

Botulinum Toxin Time-Resolved Absorption and Resonance FT-IR and Raman Biospectroscopy and Density Functional Theory (DFT) Investigation of Vibronic-Mode Coupling Structure in Vibrational Spectra Analysis

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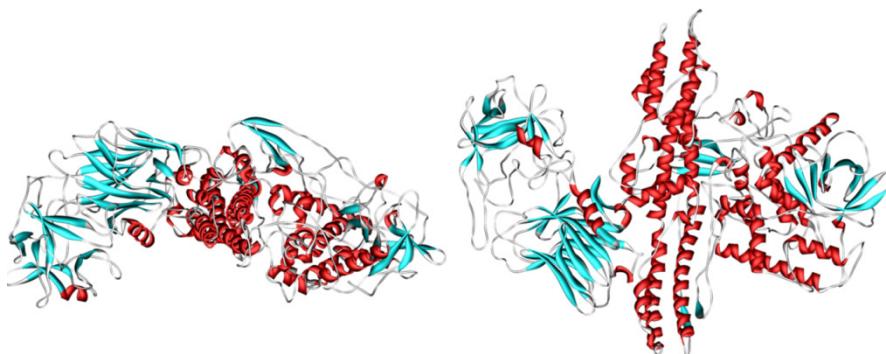
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Abstract Parameters such as FT-IR and Raman vibrational wavelengths and intensities for single crystal Botulinum Toxin are calculated using density functional theory and were compared with empirical results. The investigation about vibrational spectrum of cycle dimers in crystal with carboxyl groups from each molecule of acid was shown that it leads to create Hydrogen bonds for adjacent molecules. The current study aimed to investigate the possibility of simulating the empirical values. Analysis of vibrational spectrum of Botulinum Toxin is performed based on theoretical simulation and FT-IR empirical spectrum and Raman empirical spectrum using density functional theory in levels of HF/6-31G*, HF/6-31++G**, MP2/6-31G, MP2/6-31++G**, BLYP/6-31G, BLYP/6-31++G**, B3LYP/6-31G and B3LYP/6-31-HEG**. Vibration modes of methylene, carboxyl acid and phenyl cycle are separately investigated. The obtained values confirm high accuracy and validity of results obtained from calculations.



Molecular structure of Botulinum Toxin [1-42].

Keywords: Vibronic Structure, Vibrational Spectra Analysis, Density Functional Theory (DFT), Botulinum Toxin, Non-Focal Functions of Becke, Correlation Functions of Lee-Yang-Parr, Time-Resolved Absorption and Resonance, FT-IR and Raman Biospectroscopy

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1. Introduction

Botulinum toxin (BTX) is a neurotoxic protein produced by the bacterium *Clostridium botulinum* and related

species. It prevents the release of the neurotransmitter acetylcholine from axon endings at the neuromuscular junction and thus causes flaccid paralysis. Infection with the bacterium causes the disease botulism. The toxin is also used commercially in medicine, cosmetics and research. Density Functional Theory (DFT) is one of the

most powerful calculation methods for electronic structures [5,6,7]. Numerous results have been previously studied and indicate successful use of these methods [8,9,10]. The theory is one of the most appropriate methods for simulating the vibrational wavenumbers, molecular structure as well as total energy. It may be useful to initially consider the calculated results by density functional theory using HF/6-31G*, HF/6-31++G**, MP2/6-31G, MP2/6-31++G**, BLYP/6-31G, BLYP/6-31++G**, B3LYP/6-31G and B3LYP6-31-HEG** approach [11-16]. It should be noted that calculations are performed by considering one degree of quantum interference as well as polarization effects of 2d orbitals in interaction [17-343].

2. Details of Calculations

All calculations of molecular orbital in the base of ab are performed by Gaussian 09. In calculation process, the structure of Botulinum Toxin molecule (Figure 1) is optimized and FT-IR and Raman wavenumbers are calculated using HF/6-31G*, HF/6-31++G**, MP2/6-31G, MP2/6-31++G**, BLYP/6-31G, BLYP/6-31++G**, B3LYP/6-31G and B3LYP6-31-HEG** base. All optimized structures are adjusted with minimum energy.

