LABORATORY OF ELEMENTARY BIOPHYSICS

Experimental exercises for III year of the First cycle studies

Field: “Applications of physics in biology and medicine”

Specialization: “Molecular Biophysics”

PPB2a

Identification and assessment of organic compounds concentrations on the basis of infrared spectra.

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I. Introduction

Infrared (i.e. electromagnetic radiation in the wavelength range from 780 nm to 1 mm) was discovered in 1800 by William Herschel. This area is divided into the following scopes:

- near infrared (NIR), 0.7-5 µm
- mid infrared (MIR), 5-30 µm
- far infrared (FIR), 30-1000 µm.

Spectra of chemical molecules observed in the NIR region are directly connected to their composition. Therefore research method based on the analysis of infrared spectra is used to study the structures of molecules and check their purity. Infrared spectroscopy is routinely used in research laboratories and industry.

The aim of the experiment is:

- to learn the research method based on infrared spectroscopy
- to learn the principle of FTIR - spectrometer operation and rules of the work with the materials used
- to carry out a typical experiment involving the record of infrared spectra
- to analyse of the spectra obtained during the experiment.

I. Basic Definitions

The electromagnetic radiation consists of waves of oscillating intensities of electric and magnetic fields. These waves propagate at the speed of light and have a specific energy, length and frequency. The wavelength is related to the frequency by relationship:

$$\nu = c/\lambda,$$

and energy:

$$E = hc/\lambda,$$

Where:

λ- wavelength,

c- light velocity,

h-Planck's constant,

ν-frequency

E-energy.

The wave number is defined as the inverse of the frequencies:

$$\tilde{\nu} = 1/\nu.$$

The electromagnetic radiation may be absorbed or emitted by atoms. This absorbed or emitted portion of energy must correspond to the difference between the energy levels of the atom with which it interacts - is quantized.

The total energy of the molecule consists of translation, rotation, oscillation (vibration) and electron energy ($E_{tot} = E_{el} + E_{osc} + E_{rot} + E_{trans}$). Absorption of quantum energy in the range of microwave results in a change of rotation energy. Absorption of quantum energy in the infrared range causes the change of the oscillation mode and the absorption of visible or ultraviolet radiation leads to changes in electron energy of molecules.

As mentioned, transitions between rotation states can be tested in the microwave range. Observation in infrared region of rotation transitions between states is possible by coupling the rotations with the oscillations. Energy states of molecules corresponding to the rotations are recorded only in the gas phase. This is not possible in the liquid, due to frequent collisions of molecules and, therefore, the inability to perform their full turns (Fig.1). Recording of vibration spectra is possible for substances in a liquid and solid phase.

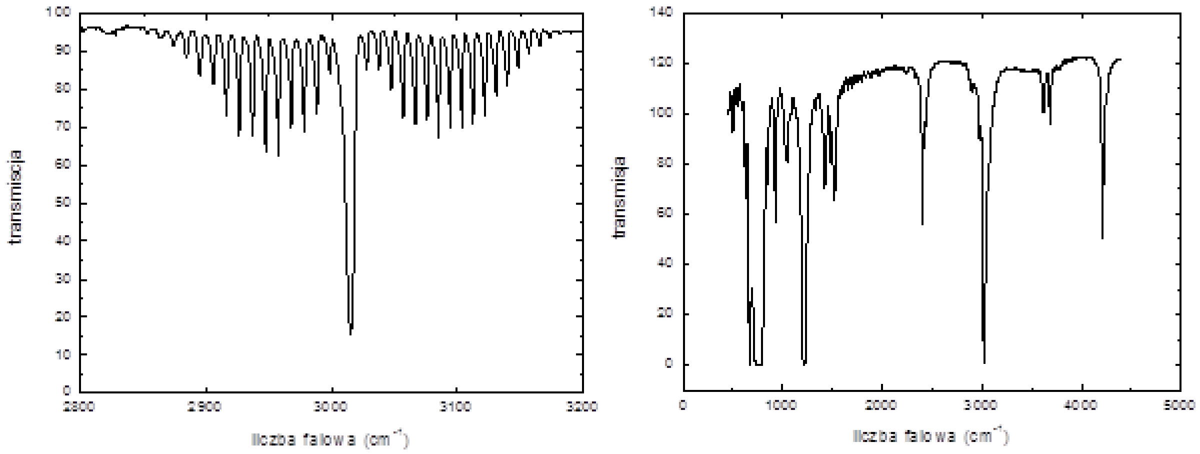

Figure 1. Vibration-rotation spectrum of methane in the gas phase and the vibration spectrum of chloroform in the liquid phase.

