust calibration it is better to work on a population covering a good variability.

Statistical characteristics of the calibration model

SEC (standard error of calibration) and R^2 (Coefficient of determination) are indexes of the precision of the calibrations and of adjustment of data.

A "good" model is obtained when the SEC is reasonably close to the repeatability of the analysis by the reference method.

Terms = Number of variables in the calibration equation.

Criteria of model validation

SECV[1] and SEP[2] are indicators of the precision that can be expected when the calibration model will be used with unknown samples. The difference is that SECV estimates this capacity on samples from the population used during
model development, whereas SEP uses completely new samples. These two criteria should have values as close as possible to the repeatability of reference analysis. Also a large difference with SEC can indicate a lack of stability of the calibration.

RPD[3] is the ratio between the variability of the population (Standard Deviation SD) and the precision of calibration (SECV or SEP). This ratio is therefore an indication of the information brought by the model. It is often considered that a model is valuable when RPD values are above 3-4, and very good above 6 … but this judgement has to consider the variability of the initial population and the precision of reference analysis. A "good" model is basically a model which is useful in practice !

[1] Standard error of cross-validation
[2] Standard error of prediction
[3] Ratio performance / deviation

Examples of applications

Nutritional value of tropical forages

Thanks to the thousands of samples available in the collections of animal feed laboratory of Cirad, it was possible to compute calibrations for the chemical composition and nutritional value of several tropical forages. The development of calibrations adapted to specific cases can be based on this important existing information.

The size of the database allows to use “local calibrations”: for a given sample, it is possible to select a subset of similar samples in order to compute a prediction equation exactly adapted to this sample.

Digestibility studies

In animal feeding the digestibility of forages and feeds is an extremely important information. In some cases NIRS allows an estimation of digestibility parameters from the faeces of animals[1]. Some studies concern the feeding of domestic or wild animals in rangelands. The reduction of analytical work thanks to the use of NIRS analysis allows much more powerful study protocols, with in particular surveys on a higher number of animals through space and time. Classical analytical methods would not allow to get results on the hundreds – sometimes thousands – of samples required for such protocols.
In another area, NIRS allowed, in collaboration with INRA, to measure the digestibility of feeds on thousands of chicks and to study the individual variability and heritability of this parameter[2].

Variability and quality of by-products used in animal feeding

Studies on composition and nutritional value of crop by-products (sorghum) have been done to estimate the genetic variability of these parameters. Indeed, with a same production of grain, quantity and quality of sorghum stems and leaves can vary widely – which make it useful to integrate these parameters during the selection of varieties. But these studies involve a very high number of measurements and the use of NIRS allows an amplification of selection protocols[3].

[1] Lecomte P, Fall S, Friot D. Richard D, Ickowicz A, Guerin H et Bonnal L. 2003. Calibration de critères de valeur alimentaire *in vivo* sur les données spectrales proche Infrarouge des fèces émises par l’animal. Cas de rations distribuées à des ruminants tropicaux (Sénégal). Présented at 10th congress « Recherches Rencontres Ruminants ».
[2] Mignon-Grasteau S, Muley N, Bastianelli D, Gomez J, Péron A., Sellier N, Millet N, Besnard J, Hallouis JM, Carré B., 2004. Heritability of digestibilities and divergent selection for digestion ability in growing chicks fed on a wheat diet. Poultry Science. 83:860-867.
[3] Kondombo C.P. 2001. Evaluation agronomique et fourragère de 194 lignées recombinantes de sorgho. Mémoire de fin d'études pour l'obtention du diplôme d'ingénieur du développement rural. Université Polytechnique de Bobo-Dioulasso.

The Animal Feed Laboratory of Cirad

- Proposes calibrations for various products: principal tropical forages, raw materials, feeds, …
- Develops calibrations in the framework of research projects in partnership with various organisations. The close link between NIRS activities and chemical laboratory allows the adaptation of existing calibration to new series of samples.
- Provides a methodological support for the integration of the technique in research protocols or development projects, with the objective of reducing analytical work / costs and allow measurements to be done on a large number of samples.
- Can advice and help its partners for the technology transfer, network works and skill development.
- Proposes theoretical and practical training to NIRS technique.

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