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FINAL REPORT ON THE START PROGRAMME

High-pressure effect on the vibrational spectra of pharmaceutical compound sulindac

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Abstract

Sulindac is a widely used non-steroidal anti-inflammatory drug known for its polymorphism. The high-pressure experiments provide important information, necessary for enhancing the pharmaceutical compounds stability. Thus, the research represents an important milestone on the way towards novel improved drug formulations.

In this work, the effect of high pressure on the vibrational spectra of sulindac was studied by Raman spectroscopy technique.

It was found that an increase in pressure leads to noticeable changes in the spectra, including a shift of bands towards higher frequencies, changes in intensity. The data obtained indicate significant changes in the molecular structure of sulindac above ~ 4.6 GPa. Those anomalies can indicate the phase transition to high pressure form of sulindac.

The study of spectral changes under high pressure can be a useful tool to investigate the effect of pressure on the pharmacological properties of drugs and the development of new dosage forms.

1. Introduction

A molecular crystal is a type of solid in which the structure and properties are determined mainly by the arrangement of molecules rather than by intramolecular forces. These crystals are held together by weak intermolecular forces such as electric dipoles or van der Waals interactions [1].

The molecular crystal of sulindac, like many other organic compounds, has a complex structure and properties that can change due to external factors such as pressure. During the drug manufacturing process, sulindac and other pharmacological components may be subjected to various technological influences such as compression, loading, localized heating. These influences can lead to new, undesirable chemical-physical and pharmacological properties in the final product.

For example, during tablet formation, pressures can reach 0.6 GPa, which is sufficient to develop irreversible polymorphic phase transitions. As a result of these transitions, new stable forms of molecular compounds are formed, which may differ from the original ones in their pharmacological activity.

Polymorphism of drugs is a common phenomenon that has a significant impact on their biological activity, solubility, dissolution rate as well as efficacy. Therefore, the study of polymorphic phase transitions in complex molecular components of drugs under high pressure is a relevant area of condensed matter state physics and applied pharmacology.

Sulindac is a new non-steroidal anti-inflammatory drug with analgesic and antipyretic activity. It is a yellow, odorless crystalline powder. Like many other drugs, it has polymorphism, that is, the ability to crystallize into different forms. Different polymorphic forms of the same drug, in this case sulindac, may have different physical properties. Thus, three polymorphic forms of sulindac are known to date [2]. But the effect of high pressure on its crystal structure has not yet been studied.

The aim of this work is to study the pharmaceutical compound sulindac by experimental method. The object of the study is the pharmaceutical compound

sulindac. The subject of the study is the effect of high pressure on the vibrational spectra of sulindac and changes in its structure and properties under high pressure.

Raman spectroscopy allows us to study changes in vibrational spectra of sulindac at high pressures, which may indicate changes in molecular structure and phase transitions.

Studying sulindac at high pressures using these methods will help to better understand the effect of pressure on its crystal structure, which can optimize the drug production process and improve its quality and efficacy.

The main objectives of the study are:

- Acquisition of skills of working with Raman spectroscopy unit LabRAM HR Evolution (France) in backscattering configuration;
- Investigation of the effect of high pressure on vibrational spectra of pharmaceutical compound sulindac using Raman spectroscopy;
- Obtaining and further processing of spectra in Origin program;
- Analysis and interpretation of experimental results.

2. Experimental

2.1. Materials and samples

The molecular formula of sulindac is $C_{20}H_{17}FO_3S$, and the chemical name is cis-5-fluoro-2-methyl-1-[(para-methylsulfinyl)benzylidene]inden-3-acetic acid. It is a non-steroidal anti-inflammatory drug with analgesic, antipyretic, and anticancer properties. The sulindac molecule is characterized by two cyclic systems (a phenyl ring carrying a para-methyl sulfoxide group; an indene ring having a fluorine atom and a methyl group as substituents and carrying a carboxymethyl group (almost perpendicular to the plane of the condensed rings)). The two ring systems are linked by a double bond, forming an ethylidene bond, which is also attached to the sulfoxide group.

This spatial configuration gives the sulindac molecule a characteristic geometry that is key to its pharmacological properties. Due to its structure, sulindac is able to interact with the enzyme cyclooxygenase (COX), blocking the synthesis of prostaglandins, which are mediators of inflammation and pain [2].

