NMR Laboratory 1

Place: NMR laboratory, room **A28**, then computer room A276

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Content of laboratory work

1. Introduction to the JEOL ECZ 500R instrument (11.73 Tesla, 500 MHz) and the measurement of ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra. Measurement of the ^{1}H NMR spectrum of a spirit sample (your own sample in the amount of 0.1 mL would be welcome).
2. Processing of the measured spectra using the MestreNova program.
3. Calculations of the empirical formula and the degree of unsaturation of unknown substances using the MS Excel spreadsheet. Evaluation of ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra of two unknown organic compounds. Estimation of the structures of unknown compounds. Confirmation of the correct estimation with the help of chemical shift prediction with MestreNova, ChemDraw or SpecTool.
4. Evaluation of the ^{1}H NMR spectrum of the spirit. Assignment of ethanol, methanol and water signals and their integration. Calculation of molar, mass and volume fractions of ethanol, methanol and water in spirits using MS Excel spreadsheet. Assessment of the safety of spirits.

Prerequisites

1. Basic knowledge of ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra of simple compounds, i.e. number of signals, integral intensity of signals, multiplicity of signals, chemical shifts of signals (tables are allowed).
2. Knowledge of basic analytical calculations, i.e. calculations and conversions of molar, mass and volume fractions, calculation of molar mass and elemental analysis from molecular formula, calculation of empirical formula from elemental analysis.
3. Knowledge of the valency of the elements listed below. Calculation of the degree of unsaturation from the structural formula and from the molecular formula.
4. Work with MS Excel spreadsheet.

Procedure for determining the structure of an unknown organic compound

1. Create an MS Excel spreadsheet file named YEAR-MONTH-DAY-LASTNAME. This file will be submitted as a protocol.
2. Prepare in a spreadsheet the calculation of the molar mass, elemental analysis and degree of unsaturation of compounds containing C (A_r = 12.011), H (A_r = 1.008), N (A_r = 14.007), O (A_r = 15.999), Cl (A_r = 35.450) and Br (A_r = 79.904).[1] Consider only trivalent nitrogen.
3. Prepare the calculation of the empirical formula from elementary analysis in a spreadsheet. The oxygen content is usually not determined, but added up. Therefore, if the sum of the contents of the determined elements is not approximately 100%, assign the remaining value to oxygen.
4. From the elemental analysis of the unknown samples, calculate the empirical formula and calculate its degree of unsaturation.
5. From the information from points 3 and 4, propose a molecular formula.
6. Process the FID records of the ^{1}H and $^{13}C\{^{1}H\}$ NMR experiment in the MestreNova software. Adjust the phase for the obtained spectra, correct the baseline, use the standard or residual solvent as a reference. Determine the number of signals in the ^{1}H NMR spectrum and their integral area, and subsequently the signal ratio. For separated signals, calculate the values of the spin-spin coupling constants. Determine the number of signals in the ^{13}C NMR spectrum.
7. Considering the elements present and the chemical shifts of the ^{1}H and ^{13}C nuclei, propose the structural fragments of the molecule with the help of chemical shift tables or the MestreNova program. Redesign the molecular formula and verify its meaningfulness by calculating the degree of unsaturation.
8. Based on the multiplicity of signals in the ^{1}H NMR spectrum, refine the fragments of the molecule; consider the possible symmetry of the molecule.
9. Propose the structure of an organic compound and predict the chemical shifts of ^{1}H and ^{13}C nuclei in ChemDraw or MestreNova. Compare the predicted chemical shifts with the experimental ones. In case of small differences, consider the structure as correct.
10. A small difference is considered approx. 0.3 ppm for chemical shifts of ^{1}H nuclei and approx. 2 ppm for chemical shifts of ^{13}C nuclei. If the differences are large, review the previous procedure and propose a different structure.
11. Write down the proposed structure, the chemical shift values and differences of the prediction and experiment in a spreadsheet file.

Spirit safety assessment procedure

1. Calculate the molar masses of ethanol, methanol, and water.
2. Process the FID records of the ^{1}H NMR experiment in the MestreNova program. Adjust the phase for the obtained spectra, correct the baseline, use the standard or residual solvent as a reference.
3. Determine the ethanol, methanol and water signals and read their integral values; do not forget the presence of hydroxyl groups of ethanol and methanol. Normalize the integral signal intensity of the –OH group of methanol to a value of 10. For the separated signals, calculate the values of the spin-spin coupling constants.
4. In a spreadsheet, create a calculation to determine the mole, mass, and volume fraction of methanol, ethanol, and water. Neglect aromatic components and impurities if they are negligible.

5. In the spreadsheet, discuss whether the analyzed alcohol is safe in terms of methanol content. Consider 12 g of methanol per 1 L of pure ethanol as the maximum allowed amount.
6. Save the spreadsheet file to your storage media. You can edit the file further. Send the final version to the email of the assistant by midnight.

Reference

1. For more information on molar masses, see the Commission on Isotopic Abundances and Atomic Weights IUPAC website (CIAAW, www.ciaaw.org).

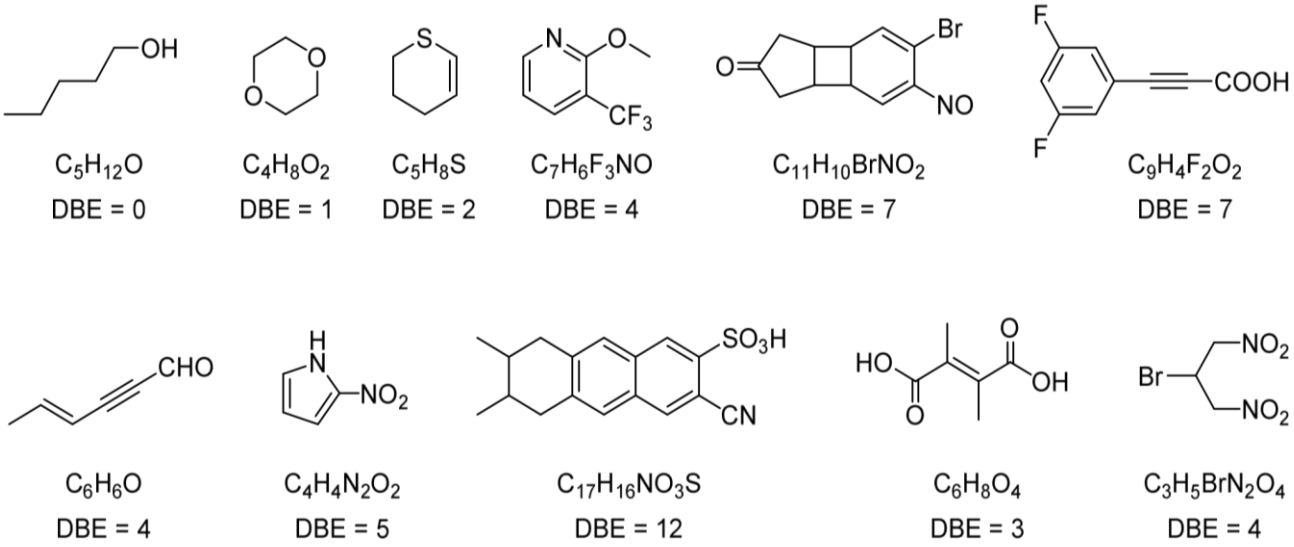
CONTENTS

Double bond equivalent - degree of unsaturation of molecule

The degree of unsaturation of a compound is usually expressed by **double bond equivalent** (DBE, or UN, unsaturation number). The DBE value of an organic compound indicates the minimum number of "reduction operations" necessary to convert a molecule of that compound into a saturated and noncyclic one. If the structure of the molecule is known, the DBE can be calculated as the sum of the number of double bonds, the number of cycles, and twice the number of triple bonds.

DBE = number of double bonds + number of cycles + 2 * number of triple bonds

Examples:



$C_5H_{12}O$ DBE = 0

$C_4H_8O_2$ DBE = 1

C_5H_8S DBE = 2

$C_7H_6F_3NO$ DBE = 4

$C_{11}H_{10}BrNO_2$ DBE = 7

$C_9H_4F_2O_2$ DBE = 7

C_6H_6O DBE = 4

$C_4H_4N_2O_2$ DBE = 5

$C_{17}H_{16}NO_3S$ DBE = 12

$C_6H_8O_4$ DBE = 3

$C_3H_5BrN_2O_4$ DBE = 4

For structural analysis, the calculation of the degree of unsaturation from the molecular formula, which is usually obtained from mass spectrometry or estimated from an empirical formula obtained from elemental analysis, is essential. The calculation of DBE from the molecular formula results from the valency of the elements that the molecule of the compound contains. Valency in this case means the maximum number of single bonds that a given element in a given oxidation state is theoretically able to form. In the molecules of stable organic compounds, the valency of some elements is unambiguous (e.g. hydrogen, lithium, fluorine, chlorine, bromine and iodine are monovalent, oxygen divalent, carbon tetravalent). However, some elements can have different valences (e.g. nitrogen is trivalent or pentavalent, sulfur is divalent, tetravalent or hexavalent) and the DBE calculation then leads to several possible values.

Calculation of DBE from the molecular formula

The DBE calculation from the molecular formula can be derived by considering a linear (acyclic) and fully saturated molecule:

1. Valency and the number of multi-bonding elements of a given formula indicate the maximum number of bonds they can participate in. If the molecular formula $C_7H_6F_3NO$ is correct and nitrogen is trivalent, then the maximum number of bonds of multivalent elements is:

$$4 \cdot 7_C + 3 \cdot 1_N + 2 \cdot 1_O = 33$$

2. In a saturated acyclic structure, all multivalent elements are linked to each other by exactly one bond. Each of the multivalent elements is therefore part of two bonds, with the exception of the outer elements (otherwise a monocyclic structure would result). The number of possible bonds is thus less than twice the number of multivalent elements plus one bond of the first and one bond of the last element of the chain:

$$-2 \cdot (7_C + 1_N + 1_O - 1) = 33 - 16 = 17$$

3. The calculation of the number of possible bonds can be simplified by combining the previous two steps by reducing the valency of these elements by two and increasing the number by two for the outer elements. This reduces the numbers and eliminates the divalent elements entirely:

$$2 \cdot 7_C + 1 \cdot 1_N + 0 \cdot 1_O + 2 = 17$$

4. The number of possible bonds must be further reduced by the number of singlevalent elements that must be bound to multivalent elements. Since two elements are always involved in the formation of a normal covalent bond, half of this difference is equal to the DBE of the compound of the given molecular formula:

$$DBE = \frac{(17 - 9)}{2} = 4$$

5. If the compound contains elements with multiple possible valences, it is important to consider all the possibilities. If we consider nitrogen as pentavalent, then for the molecular formula meantioned above the DBE = 5:

$$DBE = \frac{(2 \cdot 7_C + 3 \cdot 1_N + 2 - 6_H - 3_F)}{2} = \frac{(19 - 9)}{2} = 5$$

Elemental analysis

Elemental analysis - proof of the presence, or determination of individual elements. The prerequisite for further procedures is the mineralization of the organic sample (oxidative or reductive decomposition).

Subsequently, substances are determined by inorganic analysis methods.

Mineralization of the organic sample is carried out by:

- high temperature combustion
- boiling in mineral acids

The amount of oxygen is not determined experimentally, but adds up to 100%. Because combustion creates compounds containing oxygen and it is not possible to distinguish between the oxygen in the original unburned sample and the oxygen that comes from the air.

Example:

During the quantitative analysis, 70 mg of the substance was burned. 102.8 mg of carbon dioxide and 41.6 mg of water were formed. During the combustion of a substance, following reactions take place:

$$C + O_2 \rightarrow CO_2$$

$$2\,H_2 + O_2 \rightarrow 2\,H_2O$$

Now, we calculate how many mg of carbon and hydrogen produced the detected amounts of carbon dioxide and water:

from x mg of C → 102.8 mg of CO_2

from y mg of H → 41.6 mg of H_2O

Using the relative atomic masses M(C) = 12.01 g/mol, M(CO_2) = 44.01 g/mol, M(H) = 1.01 g/mol, M(H_2O) = 18.02 g/mol, we calculate the mass of each element in the sample.

m(C) = 28 mg

m(H) = 4.66 mg

Next, we determine the elemental composition. We calculate the mass percentage of the elements in the sample ($m_{element}/m_{sample} \times 100$).

x = 40 %

y = 6.66 %

The rest up to 100% is attributable to elements or impurities that were not determined. In our case, we will consider that this entire remainder belongs to oxygen (i.e. 53.34%).

Calculation of stoichiometric and molecular formula

Stoichiometric formula

- expresses the basic composition of the compound
- indicates which elements the compounds consists of and in what proportion the atoms of these elements are represented in the compound

Empirical formula

- indicates the type and number of atoms in a molecule of given compound
- can be identical to the stoichiometric formula or an integer multiple thereof

Example: hydrogen peroxide has stoichiometric formula HO and empirical formula H_2O_2

The atomic ration of the elements in the sample can be found by dividing the percentage of each element by its relative atomic mass.

The stoichiometric formula is obtained by dividing the ratio by the smallest of the numbers.

Since in a compound (in an empirical formula) the elements are in the ratio of whole numbers, we try to achieve the the ratio of whole numbers by mathematical operations (multiplication of integers).

To verify the "correctness" of the calculated formula, the following can be used: a) back-calculation of the elemental content; b) the DBE value, which must be an integer; c) NMR spectra.

Calculation of elemental content

From the empirical formula of a substance, we can calculate the elemental composition by dividing the mass of each element (the number of moles of that element multiplied by the molar mass) by the total mass of the molecule (1 mole × molar mass of the molecule). The number obtained must be multiplied by 100 to give the result in %.

E.g. $C_{12}H_{11}F_3N_2O_2$

$$\%C = \frac{12 \cdot A_r(C) \cdot 100}{M_r(C_{12}H_{11}F_3N_2O_2)} = 52.93$$

$$\%H = \frac{11 \cdot A_r(H) \cdot 100}{M_r(C_{12}H_{11}F_3N_2O_2)} = 4.07$$

$$\%F = \frac{3 \cdot A_r(F) \cdot 100}{M_r(C_{12}H_{11}F_3N_2O_2)} = 20.95$$

$$\%N = \frac{2 \cdot A_r(N) \cdot 100}{M_r(C_{12}H_{11}F_3N_2O_2)} = 10.29$$

$$\%O = \frac{2 \cdot A_r(O) \cdot 100}{M_r(C_{12}H_{11}F_3N_2O_2)} = 11.76$$

Nuclear magnetic resonance (NMR)

Introduction

NMR (nuclear magnetic resonance) spectroscopy is a non-destructive method of structural analysis. Together with infrared spectroscopy and mass spectrometry, they are the basic methods for **identifying substances**.

The basic building blocks of atomic nuclei, protons and neutrons, rotate around their own axis and therefore have a momentum *p*, referred to as **spin**. Protons and neutrons have spin quantum number $I = ½$. In the nuclei of isotopes with an even number of both protons and neutrons, the spins of the particles are paired so that the resulting nucleus spin $I = 0$. Such nuclei (e.g. ^{12}C, ^{16}O) have zero spin and do not give NMR signals. Nuclei with an odd number of protons, neutrons or both types of particles do not have paired spins and their $I > 0$. Since the movement of an electrically charged particle along a closed path is associated with the creation of a magnetic field, nuclei with $I > 0$ (e.g. ^{1}H, ^{13}C, ^{15}N, ^{19}F, ^{31}P) have their own magnetic moment μ

$$\mu = \gamma \cdot p \tag{1}$$

where the constant γ is the **gyromagnetic ratio** characteristic of each isotope.

If we place a sample containing an isotope with a non-zero magnetic moment in a strong magnetic field with the **induction of a magnetic field B_0**, the energy levels of its nuclear spins will be split.

The potential energy of the nucleus *E* is given by

$$E = -\mu \cdot B_0 \tag{2}$$

For the component of the magnetic moment in the direction of the B_0 field, the relation applies

$$\mu = \frac{m \cdot h \cdot \gamma}{2\pi} \tag{3}$$

where *h* is Planck's constant, *m* is the magnetic quantum number, which takes the values $m = I, I - 1, \ldots, -I + 1, -I$. The number of generated energy levels is determined by the value of the spin quantum number *I* (number of states = $2I + 1$), therefore, for isotopes with $I = ½$, such as ^{1}H and ^{13}C, the nuclear spins occupy two energy states with magnetic quantum numbers $m = +½$ and $m = -½$. Their energy can then be calculated from the relation

$$E_{+½} = -\frac{1}{2} \cdot \left(\frac{h \cdot B_0 \cdot \gamma}{2\pi}\right)$$

$$E_{-½} = +\frac{1}{2} \cdot \left(\frac{h \cdot B_0 \cdot \gamma}{2\pi}\right) \tag{4}$$

However, according to the laws of quantum mechanics, each nucleus can only be permanently in one energy level, and the spin can therefore have only one of two possible orientations. Transitions between these levels can be caused by radiation whose frequency meets the condition

$$\Delta E = E_{-½} - E_{+½} = h\nu \tag{5}$$

where ΔE is the energy difference between the levels. Using the relations (4) and (5) we can then write

$$\nu_0 = \frac{\Delta E}{h} = \frac{h \cdot B_0}{2\pi} \tag{6}$$

The given relationship is known as **the resonance condition**, the frequency ν_0 is the so-called **Larmor frequency**. However, it is often given in the form of $\omega = \gamma \cdot \boldsymbol{B_0}$, where ω is the angular velocity. Since the level difference ΔE is small, only the energy of radio frequency radiation (with a frequency of the order of MHz) is sufficient to excite these nuclei.

