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A hybrid method for estimation of molecular dynamics of diazepam-density functional theory combined with NMR and FT-IR spectroscopy

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ABSTRACT

Reorientation of the molecule of diazepam was investigated by calorimetric methods, IR absorption and NMR. The investigation of dynamics was complemented by density functional study (DFT) of vibrational frequencies and infrared intensities, calculations of steric hindrances and Monte Carlo simulations. The results indicated the occurrence of reorientation jumps of the CH_3 group and conformational motion of the benzodiazepine ring. The activation parameters of the methyl group reorientation were determined and the activation barrier obtained was in good agreement with the theoretically estimated value. The FT-IR spectra were assigned using results of DFT calculations.

1. Introduction

Diazepam belongs to benzodiazepine (BZ) derivatives, a group of medicaments of a wide spectrum of activity along the four main lines: antianxiety, sedative and sleep-inducing, anticonvulsive and reducing muscle tension (Qashu et al., 2010; Igarashi et al., 2009; Riss et al., 2008). Diazepam, after oral administration, is well absorbed from the alimentary tract in the unchanged form and is metabolised in the liver (Acikgoz et al., 2009; Inaba et al., 1988). In the first stage, as a result of N_1-demethylation, it undergoes biotransformation to the active metabolite – desmethyldiazepam. The demethylation can be accompanied by C_3-hydroxylation, leading to formation of the active metabolite – temazepam. Both desmethyldiazepam and temazepam undergo transformation to oxazepam, another active metabolite, which is then transformed into the inactive metabolite 4-hydroxyoxazepam. As a result of biotransformation many psychotropic active metabolites are formed, showing different and longer-lasting effects than those of the parent drug, and the final clinical result depends on the combined influence of diazepam and its active metabolites. The anticonvulsive effect is a result of the combined influence of diazepam, desmethyldiazepam and oxazepam, while the calming effect is mainly related to the activity of desmethyldiazepam. The long-lasting pharmacological effect of diazepam is related to the accumulation of oxazepam in the brain (Klotz, 2007).

Although benzodiazepine derivatives are not a new group of drugs, they belong to the widespread and most often prescribed medications in the world; over 30 preparations are currently in use. Because of the wide range of their activity, they have become overused and their uncontrolled application often leads to addiction (Thibaut et al., 2009; Cook et al., 2007). Moreover, increasing number of acute poisonings with BZ has stimulated attempts at explaining the mechanism of activity of this group of compounds (McConnell, 2008; Rada and Hoebel, 2005).

At the present state of knowledge it is assumed that the therapeutic activity of BZ involves intermediacy of two types of receptors: (i) the central one, GABA-A, in the central nervous system, activated by gamma aminobutyric acid and (ii) peripheral one (PRB = peripheral-type benzodiazepine receptor), localised mainly in the cell membrane and membranes of cell organelles. PRB has been found mainly in the adrenal glands, cardiac muscle, kidneys, lungs and liver. Physiological significance of this receptor has not been unambiguously established yet. Diazepam and flunitrazepam belonging to BZ, show the same affinity to the receptors of both types, while the other members of BZ group show the specificity of bonding.

The study on the significance and role of PRB receptor was performed with 4-chlorodiazepam, a diazepam derivative, as a ligand (Lonergan et al., 2009). This compound shows micromolar affinity towards GABA-A, while its bonding with PRB is species dependent.

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The BZ bond to their receptors results in an increase in the frequency of opening of the chloride channel in response to GABA. This effect can be selectively neutralised by the substances being antagonists of BZ receptors, like, e.g. flumazenil, which can neutralise the majority of effects observed after administration of BZ (Ren et al., 2010). This is the basis of specific antidotes applied in the cases of overdose (Dubrovina and Zinov'eva, 2009; Biggio et al., 2007).

There is rich literature on the clinical, pharmacokinetic, neurochemical properties of BZ and on the possible addiction to them; however, the opinions on the effectiveness of their activity and development of addiction are much different. Because of this diversity of opinions, in 1996, WHO issued a special report on the proper use of BZ and the threats following from the addition to BZ, prepared on the basis of opinions of a group on international experts (Longo and Johnson, 2000). Taking into regard the wide range of activity and great demand for BZ, related to increasing rate of depressions and anxiety-related disorders, the information on the proper use if BZ and the effects of their overuse is still needed (Khong et al., 2004). It should be mentioned that recently many authors have been working on explanation of the function of the peripheral BZ receptor, which also takes part in many other important processes taking place in the cell, such as, e.g. steroidogenesis, energy metabolism or calcium transportation (Malcolm, 2003; Lilja et al., 2001; Livingston, 1994). For this reason, exact explanation of the physiological functions of PRB should prove much helpful in diagnostics and therapy of many diseases.

One of the approaches to extend the knowledge on PRB is based on determination and analysis of permitted molecular reorientations of benzodiazepine derivatives being agonists of these receptors. For this purpose the study was undertaken with the help of calorimetric methods, IR absorption and NMR. The investigation was complemented by density functional study (DFT) of vibrational frequencies and infrared intensities, calculations of steric hindrances and Monte Carlo simulations.