The chemical structure of sulindac is shown in Figure 1.

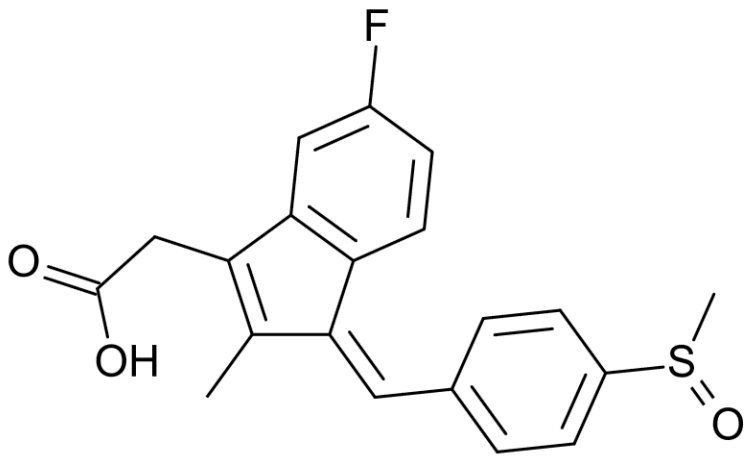


Figure 1: Chemical structure of sulindac [4]

3. Methods

3.1. Raman spectroscopy

Raman spectroscopy is an important part of optical spectroscopy, which studies the interaction of monochromatic radiation, usually from a laser source, with matter. It is accompanied by a change in the energy of the scattered radiation compared to the energy of the incident exciting radiation. Raman scattering of light is caused by inelastic collisions of photons with molecules, in which they exchange energy. The frequency of photons shifts up or down, which contains information about vibrational, rotational and other low-frequency transition modes in molecules. Accordingly, Raman spectroscopy can be used to study solid, liquid and gaseous samples.

The phenomenon of inelastic scattering was predicted in the 20s of the last century, and then proved independently by two groups of scientists: Raman S. V., Krishnan K. S. [5] and Landsherg G. S., Mandelstam L. I. It remains one of the most important analytical and research methods of structural studies to this day. It has many applications in many fields: physics, biology, medicine, chemistry, pharmaceuticals, forensics, etc. This type of analysis is incredibly informative for chemical identification and molecular structure research. Raman spectra are very sensitive to the nature of chemical bonds - both in organic molecules and polymeric materials, and in inorganic lattices. Due to this, each substance and material has its own individual spectrum (fingerprint). The spectra mainly consist of lines corresponding to deformation and stretching vibrations of chemical bonds of carbon (C) with, as a rule, nitrogen (N), oxygen (O) and hydrogen (H) and some others. These lines appear in the range from 600 cm^{-1} (stretching vibrations of single bonds C-C) to 3600 cm^{-1} (vibrations of the hydroxyl group -OH) [6].

Raman spectroscopy is characterized by a change in the frequency of scattered radiation compared to the frequency of the primary (exciting) radiation. In this case, unlike luminescence, which is also secondary radiation with a changed frequency, in Raman spectroscopy the scattering system does not pass into an

excited state for finite (even small) time intervals. Such excited states in scattering processes play the role of only virtual states.

During scattering, a change in the frequency of the primary radiation is accompanied by a transition of scattering structural units to other vibrational or rotational levels. Raman spectroscopy can be considered as a process consisting of two related acts - the absorption of a quantum of primary light frequency ħ and the emission of a quantum of frequency ħ', however, as already noted, in Raman spectroscopy the system under the action of a quantum with energy ħ does not go into an excited electronic state even for a very short time, therefore the energy ħ of the primary quantum can be significantly less than the energy $ħ_e$ of a quantum capable of transferring a molecule from the ground electronic state 0 to an excited electronic state 1 (Figure 2, a).

