JOINT INSTITUTE FOR NUCLEAR RESEARCH
Frank Laboratory of Neutron Physics

FINAL REPORT ON THE START PROGRAMME

Characterization of ibuprofen by X-ray diffraction and spectroscopy methods

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Abstract

The primary objective of this study is to examine the characteristics of the pharmaceutical substance known as ibuprofen. Investigating medications is crucial for advancing and enhancing healthcare practices. The aim of the research is to verify the purity of the ibuprofen sample obtained from a pharmacy and to analyze it in comparison with information presented in relevant literature. Diverse research methodologies are employed in the study of ibuprofen.

During the START student program in JINR main features were mastered for the following experimental methods: differential scanning calorimetry (DSC), X-Ray powder diffractometry (XRPD), FTIR spectroscopy. The report provides an introductory description of these methods, as well as the obtained experimental results for investigated sample of ibuprofen. Using the powder X-ray diffraction technique, it was proven that the ibuprofen sample under investigation exists in a crystalline form. The vibrational frequencies obtained through experimental measurements closely matched the values calculated using the Density Functional Theory (DFT) approximation, demonstrating a high degree of agreement. Linear correlations were found between the experimental vibrational frequencies and those calculated using the DFT method.

1. Introduction

As nonsteroidal anti-inflammatory drugs (NSAIDs) prevail as pain relievers, they have become highly popular drugs in the pharmaceutical market. From a structural viewpoint, NSAIDs skeletons are usually classified as being an aromatic acetic acid or propionic acid skeleton that contains an additional chiral center. Among the aromatic propionic acid skeletons, ibuprofen is the most widely used. Considering that ibuprofen can be purchased without a prescription, it is believed that their medicinal benefits have significantly improved patients' lives. An important role for the assimilation of medicines is played by the acidity (or pH) of the medium into which the drug enters. Depending on the acidity of the medium, the mechanism of interaction of drugs with cell receptors may change, which greatly affects the effectiveness of drugs (exposure time, excretion from the body). There is quite a lot of information in the literature devoted to the study of this kind of medicines, but we have not found articles concerning the consideration of the influence of the acidity of the medium on the properties of medicines. It should be noted that when the acidity changes, the shape of the molecules also changes, taking different forms from neutral and protonated too anionic. For a comprehensive study of the effects of the acidity of the medium on drugs, it is necessary to conduct both an experiment (X-ray powder diffraction, IR spectra, Raman spectra, DSC method) and computer modeling of the obtained spectra. At the first stage, a detailed characterization of the test sample by available experimental methods is required.

Purpose of the study is the complex investigation of ibuprofen by experimental and quantum-chemical methods in order to demonstrate the sample quality and to choose the characteristic parameters that can be used for the following investigations.

Object of research is an ibuprofen sample. Subject of research is key structural parameters that characterize the ibuprofen properties in the condensed matter.

The main tasks of the study are:

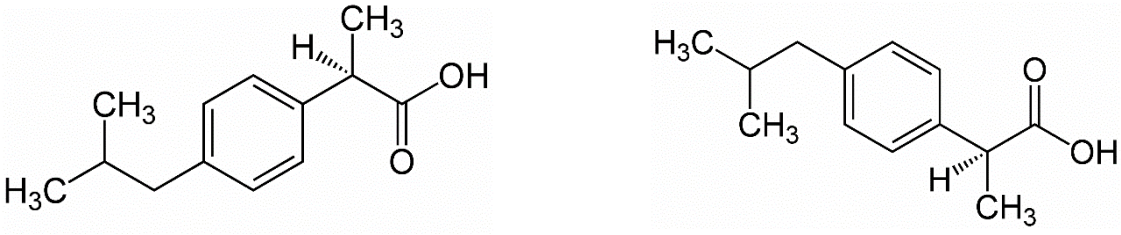
- characterization of ibuprofen by DSC method;
- determination of the main characteristics of ibuprofen by powder X-ray diffraction;
- investigation of ibuprofen sample by FTIR spectroscopy;
- *in silico* calculations of vibrational frequencies of ibuprofen enantiomers by semiempirical quntum-chemical and DFT methods
- joint analysis of the results of experimental studies and molecular modeling.

