RAMAN SPECTROSCOPY

Lucia Becherová, Marcela Dendisová, Martin Člupek, Marie Švecová

Introduction – principles of Raman spectroscopy

Raman spectroscopy belongs to the group of vibration molecular spectroscopies and was discovered by Indian physicist Chandrasekhara Venkata Raman (Nobel Prize 1930). In the year 1928, Professor Raman together with K. S. Krishnan described observation of inelastic optical scattering, which is a fundamental effect of the method. This spectroscopic method suits very well for identification of substances, determination of their composition, and their molecular structure. It is used for analyses of solid samples (both crystalline and amorphous materials; metals, semiconductors, polymers etc.), liquids (pure substances, solutions – both aqueous and nonaqueous), gases, moreover for analyses of surfaces (e.g., sorbents, electrodes, sensors) or for analyses of biological systems (from biomolecules to organisms analyzed *in vivo*). The usage of Raman spectroscopy can be found from mineralogy and geochemistry, through chemical and pharmaceutical industry to biology and medicine.

The nature of Raman scattering is a radiative two-photon transition between two stationary vibration states of a molecule, which energies are E_1 a E_2. This phenomenon is induced by an interaction with a photon of incident radiation with frequency $\nu_0 > | E_2 - E_1 | / h$, where h is Planck constant, and accompanied by a formation of a photon of scattered radiation with frequency ν_R (see Fig. 1). This scattering effect can be viewed in a simplified way as simultaneous absorption of a photon of an exciting radiation by a molecule (when the molecule excites to a virtual energy level) and emission of secondary photon of scattered radiation under the condition of energy conservation:

$$h \nu_R = h \nu_0 \pm (E_2 - E_1) \qquad (1)$$

There are several possibilities how to execute such a transition depending on the position of a virtual energy level compared with the eigen-levels (eigen-states) of the molecule (e.g., the normal Raman effect and the resonance one).

The Raman scattering effect should be described precisely using quantum theory, the fundamentals of this effect can be also described in a classical approximation. In the case of classical approximation, an equation for induced dipole moment $\boldsymbol{p}$ can be given for a molecule interacting with incident radiation:

$$\boldsymbol{p} = \alpha \boldsymbol{E} \cos\left(2\pi \nu_0 t\right) + \frac{1}{2}\frac{\partial \alpha}{\partial q} q \boldsymbol{E} \left\{\cos\left[2\pi\left(\nu_0 - \nu_{vib}\right) t\right] + \cos\left[2\pi\left(\nu_0 + \nu_{vib}\right) t\right]\right\} \qquad (2)$$

where ν_0 is the frequency of incident radiation, ν_{vib} is a vibration frequency, $\boldsymbol{E}$ is a vector of electric field intensity of incident radiation, q is an internal coordinate of a molecule and α is the polarizability of the molecule (the polarizability represents a measure of "difficultness" to deflect (to distort) negative charges from its normal shape by an external electric field). From

the equation (2), it is evident that the molecule emits radiation (i) with the unchanged frequency (ν_0 – called Rayleigh scattering ——), (ii) with higher frequencies ($\nu_0 + \nu_{vib}$ ——) and (iii) with lower frequencies ($\nu_0 - \nu_{vib}$ ——). The emitted radiation of changed frequencies is collectively called Raman scattering; the lower frequencies ($\nu_0 - \nu_{vib}$) correspond to Stokes scattering (Stokes spectral region), while the higher frequencies ($\nu_0 + \nu_{vib}$) are attributed to anti-Stokes scattering (anti-Stokes spectral region). From the equation (2) it follows that to observe any Raman line , a given vibration motion has to be accompanied by a change of polarizability, that means

$$\frac{\partial \alpha}{\partial q} \neq 0 \qquad (3)$$

If the change of polarizability during vibration motion is zero, both terms describing Raman scattering are zero in equation (2); only the term related to Rayleigh scattering (equation (2)) remains non-zero. Equation (3) is denoted as a fundamental selection rule for Raman spectroscopy, which is on principle different compared to observation of vibration modes in the infrared spectroscopy, where the fundamental selection rule is a change of dipole moment during the corresponding vibration motion.

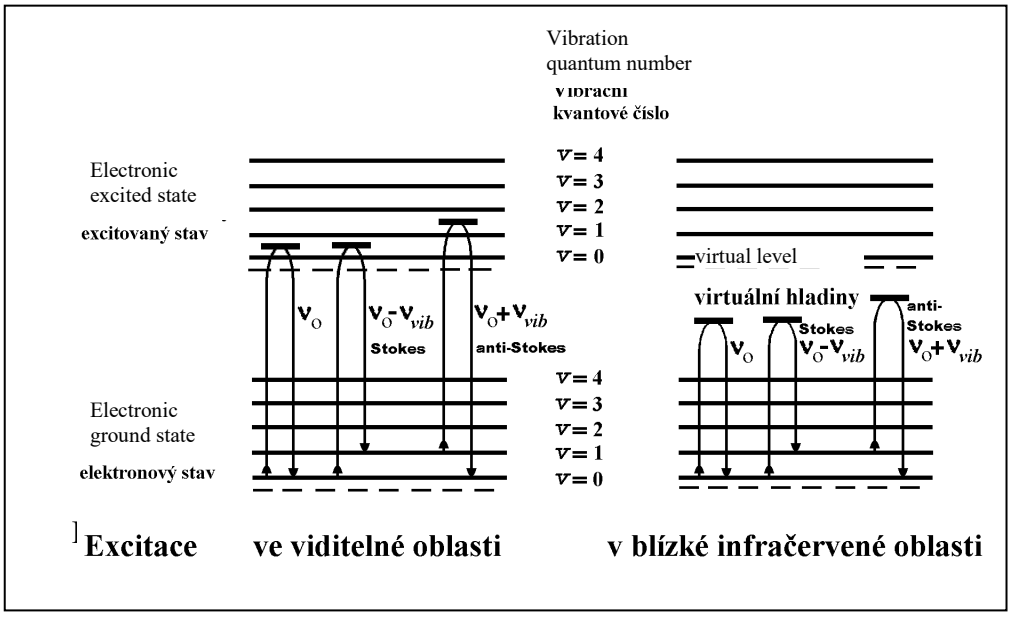

Fig. 1. **Scheme of two-photon transitions of Raman and Rayleigh scattering with marked excitation in visible and near infrared range**, ↑ - denotes excitation, ↓ - denotes emission of a photon

If a given vibration mode is active in Raman spectrum, it is possible in principle to observe two lines, which are symmetrically placed around the line of Rayleigh scattering – the first one in Stokes region ($\nu_R = \nu_0 - \nu_{vib}$) and the second one in anti-Stokes region ($\nu_R = \nu_0 + \nu_{vib}$). In many practical cases the spectra are measured only in Stokes scattering region, considering the necessity to reject Rayleigh scattering, which intensity is ca 10^5-10^{12}-times higher than the intensity of normal Raman lines.

Structural analysis and interpretation of spectra

Raman spectra as well as infrared spectra provide information on vibration (and rotation) motion of polyatomic particles (molecules, crystals etc.). The frequency of normal vibration mode depends predominantly on weights of involved atoms and on corresponding bond strength; that means on fundamental parameters that describe the structure of a molecule. In general terms, identification possibilities of Raman spectroscopy are comparable with the potential of infrared spectroscopy. It should be emphasized that **vibration frequencies of any molecule are independent on the vibration spectroscopic method used (i.e., either infrared or Raman spectroscopy)**, but intensities of spectral lines have to be apparently different for both spectroscopic techniques. **In the case of Raman spectrum, the intensities of bands are proportional to the square power of the change of polarizability during vibration motion $(\delta\alpha/\delta q)^2$, while in the infrared spectrum they are proportional to the square power of the corresponding change of dipole moment.** Assignment of bands to individual vibration modes is performed in a similar way, which is used ordinarily in the case of interpretation of absorption spectra in the mid infrared region. For assignment of individual signals, combined tables of characteristic vibration frequencies of functional groups contain a common value of band position (wavenumber, cm^{-1}) and separate information on band intensities either in infrared or in Raman spectra. While in the infrared spectra there are intense bands assigned to vibrations accompanied by significant changes of dipole moment (vibrations of polar groups, e.g. –OH, -C=O, $-NO_2$), band intensities in Raman spectra are related to changes of polarizability (more intense bands are observed for symmetric vibrations and in-phase vibrations than for antisymmetric vibrations and in-phase opposition. Especially intense bands in Raman spectra are typical for vibrations of multiple symmetric bonds – e.g., -C≡C-, -C=C-, -N=N-). The appearance of both infrared and Raman spectra is strongly affected by a general symmetry of molecules (cells, crystals) and a local symmetry of individual vibration modes. For molecules with low symmetry (characterized only by symmetry element "identity"), bands of all vibration modes are observed in both types of spectra, of course with profoundly different intensities. For molecules with high symmetry, the infrared spectrum and the Raman one are mutually complementary. E.g., for molecules with the inversion centre of symmetry, the law of alternative forbiddance is valid, i.e., vibration bands observed in the Raman spectrum are forbidden in the infrared spectrum and vice versa. (Totally symmetric vibrations are active in Raman spectrum and they are inactive in infrared spectrum.)

In contrast to infrared spectra, it is possible to easily identify many symmetrically substituted groups and/or molecular skeletons. For example, symmetrically substituted C≡C triple bond manifests typically as a very strong band of stretching C≡C vibration in a range from ca 2260 to ca 2160 cm^{-1}. Hence, it is possible to distinguish it unambiguously from nonsymmetrically substituted C≡C bond (ca. 2180 to 2100 cm^{-1}). Furthermore, it is possible to differentiate various types of triple bonds (with various types of substituents and neighbouring groups) and to identify multiple triple bonds separately in one molecular structure.

In addition, the double C=C bond is represented in Raman spectra by a very characteristic, intense band of stretching vibration in a region ca. 1690 – 1630 cm^{-1} for nonconjugated alkenes. The conjugation effect lowers the frequency; therefore, the characteristic range is 1660 – 1580

cm^{-1} for conjugated alkenes. On the contrary the stretching vibrations of C=O bonds exhibit only weak bands in Raman spectra of various types of carbonyl and/or carboxyl compounds (ketones, esters, amides etc.). Using combination of Raman and infrared spectroscopy it is possible to clearly assign individual given bands in the region 1750 – 1580 cm^{-1} (either to C=O stretching vibration or to C=C stretching modes) and to elucidate different origins of several bands of different intensities and shapes. In contrast to infrared spectroscopy, Raman spectroscopy allows to successfully characterize various technically important elemental materials, e.g., carbon materials (graphitic layers, soot particles, natural and synthetic diamonds, graphenes, etc.) or silicon materials (electronics). Various industrial applications use Raman spectroscopy to study different inorganic materials containing heavy metals, e.g., corrosion oxidic, /di/sulfidic layers on alloys of heavy metals (corrosion of materials under natural or production conditions). In the case of study of polypeptides and proteins, Raman spectroscopy enables to monitor symmetric stretching S-S vibration of disulfidic bridges (e.g., equilibrium cystine – cysteine, or formation of different more soluble disulfides with cysteine for medication of cystinuria).