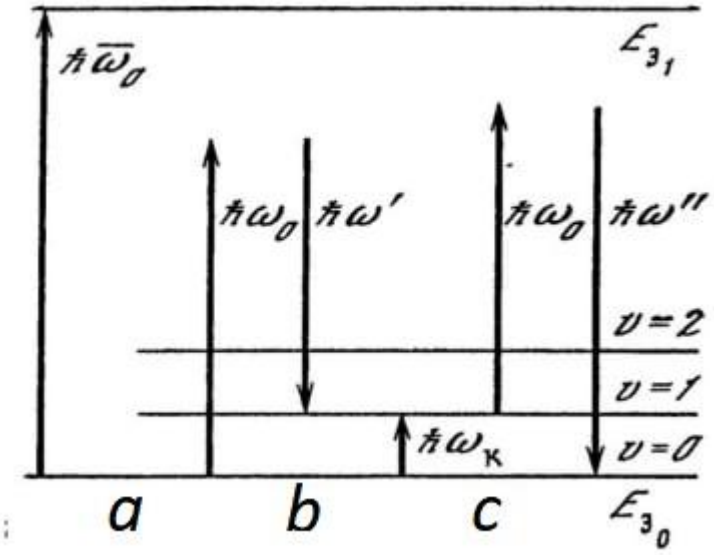


Figure 2: Schematic of transitions in absorption and Raman scattering of light [7]

In the process of Raman spectroscopy, a quantum of light ħ affects an electronic system, transferring to it (or receiving from it) a part of energy, with the scattering object changing to another vibrational state, and the quantum scattered by it having a changed energy ħ' ≠ ħ. Raman spectroscopy, which occurs when a molecule transitions from an unexcited vibrational state characterized by vibrational quantum number ν = 0 to an excited vibrational state with ν = 1, ν = 2, etc., is called Stokes Raman spectroscopy (Figure 2, b).

If, however, before exposure to light the molecule was in an excited vibrational state, e.g., characterized by ν = 1, it can move to an unexcited vibrational state with ν = 0 in Raman spectroscopy, with the energy of the scattered light quantum ħ" > ħ - anti-Stokes Raman spectroscopy (Figure 2, c).

All of the above also applies to Raman spectroscopy with a change in the rotational state of the molecule characterized by rotational quantum numbers. The relation between the energies of incident and scattered photons in the case of Stokes Raman spectroscopy is as follows:

$$\hbar\omega' = \hbar\omega - \hbar\omega_k \qquad (1)$$

and in the case of anti-Stokes Raman spectroscopy –

$$\hbar\omega'' = \hbar\omega + \hbar\omega_k \qquad (2)$$

value represents the energy of the excited vibrational (or rotational) state of the molecule.

Relations (1), (2) explain the main regularities in the vibrational spectra of Raman spectroscopy. Raman lines are arranged symmetrically with respect to the unbiased (Rayleigh) line, whose frequency coincides with the frequency of the excitation light. The frequency of each satellite is a combination of the frequency of the excitation light and the frequency of vibrational or rotational transitions of the scattering molecules. Each satellite with a frequency (red, or Stokes satellite) corresponds to a satellite with a frequency (violet, or anti-Stokes satellite). In the case of vibrational Raman spectroscopy, by measuring the frequencies of the satellites, the natural frequencies of vibration of the molecule can be determined.

From the above it is clear that with the help of Raman spectroscopy spectra it is possible to measure the frequencies of natural vibrations of molecules and crystals. This opens wide possibilities for the identification of substances and the study of transformations occurring in them under the influence of external influences.

A crystal, on the other hand, is characterized by symmetry and a regular arrangement of atoms, whereby they are located at small distances from each other

and cannot shift much from their average positions. Since each band in the spectrum corresponds to a certain vibration of a molecule, and its frequency is extremely sensitive to the spatial orientation of bonds and the mass of atoms, Raman spectroscopy can be used to obtain important information about the dynamics of atoms and molecules [8].

Changes in temperature, pressure and other external factors lead to changes in the lattice symmetry of some crystals (structural phase transformations). The rearrangement of the crystal lattice naturally leads to a change in its vibrational spectrum, so this method is a fine tool for analyzing these transformations as well.

3.2. Raman spectrometer for studying atomic dynamics of condensed matter at high pressure

Registration of Raman spectra is a difficult task. First, this is due to the low intensity of Raman spectroscopy lines (10^{-5}- 10^{-6} of the intensity of the excitation line) [5]. Secondly, the difficulty arises from the fact that simultaneously with weak Raman spectroscopy lines the excitation radiation is also registered. All this imposes a number of requirements on the equipment designed for recording Raman spectroscopy. A schematic of a typical setup is shown in Figure 3.

