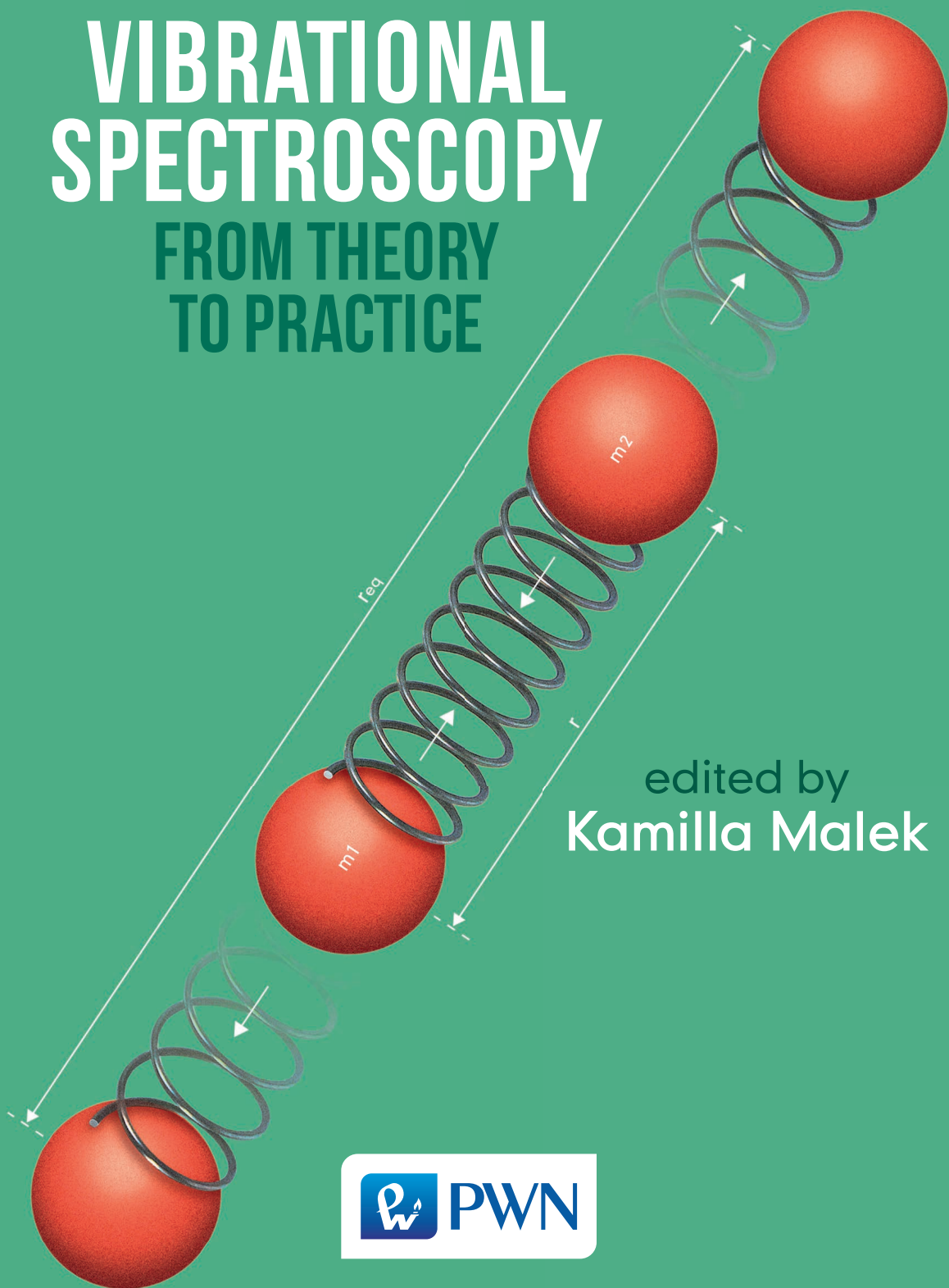


# VIBRATIONAL SPECTROSCOPY

FROM THEORY  
TO PRACTICE



edited by  
**Kamilla Malek**

 PWN

**Vibrational spectroscopy. From theory to practice**  
**Editor Kamilla Malek**

AUTHORS

Barańska Małgorzata, Prof., PhD, DSc	Faculty of Chemistry, Jagiellonian University in Krakow
Bukowska Jolanta, Prof., PhD, DSc	Faculty of Chemistry, University of Warsaw
Chmura-Skirlinińska Antonina, PhD	Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University in Krakow
Chruszcz-Lipska Katarzyna, PhD	AGH University of Science and Technology, Faculty of Drilling, Oil and Gas
Czamara Krzysztof, MSc	Faculty of Chemistry, Jagiellonian University in Krakow
Dybaś Jakub, MSc	Faculty of Chemistry, Jagiellonian University in Krakow
Gąsior-Głogowska Marlena, PhD, Eng.	Faculty of Fundamental Problems of Technology, Wrocław University of Science and Technology
Jaworska Aleksandra, PhD	Faculty of Chemistry, Jagiellonian University in Krakow
Kaczor Agnieszka, PhD, DSc	Faculty of Chemistry, Jagiellonian University in Krakow
Kochan Kamila, MSc	Faculty of Chemistry, Jagiellonian University in Krakow
Królikowska Agata, PhD	Faculty of Chemistry, University of Warsaw
Lipiński Piotr, F.J., PhD	Mossakowski Medical Research Centre, Polish Academy of Sciences
Majzner Katarzyna, PhD	Faculty of Chemistry, Jagiellonian University in Krakow
Malek Kamilla, PhD, DSc	Faculty of Chemistry, Jagiellonian University in Krakow
Marzec Katarzyna M., PhD	Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University in Krakow
Miśkowiec Paweł, PhD	Faculty of Chemistry, Jagiellonian University in Krakow
Oleszko Adam, MSc, Eng.	Faculty of Fundamental Problems of Technology, Wrocław University of Science and Technology
Pacia Marta Z., MSc	Faculty of Chemistry, Jagiellonian University in Krakow
Rode Joanna, PhD	1. Institute of Nuclear Chemistry and Technology 2. Institute of Organic Chemistry, Polish Academy of Sciences
Ryguła Anna, PhD	Jagiellonian Centre for Experimental Therapeutics (JCET), Faculty of Chemistry, Jagiellonian University in Krakow
Staniszewska-Ślęzak Emilia, MSc	Faculty of Chemistry, Jagiellonian University in Krakow
Wiercigroch Ewelina, MSc	Faculty of Chemistry, Jagiellonian University in Krakow
Wróbel Tomasz, PhD	Faculty of Chemistry, Jagiellonian University in Krakow
Zajac Grzegorz, MSc	Faculty of Chemistry, Jagiellonian University in Krakow

Cover and title page design:

**Marek Goebel**

Project editor:

**Katarzyna Włodarczyk-Gil**

Editorial coordinator:

**Iwona Lewandowska**

Copy editor:

**Łukasz Boda**

Production coordinator:

**Mariola Grzywacka**

Edited by:

**Kamilla Malek, Faculty of Chemistry, Jagiellonian University**

DTP:

