Portable infrared spectrometers: analytical strategies, challenges, and perspectives

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Analytical Chemistry and Chemometrics in Como





Smart analytical methods and applied chemometrics group



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University of Insubria Como







Activities

- Development of analytical methods (possibly smart and non destructive) for real matrices
 - Spectroscopic techniques mainly ATR and portable MIR and NIR spectroscopy
 - Chromatographic techniques
- Implementing control process systems based on multivariate analysis

Activities

- Teaching:
 - Analytical chemistry lab
 - Application of analytical chemistry (cooperative learning)
 - Chemometrics
 - Experimental design for PhD students





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Portable spectroscopy

- ATR-FT-MIR 4100 ExoScan FTIR (4000 650 cm⁻¹, Agilent)
- MIR Alpha (7500 and 375 cm^{-1} , Bruker)
- NIR
 - NeoSpectra Development kit (1350-2558 nm -Si-Ware)
 - NeoSpectra Scanner (1350-2550 nm -Si-Ware)
 - SCiO (740 1070 nm -Consumer Physics)









Visiting professor at URV

Wine fermentation process

Miniaturized IR sensors

Potentiometric sensors



Why portable spectrometers?

The lab goes to the sample



Adaptability to on-line requirements





The lab goes to the sample

Cultural heritage: samples often cannot be moved to the lab

Forensic sciences: need for rapid on-site analyses, to be confirmed later in the laboratory (if necessary)

Agriculture: follow the maturation of a product directly on the plant

... just to name a few examples...







On-line measurements

Process control practices are becoming more and more fundamental today, in some cases mandatory.

Portable sensors adapt more easily to processes and are also cheaper than the benchtop ones.







Advantages of portable spectrometers (when used in optimal conditions)

Almost no sample pre-treatment needed: the analysis is often performed on the sample as it is

Less chemicals used (often just to clean the spectrometer) and less waste (or none)

Less analysis expenses (no sample transport and storage)

Cheaper instrumentation if compared with benchtop one

Less waste at the end of their life, when they break down and can no longer be used.

Drawbacks

Reduced spectroscopic range and spectral resolution

- Less instrumental parameters that can be optimized
- Equipment are not so optimized (they are quite new in the market...)

The measurements could be very different from the benchtop spectrometers -> need for new analytical strategies

ALL THAT

GOLD





Portable, handheld or miniaturized?

There is no consensus in the literature about the distinction between portable and miniaturized devices.

Focusing on the size of the device, field spectrometers can be divided into transportable (deployable on field while mounted in a car), portable in a suitcase (>4 kg of total weight) and handheld (<1 kg) ones*.

Miniaturized instruments would be those having the size of a book or less.

*Crocombe, R. A. Portable Spectroscopy. Appl. Spectrosc., 2018, 72, 1701-1751

Infrared radiation

The infrared range is usually divided into three regions:

near infrared (NIR - nearest the visible spectrum), with wavelengths 0.78 to about 2.5 micrometres

middle infrared (MIR), with wavelengths 2.5 to about 50 micrometres

far infrared (FAR), with wavelengths 50 to 1000 micrometres.

Molecular vibrational frequencies lie in the IR region of the electromagnetic spectrum.





Electromagnetic Spectrum

Vibrational spectroscopy

Fundamental molecular vibrations

- Mid IR (less energetic IR radiation)
- Raman

Overtones and combination bands

• NIR (more energetic IR radiation)



How to choose a portable IR sensor?

Let's start by analyzing some characteristics of the sample: solid, powder, liquid?

NIR PORTABLE SPECTROMETERS: the majority of them are not made for liquid/powder analysis













908-1676

nm

64 g

(4.5 *x* 5.0 cm)

< 30,000 €



1596–2396
nm
1.2 kg
, 0 (a

(24.4 x 23.2 x 9.7 cm) < 50,000 € 17

740–1070 nm

35 g (0.315 × 0.95 × 2.75 cm) < 5,000 €

Way of measurements – in some cases, a certain inventiveness is needed



Kefir fermentation



How can we analyze a sample whose chemical and physical properties change over time?

How to choose a portable IR sensor?

Let's start by analyzing some characteristics of the sample: solid, powder, liquid?

Is the sample thin or thick?

Surface or bulk analysis?

Penetration depth

In general, the shorter the wavelength of infrared radiation, the greater the penetration depth.

NB: Exposure to IR radiation is perceived as heat!