From the point of view of qualitative information, it is possible to compare measured spectra of pure substances with spectral libraries, thus performing chemical identification of substances and materials. Raman spectrum can be used for identification as an excellent „finger-print“, especially in the case where data stored in the spectral library were measured under the same experimental conditions as the sample tested (the same state of matter is extremely important). In comparison with an infrared spectrum, the Raman spectrum is usually simpler and more transparent. In commercially available electronic systems, spectral libraries are implemented, for example, for flammable compounds, explosives, addictive drugs or pharmaceutically important chemicals. In many situations, it is possible to identify multiple components in quite complex mixtures without any chemical separation step used. Relatively large databases of Raman spectra (up to tens of thousands of spectra) are available commercially, but it is very useful to create dedicated spectral libraries by processing of own measured data to solve specific problems in the application area given.

In many cases, the spectra obtained have to be unscrambled and classified using various multivariate chemometric methods. Furthermore, Raman spectra are used for quantitative analysis. Raman spectroscopy gains ground as a new method for environmental analyses or as an analytical tool for medicinal chemistry. The measurement itself is relatively fast[1], often nondestructive and usually it does not require any special sample preparation. Thus, the consumption of both chemicals and disposable analytical sets is minimized; thereby, the generation of environmentally hazardous wastes is prevented. It is possible to measure samples in glass and some other transparent packages. Water exhibits only weak Raman bands; thus, it is easier to analyze aqueous solutions than in the case of infrared spectroscopy. Furthermore, the optical materials used in Raman spectroscopy are not sensitive to any humidity. The steps of spectral data processing and evaluation of measured spectra are mostly more laboured and time-consuming than the spectral measurement itself.

[1] Several seconds can be sufficient to record individual spectrum, several minutes of data accumulation are frequently used to improve signal/noise ratio.

Quantitative analysis using Raman spectroscopy

As noted above, Raman spectroscopy is now used in quantitative analysis. Nevertheless, it is absolutely necessary to consider many specific factors of Raman spectroscopy (e.g., proper and stabile value of laser power, minimization of effects of possible re-absorption of scattered radiation, fixed penetration depth of exciting radiation into sample, and so on).

We have to consider that Raman spectroscopy is not an absorption spectroscopy, the band overlaps of various components have to be taken into account and mutual influences of varying concentrations of individual components affects both shapes and intensities of corresponding Raman bands. Hence, a simple principle of linear dependence of band intensity on an analyte concentration is not fulfilled frequently in the case of Raman spectral analysis. For calibration in Raman spectroscopy, it is mostly essential to develop complex calibration models using advanced chemometric algorithms, which commonly require large sets of standards (often more than 30 calibration samples). A proper set of standards has to be sufficiently representative, it should cover the whole interval of expected or predicted variability of sample characteristics, which have to be analyzed quantitatively; not only variability of monitored analytes, but also other types of variability (both of physical and chemical nature) have to be considered. The preparation of the appropriate set of calibration samples requires very careful planning of experiments. When advanced regression methods are used to create calibration models, the broad spectral regions or whole Raman spectra (in many cases the range of Stokes scattering) are evaluated instead of values of intensity in maxima of several selected bands. Thus, the aim is to find out any relation of multidimensional spectral information (represented by a matrix of values of scattering intensity in selected spectral intervals for the set of calibration samples) and sample composition data (represented by matrix of concentration values for a group of monitored analytes in the set of calibration samples). The experimental conditions of measurement of all spectra, the methods of spectral corrections, processing and evaluation have to be preserved for all data from a calibration step, over validation procedure to analysis of unknown studied samples.

Only in very simple cases of pellucid solutions measured under stable experimental conditions it is possible to perform relatively easy calibration procedure based on processing of values of corrected peak areas (after spectral background subtraction) for one selected band or several ones. The peak areas of analyte bands are related frequently to the reference values of the peak areas assigned to an added internal standard, eventually to the solvent.

Raman spectrometer and measurements techniques of Raman spectra

The Raman spectra can be recorded using either dispersive spectrometers or Fourier transformation (FT) spectrometers. The main parts of spectrometers are following: (i) source of excitation radiation (laser), (ii) sample compartment (sample holder), (ii) collection optics, (iii) dispersive element (dispersive spectrometers) / interferometer (FT spectrometers) and (iv) detector.

For Raman spectroscopy it is obvious to use as radiation sources various types of lasers, which cover both the visible (VIS) and near infrared (NIR) range (rarely ultraviolet - UV lasers are used). In the case of simple routine spectrometers, the source is a fixed laser with one emitted laser beam of a given wavelength. Usually, it is a quite cheap and durable solid-state or diode laser, working in continuous or quasi-continuous operation mode. In the case of advanced scientific systems, there are pre-aligned optical benches for several lasers, which allow optimizing the excitation wavelength to the issues solved. The advantage of an excitation in UV-VIS region is apparently higher scattering intensity (the scattering intensity decreases with the fourth power of the wavelength of excitation radiation). On the contrary, the crucial disadvantages are risks of very intense fluorescence and/or unwanted photochemical reactions. These risks are given by the position of actual virtual level in the region of electronic excited levels (Fig. 1). The suppression of risks of unwanted photochemical and photophysical processes is just the key advantage of Raman effect excitation in NIR region, where the virtual level is undoubtedly under the level of electronic excited states (Fig. 1). Considering the lower scattering intensity in the case of NIR excitation the FT Raman spectrometer requires optical systems of high luminosity, minimal loss of radiant flux and very sensitive (cooled) detectors. The flux of Raman scattered radiation increases generally with the increase of excitation laser radiation flux; that means with increasing value of the **laser power**. Nevertheless, the maximal value of laser power used is limited by risks of sample (over)heating, its degradation, eventually by risks of additional undesirable photochemical and photophysical processes. The value of laser power is possible to set in the operation software and to optimize it to the sample properties, requirements on speed of analysis and on the value of signal/noise ratio achieved in the spectrum recorded.

Raman spectra are measured mostly for samples placed in a closed dark sample compartment. The compartment is usually adapted for measurement of samples in various glass vessels (tubes, ampoules, vials, cells for UV-VIS spectrophotometry or cells for NMR spectroscopy). Thus, liquids and powdered solids can be measured easily decreasing their volume to hundreds of microliters. Furthermore, there exist many various special holders, for example for macroscopic measurement of solid piece samples, for spectral mapping of thin-layer chromatograms, or for analysis of multiple samples on spotting plates. An important aspect of sample location in a sample compartment is the possibility of sample precise positioning toward the excitation beam and the collection optics of scattered radiation. The instruments are frequently equipped with **x-y-z positioning device** either with manual control or with stepwise moving motors controlled using a joystick, special buttons or operation software. The key parameter is the distance of the sample from the collection optics, in order to focus the scattered radiation optimally either on the entrance slit of a dispersive Raman spectrometer or on the entrance aperture of the Raman spectrometer with Fourier transformation, eventually into collection optical fiber.

Microspectrometric instrumentation, represented by the connection of a Raman spectrometer with an optical microscope, is based on replacement of standard sample compartment with the optical microscope. Raman micro-spectrometry is used very frequently for surface analysis including surface (interface) spectral mapping.

Besides the described design of the spectrometer, where the sample is placed in an internal holder of the instrument, Raman spectrometers can be designed for *in situ* analyses using various types of remote probes usually connected to the spectrometer body via fiber optics. The Raman probes can be placed directly in various vessels (e.g., chemical or biotechnological production reactor) or they can be touched to sample surfaces.

From the scattered radiation it is necessary to reject intense radiation from Rayleigh scattering. This rejection causes in the case of routine instruments an information loss in the range of Raman shift ca. ± 100 cm^{-1} around the position of the excitation line (excitation line corresponds to the value 0 cm^{-1} of the Raman shift). Usually, the superior spectrometers allow to measure spectra more closely to excitation line up to the value of bare 10 cm^{-1}. To the rejection of Rayleigh scattering holographic filters or simple pre-monochromators are used routinely. Two different (above described) types of construction of Raman spectrometers are used for further processing and detection of scattered radiation. In the case of Raman effect excitation in VIS (eventually in UV and NIR) region the usage of a dispersive spectrometer with a grating spectrograph and „area“ (multichannel) CCD detector is standard. The system with interferometer and a highly sensitive single-channel detector is typical for recording Raman scattering spectra in the near infrared range (NIR). The primary recorded interferogram is transformed using Fourier transformation (FT) to final spectrum, thus the instrument is named FT Raman spectrometer.

In all of the spectral ranges mentioned above (UV-VIS-NIR) high-quality quartz glass is commonly used as an optical material. For the remote sensing quartz fiber optics is applied.

Two dispersive Raman spectrometers iRaman Plus (B&W Tek, USA) are installed in the analytical laboratory and they are equipped with accessories designed for measuring liquid and solid samples. For excitation of Raman scattering, thermoelectrically cooled diode lasers are used, the output radiation is at the edge between Vis and NIR ranges at the wavelength of 785 nm with maximal laser power of 450 mW (approximately 350 mW at the sample as the input from the fiber optic). The sample holders are located outside of the instrument body and the excitation radiation is focused on the sample through the probe head (“Raman probe”) connected to the instrument using optical fibers, where one fiber “conduct” excitation radiation and the second one serves to transfer the scattered radiation from the sample. Rayleigh scattering is rejected by a high-quality filter, and the sophisticated design of spectrometer gives the opportunity of working range of the instrument from ca. 65 cm^{-1} to 3350 cm^{-1} in the Stokes region. The range is fixed and cannot be changed, because the instrument does not contain any moving parts, both the dispersive grating and multichannel thermoelectrically cooled detector are fixed in prealigned positions.

Laboratory exercise – Analysis of liquid and solid samples using Raman spectrometry

Tasks

A) Quantitative analysis of a liquid sample

1) Prepare a set of standard solutions for measurement of a calibration dependence to determine the content of ethanol in water in the range from 0 to 40 vol. % (step 5 %).
2) For an appropriately selected standard solution, you have to test the influence of the varying values of laser power (Table I) and of integration time of the signal (Table II) on the signal/noise ratio. Optimize the experimental conditions for the measurement of the set of calibration samples.

Table I: Laser power

Laser power (%)	50	60	70	80	90	100
time (s)	1	1	1	1	1	1

Table II: Integration time of signal

time (s)	1	2	3	5	10

3) Measure the spectra of the whole set of calibration samples under optimal experimental conditions. (Do not change any parameter during this experiment.) Measure the spectra of unknown samples – model and real one, and then spectra of a real sample with 4 standard additions under the same conditions.
4) Create a calibration graph by calculating areas of an appropriately selected band in the series of calibration spectra and estimate the content of ethanol in the unknown samples.
   Estimate the content of ethanol in the real sample graphically.