Harmonic vibrational wavenumbers are calculated using second degree of derivation to adjust convergence on potential surface as good as possible and to evaluate vibrational energies at zero point. In optimized structures considered in the current study, virtual frequency modes are not observed which indicates that the minimum potential energy surface is correctly chosen. The optimized geometry is calculated by minimizing the energy relative to all geometrical quantities without forcing any constraint on molecular symmetry. Calculations were performed by Gaussian 09. The current calculation is aimed to maximize structural optimization using density functional theory. The calculations of density functional theory are performed by HF/6-31G*, HF/6-31++G**, MP2/6-31G, MP2/6-31++G**, BLYP/6-31G, BLYP/6-31++G**, B3LYP/6-31G and B3LYP6-31-HEG** function in which non-focal functions of Becke and correlation functions of Lee-Yang-Parr beyond the Franck-Condon approximation are used. After completion of optimization process, the second order derivation of energy is calculated as a function of core coordination and is investigated to evaluate whether the structure is accurately minimized. Vibrational frequencies used to simulate spectrums presented in the current study are derived from these second order derivatives. All calculations are performed for room temperature of 343 (K).

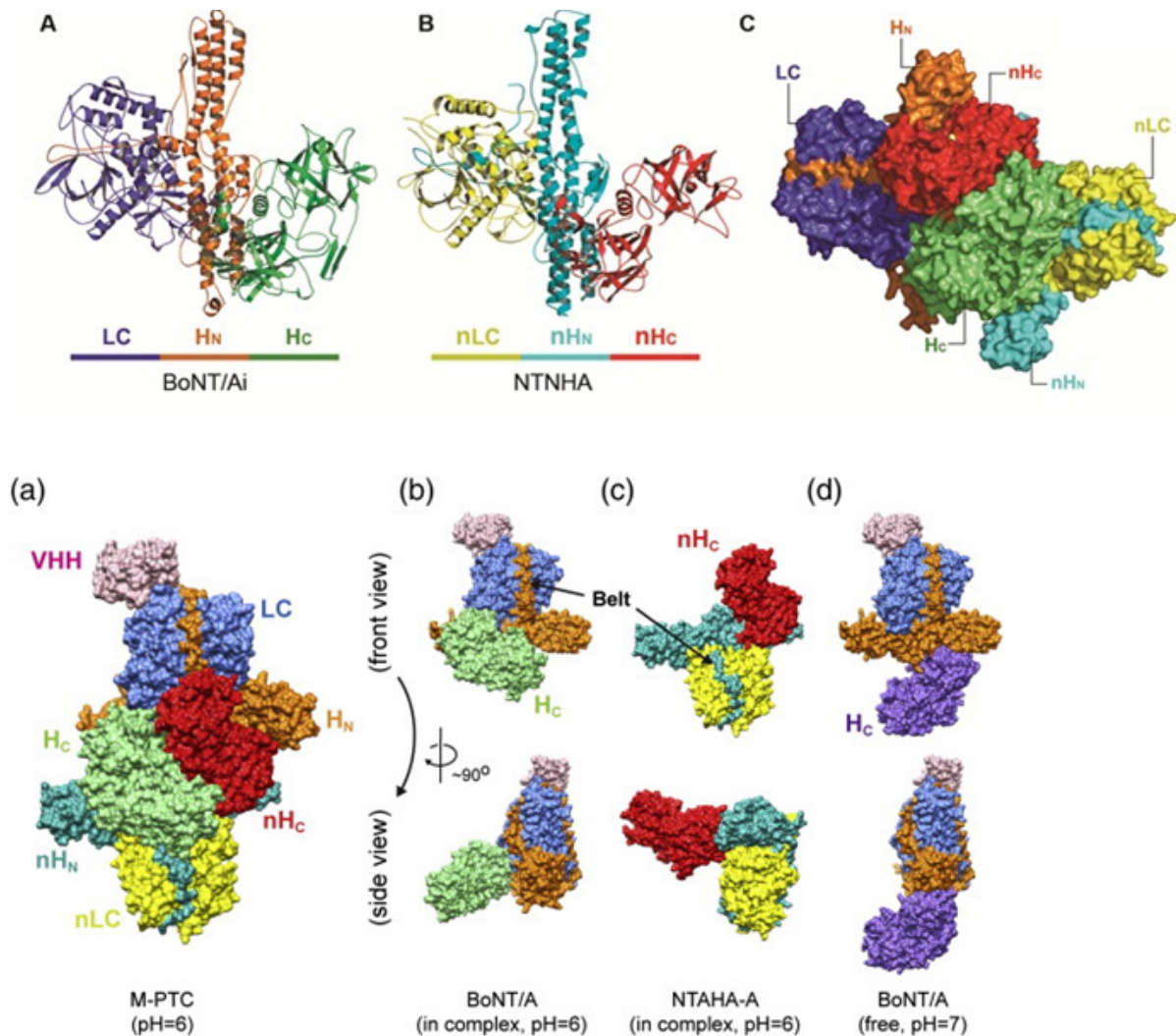


Figure 1. Different sections of the Botulinum Toxin [43-93]

