IJPAS | INTERNATIONAL JOURNAL PURE AND APPLIED SCIENCES
ISSN: 2149-0910

Vol. 10(1); 1-11 (2024)
DOI: 10.29132/ijpas.1376725

Research Article

Nuclear Magnetic Resonance and Quantum Chemical Calculations of Ca^{+2} Doped Norepinephrine Molecule by Using DFT and HF Methods

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Received: 16.10.2023

Accepted: 08.05.2024

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Abstract: Norepinephrine plays an important role in many processes such as stress response, attention, alertness, blood pressure regulation, neurotransmission and emotional states. In this study, the molecule was optimized in order to get knowledge about the formation of the Ca^{+2} doped norepinephrine complex and the functions of norepinephrine in neurotransmission or other cellular processes by interacting with calcium. Quantum mechanical calculations such as FT-IR, Nuclear Magnetic Resonance (NMR), HOMO-LUMO structure with the energy level diagram, UV-visible absorption, The density of states (DOS) of the optimized molecule were performed. The physical and chemical structure characteristics of the norepinephrine molecule and the change of its structural properties by molecular bonding with Ca^{+2} were investigated.

Keywords: NMR; DFT; HF; FT-IR; UV- Vis

Ca^{+2} Katkılı Norepinefrin Molekülünün DFT ve HF Yöntemleri Kullanılarak Nükleer Manyetik Rezonans ve Kuantum Kimyasal Hesaplamaları

Özet: Norepinefrin stres tepkisi, dikkat, uyanıklık, kan basıncının düzenlenmesi, nörotransmisyon ve duygusal durumlar gibi birçok süreçte önemli bir rol oynamaktadır. Bu çalışmada, Ca^{+2} katkılı norepinefrin kompleksinin oluşumu ve norepinefrinin kalsiyum ile etkileşime girerek nörotransmisyon veya diğer hücresel süreçlerdeki işlevleri hakkında bilgi edinmek amacıyla molekül optimize edilmiştir. FT-IR, Nükleer Manyetik Rezonans (NMR), enerji seviyesi diyagramı ile HOMO-LUMO yapısı, UV-görünür emilim, optimize edilmiş molekülün durum yoğunluğu (DOS) gibi kuantum mekaniksel hesaplamalar yapılmıştır. Norepinefrin molekülünün fiziksel ve kimyasal yapı özellikleri ve Ca^{+2} ile moleküler bağlanarak yapısal özelliklerinin değişimi incelenmiştir.

Anahtar Kelimeler: NMR; DFT; HF; FT-IR; UV- Vis

1. Introduction

Norepinephrine is a hormone that enhances levels of attention and alertness, which improves focus and response abilities. Therefore, norepinephrine levels are thought to be associated with conditions such as sleep patterns, attention disorders, and hyperactivity. It is also a neurotransmitter that helps with memory encoding and maintenance [1]. Especially it plays an important role in the recording of emotionally intense experiences in memory. Norepinephrine regulates transmission between nerve cells. The release of norepinephrine from one particular nerve cell to another can increase or decrease nerve transmission [2]. Norepinephrine increases blood pressure by causing blood vessels to constrict. This effect plays an important role in regulating blood pressure in the body. Epinephrine (adrenaline), a drug used to raise blood pressure, increase heart rate and relieve asthma symptoms, is a synthetic derivative of norepinephrine. A well-balanced level of norepinephrine in the body is important to provide a healthy functioning neurotransmission and a harmonious stress response [3]. However, unbalanced levels of norepinephrine can cause some health problems [4]. For example, low levels of norepinephrine can be associated with depression, fatigue and loss of motivation, while high levels of norepinephrine can be associated with conditions such as anxiety, hypertension (high blood pressure) and panic disorder [4, 5]. Some drugs can affect norepinephrine levels or act on the nervous system by acting on norepinephrine receptors. The study of the physical and chemical structure of this molecule is very important because it is an active component in many drugs and it is very important to study the changes in its structural properties after it bonds with other molecules. Norepinephrine can be considered as a precursor to the dopamine molecule [6]. Norepinephrine is synthesized from dopamine by the enzyme dopamine beta-hydroxylase [7]. Norepinephrine is a molecule known as 4-(2-amino-1-hydroxyethyl) benzene-1,2-diol. Its chemical formula is C8H11NO3 and belongs to the catecholamine group [8].

