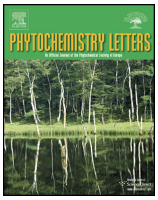
Phytochemistry Letters xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol



Advances of infrared spectroscopy in natural product research

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ARTICLE INFO

Article history:
Received 1 October 2014
Accepted 23 October 2014
Available online xxx

Keywords:
IR spectroscopy
Near infrared
Mid infrared
Imaging spectroscopy
Natural products

ABSTRACT

Natural product's properties are related to certain classes of compounds such as alkaloids, flavonoids, essential oils and others. Traditionally, separation techniques including thin layer chromatography (TLC), liquid chromatography (LC), gas chromatography (GC) and capillary electrophoresis (CE) even hyphenated to mass spectrometry (MS) were used for the elucidation, qualitative and quantitative analysis of individual compounds.

In food industry, spectroscopic investigations using infrared radiation have been used to monitor and evaluate the composition and quality already since the early sixties. During the last four decades near-infrared spectroscopy (NIR; 800–2500 nm; 12,500–4000 cm^{-1}) has become one of the most attractive and used methods for analysis for the following reasons: it represents a non-invasive analytical tool allowing a fast and simultaneous qualitative and quantitative characterization of natural products and their constituents. Additionally, the development of custom-made hand-held instruments enables in-field measurement for determining the optimum harvest time.

Attenuated total reflection (ATR) and Fourier transform infrared (FTIR) spectroscopic imaging are suitable not only for the differentiation of different plant species, but also to distinct various ingredients within a plant. FTIR spectroscopic microscopy enables molecular imaging of complex botanical samples and therefore the detection and characterization of the molecular components of biological tissue.

In the present contribution, the principle, technique and methodology of the different infrared spectroscopic methods are described followed by a discussion of quantitative and qualitative application possibilities in the field of natural product analysis.

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1. Introduction

Infrared (IR) radiation is the region of the electromagnetic spectrum between the visible (VIS) and the microwave wavelength (Mcclure, 2003). In near-infrared (NIR) spectroscopy excitation of molecules is accomplished in a wavelength range between 750 and 2500 nm, corresponding to a wavenumber range between 4000 and 13,000 cm^{-1} (Herschel, 1800). Solid, liquid or gaseous samples can absorb parts of the incoming IR radiation at specific wavelength resulting in a fingerprint or a spectrum (Blanco and Villarroya, 2002). In this spectral region molecules containing C–H, C–O, C=O, N–H and O–H functional groups are excited to perform stretching-, deformation- and scissor-vibrations. In comparison to the mid-infrared (MIR; 4000–400 cm^{-1}) region, where only fundamental vibrations ("signatures") can be observed, overtones and combinations can be found in the NIR region containing a manifold of information compared to MIR (Barton, 2002). The result is often a crowded spectrum containing overlapping peaks. Although NIR-intensities are 10–1000 times lower than for the MIR, highly sensitive spectrometers can be built through several means including the use of efficient detectors (Mcclure, 2003). The light recorded by the detector contains compositional information, which can be unraveled by a computer to report multiple analyses almost instantaneously. NIR spectroscopy can provide simultaneous, rapid and non-destructive qualitative and quantitative analysis of major components in many organic substances (Guggenbichler et al., 2006; Huck-Pezzei et al., 2013; Schönbichler et al., 2014). The NIR spectrum is represented by a huge number of partially overlapping overtones and combination vibrations. Additionally to these vibrations, scattering effects, instrumental noise and/or sample in-homogeneities might occur (Mcclure, 2003). As a consequence it is in

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Abbreviations: ATR, attenuated total reflection; CA, cluster analysis; CE, capillary electrophoresis; CEC, capillary electro chromatography; DR, diffuse reflection; FPA, focal plane array; FT, Fourier transform; LC, liquid chromatography; MCT, mercury cadmium telluride; MEIRS, material enhanced infrared spectroscopy; MIA, multivariate image analysis; MLR, multiple linear regression; MVA, multivariate analysis; NIR, near infrared spectroscopy; PCA, principal component analysis; PLSR, partial least square regression; TCM, Traditional Chinese Medicine; Vis, visible.

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http://dx.doi.org/10.1016/j.phytol.2014.10.026
1874-3900/© 2014 Published by Elsevier B.V. on behalf of Phytochemical Society of Europe.

Please cite this article in press as: Huck, C.W., Advances of infrared spectroscopy in natural product research. Phytochem. Lett. (2014), http://dx.doi.org/10.1016/j.phytol.2014.10.026

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many applications impossible to correctly assign the corresponding vibration bands. NIR in combination with multivariate statistical analysis (MVA) is a powerful combination enabling the extraction of the required information from the spectrum (Blanco and Villarroya, 2002). The most appropriate chemometrical procedure is principal component analysis (PCA) for reducing the number of variables facilitating both qualitative and quantitative analysis. For establishing suitable quantitative models, a selected set of samples has to be analyzed by highly efficient reference methods, mainly based on separation technologies including liquid chromatography (LC) (Stöggl et al., 2005, 2004), capillary electrophoresis (CE) (Huck et al., 2011), solid-phase extraction (SPE) (Sultan et al., 2005) and mass spectrometric (MS) techniques (Najam-ul-Haq et al., 2007; Vallant et al., 2007).