**Dariusz Ziach**

The publication was co-funded by the Faculty of Chemistry  
of the Jagiellonian University in Krakow

The book that you have purchased is the work of its author and publisher. Please respect their rights. You may make this book available free of charge to your family and friends, but do not publish it on the net. If you quote excerpts from it, do not change the original and always be sure to mention where they come from. And if you copy parts of it, do so only for your own use.

It is imperative that we respect other people's rights and property.

See more on [www.legalnakultura.pl](http://www.legalnakultura.pl)

*Polska Izba Książki*

Copyright © by Wydawnictwo Naukowe PWN SA  
Warszawa 2016

ISBN 978-83-01-18885-6

First edition

Wydawnictwo Naukowe PWN SA  
02-460 Warszawa, ul. Gottlieba Daimlera 2  
tel. 22 69 54 321, faks 22 69 54 288  
infolinia 801 33 33 88  
e-mail: [pwn@pwn.com.pl](mailto:pwn@pwn.com.pl); [reklama@pwn.pl](mailto:reklama@pwn.pl)

Printed and bound by

# Content

<b>1. Fundamentals of infrared spectroscopy (FT-IR)</b> .....	11
<i>(Kamilla Malek, Emilia Staniszewska-Ślęzak, Kamila Kochan, Katarzyna Majzner)</i>	
1.1. Quantum description – models of the harmonic and anharmonic oscillators. .	12
1.2. Normal coordinates .....	13
1.3. Construction of a FT-IR spectrometer. ....	15
1.3.1. Light source .....	15
1.3.2. Interferometer .....	16
1.3.3. Sample chamber. ....	17
1.3.4. Detector .....	17
<b>2. Fundamentals of Raman scattering spectroscopy</b> .....	19
<i>(Kamilla Malek, Małgorzata Barańska, Kamila Kochan)</i>	
2.1. Quantum description of Raman scattering .....	20
2.2. Instrumentation in Raman spectroscopy .....	22
2.2.1. Laser .....	23
2.2.2. Sample compartment. ....	23
2.2.3. Optical element splitting the beam of electromagnetic radiation . . .	24
2.2.4. Detector .....	25
<b>3. Special FT-IR techniques</b> .....	26
3.1. Sampling techniques in FT-IR spectroscopy <i>(Paweł Miśkowiec)</i> .....	26
3.1.1. Transmission technique. ....	26
3.1.2. Reflexive techniques .....	27
3.1.2.1. Attenuated total reflection, ATR. ....	28
3.1.2.2. Diffuse reflection in the mid-infrared range, DRIFT .....	30
3.1.2.3. Reflection – absorption infrared spectroscopy, IRRAS. ....	32
3.1.3. Photoacoustic spectroscopy, PAS. ....	34
3.1.4. Infrared emission spectroscopy, ES (IRES) .....	35
3.2. FT-IR microscopy and imaging <i>(Ewelina Wiercigroch, Kamilla Malek)</i> .....	36
3.3. Vibrational circular dichroism <i>(Piotr F. J. Lipiński)</i> .....	40
3.3.1. Chirality. ....	40
3.3.2. What is Vibrational Circular Dichroism? .....	40
3.3.3. How is VCD measured? .....	41
3.3.4. Applications of VCD spectroscopy .....	42