2. Materials and methods

2.1. Materials

Diazepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4 benzodiazepin-2-one) was purchased in a hermetically sealed containers from GlaxoSmithKline Pharmaceuticals S.A., Poznań. Molecular formula: $C_{16}H_{13}ClN_2O$, m.w. = 284.7 g/mol. Chemical structure of diazepam is presented in Fig. 1.

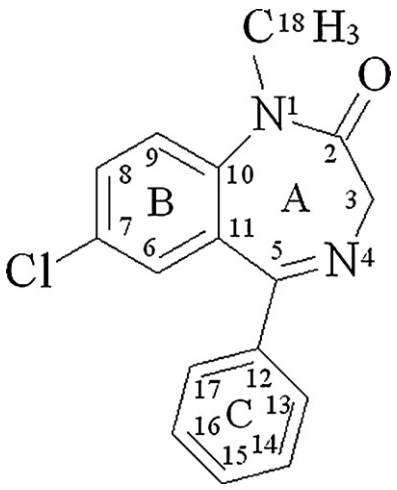

Fig. 1. The molecule of diazepam: A, diazepine ring; B, benzene ring; C, phenyl ring.

2.2. Methods

2.2.1. DSC measurements

DSC measurements were performed on a Thermal Analysis Q2000 type calorimeter, at the heating and cooling rate of 10.0 K/min in the range 100–470 K.

2.2.2. FT-IR measurements

In the mid infrared region the FT-IR spectra of diazepam-KBr were recorded. The spectra were taken in the region 4000–400 cm^{-1} with an IFS 113v FT-IR spectrophotometer (Brucker, Karlsruhe) equipped with a DTGS detector; resolution 2 cm^{-1}, NSS = 64. He Happ–Genzel apodization function was used. Diazepam (2 mg) was compressed into disks after being mixed with KBr (200 mg).

All the measurement was performed in dry atmosphere to avoid any unwanted contribution.

2.2.3. NMR studies

The sample for NMR studies was degassed and sealed off under vacuum in glass ampoules.

The second moment of NMR line for proton was measured in a function of temperature by a continuous wave spectrometer working on protons at the frequency of 28 MHz. Its values found by numerical integration of the absorption curve derivatives were corrected for the finite modulation field. The proton spin-lattice relaxation time T_1 was measured as a function of temperature on a pulse spectrometer working at 58.9 MHz. The measurements were performed by using the saturation recovery method. Both spectrometers used were constructed at the Radiospectroscopy Laboratory at the Faculty of Physics, AMU. The temperature of the sample was controlled by means of a gas-flow cryostat and monitored with Pt resistor to an accuracy of 1 K. NMR measurements at 200 MHz were performed using a Tecmag HF1 Apollo NMR console incorporating an Oxford Instruments magnet of 4.7 T. The proton spin-lattice relaxation times were measured by the saturation-recovery technique. The temperature of the sample was stabilized in a dynamic Oxford helium flow cryostat and controlled by Oxford ITC 503 with the accuracy and stability of ±0.1 K.

2.3. Calculation details

The steric hindrances for the isolated molecule and crystal of diazepam were calculated by the atom–atom potential method (Kitajgorodski, 1973). Simulations of the second moment of NMR line for protons were performed by the Monte Carlo method using the program described in (Goc, 2004). Optimisation of the diazepam molecule structure, calculations of energy barrier for CH_3 group reorientation and intensities of infrared vibrations were made by the DFT method (density functional theory) with Becke 3-Lee-Yang-Parr functional (Becke, 1993, 1996; Lee et al., 1988) and basis set 6-311G**. All calculations were performed using the GAUSSIAN 03 program (Frisch et al., 2004).

3. Results and discussion

3.1. DSC measurements

The calorimetric measurements are important supplement of the hitherto published results as to the best of our knowledge there are no literature data on thermal characterisation of diazepam below 260 K. According to the DSC study carried out from room temperature to the melting point T_m = 404.5 K, diazepam was found to show no phase transition (Van den Mooter et al., 1999; Zayed et al., 2005). Van den Mooter et al. (1999) reported the presence of a glassy phase with T_g = 315.2 K, upon melting of the compound

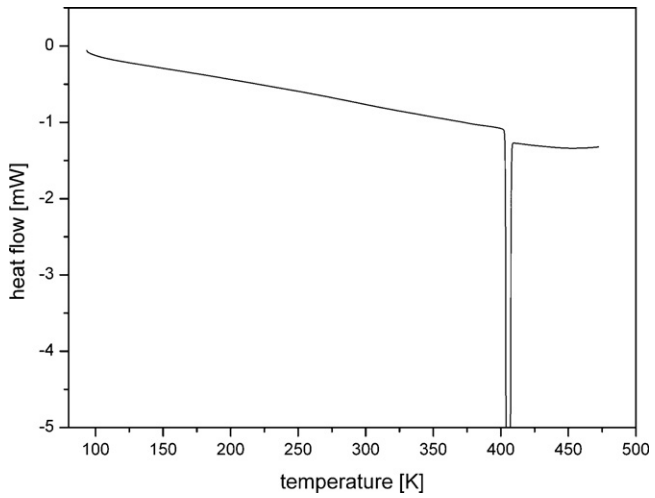

Fig. 2. Temperature dependence of heat flow for diazepam.