2. Experimental

2.1. Materials and samples

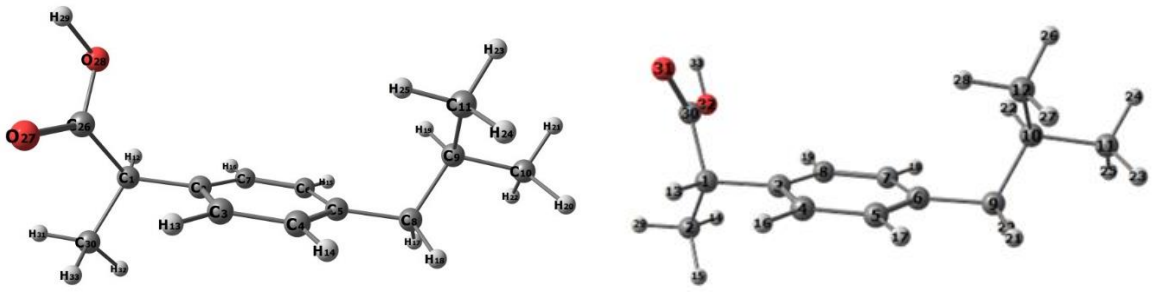
Ibuprofen has a molecular formula $C_{13}H_{18}O_2$ and a chemical name 2-[4-(2-methylpropyl)phenyl] propanoic acid [1]. Its molecular weight is 206.28 g/mol. Ibuprofen in its structure is a stable solid crystalline colorless substance with a characteristic odor. The boiling point of ibuprofen is 157 °C, and the melting point is 76-77.5 °C. Solubility of ibuprofen in water is 21 mg/l (at 25 °C). It easily soluble in most organic solvents and highly soluble in alcohol [1].

Ibuprofen has two enantiomers S(+) and R(-) the chemical structure of which is shown in Figures 1 and 2 (visualization was performed by ChemCraft software [2]).



(a) (b)

Figure 1: 2D structure of S(+)-ibuprofen and R(-)-ibuprofen under letters (a) and (b) respectively



(a) (b)

Figure 2: 3D structure of S(+)-ibuprofen and R(-)-ibuprofen under the letters (a) and (b) respectively

2.2. Methods

2.2.1. X-Ray powder diffractometry (XRD)

X-ray diffraction (XRD) is a universal non-destructive analytical method for analyzing the properties of materials such as phase composition, structure, texture and more, solid samples or even liquid samples.

The XRD method is based on obtaining and subsequent analysis of the diffraction pattern resulting from the diffraction of X-rays scattered by electrons of atoms of the irradiated polycrystalline sample. Having calculated the obtained radiograph, information about the interplane distances in the crystal is obtained. The value of the interplane distances for each substance is strictly individual, so the radiograph uniquely characterizes the substance under study. As a result, the diffraction pattern is a kind of "fingerprint" of a chemical compound, according to which it is possible to determine which of the previously known compounds corresponds to the obtained radiographic [3].

X-ray diffraction analysis of the ibuprofen powder sample was done at room temperature using a type PAN Analythical Diffractometer (The Netherlands). The measurement condition is described as follow: Co metal target, Kα filter, voltage 40 kV, 40 mA current, the analysis performed within the 2θ˚ range 5-60°. The sample was placed on the sample holder and leveled to prevent particle disorientation during sample preparation. The cuvette was made of monocrystalline Si.

2.2.2. Differential Scanning Calorimetry (DSC)

The Differential Scanning Calorimetry (DSC) is main techniques of thermal analysis. DSC detects endothermic and exothermic transitions like the determination of transformation temperatures and enthalpy of solids and liquids as a function of temperature. A typical calorimeter is an isolated chamber where a sample is placed in a surrounding medium. Then the sample is heated with a definite amount of heat. The difference in temperature between sample and surrounding medium gives the heat capacity of the sample and information about

heat release and consumption of the sample. Besides that, the differential scanning technique uses a sample and reference that are facing the same conditions and their signal is directly subtracted from each other [4].

Differential Scanning Calorimetry (DSC) experiments of ibuprofen sample were carried out in a DSC 204 F1 from Netzsch with a heating rate of 10 °C/min. A small amount of sample (3.3 mg) was enclosed in a hermetic aluminum pan. The measurements were carried out in an argon atmosphere. Heating was carried out up to 100 °C.

2.2.3. Fourier-transform infrared spectroscopy (FTIR)

Infrared spectroscopy is a branch of spectroscopy that studies the interaction of infrared radiation with substances. When infrared radiation is passed through a substance, the vibrational movements of molecules or their individual fragments are excited. At the same time, there is a weakening of the intensity of the radiation that has passed through the sample. However, absorption does not occur in the entire spectrum of incident radiation, but only at those wavelengths whose energy corresponds to the excitation energies of vibrations in the studied molecules. Consequently, the wavelengths (or frequencies) at which the maximum absorption of IR radiation is observed may indicate the presence of certain functional groups and other fragments in the sample molecules.

Fourier-transform infrared spectroscopy (FTIR) is a technique used to obtain an infrared spectrum absorption or emission of a solid, liquid, or gas. An FTIR spectrometer simultaneously collects high-resolution spectral data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer, which measures intensity over a narrow range of wavelengths at a time [5].

Infrared spectrum of ibuprofen in KBr disks (ca. 0.3% (w/w)) was recorded at room temperature within 400–4000 cm^{-1} on a Nicolet IS5 FTIR spectrometer. The spectrum of IR disturbed total internal reflection was measured at room temperature on a spectrometer Nicolet iS50 FTIR within 400–4000 cm^{-1}.

2.2.3. Quantum chemical modeling

In quantum chemistry, there are various calculation methods that allow us to study the structure and transformations of complex molecules. Using calculations in quantum mechanics, it is possible:

- to obtain information about the geometric structure of a molecule and the relative energies of its various configurations;
- to estimate the rate of transformation of some molecules into others;
- to determine such characteristics of the molecule as the dipole moment, polarizability and spin interaction constants;
- to predict the existence of new chemical structures, their properties, geometric structure, etc.