On the energetically lower level there are nuclei whose projection of the nuclear magnetic moment μ is oriented in agreement with the external magnetic field B_0. This orientation corresponds to the magnetic quantum number $m = +½$. Nuclei at a higher energy level have a resulting magnetic moment oriented against the direction of the magnetic field $m = -½$.

In the equilibrium state at temperature $T = 300$ K and magnetic field induction $B = 1$ T, according to Boltzman's distribution law, the ratio of the number of spins of protons at energy levels $n_{+½}$ ($m = +½$) and $n_{-½}$ ($m = -½$)

$$\frac{n_{+½}}{n_{-½}} = e^{\left(\frac{\Delta E}{k \cdot T}\right)} = 1.000006 \tag{7}$$

where k is Boltzmann's constant.

The population of nuclei at a lower energy level is therefore only slightly larger than at a higher energy level. The result of this excess is the vector sum of all nuclear spin magnetic moments – **nuclear magnetization $\boldsymbol{M_0}$**. Its size is proportional to the excess of nuclei at a lower energy level.

When measuring NMR spectra by pulse methods, the population of both energy levels is equalized by a suitable radio frequency pulse and the magnetization M_0 deviates from its equilibrium position (from the direction of the axis of the external magnetic field B_0). Since the magnetization is associated with the angular momentum of the nuclei, it does not immediately return to the direction of the magnetic field after the end of the pulse, but behaves similarly to a flywheel fixed outside the center of gravity, whose axis of rotation is inclined to the gravitational field. The result is precession, during which the magnetization maintains its magnitude and inclination with respect to the magnetic field and rotates around the direction of the magnetic field B_0 with the Larmor frequency (**Fig. 1**).

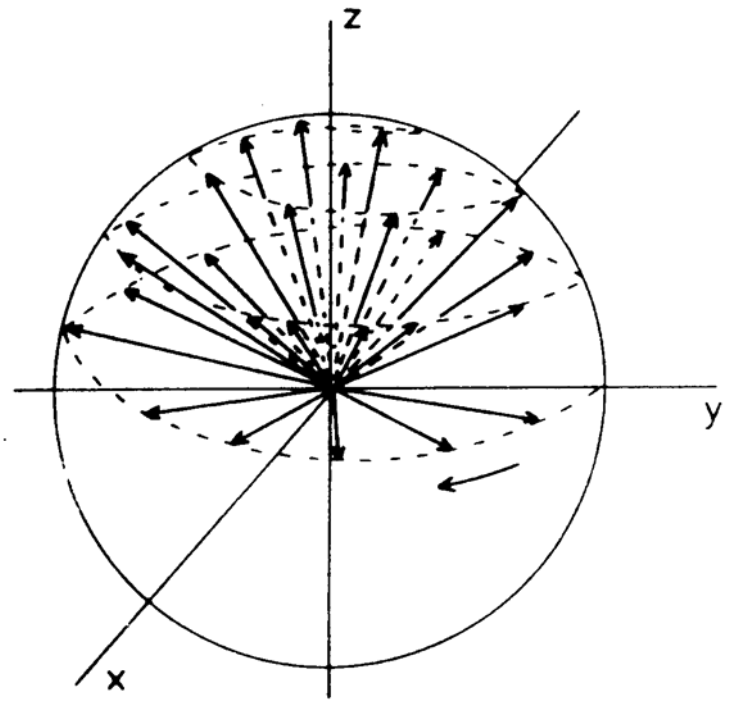


Fig. 1: Return of the magnetization vector *M* to the equilibrium position after deflection by the radio frequency pulse.

In addition, the disturbed state of equilibrium is gradually restored through relaxation processes. After a certain time, characterized by the so-called **spin-lattice relaxation time T_1**, the equilibrium distribution of spins on both energy levels stabilizes again. This restores the magnetization component in the direction of the field. The component of magnetization perpendicular to the direction of the magnetic field, on the other hand, decreases to zero with the so-called **spin-spin relaxation time T_2**.

Just as a rotating magnet generates an alternating voltage in the alternator stator coils, the rotating magnetization vector induces an alternating voltage in the receiver coil, which is wound around the sample in the NMR spectrometer. The time course of the voltage (signal) induced by the nuclear magnetization in the coil, which is commonly referred to as **FID** (free induction decay), is indicated in **Fig. 2** and can be imagined as a decaying note after pressing a piano key.

If the sample contains only one type of atoms of one isotope, e.g. $CHCl_3$ (**Fig. 2a**), the decrease in the amplitude of the damped oscillations is exponential and is characterized by the spin-spin relaxation time T_2. The frequency of oscillations is the Larmor frequency. For more complex samples, the resulting FID is an interferogram or superposition of FIDs originating from different nuclei of the same isotope (**Fig. 2b**). In order to obtain the resonance frequencies of individual types of nuclei and the intensity of their signal response, it is first necessary to convert the FID, i.e. the time dependence of the signal to the frequency dependence of the signal, or to the **NMR spectrum**. This conversion is carried out on a computer by the so-called Fourier transformation.

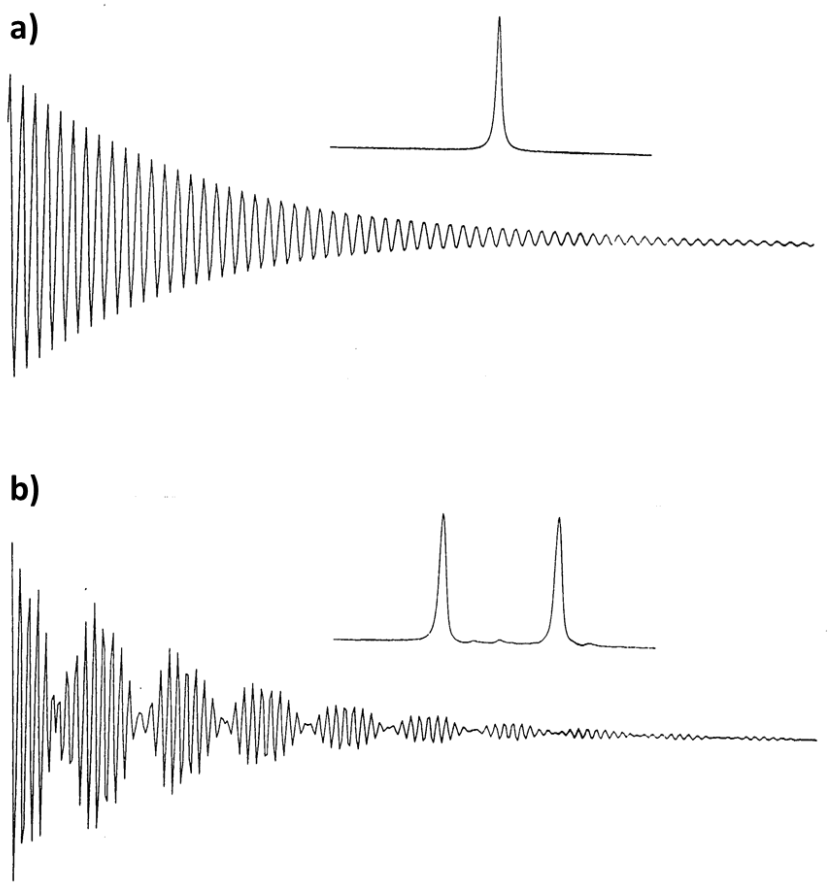


Fig. 2: FID – dependence of signal *s* on time *t* for a) one or b) two types of nuclei, to which one or two signals correspond in the spectrum, resp.

NMR spectrometer

The NMR spectrometer can be divided into three basic parts: the magnet (**Fig. 3**), the electronics of the spectrometer and the computing system with peripherals. The basis of an NMR spectrometer is a superconducting magnet, which is formed by a solenoid coil made of superconducting material. This is placed in a container with liquid helium at a temperature of 4 K. At this temperature, the material of the coil exhibits superconductivity. Outside the container with liquid helium, there is still a container with liquid nitrogen at a temperature of 77 K, and the rest of the cryostat space is evacuated. The cryostat as such has the shape of a hollow cylinder.

The sample of the measured substance, which is in a glass cuvette, is placed inside the cavity of the cryostat, i.e. in a space surrounded by a superconducting coil. In this space, where there is a laboratory temperature, there is also a so-called measuring probe. The main part of the probe are the electrical circuits that serve to deliver energy into the sample via a radio frequency pulse during excitation, and to detect the frequencies emitted by the sample during relaxation.

Superconducting correction coils placed in the cryostat and so-called warm correction coils outside the cryostat serve to achieve homogeneity of the magnetic field.

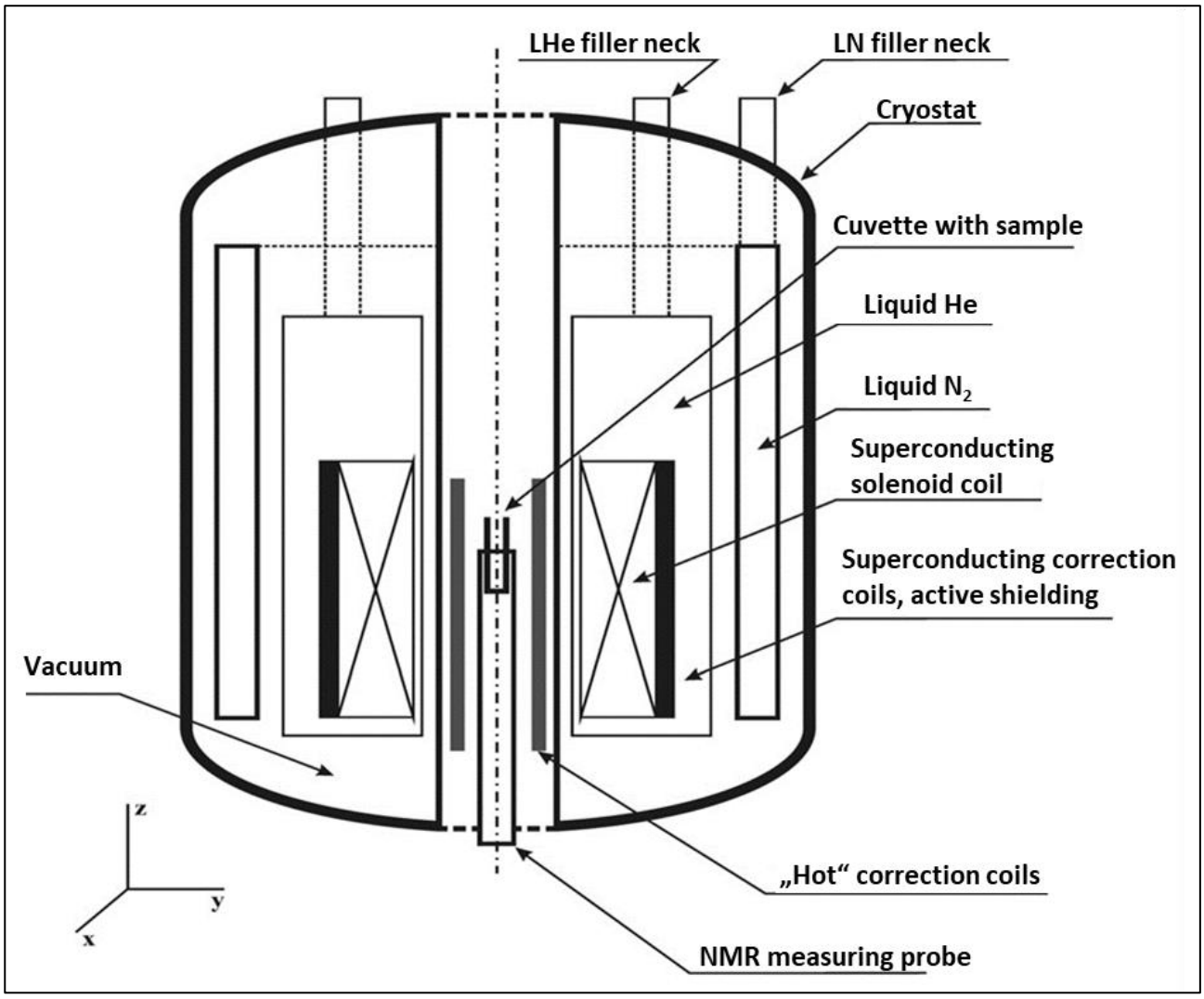


Fig. 3: Schematic illustration of a superconducting magnet.

Interpretation of the NMR spectrum

The relationship between the frequency of the absorbed radiation and the magnetic induction B_0 is expressed in the resonance condition (6). According to the stated condition, the resonance frequency should be constant for all nuclei of the same isotope. In a molecule, however, the individual nuclei are bound into groups and are thus influenced by a different electronic environment. The electron environment changes ("shields") the magnetic field at the location of the nucleus B_{loc}, so that at the location of the nucleus the magnetic induction is given by relation:

$$B_{loc} = B_0(1 - \sigma) \tag{8}$$

where σ is the **shielding constant**. Both ^{1}H and ^{13}C nuclei are always shielded in the molecule, so the values of the shielding constant are always positive.

Determining the shielding constant is experimentally demanding, which is why in practice relative values are given relative to the standard. Tetramethylsilane – TMS is used as a standard, which is directly added to the measured sample as a so-called **internal standard**. The distance of the resonance signals from the TMS signal is measured in hertz (Hz). Since this value is dependent on B_0, the **chemical shift** δ has been defined as a quantity that is independent of the induction of the magnetic field:

$$\delta = \frac{\nu - \nu_{std}}{\nu_{std}} \cdot 10^6 \; [ppm] \tag{9}$$

where ν and ν_{std} are the frequencies of the measured nucleus and standard. Chemical shift is expressed in *ppm* (parts per million). The TMS chemical shift value is equal to 0 ppm according to relation (9).

Example: In the ^{1}H NMR spectrum there is a signal with a frequency of 300002020 Hz. What is its chemical shift given that the TMS frequency is 300000300 Hz? Substituting into relation (9) we obtain:

$$\delta = \frac{300002020 - 300000300}{300000300} \cdot 10^6 = 5.73 \; ppm$$

^{1}H NMR spectroscopy

From the ^{1}H NMR spectrum, the following data can be determined for each signal:

- chemical shift
- integral intensity
- multiplicity

Chemical shift

The range of chemical shifts in ^{1}H NMR spectra is most often in the range of 0-20 ppm. **Table I** (see Appendix) lists the proton chemical shifts by which the proton signal can be assigned to a particular structural grouping. If the protons are bound in the same way, and thus have the same "surroundings", they show the same resonance frequency. Such nuclei are called **magnetically equivalent** and are also always **chemically equivalent** (but not vice versa). Thus, for example, in benzene or cyclohexane, due to symmetry, all protons are equivalent and will provide only a single signal in the ^{1}H NMR spectrum. Similarly, in the $-CH_3$ group, due to the free rotation around the single bond, all three protons are equivalent, and only one signal will correspond to them in the spectrum. Also, the compounds tetramethylsilane $-Si(CH_3)_4$ and symmetrically substituted ethane $X-CH_2-CH_2-X$ (both free rotation around the single bond and symmetry of the molecule are applied here) will give only a single signal in the ^{1}H NMR spectrum, corresponding to twelve (in the case of tetramethylsilane) and four (in the case of ethane) equivalent protons.

Chemical shift is influenced by a number of factors (electronegativity of substituents, magnetic anisotropy, steric effects, temperature, concentration, solvent, etc.). The most important of them is the electronegativity of the substituents. As the electronegativity of neighboring atoms or groups increases, shielding decreases and the value of the chemical shift δ increases. Temperature, concentration and solvent significantly affect the value of the chemical shift of protons bound to heteroatoms (–OH, –NH–, –SH).

Figure 4 shows the ^{1}H NMR spectrum of ethyl formate. There are three signals (1.3 ppm, 4.2 ppm and 8.0 ppm) in the spectrum, corresponding to the three groups of protons contained in ethyl formate, i.e. $–CH_3$, $–CH_2–$ and H–CO–. These differ in their chemical shifts, which are consistent with the values shown in **Table I**. The signal with a chemical shift of 7.26 ppm, also found in the spectrum, corresponds to the residual signal of $CHCl_3$, which was used as a solvent in this case.

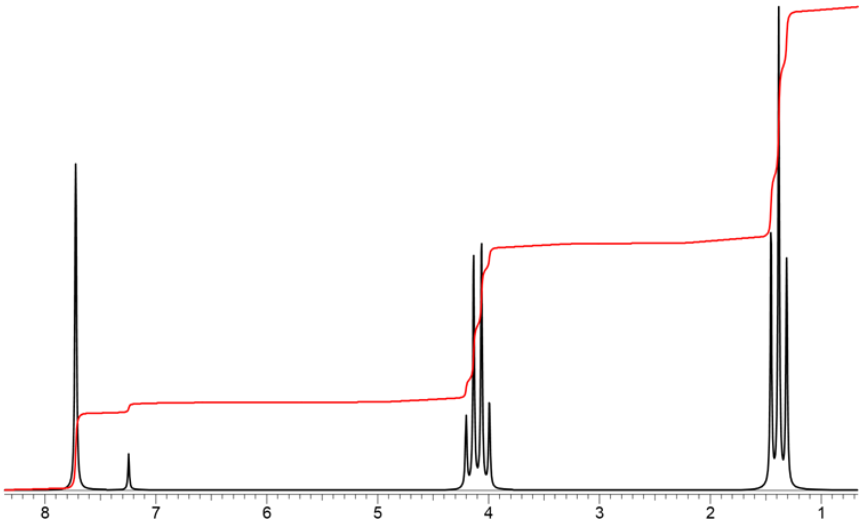


Fig. 4: ^{1}H NMR spectrum of ethyl formate

Note: Solvents used in NMR spectroscopy are not 100% deuterated, and therefore signals of non-deuterated solvents are always found in ^{1}H NMR spectra.