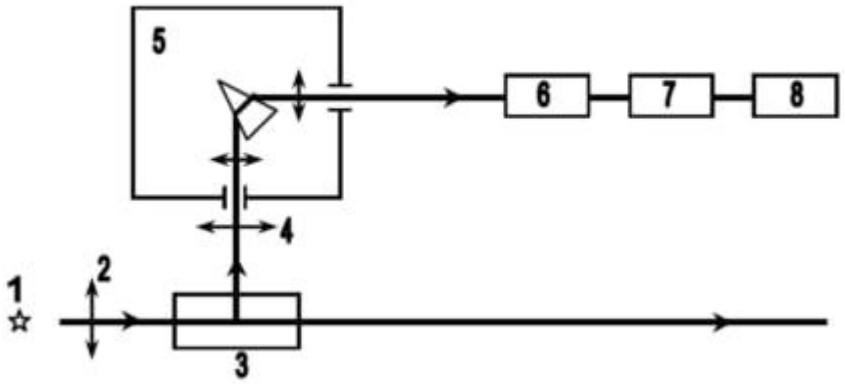


Figure 3: Block diagram of the spectrometer for Raman spectroscopy observation [7]

Light from the radiation source 1 is focused by lens 2 onto the cuvette with the substance under study 3. The scattered light is directed by lens 4 to the input slit of monochromator 5. At the output of the monochromator is a radiation receiver 6, the signal from which is fed through an amplifier 7 to the recording device 8 (microammeter). Argon and helium-neon lasers are used as excitation radiation sources.

The study of the vibrational spectra of sulindac in the present work was carried out using a LabRAM HR Evolution spectrometer (France) in a backscattering configuration (Figure 4). The laser wavelength was 633 nm/17 W. For the excitation line of the laser used, an appropriate bandpass filter was applied. An aperture with a slit size of 200 μm and an objective with 20x magnification were used in the experiment. The characteristic measurement time of one spectrum was 10 minutes.