Quantum chemistry methods can be divided into nonempirical, semi-empirical and density functional theory (DFT) methods. The main difference between the methods is the method of approximation of the Schrodinger equation.

In present work the density functional theory (DFT) method and the semi-empirical PM6 method were used. Using these methods, the geometric parameters of the ibuprofen molecule and calculated IR-spectra for two enantiomers were optimized. The calculation by the DFT method was performed by the ORCA software [6] with the BP86/def2-TZVP theory level [7], since it is one of the more optimal and does not take much time to calculate.

The calculation by the PM6 method [8] was done in the MOPAC2016 software [9]. In addition to optimizing the geometry and calculating the spectrum, dependences of changes in parameters such as enthalpy of formation and some frequencies were obtained from the rotation of the –COOH functional group by 15° by selecting a dihedral angle, as shown in Figure 3.

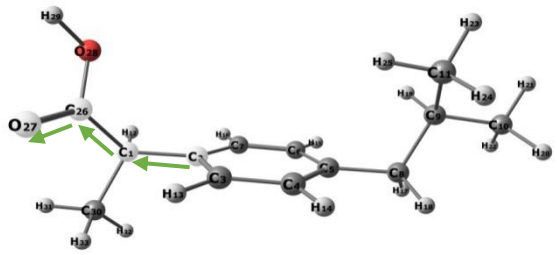


Figure 3: Selecting a dihedral angle in the ibuprofen structure

3. Results and discussion

3.1. X-Ray powder diffractometry (XRD)

A sample of ibuprofen was examined for crystallinity by powder X-Ray diffraction. The resulting diffractogram is shown in the Figure 4.

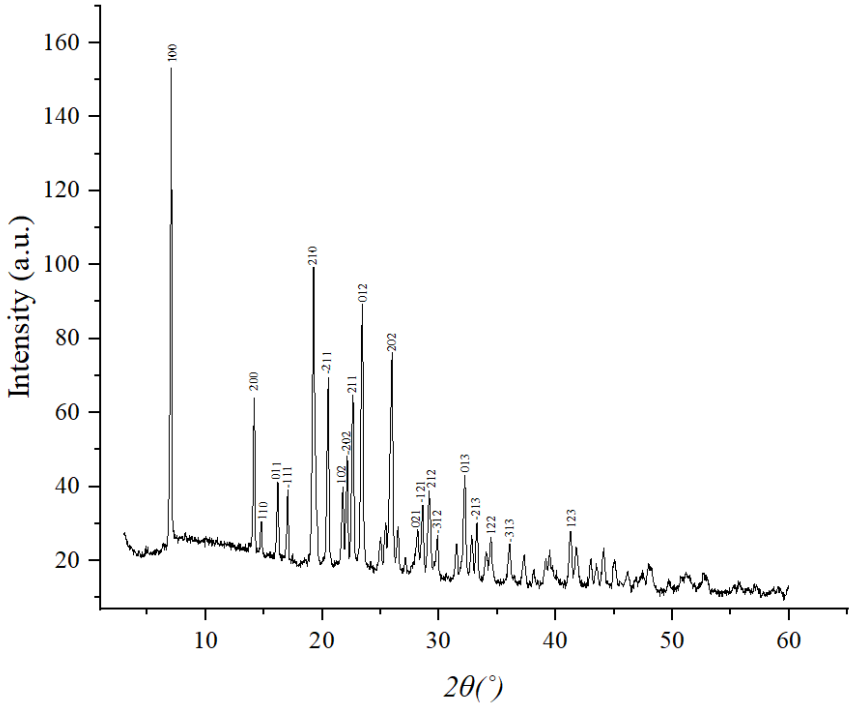


Figure 4: Experimental X-ray diffractogram of ibuprofen sample

With the help of the PowderCell Program [10] a theoretical diffractogram was calculated, which is shown in the Figure 5.

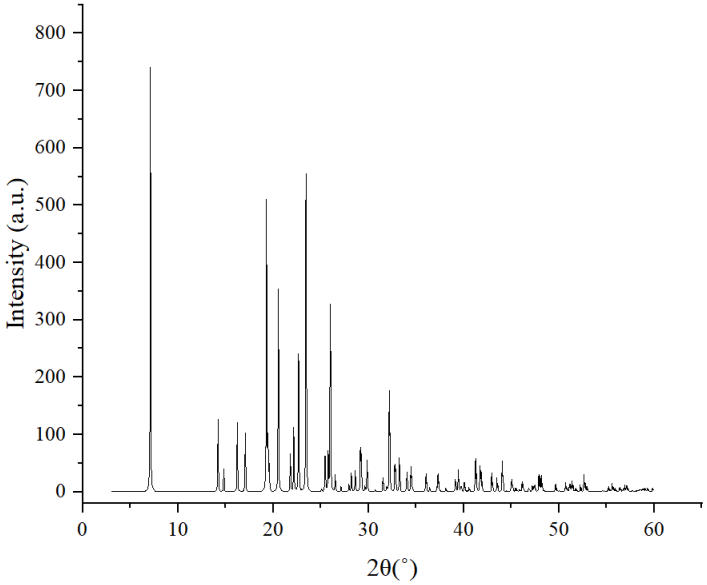


Figure 5: Ibuprofen diffractogram calculated in the program PowderCell [10]

Then we conducted a comparative analysis of the results obtained with data from the ICDD PDF-4 database [11] (Table 1). We used the card 00-034-1728 with data for ibuprofene. Miller indices were assigned with experimental peaks and are listed in Table 1 as well as on Figure 4.