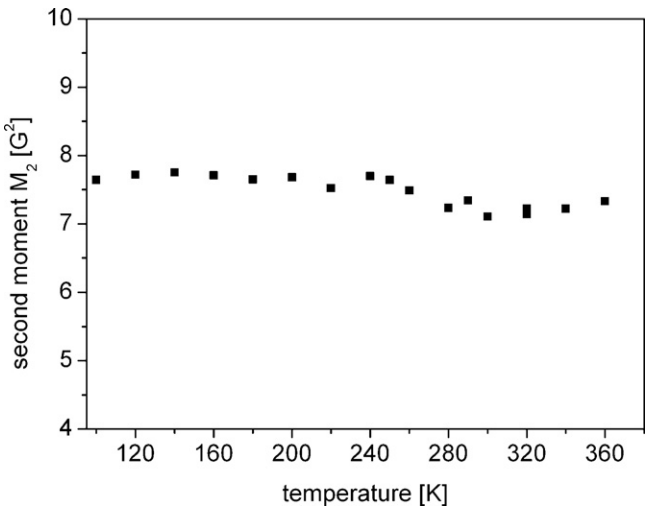

Fig. 3. Temperature dependence of the NMR second moment M_2 for diazepam.

and its fast cooling to 268 K. Our DSC measurements performed in the range 100–470 K did not show phase transition to the melting point and confirmed the literature melting point value (Fig. 2).

3.2. FT-IR studies

The FT-IR spectra for diazepam in the range 4000–400 cm^{-1} have been already reported (e.g. Neville and Shurvell, 1990; Gunasekaran et al., 2006). In Neville and Shurvell (1990) the assignment was made in the conventional way, while in Gunasekaran et al. (2006) it was made by the FG matrix method proposed by Wilson. The IR absorption spectrum of diazepam is composed of two parts of the bands covering two ranges of wavenumbers: from 450 cm^{-1} to 1750 cm^{-1} and from 2750 cm^{-1} to 3200 cm^{-1}. The bands observed in the lower wavenumbers are assigned to the heavy atom in-plane (stretches and bends), out-of-plane and torsional modes, while the higher wavenumbers bend – to the localised hydrogen stretches.

The purpose of the work is to study the vibrational dynamics of diazepam and to propose an assignment of the infrared bands on the basis of experimental and theoretical data. For this reason the FT-IR spectra are characterised taking into account the high-level quantum chemical calculations. The vibrational frequency and approximate description of normal modes was made by the DFT method. The DFT calculations are computationally less demanding than the conventional *ab initio* correlation techniques. It should also be noted, that the calculation of the vibrational frequencies by DFT method give good agreement with experiment and rather better results than to the Hartree–Fock theory (Ziegler, 1991; Finley and Stephens, 1995; Rauhut and Pulay, 1995).

3.3. Second moment NMR (M_2) and spin-lattice relaxation time (T_1) measurements

To identify the molecular reorientations taking place in diazepam, second moment of NMR line M_2 and spin-lattice relaxation time T_1 were measured for protons in a wide range of temperatures. Results are presented in Figs. 3 and 4.

As follows from Fig. 3, the second moment value M_2 at 100 K is 7.7 G^2 and does not change up to 250 K. From this temperature the value of M_2 slightly decreased and above 320 K it was constant and equal to 7.2 G^2. The spin-lattice relaxation time T_1 for protons was measured for the range 77–400 K at 58.9 MHz and in the range 74–300 K at the frequency of 200 MHz. For the two series of measurements the dependence of T_1 time on reversed temperature revealed a minimum of 0.71 s at 125 K and of 0.18 s at 110 K for 58.9 MHz and 200 MHz, respectively. For the system in which molecular reorientations occur the T_1 time is described by the following formula (Abragam, 1961; Slichter, 1996):

$$\frac{1}{T_1} = C \cdot \left[\frac{\tau_c}{1+\omega_0^2\tau_c^2} + \frac{4\tau_c}{1+4\omega_0^2\tau_c^2} \right] \quad (1)$$

where C is the relaxational constant; ω_0, the resonance frequency; T, temperature; $\tau_c = \tau_0 \exp(E_A/RT)$, the correlation time (the Arrhenius relation); τ_0, the preexponential factor; E_A is the activation energy; R is the gas constant. The inversed correlation time τ_c is the frequency of jumps of a molecule or its fragment through the energy barrier E_A, while the inversed pre-exponential factor τ_0 is the frequency of vibrations of a molecule or its fragment at the potential energy minimum.

The best fit of the expression describing the temperature dependence of T_1 (Eq. (1)) to the experimental values permitted determination of the activation parameters presented in Table 1.