Mid - IR

• microns: suitable for analyzing the surface of a solid sample

NIR

• millimeters: suitable for analyzing the bulk of a solid sample

Penetration depth

if it is necessary to determine the ripeness of an apple directly on the plant, the radiation cannot stop at the peel but must penetrate inside the fruit to give useful information -> NIR probably is the best solution!

PS thickness but also absorptivity have to be considered



There is another aspect that needs to be considered, which is the homogeneity or heterogeneity of the sample.

Heterogeneity of the sample is also one of the main reason to perform sample pretreatment when using portable spectrometers. Why does sample homogeneity influence the choice of a specific IR spectrometer?

It depends on the size of the analysis window, i.e. on the surface of the analyzed sample.

In these pictures two portable NIR spectrometers that work in external reflection mode are reported.

The NIR spectrometer in the upper figure has a10mm spot collection surface, useful for not homogeneous samples, while the sensor in the lower figure has a 3mm spot size, more appropriate to homogeneous samples (e.g. extremely fine powder).





How can we deal with sample homogeneity?





Can the proposed analytical method be considered portable if sample pretreatment is required?

Necessary to consider the pros and cons of each pretreatment the sample is subjected to: e.g. grinding a sample could heat it and thus change its physicochemical characteristics To capture the variability of heterogeneous samples it is necessary to analyze the sample in different points (or different aliquots) by repositioning it each time on the instrument.

Replicates



Warning!



The window of the instrument can get hot... very hot!

Wet samples could change their humidity, and thermolabile samples could even change their chemical form!



Warning!



This can also affect your choice of number of scans and scan time!

NB: Almost all NIR sensors are equipped with tungsten halogen lamps, except for some (e.g. SCiO), which has an LED source.

Humidity change

The image shows the forage NIR signal during analysis, leaving the sample stationary. The instrument window heats up and the peak of the water changes.

The sample is drying!





How to choose a portable IR sensor?

Another thing to consider:

Need of interpret the spectra?

Need to obtain information from the signal?

Signals obtained: ultramarine pigment in linseed oil on canvas

MIR spectra



MIR signals show sharp peaks, attributable to the vibrations of the functional groups.

NIR peaks are large, overlapped and difficult to interpret.

NIR spectra



Way of analysis... and strategies developed by our group



External reflection mode



Widely applicable and allows to acquire IR spectra in a non-destructive way with no sample preparation.

Sensitivity to the sample surface properties, e.g. texture or external defects, needs to be noted.

The scattering effects caused by sample morphology may be manifested in the spectrum as well.

There are several configurations: specular, diffuse...





The spectrometer does not work in contact with the painting. It is necessary to correct the recorded signal by making a background that considers the ambient light.

Since the purpose of the analysis is to analyze the pigments, which is the most superficial part of the painting, a MIR spectrometer working in external reflection mode is used.



This miniaturised NIR sensor in this case is analyzing a sample of red rice without any sample pretreatment.

Need to optimize the distance from the sample (in this case in contact but it is not the standard - in some cases, best spectra are recorded at a certain distance from the sample), number of replicates, and considering the tilting of the sensor that can affect the measurements 36



This NIR sensors is used here for forage analysis. The forage has been ground and placed in contact with a window from which the radiation comes out through the sample holder cell shown in the figure.





A home-made cell has been developed for food powder analysis (among them, insect powder analysis) without apply any pressure on the powder

In connection with the PC

In the upper photo, a milk sample is measured directly in a beaker during fermentation. In the bottom one, samples of olive oil are measured with different configurations.

The choice of the container in which to place the sample can influence the analysis: the material of the container could absorb some of the radiation, as in this case.













light Source detector sample - K. Reflective Coating

Transflection

If sample thickness and/or absorptivity is not high enough to yield a spectrum with an adequate SNR, transflectance (transflection) mode may be applied.

Use an IR reflective element.

Liquid or thin solid samples.

The beam transmitted through sample is reflected to pass through the sample again before reaching the detector.

The optical path is effectively doubled in this mode.



Transflection

In this case, we have studied a "homemade" cell to analyze olive oil in transflection.

A plastic ring was used to contain the sample, which was glued with Loctite Super Glue 3 to a borosilicate cover slip. A 200 μ L drop of the sample was deposited and covered with another borosilicate cover slip on the top of the plastic ring. This volume was enough to cover the whole surface of the optical window included in the plastic ring, ensuring a reproducible optical path in all the measurements. The homemade cell was placed on the top of the NeoSpectra optical window, and the Spectralon reference (reflective material) was placed on the top of the home-made cell.



