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Executive Summary of the Thesis

Computational model of drugs by DFT and Raman spectroscopy: a study on Perampanel

Laurea Magistrale in Materials Engineering and Nanotechnology - Ingegneria dei Materiali e delle Nanotecnologie

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1. Introduction

Perampanel (PER) is an anti-epileptic drug, sold under the commercial name of Fycompa. It is used to treat patients affected by epilepsy to prevent seizures. The precise and constant tuning of Perampanel dosage, to date, is done by *Therapeutic Drug Monitoring* (TDM) which is a very reliable but expensive and time-consuming technique. PER studies focusing on non-invasive, and cheaper, monitoring methods (*e.g.*, Raman spectroscopy analyses on saliva slabs), evidenced that Perampanel could be analysed in SERS conditions [1]. Moving from this consideration, the research work presented here is a computational effort to understand the molecular and vibrational structure of PER, in view of the possible application of SERS to analyse PER.

2. Methods

It is here presented the computational simulation of a PER model using molecular mechanics. Given the Born-Oppenheimer approximation, molecular mechanics relies on both quantum physics and classical mechanics to describe the potential energy of the systems with force fields. To quantify these energies different strategies can be adopted.
This research relied on the *Density Functional Theory* (DFT) method and the computational work was performed with the aid of Gaussian 09. More specifically, the geometry optimization calculations, executed under *very tight* convergence criteria, were carried out with the B3LYP hybrid functional while the basis functions belong to the 6-31G(d,p) basis set. The effect of the Van der Waals interaction was taken into account by the empirical dispersion scheme devised by Grimme, including Becke–Johnson Damping.

3. Gas-phase model of Perampanel

Perampanel (Fig. 1) counts forty-two atoms thus more than one hundred vibrational degrees of freedom: a systematic exploration of the Potential Energy Surface (PES) is only feasible upon some approximations.

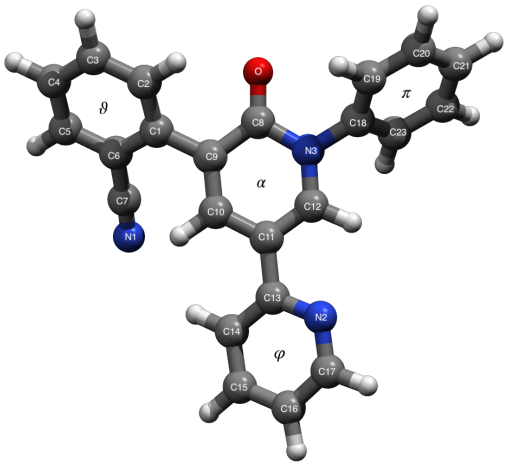
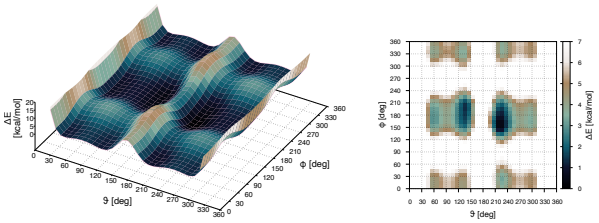


Figure 1: Structure of PER molecule in CPK coloring scheme with atom labelling and ring notation.

Firstly, the PES was scanned by rotating the two dihedrals angles between the planes of the α ring and the ϑ and ϕ rings, respectively. These calculations allowed us to retrieve twelve local minima in the PES (represented in Fig. 2), whose corresponding atomic coordinates were used as input for the subsequent geometry optimization calculations.



(a) (b)

Figure 2: 3D PES (a) and its projection onto ϑ and ϕ axis (b).

Of all the conformers determined by the full geometry relaxation of the aforementioned minima, I focused on those that were interesting from an energetic point of view (PER_A and PER_B, *i.e.*, the absolute and the neraby local minimum) and on PER_H, which was found to be very similar to the ligand 6ZP of the 5L1F query in the *RCSB Protein Data Bank* [5]. Again, an energy scan was carried out on the aforementioned conformers, by rotating the last aromatic ring (π). The atomic coordinates of the minima retrieved by these calculations were collected and used to obtain three relaxed conformers in stable conformation. In the following table (Table 1) the characteristic values of the dihedrals ϑ, ϕ and π are reported for the three PER conformers just mentioned, with the respective values of the potential energy difference with respect to the absolute minimum (PER_A).