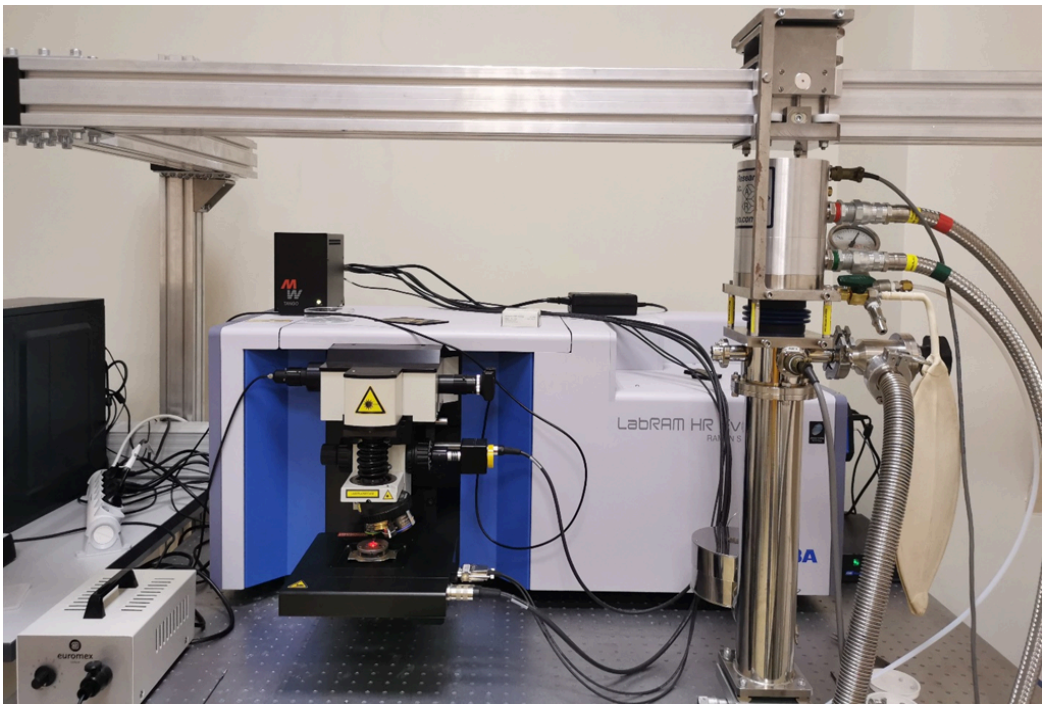
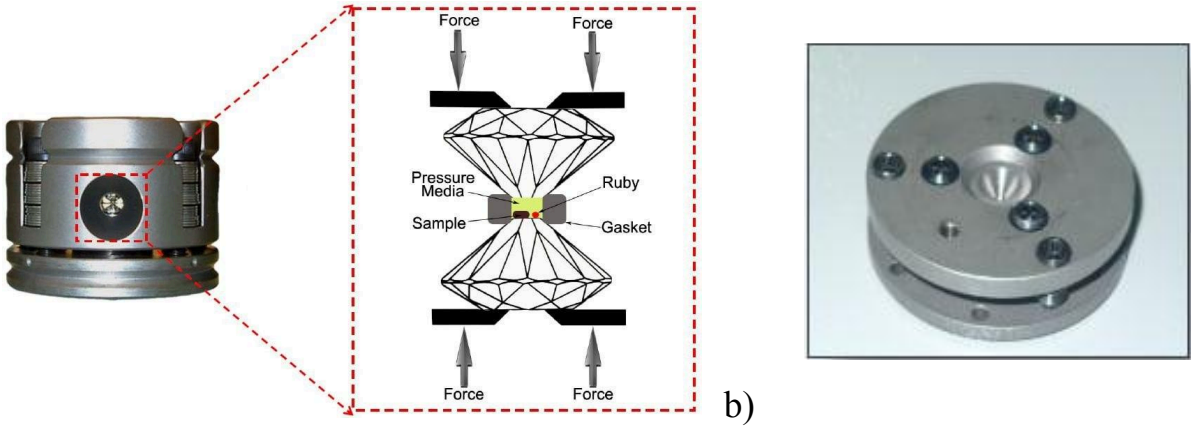


Figure 4: General view of the LabRAM HR Evolution Raman spectrometer (Horiba, France)

A high-pressure high pressure cell with diamond anvils was used to create high pressure on the sample in the experiments (Figure 5).



a) b)

Figure 5: Schematic (a) and general view (b) of the diamond anvil cell [9]

4. Results and discussion

An important contribution to the understanding of the behavior of the crystal structure of a substance under high pressure is the change of vibrational spectra during compression. The observed vibration frequencies are shown in Figure 6 with the corresponding labeling of the vibrations of the different functional groups present in the molecule.