Calcium signals are used in many biological processes such as neurotransmitter release in cells and muscle contractions. Generally, Ca^{+2} ions play an important role in many biological processes inside and outside the cell. Calcium signals are used in neurotransmitter release and muscle contractions to enable communication between nerve cells [9]. When a calcium (Ca) atom is bound to the norepinephrine molecular structure, a norepinephrine-calcium complex is formed (Fig.1). This complex is a structure formed when the norepinephrine molecule combines with a calcium ion. The norepinephrine-calcium complex may play a potential role in nerve conduction or other cellular processes through the interaction of norepinephrine with calcium.

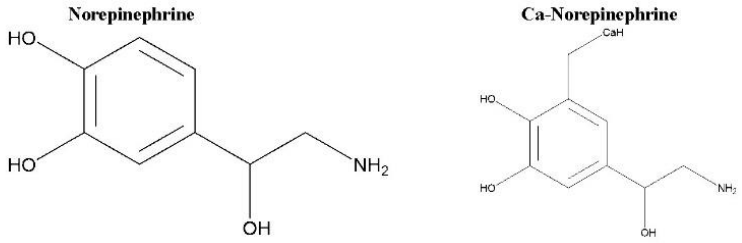


Figure 1. Skeletal formula of norepinephrine and Ca doped norepinephrine by ChemOffice drawing

In this study, Stuttgart/Dresden (SDD) was selected as the most optimal basis set for the norepinephrine molecule using the DFT method. The LanL2MB complex best represented the basis set for this method. The aim is to study the structural state of calcium in the complex by molecular bonding of the norepinephrine molecule with Ca^{+2}.

2. Materials and Methods

2.1. Calculation Method

The GaussView 6.0.16 program was used to plot the molecules of norepinephrine and Ca^{+2} doped norepinephrine [10]. The Gaussian 09: AS64L-G09RevD.01 program was used for all calculations [11].

Calculations were made with the DFT and HF approach. In the norepinephrine molecule, SDD was chosen as the most suitable basis set using the DFT method. The HF approach was used for Ca^{+2} doped norepinephrine. The complex represented the most optimal basis set LanL2MB. Quantum chemical descriptors such as hardness, softness, chemical potential, and electronegativity were made using the DFT method for the norepinephrine molecule and the HF approach for the Ca-doped norepinephrine complex. To examine the band structure of the norepinephrine and Ca-doped norepinephrine optimized data, density of state (DOS) cards were examined with the GaussSum 3.0 tool [12]. Molecular trajectory data were mixed with Gauss's HOMO-LUMO diagram curves.

2.2. Spectroscopy

Fourier Transform Infrared (FT-IR), Nuclear Magnetic Resonance (NMR) and UV-Visible Spectroscopy were used to obtain information about the structure analysis of norepinephrine and Ca-norepinephrine molecules. FT-IR spectroscopy examines the vibrational energy transitions of molecules. Because the IR spectrum is characteristic of the molecule, it gives important information about the structure. To obtain specific information about norepinephrine and the Ca-doped norepinephrine complex, the frequency, intensity and half-bandwidth changes were analyzed by FT-IR spectroscopy. In accordance with the data obtained, it is possible to have information about the structure analysis and conformations of biologically active molecules. NMR analysis is also needed as FT-IR is not sufficient for precise structure analysis. NMR spectroscopy studies the behavior of nuclei in a magnetic field, obtaining characteristic resonance signals depending on the magnetic character of the atomic nucleus. These signals provide information about the molecule's skeleton. In addition, UV-Visible Spectroscopy is based on the principle of measuring the light transmitted or reflected from the molecule using UV light (200-400 nm) and visible light (400-800 nm). It obtains the spectrum by measuring the light passing through or emitted by the molecule at a specific wavelength.

3. Result and Discussion

3.1. Calculation Method

The norepinephrine molecule for theoretical calculations was optimized using Gaussian 09: AS64L-G09RevD.01 program package [11]. Figure 2 shows the optimized structures of the two molecules. The 9H hydrogen at 7C of the optimized molecule in Figure 2a was removed and the structure of the molecule was optimized by attaching 23Ca atoms to the structure as shown in Figure 2b.

Energy change occurs at each step in the optimization process of molecules. The energy change of the molecules in the process of optimization is shown in Figure 3. In the figure, the beginning is indicated by a blue dot. Each step is marked with green symbols. The red dot represents the end point. As seen in Figure 3, the norepinephrine molecule started at about -3.4 eV and ended at -3.99 eV in 8 optimization steps. In the Ca-doped norepinephrine molecule, the optimization steps started at about -4.5 eV and ended at -5.11 eV in 9 optimization steps.