3.3.5. Calculations of VCD spectra and their problems . . . . .	43
3.3.6. Very short summary of current developments . . . . .	44
<b>4. Special Raman techniques . . . . .</b>	<b>46</b>
4.1. Resonance Raman scattering spectroscopy ( <i>Katarzyna M. Marzec, Jakub Dybaś</i> ) . . . . .	46
4.1.1. Resonance <i>versus</i> normal Raman scattering and fluorescence . . . . .	46
4.1.2. Phenomenon of resonance Raman scattering . . . . .	47
4.1.3. Application and potential of RRS . . . . .	49
4.1.4. Instrumentation. . . . .	50
4.2. Surface-enhanced Raman scattering spectroscopy (SERS) ( <i>Agata Królikowska, Jolanta Bukowska</i> ) . . . . .	52
4.2.1 Mechanism of surface enhancement . . . . .	53
4.2.2 Types of the substrates used in SERS spectroscopy . . . . .	55
4.2.3. SERS spectral features . . . . .	56
4.2.4. Applications of SERS spectroscopy . . . . .	57
4.3. Raman optical activity (ROA) ( <i>Joanna E. Rode</i> ) . . . . .	57
4.3.1. Schematic diagram of ROA phenomenon . . . . .	58
4.3.2. Theoretical description of ROA phenomenon. . . . .	59
4.3.3. Instrumentation. . . . .	61
4.3.4. Applications of ROA . . . . .	63
4.3.5 Summary . . . . .	64
4.4. Raman imaging ( <i>Agnieszka Kaczor</i> ) . . . . .	67
<b>5. Chemometric analysis of FT-IR and Raman spectra</b> ( <i>Katarzyna Majzner, Kamila Kochan, Małgorzata Barańska</i> ) . . . . .	<b>74</b>
5.1. Analysis of marker bands. . . . .	74
5.2. Cluster analysis (CA). . . . .	76
5.2.1. Hierarchical cluster analysis (HCA) . . . . .	77
5.2.2. Non-hierarchical cluster analysis. . . . .	81
5.2.3. Comparison of HCA, KMCA and FCA methods . . . . .	83
<b>6. Selected applications of FT-IR spectroscopy . . . . .</b>	<b>85</b>
6.1. An effect of molecular symmetry and isotopic substitution on IR and Raman spectra of chloromethane derivatives ( <i>Kamilla Malek, Katarzyna M. Marzec</i> ) . . . . .	85
6.2. Peak-fitting process by an example of ATR FT-IR spectra of soft tissues ( <i>Marlena Gąsior-Głogowska, Adam Oleszko</i> ) . . . . .	89
6.2.1. Skin . . . . .	89
6.2.2. Skin's proteins . . . . .	90
6.2.3. Infrared spectroscopy of tissues . . . . .	90
6.3. Synthesis and spectral characteristics of hydroxyapatites ( <i>Marlena Gąsior-Głogowska, Adam Oleszko</i> ) . . . . .	96
6.3.1. Hydroxyapatites. . . . .	96
6.3.2. Infrared spectroscopy of hydroxyapatite . . . . .	97

6.4.	The application of infrared spectroscopy for the determination of petroleum hydrocarbons in surface water and wastewater ( <i>Paweł Miśkowiec</i> ) . . . . .	100
6.4.1.	The composition of crude oil; environment pollution with petroleum products . . . . .	101
6.4.2.	The toxic properties of the components of crude oil. . . . .	102
6.4.3.	Legislation concerning problems of environmental pollution with petroleum products and methods of its measurement . . . . .	103
6.4.4.	An application of IR spectroscopy in analytical chemistry . . . . .	104
6.4.5.	Nernst distribution law . . . . .	105
6.5.	Determination of absolute configuration using vibrational circular dichroism ( <i>Piotr F. J. Lipiński</i> ) . . . . .	108
6.5.1.	Determination of absolute configuration <i>via</i> VCD . . . . .	108
6.6.	The identification of painting materials and degradation products. FT-IR imaging of paint layers ( <i>Emilia Staniszevska-Ślęzak, Kamilla Malek</i> ) . . . .	113
6.6.1.	Chemical characteristics of a paint layer and products of its degradation. . . . .	113
6.6.2.	FT-IR spectroscopy as a technique used in the identification of chemical composition of artworks. . . . .	116
6.7.	Structural analysis of proteins by means of FT-IR spectroscopy ( <i>Katarzyna Majzner</i> ) . . . . .	119
6.7.1.	Anatomy of proteins . . . . .	119
6.7.2.	Application of FT-IR spectroscopy to studying proteins. . . . .	121
6.8.	Structural analysis of lipids by using infrared spectroscopy ( <i>Tomasz P. Wróbel</i> ) . . . . .	126
6.8.1.	Characteristics and occurrence of lipids . . . . .	126
6.8.2.	Application of FT-IR spectroscopy to studying lipids . . . . .	127
6.9.	Structural analysis of carbohydrates by means of FT-IR spectroscopy ( <i>Kamilla Malek, Ewelina Wiercigroch</i> ) . . . . .	130
6.9.1.	Molecular structure of carbohydrates . . . . .	130
6.9.2.	Characteristic IR bands of carbohydrates . . . . .	133
6.10.	An analysis of ATR FT-IR spectra of animal tissues ( <i>Emilia Staniszevska-Ślęzak, Kamilla Malek</i> ) . . . . .	135
6.10.1.	Biochemical features of selected animal tissues . . . . .	135
6.10.2.	FT-IR spectra of animal tissues . . . . .	136
6.11.	Diagnostics of disease development by FT-IR imaging of tissue ( <i>Kamila Kochan, Małgorzata Barańska</i> ) . . . . .	141
<b>7.</b>	<b>Selected applications of Raman spectroscopy</b> . . . . .	<b>145</b>
7.1.	The identification of proteins secondary structure in Raman spectra ( <i>Anna Rygula</i> ) . . . . .	145
7.1.1.	Characteristics of protein in Raman spectrum . . . . .	145
7.1.2.	Marker bands of amino acid residues . . . . .	146