Integral intensity

In ^{1}H NMR spectra, the areas of resonance signals are proportional to the number of protons in the molecule. For this purpose, an integral record is taken, which usually has a waveform over the individual signals. The height of the integral wave corresponds to the areas of the individual signals, and the values of these areas are located below the scale of chemical shifts. In order to obtain the number of protons contained in the individual signals, the ratio of the area sizes must be adjusted to a ratio of small integers.

Thus, for example, in the case of ethyl formate (**Fig. 4**), the area (wave height) under the signal at 1.3 ppm must be considered as equivalent to three protons. In this region, only the signals of $–CH_3$ groups are practically always present. So we can easily figure out how much area corresponds to one proton, divide the other areas by that value, and then round the numbers off. In the spectrum of ethyl formate in **Fig. 4**, this ratio after rounding from right to left is 3:2:1. From this ratio, it follows that the signal with relative intensity 3 corresponds to the $–CH_3$ group, the signal with relative intensity 2 corresponds to the $–CH_2–$ group and the last signal with relative intensity 1 corresponds to the H–CO– group. The signal at 7.2 ppm is from $CHCl_3$, which is always present together with $CDCl_3$.

Multiplicity

In the ^{1}H NMR spectrum of ethyl formate, the signals differ not only by the different position, given by the different chemical shift of the individual groups in the molecule, by the different intensity, given by the number of protons in the individual groups of the molecule, but also by the fine structure (splitting, multiplicity) of the signals. The cause of this splitting is the mutual interaction of the spins of individual protons - **spin-spin coupling**. It is an interaction that is transmitted by bonding electrons (not through space). It is therefore usually most significant for the closest neighboring groups. The spin-spin coupling is mutual, i.e. if the *A* proton signal is split by the protons corresponding to the *B* signal, the *B* proton signal must also be split by the protons corresponding to the *A* signal.

The spin-spin coupling is characterized by the value of the **spin-spin coupling constant *J***. Its value is expressed in Hz and does not depend on the induction of the external magnetic field. From the spectra, the value of *J* can be calculated as the difference in the frequencies of the individual lines of the multiplets. The positions of the multiplet lines in *Hz* are indicated above these multiplets. The values of some spin-spin coupling constants are shown in **Fig. 5**. Assuming that the interpreted spectrum is a so-called **1st-order spectrum**, this means that the condition $\Delta\nu / J > 6$ is fulfilled, where $\Delta\nu$ is the difference of resonant frequencies of mutually interacting nuclei, we can calculate the coupling constants as the difference of the line frequencies of individual multiplets.

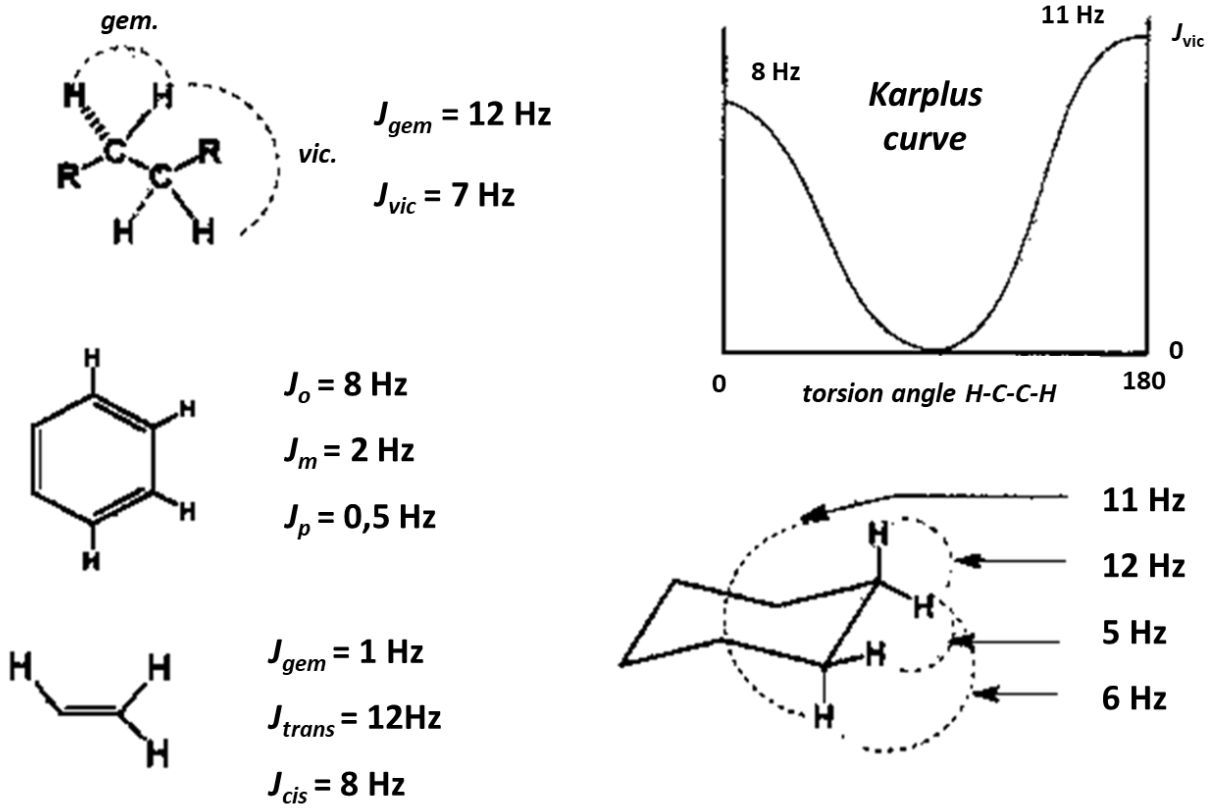


Fig. 5: Coupling constants (*J*) in different functional groups.

For protons and, in general, for nuclei with spin quantum number $I = ½$, simple rules apply to the shape of the multiplet in the 1st-order spectra:

1. If in the neighborhood of the observed nucleus there are groups with nuclei having a different coupling constant with the observed nucleus, the signal of the observed nucleus is split into a maximum of $(n_1 + 1) \cdot (n_2 + 1)$ components, where n_1 or n_2 are the numbers of nuclei of neighboring groups with coupling constant J_1 or J_2. However, if $J_1 = J_2$, then the signal is split into $(n + 1)$ components, where n is the number of nuclei of the adjacent group or groups with the same mutual coupling constant.
2. The relative intensities of the lines of the multiplet, formed by the interaction with a group of n magnetically equivalent nuclei, are in the ratios of the binomial development coefficients $(a + b)^n$, which can be determined from Pascal's triangle, i.e. doublet 1:1, triplet 1:2:1, quartet 1:3:3:1, etc. Splitting options for one to three coupling constants are shown in **Fig. 6**.

In the spectrum of ethyl formate (**Fig. 4**) we find two multiplets, a triplet with a chemical shift of 1.3 ppm and a quartet with a chemical shift of 4.2 ppm. The triplet corresponds to the $–CH_3$ group because this group is directly adjacent to the two equivalent protons of the $–CH_2–$ group. The quartet, on the other hand, corresponds to the $–CH_2–$ group, because the methylene group is directly adjacent to the three equivalent protons of the $–CH_3$ group. The proton in the H–CO– group has no proton directly next to it, therefore the signal with a chemical shift of 8.0 ppm corresponding to this group is a singlet in the spectrum of ethyl formate.

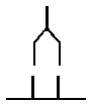
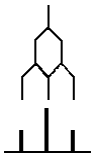
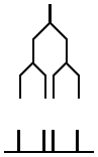
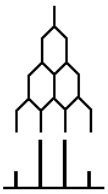
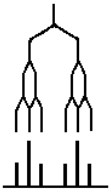
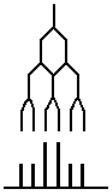
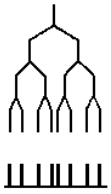
J_1	$J_1 = J_2$	$J_1 > J_2$	$J_1 = J_2 = J_3$	$J_1 > J_2 = J_3$	$J_1 = J_2 > J_3$	$J_1 > J_2 > J_3$
						
doublet	**triplet**	**doublet of doublets**	**quartet**	**doublet of triplets**	**triplet of doublets**	**doublet of doublets of doublets**
d	**t**	**dd**	**q**	**dt**	**td**	**ddd**

Fig. 6: Splitting options.

The value of the spin-spin coupling constant in ^{1}H NMR spectra decreases with the number of bonds between the interacting nuclei. That is why we distinguish **geminal** coupling constants, denoted ^2J (H–C–H), **vicinal** ^3J (H–C–C–H), and **long-range** coupling constants ^4J to ^9J. The peculiarity of aromatic protons should be emphasized. In a substituted aromatic ring, the hydrogen atoms are not equivalent. Depending on the nature of the substituent present, the condition for a 1st-order spectrum is often not met (differences in chemical shifts are often small), and spectrometers with a lower operating frequency cannot distinguish these hydrogen atoms. In the spectrum, we find a series of signals with a fine structure in the region of 6-9 ppm. Since these signals can be confused with double bond signals (chemical shift ranges overlap), the relative intensities of the multiplets and the values of the spin-spin coupling constants (**Fig. 5**), which are smaller compared to the double bond, must also be considered when analyzing the spectrum.

Note: In the case of multiple multiplets, the outer, weakest, signals of the multiplet may be obscured by noise (e.g. for nonet, the theoretical ratio between the weakest and strongest signal of the multiplet according to Pascal's triangle is 1:70).

^{13}C NMR spectroscopy

Since the isotope ^{12}C is not magnetically active, only the isotope ^{13}C, whose spin quantum number is the same as that of protons, i.e. I = ½, can be measured in the case of carbons.

Compared to ^{1}H NMR spectra, ^{13}C NMR spectra have a number of differences:

1. The range of chemical shifts, in contrast to ^{1}H NMR spectra, is approximately 300 ppm.

   However, usually the signals of carbon atoms of organic compounds occur in the interval 0-220 ppm. This improves clarity and facilitates the interpretation of these spectra, as there is not as much signal overlap as in the case of proton spectra. The chemical shift of carbons is related to TMS as is the case for protons. Even in this case, the chemical shift is influenced by a number of factors. Its value depends primarily on the hybridization of the carbon atoms, with the chemical shift decreasing in the following order:

   $$sp^2 > sp > sp^3$$

2. However, the quaternary and carbonyl carbon atoms have the largest chemical shifts. Furthermore, the chemical shift is influenced by the inductive effect of substituents. Electron-withdrawing substituents cause the C_α and C_β carbons to shift to lower field, i.e. to higher chemical shifts, while for C_γ the effect is the opposite and usually negligibly small for C_δ. Steric, conjugation, and other effects also affect chemical shift. A basic overview of ^{13}C chemical shifts is summarized in **Table II**.
3. The intensity A of the signals of ^{13}C nuclei depends on the gyromagnetic ratio, the spin quantum number I and the natural occurrence of the nucleus n (in %) according to the relation:

$$A = \gamma^3 \cdot n \cdot I(I+1) \qquad (10)$$

Due to the natural occurrence of ^{13}C nuclei, which is only 1.11%, and due to the four times smaller gyromagnetic ratio compared to the ^{1}H nucleus, the relative sensitivity of carbons is approximately 5700 times lower than that of protons. Therefore, ^{13}C NMR spectroscopy became a common method only after the introduction of pulsed FT NMR spectrometers, which allow rapid accumulation of spectra. Since the signal-to-noise ratio increases with the square root of the number of accumulations (scans), in order to obtain high-quality carbon spectra, hundreds to several tens of thousands of accumulations must be performed, depending on the structure of the molecule and the concentration of the sample.

In comparison to proton spectra, the intensities in ^{13}C NMR spectra are not always proportional to the number of corresponding carbon nuclei. This makes it difficult to use ^{13}C NMR spectroscopy for quantitative purposes and sometimes also to interpret the spectra. The reason is the unequal relaxation times of different carbon nuclei, which depend on the surroundings of the atoms in question. It is useful to know that signals from quaternary carbons give weaker signals than carbons to which hydrogen atoms are attached. In addition, the intensity of the signals in the ^{13}C NMR spectra depends on how (according to the pulse sequence used) the spectrum was obtained.

4. Unlike proton spectra, ^{13}C-^{13}C interactions practically do not appear in carbon spectra due to the low probability of two ^{13}C isotope nuclei in one molecule in close proximity.

On the contrary, the spin-spin interaction ^{1}H-^{13}C is prominent in the carbon spectra. The most significant are the direct $^{1}J_{CH}$ coupling constants, which depend on the hybridization of the carbon atom and whose value varies in the following order: sp^3 (120-150 Hz), sp^2 (150-250 Hz), and sp (250-320 Hz). This fact makes it easier to assign the signals of the carbons of the molecule, but on the other hand, it worsens the clarity of the spectra if there is a small difference in the chemical shifts of the individual carbons. Therefore, a method called **decoupling** is used. ^{13}C NMR spectra are measured with simultaneous irradiation of all protons in the molecule. This will eliminate all splitting caused by the spin-spin carbon-proton interaction. In addition, there will also be an increase in the intensity of the signals of carbon atoms in the spectrum, because all the intensity is then concentrated in a single singlet signal peak.

This is documented in **Fig. 7**, where the non-decoupled and decoupled spectrum of butan-2-ol measured in $CDCl_3$ is shown (the signal at 77 ppm is from $CHCl_3$, which is always present together with $CDCl_3$). In the non-decoupled spectrum (**Fig. 7a**), two quartets corresponding to two $–CH_3$ groups, a triplet corresponding to $–CH_2–$ group and a doublet corresponding to –CH– group can be observed. The quartet with a higher chemical shift belongs to the methyl group, which is closer to the –OH group. In the decoupled spectrum (**Fig. 7b**), there are only four unsplit signals characterized by different chemical shifts and corresponding to four types of carbon atoms in the butan-2-ol molecule.

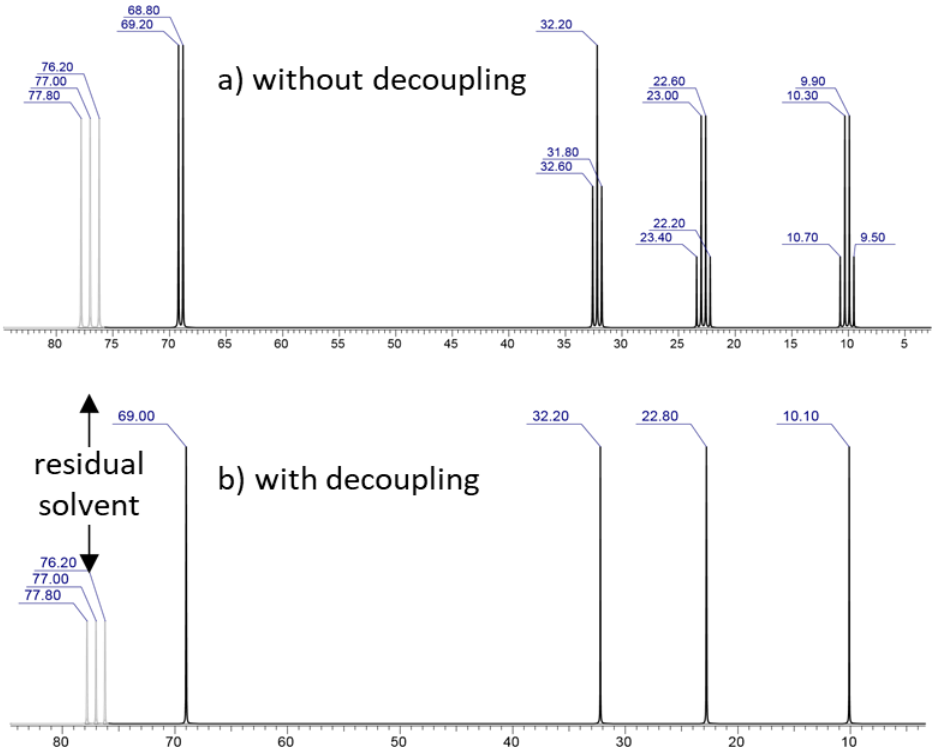


Fig. 7: a) ^{13}C NMR spectrum (without decoupling) and b) $^{13}C\{^{1}H\}$ NMR spectrum (with decoupling) of butan-2-ol.

Example of ^{1}H NMR spectrum interpretation

Interpretation of the NMR spectrum means evaluation of all parameters, i.e. chemical shift, integral intensity and spin-spin coupling constant. In practice, when determining the structure of an unknown substance, the conclusions from the NMR spectrum are then compared with other physicochemical data of the substance and with the results of other spectral methods, especially mass, infrared and ultraviolet spectroscopy, or with the results of X-ray structural analysis.