Table 1: ϑ, ϕ and π values tabulated with corresponding ΔE.

Name	ϑ	ϕ	π	ΔE [kcal/mol]
PER_A	141.37°	-157.68°	-55.14°	0.0
PER_B	-141.37°	158.67°	55.14°	0.0
PER_H	56.71°	-17.02°	-55.32°	2.38

What became apparent by these last calculations was the one-to-one correspondence between the sub-conformers of PER_A and PER_B: the values assumed by ϑ, ϕ and π returned four enantiomeric couples.

Comparison with experimental data

Recalling the aim of this research, in this section I report a comparison between the computational results and available experimental Raman data. There were minimal to none variations in the spectra of one conformer with respect to the other (PER_A and PER_B spectra are exactly the same). But, to make a comparison with the experimental data let us use the PER_H spectrum which is supposedly most similar to the ligand as seen in the previous paragraphs. Experimental data come from the Raman spectroscopical analyses of powdered Perampanel [4].

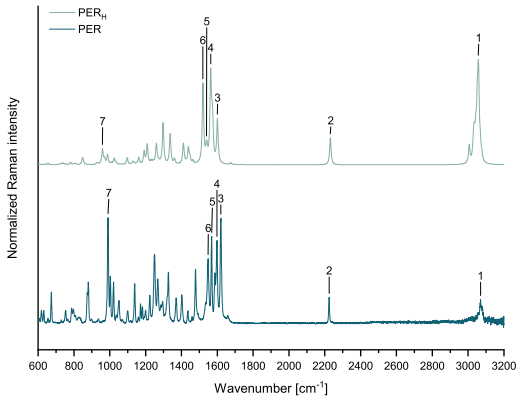


Figure 3: Comparison between simulated PER_H spectrum from DFT calculations (top) and experimental data on solid Perampanel (bottom)

In Fig. 3 it can be observed how the theoretical data fit closely the experimental data. With an empirical uniform frequency scaling factor of

0.95, all the marker peaks of Perampanel find confirmation in the experimental spectrum.

Table 2: Assignment of the main Raman peaks of PER

Label	Mode	Wavelength [cm^{-1}]
1	C-H stretching	3057
2	C≡N stretching	2231
3, 4, 5, 6	C-C stretching	1600, 1563 1540, 1520
7	Ring breathing	959

4. Model of protonated Perampanel

The strategy for investigating protonated Perampanel was the same as the one described above: firstly, the definition of the equilibrium geometries of possible isomers, then the analysis of the theoretical Raman spectra.
Specifically, starting from the structures retrieved previously, namely PER_A (PER_B was excluded in virtue of its enenatiomeric symmetry with PER_A) and PER_H, eight isomers were simulated: one for each of the four protonation sites (the three nitrogen and the oxygen atoms) for each conformer. Compared with the associated neutral forms, all of these isomers showed a decrese of energy by circa 200 kcal/mol. More to the point, the isomers that were found to be most energetically convenient were the ones corresponding to $H^+N_1PER_A$ and $H^+N_2PER_H$ (*i.e.*, the hydronated molecules with the proton placed near the N_1 nitrogen the former, and near the N_2 atom the latter, see Fig. 1 for reference) with a ΔE (with respect to the non-protonated molecule) of -244.02 and -242.61 kcal/mol respectively. In both cases the second least energetic isomers were the ones with the H^+ ion bound to the oxygen (H^+OPER_A ΔE = -240.17 kcal/mol and H^+OPER_H ΔE = -241.30 kcal/mol).
The addition of the proton to the conformers, of course, generated modifications in the equilibrium geometries: for example, placing the hydrogen ion near N_3 induced a distortion from the planarity making the molecule assume an almost tetrahedral coordination in its proximity. What was found to be very peculiar is the molecular system, here nameds $H^+N_1PER_H$, which is a non-conformational isomer of protonated Perampanel with the presence of three fused rings (Fig. 4).