3. Vibration Analysis

Analysis of vibrational spectrum of Botulinum Toxin is performed based on theoretical simulation and FT-IR empirical spectrum and Raman empirical spectrum using density functional theory in levels of HF/6-31G*, HF/6-31++G**, MP2/6-31G, MP2/6-31++G**, BLYP/6-31G, BLYP/6-31++G**, B3LYP/6-31G and B3LYP6-31-HEG**. Vibration modes of methylene, carboxyl acid and phenyl cycle are separately investigated.

C-H stretching vibrations in single replacement of benzene cycles are usually seen in band range of 3180-3430 cm^{-1} . Weak Raman bands are at 3169 cm^{-1} and 3182 cm^{-1} . C-C stretching mode is a strong Raman mode

at 1179 cm^{-1} . Raman weak band is seen at 1673 cm^{-1} , too. Bending mode of C-H is emerged as a weak mode at 1378 cm^{-1} and 1177 cm^{-1} and a strong band at 1261 cm^{-1} in Raman spectrum. Raman is considerably active in the range of 1180-1430 cm^{-1} which 1173 cm^{-1} indicates this issue.

C-H skew-symmetric stretching mode of methylene group is expected at 3165 cm^{-1} and its symmetric mode is expected at 2979 cm^{-1} . Skew-symmetric stretching mode of CH_2 in Botulinum Toxin has a mode in mid-range of Raman spectrum at 3080-3200 cm^{-1} . When this mode is symmetric, it is at 3075 cm^{-1} and is sharp. The calculated wavenumbers of higher modes are at 3043 cm^{-1} and 3073 cm^{-1} for symmetric and skew-symmetric stretching mode of methylene, respectively.