II. Oscillations of macromolecules

Considering the classical model of the diatomic molecule vibrating in a harmonic potential we treat it as a system of two masses connected by a spring. They can perform oscillations with a frequency that depends on the reduced weight of the system (m_{red}), and force constant (k) according to equation:

$$\nu = \frac{1}{2\pi} \cdot \sqrt{\frac{k}{m_{red}}}$$

In the case of the quantum model an energy of particle can take only certain values

$$E_n = \left(n + \frac{1}{2} \right) \cdot h\nu$$

which are determined by the selection rule Δn = ± 1. The molecule can absorb or emit energy equal to hν.

Real vibrating molecules are described by the relationship for anharmonic oscillator:

$$E_n = h\nu_o \left(n + \frac{1}{2} \right) - h\nu_o x \left(n + \frac{1}{2} \right)^2$$

for which, in the case of low-energy vibrations, harmonic potential approximation can be used but for the higher energy this approximation is improper.

Selection rule allows the emergence of weaker uppertones Δn = ± 2.3 ... which correspond to transitions ΔE = ± 2hν, 3hν. In the case of extremely high energy the dissociation of the molecule takes place.

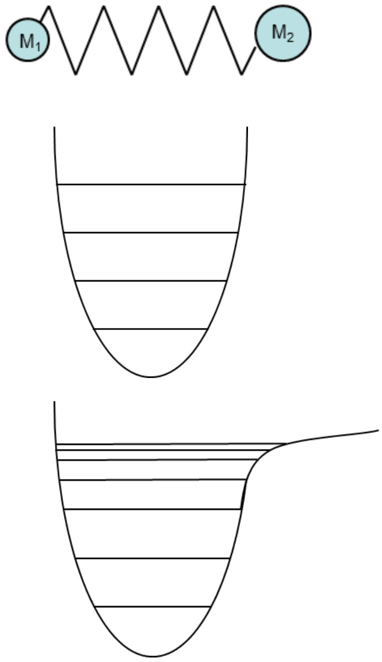

Figure 2. Diatomic vibrating molecules model, and the wells of harmonic and anharmonic potential.

III. Vibrational modes of oscillation and group frequency

Oscillations of molecules can be divided into stretching and bending vibrations. Even the most seemingly complex vibrations can be decomposed into so-called normal modes. For example H_2O molecule has three modes of vibrations: (1) bending, (2) symmetric stretching and (3) anti-symmetric stretching. All non-linear molecules containing N atoms have 3N-6 normal modes of vibrations. The linear molecules have 3N-5 normal modes. Each of these oscillations corresponds to a potential well with a set of energy levels. An oscillating molecule absorbs a quantum of energy, corresponding to the absorption bands visible in the infrared spectrum.

The oscillation modes visible in the infrared spectrum must be accompanied by a change of the dipole moment of the molecule. Therefore not all vibration modes (i.e. modes without change of dipole moment) can be seen in the infrared spectrum. For example, the linear molecule CO2 has 4 normal modes and in the spectrum two bands can be seen because one of the vibration is degenerate, and the second is not active in the infrared (is not accompanied by the change of the dipole moment).