B) Analysis of a solid-state sample in the form of a tablet

1) Optimize the experimental conditions for the measurement of a solid-state sample in the form of a tablet.
2) Measure a solid sample on x-y-z positioning device at least at 5 different points. Evaluate the homogeneity/heterogeneity of the unknown sample.
3) Try to homogenize the sample and evaluate the effectiveness of the homogenization step.

C) Structure analysis of liquid or powdered solid sample

1) Measure a given sample in liquid form in a glass vial. Optimize the value of the accumulation time and the laser power.
2) Describe the positions and intensities of important peaks (band maxima) in tabular form.
3) Try to assign important spectral peaks to characteristic vibration modes of the molecular skeleton and to present functional groups in the molecule. Propose the possible structure of the molecule.

Measurement of Raman spectra

Raman spectra are measured in the software BWSpec (B&W Tek, USA). The basic setup and adjustment of the instruments and computers is done by the teacher and at the beginning you use the parameters adjusted by the teacher. The teaching person informs you about the manipulation with the spectrometers and about the operation with the software. The Raman spectrometer iRaman Plus (B&W Tek, USA) is a dispersive spectrometer that is controlled entirely using software BWSpec except for the switch-on/off the power supply using rocker-dolly switch and the switch-on/off laser supply using a special key. The probe head ("Raman probe") is connected to the body of the spectrometer through fiber optics. The Raman probe is fixed either in a versatile sample holder for vials and cells, or mounted to the body of the x-y-z positioning device. The spectrometer parts are presented in Fig. 2.

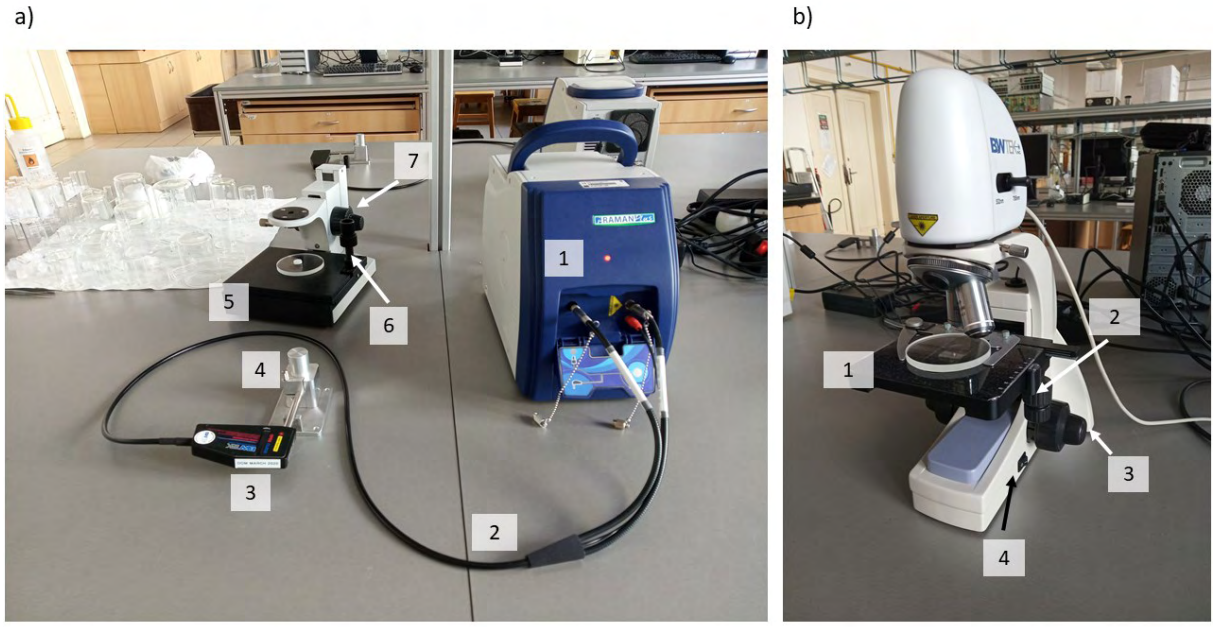

Fig. 2: **A photo of an iRaman Plus spectrometer with accessories for a) macroscopic and b) microscopic sampling**; parts labelling a) 1- body of the spectrometer, 2 – fiber optic, 3 - Raman probe, 4 – sample compartments for vial, 5 – x-y adjustable table for solid-state samples, 6 – table moving in the x-y directions, 7 – focus; parts labelling b) 1 – x-y adjustable table of microscope, 2- table moving in x-y directions, 3 – focus, 4 - lightning

The versatile sample holder (Fig. 3a) contains a sample compartment which is designed for vials with a proper circular diameter. In the case of sample measurements in vials you should insert a carefully-capped vial containing suitable volume of prepared sample (the glass vial should be filled in more than a half) into the circular hole of the sample holder and then you have to close it by the grey cap of the versatile holder. (Do not manipulate with the position of the Raman probe, the position is pre-adjusted to focus the optical beams into the vial, and not onto its outer wall.)

The measurement of a sample in the form of tablets is very easy; you place only the given sample on the stage of the x-y-z positioning device (Fig. 3b) in a central part so that the tablet is under the optical head of the Raman probe. Optimization of the z-focus and sample position is performed in the mode of continual recording of spectra after setting essential parameters

in program BWSpec (function of "acquire continuously"). Any contact of the optical head and the sample has to be prevented during the manipulation with the x-y-z positioning device.

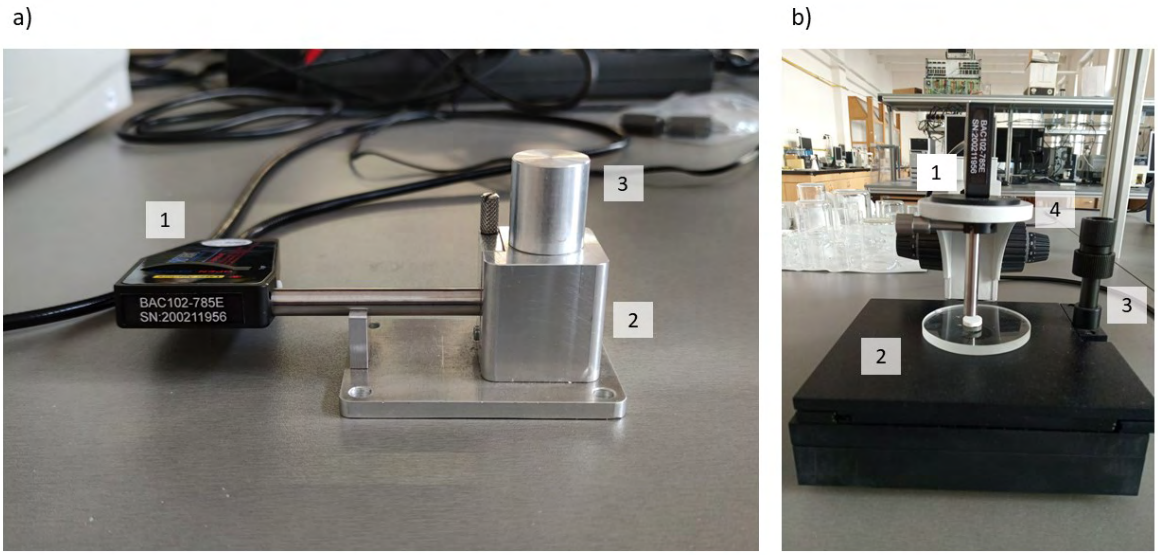

Fig. 3: **Raman probe connected to a) the vial sample holder, b) the mapping table for solid-state samples**; parts labelling a) 1 - Raman probe, 2 – body of the sample holder, 3 – cover; parts labelling b) 1 – Raman probe, 2 - x-y adjustable table for solid-state samples, 3 - table moving in the x-y directions, 4 – focus

After introducing the sample into the compartment (a versatile sample holder or x-y-z positioning device), you have to set up the experimental parameters in the software BWSpec: laser power/level, accumulation (integration) time, number of repeated accumulations.

The setting of the individual parameters is quite user-friendly and is placed in the upper part of the working window (Fig. 4). The first field serves for setup of integration time (in the next field you can select the time unit – options are milliseconds, seconds, and minutes), the second one is for setup of number of acquisitions ("time average") and you fill in the laser power/level value in the last field. It should be reminded that the values for laser power are set up as percentages of the maximal possible laser power (approximately 350 mW according to accessories used), absolute value is not used. The next series of buttons allows us to control the measurement. The first button starts the individual measurements (acquire one spectrum), the second one allows us to display spectra overlayed after recording (acquire overlay), the third one then continuous collecting of spectra (acquire continuously – useful for focusing on tablets samples), the square button is for stopping measurement (stop), and the last one in the block is for recording so-called "dark" with switched off laser (acquire dark – function explained below). In the panel on the right-hand side, it is necessary to check several parameters. X-axis should be plotted in the Raman shift values and y-axis with applied dark corrections ("dark subtraction"). In the basic setting, options such as "external trigger" and "auto time" are turned off.

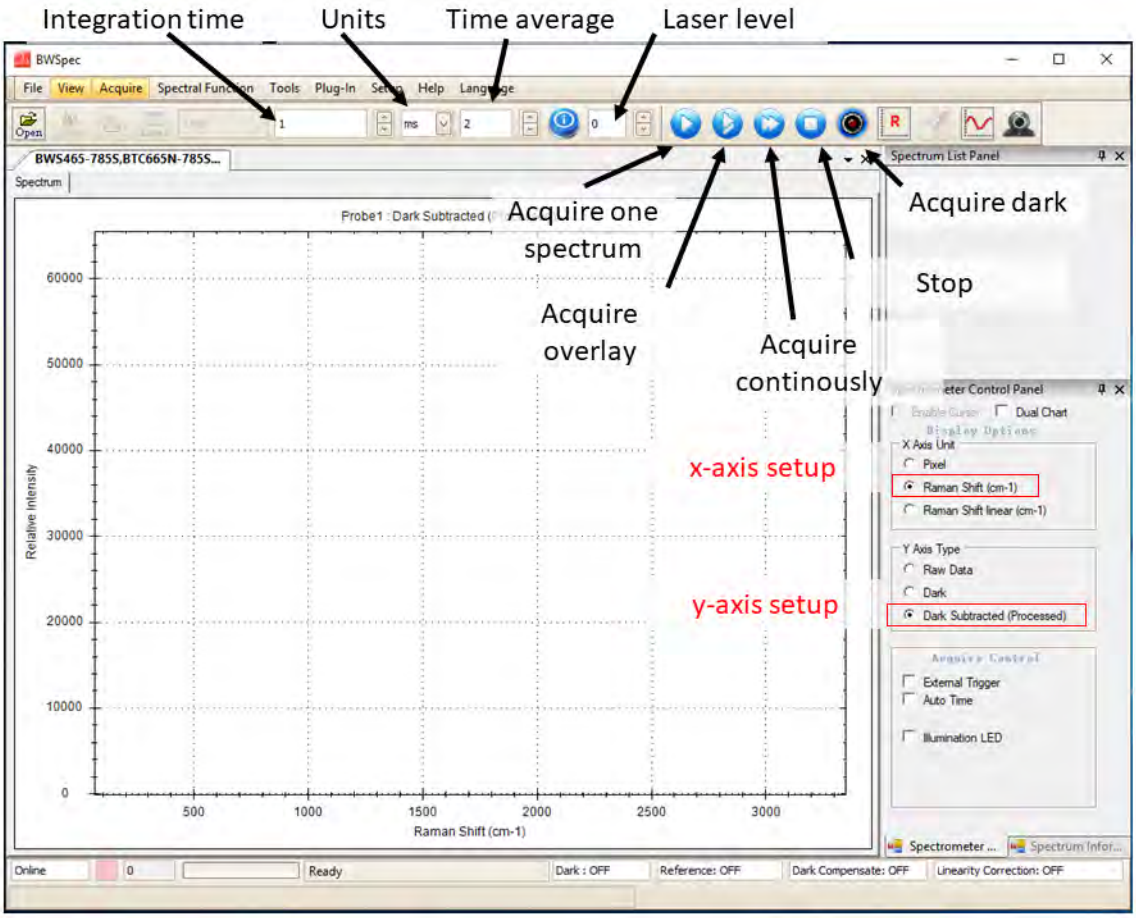

Fig. 4: **Main working window of the BWSpec software and introduction of its basic functions**