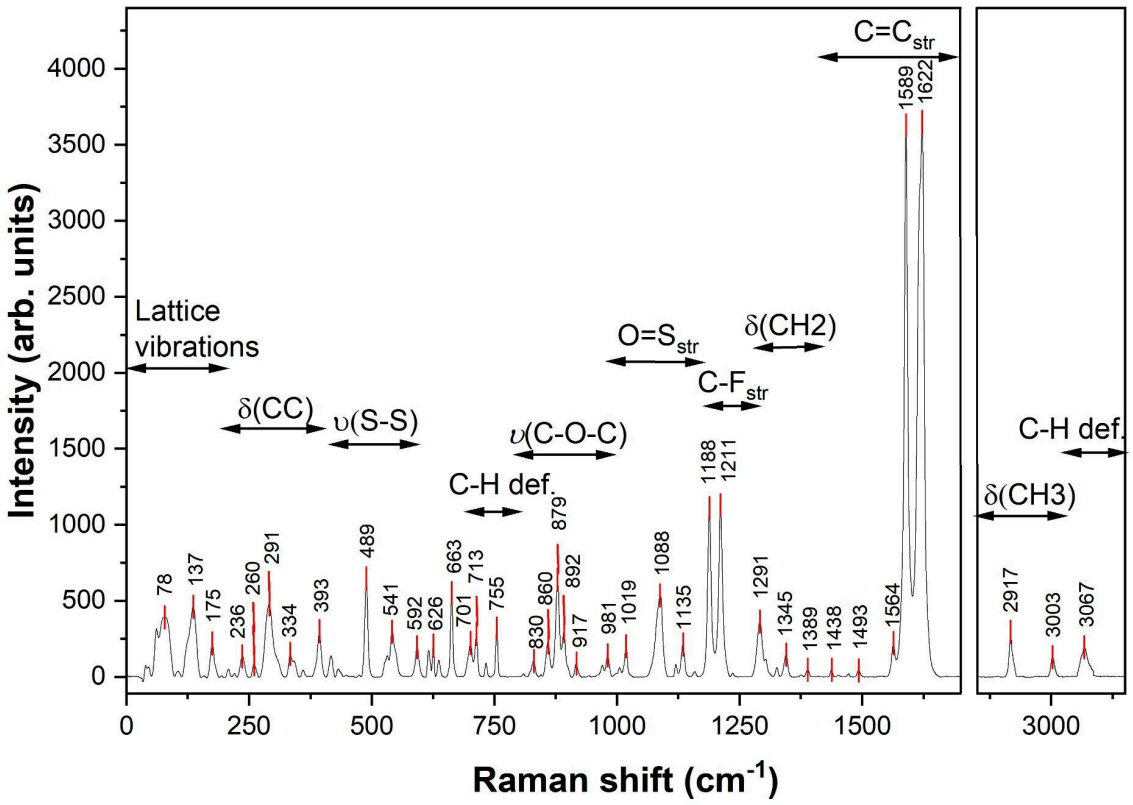


Figure 6: Raman spectrum of sulindac at room temperature and normal pressure

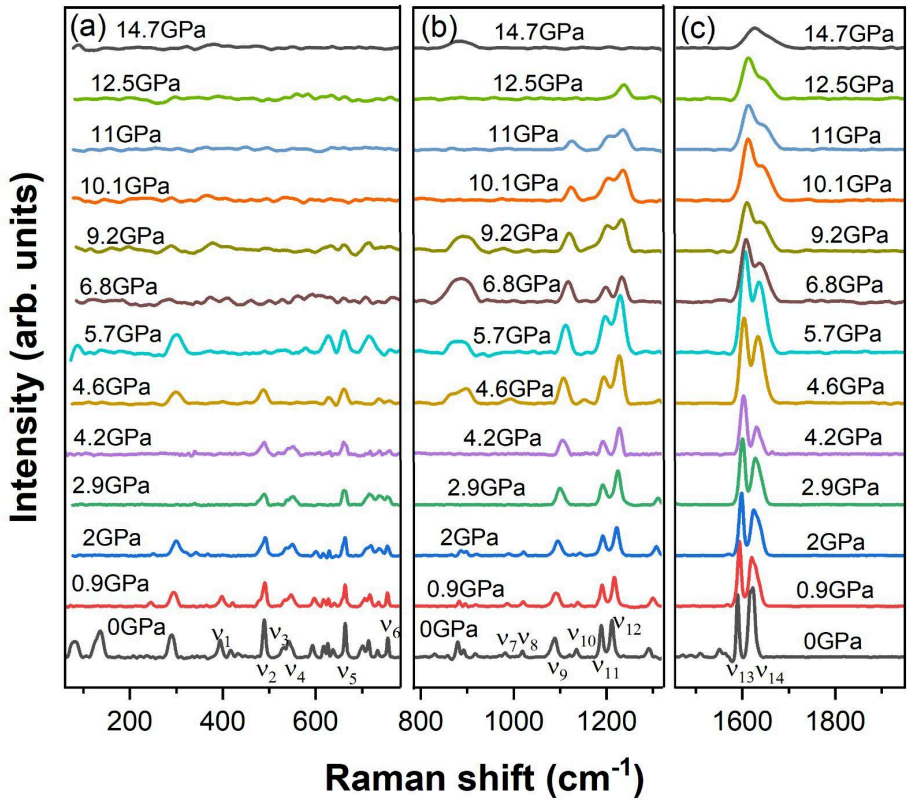
The observed vibrational frequencies are presented in Table 1 with the corresponding labeling of the vibrations of the different functional groups present in the molecule. Five different vibration groups can be clearly distinguished: a stretch of vibration modes up to 200 cm^{-1}, vibration groups located at 1088, 1600 and 2920 cm^{-1}. The bands in the frequency region up to 200 cm^{-1} under normal conditions correspond to intermolecular phonons, while the peaks located higher in energy correspond to intramolecular phonons. The bands in the frequency region

above 300 cm^{-1} belong to intramolecular phonons associated with elastic vibrations of hydrogen atoms.

