Raman spectroscopic characterization of anti-diabetic drug metformin hydrochloride

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Abstract. Raman scattering spectra of metformin hydrochloride, one of the most widely used oral anti-diabetic drugs which also possesses anti-aging and anti-tumor properties, have been obtained by high-resolution and high-sensitivity Raman-scattering technique. It is established that rather narrow (4–10 cm⁻¹) spectral lines of molecular vibrations are observed in the metformin hydrochloride pressed powder sample in the important spectral range of 50–1700 cm⁻¹. These “fingerprints” can be applied to reveal which chemical bonds produce spectral components corresponding to the vibrations of particular functional groups and the presence of crystalline state of the metformin hydrochloride without evidence of an amorphous phase. This observation makes the metformin hydrochloride as promising model system for development and application as molecular biosensors.

1. Introduction
Metformin hydrochloride — an organic compound (chemical formula, C4H11N5 · HCl) containing two methyl groups at one end of the molecule — was originally discovered in the Galega officinalis herbaceous plant and proved to be one of the most effective anti-diabetic drugs. The metformin hydrochloride belongs to the class of biguanides representing derivatives of colorless crystalline compound guanidine (CH5N3) [1, 2]. It is widely used as the main first-line oral drug of choice in the therapy of patients with diabetes mellitus type II diagnosis in combination with obesity. The metformin hydrochloride is one of the two oral anti-diabetic drugs included in the World Health Organization (WHO) Model List of Essential Medicines [3]. It important that in addition, this drug
also favors improved utilization of glucose in tissues, reduction in the use of fatty acids as an energetic substrate in the organism, and a decrease in the levels of blood cholesterol, triglycerides, and insulin, as well as loss of excess body weight [1–4]. Furthermore in recent years, it has been also shown that the metformin hydrochloride produces geroprotective (anti-aging), anti-carcinogen, and anti-tumor effects [4–8] and is among the best agents for the prophylaxis of cardiovascular complications of diabetes mellitus [1–5, 9]. On the other hand, the drug can produce a limited number of undesirable side effects [5, 9]. Consequently there is a need for significance feedback to be more effectively and accurately monitored. Despite the broad range of biological activity and good results of the metformin hydrochloride therapy, the complicated physicochemical molecular mechanisms of the drug’s action are still not completely clear [1, 4, 6, 7]. One of the reasons is so little knowledge about its molecular interaction properties with other binding partner components, including proteins, DNAs, etc. New investigations are also necessary including the development of effective methods for the monitoring of a major chemical activity processes on the base of the control of its molecular structure.

Current literature data have reported several approaches to study molecular structure of the metformin hydrochloride obtained by using melt granulation technology to develop a high drug loaded matrix tablet of the metformin hydrochloride with a hydrophilic binder and stearic acid as an extrusion aid for producing cohesive granules and using an unidentified Raman scattering lines to show the uniform distribution of the metformin hydrochloride in the tablet [9]. In the case of other recent Raman study of the structural dynamics of metformin drug [10] the effective spectral slit width was limited by ~5 cm⁻¹. Moreover, the Fourier-transform infrared measurements demonstrated nonexistence of chemical interaction between the metformin hydrochloride drug and the other polymer excipients [11]. In other recent report surface enhanced Raman scattering is used to study the metformin hydrochloride aggregated with Ag nanoparticles [12]. For large response of the system, aggregating metallic nanoparticles should form discrete clusters. In addition, for chemisorbed molecules that are in direct contact with the metallic surface, additional enhancement can occur because of the appearance of the coupling between the electronic orbitals of the molecule and states of the conduction band of the metal. This enhancement can reach one or two orders of magnitude. At the same time, however, we note that the adsorbed molecule and metallic nanoparticle can form a new complex. As a result of these effects, the appearing internal giant electric fields and the formation of molecular complexes depend strongly on a number of physicochemical properties of the system under consideration and can significantly affect the spectral parameters of the corresponding lines of scattered light for the metformin hydrochloride. Therefore, the analytical capabilities of the surface enhanced Raman scattering spectroscopy became limited if the aim of such studies is to analyze the undistorted molecular structures of the metformin hydrochloride. Thus, a key to obtaining more correct and precision results for the determination molecular structure and mechanisms of intermolecular interactions it is necessary also development of highly sensitive methods of molecular spectroscopy of the pure metformin hydrochloride molecules without additional molecular probes containing metallic nanostructures and fluorescent labels.

Here we present results of the investigation of the metformin hydrochloride by the developed method of the non-resonant Raman spectroscopy at high spectral and spatial resolution and high level of detection sensitivity [13]. These measurements revealed complicated spectra of the metformin hydrochloride complexes and, in particular, allowed narrow spectral lines corresponding to separate molecular groups in the metformin hydrochloride structure to be resolved. The obtained results showed that the optimized high-resolution non-resonant Raman-scattering measurements can be successfully used for studying the molecular structure and elucidating the chemical nature of inter- and intramolecular interaction in the pressed metformin hydrochloride powder.