7.1.3.	Other techniques of Raman spectroscopy for the analysis of proteins . . . . .	148
7.2.	Raman analysis of fatty acids ( <i>Aleksandra Jaworska, Małgorzata Barańska</i> ) . . . .	150
7.2.1.	Occurrence and characteristics of fatty acids. . . . .	150
7.2.2.	An application of Raman spectroscopy in an analysis of fatty acids. . . . .	152
7.3.	Raman spectroscopy as a method to analyze lipids in animal tissues and mixtures ( <i>Krzysztof Czamara, Agnieszka Kaczor</i> ) . . . . .	155
7.3.1.	Classification of lipids . . . . .	155
7.3.2.	Spectroscopic characteristics of lipids . . . . .	157
7.4.	Polymorphism of model triacylglyceroles ( <i>Marta Z. Pacia, Krzysztof Czamara, Agnieszka Kaczor</i> ) . . . . .	161
7.4.1.	Polymorphism of lipids . . . . .	161
7.4.2.	Spectroscopic characteristics of TAGs polymorphs . . . . .	162
7.5.	Identification of carotenoids in plants by means of Raman spectroscopy ( <i>Aleksandra Jaworska, Małgorzata Barańska</i> ) . . . . .	166
7.5.1.	Structure, functions and occurrence of carotenoids . . . . .	166
7.5.2.	Raman bands of carotenoids . . . . .	167
7.6.	Identification of terpenes in citrus oils by means of Raman spectroscopy ( <i>Aleksandra Jaworska, Małgorzata Barańska, Kamilla Malek</i> ) . . . . .	170
7.6.1.	Essential oils – occurrence and composition. . . . .	170
7.6.2.	Identification of essential oils by means of gas chromatography and Raman spectroscopy . . . . .	171
7.6.3.	Chemometric analysis of Raman spectra of essential oils . . . . .	172
7.7.	An analysis of pigments and painting materials in Raman spectra ( <i>Anna Rygula, Kamilla Malek</i> ) . . . . .	174
7.7.1.	Raman spectroscopy as an analytic technique in conservation of art works . . . . .	174
7.7.2.	Structure of paint layers . . . . .	176
7.7.3.	Methodology of a Raman analysis of works of arts . . . . .	176
7.8.	Detection and determination of glucose in pharmaceuticals and body fluids ( <i>Agnieszka Kaczor</i> ) . . . . .	180
7.9.	Resonance Raman scattering spectroscopy in hemoglobin structure studies ( <i>Jakub Dybaś, Antonina Chmura-Skiriłńska, Katarzyna M. Marzec</i> ) . . . . .	185
7.9.1.	Structure and physiology of hemoglobin. . . . .	185
7.9.2.	Resonance Raman scattering spectroscopy in hemoglobin studies . . . . .	188
7.9.3.	UV-Vis absorption spectrophotometry in hemoglobin studies . . . . .	189
7.10.	Identification of enantiomers of bornyl acetate and $\alpha$ -pinene in essential oils from the Siberian fir needles ( <i>Katarzyna Chruszcz-Lipska</i> ) . . . . .	192
7.10.1.	Terpenes. . . . .	192
7.10.2.	Stereochemistry of terpenes . . . . .	193
7.10.3.	Enantiomeric contents of terpenes in essential oils . . . . .	194
7.10.4.	Raman optical activity spectra of terpenes . . . . .	195