The activation barrier of the processes observed was 8.10 kJ/mol and 8.25 kJ/mol for 58.9 MHz and 200 MHz, respectively. Similar values of the activation energy indicate that for both frequencies the same relaxational process was observed. The magnitude of

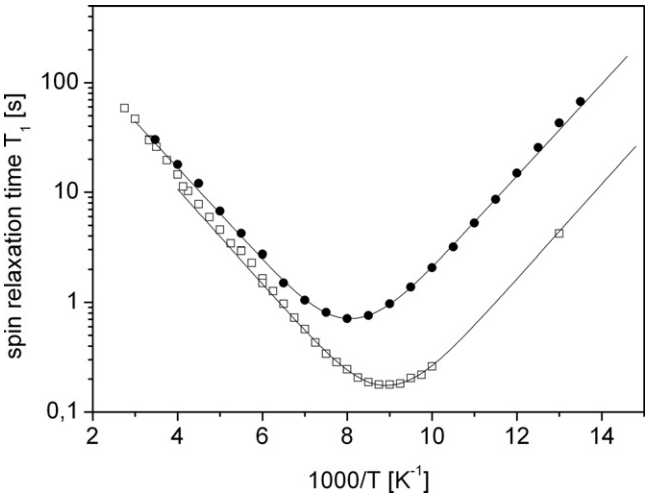

Fig. 4. Temperature dependencies of spin-lattice relaxation times T_1 for diazepam, (□) T_1 measured at 58.9 MHz, (●) T_1 at 200 MHz. The solid lines are theoretical fits to Eq. (1).

Table 1
The fit activation parameters and relaxation constant C obtained for diazepam at 200 MHz and 58.9 MHz.

	T_1 200 MHz	T_1 58.9 MHz
E_a [kJ/mol]	8.10	8.25
τ_0 [s]	1.99×10^{-13}	2.38×10^{-13}
C [1/s]	1.24×10^{9}	1.48×10^{9}

the activation energy suggests that this process is reorientation of the methyl group. Similar values of the activation energies were obtained for reorientation of the methyl group in other biologically active compounds, e.g. β-estradiol 9.3 kJ/mol (Andrew et al., 1999), 11.9 chlorpropamide (Wasicki et al., 2009), 8.3 kJ/mol L-adrenaline (Andrew et al., 1998).

Interpretation of the process manifested as a minimum of the relaxation time and the process causing a small change in the second moment of NMR line is given further in the text.

3.4. Steric hindrance calculations

To give a detail interpretation of the results and identify the molecular reorientations at first analysis should be made of the possible types of motions.

Steric hindrances are inferred from analysis of the potential energy curves for rotation of molecules or their fragments about specific axes. If the potential energy assumes anomaly high value, it means that the distances between atoms are shorter than the allowed ones and such reorientation is impossible. The energy of interaction between two not bonded atoms can be described by the formula (Buckingham and Corner, 1947; Kitajgorodski, 1973):

$$E_{ij} = -\frac{A}{r_{ij}^6} + B\exp(-C \cdot r_{ij}) \quad (2)$$

where r_{ij} is the distance between atoms i and j; A, B, C are the constants characteristic of given atoms. The calculations were performed for the constants A, B, C given in Daszewski (1982), which were earlier used for the calculations for chlorpropamide (Wasicki et al., 2009).

For the crystal, the potential energy E is a sum of E_{ij} calculated for all pairs of interacting atoms (Kitajgorodski, 1973):

$$E = \sum_{ij} E_{ij} = \frac{1}{2}\sum_{ij}\left[-\frac{A}{r_{ij}^6} + B\exp(-C \cdot r_{ij})\right] \quad (3)$$

The crystal structure of diazepam for the calculations was taken after Camerman and Camerman (1972). According to these authors diazepam crystallises at room temperature in the space group $P2_1/a$, with four independent molecules in the elementary cell and the parameters of the cell are $a = 12.928$ Å, $b = 13.354$ Å, $c = 7.976$ Å, $\beta = 90.01°$.

As, according to Eq. (2), the potential energy considerably decreases with the distance between atoms, we chose for the calculations a cluster made of three layers each of them composed of 9 elementary cells (27 cells in total) of the size 38.78 Å × 40.06 Å × 23.93 Å, containing 108 molecules (3564 atoms). The calculations were also made for the isolated molecule of diazepam.

Three possible motions of the fragments of the diazepam molecule were considered:

- Rotation of the methyl group about the axis passing through N(1)–C(18).
- Rotation of the phenyl ring about the axis passing through C(5)–C(12).
- Conformational motion of the diazepine ring about the axis passing through C(2)–N(4), in which the position of CH_2 group relative to the plane made by C(2), N(4), N(1) changes.

The selected fragment of the molecule was rotated at the step of 1° in the range from −180° to 180° and at each step the energy was calculated from Eq. (3). The potential energy curves determined in this way for the isolated molecule and for the crystal are shown in Fig. 5.