2. Experiment
The spectra of non-resonant Raman scattering were measured at room temperature using specially pressed tablets of the metformin hydrochloride powder (1, 1-dimethylbiguanidine from MP Biomedicals Co., Solon, OH, United States). The Raman spectra were excited by second-harmonic
radiation of an yttrium aluminum garnet laser operating at $\lambda_i = 532$ nm with linewidth below 0.00001 nm [14, 15]. The spectrum of scattered light was analyzed by a LabRAM HR800 spectrometer (Horiba Jobin-Yvon, France). The spectrometer was equipped with a holographic narrow band notch filter blocking high Raleigh light scattered component, a 1800 groove/mm holographic grating and with a Peltier-cooled charge coupled device detector. The optimized spectral resolution was 2 cm$^{-1}$. The laser excitation was focused on the sample using a chromatic Olympus micro objective of 100x size in a back scattering geometry. The diameter of the spot of the laser radiation at the focus was $\sim 900$ nm that determined also the spatial resolution.

3. Results and discussions

Figure 1 show the typical spectrum of a non-resonant Raman scattering from the metformin hydrochloride powder obtained at room temperature in the important spectral range of 60–1700 cm$^{-1}$. The background intensity was rather small, as well as the contribution of signal from a glass substrate. The experimental results demonstrate that the achieved optimized high spectral and spatial resolution and high level of detection sensitivity are sufficient to detect high quality the relatively intense Raman spectrum necessary to reveal clearly pronounced sharp spectral lines. It is established that rather narrow spectral lines with a halfwidth (the full width at the half-maximum, FWHM) 4–10 cm$^{-1}$ are observed in the whole detection spectral range.

![Figure 1](image-url)

**Figure 1.** Room-temperature Raman spectrum of the pressed metformin hydrochloride powder obtained in the 50–1700 cm$^{-1}$ frequency range. The spectral resolution $R = 2$ cm$^{-1}$.

The Raman spectra of the metformin hydrochloride as other macromolecules are determined by their spatial structure, which is specified by a large number of atoms vibrating around the equilibrium positions. It is known that the metformin hydrochloride crystallize in monoclinic crystal system, space group $P2(1)/n$ with the following lattice parameters: $a = 7.991(3)$, $b = 13.950 (5)$, $c = 8.020 (2)$ nm, $\beta = 114.98 (3)$ $^\circ$ [16]. At the same time, an exact number of formula units in a unit cell of the metformin
hydrochloride is not known. Usually for this type of compounds there are at any case four formula units. Therefore it is clear that for such a complicated molecule structure composed of the twenty constituent atoms in the formula unit the number of normal vibrational modes active in the first-order Raman scattering is rather high (several hundreds). Moreover, the spectral range for the frequency shifts of these modes is limited and, correspondingly, overlapping spectral contributions generated by such a large set of atoms and molecules should be observed. Therefore the experimental observation in the obtained Raman spectrum of the the metformin hydrochloride powder pretty narrow spectral lines is rather unexpected. For example, the FWHM of the isolated individual spectral line at 936 cm$^{-1}$ assigned to pendulum stretching vibrations of N–H bonds [11] was 5 cm$^{-1}$. Spectra detected with the spectral resolution of 0.5 cm$^{-1}$ demonstrated almost the same FWHM, but with fairly less signal/noise ratio. Therefore the unsmoothed spectrum in Fig. 1 obtained with the spectral resolution of 2 cm$^{-1}$ (at the excitation 532 nm laser-line) has proved to be sufficient for the separating spectral components corresponding to the vibrations of particular constituent molecular groups. For example, some other spectral lines can be tentatively assigned to C-N-C deformation at 424 cm$^{-1}$, at 739 and 935 cm$^{-1}$ to N-H wagging, and at 1571 and 1653 cm$^{-1}$ to C = N bonds in reasonable agreement with the theoretical calculations using the \textit{ab-initio} Hartree–Fock and Density Function Theory methods [11].

Observation of the sharp narrow spectral lines with a small background intensity in the spectrum of Fig. 1 also directly indicate reveiling microstructure nature of the metformin hydrochloride powder, namely the presence of crystalline state of the metformin hydrochloride without evidence of an amorphous phase. This observation may be useful as accurate technique for determining the degree of crystallinity of the metformin hydrochloride powder. Variations of the spectral parameters must also be observed for all functional groups influenced by pair interactions and the environment including in a different physical mixture forms, for example the crystalline state of the metformin hydrochloride as a drug in the matrix tablet. Our conclusion on the crystalline properties is in a good agreement with the recent X-ray diffraction data of the pure metformin hydrochloride powder [11]. These observations draw special attention to the metformin hydrochloride for its practical use for development and application as molecular biosensors.

4. Conclusions
On the whole, the experimental data obtained in the Raman scattering of the metformin hydrochloride powder sample showed that the optimized high spectral and spatial resolution were sufficient to reveal clearly pronounced narrow spectral lines with the rather small background intensity. These observations allows to separate spectral components corresponding to vibrations of particular molecular groups in the metformin hydrochloride molecules containing relatively large numbers of atoms per unit cell. In addition obtained data permitted to reveal presence of crystalline state of the metformin hydrochloride powder sample without evidence of amorphous phase. In conclusion, obtained results may find useful applications in developing spectrometric biomarkers with high chemical specificity and to investigate open questions of molecular mechanisms of interactions that may occur between the metformin hydrochloride and biological tissues important for emerging precision medical diagnosis.

Acknowledgments
The authors acknowledge the partial support from the Presidium of the Russian Academy of Sciences Program No. 5: Photonic technologies in probing inhomogeneous media and biological objects.

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