7.11. Determination of absolute configuration of $\alpha$ -pinene enantiomers by means of Raman optical activity and quantum chemical calculations (Grzegorz Zając, Małgorzata Barańska) . . . . .	198
7.11.1. Theoretical calculations of ROA . . . . .	198
7.12. Estimation of surface enhancement factor and adsorption studies of 3-amino-5-mercapto-1,2,4-triazole (AMT) on silver using surface-enhanced raman scattering spectroscopy (SERS) (Agata Królikowska, Jolanta Bukowska) . . . . .	204
7.12.1. Surface enhancement factor in SERS. . . . .	204
7.12.1.1. Types of definition of enhancement factor. . . . .	205
7.12.1.2. Enhancement factor for a given substrate (EF) . . . . .	205
7.12.1.3. Analytical enhancement factor (AEF) . . . . .	206
7.12.1.4. Single molecule enhancement factor (SMEF) . . . . .	206
7.12.2. The most common sources of errors in the determined values of SERS enhancement factors . . . . .	207
7.12.3. Structure and properties of 3-amino-5-mercapto-1,2,4-triazole (AMT) . . . . .	207
7.12.4. SERS spectrum of 3-amino-5-mercapto-1,2,4-triazole (AMT) on silver substrate . . . . .	208
7.13. Identification and studying of distribution of caffeine in drug samples <i>in situ</i> (Małgorzata Barańska, Agnieszka Kaczor, Kamilla Malek) . . . . .	212
7.14. Characteristics of the cell organelles based on analysis of marker bands and cluster analysis (Katarzyna Majzner) . . . . .	215
7.14.1. The assignment of the major Raman bands in the cell spectra . . . . .	216
7.14.2. An analysis of Raman imaging of cells . . . . .	218
7.15. <i>In vitro</i> and <i>in vivo</i> Raman imaging of unicellular carotenoid producers (Marta Z. Pacia, Agnieszka Kaczor) . . . . .	220
7.15.1. Spectral characteristics of cells compartments . . . . .	221
<b>List of figures</b> . . . . .	227
<b>List of tables</b> . . . . .	234





# Fundamentals of infrared spectroscopy (FT-IR)

*Kamilla Malek, Emilia Staniszewska-Ślęzak, Kamila Kochan, Katarzyna Majzner*

The interaction of matter with electromagnetic radiation is fundamental in optical spectroscopy and it relies on absorption, emission and scattering of light photons. Due to such interactions one registers a spectrum consisting of bands of a given frequency, shape and intensity. As the title of this Chapter indicates infrared absorption spectroscopy results from absorption of light quantum from IR region, which matches to energy difference between vibrational levels. The IR spectrum is usually presented in a scale of wavenumbers [ $\text{cm}^{-1}$ ] and can be collected in three regions of IR radiation. It should be emphasized that IR and Raman techniques (described in Chapter 2) are complementary to each other, as both rely on registration of transitions between vibrational levels, but information that they provide is complementary to each other. This can be illustrated by the rule of mutual exclusion, which states, that if a molecule has a center of symmetry, vibration active in infrared absorption spectrum is inactive in Raman and *vice versa*. The most frequently used IR region is mid-infrared radiation (MIR, *Mid InfraRed*), which covers the region of 400 – 4000  $\text{cm}^{-1}$ . Within this IR spectrum, a region from 400 to 1500  $\text{cm}^{-1}$ , called “fingerprint”, is specific for each chemical species and this fact strictly results from characteristics of vibrational motion described by normal modes. A region of far infrared radiation (FIR, *Far InfraRed*, 50 – 400  $\text{cm}^{-1}$ ) also registers normal modes while a region above 4000  $\text{cm}^{-1}$  (NIR, *Near InfraRed*) provides information about overtones and combination modes. The abbreviation *FT* in the name of the technique means *Fourier transform* – the mathematical operation that decomposes a function of time (a *signal*) into the function of frequency. FT-IR spectroscopy is applied for the identification and quantitative analysis of chemical compounds or their mixtures as well as the determination of physicochemical features such as molecular structure and its changes due to a stress/reaction, kinetics of reactions and intramolecular dynamics. IR spectra of a sample in any state can be collected.

## 1.1. Quantum description – models of the harmonic and anharmonic oscillators

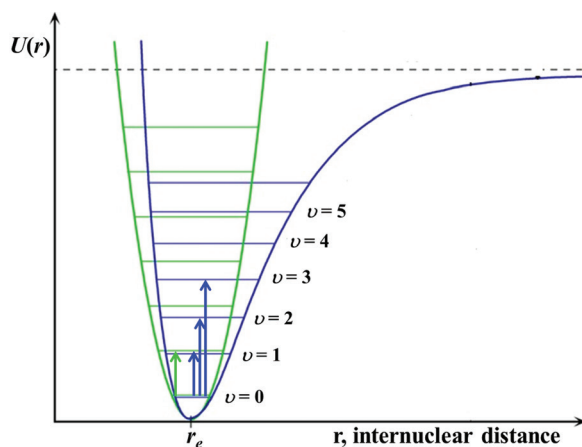
Molecules consisting of nuclei and electrons, have a certain reserve of internal energy, which manifests itself in various forms of movement, e.g. vibrations. A molecule containing  $N$  atoms can perform  $3N - 6$  vibrations or  $3N - 5$ , if it is linear. An exceptional feature of oscillations is non-zero value of their potential energy in the ground vibrational state, therefore vibrations of molecules take place in every phase and do not stop even at temperature of 0 K. As any energy in the micro-world, vibrational energy is also quantized, what means that its values are limited to certain discrete values called vibrational levels. Therefore, each chemical species adopts or donates energy only in characteristic portions called quanta. The energy of discrete vibrational levels is determined by solving the Schrödinger equation for a model of a harmonic oscillator. The eigenvalues of the vibrational Schrödinger equation for diatomic molecule are given by (Eq. 1.1):