Let us now show how to interpret the spectrum of an unknown sample. In **Fig. 8** is the ^{1}H NMR spectrum of a substance that was measured as a solution in $CDCl_3$.

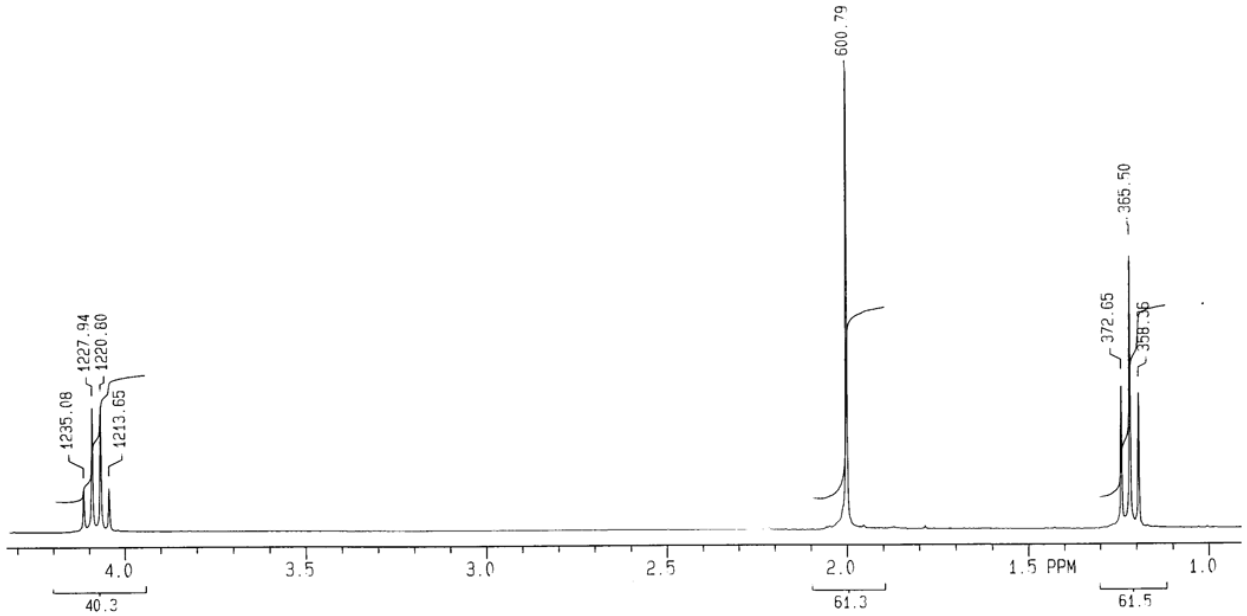


Fig. 8: ^{1}H NMR spectrum of an unknown compound.

From the results of elemental analysis, the general formula C_4H_8O was determined. First, we calculate the DBE (double bond equivalent) according to the relation:

$$DBE = n(C) - ½\, n(H) + ½\, n(N) + 1 \qquad (11)$$

where $n(C)$ is the number of carbon atoms (generally tetravalent atoms), $n(H)$ is the number of hydrogen atoms (generally monovalent atoms, i.e. also halogens) and $n(N)$ is the number of nitrogen atoms (generally trivalent atoms). Double-bonded atoms (e.g. oxygen) do not affect the DBE.

The DBE indicates how many multiple bonds or cycles a structure with a given general formula must contain in order to comply with the basic rules about the bonding of atoms. For the general formula C_4H_8O, DBE = 1 results and this means that this substance contains either one double bond or one ring. Similarly, for UN = 2 there are four variants: the substance contains a) two double bonds, b) one triple bond, c) two rings, or d) a ring and one double bond. Conversely, for the benzene core, the double bond equivalent must be DBE = 4, since benzene represents three double bonds and one ring.

There are three signals in the spectrum (from right to left, a triplet with a chemical shift of 1.22 ppm, a singlet with a chemical shift of 2.00 ppm, and a quartet with a chemical shift of 4.08 ppm). From the chemical shift table (**Table I**, Appendix), the signal at 1.22 ppm can be assigned to the $H_3C–C–$ group, the signal at 2.00 ppm to the $H_3C–CO–$ or $H_3C–C=C–$ group, and the signal with a chemical shift of 4.08 ppm to the $–CH_2–O–$ or $–CH_2–X$ group.

Let's have a look at what the integral intensity of the individual signals is. The signal with a chemical shift of 1.22 ppm according to the chemical shift tables most likely corresponds to the $–CH_3$ group. In that case, the area indicated under this signal corresponds to three protons, and with a simple mathematical adjustment we find that the areas of the individual signals are (from right to left) in the ratio 3:3:2. This is also in agreement with the results obtained from chemical shifts.

The signal with a chemical shift of 1.22 ppm is a triplet. This means that it is adjacent to a group containing two protons, i.e. $–CH_2–$ group. In the spectrum, the $–CH_2–$ group corresponds to a signal with a chemical shift of 4.08 ppm. The latter, if $–CH_2–$ group is attached to the $–CH_3$ group and assuming that it is attached to oxygen or halogen from the other side (as follows from **Table I**), must be split into a quartet. Such a signal is found in the spectrum. The variant that the $–CH_2–$ group is attached to the halogen can be ruled out due to the results of the elemental analysis. The compound must therefore contain an ethyl group bound to an oxygen atom ($–O–CH_2–CH_3$). A signal with a chemical shift of 2.00 ppm is a singlet. It therefore corresponds to the $–CH_3$ group, which is not directly attached to a group containing a proton. According to **Table I**, this group is attached to a carbonyl group or to a group with a double bond. We can rule out the second possibility, because if a proton were bound to the double bond, such a signal would have to be found in the spectrum. If only carbon or other atoms were attached to the double bond, it would not agree with the general formula of the compound.

The compound thus contains $H_3C–CO–$ and $–O–CH_2–CH_3$ groups. This is also consistent with the DBE (there is a double bond in the C=O group in the compound). Since other signals are not found in the spectrum, and due to the general formula, we can combine these two groups. The ^1H NMR spectrum in **Fig. 8** is therefore the spectrum of **ethyl acetate**.

Example of ^{13}C NMR spectrum interpretation

In **Fig. 9** are the ^{13}C NMR and ^{13}C{^{1}H} NMR spectra of a substance that was measured as a solution in $CDCl_3$.

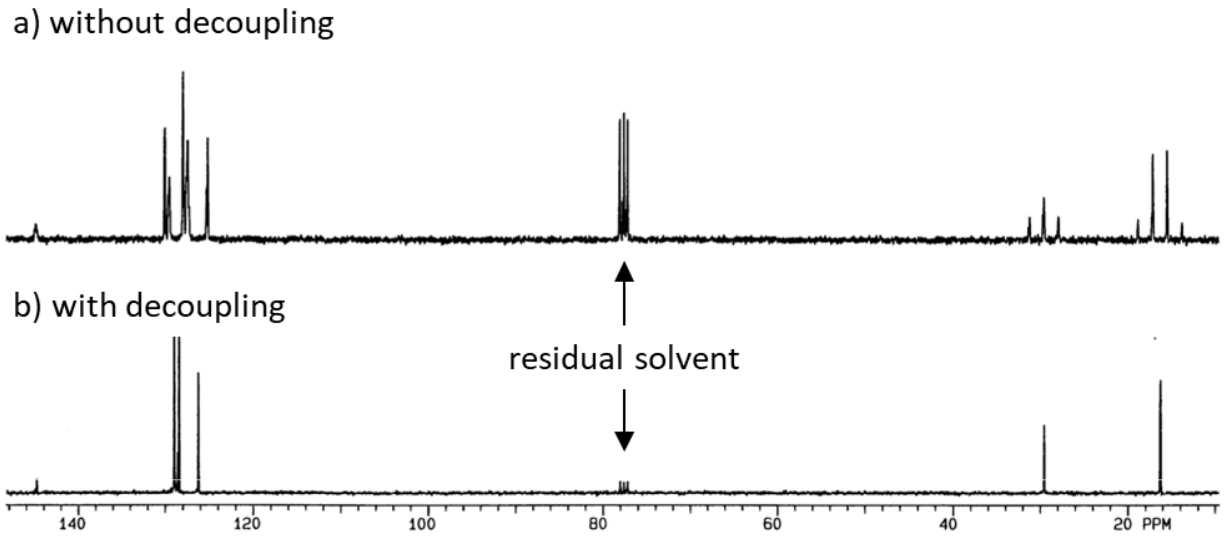


Fig. 9: a) ^{13}C NMR spectrum (without decoupling) and b) ^{13}C{^{1}H} NMR spectrum (with decoupling) of an unknown compound.

The general formula C_8H_{10} was determined from the elemental analysis. Substituting into relation (10) for the double bond equivalent, we calculate DBE = 4. In both carbon spectra (**Fig. 9**), a signal with a chemical shift of 77 ppm can be observed, corresponding to the signal of the solvent. The multiplicity of this signal is caused by the spin-spin interaction of carbon (^{13}C) with deuterium (^{2}D). Since deuterium has a spin quantum number $I = 1$, according to the general rule, the signal will be $2 \cdot n \cdot I + 1$, split into $2 \cdot n + 1$ components, i.e. a triplet (n is the number of nuclei, in our case deuterium).

In the spectrum in **Fig. 9**, in addition to the solvent signal, there are a total of 6 signals with a chemical shifts: 16.2; 29.5; 126.2; 128.5; 128.9 and 144.9 ppm. The first signal, with a chemical shift of 16.2 ppm, has a multiplicity of 4, i.e. a quartet, in the non-decoupled spectrum. The latter belongs to the $–CH_3$ group. The second signal is a triplet, i.e. it corresponds to the $–CH_2–$ group. The last signal on the left is a singlet. Due to its high chemical shift value (144.9 ppm) and low intensity, it can be concluded that it is a quaternary carbon atom. According to the general formula, however, the compound contains 10 carbon atoms. The molecule must therefore contain an element of symmetry, or two pairs of equivalent carbons. Considering the number of unsaturation and the presence of signals in the range of 125-130 ppm, it can be concluded that the molecule contains an aromatic core. Since there is only one quaternary carbon in the spectrum, it is likely to be a monosubstituted aromatic core. The monosubstituted aromatic core also has the already predicted two pairs of equivalent carbons. Since we determined the remaining two carbons as $–CH_2–$ and $–CH_3$ groups, the conclusion is clear. The ^{13}C NMR spectrum in **Fig. 9** is the spectrum of **ethylbenzene**.

Control questions:

1. Which isotopes give NMR signals?
2. What quantities are shown on the *x* and *y* axes in the NMR spectrum?
3. Which basic parameters are obtained from the NMR spectrum?
4. What is a chemical shift and what affects its value?
5. What is the unsaturation number and how is it determined?
6. What is the unsaturation number of hexane, cyclohexane, cyclohexene, and benzene?
7. How many signals are there in the ^{1}H NMR spectrum of compounds containing the groups CH_3O–, CH_3CH_2O–, or $CH_3CH_2CH_2O$–? (In the case of multiple signals, also determine their relative intensities.)
8. What controls the intensity of multiplet lines?
9. What is the multiplicity of the groups listed in question 6?
10. What signals are there in the ^{1}H NMR spectrum of compounds containing symmetrically or asymmetrically *p*-substituted aromatic core?
11. How does the presence of an electronegative element (e.g. oxygen) affect the value of the chemical shift of the adjacent carbon?
12. What is decoupling and why is it used?
13. How do the ^{1}H NMR spectra of *trans*-but-2-ene and *cis*-but-2-ene differ?

Appendix

Example of interpretation of ^{1}H NMR and ^{13}C NMR spectra of an unknown compound

In the **Fig. 10a,b** is the ^{1}H NMR spectrum of an unknown compound and in the **Fig. 11a,b** is its decoupled and undecoupled ^{13}C NMR spectrum.

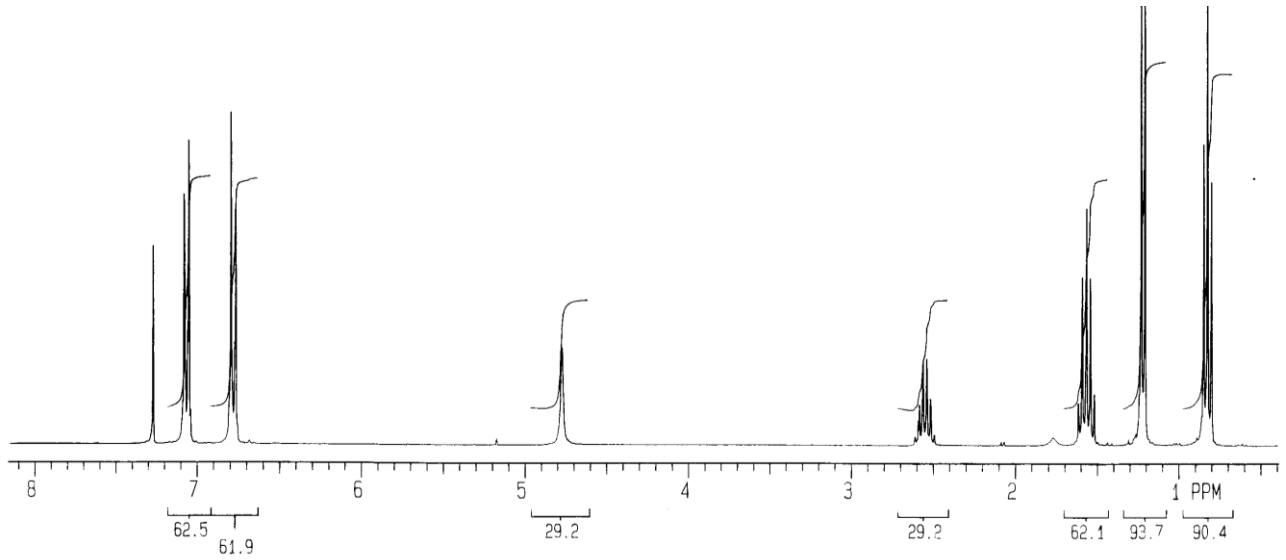


Fig. 10a: ^{1}H NMR spectrum of an unknown compound.

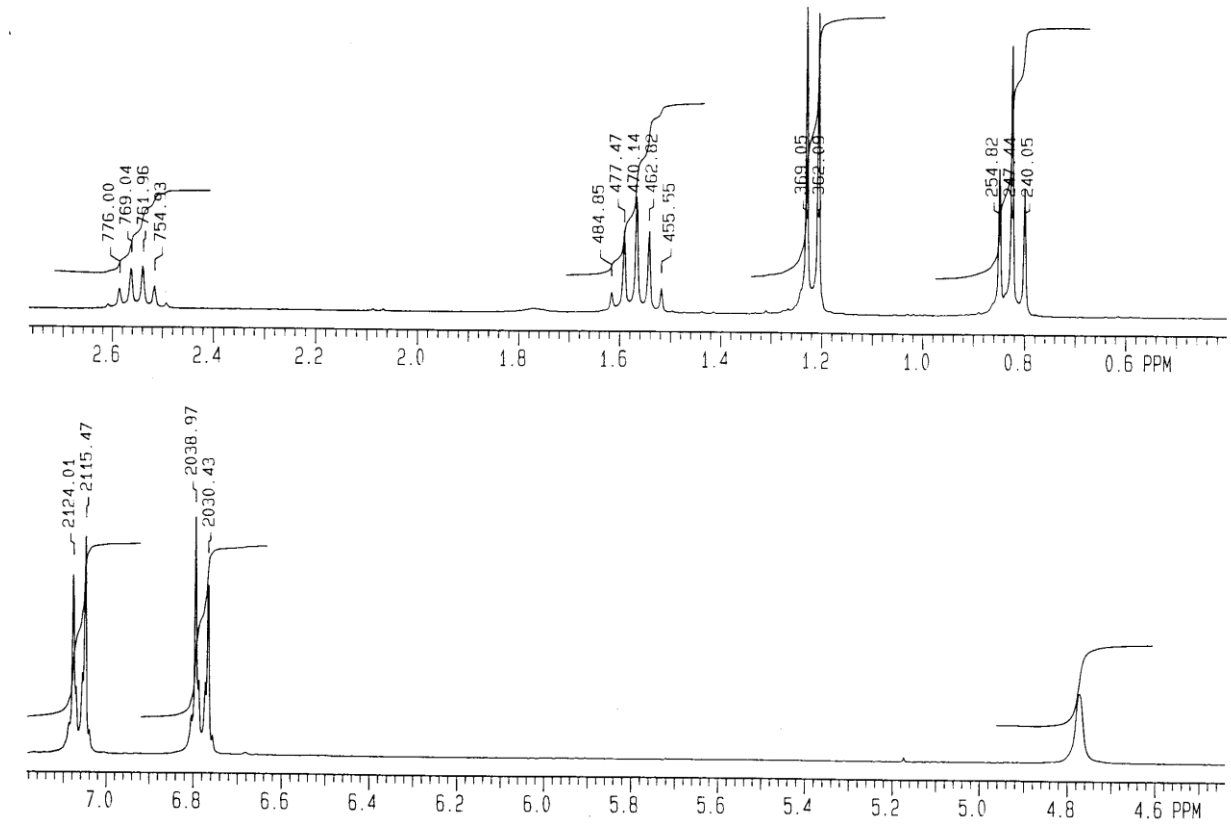


Fig. 10b: ^{1}H NMR spectrum of an unknown compound (zoomed).

