Structure determination by applying vibrational spectroscopy

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Introduction
Vibrational spectroscopy (infrared and Raman) belongs to those structure sensitive techniques which can probe samples in gas, liquid and solid state. It provides structural information for molecules that cannot be probed by conventional high resolution structure dependent technique such as scatterings (X-rays, neutrons) and NMR spectroscopy. However, the core problem of the vibrational spectroscopy is interpretation of spectra. In general, the extraction of the structural data from the vibrational spectra is case dependent. That means that band assignment is followed by the search of structural dependent normal vibrations and their interpretations. The vibrational spectra is composed of absorption, reflection or transmission (type of bands depends on applied technique) of 3N-6 (3N-5 when molecule is linear) normal modes, which are further combined with the anharmonic features such as overtones, combination bands and Fermi resonances. In gas phase are vibrational levels coupled with the rotational degree of freedom which further split the vibrational bands. However, this additional information allows the most accurate determination of the structure, particularly of small gas molecules.

Although the vibrational spectra possess all information about the structure and dynamics of the probed system, the high density of vibrational states encumbers their application. For small molecules, especially when corresponding theoretical treatment is available, these structural vibrational parameters can be retrieved from the spectra. For larger systems only partial information is available. Therefore, we will present, on selected examples (Hydrogen bond, Proton transfer, Structure of dipeptides in water, Protein hydration and Structural studies on graphene), how to retrieve as much as possible information from the vibrations spectra and correlates it with the structure of probed molecule.

Hydrogen bonds
The structural investigations of hydrogen bonds (H-bonds) represent the heydays of vibrational and especially infrared spectroscopy. Its tremendous sensitivity on structural parameters of established H-bond persuades a numerous studies that represent the foundation of the understanding spectral characteristics. These studies on model system enable exploitation of the H-bond patterns in more complex environments such as biomolecules or composite materials.

H-bond (A-H-B) is a donor-acceptor interaction which specifically involves hydrogen atoms. It is formed when electronegativity of A relative to hydrogen in an A-H covalent bond is such as to withdraw electrons and leave proton partially unshielded. To interact with such donor A-H bond, the acceptor B must have lone pair electrons or polarizable π electrons. The formation of H-bond shifts the stretching mode of proton donor group (A-H) to lower frequencies. This frequency downshift is known also as a “redshift”. The magnitude of redshift correlates with H-bond strength and thus with the geometry (length and angle) of the H-bond formation. Frequency redshift is associated with extensive line broadening of the A-H stretching mode due to coupling with low frequency A B mode and anharmonic effects.
As an example of typical H-bond vibrational features we will introduce the spectra of oxalic acid dihydrate\textsuperscript{14,15}. Crystalline solids containing strong H-bond with O–O distances in the range of, say, 2.45Å to 2.65Å, often exhibit interesting and useful physical properties such as ferroelectricity, antiferroelectricity and ionic conductivity. These properties are connected with the hydrogen dynamics that is governed by potential surfaces in the shaping of which the crystal lattice is involved. Oxalic acid dihydrate belongs to this class of materials; it contains a strong H-bond connecting acid's OH with a water molecule, O–O distance ~ 2.50 Å and also exhibits ac conductivity with temperature and frequency indicatives of polaron formation\textsuperscript{16}. A typical infrared spectrum of oxalic acid dihydrate is presented in Fig. 1.

**Figure 1.** Infrared spectrum of oxalic acid dihydrate (left) and iron oxalate dihydrate (right).

Broad absorption extended between 3000 cm\(^{-1}\) and 750 cm\(^{-1}\) (Fig.1 left spectrum, blue shaded) is due to vibration of OH groups of oxalic acid H-bonded with water oxygens. On contrary this absorption completely vanished in the case of oxalate dihydrate, presented in the right spectrum of Fig. 1. Bands with red pattern belong to OH stretching of hydrating water. Raman spectrum of oxalic acid dihydrate is presented in Fig. 2. The characteristic pattern of OH stretching involved in strong H-bond, significantly losses the intensity. Substantial changes in relative intensities of the spectra bands can be observed also for other vibrations in Raman spectrum\textsuperscript{14,15}. These changes with respect to infrared spectra are in general consequence of diverse origin of exciting vibrations which involves different nature of interaction between the electromagnetic waves with matter that leads to different selection rules.