Table 1: Peak data list for ibuprofen

2θ,°	**Peak max., %**	**d_{exp}, Å**	**d_{ref} [11], Å**	**hkl**
7.04		14.568	14.680	*100*
14.21	41.32	7.231	7.290	*200*
14.79	9.50	6.949	7.030	*110*
16.2	17.21	6.348	6.400	*011*
17.05	15.63	6.034	6.076	*-111*
19.25	100.00	5.350	5.378	*210*
20.51	48.91	5.024	5.061	*-211*
21.75	15.60	4.741	4.750	*102*
22.11	22.66	1.106	4.679	*-202*
22.41	69.77	4.603	4.578	*211*
23.4	81.08	4.411	4.430	*012*
25.9	58.85	3.991	4.009	*202*
26.5	8.59	3.902	3.925	*-302*
27.34	3.45	3.785	3.825	*120*
28.15	8.24	3.678	3.687	*021*
28.8	12.41	3.597	3.636	*-121*
29.15	15.36	3.554	3.567	*212*
29.89	7.30	3.468	3.496	*-312*
32.23	18.12	3.222	3.241	*013*
32.78	6.72	3.170	3.175	*022*
33.25	9.35	3.126	3.137	*-213*
34.04	5.09	3.056	3.061	*113*
34.4	6.32	3.025	3.039	*122*
36.06	6.14	2.890	2.904	*-313*
37.27	4.90	2.799	2.808	*213*
38.16	3.12	2.736	2.744	*510*
39.47	5.27	2.649	2.677	*-421*
41.28	7.99	2.537	2.545	*123*
41.8	5.13	2.507	2.518	*-422*

We also obtained the unit cell parameters, which we also compared with the parameters from the database [11] (Table 2).

Table 2: Unit cell parameters for ibuprofen			
Crystallographic parameters	**Values from ref. [11]**	**Experimental values***	**Absolute error**
a (Å)	14.6670	14.6900	0.023
b (Å)	7.8990	7.9169	0.0179
c (Å)	10.7310	10.7543	0.0233
Alpha (˚)	90.0000	90.0000	0
Beta (˚)	99.4600	99.4340	0.026
Gamma (˚)	90.0000	90.0000	0

*estimated with PowderCell on the base of experimental diffraction pattern

Based on the data obtained, it can be concluded that the studied ibuprofen sample is in a crystalline state and does not contain amorphous phases. Crystal structure parameters obtained from experimental diffraction pattern confirmed that the ibuprofen powder has a monoclinic type of structure and a spatial group of 14.

In the diffractogram of the ibuprofen sample 3 peaks corresponded to the following interplane distances: d = 5.35 Å (*210*), d = 4.411 Å (*012*), d = 3.991 Å (*202*) are may be used for identification purposes as pointed out in reference [12]. The first peak of intensity (d = 14.568 Å) is influenced by the morphology of the sample itself, so it is not one of the main peaks and it should not be taken into account for identification purposes [12].

3.2. Differential Scanning Calorimetry (DSC)

A sample of ibuprofen was examined for crystallinity by differential scanning calorimetry. The result is shown in Figure 6. The sample was heated from 26 °C to 100 °C and after that was cooled down to 26 °C. The curve obtained as a result of heating (curve 1.2 in Figure 6) contains a peak corresponding to the melting point of ibuprofen. No extrema were observed on the cooling curve (line 1.3 in Figure 6) in the studied range.