The methyl group can perform reorientations over the threefold potential energy barrier in which the minima are distanced by 120°. Assuming that the difference between the minimum and maximum of the energy curve is the height of the energy barrier for a given motion, the barriers for the methyl group in the isolated molecule and in the crystal are 10.3 kJ/mol and 11.6 kJ/mol, respectively. The small difference in the height of the energy barriers implies that the barrier is mainly intramolecular. The calculated values of the barriers are slightly higher than that obtained from T_1 experiment. The difference can be attributed to the limitations of the calculation method applied which assumes that the molecules surrounding a given molecule are motionless. In the real crystal the reorientation of neighbouring molecules decreases the potential barrier.

The possibility of the phenyl ring rotation was also considered. The calculations proved that the potential energy related to the rotation of the phenyl ring takes very high values (thousands of kJ/mol) for certain angles, which means that the distances between atoms are below the allowed values. The calculations implied that the phenyl ring cannot rotate by 360° and can change its position in certain ranges of angles. Fig. 5b presents the potential energy curve for the angles at which its value does not exceed 4000 kJ/mol. For the phenyl ring the potential energy dependence on the rotation angle is significantly different from that obtained for the crystal. The potential energy of the phenyl ring shows only one minimum and it can be concluded that in the real system only oscillations of the phenyl ring within ±20° are probable.

We also analysed the conformational motion of the diazepine ring (Fig. 5c). The potential energies calculated for the isolated molecule and the crystal do not differ much. Analysis of the curves indicates a possibility of conformational motion causing reorientations of the CH_2 group by about 60°. The results obtained in our study are consistent with those reported in Paizs and Simonyi (1999), in which the energy barrier for conformational motion of ring of the isolated diazepam molecule was determined by DFT.

3.5. Monte Carlo simulations of second moment

According to our calculations discussed in Section 3.4, the following types of molecular motion are possible: reorientation of CH_3 group by 120°, oscillations of the phenyl ring by ±20° and conformational change of the diazepine ring related to reorientation of CH_2 group by 60°. At the next stage of our analysis we performed a Monte Carlo simulation of the second moment of NMR line M_2 for the diazepam molecule in the crystal performing the abovementioned molecular motions. The results were compared with experimental data.

The second moment can be seen as the square of the average local magnetic field B, induced by dipole–dipole interaction at the position of resonant nuclei by all nuclei in the sample possessing dipolar magnetic moment (Van Vleck, 1948). The theoretical value of the second moment of the NMR line calculated on the basis of the crystallographic data obtained at room temperature (Camerman and Camerman, 1972), corresponding to the rigid lattice, is equal to 14.0 G^2 and is much higher than the experimental value of 7.7 G^2 obtained at $T = 100$ K (Fig. 3). It means that below 100 K the molecule