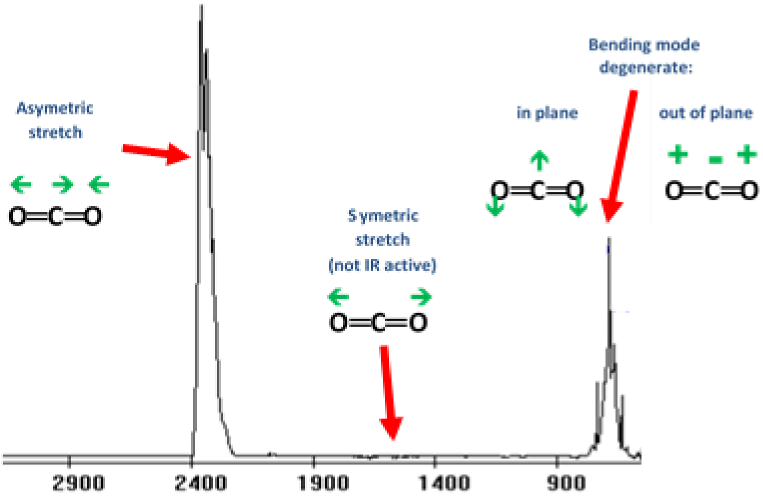

Figure 3. Oscillation spectrum of CO_2.

It is difficult to analyse every vibration mode for complex molecules. It is assumed that each functional group in a molecule can be analysed independently, for example. CH_3 group has the same energy of oscillation (wavelength of absorption) no matter how does the molecule possessing this group look.

Each functional group has a characteristic set of frequencies corresponding to its vibrations. In Fig. 4 there are shown examples of the functional groups and ranges of the infrared spectrum in which we should search for the corresponding absorption bands.

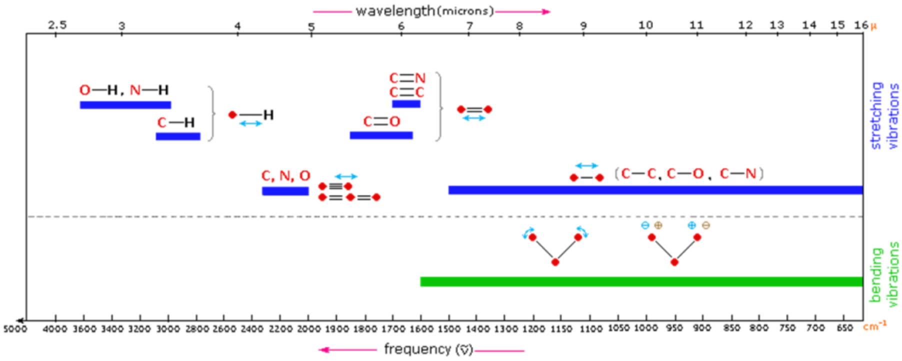

Figure 4. Examples of functional groups of molecules and corresponding ranges of the oscillation frequencies. http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/InfraRed/infrared.htm

The rules that govern the oscillation frequency of functional groups in molecules:

- stretching vibration frequencies are higher than the corresponding bending vibration.
- bond with hydrogen has a higher frequency than the corresponding bond with the heavier atom.
- triple bonds have a higher frequency of stretching vibrations than the corresponding double bonds and the double bonds have a higher frequency than the corresponding single bond vibrations (except for bonds with hydrogen).

Knowledge of the characteristic group modes of vibration allows for identification of chemical molecules and/or their pollution, by examination of the infrared spectra.

IV. Beer-Lambert Law

The intensity of the radiation passing through the absorbing medium is attenuated according to the formula:

$$I = I_0 e^{-\varepsilon \cdot c \cdot l}$$

where Io is the incident beam,

I - intensity of the radiation beam after passing through the absorbing solution,

l - the distance the light travels through the material (the path length),

c - the concentration c of attenuating (absorbing) species in solution,

ε - absorption coefficient.

We define transmittance as:

$$T = \frac{I_0}{I}$$

and absorbance as:

$$A = -\ln T = \varepsilon \cdot c \cdot l$$

Thus, by measuring the transmittance (T) of the sample and calculating the absorbance (A), for known absorption coefficient (ε) and the optical path length (l) we can determine the concentration of the absorbing substance (c).

The absorbance depends linearly on the concentration at a fixed optical path length and wavelength. Drawing, a calibration curve by plotting the absorbance as a function of concentration A(c) for known concentrations of the substance we can calculate the content of the substance in a sample of unknown concentration.

V. The measurement technique

The infrared spectrophotometer with a Fourier transformation (FTIR) will be used in the measurement.