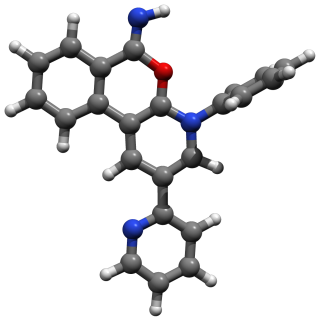


Figure 4: The non-conformational isomer $H^+N_1PER_H$.

Hereunder, in Fig. 5, the Raman spectra of the four protonated PER_H isomers are shown in comparison: all spectra are, of course, characterized by the presence of peaks specific of Perampanel (*e.g.*, the in-plane ring deformation modes between circa 1700 and 1550 cm^{-1} and the breathing normal modes at about 850 cm^{-1}) and a peak around 3200 cm^{-1} corresponding to the N-H^+ stretching normal mode (label 2 in the graph in Fig. 5). Unfortunately, this frequency range is difficult to be tested in experimental SERS, so the N-H^+ stretching vibrational mode is just a computational indication of the protonation process. Hence, in the perspective in which one wants to point out what differentiates the isomers on the basis of the protonation sites, it is more worthwhile to compare the different spectra since it is not much the presence while the absence of certain peaks to give the desired insights. For instance, the absence of the C=O stretching mode peak tells that the proton is bound to the oxygen, and that the double bond was changed into a single bond.

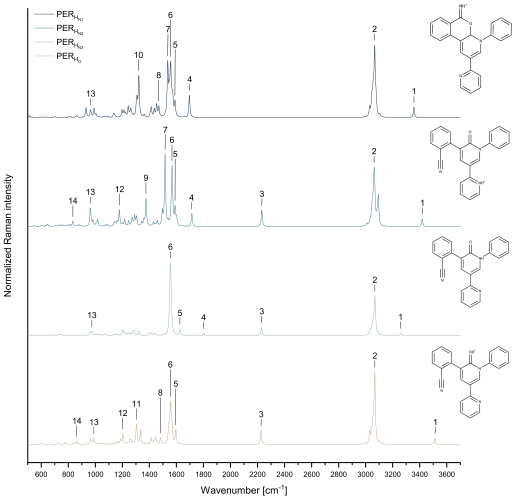


Figure 5: Simulated Raman spectra of H^+PER_H isomers.

Depending on the specific protonation site, the spectrum of each molecule is subject to distinctive modifications. Let us go through each spectrum to check these indicators: $H^+N_1PER_H$ had both the C=O double bond and the C≡N triple bond broken thus, in its spectrum, the peaks indicative of the C-O and the C=N bonds stretching are present. What is distinctive is the shift of the C=N double bond stretching normal mode to wavenumbers in the range of a C=O stretching mode (from circa 2200 to 1696 cm^{-1}), though absent in this molecule. This fact makes $H^+N_1PER_H$ more difficult to be detected experimentally and differentiated from the other isomers. To the protonated structure $H^+N_2PER_H$ corresponds the presence of two, intense and distinct, peaks (peaks 6 and 7) where the other isomers only showed one (7). $H^+N_3PER_H$ exhibits a very intense peak (7) around 1600 cm^{-1} (the frequency region of the C-C stretching and ring deformation modes). When the protonation occurs on oxygen, in H^+OPER_H spectrum, instead of the C=O bond stretching peak (peak number 4), there is the one related to the stretching of the C-O bond C-O-H^+ moiety.
The above considerations also apply for the protonated isomers of PER_A which spectra are very similar to their duals.

Comparison with experimental data

Let us now compare the experimental SERS data with the findings retrieved from the analysis of the protonated species.
Fig. 6 shows two Raman spectra: at the bottom there is the experimentally recorded SERS spectrum of a protonated Perampanel sample [4] and at the top there is the curve given by the sum of the spectra of $H^+N_1PER_H$, $H^+N_2PER_H$, $H^+N_1PER_H$ and H^+OPER_H. H^+PER_H was used for the comparison with respect to the experimental data in virtue of the similarity of the neutral structure PER_H with the ligands of the *Protein Data Bank* archive [5]. Of course a more rigorous approach would have required a summation with weighted coefficients based on the Boltzmann's statistics, but given the exploratory nature of this approach and the minor energy difference among the isomers the algebraic sum of the spectra is an approximation good enough for the comparison with the experimental data, which actually show a very promising match with the theoretical spectrum.