$$E_{\text{osc}} = h\nu \left( \nu + \frac{1}{2} \right), \quad (1.1)$$

where:  $\nu$  is frequency of the harmonic oscillator  $\nu = \frac{1}{2\pi} \sqrt{\frac{f}{\mu_{\text{red}}}}$ ,  $f$  – force constant,  $\mu_{\text{red}}$  – reduced mass  $\mu_{\text{red}} = \frac{m_1 m_2}{m_1 + m_2}$ , and  $\nu$  – a vibrational quantum number ( $\nu = 0, 1, 2, 3, \dots$ ).

The value of the vibrational quantum number in Eq. 1.1 determines quantum nature of vibrational motion and its energy also depends on the type of the molecule since this expression includes the value of force constant and reduced mass of the chemical species.

During oscillations, potential energy reaches the maximum value at the largest deflection from the equilibrium, the potential energy curve change is illustrated by a parabola (Figure 1.1).



**Fig. 1.1.** Potential energy  $U$  curve for the models of the harmonic oscillator (green line) and of the anharmonic oscillator (blue line). Vertical arrows label allowed transitions between vibrational levels  $\nu$

Potential energy curve in Figure 1.1. shows that energy of the harmonic oscillator never reaches zero value, since the energy is  $\frac{1}{2}h\nu$  for the lowest allowed vibrational level  $\nu = 0$ . The vibrational levels of the harmonic oscillator are equally separated by  $h\nu$  and therefore energy difference does not depend on the vibrational quantum number  $\nu$  and is affected by chemical species only. Vibrational spectrum of the harmonic oscillator exhibits only one band since it represents transition for a diatomic molecule. A required condition to record the IR absorption spectrum is a change of dipole moment of a molecule during vibration, wherein the change of the vibrational level can be only  $\Delta\nu = 1$  (a green arrow in Fig. 1.1).

The transition moment of absorption must be non-zero and is given by (1.2):

$$\mu_{nm} = \langle \Psi_n | \hat{\mu} | \Psi_m \rangle \neq 0, \quad (1.2)$$

and its operator is the change of the dipole moment  $\hat{\mu}$  due to transition from  $n$  to  $m$  vibrational level. This transition is called a fundamental tone. Due to the fact that the most occupied vibrational level at room temperature (according to the Boltzmann energy distribution) is  $\nu = 0$ , bands observed in IR spectrum mainly originate from transitions from  $\nu = 0$  to  $\nu = 1$  (the green arrow in Fig. 1.1).

The model of the harmonic oscillator is an idealized model, whereas in fact, oscillations are anharmonic, as the Hooke's law is not satisfied. In this case the potential energy curve is illustrated by the Morse curve (Fig. 1.1.B) and energy of the anharmonic oscillator is expressed by an equation 1.3:

$$E_{osc}^{anh} = h\nu \left( \nu + \frac{1}{2} \right) - h\nu x \left( \nu + \frac{1}{2} \right)^2, \quad (1.3)$$

while neighboring vibrational levels are separated by (1.4):

$$\Delta E_{osc}^{anh} = E_{\nu+1} - E_{\nu} = h\nu[1 - 2x(\nu + 1)], \quad (1.4)$$

where the parameter  $x$  is an anharmonic constant that describes deviation of anharmonic oscillator from the ideal harmonic model. It takes considerable values for vibrations of a high energy.

It is clear from Figure 1.1.B and equation (1.4) that the intervals between vibrational levels are not constant, but they decrease along with increasing quantum number  $\nu$ . The Morse potential also shows dissociation of a bond which appears for large values of  $\nu$ . In addition, the model of the anharmonic oscillator extends the selection rules. Transitions become allowed not only for  $\Delta\nu = 1$  (fundamental tones) but also for  $\Delta\nu = 2, 3, \dots$ , sequentially called the first, second (etc.) overtone (blue arrows in Fig. 1.1). Their intensities are much lower than intensity of the fundamental tone.

## 1.2. Normal coordinates

In general, a molecule built from  $N$  atoms has  $3N - 6(5)$  internal degrees of freedom. They describe oscillations, for which a concept of the normal coordinates/

