Chapter 5
The Role of Multivariant Analysis on the Interpretation of FTIR and Raman Spectra

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ABSTRACT

FTIR and Raman spectroscopy are complementary spectroscopic techniques that play an important role in the analysis of molecular structure and the determination of characteristic vibrational bands. Vibrational spectroscopy has a wide range of applications including mainly in physics and biology. Its applications have gained tremendous speed in the field of biological macromolecules and biological systems, such as tissue, blood, and cells. However, the vibrational spectra obtained from the biological systems contain a large number of data and information that make the interpretation difficult. To facilitate the analysis, multivariant analysis comprising the reduction of the dimension of spectrum data and classification of them by eliminating redundancy data, which are obtained from the spectra and does not have any role, becomes critical. In this chapter, the applications of Principal Component Analysis (PCA), Linear Discriminant Analysis (LDA), and their combination PCA-LDA, which are widely used among multivariant techniques on biological systems will be disclosed.

DOI: 10.4018/978-1-7998-1518-1.ch005

INTRODUCTION

Infrared Spectroscopy

Infrared spectroscopy, which characterizes the chemical structure of the molecules and is based on molecular vibrations, is one of the most important techniques commonly used (Christy et al., 2001; Smith, 2011; Ciurczak and Igne, 2015; Alvarez-Ordonez and Prieto, 2012; Stuart, 2004). When a molecular system is irradiated by infrared (IR) radiation, that has all IR frequencies, the molecules absorb characteristic frequencies, that correspond to energies equal to the difference between their vibrational energy levels. There are selection rules for absorbing infrared radiation: The obvious selection rule for vibrational transitions is that the electric dipole moment of the molecule must change during the vibrational motion. Identification of the structure of molecules in the sample is performed by the determination of functional groups and chemical bonds, using IR spectroscopy. In addition, the surroundings of the functional group can also be examined. In IR transmission spectroscopy, the sample is placed on the path through which the beam passes, and the molecules in the sample are excited by incident beam at different wavenumbers in the range of 4000 to 400 cm^{-1}. The wavenumber at which the radiation is absorbed is measured and a spectrum in which x-axis is wavenumber and y-axis is percent transmittance (or percentage absorbance) is obtained.

One of the most commonly used sampling techniques for FTIR is ATR based on the presence of evanescent wave in the solid or liquid sample and ATR unit contains a crystal with high refractive index. In ATR technique, information on the surface structure of the material to be analyzed is obtained by the high-quality spectra in a short time without sample preparation for materials which have generally strong absorption (ie low transmittance in the IR range) or are too thick (Crompton, 2006; Theophanides, 2012; Larkin, 2011; Bart, 2006; Settle, 1997).

One of the sampling methods used for FTIR analysis of solid materials is the KBr disk method; the sample is ground into powder form and then ca. 1 mg sample is mixed well with powdered 100 mg KBr which is fully transparent in the IR range down to 400 cm^{-1}, and is finally pressed into a pellet under to 10 tones to prepare the sample for analysis. Because of high hygroscopic nature of KBr, the presence of water in KBr can mask peaks in the IR spectrum, therefore KBr should be dried in a vacuum oven before the pellet preparation (Griffiths and Haseth, 2007; Yadav, 2005; Nyquist, 2001; Kenkel, 2014, Hansen et al., 2012). Solid samples can also be analyzed by mull technique; For this process, a small amount of nujol (mineral oil) is dropped into the crushed sample in a agate mortar, and a paste is prepared. After then a thin film of nujol paste is applied onto the salt plates (mostly KBr plates are used), and the plates are mounted in a path of IR beam for recording the IR spectrum. (Anderson et al., 2004; Hayes, 2008). Liquid and solution samples can also be analyzed by FTIR by placing them between NaCl or KBr plates which are transparent to IR above 700 cm^{-1} or 400 cm^{-1}, respectively (Hollas, 2004; Kelsall et al., 2005; Ghatak, 2010; Pavia et al., 2018; Dean, 2003).

Raman Spectroscopy

In Raman spectroscopy, when the sample is illuminated in the UV-visible region, the incident photons are scattered from the sample molecules (Ferraro and Nakamoto, 1994; Lewis and Edwards, 2001; Tu, 1982; Vandenabeele, 2013; Smith and Dent, 2005; Gardiner and Graves, 1989). Depending on the elastic or inelastic collisions between the photons and the molecules, the scattered light is called Rayleigh or