After introducing the sample into the sample compartment, the first step is to measure dark correction. Dark is a type of correction including information about the amount of recording light by detector in the case that the laser is switched off – it means information about any interfering light incoming from the outside to the system. The main effect on the dark has integration time and number of acquisition (time average), hence after every change of these parameters as well as change of samples (reflection from the glass walls or sample surface may differ), it is crucial to record new one. Information about the current state of dark is signalized in the middle of the panel at the bottom of the working window (Fig. 5). Another useful button is located on the right side on the top of the working window and serves to remove all spectra from the working window (in some cases it is necessary to use this function for activation of dark acquisition).

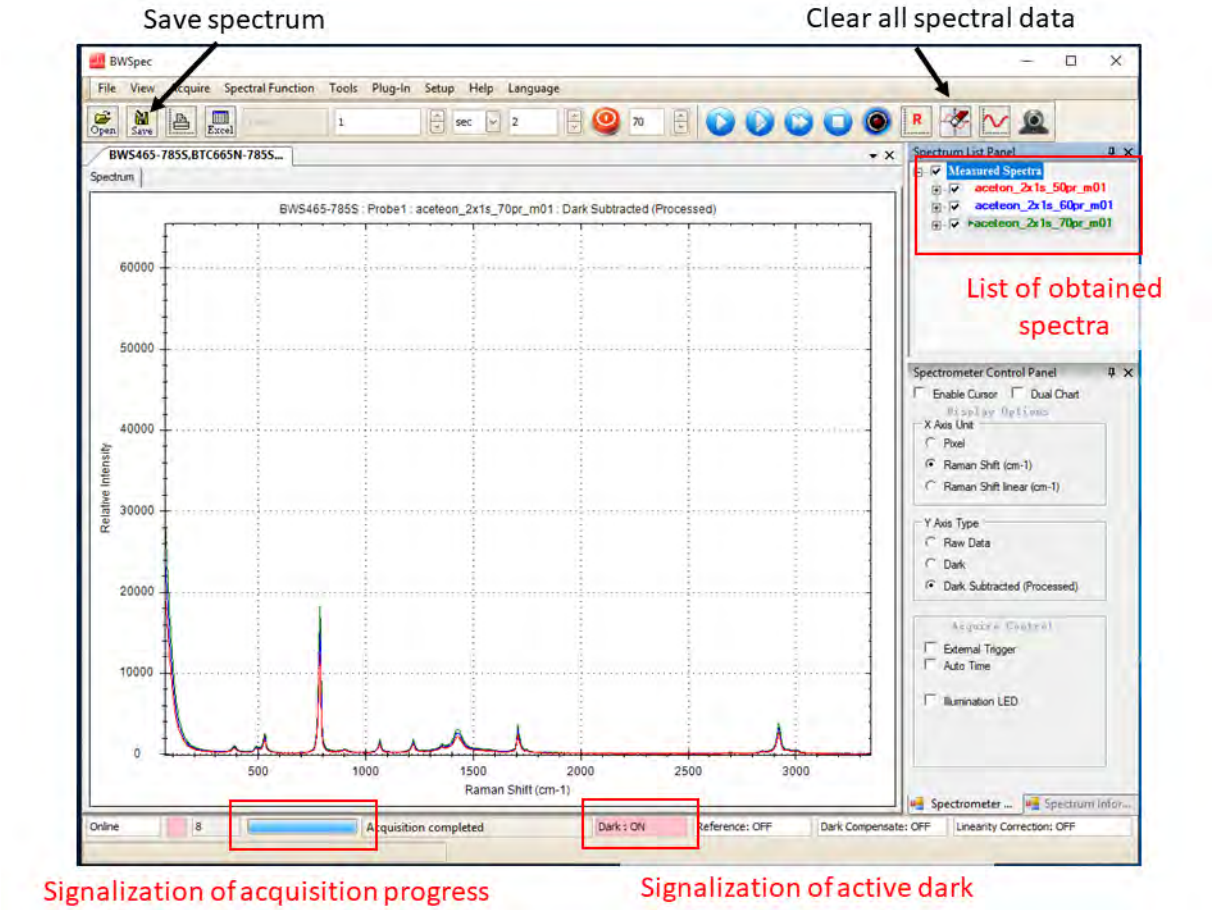

Fig. 5: **Main working window of the BWSpec software and introduction of its other basic functions**

The disadvantage of the BWSpec software is that the spectra are not saved automatically. For this reason, it is highly recommended to save manually each spectrum immediately after its measurement with current (and right) dark correction. Select the spectrum from the list on the right-hand side of the working window, click on it by the right mouse button and rename the spectrum. For the naming spectra, form systematically and clear acronyms based on the short title of the sample and experimental conditions to easily identify individual spectra and experiments. Use only characters from the English alphabet and avoid using special characters. Instead of typing spaces, insert dashes. Then save the precisely named spectrum by the button in the left-top corner of the working window (Fig. 5). In the pop-up window, check and select the correct path for saving the spectrum, spectrum title, and also file format (*.spc – common spectroscopic data format compatible with other software). After checking the mentioned parameters, save the spectrum using the appropriate button.

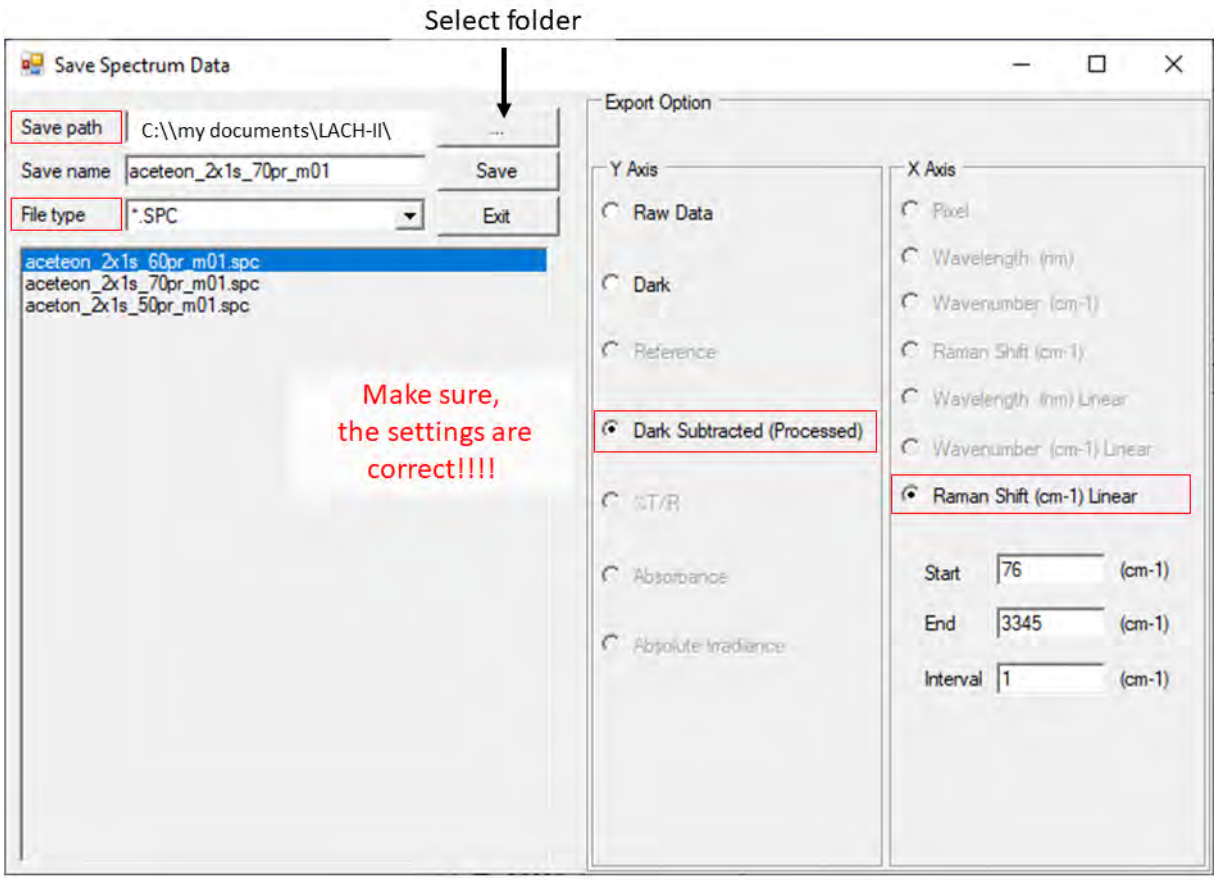

Fig. 6: **Example of saving the spectrum**

The possibilities of data processing are really limited in the BWSpec software, so another evaluation will be performed in the software Omnic.

Raman spectra evaluation

Software Omnic offers a wide range of functions for spectral data processing and evaluation. It is known for its easy and user-friendly working interface. Spectra can be imported by the button “open file” on the left side on the top part of the working window (Fig. 7) or you can also use the menu “file” -> “open” or the keyboard shortcut “Ctrl + O”. If you open several spectra in one window, it is very useful to copy the file title and paste it into the line showing the title of the currently selected (and active) spectrum (the spectrum title is not automatically transferred) and confirm by “enter”.