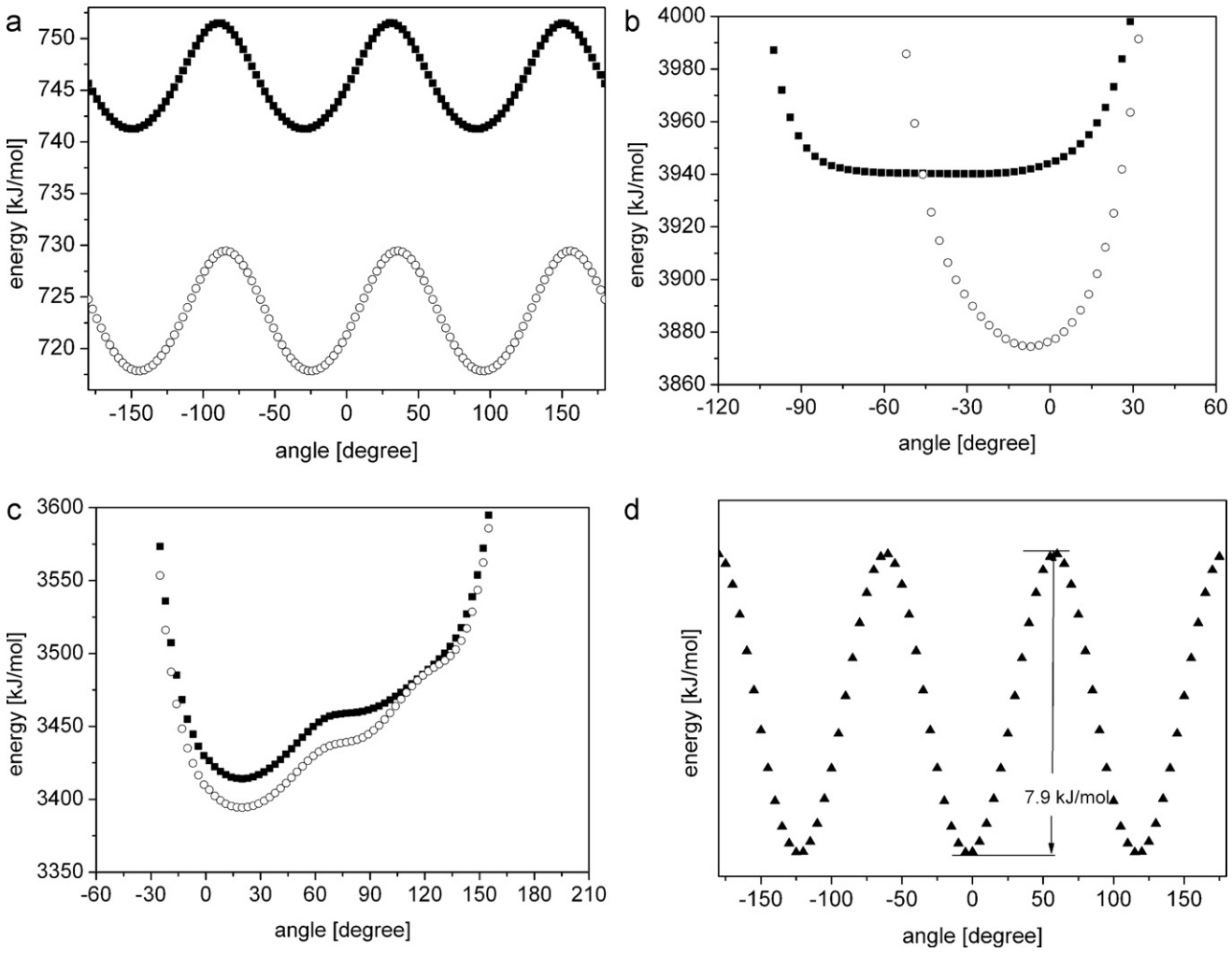

Fig. 5. The potential energy curves corresponding to rotations of particular fragments of the diazepam molecule (calculated according to Eq. (3)) for the isolated molecule (■) and for the crystal (○). The energy values were presented for rotations with a step of 3°. (a) CH_3 group, (b) phenyl ring, (c) CH_2 group related to conformation changes of the diazepine ring. (d) The potential energy curve calculated by DFT taking into regard the rotation of CH_3 group in the optimised molecule.

performs reorientation whose frequency is greater than the absorption line width for the rigid structure. Internal motion of any type would influence the local magnetic fields through the *B* term which depends on relative orientation of interacting spins with respect to the direction of the external magnetic field, as well as on the distance between the interacting spins (Slichter, 1996; Goc, 1998). To explain the complex motion of the diazepam molecule it was necessary to perform to the numerical methods of M_2 calculations. In this paper we present the results of a Monte Carlo simulation of the second moment M_2. The calculations were carried out for the same cluster as described above made of 27 elementary cells. The simulations were performed for all the above-mentioned molecular motions taking into account the possibility of simultaneous reorientations and oscillations about different axes. Selected results of M_2 simulations and the corresponding experimental values are given in Table 2.

Analysis of the values from this table shows that the oscillations of the phenyl ring and the conformational motion of the diazepine ring have little effect on M_2, reducing it to 13.5 and 11.7 G^2, respectively. The methyl group reorientation is responsible for a greater change in M_2 reducing it to 8.6 G^2, which is only by about 1 G^2 higher than the experimental value at 100 K. Therefore, it is reasonable to conclude that the motion observed experimentally below 100 K is related to CH_3 group reorientations. The difference of about 1 G^2 can be a consequence of contractions imposed by the crystal lattice. The change in the second moment observed in the range 250–320 K can be explained by the conformational motion of the diazepam molecule. Similar conclusions were drawn by Andrew et al. (1999) for β-estradiol.

Table 2
Experimental values of the second moment of NMR line M_2 (a) and results of the Monte Carlo simulations of M_2 (b) for diazepam.

	M_2 [G^2]
(a)	
M_2 experimental T = 100 K	7.7
M_2 experimental T = 340 K	7.2
(b)	
Type of motion	
Rigid structure	14.0
Oscillation of phenyl ring (±20°)	13.5
Conformational motion of diazepine ring causing jumps of CH_2	11.7
Reorientation of methyl group	8.6
Combination of reorientation of methyl group and oscillation of phenyl ring (±20°)	8.4
Combination of reorientation of methyl group and Conformational motion of diazepine ring causing jumps of CH_2	7.1
Combination of reorientation of methyl group, oscillation of phenyl ring (±20°) and conformational motion of diazepine ring causing jumps of CH_2	6.9

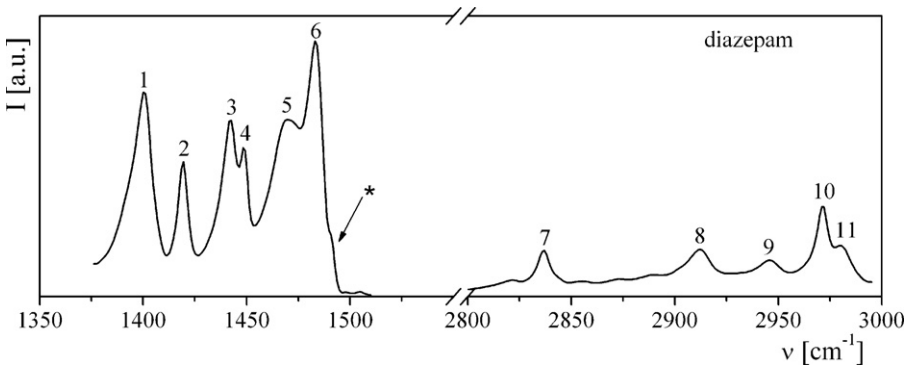

Fig. 6. Experimental FT-IR spectrum of diazepam. Bands numbers are the same as in Table 3.