Transflection





Transmission mode

Typically used for acquisition of the spectra of liquid or sufficiently thin solid samples.

Liquid samples are placed in a cell (cuvette) made of optical material transparent for IR radiation.

The effective optical path needs to be in the range of micrometers to acquire the spectra (organic materials have high IR absorptivity).

Most portable IR instruments are not designed to work in transmission mode.

The sensor in the picture is sold with an accessory that allows the analysis of liquids in transmission mode with the use of a cuvette.



ATR mode

The infrared light passes through a crystal of a certain material (diamond, ZnSe or germanium) and interacts with the sample, which is in contact with the crystal.

Light undergoes multiple internal reflections in the crystal of high refractive index. The number of reflections may be varied by varying the angle of incidence.

The penetration depth into the sample is typically between 0.5 and 2 micrometres.







ATR – MIR portable spectrometer



This spectrometer is used here to follow the progress of wine fermentations. A drop of sample is placed on the crystal of the instrument and the spectrum is recorded.

ATR – MIR portable spectrometer



For powder samples it is necessary to apply constant pressure and therefore an appropriate accessory.



Multivariate data analysis

Collect measurements on samples to be used to calibrate the system -> build the calibration model (classification/prediction)

Spectroscopic data pretreatment and variable selection

Model validation -> test of the model on new samples

Study of the system performances (errors made by the model)



error in the raw data? Intrinsic analytical and instrumental errors? Data uncertainty?

Collect measurements on samples to be used to calibrate the system -> build the calibration model (classification/prediction)

Spectroscopic data pretreatment and variable selection

Model validation -> test of the model on new samples

Study of the system performances (errors made by the model)



On the importance of investigating data uncertainty

Understanding the sources of errors in raw data ightarrow improve the quality of measurements

Knowing the uncertainties → manage and treat the data suitably (preprocessing, extraction of chemical information, diagnostics)

Identifying the error shape \rightarrow monitor instrumental performances

Multivariate measurement error

Estimation through replication Theoretical prediction Empirical modeling



Wentzell et al. Implications of measurement error structure on the visualization of multivariate chemical data: hazards and alternatives. Can. J. Chem. 2018, 96, 738–748, doi:10.1139/cjc-2017-0730



The outer product of the measurement error vector **e** with itself gives rise to the error covariance matrix $\boldsymbol{\Sigma} = E(\boldsymbol{e} \cdot \boldsymbol{e}^T)$

$$= \begin{bmatrix} \sigma_1^2 & \sigma_{12} & \sigma_{13} & \dots & \sigma_{1n} \\ \sigma_{12} & \sigma_2^2 & \sigma_{23} & \dots & \sigma_{2n} \\ \sigma_{13} & \sigma_{23} & \sigma_3^2 & \dots & \sigma_{3n} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ \sigma_{1n} & \sigma_{2n} & \sigma_{3n} & \dots & \sigma_n^2 \end{bmatrix}$$

The error covariance matrix is a $n \times n$ square symmetric matrix with **diagonal elements**, $\sigma_{j=k}$, containing the **variance at each channel** and the **off-diagonal elements**, $\sigma_{j=k}$, containing the **covariance** of the measurement errors at channels *j* and *k*



all of the diagonal elements are equal to 1 and all of the off-diagonal elements have values that can range from -1 to 1

The error correlation matrix provides information that may be qualitatively hidden in the covariance matrix due to scale

Error covariance matrix gives information regarding the magnitude of the errors and their relationships By comparing the error variances of each channel is possible to determine whether the errors are independent or related to one another Error correlation matrix shows these relationships independent of scale

Wentzell, P.D. Measurement Errors in Multivariate Chemical Data. J. Braz. Chem. Soc. 2014, , https://doi.org/10.5935/0103-5053.20130293.