Table 1. Vibrational modes of the sulindac molecule (cm^{-1}) obtained by Raman spectroscopy

Raman, cm^{-1}	**Vibrational modes assignment**
78, 137, 175	Lattice vibrations in crystals, LA modes
236, 260, 291, 334, 393	δ(CC) aliphatic chains
489, 541, 592	v(S-S)
701, 713, 755	C-H deformation
830, 860, 879, 892, 917, 981	v(C-O-C)
1019, 1088, 1135	$O = S_{str}$
1188, 1211	$C - F_{str}$
1291, 1345, 1389	δ(CH2)
1438, 1493, 1564, 1589, 1622	$C = C_{str}$
2917, 3003	CH_3
3067	C-H deformation

With increasing pressure, all bands in the Raman spectrum shift towards higher energies, with the increase in frequency depending on the nature of atomic vibrations. Also, the intensity of the bands slightly changes with increasing pressure, as the conditions of resonance excitation of Raman spectroscopy change due to the shift of electronic levels [10]. Raman spectra for sulindac at different pressures are presented in Figure 7.



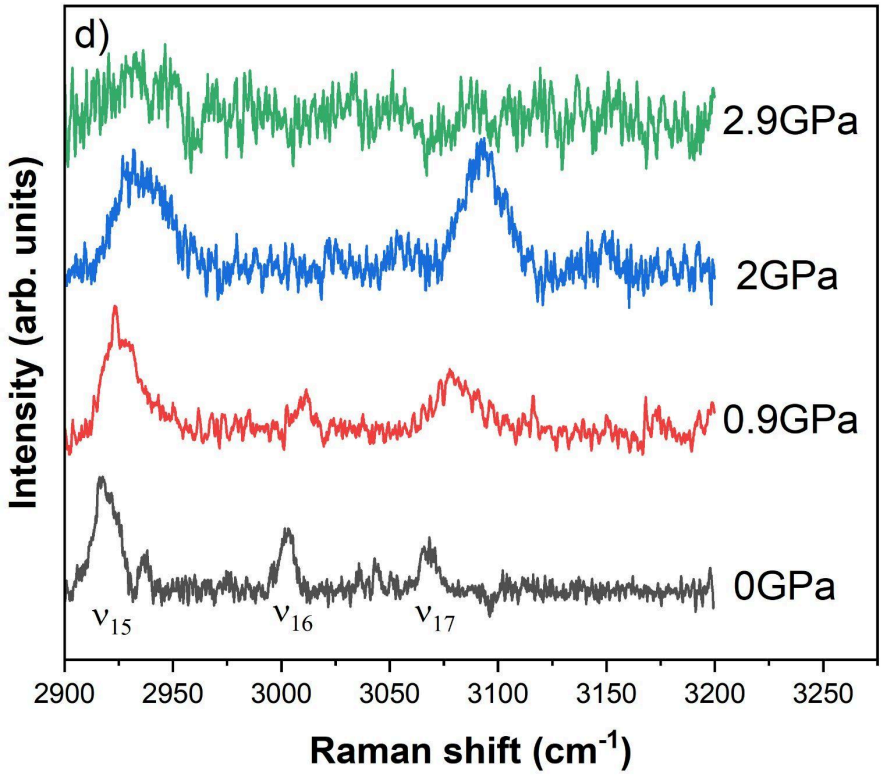


Figure 7: Raman spectroscopy spectra of sulindac at different pressures and room temperature in the range: a) 60 to 780 cm^{-1}, b) 785 to 1319 cm^{-1}, c) 1458 to 1950 cm^{-1} d) 2900 to 3200 cm^{-1}

The spectra in Figure 8 show the changes in the selected frequencies of sulindac oscillations with increasing pressure.