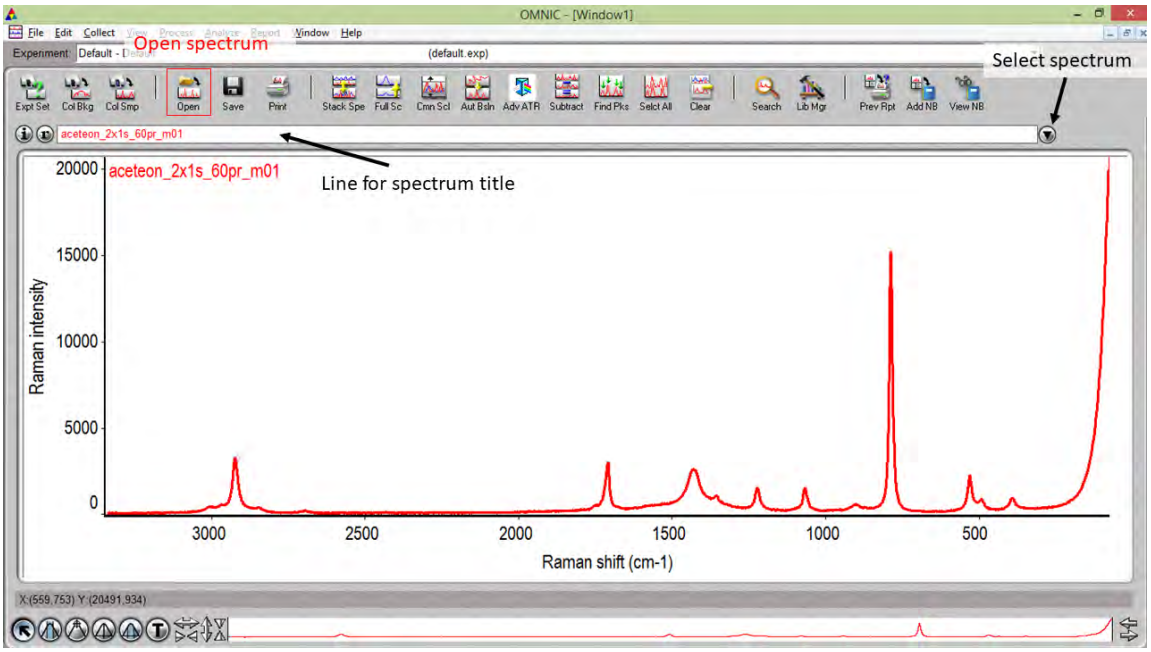

Fig. 7: **Basic working window of software Omnic**

In the menu "view" (Fig. 8), you can find several options for displaying the spectra. You can select "full scale" (every opened spectrum is maximally intense), "common scale" (values on the y-axis are mutual for all spectra and one can compare spectral intensities), or "off set" (spectra have shifted baseline to each other). Different view modes or scale will suit individual functions and each of you in the next steps of data processing.

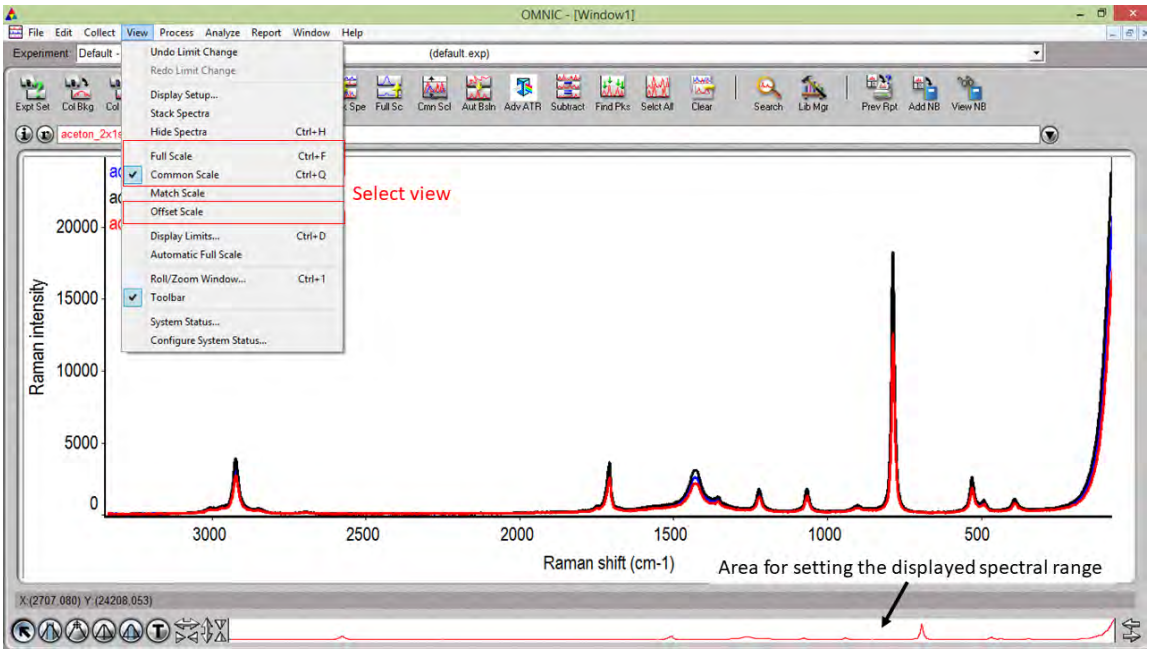

Fig. 8: **Examples of spectral view modes in software Omnic**

To choose appropriate experimental conditions and parameters like integration time and laser power, the value of signal to noise ratio (S/N) is calculated. For S/N values (will be compared) calculation, it is needed to obtain the height of the symmetrical and separated band.

To zoom in on the selected band, just draw a rectangle around the band by the left mouse button and click inside. To zoom out and again display the whole spectrum, double-click into the white rectangle at the bottom part of the working window. For obtaining a peak height, we will use the appropriate tool from the panel in the left-bottom part of the working window (Fig. 9). Triangle marks serve to set up the borders of baseline correction (the spectral background in the Raman spectra can be increased by several effects, e.g., fluorescence or thermal heating, which is unwanted) and square marks are moved to the peak maximum. In the left-bottom corner you can find the value of corrected peak height for the red-displayed spectrum (red line is for active and processed spectrum). If you open whole spectral series (all spectra to be processed to choose the best option of one experimental parameter, e.g., all spectra measured with different value of laser power) and set it correctly, you can simply hide the currently processed spectrum by keyboard shortcut "Ctrl + H" and note the corrected peak values. Using another function "spectral cursor tool" (Fig. 10) note the height of the spectral noise on the baseline. At five different positions, note the "top and down" height value of the noise and subtract them from each other. Average subtracted values of the noise height and use them for S/N value calculation. Finally, compare the S/N values and choose the highest one as the best option for this experiment.

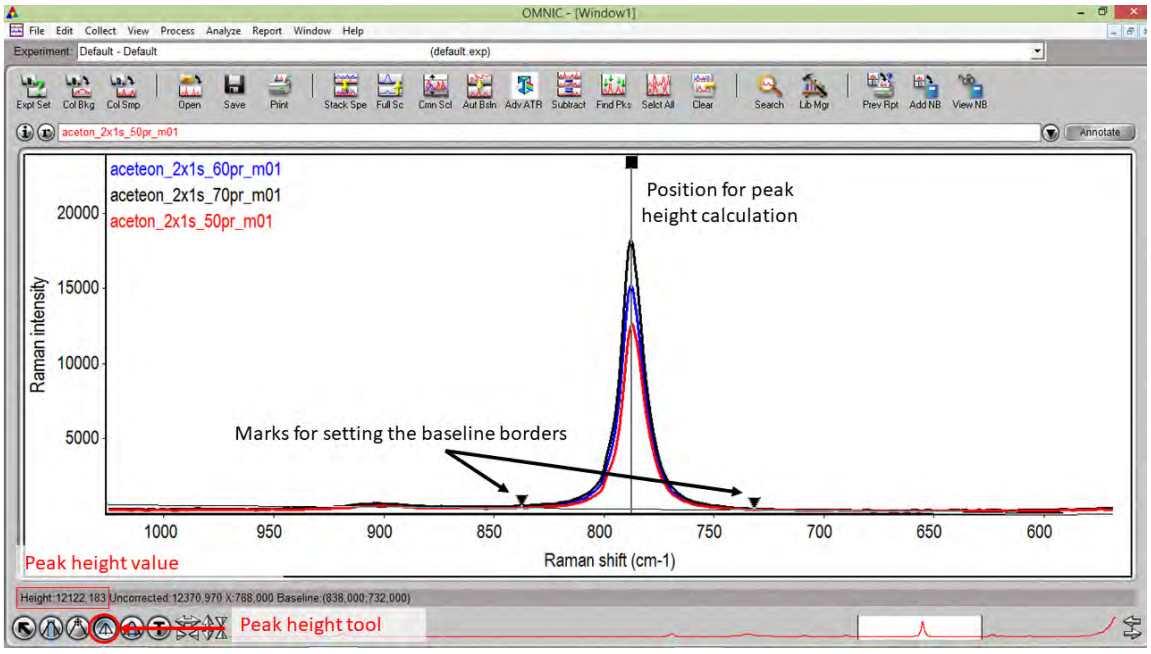

Fig. 9: **Example of setting of the peak height calculation**

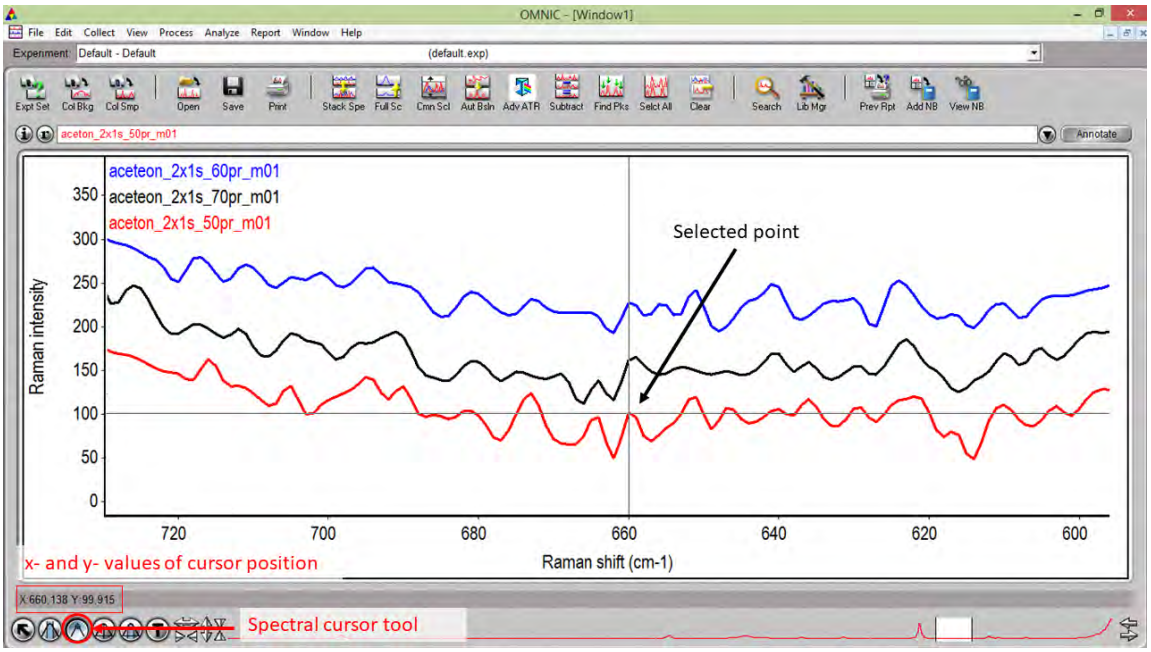

Fig. 10: **Example of spectral cursor tool**

In a similar way, the peak area will be obtained for the development of calibration models and the evaluation of the standard addition method. We use the function "peak area tool" in software Omnic placed in the left bottom corner (Fig. 11). This function again possesses triangle marks for the baseline setting and another pair of triangle marks for the setting of the region borders, which will be used for the area calculation. In the left bottom corner, you can also see all information about the current setting of individual parameters and final peak area. Write the peak area values in the Excel sheet, develop the calibration curve, and in its equation fill the peak area values of unknown samples (model and real samples). Also use the peak area tool for a graphical evaluation of the standard addition method applied to a real sample.