Type of replicates:

Instrumental replicates

Subsequent acquisitions without sample displacement

Experimental replicates

Acquisition after resampling and positioning on the instrument





Comparison between instrumental and experimental replicates



5 samples with different properties (raw protein content, ashes, neutral detergent fiber content, dry matter)





Spectra preprocessing



174 samples with different properties (raw protein content, ashes, neutral detergent fiber content, dry matter) Each sample is represented by the mean of three experimental replicates spectra



Raw spectra

the error covariance matrix become more normal distributed --> A condition for PLS regression models was better satisfied

Different package forms and instruments



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ECMs shapes typical for NIR

Main errors present: Offset noise \rightarrow error values above 0

Multiplicative noise \rightarrow error proportional to the wavelength Shot noise \rightarrow electronic error

Low independent error in general → high correlation of the error

ECMs differ between instrument → different technologies, spectral ranges, resolution, window width

ECMs differ within instruments is influenced by sample compactness

Measurement error covariance and correlation matrix for sugar lump spectra (a) and granulated sugar spectra(b) acquired with AvaSpec-Mini-NIR(a) and for sugar lump spectra (c) and granulated sugar spectra(d) acquired with NeoSpectra Scanner.

Independent session of acquisition

ECM calculated on experimental replicates according to the session of acquisition

For NeoSpectra Scanner absolute values are on average lower for sugar lumps than for granulated sugar → combination of sessions

Diagonal of the error covariance matrices calculated over session replicates for AvaSpec-Mini-NIR data: (a) sugar lump spectra (b) granulated sugar spectra and for NeoSpectra Scanner data: (c) sugar lump spectra (d) granulated sugar spectra

Variance at each channel is the typical for NIR errors

Absolute values are different for distinct sessions within samples

Discrepancy is bigger when considering granulated sugar than for sugar lumps







Multivariate Limit of Detection (LOD)







Predicted vs measured plot for fats content model. a) Results for SCiO data, b) Results for NeoSpectra data

 h_0 = leverage that quantifies the distance of the predicted sample at zero concentration to the mean of the calibration set

v = degree of freedom

RMSEC = Root Mean Squares Error in Calibration

 $\Delta(\alpha, \beta, v) = \alpha$ and β probabilities of wrongly concluding the presence/absence of analyte, when $v \ge 25$, $\Delta(\alpha, \beta, v) \rightarrow 2$

$$\Delta(\alpha, \beta, v) \rightarrow 2$$

 $LOD = \Delta(\alpha, \beta, v) \cdot RMSEC \cdot \sqrt{1 + h_0}$

Klaas, N.; Faber, M.; Bro, R. Standard error of prediction for multiway PLS: 1. Background and a simulation study. Chemom. Intell. Lab. Syst., 2002, https://doi.org/10.1016/S0169-7439(01)00204-0

And what about other effects?

Order of replicates Session of analysis Timing of background Charge ... and any interaction between them?



Reading suggestion





Article

Miniaturized NIR Spectrometers in a Nutshell: Shining Light over Sources of Variance

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Abstract: The increasing portability and accessibility of miniaturized NIR spectrometers are promoting the spread of in-field and online applications. Alongside the successful outcomes, there are also several problems related to the acquisition strategies for each instrument and to experimental factors that can influence the collected signals. An insightful investigation of such factors is necessary and could lead to advancements in experimental set-up and data modelling. This work aimed to identify variation sources when using miniaturized NIR sensors and to propose a methodology to investigate such sources based on a multivariate method (ANOVA—Simultaneous Component Analysis) that considers the effects and interactions between them. Five different spectrometers were chosen for their different spectroscopic range and technical characteristics, and samples of worldwide interest were chosen as the case study. Comparing various portable sensors is interesting since results could significantly vary in the same application, justifying the idea that this kind of spectrometer is not to be treated as a general class of instruments.

Reading suggestion

.: Miniaturization in NIR Spectroscopy Reshapes Chemical Analysis

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Smartphone-based sensor technology, in conjunction with chemometrics and machine learning, is advancing applications such as agricultural and environmental field monitoring.

KRZYSZTOF B. BEC AND CHRISTIAN W. HUCK, UNIVERSITY OF INNSBRUCK

Near-infrared (NIR) spectroscopy has been used in groundbreaking research in many of the life sciences¹ due to its capacity to rapidly determine and analyze the composition of materials. Bulky instruments with limited portability, however, have kept the technology from being used in field applications. Current trends in miniaturization, however, have opened up new realms in which NIR spectroscopy would never have functioned years ago, providing on-site, flexible, and accurate tools for agricultural and environmental analysis and food quality inspection. This is especially true in the herbal medicine and food industries, where smartphone-operated sensors can be easily used in remote locations for rapid on-site analysis — for example, for in-field monitoring of medicinal plants.



Reading suggestion



Analytical Chemistry Strategies in the Use of Miniaturised NIR Instruments: An Overview

Barbara Giussani, Giulia Gorla & Jordi Riu

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Giulia Gorla and Dani Schorn, and all my students



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