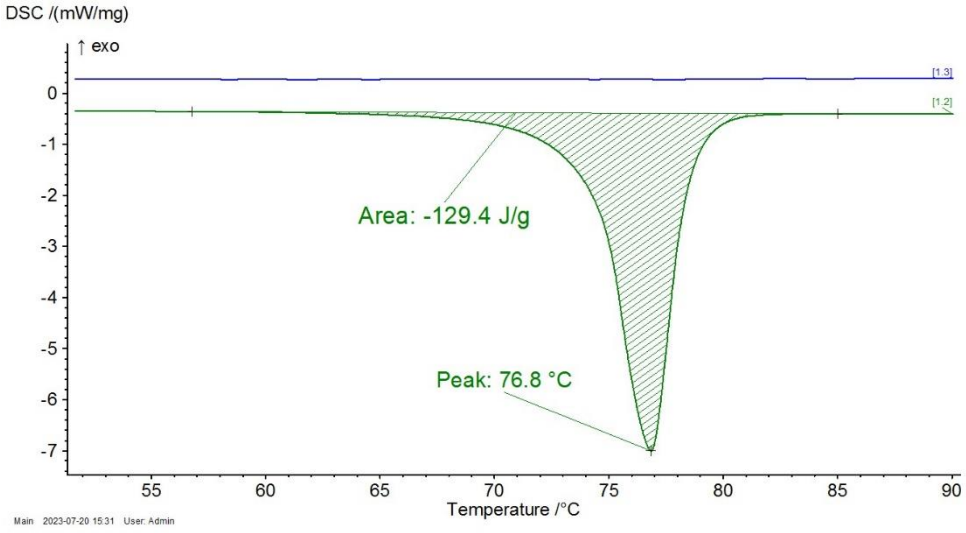


Figure 6: DSC curve of ibuprofen

The melting point obtained for ibuprofen ($T_{peak\ fusion}$ = 76.8 °C) was compared with that from the NIST database [13]. The obtained experimental value coincide with the ones indicated in the database.

3.3. FTIR spectroscopy and DFT calculation of ibuprofen

The ibuprofen sample was examined by IR-spectroscopy. For the purpose of comparative analysis, the experiment was carried out by two methods: ATR and in potassium bromide disks. The experimentally obtained spectra (Figure 7) are concordant. The difference in the observed peak intensities is due to the experimental conditions and the features of the methods used. In the case of the ATR method, the resulting peak is not affected by the environment. In the second case, potassium bromide used as a dispersion medium is hygroscopic. Absorbed water causes broadening of the peaks and an increase in their intensity in the region of 2500-3500 cm^{-1}. The assignment of the main bands in the obtained experimental spectra is given in the Table 3.

For more detailed description of experimental IR-spectra their comparison with DFT-calculated modes can be used. With the help of such a description, it is possible to determine the cause of the divergence of the spectra. Such a description for ibuprofen was performed.

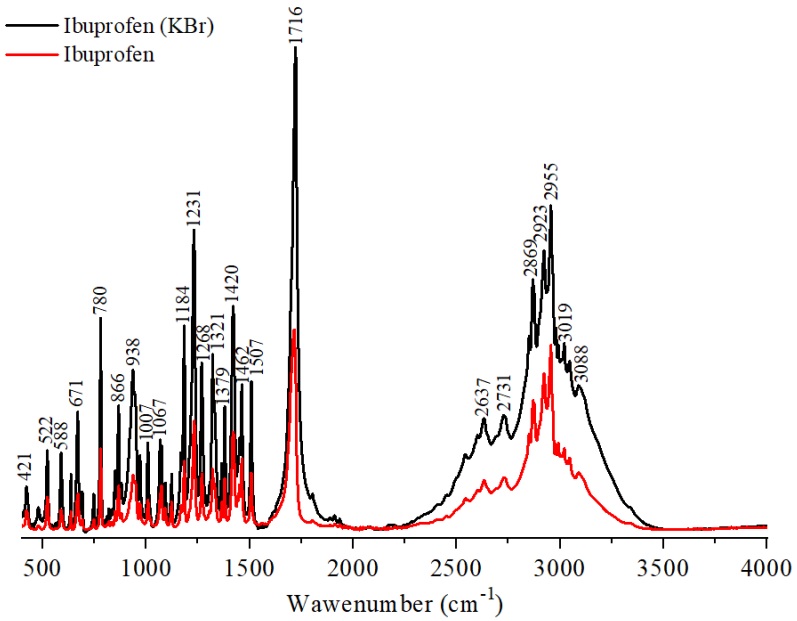


Figure 7: Vibrational optical spectra for solid ibuprofen

DFT molecular geometry optimization was performed for two enantiomers of ibuprofen on the BP86/def2-TZVP level of theory. Obtained equilibrium configurations were used for the vibrational frequencies calculations. Corresponded wave numbers for two enantiomers of ibuprofen are listed in Table 3. It should be noted that the values of vibrations for enantiomers are very close.

Table 3: Experimental (FTIR) and DFT-calculated harmonic wave numbers (cm^{-1}) for ibuprofen

Exp	**Enantiomer S**	**Enantiomer R**	**Approximate descriptions**
421	410	411	twist out-of-plane CH in ϕ; symmetric deformation $(CH_3)_2CHCH_2$
588	611	611	w OH in COOH
1007	999	999	ϕ CH in-plane bending
1131	1131	1131	v C-O
1364	1365	1365	v_{asym} ϕ; t CH_2
1716	1783	1783	v C=O
2850	2936	2936	v C-H in $(CH_3)_2CHCH_2$
2955	2997	2998	v C-H in $CH_3CHCOOH$
3046	3085	3087	v_{asym} C-H in ϕ
3307	-	-	v O-H (H-bonded)
-	3606	3606	v O-H (free)

Decoding of notations: v – stretching, v_{sym} – symmetric stretching, v_{asym} – antisymmetric stretching, w – wagging deformation, twist – torsional deformation.