**Figure 2.** Raman spectrum of oxalic acid dihydrate (left) and deuterated analogue (right)
**Proton transfer**

Proton transfer is a crucial mechanism of many chemical and biological processes and has been a subject of intensive studies[17-21]. Proton transfer is a part of fundamental biological reactions, such as enzyme catalysis, respiration and photosynthesis.[22-24]. However, it is an ultrafast process, with a timescale of pico- and femtoseconds which can be directly studied only by the most advanced time-resolved spectroscopic methods. The protonated cations have been extensively studied due to their role in proton transfer and protein pumps and channels where they were detected experimentally. The Zundel cation, H$_5$O$_2^+$, is present in aqueous solutions and comprises a short, strong hydrogen bond - the distance between O atoms is about 2.40 Å. It is formed during the proton transfer between a hydronium ion, H$_3$O$^+$, and a water molecule. The existence of such a cation was theoretically predicted[25] already in 1936 and was experimentally confirmed by G. Zundel[20,21]. However, an issue of stability of the hydronium and Zundel cation is still open. It appears that they are close in energy and that the energy barrier for the proton transfer is either very low or altogether nonexistent[18,24].

![Figure 3. The structure of Zundel cation in crystalline nitranilic acid hexahydrate. Infrared spectra on right represent the nitranilic acid hexahydrate recorded at RT (black) and T=-150°C (red). The blue spectrum is probed nitranilic acid when Zundel cation is not present (sodium salt dihydrate).](image)

An ideal system for study of the proton dynamics in a Zundel cation is nitranilic acid hexahydrate (2,5-dihydroxy-3,6-dinitro-2,5-cyclohexadiene-1,4-dione, Fig. 3). Due to its extremely low pKa values (-3.0 and -0.5, respectively), it actually crystallises as a hydronium salt.

Infrared spectrum of nitranilic acid hexahydrate shows[26] broad complex patterns which are due to vibration of stretchings of OH groups involved in H-bonding with various strength. The bands of three distinct OH groups are located in well separated regions (Fig. 3.). The first one from the left is assigned to vibration of loosely bound water molecules. The position and the band shape resemble to symmetric and antisymmetric stretchings of water molecules in oxalic acid dihydrate. We ascribed both vibrations to OH groups (O$_7$-H-O$_5$) of two water molecules which participate in the Zundel cation. The next feature associated with the frequency shift and broadness of the vibrational band suggests that this band originates from the OH groups, which are involved in the stronger H-bond formation.

Apparently candidates for such type of H-bonding are the OH groups (O$_6$-H$_{6AB}$-O$_7$) of water molecules that are a part of the Zundel cation. The last region between the 2000 cm$^{-1}$ down to far infrared represents so called Zundel continuum (magenta ellipsoid). This broad
region topped with the internal modes of nitranilic acid and Fermi transmissions confirms the existence of very strong H-bond of proton spanned between two oxygens (O₅-H₁-O₆).

**Structure of dipeptides in water**

A basic unsolved problem in protein folding is creating an accurate calculation of the folding energetics of flexible peptides. Accurate experimental results for the major backbone conformations are needed to test prediction methods. Even the simple problem of calculating the \( \phi, \psi \) Ramachandran map of the alanine dipeptide is beyond the reach of standard force fields used in molecular dynamics simulations. There are two probable reasons: one is that the energy differences between the major backbone conformations are small, and the second is that standard force fields contain so many parameters that errors cannot be found readily by comparing simulations with experimental results. The ability to calculate accurately the relative energies of the various backbone conformations is needed for simulating early stages of the protein folding process, which is an important current problem in molecular biophysics.