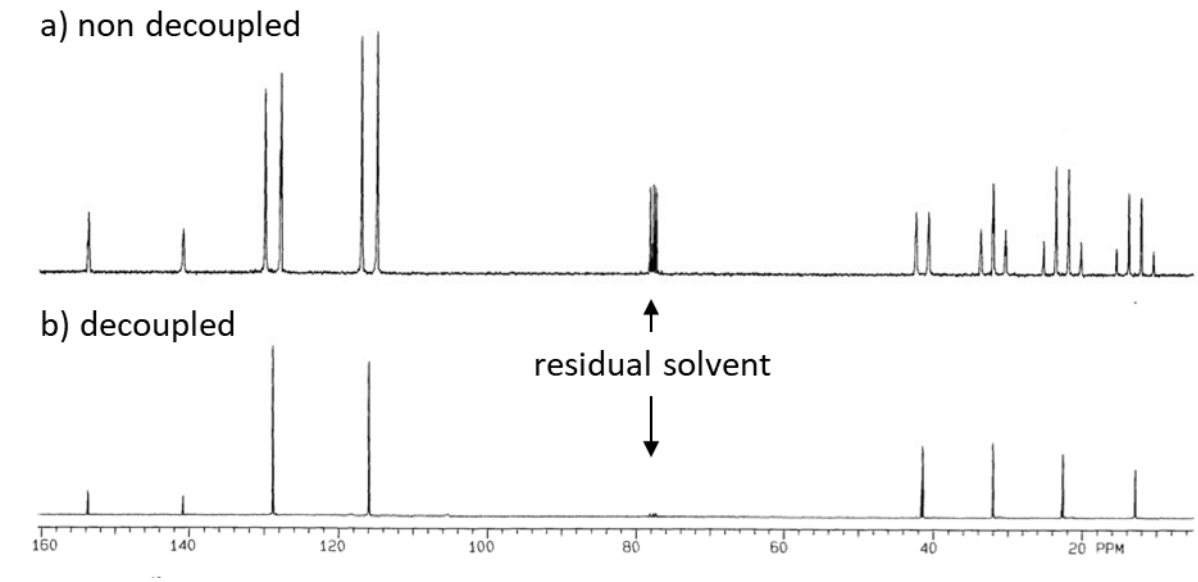


Fig. 11: ^{13}C NMR spectra of an unknown compound.

The molecular formula of the compound $C_{10}H_{14}O$ is deduced from the results of the elemental analysis. Substituting n(C) = 10 and n(H) = 14 into formula (10), we calculate UN = 4. This implies that the molecule contains four double bonds or two triple bonds or four rings, or some combination of these possibilities, e.g., an aromatic core.

Eight resonance signals can be found in the ^{1}H NMR spectrum (the signal on the left with a chemical shift of 7.26 ppm belongs to $CHCl_3$, which is contained in $CDCl_3$). We will not consider the signal of $CHCl_3$ further. Now we determine the chemical shift and multiplicity of each signal. From right to left: triplet (0.81 ppm), doublet (1.21 ppm), quintet (1.57 ppm), sextet (2.55 ppm), singlet (4.77 ppm), doublet (6.78 ppm) and doublet (7.06 ppm).

In the decoupled ^{13}C NMR spectrum (**Fig. 11a**), there are a total of 8 signals with chemical shifts of 12.89, 22.65, 32.00, 41.51, 115.92, 128.80, 140.83 and 153.73 ppm, in addition to the solvent signal with a chemical shift of 77 ppm. The first two signals (12.89 and 22.65 ppm) occur as quartets in the undecoupled spectrum and thus correspond to methyl groups. The third signal with chemical shift of 32.00 ppm corresponds to a triplet in undecoupled spectrum, thus it is a methylene group. The signals with chemical shifts of 41.51, 115.92 and 128.80 ppm correspond to doublets in the undecoupled spectrum and are therefore –CH– groups. The last two signals belong to either quaternary carbons or carbonyls, because they are observed as singlets in the undecoupled spectrum. If we compare the number of signals in the decoupled spectrum with the number of carbons in the molecular formula, we find that there are two less signals in the spectrum. This suggests that the molecule contains an element of symmetry.

Next, we find the relative intensity values of the individual signals from the ^{1}H NMR spectrum. In the spectrum, the area sizes of the individual integrated regions are given below the x axis. Supposing that the triplet with the chemical shift of 0.81 ppm corresponds to the $–CH_3$ group (i.e., three protons), a simple calculation from the area sizes listed below the individual signals and rounding integral values to whole number can provide a ratio (from left to right again) of 3:3:2:1:1:2:2.

Note: If the division does not provide numbers, that can be easily rounded, the signal of some other group should be used for this calculation. Particular attention should be paid to the integral intensity of broad signals, which may be distorted by error, and it is not correct to relate the other intensities to this one. Usually, these are signals of protons on heteroatoms (O, S, N), which may contain a signal of water corresponding to the moisture content in the sample. In addition, the chemical shift of these signals is highly dependent on temperature.

Now we try to assign groups or the individual groups to the individual signals in the ^{1}H NMR spectrum. From **Table I** of the chemical shifts results that the first two signals with relative intensity 3 probably correspond to two non-equivalent $–CH_3$ groups (due to differences in multiplicity and chemical shift) which are bound to the carbon chain. A signal of relative intensity 2 with chemical shift 1.57 ppm could correspond to the $–CH_2–$, but also to the $–NH_2$. As a result of quadrupole moment of the isotope ^{14}N, the signals of protons in the –NH– and $–NH_2$- are broad and do not show fine structure. The $–CH_2–$ group is also already confirmed from ^{13}C NMR spectrum. The signal with a chemical shift of 2.55 ppm fits the –C–CH–Ar group according to **Table I**. The signal with a relative intensity 1 and chemical shift of 4.77 ppm probably corresponds to the –OH or –NH– group. Considering the results of the elemental analysis, the –NH– group can be excluded. The last two signals with relative intensity 2 occur in the aromatic proton region. The signals of conjugated alkenes also occur in this region. However, in this case these can be excluded considering the multiplicity of signals. In contrast, the multiplicity is characteristic for *para*-disubstituted aromatic core. The aromatic system also corresponds to $UN = 4$.

The presence of signals in the region around 120 ppm in ^{13}C NMR spectra also proves the presence of an aromatic system (**Table II**). Since this is a *para*-disubstituted aromatic core, only two signals are present and correspond to a pair of equivalent –CH= carbon atoms of the aromatic ring. The last two signals with chemical shift 140.83 and 153.73 ppm correspond to quaternary carbon atoms of the aromatic ring. The presence of carbonyl can now be excluded due to the unsaturation number.

Thus, we can summarize the partial results: the organic compound contains two non-equivalent $–CH_3$ groups, $–CH_2–$, –CH– and –OH group and *para*-disubstituted aromatic ring. A total of 7 possible structures of the unknown molecule can be proposed (**Fig. 12**).

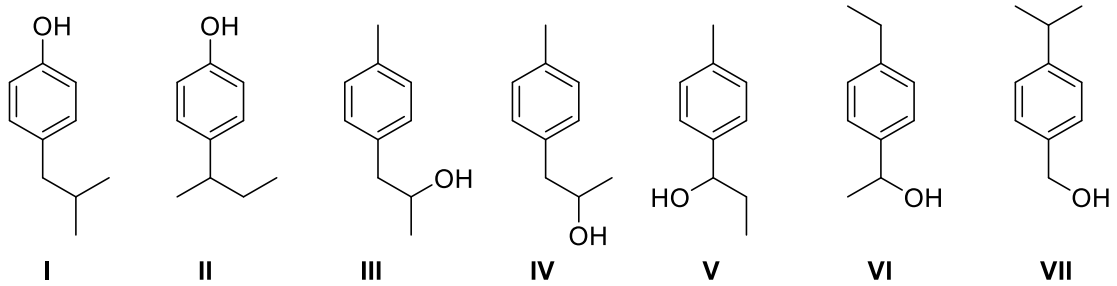


Fig. 12: Proposed structures of an unknown substance.

Now we use the multiplicity of the signals in the ^{1}H NMR spectrum to select a single correct structure. Let us try to propose theoretical spectra for each structure. Assume that these are the 1st-order spectra.

Structures **I** and **VII** contain –CH– $(CH_3)_2$ group, where there are two equivalent $–CH_3$ groups. These would give a single signal, a doublet (interaction with the neighbouring –CH– group) with a relative intensity 6. There is no such signal in our spectrum, so we can exclude these two structures.

Structure **IV** contains an ethyl group attached directly to the aromatic core. This group would give two signals: triplet and quartet in 3:2 ratio. Since this pair of signals is also not present in the spectrum, we can exclude structure **IV**.

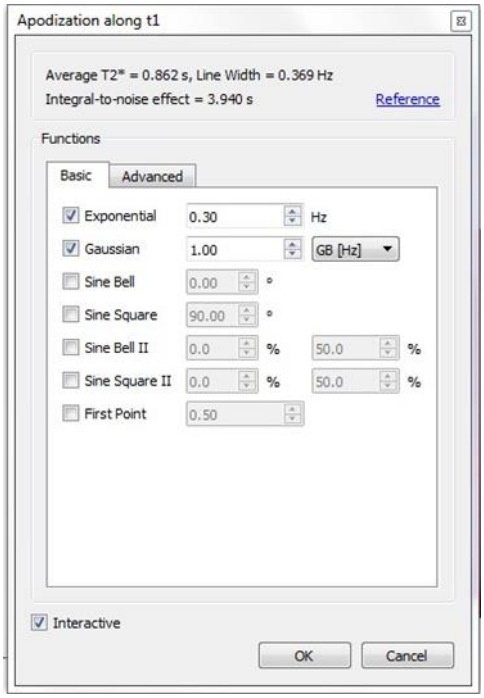
In structures **III**, **IV** and **V** there is a methyl group bound to the aromatic core, which would give a singlet with chemical shift in range 2-3 ppm and relative intensity 3. Again, this signal is not present in the spectrum, so we can also exclude these three structures. Thus, structure **II** remains.

Now we try to propose the multiplicity of individual signals for structure **II** in the ^{1}H NMR spectrum. The $–CH_3$ group next to the $–CH_2–$ group must provide a triplet. The other $–CH_3$ group bound to the –CH– group must provide a doublet. The $–CH_2–$ group which is between the –CH– and $–CH_3$ has a total of 4 protons in its immediate neighbourhood and according to the $n + 1$ rule should give a quintet. Finally, the –CH– group has a total of five protons in the immediate neighbourhood and by the same rule should give a sextet. All four signals with the corresponding relative intensities and multiplicities can be found in the spectrum. Thus, ^{1}H NMR (**Fig. 10 a,b**) and ^{13}C NMR (**Fig. 11**) spectra belong to ***p*-(2-butyl)phenol** (structure **II**).

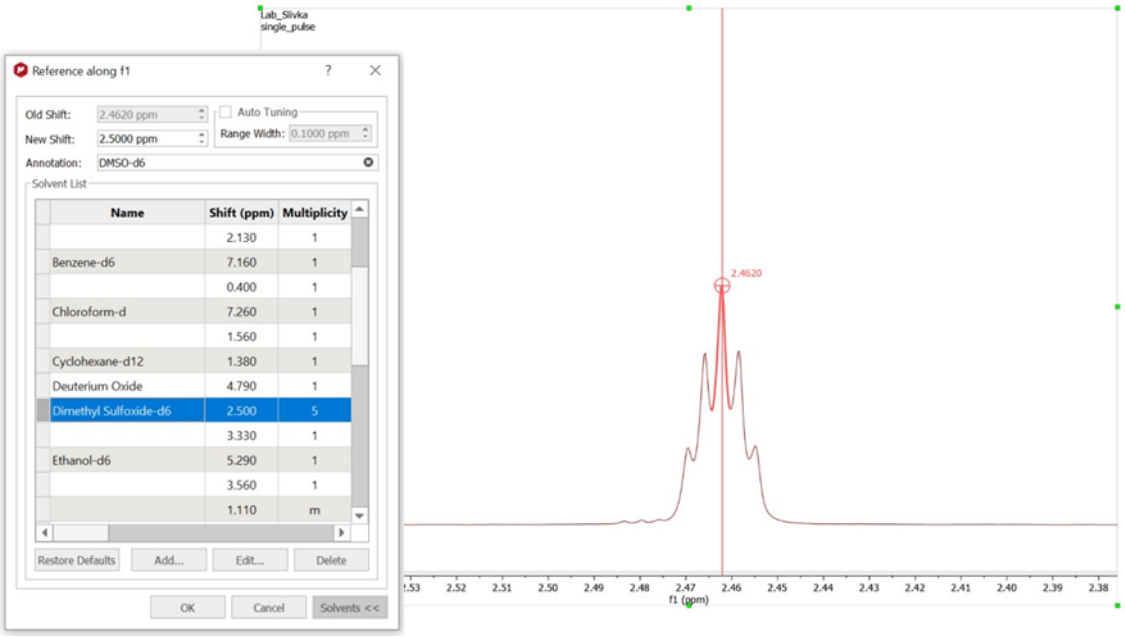
MestreNova (MNova) Software

Procedure for evaluation of measured 1D NMR spectra in Mnova software:

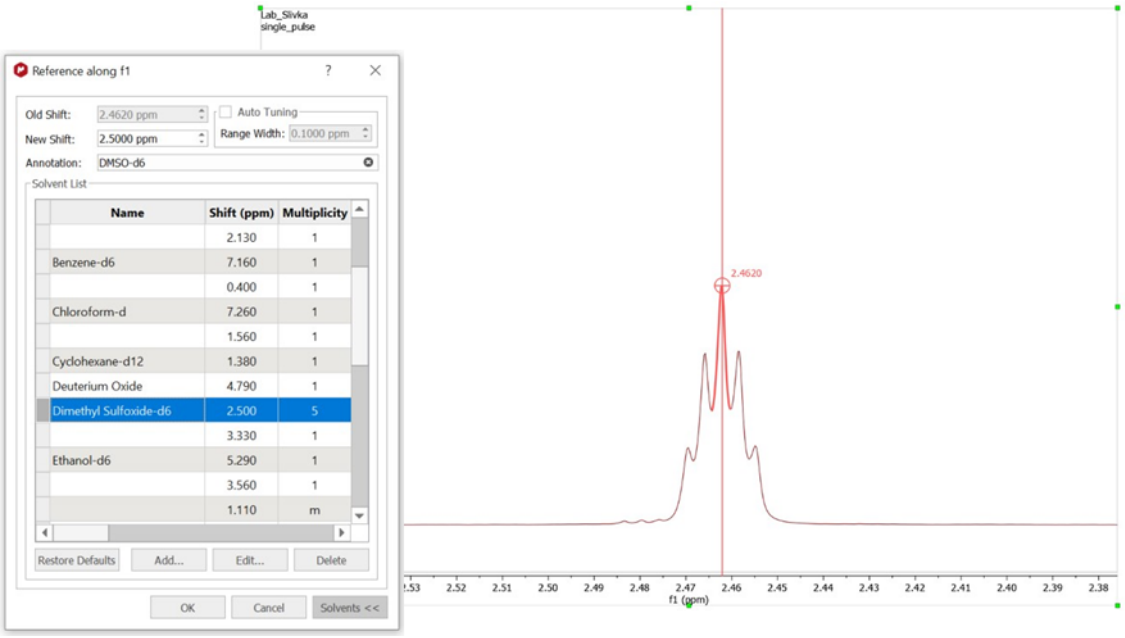
1. Open the measured NMR spectra in ***Mnova*** (or you can drag them into the open software).
2. Increasing/decreasing the intensity of signals in measured spectra using the mouse wheel.
3. Adjust the spectrum using the window function "***W***" – setting the ***Exponential*** to 0.3 and ***Gaussian*** to 1.0 (or similar values depending on the resolution and quality of the measure spectra).



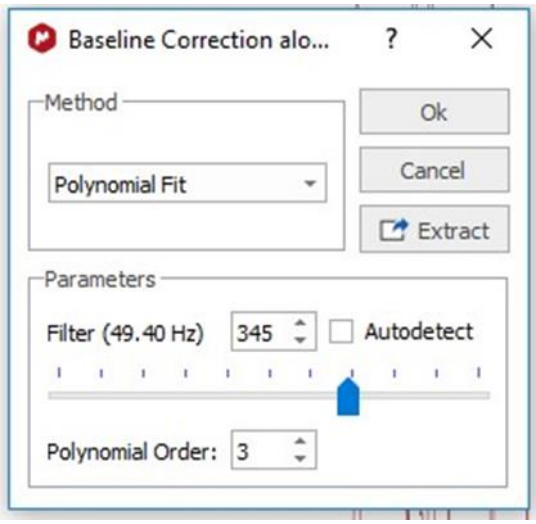
4. Solvent signal reference: "***L***" – select the signal of dimethyl sulfoxide (DMSO, the selected signal is highlighted in red) and click on it. A table appears, click the "***Solvents***" button and select the solvent and the chemical shift of its signal.