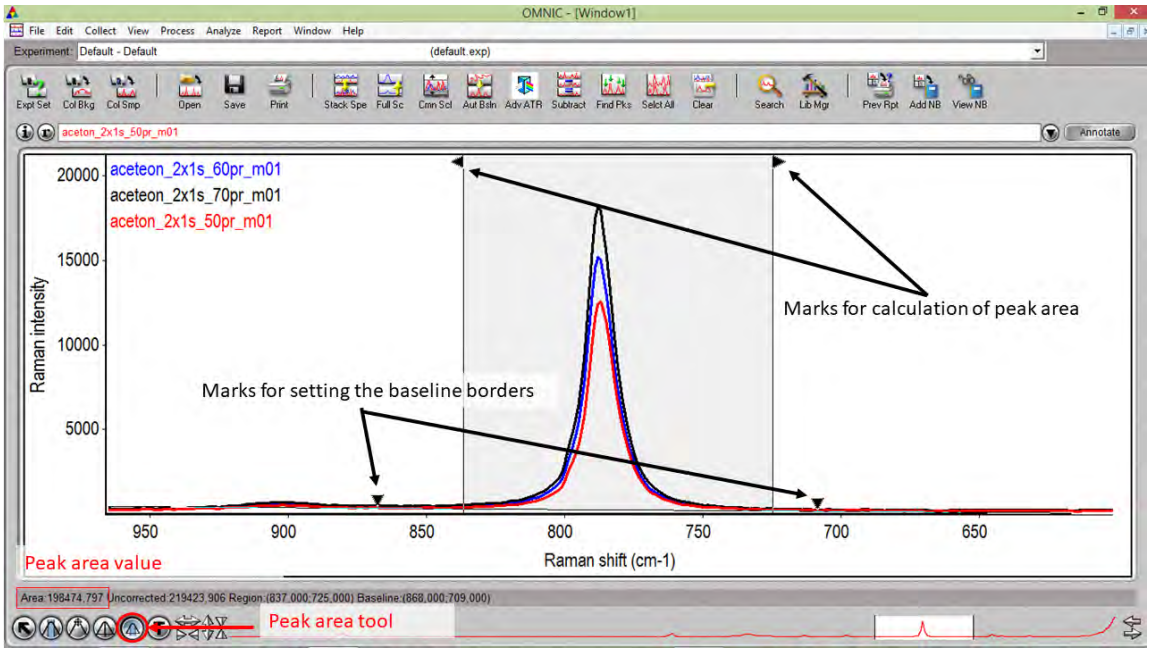

Fig. 11: **Example of setting of a peak area tool**

It is very helpful to know the exact position of band maxima for Raman spectra interpretation and band assignment to the individual functional groups. The tool "find peaks" is used for band annotation. This tool is placed in the menu "analyse" -> "find peaks" (you can also press the keyboard shortcut "Ctrl + K", Fig. 12). The black line defines the threshold under which no bands are annotated. By this line you can minimize the number of annotated bands in the area with high noise level. In the left part of the screen, you can see the tool for improvement of the sensitivity of band annotation (if also weak bands are annotated, just decrease the sensitivity, and on contrary, if all important bands are not annotated, increase the sensitivity – it is recommended to consult the changes with the teacher). Subsequently, you can replace the original spectrum or add to the used or new working window. The number of annotated bands can be improved (add or delete) using the "annotation tool" located in the left bottom corner of the working window.

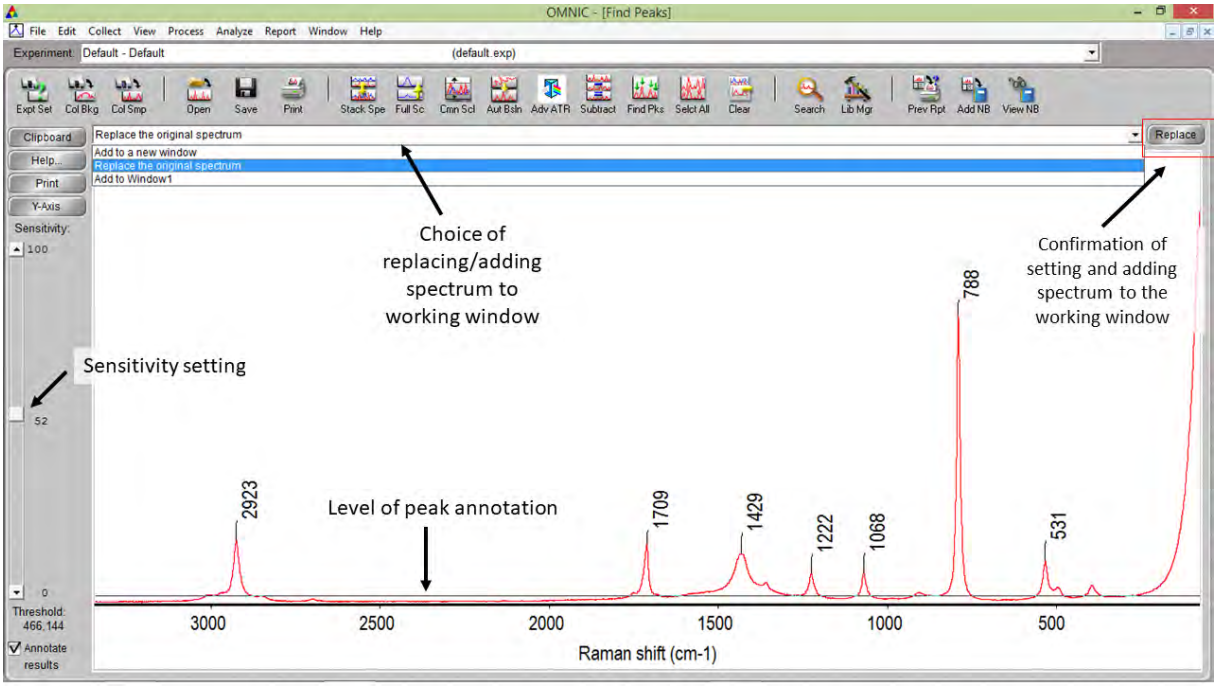

Fig. 12: **Example of setting of the "find peaks" tool**

Knowledge test questions

1. What is physical nature of Raman scattering?
2. What is the fundamental selection rule for the observation of Raman band for a given vibration mode?
3. What is the difference between Stokes and anti-Stokes scattering?
4. Describe the two principal types of construction of Raman spectrometers.
5. What radiation sources are used for excitation of Raman scattering?
6. Describe main parts of dispersive Raman spectrometer.

7. Summarize the common characteristics of infrared and Raman spectroscopy; specify the differences between these two methods; describe the principle of their complementarity.

Used and recommended literature

1. B. Strauch: *„Možnosti laserové Ramanovy spektrometrie“* v Nové směry v analytické chemie (J. Zýka, uspořadatel), kap. 8, (str. 171 – 203), svazek III, SNTL Praha 1988.
2. Raman Spectroscopy – http://cctr.umkc.edu/www/w3/dept/physics/ramanderivation.html
3. The Photonics Dictionary *Raman effect* – http://www.laurin.com/datacenter/dictionary/cd/dr/ramaeffe.htm *Raman spectroscopy* – http://www.laurin.com/datacenter/dictionary/cd/dr/ramaspec.htm
4. P. Matějka: *„Infračervená spektrometrie“* v Návody pro laboratorní cvičení z analytické chemie II (J. Krofta a kol.), VŠCHT Praha 1997 (vydání páté), 2001 (vydání šesté).
5. P. Matějka: „*Ramanova spektroskopie*“ v Návody pro laboratorní cvičení z analytické chemie III, (Matějka P. a kol.), VŠCHT Praha 2002, vydání první.

List of symbols and abbreviations

E	energy
$\boldsymbol{E}$	vector of intensity of electric field
I	intensity
k	Boltzmann’s constant
v	vibration quantum number
h	Planck’s constant
$\boldsymbol{p}$	vector of induced dipole moment
q	internal coordinate of a molecule
T	temperature
α	polarizability
ν	frequency

CCD	„Charge-Coupled-Device“ – frame, field detector
FT	Fourier transformation
FTIR	Fourier Transform Infra-Red
NIR	Near Infra-Red
UV	Ultra-Violet
VIS	Visible
UPS	Uninterrupted Power Supply

APPENDIX – INTERPRETATION OF SPECTRA

The frequencies of individual vibration modes are independent of the spectroscopic method used (either infrared or Raman spectroscopy). Differences in both types of spectra are observed in band intensities, which are related to different selection rules for these two types of vibration spectroscopy.

In the below given Table I, an overview of some selected bands of important types of substances with values of relative intensities of bands in Raman spectra is presented.

For the proper use of the table, you have to go from higher wavenumbers to lower ones. In the column "Next characteristic band" you will find the range of next spectral band (or multiple bands) characteristic for the investigated functional group, which have to be confirmed or rejected in the spectrum/molecular structure. In some cases, there is a reference on a rather broader region, which is finally subdivided on separate sub-regions considering the influence of neighbouring groups, skeleton branching, position of the substituent etc. If there is no further reference on the next wavenumber value (wavenumber range), the list of typical bands is completed and the characterization of a given functional group is finished. In the cases of band overlaps of various functional groups in a selected range, it is absolutely necessary to inspect every possibility, which can be taken into account for the bands' assignment. It is essential to examine mutual band overlaps and to consider the possible distribution of various functional groups in a given molecule. When a specified functional group is only weakly abundant in a molecule, all its bands should be weaker than the bands of the dominant type of skeleton or the prevailing type of functional groups.