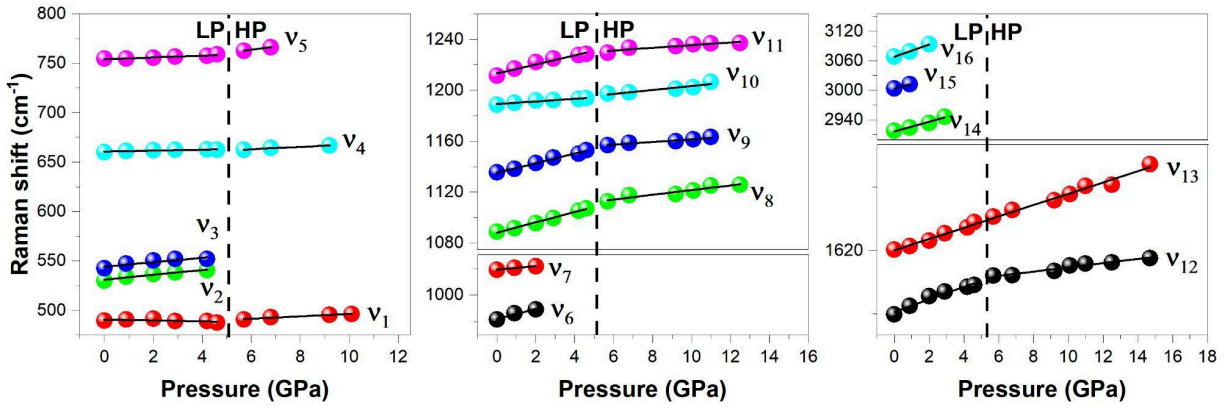


Figure 8: Pressure dependence of the frequencies of selected vibrational modes of the ambient and pressure-induced form-HP of the sulindac on pressure.

The assumed errors are within the limits of the symbol sizes. Solid lines represent linear interpolations of the experimental data. The pressure region associated with the pressure-induced phase transition is labeled.

Despite the fact that most of the modes show a nonlinear character of the frequency change, a noticeable shift towards higher frequencies is observed in the range 2900-3200 cm^{-1}. This may be due to changes in intermolecular interactions.

At pressures above 4.6 GPa, anomalies in the spectra are observed, which may indicate the presence of a phase transition from initial low pressure phase (LP-form) of sulindac into a new high-pressure sulindac form (HP-form). A gradual broadening of Raman lines near 14.7 GPa is followed by their disappearance up on further compression. Such a behavior corresponds to a gradual phase transition to the amorphous phase of sulindac.

The changes in vibrational modes can be used to distinguish sulindac polymorphs, especially in the outer mode region.

It is important to note that the observed changes in the spectra do not indicate a change in the space group of sulindac, but indicate structural rearrangements within the molecule.

5. Conclusion

The crystal structure of the complex molecular crystal of sulindac $C_{20}H_{17}FO_3S$ was investigated by Raman spectroscopy at high pressures up to 14.7 GPa and at room temperature. At normal pressure, sulindac is in polymorphic form II, the crystal structure of which has monoclinic symmetry with space group P 1 21/c 1. The vibration spectra of sulindac obtained by Raman spectroscopy were investigated. In the course of the work, a table with observed vibration frequencies with the corresponding designation of vibration modes of the sulindac molecule was formed. The obtained spectra show significant differences between the observed frequencies with increasing pressure.

The results of Raman spectroscopy measurements indicate the presence of the transition, which is determined by the appearance of anomalies in the high-pressure behavior of the frequencies of the bending vibrational modes. At pressures above ~4.6 GPa transition from initial low pressure phase (LP-form) of sulindac into a new high-pressure sulindac form (HP-form) was found.

A gradual broadening of Raman lines near 14.7 GPa followed by their disappearance up on further compression was observed. Such a behavior corresponds to a gradual phase transition to the amorphous phase of sulindac.

Thus, our experimental data indicate the development of a polymorphic phase transition in sulindac at relatively low pressures. This fact determines the fundamental possibility of breaking the stability of the initial form II of sulindac in the process of tabletting or milling.

Acknowledgement

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