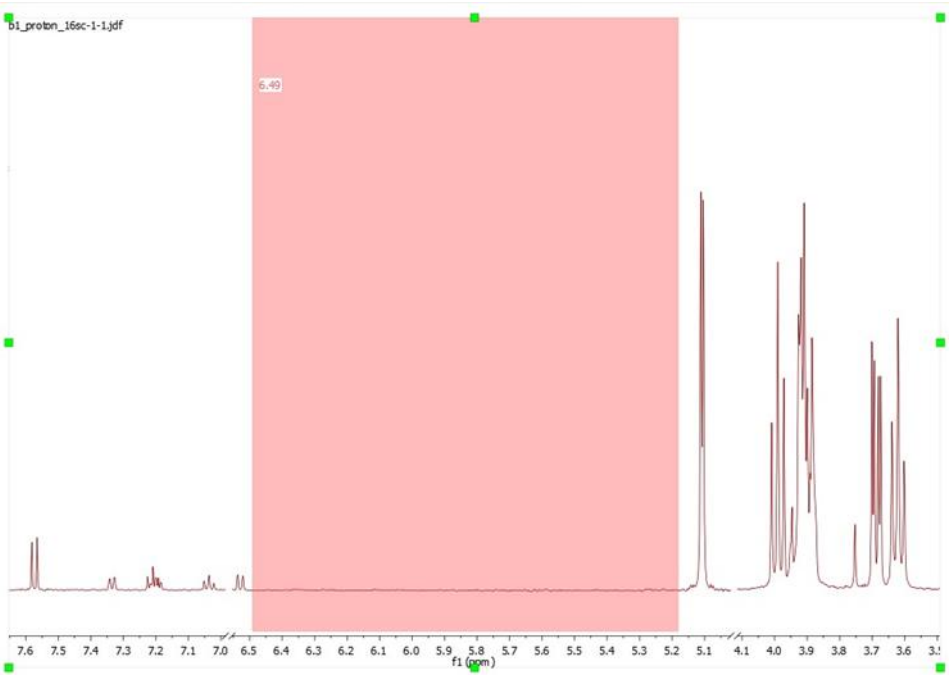
5. Spectrum phasing: "***Shift+P***" – table and pivot will appear. Move the pivot to the centre of one of the outermost signals. Phase this signal so that its baseline is in the plane. Phasing procedure: in the pop-up table, left-click in the purple box and drag the mouse to one side or the other. When the signal below the pivot is aligned, phase the other side of the spectrum by right-clicking in the purple box and dragging to either side. For a finer phasing step, hold down "***Ctrl***" along with the mouse button.



6. Baseline correction: "***B***" – select the ***Polynomial Fit*** method in the pop-up window. If you need to fine-tune the baseline accuracy, you can change the ***Filter*** value or the ***Polynomial Order*** value in the ***Parameters*** window (the ***Autodetect*** box must be unchecked).

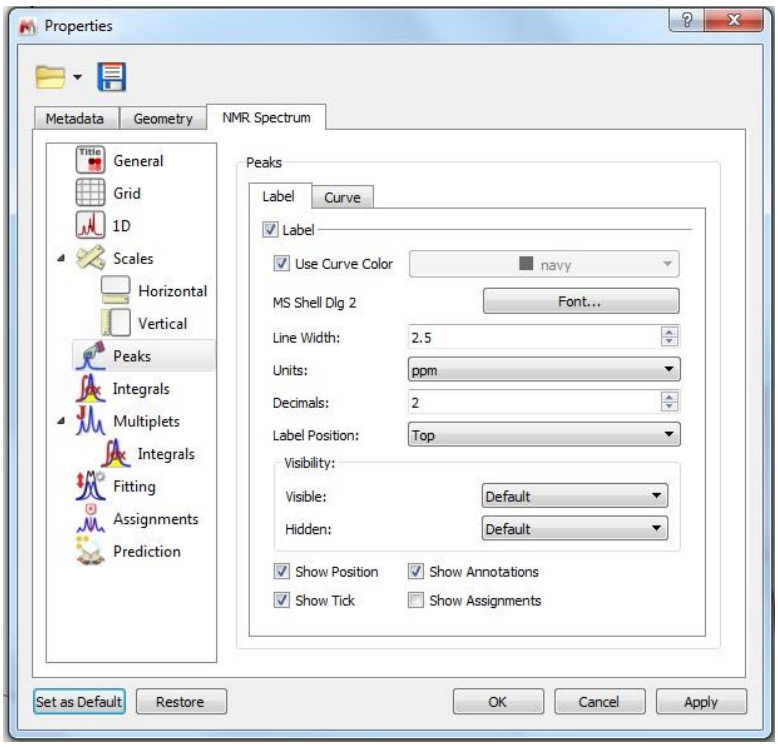


7. Peak area integration: "***I***" – use the mouse to select the entire peak area from its start to the end. The first integral area of the peak has normalized value of 1, all other integral area values are proportional to the first area. The normalized integral value of the peak areas can then be edited in the "***Integral Manager***" panel, which can be displayed via "***View***" – "***Tables***" – and select the "***Integral Manager***" checkbox. You can set the number of displayed decimals by right-clicking on the spectrum – "***Properties***" – "***Integrals***".
8. Cropping (removing) the part of the spectrum without signals: "***X***" – use the mouse to select the area to be cut from the spectrum. You will find it easier to work with the spectrum. If you want to view the cut out areas of the spectrum, press "***V***" – to view the area, select the area of the spectrum with the mouse. Or click on "***View***" – "***Cuts***" – "***Restore All***" to see the original spectrum.



9. Peak picking: "***Ctrl+K***" – use this tool to select the signals for which you want to know the chemical shift value. In this mode, the cursor selects only the peaks of well-separated signals. For finer manipulation press "***Shift***" – in this mode you can mark any point of the spectrum.

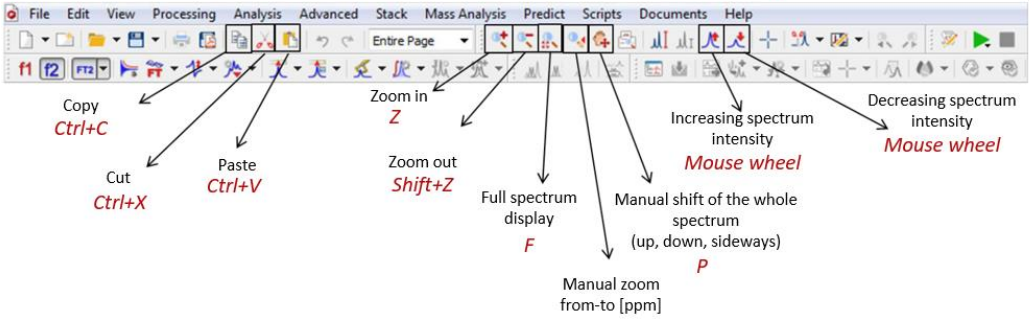
10. <u>Setting the display of chemical shift values:</u> right-click on the spectrum – select "***Properties***" – "***Peaks***" – here you can switch between displaying in *Hertz* and *ppm* units and also change the number of displayed decimals.

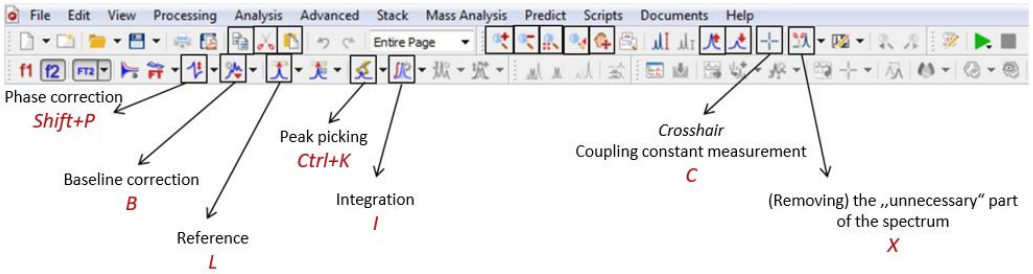


11. <u>Zoom in on the spectrum:</u> "***Z***" and select the area with the mouse
12. Full spectrum display: "***F***"
13. You can exit any function by pressing "***Esc***"

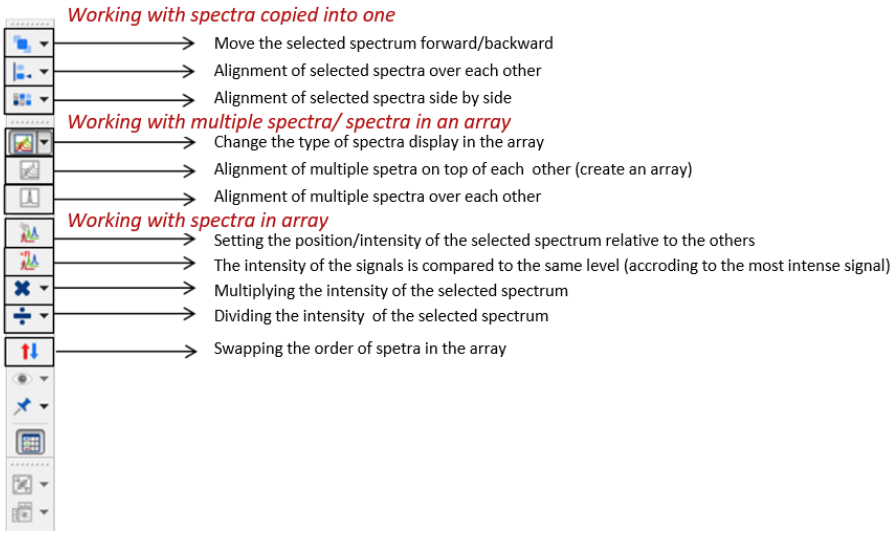
Classic toolbar display in Mnova

Description of the most commonly used tools in Mnova and their *keyboard shortcuts*





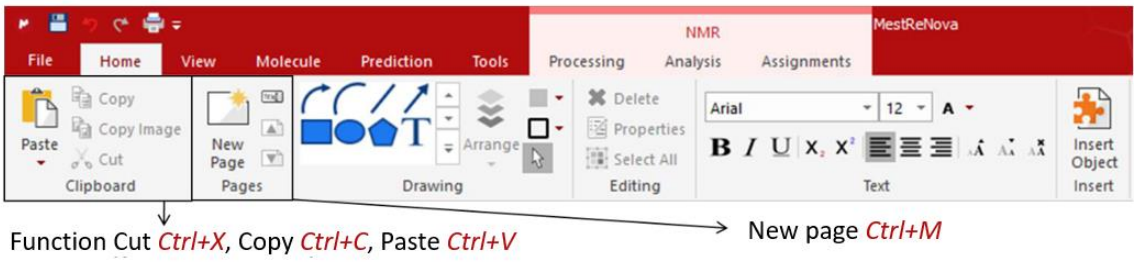
Description of the most commonly used tools in Mnova