The table I was compiled on the basis of author's experience using the data given in below referenced literature. Only important types of functional groups and key types of skeleton of organic molecules were considered and a lot of detailed information was omitted. This table is not intended for detailed interpretation of Raman spectra, its purpose is only a summary of fundamental characteristics which are important for education of principles of Raman spectroscopy, but not only for this laboratory exercise.

1/ G. Socrates: Infrared and Raman Characteristic Group Frequencies, J.Wiley, Chichester Third Edition 2001.

2/ N. P. G. Roeges: A Guide to the Complete Interpretation of Infrared Spectra of Organic Structures, J.Wiley, Chichester 1993.

3/ Spectool for Windows 2.1, A Hypermedia Book for Structure Elucidation of Organic Compounds with Spectroscopic Methods, Chemical Concepts, Weinheim 1994.

Table III: Wavenumbers of characteristic vibrational modes of several types of bonds and groups as observed in Raman spectra

Wavenumber, cm^{-1}	Intensity	Assignment	Functional group	Next characteristic band
water – in organic solvents				
3760-3580	vw-w	$\nu_{as}(H_2O)$	**H_2O**	3640-3500
3640-3500	w-m, br	$\nu_s(H_2O)$	**H_2O**	1640-1605
1640-1605	vw	$\delta(H_2O)$	**H_2O**	-
water – crystal, solvent				
3600-3100	m,br	ν(OH)	**H_2O**	1645-1615
1645-1600	v	$\delta(H_2O)$	**H_2O** ,	-
alcohols, phenols				
3670-3580	vw-w,	ν(OH)	**-OH**, isolated	1420-1260
		– without influence of hydrogen bridges, narrow		
3600-3400	vw	ν(OH)	**-OH**, intramol. H-bond	1440-1300
		– significant effect of intramolecular hydrogen bridges, wavenumber independent on concentration, broader than for isolated –OH, narrower than in the case of intermolecular bridges		
3600-3150	vw-w,br	ν(OH)	**-OH**, solids or liquids	1440-1290
		– significant effect of intermolecular hydrogen bridges , wavenumber decreases with increasing concentration		
3200-2500	w, br	ν(OH)	**-OH**, solids or liquids .	1440-1290
		- chelated –OH group		
1440-1310	m-w	δ(COH)	tert.-OH, Ar-OH	1260-1100
		(Beware of band overlaps)		
1400-1260	m-w	δ(COH)	R**OH**, R_2**OH**, (prim. and sec.)	1150-1000
1260-1170	m-w	ν(CO)	Ar-**OH**	-
1215-1100	m-s	ν(CO)	R_3**C-OH,** (tert.)	800-750
1150-1070	s-m	ν(CO)	R_2**C**H-**OH**, (second.)	900-800
1090-1000	s-m	ν(CO)	R-OH, (primar.)	900-800
900-800	s-m	ν(CCO)	R-OH, (primar.)	-
900-800	s	ν(CCO)	R-OH, (second.)	-
800-750	s	δ (CO)	R_3C-OH, (tert.)	-
carboxylic acids – non-dissociated form, isolated molecules				
3580-3500	vw	ν(OH)	-CO**OH**, monomer	1800-1740
		non-associated molecules		
1800-1740	w-m	ν(C=O)	-**CO**OH, monomeric	1380-1280
1380-1280	m-w	δ(OH)	-CO**OH**, monomeric	1190-1075
1190-1075	w	ν(CO)	-**COO**H, monomeric form	-
carboxylic acids – non-dissociated form, associated molecules				
3200-2230	vw-w, br	ν(OH)	-CO**OH**, dimer, assoc.	1725-1700
		wavenumber decreases with increasing concentration,		intermol. H-bond
1725-1700	w-m	ν(C=O)	-**CO**OH, dimer form	1440-1395
1725-1700 – saturated acids, 1710-1680 – non-saturated and Ar acids				
1440-1395	w-m	δ(OH)+ ν(CO)	-**COOH**, dimer form	1320-1210
1320-1210	v (m-s)	ν(CO)	-COOH, dimer form	970-875
			dimer form, sometimes doublet	
970- 875	m,br	γ(OH)	-COOH, dimer form	-
carboxylic acids – dissociated form (anion), associated molecules				
1655-1540	w	$\nu_{as}(COO^-)$	-**COO^-**	1440-1335
1440-1335	m-s	$\nu_s(COO^-)$	-**COO^-**	-
		broader band with shoulders (two to three peaks)		

amines – primary

3550-3280	m-w	$\nu_{as}(NH_2)$	-**NH_2**	3450-3160
wavenumber decreases with increasing concentration, intermol. H-bridges, weaker effects compared to OH broader band in the case of chemically pure substance in condensed phase				
3450-3160	vw-w	$\nu_s(NH_2)$	-**NH_2**	1650-1580
wavenumber decreases with increasing concentration, intermol. H-bridges, weaker effects compared to OH broader band in the case of chemically pure substance in condensed phase				
1650-1580	w	$\delta(NH_2)$	-**NH_2**	Ar: 1360-1240 R : 1295-1145
1360-1240	m-w	ν(CN)	Ar**NH_2**	1120-1020
1295-1145	m-w	$\rho(NH_2)$	R**NH_2**, often overlap of bands	1240-1020
1240-1020	m-s	ν(CN)	R**NH_2**, often overlap of bands	-
1120-1020	m-w	$\rho(NH_2)$	Ar**NH_2**, often overlap of bands	1120-1020

amines – secondary

3500-3300	w	ν(NH)	-**NH**-	1580-1490
1580-1490	w	δ(NH)	-**NH** -	R: 1190-1170
		for ArNH, risk of band overlaps		Ar:1360-1250
1360-1250	m-w	ν(CN)	Ar_2**NH**, Ar-**NH**-R	1280-1180
1280-1180	m-w	$\nu(C_RN)$	Ar-NH-R	-
1190-1170	m	ν_{as}(CNC)	R_2NH	1145-1130
1145-1130	m-w	ν_s(CNC)	R_2NH	1145-1130
750- 700	w,br	ω(NH)	-**NH**-	-

amides - primary

3540-3320	m-w	$\nu_{as}(NH_2)$	-CO-**NH_2**	3420-3180
lower wavenumber caused by H-bonds, broader band for chemically pure substance in condensed phase				
3420-3180	m-w	$\nu_s(NH_2)$	-CO-**NH_2**	1690-1640
lower wavenumber caused by H-bonds, broader band for chemically pure substance in condensed phase				
1690-1640	m-w	ν(C=O)	-**CO**-NH_2, **amid I**	1640-1590
1640-1590	w-m	$\delta(NH_2)$	-CO-**NH_2, amid II**	1420-1400
1420-1400	m	ν(CN)	-CO-NH_2**, amid III**	1170-1130
1170-1130	vw	$\rho(NH_2)$	-CO-**NH_2,** vague	600-550
600- 550	m	δ(N-C=O)	-CO-NH_2	-

amides - secondary

3460-3270	m-w	ν(NH)	-CO-**NH**-, **trans**	1700-1665, 3100-3070
broader band in the case of chemically pure substance in condensed phase				
3180-3140	m-w	ν(NH)	-CO-**NH-, cis**	1700-1630
3100-3070	vw	overtone	-CO-**NH-, trans**	1700-1665
		from amide II, very weak, sometimes not evident		
1700-1630	w-m	ν (C=O)	-**CO**-NH-, **amid I**	1570-1510
1570-1510	m-w	δ(NH)	-CO-**NH**-, **amid II**	1305-1200
1350-1310	s	ν(CN)	-CO-NH-, **amid III, cis**	820-780
1305-1200	s	ν(CN)	-CO-NH-, **amid III, trans**	770-620
		frequently around 1260 cm^{-1}		
820-780	m-s,br	γ(NH)	-NH-CO-, **cis**	-
770- 620	w,br	γ(NH)	-NH-CO-, **trans**	-

amides - tertiary

1670-1630	w-m	ν(C=O)	-CO-N<	870-700
870-700	s	ν_{as}(C-N-C)	-CO-N<C	620-570
620-570	m	δ(N-C=O)	-CO-N<C	-

alkynes (alkines) – alkylacetylenes

3340-3280	w-m	ν(CH)	-C≡**C-H**	2150-2100
2150-2100	s-m	ν(C≡C)	**-C≡C**-H	1020-905
1020-905	w-m	ν (C-C≡C)	H-C≡**C-C**	370-220
370-220	w-m	δ(-C≡**C-H**)	-C≡**C-H**	-

alkynes (alkiny) – dialkylacetylenes

2260-2190	vs-s	ν(C≡C)	-C≡C-	
	for symmetric molecules vs		sometimes accompanied by a band ca. 2310 cm^{-1}	

alkenes – vinyl a vinylidene derivatives

3120-3050	m	$\nu_{as}(CH_2)$	>C=**CH_2**	3050-2960
3050-2960	m	$\nu_{s}(CH_2)$	>C=**CH_2**	1685-1620
1685-1620	s	ν(C=C)	>**C=C**H_2	1440-1360
1440-1360	m-s	δ(CH_2)	>C=**CH_2**	1320-1250
	frequently bands overlap			
1320-1250	m-w	δ(CH)	>C=CH_2	1180-1010
1180-1010	m	δ (CH)	>CH=CH_2	980- 810
980- 810	w	γ(CH)	>CH=CH_2	-

alkenes, cykloalkenes and their derivatives – (internal double bonds, double bonds)

3060-2995	m	ν(CH)	=C**H**-	isolated: 1685-1620 conjugated: 1660-1580
1685-1620	s-vs	ν(C=C)	>**C=C**<, isolated	1440-1190
1660-1580	s-m	ν(C=C)	-C=C, conjugated	1440-1190
	the band position decreases with the conjugation degree, conjugation s C=C, Ar, C=O			
1440-1340	s-m	ν(C=C)	cyclic, more C=C bonds	-
	vibration of non-saturated rings			
1350-1340	w	δ(CH)	>C=**CH**-, trisub.	850-790
1350-1260	w-vw	δ(CH)	-HC=CH-, trans	1000- 910
1295-1190	s-m	ρ(CH)	-HC=CH-,cis	980- 880
1000- 910	m	γ(CH)	R-**CH=CH**-R, trans	630- 430
980- 880	m	γ(CH)	R-**CH=CH**-R, cis	730- 660
850- 790	w	γ(CH)	RR'>**C=CH**-R"	-
730- 660	w	γ(CH)	R-**CH=CH**-R, cis	-
630- 430	w	γ(CH) δ(CH)	R-**CH=CH**-R, trans	-

aromatic hydrocarbons

3105-3000	m-s	ν(CH)	Ar	1630-1590
	several bands, the number decreases with increasing substitution			
	for Ar-NO_2 derivatives 1. maximum above 3105			
1630-1590	m-s	ν(C=C)	Ar, usually 1600	1590-1575
1590-1575	v	ν(C=C)	Ar,	1525-1470
1525-1470	w	ν(C=C)	Ar, usually around 1490	1470-1425
1470-1425	w	ν(C=C)	Ar	1290- 990
				interval depends on subst. type,
for Ar, 1,2,4,5-tetrasubstit., penta- and hexasub.direct reference to interval 575- 385				
1290-1130	m	δ(CH)	Ar, 1,4-disubstit.	880- 790
1270-1220	m	δ(CH)	Ar, 1,2,4-trisubstit.	680- 610
1190-1070	w	δ(CH)	Ar, 1,2,3,4-tetrasubstit.	585- 565
1170-1120	m	δ(CH)	Ar, 1,2,3,5-tetrasubstit.	580- 505
1150-1030	m-s	δ(CH)	Ar, 1,2,3-trisubstit.	670- 500