3.6. DFT quantum mechanical calculations

3.6.1. Geometry optimisation

The paper presents for the first time the optimisation of diazepam molecule structure by the methods of quantum chemistry. The optimised geometry is used then for calculation of internal vibrations of the diazepam molecule. The calculations were carried out in the approximation of the isolated molecule. The geometrical parameters obtained from calculation (bond lengths and bond angles) show good agreement with those determined for the crystal. The Cl–C(9) bond lengths is 1.737 Å in the crystal structure and 1.758 Å in the optimized structure. The length of N(1)–C(2) changes from 1.366 Å to 1.395 Å. The bond length between nitrogen N(1) and carbon C(18) from the methyl group is 1.469 Å and 1.467 Å in the crystal and optimised structures, respectively. A comparison shows that the calculated bond lengths C(2)–O, C(3)–N(4) and N(4)–C(5) are shorter than the corresponding crystallographic. In the optimised and crystal structures these lengths are 1.218 Å and 1.212 Å, 1.461 Å and 1.453 Å, 1.286 Å and 1.281 Å, respectively. The calculations revealed small distortions of the C–C–C angles in the rings (changes below 0.5°). Although, the changes in values over 0.5° (but not larger then 1°) were noted for the bonds: C(6)–C(11)–C(10), C(9)–C(10)–C(11), C(3)–N(4)–C(5), N(4)–C(5)–C(12), C(5)–C(12)–C(13), C(5)–C(12)–C(17). Also relevant is the fact that in the optimized structure of phenyl ring rotates relative to the plane of benzene ring at the angle of 7.8°.

3.6.2. Energy of methyl group reorientation

As follows from the atom–atom calculations (Section 3.4), the influence of intermolecular interactions on the height of the energy barrier for methyl group reorientations is small. It should be noted that only the interactions between non-bonded atoms were taken into account in the calculations, whereas the contribution from other interactions between the methyl group and the rest of the molecule was disregarded. Therefore, it was important to determine this barrier for the isolated molecule by the DFT method in which all the interactions are taken into regard. Energy of the optimised molecule of diazepam was calculated for different positions of CH_3 group, which was rotated about the axis passing through N(1)–C(18) with a step of 5°. The energy dependence on the angle of CH_3 group rotation is shown in Fig. 5d. The barrier is threefold and its height is 7.9 kJ/mol, while the minimum of energy falls at the zero deviation of the CH_3 group, which confirms the correctness of the optimisation. The calculated height of the barrier is in good agreement with that found experimentally on the basis of the relaxation time T_1 measurements and slightly lower than that calculated by the atom–atom method.

3.6.3. Analysis of vibrational frequencies

For the optimised structure of the diazepam molecule, the vibrational frequencies were generated. The vibrational frequencies and approximate description of each normal mode were obtained from DFT calculations. In interpretation of the spectra their position and relative intensities were taken into account. Generally, the calculated and experimental spectra are compatible, but the calculated frequencies are higher than the experimental ones (Fig. 6). This is due to the neglect of the anharmonicity effects in the theoretical treatment (Hehre et al., 1986).

The spectrum was divided for two regions: below 1800 cm^{-1} and 2800–3200 cm^{-1}. Vibrational frequencies were scaled also in these two regions with 0.98707 and 0.94993 respectively. After considering the scaling factor, bands of the experimental spectrum were characterize. Generally, the bands' positions are in good agreement with the description proposed by Neville and Shurvell (1990). Although the results of DFT calculations allowed us to assign a few bands, which have not been characterized by Neville and Shurvell (1990) and Gunasekaran et al. (2006), for example the band at 2944 cm^{-1} was assigned to ν[C–H] in CH_3 (Table 3).

As NMR results indicated the occurrence of reorientations of the CH_3 group and a motion of the CH_2 group, we have concentrated on analysis of vibrations of these two functional groups. The bands associated with the movement of these groups were characterized in two ranges: 1375–1500 cm^{-1} and 2800–3000 cm^{-1}. As shown in Fig. 6 eleven bands were assigned as the stretching (ν) and deformation (δ) modes of CH_2 and CH_3 group or the stretching modes of C–C bonds in ring B and C. Graphical representation of CH_2 and CH_3 modes is shown in Fig. 7.

Comparison of the experimental IR frequencies with those calculated by DFT method and the appropriate assignments are presented in Table 3. For CH_2 group the δ[H–C–H] mode is found at 1471 cm^{-1} while according to DFT calculations it should be at 1475 cm^{-1}. The stretching modes of CH_2 group are assigned in IR spectrum to the bands at 2837 cm^{-1} and 2971 cm^{-1}. On the basis of

Table 3
Experimental and calculated vibration frequencies of diazepam and their assignment.