Modern toolbar display in Mnova

The most commonly used tools and their *keyboard shortcuts*

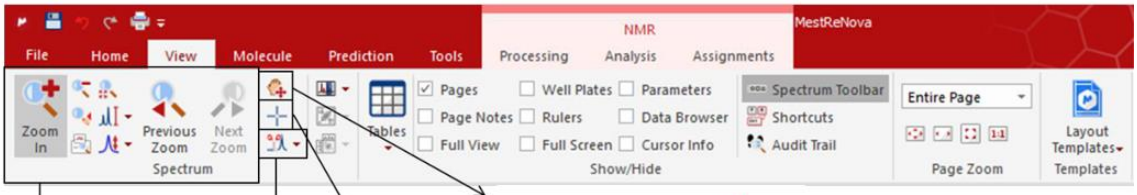
Home tab



Function Cut *Ctrl+X*, Copy *Ctrl+C*, Paste *Ctrl+V*

New page *Ctrl+M*

View tab



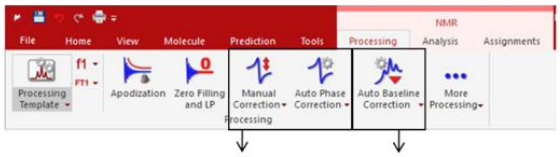
Full spectrum shift *P*

Crosshair (chemical shift indicator according to mouse position) *C*

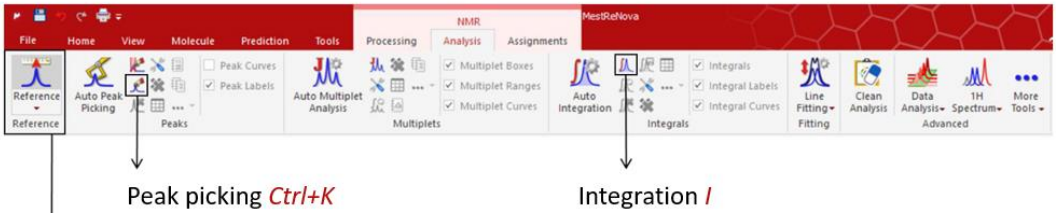
Spectrum cut-out *X*

Function Zoom in *Z*, Zoom out *Shift+Z*

Processing tab

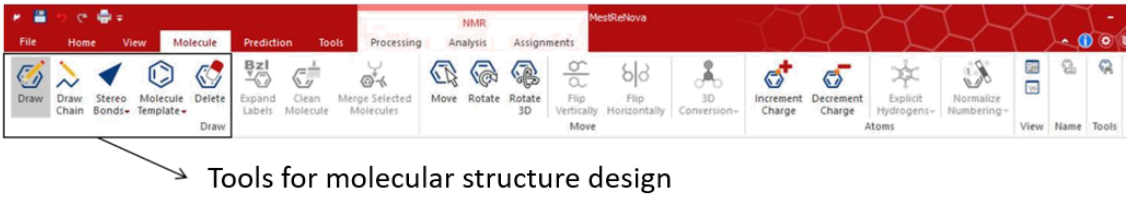


Analysis tab

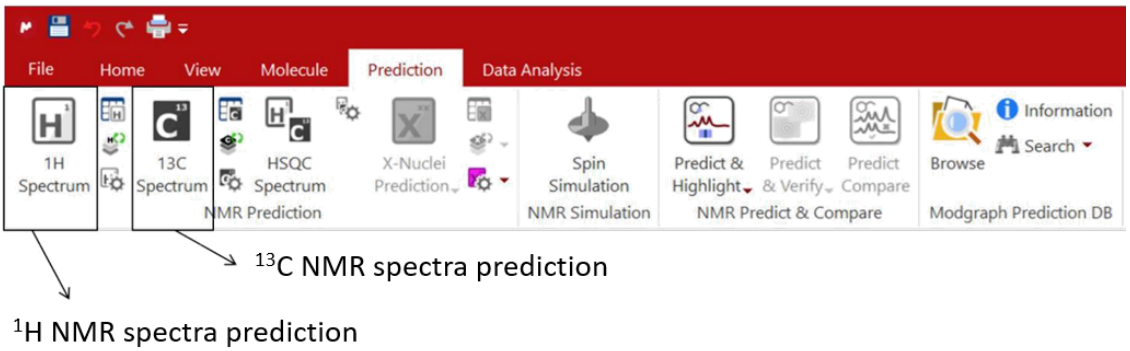


Prediction of NMR spectra of molecules

Molecule tab



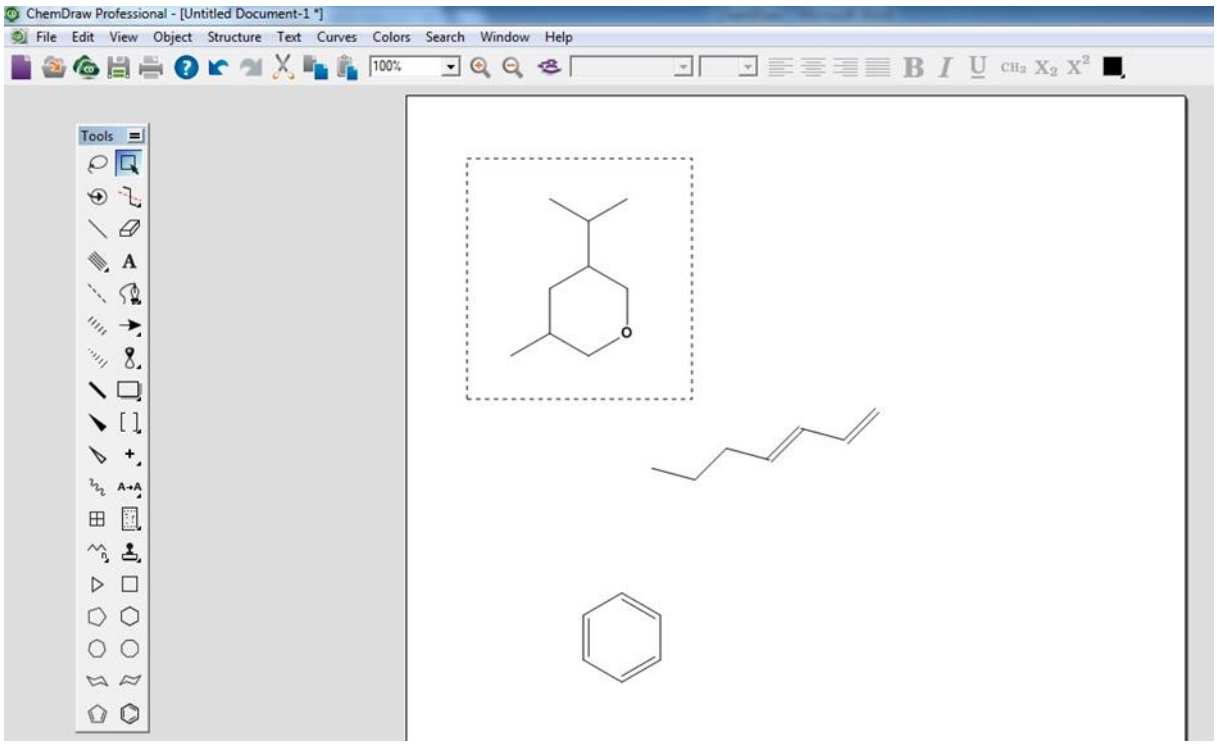
Prediction tab



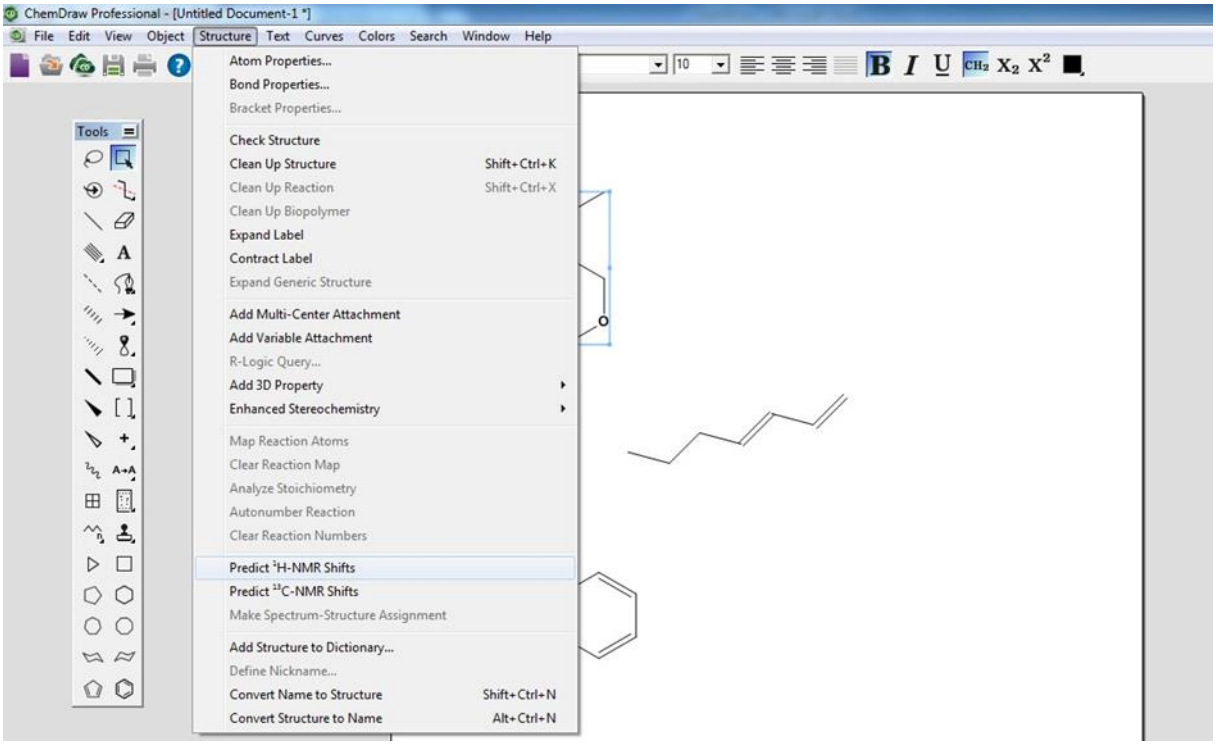
ChemDraw Software

This software is used for drawing structural formulas. Creating formulas in this software is very intuitive. In the ***Tools*** window, the desired cycle or bond can be selected and the mouse is used to create the structure. You can link the bond/cycle to any carbon or if you click on the middle of the bond you create multiple bond/multiple cyclic substances. Any carbon in the created structure can be replaced by any element. In the ***Tools*** window, select the symbol **A** (text) and select the carbon with the mouse, instead of which we enter the tag of the desired element.

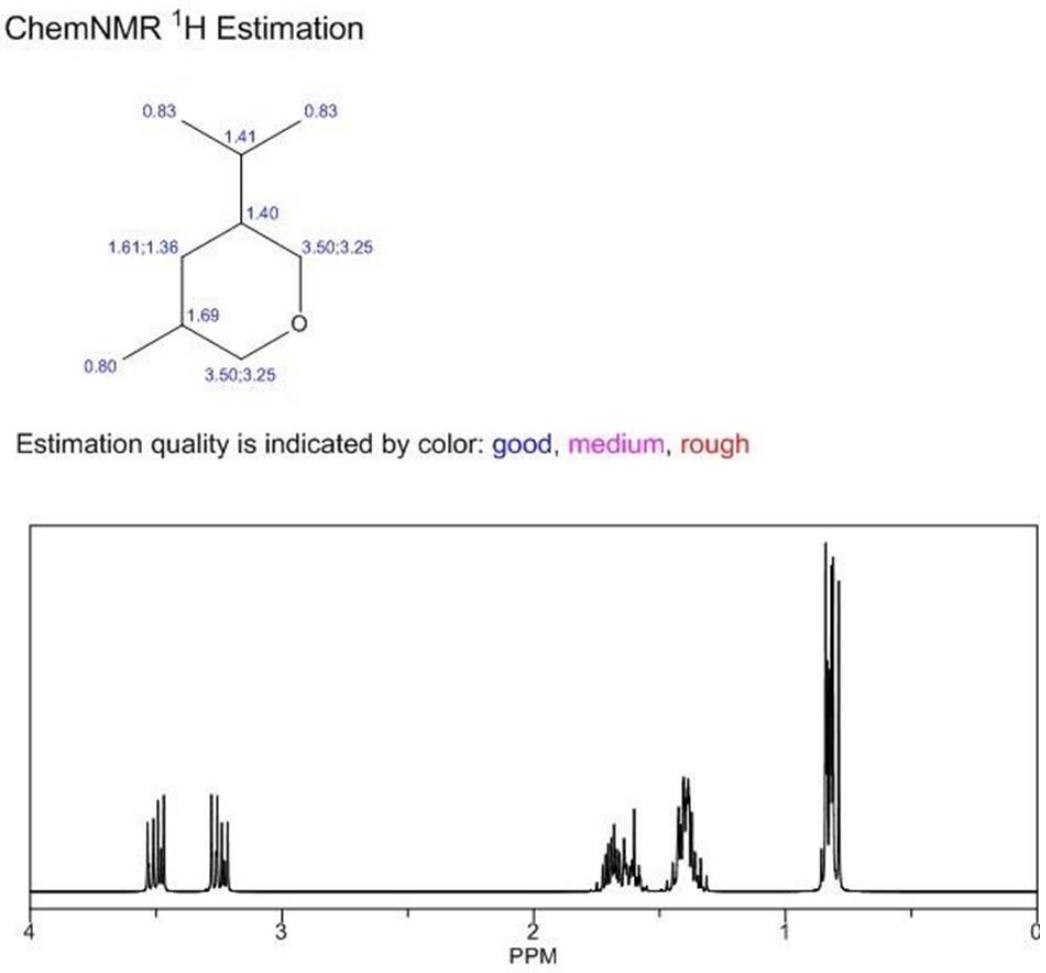
The advantage of this software is that it can predict 1D NMR spectra of both hydrogen and carbon. For prediction you need to select the desired structure (***Tools Marquee*** window and select the structure with mouse).



On the ***Structure*** tab, you can select ***Predict ^{1}H-NMR Shift*** or ***Predict ^{13}C-NMR Shift***.

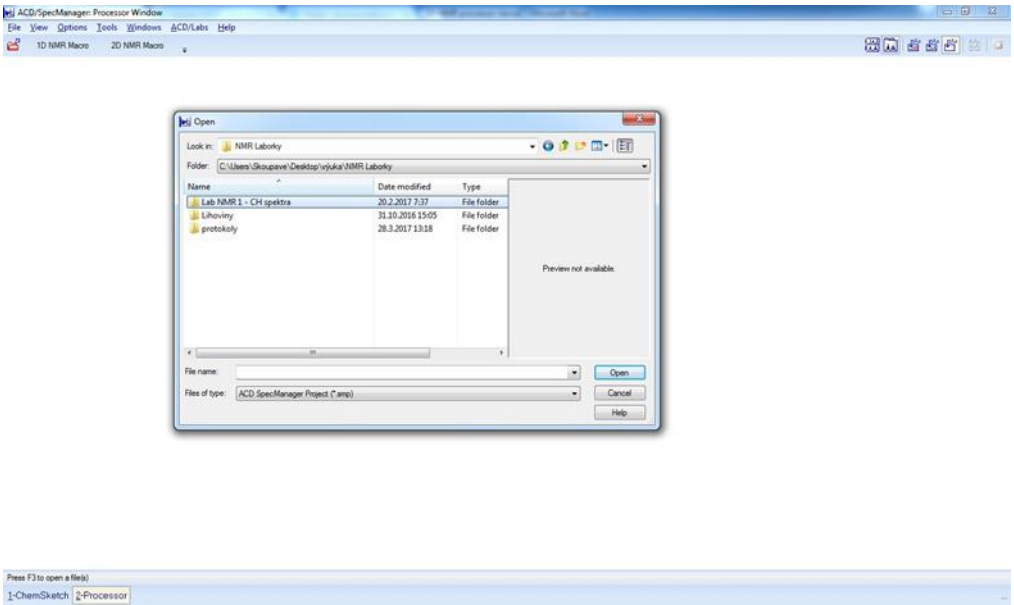


The structure with chemical shifts and the predicted spectrum is displayed. Below the spectrum there is a table with the chemical shifts of each group and comments where applicable. The structure with predicted chemical shifts and the predicted spectrum can be saved as individual images.



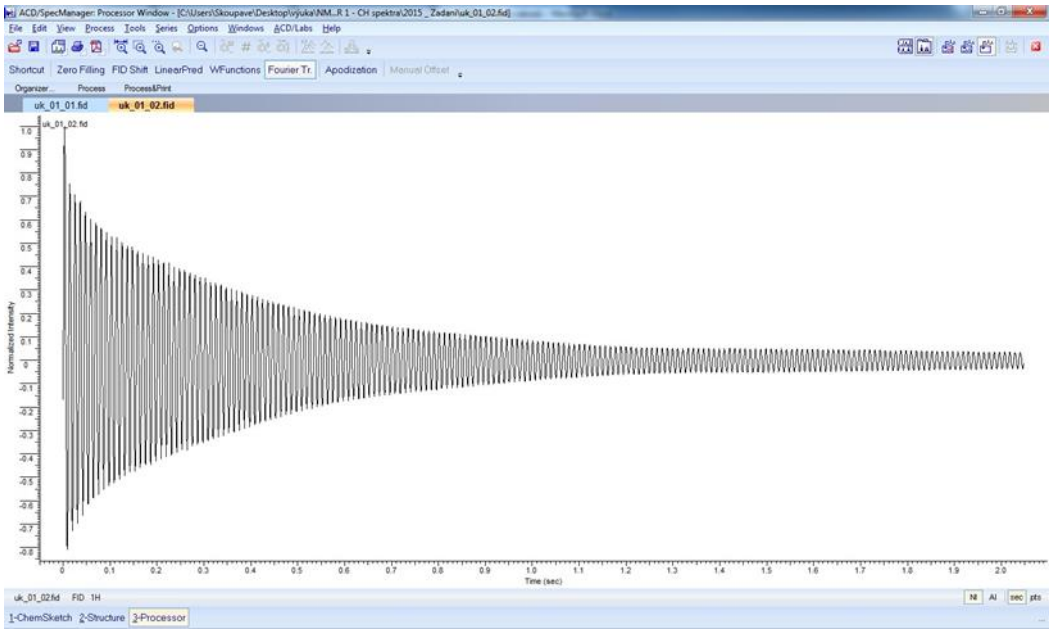
1D-NMR Processor Software

After starting the 1D-NMR processor program, select the tab ***file – open – 1 form 1D NMR directory*** and select the folder containing the files you want to open.

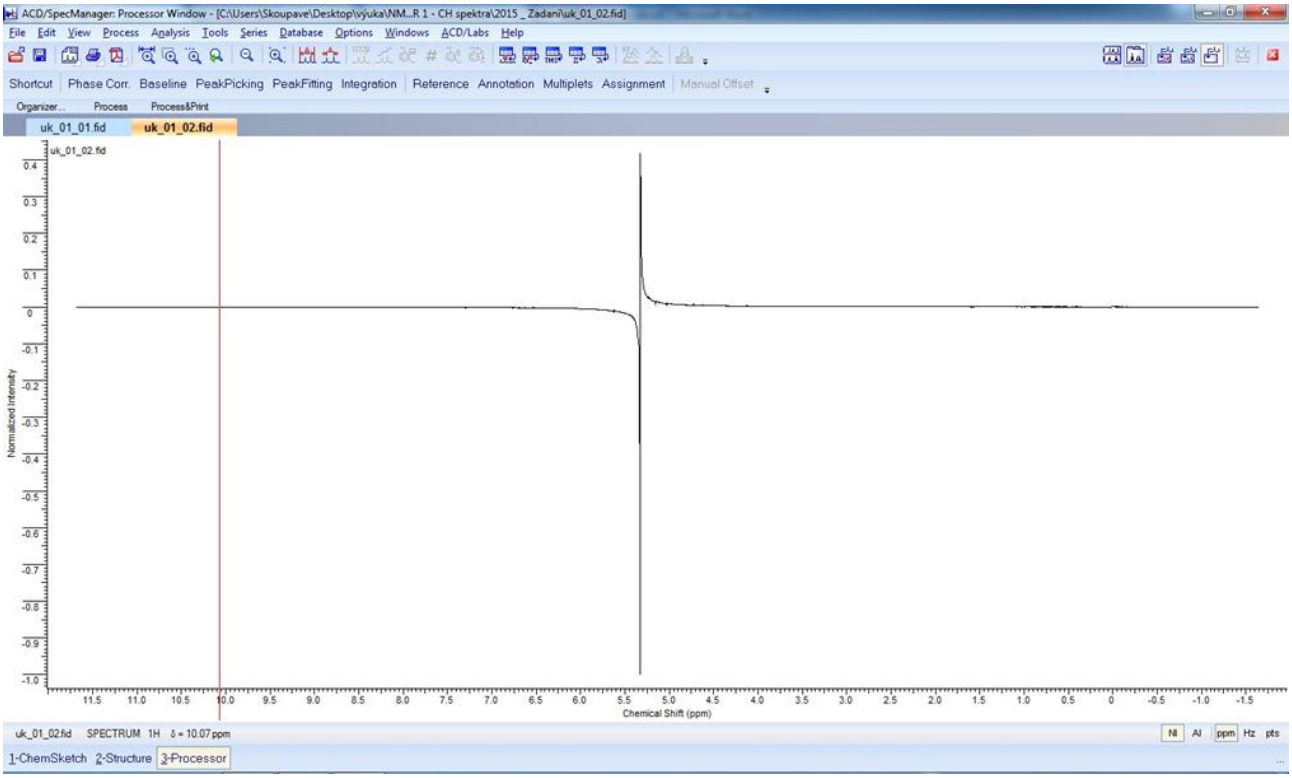


On the last line, it is necessary to select the file type ***any file*** in order to be able to open the given files. At this moment, it is possible to select one or (using the ***shift*** key) several files to open.

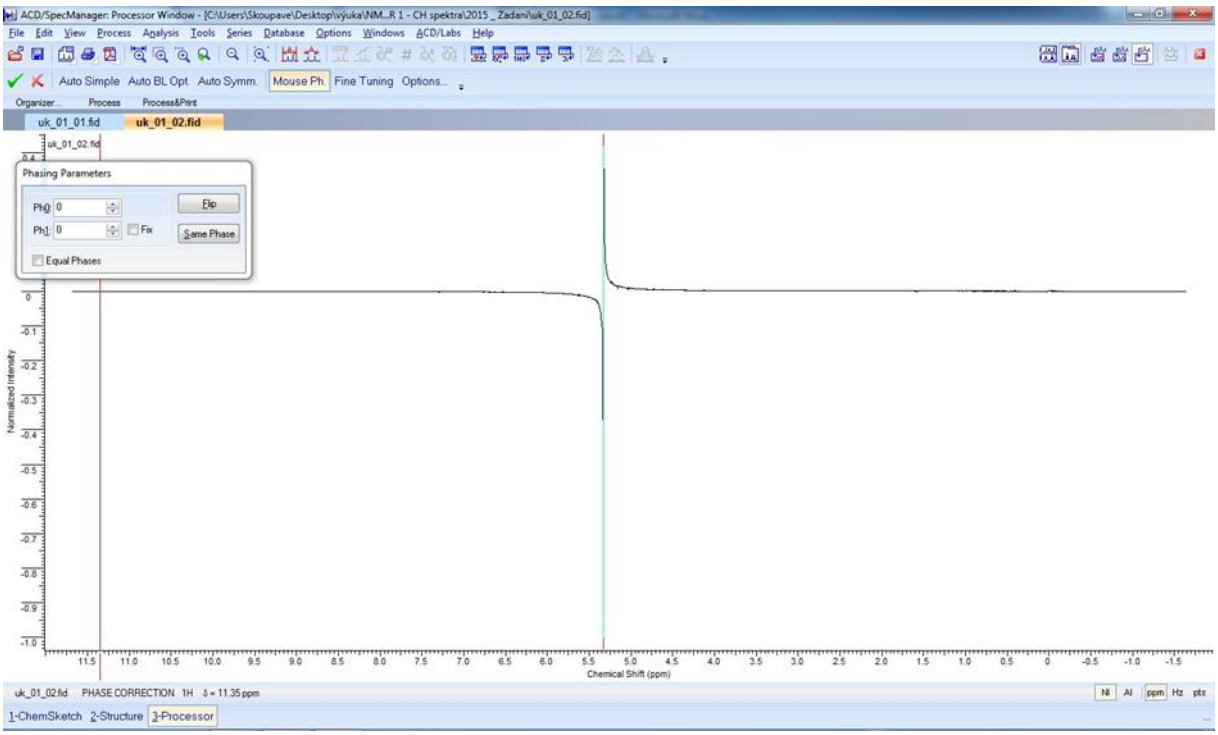
An interferogram is displayed, i.e. the dependence of the signal on time. The time record (FID) can be converted into a frequency record using the Fourier transform. In the case of NMR, the frequency record is expressed in terms of chemical shifts. We perform the conversion from the interferogram using the ***Fourier. Tr.*** button.