1140-1020	m-s	δ(CH)	Ar, 1,2-disubstit.	790-650
1050-990	m-vs	δ(CH)	Ar, monosubstit.	710-605
1025-990	vs	δ(CH)	Ar, 1,3-disubstit.,	800-660
			Ar, 1,3,5-trisubstit.	535-495
880- 790	s	γ(CH)	Ar, 1,4-disubstit.	-
800- 660	m-s	γ(CH)γ(CC)	Ar, 1,3-disubstit.	-
790- 650	s	γ(CH)γ(CC)	Ar, 1,2-disubstit.	590- 510
710- 605	m-w	γ(CC),γ(CH)	Ar, monosubstit.	-
680- 610	m-s	γ(CC)	Ar, 1,2,4-trisubstit.	-
670- 500	s	γ(CC)	Ar, 1,2,3-trisubstit.	-
590- 510	m-s	γ(CC)	Ar, 1,2-disubstit.	-
585- 565	s	γ(CC)	Ar, 1,2,3,4-tetrasubstit.	-
580- 505	v	γ(CC)	Ar, 1,2,3,5-tetrasubstit.	-
575- 545	s-vs	γ(CC)	Ar, pentasubstit.	-
535- 495	m-s	γ(CC)	Ar, 1,3,5-trisubstit.	280- 250
470- 420	s-m	γ(CC)	Ar, 1,2,4,5-tetrasubstit.	-
415- 385	s-m	γ(CC)	Ar, hexasubstit.	-
280- 250	m-s	γ(CC)	Ar, 1,3,5-trisubstit.	-

alkanes, alkyl chains

2995-2940	m	$\nu_{as}(CH_3)$	**-CH_3**	2895-2840
2955-2915	m	$\nu_{as}(CH_2)$	**-CH_2-**	2880-2835
2895-2840	m-s	$\nu_s(CH_3)$	**-CH_3**	1470-1385
2880-2830	m-s	$\nu_s(CH_2)$	**-CH_2-**	1480-1385
1480-1440	w-m	$\delta(CH_2)$	-(C)-**CH_2,** -(O)-**CH_2-**	1305-1295
		(overlap with Ar, $-CH_3$)		(reference only for –**$(CH_2)_n$-** n>2)
1470-1440	m	$\delta_d(CH_3)$	-(C)-**CH_3**, -(O)-**CH_3**	1395-1345
		(overlap with Ar, $-CH_2$-)		
1450-1390	w	$\delta_d(CH_3)$	**CH_3**-(C=O)-O-, **CH_3**-N<, **CH_3**-(C=O)-C-, **CH_3**-(S=O)-C-	1385-1300
1445-1385	m	$\delta(CH_2)$	- **CH_2**-X, X:-(C=O)-, -COOR, -C=C-, -C≡C-, Ar, -CN, NO_2, Cl, Br	785-720
1395-1345	w-m	$\delta_s(CH_3)$	**CH_3**-(C)-,**CH_3**-(O)- doublet typical for branching	1255-1130 (reference only for doublet)
1385-1330	w-m	$\delta_s(CH_3)$	**CH_3**-(C=O)-, **CH_3**-(C=O)-O-	-
1370-1310	w-m	$\delta_s(CH_3)$	**CH_3**-(N<)	-
1360-1320	w	δ(CH)	-C-H, saturated	-
1340-1300	w	$\delta_s(CH_3)$	**CH_3**-(S=O)-C-	-
1305-1295	m	$\delta(CH_2)$	–**$(CH_2)_n$-** n>2, intensity increases with n value	1100-1040
1255-1245	m	ν(CC)	$-C(CH_3)_3$	1225-1165
1225-1165	m	ν(CC)	$-C(CH_3)_3$	1020- 980
1175-1165	w	ν(CC)	$-CH(CH_3)_2$	1150-1130
1150-1130	w		$-CH(CH_3)_2$	955- 900
1100-1040	m-s	ν(CCC)	–**$(CH_2)_n$-**	900-800
1020- 980	m-s	ν(CC)	$-C(CH_3)_3$	930- 925
930- 925	m		$-C(CH_3)_3$	360-270
955- 900	m	ν(CC), δ(CCH)	$-CH(CH_3)_2$	840-790
900-800	m-s	skeletal	–**$(CH_2)_n$-**	750-720
840- 790	m	skeletal	$-CH(CH_3)_2$	495-490
785- 750	vw	$\rho(CH_2)$	$-CH_2-CH_x$, x≠2	-

750- 735	vw	$\rho(CH_2)$	propyl, $(\mathbf{CH_2})_n$-(O)-, n>4	-
735- 720	vw	$\rho(CH_2)$	$(\mathbf{CH_2})_n$-(C)-, n>3	-
495-490	m	skeletal	$-CH(CH_3)_2$	-
360- 270	m		$-C(CH_3)_3$	-

aldehydes

2850-2800	w	ν(CH)	-**CH**O	2745-2650
		sometimes shoulder below 2745 cm^{-1}		
2745-2650	s-m	ν(CH)	-**CH**O	1745-1650
		effect of Fermi resonance		
1745-1650	w-m	ν(C=O)	-CH**O**	1440-1325
		higher value for saturated aliphatic aldehydes		
		the wavenumber value decreases by conjugation of C=O bond with C=C, Ar etc.		
1440-1325	s-m	δ(CH)	-**CH**O	975-780
975- 780	m	γ(CH)	-**CH**O	-

thiols

2600-2520	s	ν(SH)	-SH	750-570
750- 570	s	ν(CS)	-C-SH	410-200
410-200	v	δ(CS)	-C-SH	-

phosphines

2460-2100	m-w	ν(PH)	-PH	1150-965
1150-965	m-w	δ(PH)	-PH	-

isocyanates

2295-2250	w	ν_{as}(N=C=O)	-N=C=O,	1460-1340
1460-1340	s	ν_s(N=C=O)	-N=C=O	650-580
650-580	w	δ(N=C=O)	-N=C=O	-

nitriles

2270-2200	s	ν(C≡N)	-C≡N	390-340
		very narrow band	reference only for aliphatic nitriles	
390-340	s	δ (C-C≡N)	-C-C≡N	200-160
200-160		skeletal	-C-C≡N	-

thiocyanates

2185-2135	m-s	ν(C≡N)	-S-**C≡N**	1090-925
		very narrow band		
1090-925	m-s	ν_s(S-C≡N)	-S-**C≡N**	700-670
700-670	s	ν_{as}(-C-S-C)	-**C-S-C**≡N	660-610
660-610	s	ν_s(-C-S-C)	-**C-S-C**≡N	-

isothiocyanates

2150-1990	m,br	ν_{as}(N=C≡S)	-**N=C=S**	1250-925
1250-925	s	ν_s(N=C≡S)	-**N=C=S**	690-650
690-650	s	δ_s(N=C≡S)	-**N=C=S**	-

β,γ- lactones

1840-1770	m-w	ν(C=O)	β,γ-lactones	1370-1160
1370-1160	w	ν(CO)	β,γ- lactones	-

esters

1800-1750	m-w	ν(C=O)	vinyl and phenylesters	1310-1250
1750-1720	m-w	ν(C=O)	saturated esters	1300-1150
1740-1705	m-w	ν(C=O)	-CO-O-, α,β-nonsatur. esters	1335-1250
1730-1705	m-w	ν(C=O)	Ar-**CO-O**-R, aromatic esters	1330-1250
1335-1250	m-s	ν_{as}(COC)	-CO-O-, α,β- nonsatur. esters, broader band than for ketones	1200-1130
1330-1250	m-s	ν_{as}(COC)	Ar-**CO-O**-R, broader than for ketones	1150-1080
1300-1150	m-s	ν_{as}(COC)	R-**CO-O**-R, saturated (broader band than for ketones)	1160-1050
1200-1130	w	ν_{s}(COC)	R-**CO-O**-R', α,β-nenasyc.	-
1200-1180	m-s	ν_{as}(COC)	H**COO**R	1165-1050
1165-1100	w	ν_{s}(COC)	H**COO**R	775-620
1160-1050	w	ν_{s}(COC)	R-**CO-O**-R', nasyc.	-
1150-1080	w	ν_{s}(COC)	Ar-**CO-O**-R	-
775- 620	m	δ(OCO)	H**COO**R	-

ketones

1750-1690	m	ν(C=O)	R-**CO**-R,' saturated ketones	1325-1175
1705-1650	m-w	ν(C=O)	ArCO-, α,β-nonsatur. ketones	1320-1280
1325-1175	m-w	ν(CC)	R-CO-R' (frequently vague)	1170-1095
1320-1280	m	δ(C-CO-C)	Ar-CO-Ar(-R), generally several bands	1225-1075
1225-1075	m	$\nu(C_{Ar}C)$	Ar-CO-	-
1170-1095	m-w	ν_{as}(CC(=O)C)	R-CO-R', several bands for longer chains	800-700
800-700	m-s	ν_{s}(CC(=O)C)	R-CO-R'	630-580
630-580	s-m	δ(CC(=O)C)	R-CO-R'	-

nitro-compounds

1570-1485	m-w	$\nu_{as}(NO_2)$	$-NO_2$	1385-1315
1385-1315	s-vs	$\nu_{s}(NO_2)$	$-NO_2$	1180-850
1180- 850	s-m	ν(CN)	$-C-NO_2$	-

Note: [(1)] Stretching vibration $\nu_{as}(CH_3)$ should be named correctly $\nu_{d}(CH_3)$. Nevertheless, the symbol ν_{as} is used very frequently. Hence, the symbol ν_{as} was preserved in this table.

Used abbreviations:

Intensity: vs - very strong, s - strong, m - medium, w - weak, vw – very weak, v - variable, br - broad band, sh - shoulder.

Description of vibration modes: ν - stretching, δ - deformation, γ – out-of-plane, ω - wagging, ρ - rocking, as – anti-symmetric, s - symmetric, d - degenerated,

amide I - III – labeling of amide bands I - III, describing strong coupling of vibration motion of amide groups, comb.b. - combination bands. R - alkyl, Ar - aryl.