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Review

Advances of vibrational spectroscopic methods in phytomics and bioanalysis

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ABSTRACT

During the last couple of years great advances in vibrational spectroscopy including near-infrared (NIR), mid-infrared (MIR), attenuated total reflection (ATR) and imaging and also mapping techniques could be achieved. On the other hand spectral treatment features have improved dramatically allowing filtering out relevant information from spectral data much more efficiently and providing new insights into the biochemical composition. These advances offer new possible quality control strategies in phytomics and enable to get deeper insights into biochemical background in terms of medicinal relevant questions. It is the aim of the present article pointing out the technical and methodological advancements in the NIR and MIR field and to demonstrate the individual methods efficiency by discussing distinct selected applications.

Contents

Abbreviations: ATR, attenuated total reflection; CA, cluster analysis; CE, capillary electrophoresis; CEC, capillary electrochromatography; DR, diffuse reflection; EPO, external parameter orthogonalization; FPA, Focal Plane Array; FT, Fourier transform; HDL, high-density lipoprotein; LC, liquid chromatography; LDL, low-density lipoprotein; MCT, Mercury Cadmium Telluride; MEIRS, material enhanced infrared spectroscopy; MIA, multivariate image analysis; MLR, multiple linear regression; MVA, multivariate analysis; NIR, near infrared spectroscopy; OSC, orthogonal scatter correction; PCA, principal component analysis; PLSR, partial least square regression; TCM, Traditional Chinese Medicine; Vis, visible.

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1. Introduction

In 2003 our group described in a review article entitled “Phytoanalysis – a challenge in phytomics” fast analytical methods for qualitative and quantitative determination of plant ingredients and phytopharmaceutical products [1]. The emphasis was primarily on conventional techniques such as liquid chromatography (LC) [2] and capillary electrophoresis (CE) [3], and the influence of the stationary phases [4] and the use of at this time non-standard analytical techniques, such as micro-liquid chromatography (μLC) [5], capillary electrochromatography (CEC) [6], mentioning for the first time near-infrared (NIR) spectroscopy as a powerful analytical alternative. Meanwhile, technical progress in separation science was limited to the development of novel stationary phases enabling low- and high-molecular weight ingredients on one hand [7], on the other site selective sample enrichment strategies became quite popular [8,9,10].

In vibrational spectroscopy, including both mid- (400–4000 cm^{-1}) and near-infrared (4000–12,000 cm^{-1}, NIR) fundamental technical and methodological progress since 2003 enabled establishing new analytical strategies allowing extreme fast quantitative analysis of a manifold of chemical and physical parameters simultaneously offering the additional advantages of short analysis time and non-invasiveness beside avoiding the necessity to employ special trained laboratory staff in the routine operation mode [11]. Additionally, especially NIR based techniques combined with algorithms based on principal component analysis (PCA) and cluster analysis (CA) can nowadays successfully be implemented to get knowledge about plant species and/or its geographic origin [12]. In this context, the implementation of miniaturized hand-held sensors is becoming of increasing importance enabling an easy direct measurement, e.g. in-field [13]. These methodological comforts are extremely helpful in the phytomics field including also Traditional Chinese Medicine (TCM), opening new possible strategies in quality control [14,15]. For the investigation of biomolecules being present in low concentrations, a combined on-line pre-concentration and following MIR/NIR analytical strategy, which is termed as “material enhanced infrared spectroscopy (MEIRS)” can be of great benefit [16,17]. Another new spectroscopic method, which is generally called Fourier transform infrared (FTIR) spectroscopic microscopy for examination of both plant and human tissue sections and for visualizing of the acquired data has been introduced [18]. 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 [19]. Imaging allows probing samples under native conditions and offers new insights into samples without the need for fixation or an additional marker [20,19].

In the present article technical achievements and data-processing methods in vibrational spectroscopy focused on near- and mid-infrared (NIR and MIR) applied in phytomics and bioanalysis are summarized, their efficiencies are discussed and compared to other more conventional analytical techniques. The use of Raman spectroscopy is not discussed in this review.

2. Technique and methodology

Let us start the discussion with a systematic approach. Therefore, in the following the technique and methodology of near-infrared (NIR), attenuated total reflection (ATR) and imaging/mapping spectroscopy will be discussed. In the ongoing step, application possibilities and selected application examples in the fields of phytomics and bioanalysis will be examined carefully.

2.1. Near-infrared (NIR) spectroscopy

The term “near” in NIR relies 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 12500 cm^{-1} covering absorptions corresponding to overtones and combinations of fundamental vibrations. Starting from the diatomic molecule as the simplest vibrating system, described by the harmonic and anharmonic oscillator, the study of more complex substances is referred to as the concept of polyatomic molecules [21].

A typical setup of a FT-NIR instrument is equipped with an e.g. tungsten-halogen light source, a polarization interferometer, light-fiber optics, integrating sphere, sample cell, PbS detector and plotting unit. Light hitting a sample can be reflected, scattered and absorbed by its molecules and partly trans-vade the sample. The ratio of the portion reflected and scattered back upon interactions, i.e. the remitted radiation power I_R, to the light intensity incident on the sample defines here the “*remittance*” R

$$R = \left(\frac{I_R}{I_0}\right) \tag{1}$$

which, for remission measurements, is easily related to the absorbance $A_{(R)}$ at the detector:

$$A_{(R)} = -\log_{10}\left(\frac{I_R}{I_0}\right) = -\log_{10}R \tag{2}$$

For measurements of light intensities transmitted, I_T, through the sample the “*transmittance*” T is introduced as

$$T = \frac{I_T}{I_0} = 10^{-\varepsilon_A cd} \tag{3}$$

with c (concentration in mol l^{-1}), d the sample thickness (cm) and hence provides for the relation

$$A = \varepsilon_A cd = -\log_{10}\left(\frac{I_T}{I_0}\right) = -\log_{10} T \tag{4}$$

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

$$A_{(T)} = -\log_{10}\left(\frac{1}{T}\right) \quad \text{and} \quad A_{(R)} = -\log_{10}\left(\frac{1}{R}\right) \tag{5}$$

“*Transflectance*” (T), a special case of transmission measurements, 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 pathlengths the light takes through the sample [22].

NIR absorption spectra of liquids, solids and semisolids, even if they are relatively simple substances or pure compounds, usually show broad and overlapping bands (in contrast to MIR absorptions). The fact that absorption signals often extend over large wavenumber ranges makes it 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