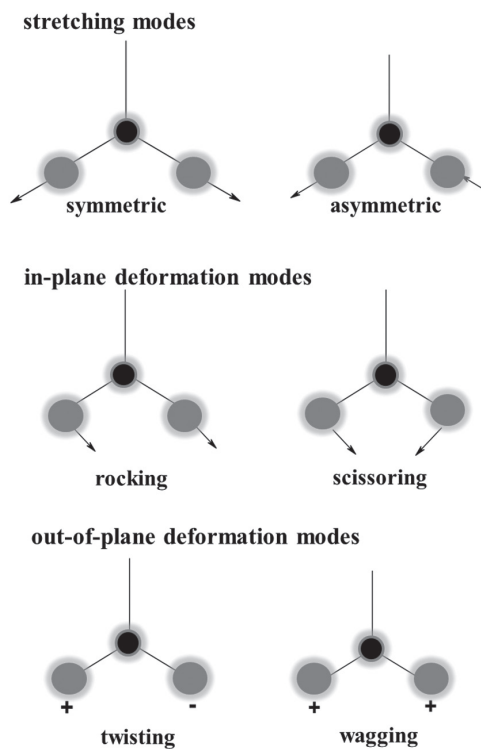
vibrations/modes was introduced. A normal coordinate describes a simultaneous movement of all atoms, occurring with the same frequency and consistent in phase, although amplitude of vibration of individual atoms can be different. Vibrations do not induce translation or rotation of the molecule. Individual normal modes are orthogonal to each other, which means that their movement is mutually independent.

Extremely helpful in the analysis of IR and Raman spectra is to consider symmetry of a molecule (and therefore – its possible molecular structure) as well as its influence on spectral features.  $3N - 6(5)$  normal modes of  $N$ -atomic molecules can be classified according to properties of a point group. For example, the molecule of  $H_2O$ , which belongs to the  $C_{2v}$  group, has two vibrations with  $A_1$  and one with  $B_2$  symmetry. The reader can get familiar with details of group theory in the literature [1].

It should be also emphasized that a number of vibrations active in IR and Raman spectra and predicted on the basis of group theory can be different from a number of bands observed in experimental spectrum. These discrepancies can result from the presence of overtones and combinatorial bands, Fermi resonance, the abolition of the degeneracy of some vibrations (due to reduction of molecular symmetry) or the presence of isomers and amorphous forms.

A helpful approach for description of vibrational spectra is to analyze them in terms of characteristic vibrations of functional groups. They occur in specific regions of a spectrum, e.g., the C-H stretching mode is observed in the region of  $2850 - 3100\text{ cm}^{-1}$ . Figure 1.2 shows typical vibrations for a functional group  $XY_2$ . Vibrations associated with the change of a bond length are called stretching vibrations, whereas those expressing change of the molecular angle are called deformational or bending vibrations. Another criterion arises from symmetry of vibrations (symmetric or asymmetric) or from motion in-plane and out-of-plane of a functional group (see Figure 1.2). Using tables of group frequencies, Raman and IR spectra can be described qualitatively. Each of these vibrations can be also considered in terms of principles of the group theory. Thus, the A-type vibrations are non-degenerate vibrations, symmetric to an axis of the highest multiplicity. The B-type vibrations are also non-degenerate vibrations, but anti-symmetric to an axis of the highest multiplicity. Finally, the E- and T(F)-type vibrations are vibration double and triple degenerated, respectively. Additional indexes placed with the symbol, such as 1, 2, 'i', 'g' (gerade) and 'u' (ungerade), determine the symmetry of vibrations with respect to an axis of multiplicity different than a major axis, a plane, the center of inversion, respectively.

Experimentally, the assignment of IR and Raman bands to vibrations of individual groups of atoms is also based on the isotopic shift (change of vibration frequency due to the substitution of a chosen atom by its isotope. This is supported by an assumption that the isotopic change does not alter distribution of electron density, and therefore it does not change the force constant of a molecule. Magnitude of this shift is determined by the equation 1.5:



**Fig. 1.2.** Types of vibrations for  $XY_2$  molecule

$$\frac{\bar{\nu}}{\bar{\nu}^*} = \sqrt{\frac{\mu_{\text{red}}^*}{\mu_{\text{red}}}}, \quad (1.5)$$

where „\*” corresponds to a molecule with a changed isotope.

It is also assumed that a considerable shift is only observed for bands that represent “isolated” vibrations of functional groups.

## 1.3. Construction of a FT-IR spectrometer

Fourier Transform IR spectrometers operate on the basis of mathematical operation called Fourier transformation. A typical spectrometer consists of a light source, an interferometer, a sample chamber and a detector.

### 1.3.1. Light source

A source of light in the IR spectrometer must be polychromatic infrared radiation due to the necessity of fitting its energy to transitions between vibrational levels of all the molecules. Typically, light sources are a global (SiC) and a Nernst rod ( $ZrO_2 + Y_2O_3$ ), which – when heated to high temperature – emit radiation similar