Figure 8 shows the calculated IR-spectrum of the S(+)-enantiomer with the indicated peaks and the types of oscillations that relate to them.

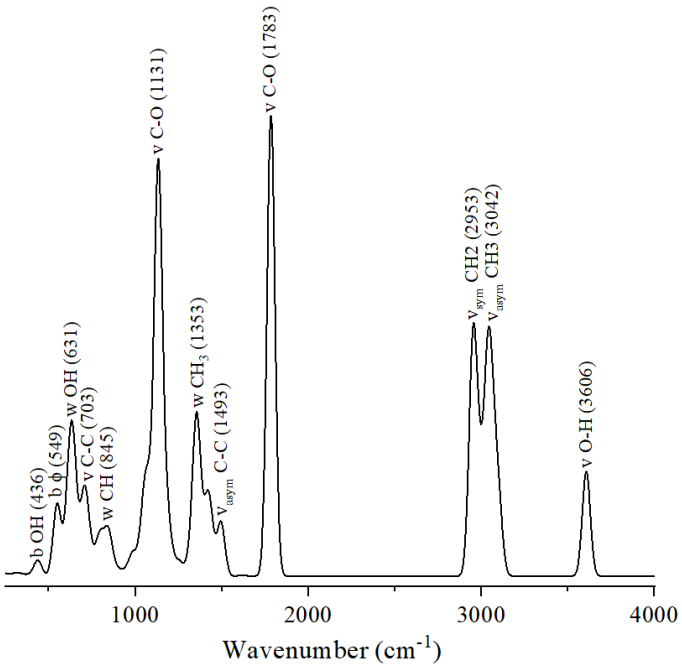


Figure 8: IR spectrum of S(+) ibuprofen calculated on the BP86/def2-TZVP level of theory (ν – stretching, ν_{sym} – symmetric stretching, ν_{asym} – antisymmetric stretching, w – wagging deformation, twist – torsional deformation)

It can be seen from the spectrum that the main peaks take values 1131, 1783 and 2997 cm^{-1}, which corresponded to motions of ν(C-O), ν(C=O) and ν(C-H) respectively. It should also be noted the peak that is responsible for the stretching of the O-H bond (3066 cm^{-1}), it is practically not visible on the experimental spectrum, although it is noticeable in the theoretical calculation.

A good agreement is observed for the experimental and calculated values. Linear regression was obtained (Figure 9) for experimental and DFT-calculated vibrational frequencies. Equation (1) describes the line on Figure 9. It should be noted the peak for the free O-H bond stretching (3606 cm^{-1} in calculations) has not appeared in experimental spectra. At the same time, the experimental spectrum contains a peak at 3307 cm^{-1}, which is characteristic of the H-bonded O-H group.

$$\nu_{exp} = (0{,}984 \pm 0{,}004)\cdot\nu_{cal};\ R = 0{,}99966 \qquad (1)$$

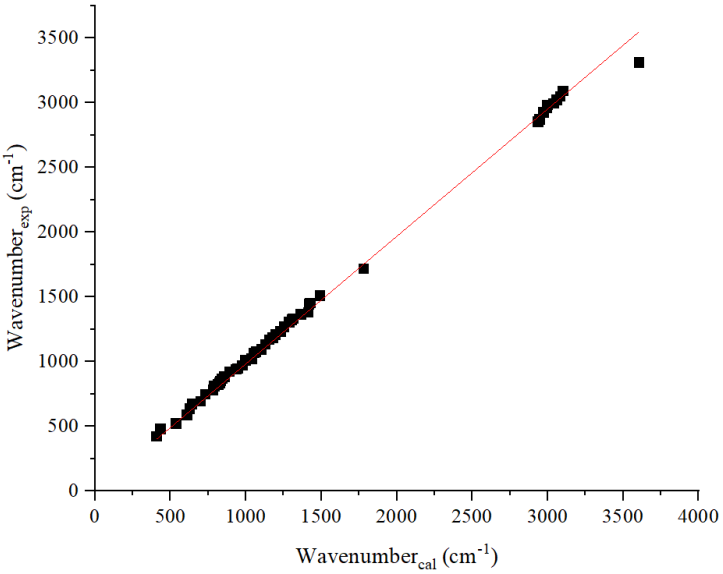


Figure 9: Plot of deviation of the theoretical calculation from the experimental one

Thus, we can conclude that the theoretically calculated spectrum agrees well with the spectrum obtained experimentally, which means that the level of theory we have chosen is well suited for predicting the IR-spectrum of ibuprofen.

3.4. PM6 calculation

Conformational mobility can greatly affect the reactivity and biological activity of organic compounds. In the structure of ibuprofen, one of the possible reaction centers is the carboxyl group. To study the intramolecular dynamics of the carboxyl group, we used the semi-empirical PM6 method [8] implemented in the MOPAC2016 software package [9]. For two enantiomers of ibuprofen, optimization of molecular geometry and calculation of vibration frequencies were performed.

A linear correlation (R = 0.9977) is observed between the values of the vibrational frequencies calculated in the approximation of the PM6 method and the DFT method at the BP86/def2-TZVP level of theory (Figure 10).