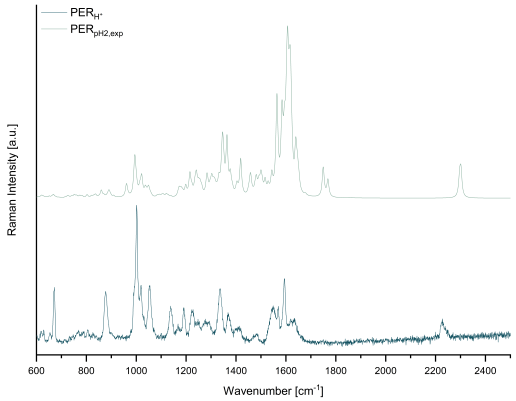


Figure 6: Raman simulated spectra of PER_H hydronated isomers.

The two graphs match qualitatively: the theoretical Raman spectrum correctly indicates the spectrum regions where the peaks of protonated Perampanel are present, but fails in predicting the fine structure in the relative intensities of the peaks. This is due to, firstly, the very nature of the computational model where an isolated molecule of protonated PER is simulated, whereas the SERS spectrum is generated by protonated PER molecules adsorbed at the gold surface. The second reason is tied to electromagnetic phenomena that occur at the surface where the SERS phenomenon occurs, *i.e.*, the orientation of the adsorbed molecule with respect to the electric field generated by the plasmon resonance should not be averaged in the same way as for a regular Raman experiment in *e.g.* solution state. This is the standard assumption by which

the Raman spectra have been simulated by DFT calculations, but it should be re-examined once a model of protonated PER on gold surface would be available in the future.

5. Conclusions

I can summarize the main findings of my research work as follows. Starting from the gas-phase PER model, the equilibrium structures of the molecule were defined through a systematic exploration of the PES. This allowed establishing the lowest energy structure among the PER conformers (*i.e.*, PER_A), and the definition of a stable structure (a PES local minimum conformation) matching the ligands of the *Protein Data Bank* archive [5] (*i.e.*, PER_H).
To explore Perampanel's PES, also a *statistical* approach was taken into consideration, specifically the EHTB (short for Extended Hückel Tight-Binding model, an approximation to the Kohn-Sham energy functional [3]) simulation run by CREST (Conformer-Rotamer Ensemble Sampling Tool) [2]. To achieve reasonable outputs the simulations became slower and slower and produced only nine equilibrium structures corresponding to five (out of ten) of the conformers that I retrieved with the systematic approach described above. However, these results were attained only because I was already aware of what I was seeking. Confirming that a systematic approach, even though challenging, is more reliable and, when feasible, as in the case of Perampanel, it is also a precious resource to set benchmarks in conformational analysis.
Moving on to the results obtained from simulating the Raman spectra of PER and H^+PER for the comparison with respect to experimental data (*i.e.*, from solid PER samples and PER in SERS conditions), the characteristic peaks of the molecule were assigned.
Such analysis and assignment (namely, the C-H stretching peaks around 3000 cm^{-1}, C-C stretching vibrational modes between 1500 and 1600 cm^{-1}, C≡N stretching at circa 2200 cm^{-1}, and the breathing and ring deformation normal modes below 1000 cm^{-1}) provide a valid and useful reference for current, and future, laboratory analyses of PER samples by Raman spectroscopy also in SERS conditions. The promising match between the theoretical spectra of protonated PER and the experimental SERS spectrum, validates both the computational approach presented here and the previous experimental work carried out by my colleagues in the laboratory.
Finally, some noticeable findings arose in the study of the molecule structure and symmetries: the isoenergetic conformers, i.e., the enantiomers PER_A and PER_B, as well as the protonated PER isomer $H^+N_1PER_H$ that was obtained from a conformer in neutral form.

References

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