Bands numbers	IR [cm^{-1}]	DFT [cm^{-1}]	Assignment
1	1400	1404	ν[C–C] in ring B
2	1419	1431	δ[N–C–H] in CH_3
3	1443	1458	ν[C–C] in ring C
4	1448	1469	δ[H–C–H] in CH_3
5	1471	1475	δ[H–C–H] in CH_2
6	1484	1490	ν[C–C] in ring B
*	~1489	1502	δ[H–C–H] in CH_3
7	2837	2835	ν[C–H] in CH_2, sym
8	2911	2872	ν[C–H] in CH_3, sym
9	2944	2951	ν[C–H] in CH_3, asym
10	2971	2973	ν[C–H] in CH_2, asym
11	2982	2998	ν[C–H] in CH_3, asym

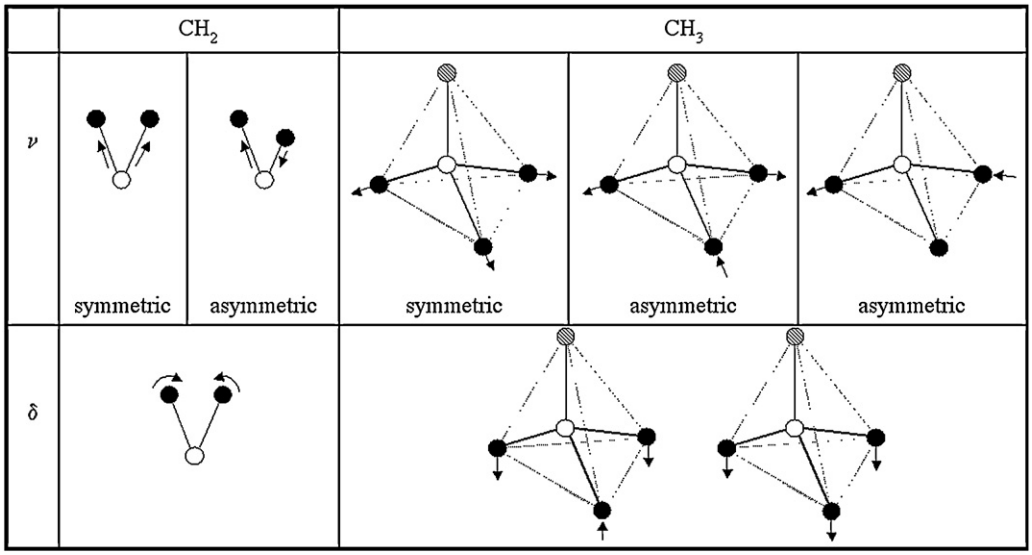

Fig. 7. Stretching (ν) and deformation (δ) modes of CH_3 and CH_2 groups.

the DFT calculations these modes occur at 2835 and 2973 cm^{-1} and are characterized as stretching symmetric and asymmetric modes of CH_2 group, respectively.

For the methyl group three types of deformation modes were defined, observed at 1419, 1448 and on the slope of the band at about 1489 cm^{-1} (marked by asterisk in Table 3). DFT calculations predicted this type of modes at: 1431, 1469 and 1502 cm^{-1}. In the IR spectrum, a band at 2911 cm^{-1} is assigned as symmetric ν[C–H], the bands at 2944 and 2982 cm^{-1} as asymmetric ν[C–H] in CH_3 while the DFT calculations gives the values 2872, 2951 and 2998 cm^{-1}, respectively.

4. Conclusions

The above presented and discussed DSC results imply that diazepam does not undergo phase transitions in temperatures from 100 K to the melting point. NMR study and the atom–atom calculations permitted identification of molecular motions taking place in the diazepam molecule. The motions included: jumps of the methyl group about the threefold axis of the N(1)–C(18) bond, conformational motions of the diazepine ring (B) causing jumps of the CH_2 group by 60° about the axis passing through the C(2)–N(4) atoms. The height of the energy barrier for the methyl group reorientation calculated by the atom–atom method and DFT provided values in agreement with the NMR results.

The frequencies of vibrations of diazepam were recorded by FT-IR and assigned with the help of DFT calculations. The stretching and deformational vibrations of CH_2 and CH_3 groups were assigned in the ranges 1400–1500 cm^{-1} and 2800–3000 cm^{-1}. The frequencies of torsional vibrations of the CH_3 group were most often in the range of the lattice vibrations so below 200 cm^{-1} (Prager et al., 2002) and could not be recorded by the FT-IR method. The inverse of the pre-exponential factor τ_0 (Table 2) indicates that the frequency of the torsional vibrations of the methyl group in diazepam is of about 160 cm^{-1} so in the range of the lattice vibration frequencies of this crystal. This conclusion is confirmed by the frequencies of vibrations calculated by the DFT method.

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