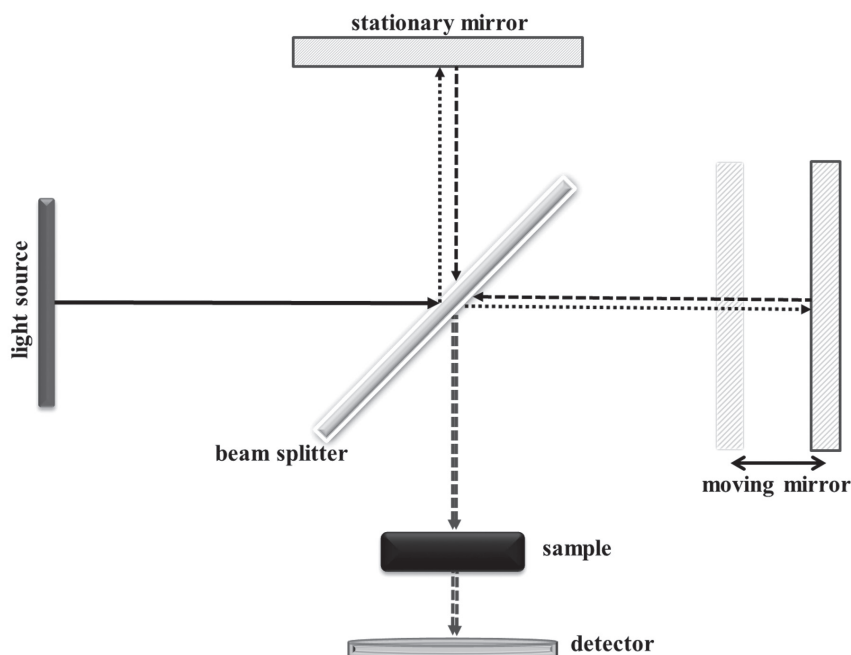
to the black body radiation. The Nerst rod emits radiation in the range from 300 to 20 000 nm (from UV to NIR), whereas the globar provides light in the range of 1 100 – 40 000 nm (NIR, MIR and FIR). The light beam from the source reaches the interferometer, then is collimated through a series of mirrors and finally directed to a sample.

### 1.3.2. Interferometer

In the Fourier Transform spectrometers, intensity of absorption is not directly recorded as a function of frequency but in a form of an interferogram, which represents relationship between a signal and time (the optical path difference). To obtain a spectrum in the frequency domain from the time domain, the interferogram is transformed by a mathematical operation called the Fourier transformation. Collection time of a spectrum is very short due to the fact that the entire spectral range is recorded simultaneously. A number of spectra registered in the time domain are averaged, and then transformed by the Fourier transformation. Advantages of the use of the interferometer in any spectrometer are as follows:

- 1/ Fellgett's advantage (multiplex advantage): recording all wavelengths simultaneously results in shortening data collection time and improvement of signal ( $S$ ) to noise ratio ( $N$ ) since  $S/N \sim n^{1/2}$  ( $n$  – number of spectra). This advantage is especially important for techniques which generate weak signal (e.g. a Raman spectrometer with laser excitation in NIR region),
- 2/ Jacquinot's advantage: the lack of slits does not limit radiation pathway,
- 3/ Connes's advantage: the movement of the moving mirror is controlled optically by a He-Ne laser and this allows to achieve high precision of the wavenumber scale in a spectrum.
- 4/ easy change of spectral resolution.

A schematic of a typical interferometer (the Michelson interferometer) used in optical spectroscopy is illustrated in Figure 1.3. The interferometer is an element splitting the electromagnetic radiation beam and its role is similar to a grating in the monochromator. It consists of two mirrors, positioned to each other at an angle of  $90^\circ$ , when one of them is stationary, whereas the second mirror moves controlled by a He-Ne laser. Between the two mirrors, a beam splitter is placed at an angle of  $45^\circ$ . The radiation falling on the beam splitter is divided into two beams transferred to both mirrors. Then light beams are reflected on the mirrors and next they are collimated on the beam splitter. Light beams interfere to each other depending on the position of the moving mirror. If the two mirrors are located at the same distance from the beam splitter, and hence the optical path difference is zero, the highest amplification of all wavelengths is observed. In case of any different positions of the moving mirror, both beams are no longer in-phase consistent and only those wavelengths for which the interference condition is satisfied will be amplified.



**Fig. 1.3.** A schematic of the Michelson interferometer along with marked pathway of beam rays

### 1.3.3. Sample chamber

A sample chamber of a FT-IR spectrometer is constructed specifically for each IR technique, discussed in Chapter 3.1. The chamber and the spectrometer are purged with dry air or dry nitrogen in order to remove water vapor and  $\text{CO}_2$  present in air. Both molecules, due to the large dipole moment, generate strong bands in spectra. Water vapor shows bands at about  $3500$ ,  $1630$  and below  $500\text{ cm}^{-1}$  whereas  $\text{CO}_2$  absorbs infrared radiation at  $2390 - 2280\text{ cm}^{-1}$  (doublet) and  $672 - 665\text{ cm}^{-1}$ . Mostly, IR spectrometers are single beam instruments and thus collection of the empty chamber or a cuvette with solvent is required before measurement of the sample.

### 1.3.4. Detector

After passing through a sample, signal is analyzed by a detector, whose function is to change intensity of radiation into an electrical signal. The standard detector in FT-IR spectrometers is DLATGS (deuterated triglycine sulfate substituted with alanine) operating in the range of  $350 - 6000\text{ cm}^{-1}$ . This detector is a pyroelectric cell sensitive to temperature changes induced by IR radiation. In FT-IR microscope, a MCT (semiconductor – Mercury Cadmium Telluride) detector is commonly used which detects radiation in the range of  $670 - 7000\text{ cm}^{-1}$  (see Chapter 3.2).



## References

1. Cotton F.A., *Chemical applications of group theory*, Wiley, 1990.