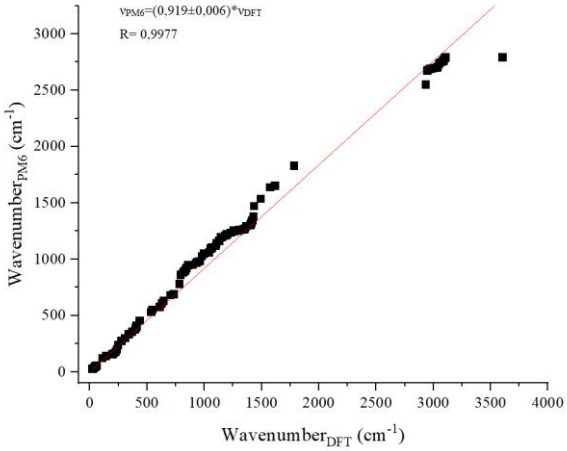
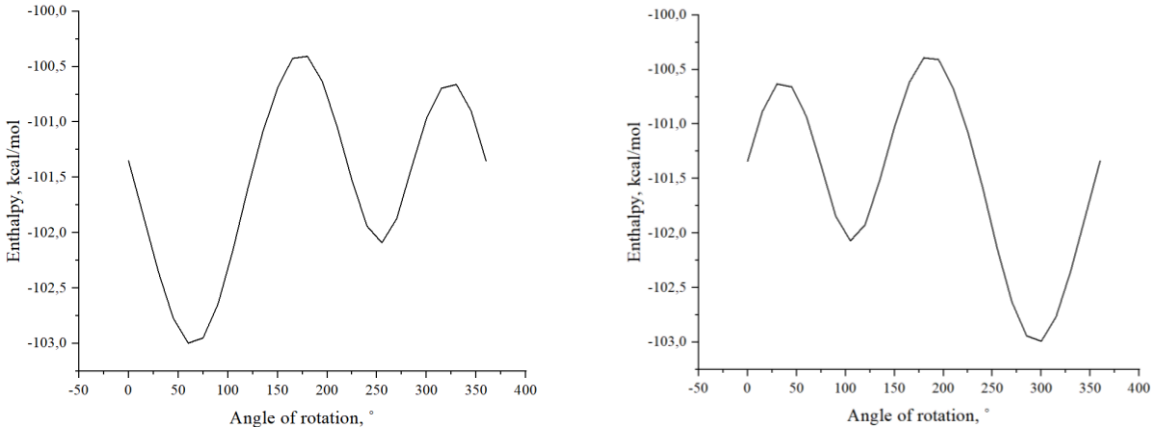


Figure 10: Linear correlation between values obtained by the PM6 and DFT methods

To further study the intramolecular dynamics of the carboxyl group of ibuprofen enantiomers, the C-C-C-O torsion angle was used as the coordinate of intramolecular rotation (see Figure 3). The change in the enthalpy of formation of the molecule during the rotation of the carboxyl group with a step of 15 ° for two enantiomers of ibuprofen is shown in Figure 11. The configurations corresponding to minima on the curves of intramolecular rotation of the carboxyl group of enantiomers are isoenergetic, but have different coordinates (60 ° and 300 °). Barriers of intramolecular rotation do not exceed 5 kcal/mol, which indicates a high mobility of the studied carboxyl group of ibuprofen.



(a) (b)

Figure 11: Plot of the dependence of the enthalpy on the angle of rotation for R(-)-ibuprofen and S(+)-ibuprofen under letters (a) and (b) respectively

Vibrational frequencies were calculated for each coordinate of the intramolecular rotation of the carboxyl group. Figure 12 illustrates the character of

the change in the frequencies of the stretching vibrations for the C-O, C=O and O-H bonds of the carboxyl group during its rotation around the C-C bond.