We utilized the infrared and Raman spectroscopy for determination of the backbone conformation of 19 dipeptides in water\(^{27-29}\). Dipeptides are in form of \( N \)-acetyl-X-\( N' \)-methylamide, where \( X \) represents an amino acid (Fig. 4). The conformation of dipeptides was determined by analysis of amide III region in Raman and infrared spectra. The examples of structure determination are presented in Figure 5. Such analysis retrieves three distinct conformers with respect to \( \phi \) and \( \psi \) dihedral angles. Due to extensive overlapping of the bands in amide III region, band fitting procedure was applied. Assignment of the bands from the amide III region was checked by temperature measurements, by changing the type and \( \text{pH} \) of solvent. The conformational changes of these measurements calculated from the vibrational spectra\(^{29}\) are in agreement with conformational changes calculated from the measured NMR \( ^3J_{\text{HN}A} \) coupling constants\(^{27}\) and with the theoretical predictions.

![Figure 4. Three distinct conformations of alanine dipeptide in water](image)

The bands that appear in both infrared and Raman spectra of solvated dipeptides are near 1313 cm\(^{-1}\), 1290 cm\(^{-1}\) and 1280 cm\(^{-1}\) and correspond to amide III vibrations of \( \text{PP}_{\|} \), \( \alpha_R \) and \( \beta \) conformers of dipeptides, respectively. The frequencies of bands, which are assigned to particular backbone conformation, slightly vary (\( \pm 5 \) cm\(^{-1}\) for \( \text{PP}_{\|} \) or \( \alpha_R \), \( \pm 7 \) for \( \beta \)) with respect to the type of the sidechain. The populations of \( \text{PP}_{\|}, \alpha_R \) and \( \beta \) conformers were found in all examined dipeptides. However, the ration of these conformers depends on the type of the sidechains. The \( \text{PP}_{\|} \) is a prevailing conformation in alanine, leucine and lysine dipeptides, while in cysteine, threonine, valine, aspartic acid, histidine, and isoleucine dipeptides \( \beta \) conformation is dominant (Fig. 6). The least populated conformation found in dipeptides in water is \( \alpha_R \). In general it is below 12 %.
Figure 5. Decomposition of the amide III region with model bands

The only exception is glycine dipeptide, where the calculated population of $\alpha_R$ is $64 \pm 2 \%$. The calculated populations of conformers of dipeptides derived from the infrared spectra, Raman spectra and NMR $^3J_{HN}$ coupling constants mutually correlates with very high correlation indexes ($R_{IR}=0.99$, $R_{IR,NMR}=0.94$). From the extrapolation of the data in correlation chart of $\beta$ population and the values of $^3J_{HN}$ coupling constant to the limit values of populations, gives very reasonable values for coupling constants for pure $\beta$ and PP$_{II}$ conformation states. These values (9.55 Hz for $\beta$ and 4.95 for PP$_{II}$) are in a good agreement with ones calculated from the NMR measurements$^{27}$.

Figure 6. The distribution of three different population for all 19 dipeptides (left) and correlation between the populations of $\beta$ conformers determined from vibrational analysis and NMR spectroscopy

Conclusions
Presented examples show that proper manipulation of the vibrational spectra retrieve structural data of probed molecules. However, the interpretation of spectra is not straightforward and in general a series of measurements should be applied to determine the structural sensitivity of the particular vibrational bands. Some examples are listed above, some other such as protein hydration$^{30-34}$ and vibrational analysis of graphene$^{35}$ will be included in oral presentation.
References:
1 Griffiths P. R. (2002). *Introduction to Vibrational Spectroscopy*. John Wiley and Sons Ltd Chichester
5 Bellamy J. L. (1968). *Advances in Infrared Group Frequencies*. Methuen and Co. Ltd