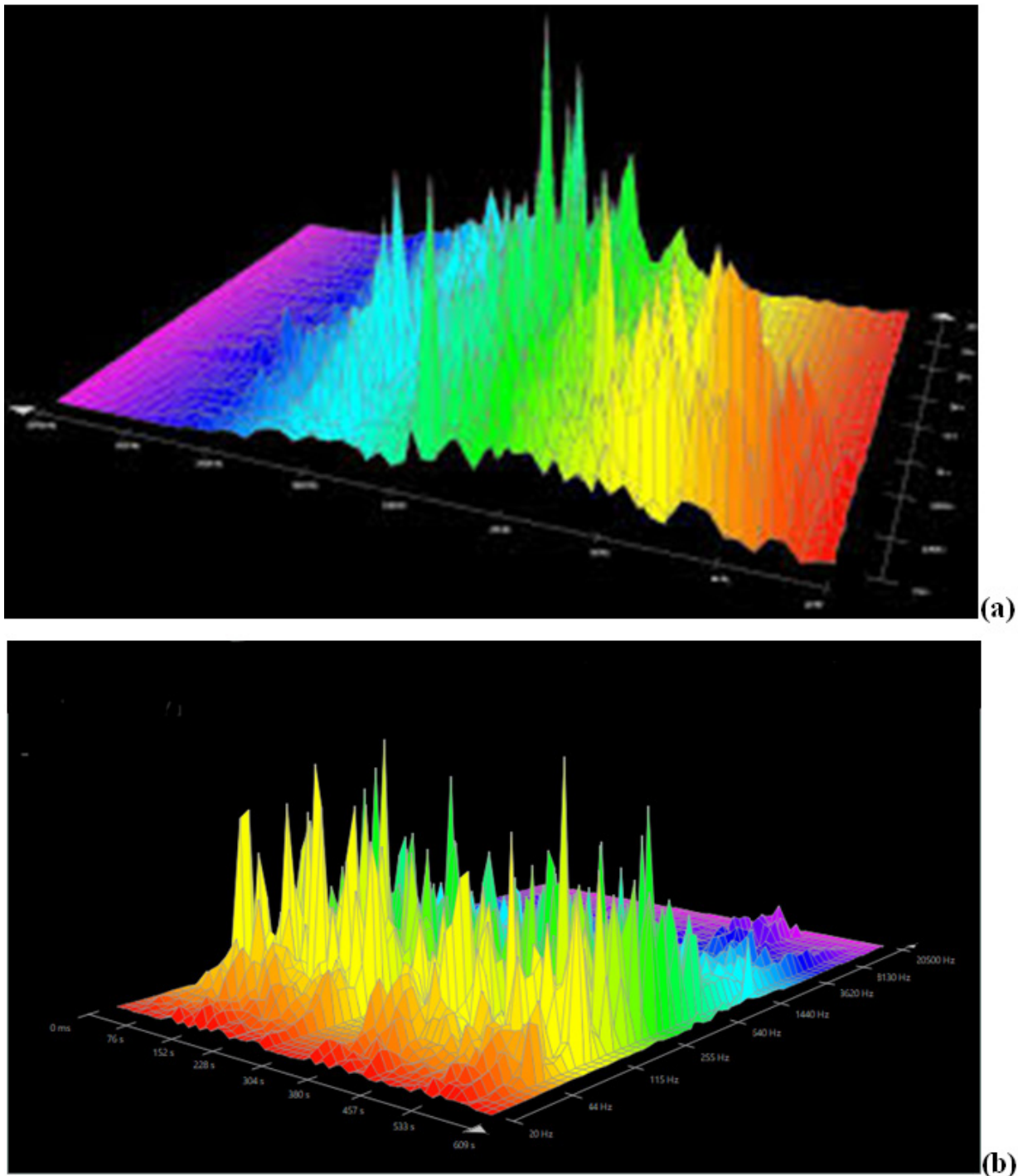


Figure 2. 3D Simulation of (a) FT-IR spectrum and (b) Raman spectrum of Botulinum Toxin

Scissoring vibrations of CH₂ are usually seen at the range of 1507–1561 cm⁻¹ which often includes mid-range bands. Weak bands at 1520 cm⁻¹ are scissoring modes of CH₂ in Raman spectrum. Moving vibrations of methylene are usually seen at 1449 cm⁻¹. For the investigated chemical in the current study, these vibrations are at 1319 cm⁻¹ were calculated using density functional theory. Twisting and rocking vibrations of CH₂ are seen in Raman spectrum at 895 cm⁻¹ and 1169 cm⁻¹, respectively, which are in good accordance with the results at 879 cm⁻¹ and 1144 cm⁻¹, respectively.

In a non-ionized carboxyl group (COOH), stretching vibrations of carbonyl [C=O] are mainly observed at the range of 1820–1868 cm⁻¹. If dimer is considered as an intact constituent, two stretching vibrations of carbonyl for symmetric stretching are at 1720–1765 cm⁻¹ in Raman spectrum. In the current paper, stretching vibration of carbonyl mode is at 1777 cm⁻¹ which is a mid-range value.

Stretching and bending bands of hydroxyl can be identified by width and band intensity which in turn is dependent on bond length of Hydrogen. In dimer form of Hydrogen bond, stretching band of O–H is of a strong Raman peak at 1347 cm⁻¹ which is due to in–plain metamorphosis mode. Out–of–plain mode of O–H group is a very strong mode of peak at 1029 cm⁻¹ of Raman spectrum. The stretching mode of C–O (H) emerges as a mid–band of Raman spectrum at 1227 cm⁻¹.

Lattice vibrations are usually seen at the range of 0–900 cm⁻¹. These modes are induced by rotary and transferring vibrations of molecules and vibrations and are including Hydrogen bond. Bands with low wavenumbers of Hydrogen bond vibrations in FT–IR and Raman spectrum (Figure 2) are frequently weak, width and unsymmetrical. Rotary lattice vibrations are frequently stronger than transferring ones. Intra–molecular vibrations with low wavenumbers involving two–bands O–H ...O dimer at 68 cm⁻¹, 173 cm⁻¹ and 229 cm⁻¹ are attributed to a rotary moving of two molecules involving in–plain rotation of molecules against each other.

4. Conclusion and Summary

Calculations of density functional theory using HF/6–31G*, HF/6–31++G**, MP2/6–31G, MP2/6–31++G**, BLYP/6–31G, BLYP/6–31++G**, B3LYP/6–31G and B3LYP6–31–HEG** levels were used to obtain vibrational wavenumbers and intensities in single crystal of Botulinum Toxin. Investigation and consideration of vibrational spectrum confirm the formation of dimer cycles in the investigated crystal with carboxyl groups from each Hydrogen molecule of acid protected from adjacent molecules. The calculated vibrational spectrum which obtains from calculations of density functional theory is in good accordance with recorded empirical values which indicates successful simulation of the problem. The obtained results indicate that the results obtained from theoretical calculations are valid through comparing with empirical recorded results.

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