The result of the Fourier transform is a spectrum that needs to be phased.

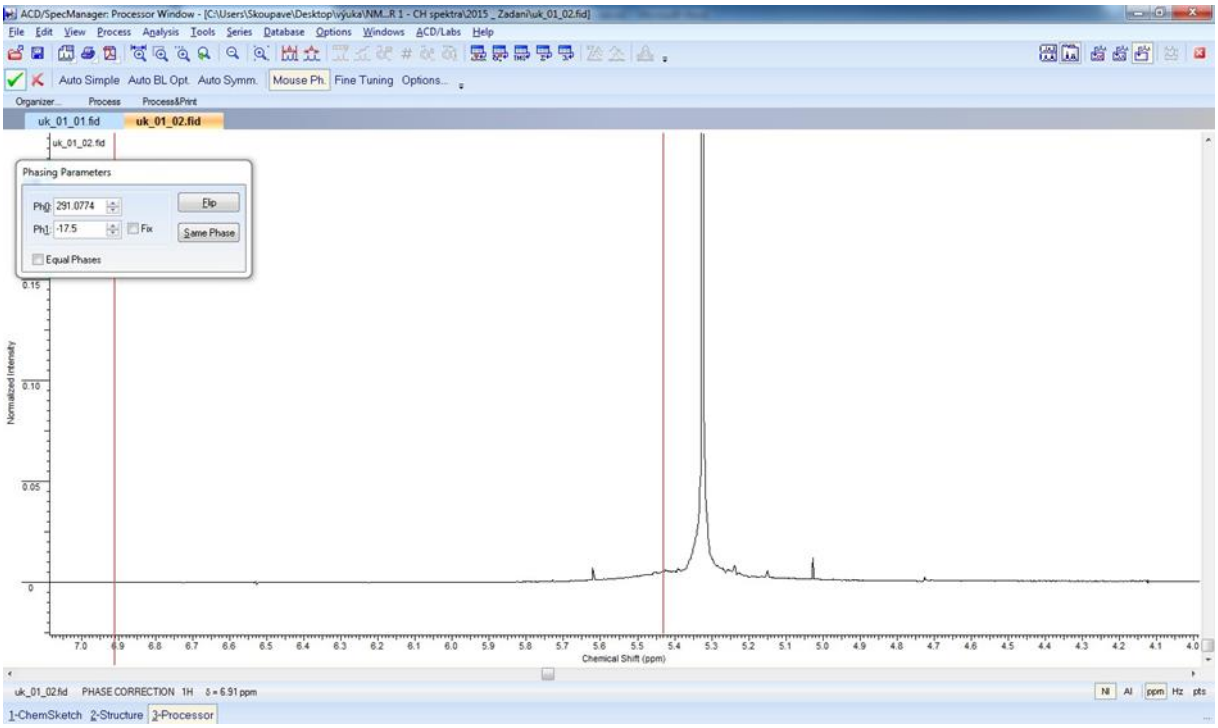


Any negative peaks must be avoided. The ***Phase Corr.*** button is used for this purpose. Clicking on this button will bring up a table, but phasing by entering numbers into it is relatively tedious. It is possible to use the auto simple button, but it does not give satisfactory results for all spectra. Mouse phasing appears practical. So we click on the ***Mouse Ph.*** button.



If the program does not automatically place the lead of the mouse, we will place it on one of the outermost peaks (this location can be moved using the left mouse button). Hold down the left mouse button and move the mouse left or right as needed. Till achieving the best result.

Further improvement can be achieved using the right mouse button. Hold the right button and move the mouse up or down. By combining these two methods, we try to achieve the best possible result. When we are satisfied with the result, it is necessary to accept the changes using the "green check mark" in the upper left corner. Otherwise, we can simply cancel all modifications with a red cross.



Integration: If necessary, before the actual integration, we first correct the baseline (***baseline*** button). With the baseline aligned, we can start integrating. We select the ***integration*** button and choose ***manual integration***. Using the mouse, click on the beginning of the band (it is recommended to zoom in on the given band) and drag the mouse with the left button pressed to the end of the band. The value of the integral will be displayed below this band. If we need to increase the number of decimal places, we select the ***options*** tab, where we select ***preferences***. In the window that pops up, select the ***integrals*** tab and increase the number of decimal places.

Spectool Software

Spectool is a software that contains data, spectra, and mathematical apparatus for interpreting molecular spectra. Information from mass spectroscopy (MS), ^{1}H and ^{13}C NMR spectroscopy (HNMR, CNMR), infrared spectroscopy (IR) and spectroscopy in the ultraviolet and visible region (UV/VIS) is covered. Since this tutorial will be used in NMR laboratory work, the use of this software will be explained particularly for this task.

After starting the software by clicking the icon labelled ***Spectool***, four windows labelled ***Manager***, ***Go***, ***Swt*** and ***Grp*** will open.

Manager with the subtitle ***Top Page*** (Fig. 13): This window is used to select spectral method and additional information, which are labelled as ***Data*** (tabulated values), ***Tools*** (where you can select tools e.g. for calculating chemical shifts, spin-spin coupling constants etc.), ***Ranges*** (ranges of chemical shifts), ***Spectra*** (spectra of some compounds) and ***SpecLib*** (spectra of different types of compounds).

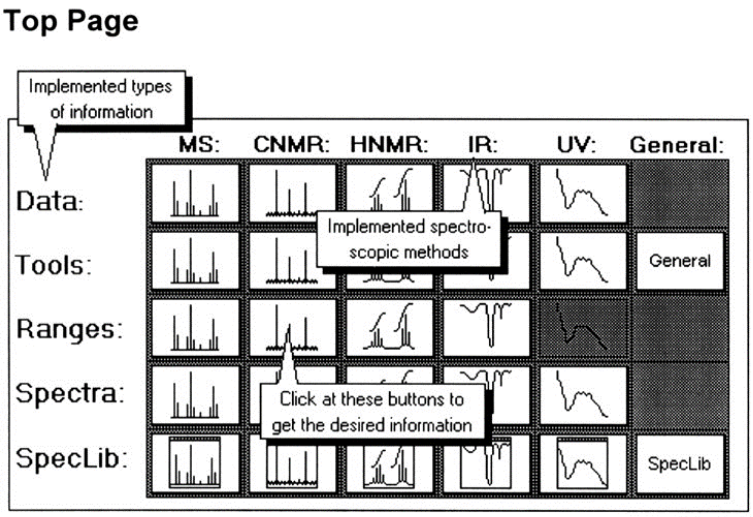


Fig. 13: ***Top Page*** window.

By selecting the type of information and the appropriate spectral method, we can progressively get the information about a specific molecule (**Fig. 14**). To go back can be accessed by using the ***Go*** button (**Fig. 15**) – by using the buttons marked as arrows. The ***Top*** button is always used to get to the starting position. ***Swt*** window (Switch – **Fig. 16**) and ***Grp*** (Group) are used to change the group or to select a different structural group, respectively.

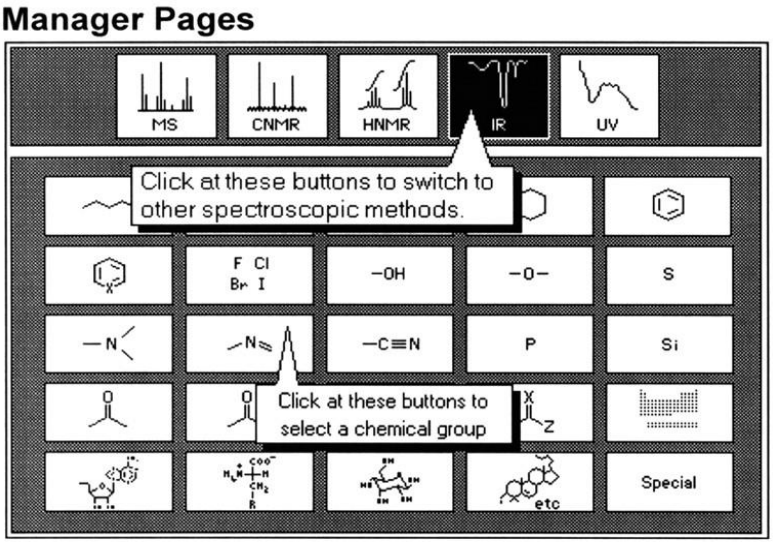


Fig. 14: ***Manager*** window.

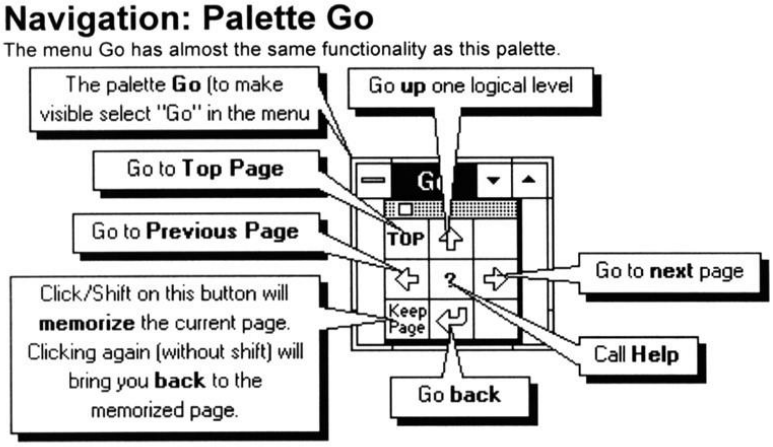


Fig. 15: ***Go*** window.

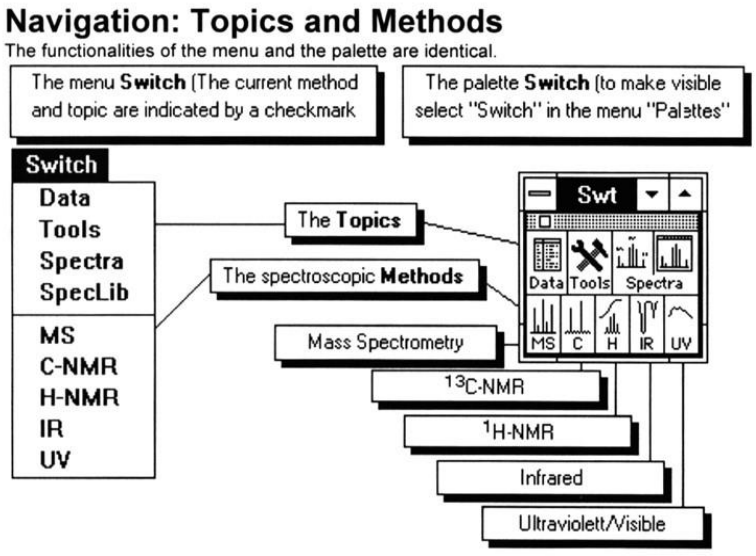


Fig. 16: ***Swt*** window.

Example of determination of ^{1}H NMR chemical shifts of toluene

In the ***Manager*** window, select ***Data HNMR***. In the new window select aromatic compounds (shown as benzene molecule), then select ***Monosubstituted Benzenes*** and in the displayed table you can read the chemical shifts of aromatic protons for different substituents, i.e. also for the $-CH_3$ group. If we click on the right arrow below the table three times, the chemical shifts of some substituted benzenes will display, with toluene at the first place.

There is another option that is suitable for more complex molecules but let us stick with toluene. Using the ***Top*** button in the ***Go*** window we will get to the starting position. Select ***Tools HNMR*** and in the next window choose ***^{1}H Shift Estimation*** and then choose ***Draw***. This will launch the ***ChemWindow*** software to draw the molecule (click on the aromatic ring and click again to place the aromatic ring on the drawing area. Click on the single bond and then create a methyl group by dragging from the selected carbon atom of the aromatic ring with the left button of the mouse). Thus, the toluene molecule is drawn. Now click on the arrow in the ***ChemWindow*** toolbar and mark the molecule by dragging around the drawn molecule with the left button of the mouse. Next you need to place it in the clipboard with ***Ctrl + C*** and click the ***Estimate*** button in the window where the ***Draw*** button was clicked. This will calculate chemical shifts and the drawn molecule will appear in a new window with the calculated chemical shifts. A similar procedure can be used to obtain the ^{13}C NMR chemical shifts.

Table I

Chemical shifts of protons ^{1}H (values are relative to TMS; concentration or temperature dependent shifts are marked with a cross).

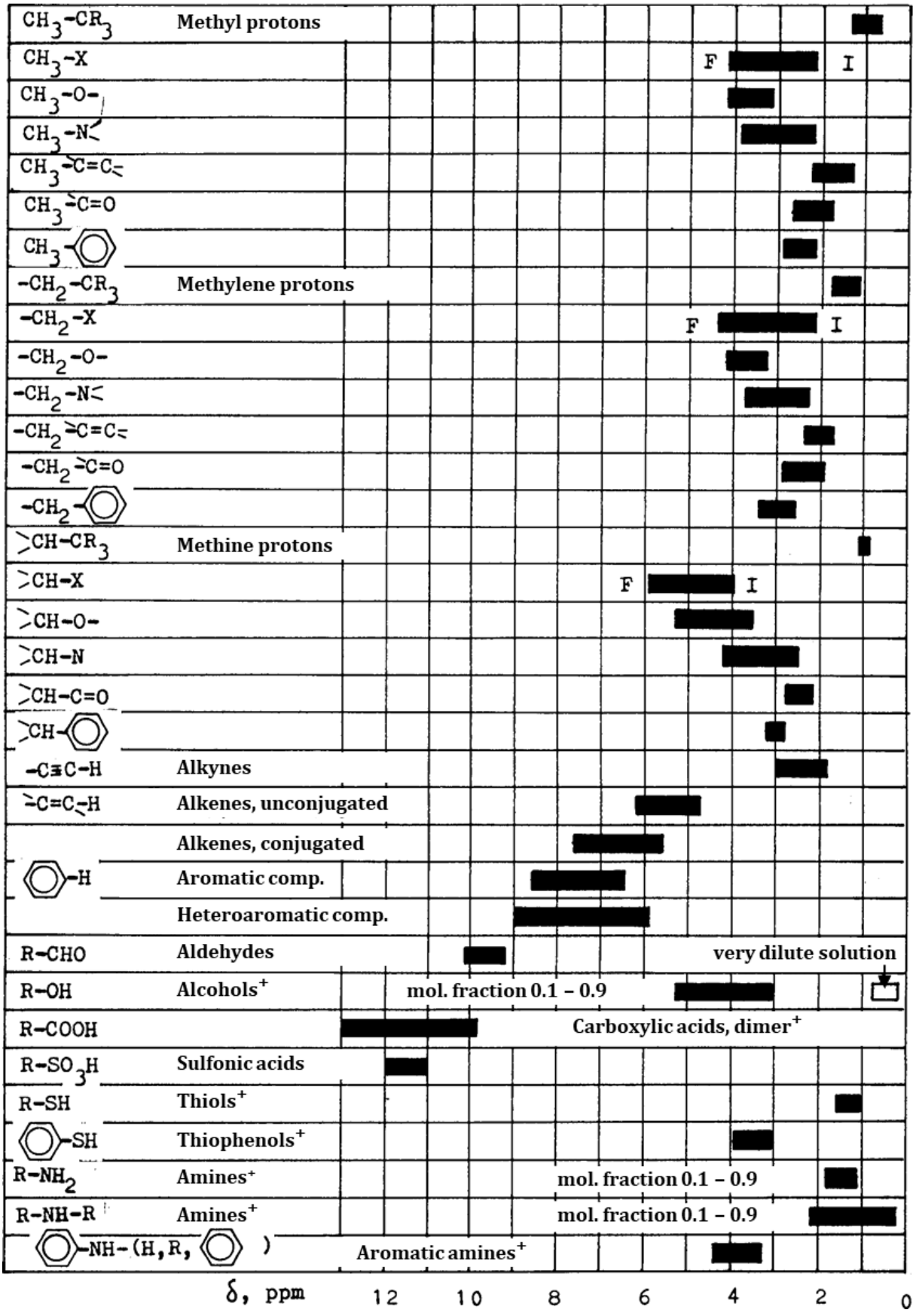


Table II

Chemical shifts of carbons ^{13}C (values are relative to TMS).