In the case of conventional measurements the sample is illuminated with a radiation beam of varying frequencies. The measurement time is 10-100 min.

In the measurement technique based on the Fourier transformation the polychromatic beam of infrared radiation interact with the sample. The absorption spectra were not observed directly, but the signal is measured as a function of time, and then transformed from the relationship y(t) into x(v). It allows to significantly reduce the time of a single measurement (to seconds). Measurements can be repeated and averaged, what improves the ratio noise to signal (N/S).

Infrared measurements require cells transparent in this area of spectrum from substances as salts: KBr, NaCl, CaF_2, and others. Because water dissolves NaCl, KBr, when you work with cuvettes made of this material, you should use solvents containing no water and work under conditions which prevents the contact between cuvettes and moisture. This applies to spectrophotometer as well, because the optical prisms are typically made of potassium bromide. Suitable solvents usually used are: chloroform, deuterated chloroform, CCl_4, etc… Cuvettes with cell windows from calcium fluoride are not soluble in water.

VI. Entry requirements

Prior to the experimental part, students must pass an preliminary test. The teacher decides about the form of this test. The material on the infrared spectroscopy, and method of the experiment performance is presented in this instruction and placed in the bibliography.

Following topics may be discussed during the test:

1. Basic definition connected to electromagnetic radiation and spectroscopic measurements: wavelength, frequency of vibrations, wavenumber, molar extinction coefficient, ground state, excited state, infrared range, forms of molecular energy, energy quantization.
2. Measurement of absorption and emission of electromagnetic radiation: principles of the absorption and emission measurements, explanation of the terms “absorption spectrum” and “emission spectrum”.
3. The absorption rotation spectra of diatomic molecules. The range of radiation in which the absorption rotational bands are observed. Scheme of energy levels and transitions allowed by the selection rules.
4. The physical description of the molecule oscillations. What types of oscillations can be performed by molecules. Which of them are visible in the infrared? Interpretation of the oscillation spectrum. Intermolecular hydrogen bonds and their detection.
5. What is the vibration-rotation spectra of diatomic molecules? The range of radiation in which the absorption vibration bands are observed. Scheme of energy levels and transitions allowed by the selection rules. Absorption spectrum, its relationship with the structure and the populations of the energetic levels. Difference between perpendicular and parallel bands in absorption vibration-rotation spectrum of molecules. The investigations, which use oscillation-rotation spectrum of molecules.
6. Apparatus. Material problems (passing IR). IR spectrophotometers, classical and with Fourier transformation.

VII. Experiment

The experiment will consist of:

A) the interpretation of the infrared spectra of selected chemical compounds, e.g.. CH_4, $CHCl_3$, and CCl_4 $CDCl_3$

B) the determination of the content of the chosen compound (e.g. diphenylamine) in a solvent (e.g. CCl_4).

After reading the safety rules students perform spectral measurements (Part A) of:

- methane in the gas phase (sample No. 1)
- selected samples of unknown content (samples No. 2, 3 and 4).

In the spectrum of methane, band (bands) associated with the vibrations of the CH group ought to be identified and their appearance in the gas phase must be discussed. Next students proceed with the identification of substances ($CHCl_3$, $CDCl_3$ and CCl_4) in the samples No. 2, 3 and 4.

In Part B of the experiment solutions (10%, 20%, 30% and 40%) of dimethylamine or chloroform in carbon tetrachloride should be prepared to register their infrared spectra. The plot for a calibration curve of the selected wave number should be drawn.
After receiving from an assistant solutions of unknown concentration of these compounds student should determine their concentration.

VIII. Report preparation

In the first part of the report (A) the interpretation of the spectra and the identification of test compound should be presented.
In the next part (B) based on the calibration curve, the content of the test substance in a solvent should be calculate.

Bibliography:

1 Z. Kęcki, “Fundamentals of molecular spectroscopy”
2 G. Barrow, “Introduction to Molecular Spectroscopy”
4 JA Barltrop and JD Coyle, “Photochemistry”
5 J. Simons, “Photochemistry and Spectroscopy”

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