Attenuated total reflectance (ATR) is a sampling technique used in conjunction with especially mid-infrared spectroscopy. The ATR technique enables samples to be examined directly in the solid or liquid state without further preparation (TCH_FTIRATR.pdf). Thereby, light undergoes multiple internal reflections in a crystal of high refractive index. The sample is in contact with the crystal and due to the penetration of IR radiation into the sample an efficient signal-to-noise (SNR) can be obtained (Schönbichler et al., 2014).

Another new spectroscopic method, which is generally called Fourier transform infrared (FTIR) spectroscopic microscopy for examination of plant, food and human tissue sections has been introduced (Pallua et al., 2011a). This technique represents a powerful tool in histological characterization and allows the investigation of the spatial distribution of proteins and small molecules within biological systems with high spatial resolution (Pallua et al., 2011b). Imaging enables probing samples under native conditions and offers new insights into samples without the need for fixation or an additional marker (Pallua et al., 2011b; Pezzei et al., 2010).

In the following chapter, technical achievements and data-processing methods in vibrational spectroscopy of natural products are summarized, their efficiencies are discussed and compared to other more conventional analytical techniques.

2. Methods

In this chapter the main principles of near-infrared (NIR), attenuated total reflection (ATR) and imaging/mapping spectroscopy are discussed. In the following part, selected applications in the fields of natural product analysis will be examined carefully.

2.1. Near-infrared (NIR) spectroscopy

"Near" in NIR is related on the position of the electromagnetic energy lying next to or near the visible (Vis) energy range. Molecular vibrations in the MIR cover absorptions in a range between 400 and 4000 cm^{-1} representing the most intense and simplest bands, whereas NIR bands arise in the interval between 4000 and 12,500 cm^{-1} covering absorptions corresponding to overtones and combinations of fundamental vibrations. The basic principle describing the vibrating system of a diatomic molecule can be described by the harmonic and anharmonic oscillator (NIS, 2008).

A FT-NIR instrument consists of an e.g. tungsten-halogen light source, interferometer, light-fiber optics, integrating sphere, sample cell, detector (e.g. PbS) and plotting unit. Light hitting a sample can be reflected, scattered and absorbed by its molecules and partly transvade the sample. The ratio of the portion reflected and scattered back upon interactions, i.e. the remitted radiation power IR, to the light intensity incident on the sample is defined as the "remittance" R

$$R = \left(\frac{I_R}{I_0}\right)$$

R can be converted to the absorbance $A(R)$ at the detector:

$$A_R = -\log_{10}\left(\frac{I_R}{I_0}\right) = -\log_{10} R$$

"Transmittance" T, for measurements of light intensities transmitted through the sample the is defined as

$$T = \frac{I_T}{I_0} = 10^{-\varepsilon_A cd}$$

which provides for the relation

$$A = \varepsilon_A cd = -\log_{10}\left(\frac{I_T}{I_0}\right) = -\log_{10} T$$

Corresponding with the transmission or remission spectra the respective absorbances can be expressed as:

$$A_{(T)} = -\log_{10}\left(\frac{1}{T}\right) \text{ and } A_{(R)} = -\log_{10}\left(\frac{1}{R}\right)$$

"Transflectance" (T) represents a special case of transmission and reflection measurement. This mode is often used for in- and on-line measurements of liquids or turbid solutions whereas the incoming light beam passes the sample, then gets reflected on a non-absorbing mirror substance (in NIRS often Teflon is taken) and is directed to the detector after penetrating the sample a second time. Due to this doubling of the pathlength, even lower concentrated analytes can be detected (Fig. 1).

Taking the absorbance log ($1/T$) according to Beer's law absorbance is rigorously valid only for non-scattering samples (visually clear liquids). In case of solid samples (powders, bulk samples), which often show strong scattering effects, the absorbance is called "pseudo absorbance" and does not exactly follow Beer's law due to reflections increasing the extinction coefficient and due to varying path lengths the light takes through the sample (Märk et al., 2010).

NIR absorption spectra of liquids, solids and semisolids usually show broad and overlapping bands (in contrast to MIR absorptions). This makes it often hard to correctly assign the vibrations to the respective chemical bonds. Therefore NIRS can, in most cases, not be used for structural determination of particular substances, but serves as a fingerprint technique revealing physical as well as chemical properties of the samples. Today chemometrics and MVA are commonly used to extract useful information from the spectra and to calibrate for quantitative properties. The next section gives an overview of available chemometrical, statistical and multivariate techniques to process NIR spectra.

2.1.1. Multivariate NIR data analysis

Multivariate data analysis (MVA) based calibration techniques are applied to link the spectral data with target parameters transferred from reference techniques. In most cases, principal component analysis (PCA) based models like principal component regression (PCR) and partial least squares regression (PLSR) are applied. Even linear regression models like multiple linear regressions (MLR) are frequently used. Both spectra interpretation techniques, band assignments and MVA, are combined into one approach to benefit from reliable and problem specific calibrations. For the choice of useful signals, wavelength selection and spectra pretreatment are also crucial. Thereby, the operator can choose between a broad range of useful spectra pretreatments that help increasing the quality of calibrations available (Faber, 1999). Especially early contributions by Savitzky and Golay have become standard for recent data pretreatment operations (Savitzky et al., 1964). For more detailed information the interested reader is

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