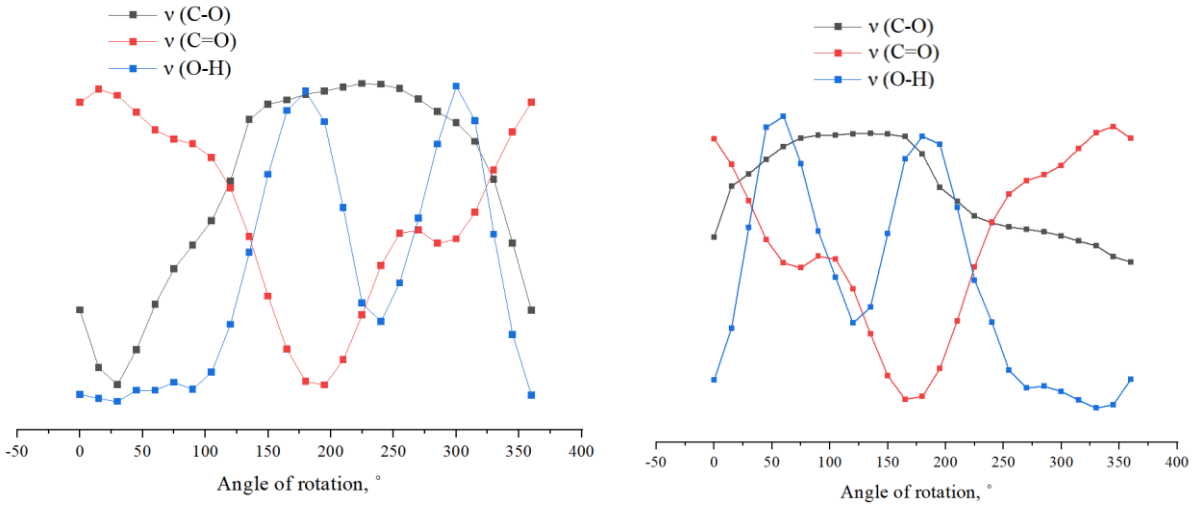


Figure 12: Plot of the dependence of the frequencies on the angle of rotation for R(-)-ibuprofen and S(+)-ibuprofen under letters (a) and (b) respectively

The obtained profiles make it possible to estimate the range of variation of the indicated vibrational frequencies for two enantiomers of ibuprofen. Obtained $\Delta\upsilon$ values are ~13 cm^{-1}, ~27 cm^{-1}, and ~3 cm^{-1} for ν(C-O), ν(C=O) and ν(O-H) vibrations respectively. Noticeable changes are observed for the C=O group.

Thus, the semi-empirical PM6 method is suitable for a preliminary estimation of the conformational mobility of ibuprofen enantiomers and the character of changes in the parameters of its IR-spectrum.

Conclusion

The experimental methods used to analyze the ibuprofen sample align with the characteristics reported in the literature.

Using the powder X-ray diffraction technique, it was proven that the ibuprofen sample under investigation exists in a crystalline form. The diffraction pattern obtained from the experiment perfectly matches the data provided in existing literature. A combined analysis of the experimental results and information from the literature reveals that the signal observed at $2\theta = 7.04°$ cannot be utilized for identifying ibuprofen, as its intensity is influenced by the orientation of the crystalline particles during sample preparation.

Using the method of differential scanning calorimetry, the melting point of ibuprofen sample (76.8 °C) was determined, which are consistent with the literature data.

The IR-spectroscopy analysis of the ibuprofen sample using both the KBr tablet and ATR method showed consistent results, indicating the reliability of the measurements. The strongest signals observed in these spectra were attributed to the vibrations of v(C-O) and v(C=O) bonds. Linear correlations were found between the experimental vibrational frequencies and those calculated using the PM6 and DFT methods. Based on these findings, the BP86/def2-TZVP level of theory is recommended for future *in silico* studies investigating the structure of ibuprofen.

Utilizing the PM6 technique, *in silico* investigation concerning the intramolecular dynamics of the carboxyl group of ibuprofen revealed alteration in the positions of crucial vibrational modes in the IR spectrum when the orientation of the -COOH group was modified.

To enhance accuracy and comprehensiveness in describing the experimental vibrational spectra, further *in silico* examinations regarding the molecular dynamics of ibuprofen in its fused state are necessary.

Acknowledgment

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References

1. Ibuprofen. [Electronic resource]: – material from PubChem / – URL: https://pubchem.ncbi.nlm.nih.gov/. – Access mode: open.
2. Zhurko G. A. Chemcraft - graphical program for visualization of quantum chemistry computations. Ivanovo, Russia, 2005. https://chemcraftprog.com
3. Hastings J. B., Thomlinson W., Cox D. E. Synchrotron X-ray powder diffraction // Journal of applied crystallography. – 1984. – V. 17. – №. 2. – P. 85-95.
4. Linseis. [Electronic resource]: – URL: https://www.linseis.com/en/. – Access mode: open.
5. Gaffney J. S., Marley N. A., Jones D. E. Fourier transform infrared (FTIR) spectroscopy // Characterization of materials. – 2002. – P. 1-33.
6. Neese F. et al. The ORCA quantum chemistry program package //The Journal of chemical physics. – 2020. – V. 152. – №. 22. – P. 224108
7. Perdew J. P. Density-functional approximation for the correlation energy of the inhomogeneous electron gas //Physical Review B. – 1986. – V. 33. – №. 12. – P. 8822.
8. Stewart J.J.P. // Journal of Molecular Modelling. 2007, 13(12), 1173–1213. 4
9. MOPAC2016, James J. P. Stewart, Stewart Computational Chemistry, Colorado Springs, CO, USA, HTTP://OpenMOPAC.net (2016)
10. PowderCell 2.3. [Electronic resource]: – URL: http://mill2.chem.ucl.ac.uk/ccp/web-mirrors/powdcell/a_v/v_1/powder/e_cell.html – Access mode: open.
11. ICCDD PDF-4. [Electronic resource]: – URL: https://www.icdd.com/. – Access mode: close.
12. Kountourellis J. E. et al. X-ray diffraction data for six analgesics //Talanta. – 1986. – V. 33. – №. 7. – P. 631-632.
13. NIST. [Electronic resource]: – URL: https://www.nist.gov/. – Access mode: open.