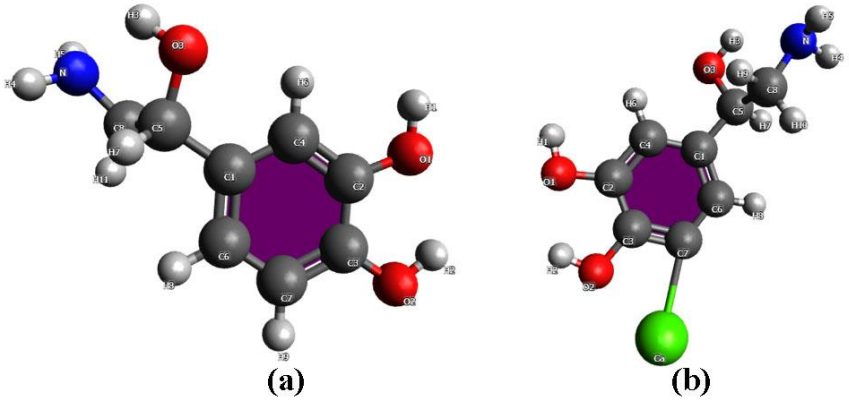


Figure 2. Optimize structure of **(a)** norepinephrine **(b)** Ca-norepinephrine

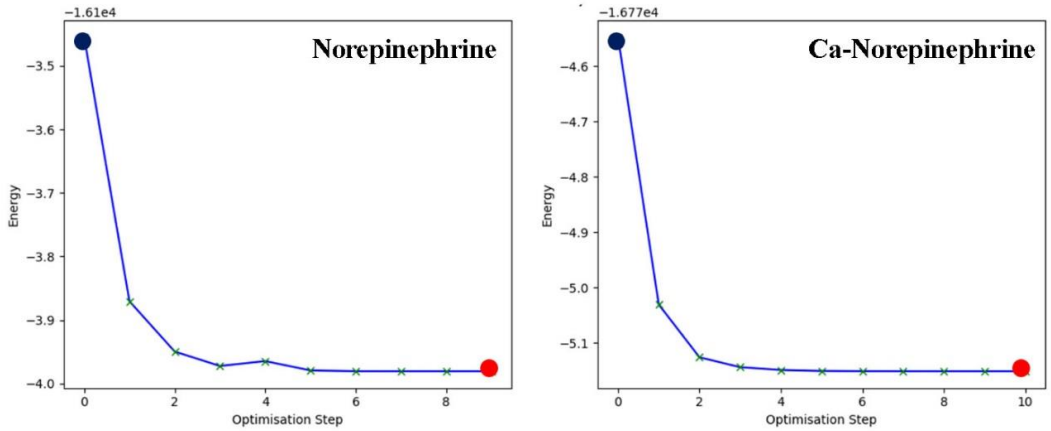


Figure 3. Energy change of molecules in the optimization process

3.2. Frontier Molecular Orbital Analysis (FMO)

The E_{HOMO} and E_{LUMO} energy values of the optimized norepinephrine molecule and Ca-doped norepinephrine complex were calculated using the DFT/B3LYP (SDD) basis set and HF method, respectively, and the Gaussian 09:AS64L-G09RevD.01 program in the LanL2MB set. The parameters of energy difference (ΔE), ionization potential (I), electron affinity (A), electronegativity (χ), chemical hardness (η), nucleophilicity (ε), electrophilicity (ω), dipole moment (μ) and chemical softness (σ) were calculated from the electron density distribution [13]. The calculated electronic structure values of norepinephrine and Ca-norepinephrine molecules are shown in Table 1.

Table 1. *Norepinephrine and Ca-doped norepinephrine electronic structure values*

Compound	Norepinephrine	Ca-Norepinephrine
E_{HOMO} (eV)	-5.741	-5.082
E_{LUMO} (eV)	-0.068	1.272
ΔE (eV)	5.673	6.354
η (eV)	2.836	3.177
I (eV)	5.741	5.082
A (eV)	0.068	-1.272
σ (eV^{-1})	0.352	0.314
χ (eV)	2.904	1.905
μ (eV^{-1})	-2.904	-1.905
ω	1.486	1.588
ε	0.672	0.629

The forbidden energy gap can change the energy levels of electrons when a substance is energized, affecting its electronic and electrical properties such as conduction or conductivity. When Ca was bound to the norepinephrine molecule, the difference between the ΔE energy range increased by 0.681 eV (Fig. 4). Increasing the forbidden energy range of the molecule reduces the probability of electrons jumping to higher energy levels, as the energy levels of electrons in the molecule have more energy. Ca bonding to the norepinephrine molecule caused changes in the molecular structure, geometry or bond lengths of the molecule.

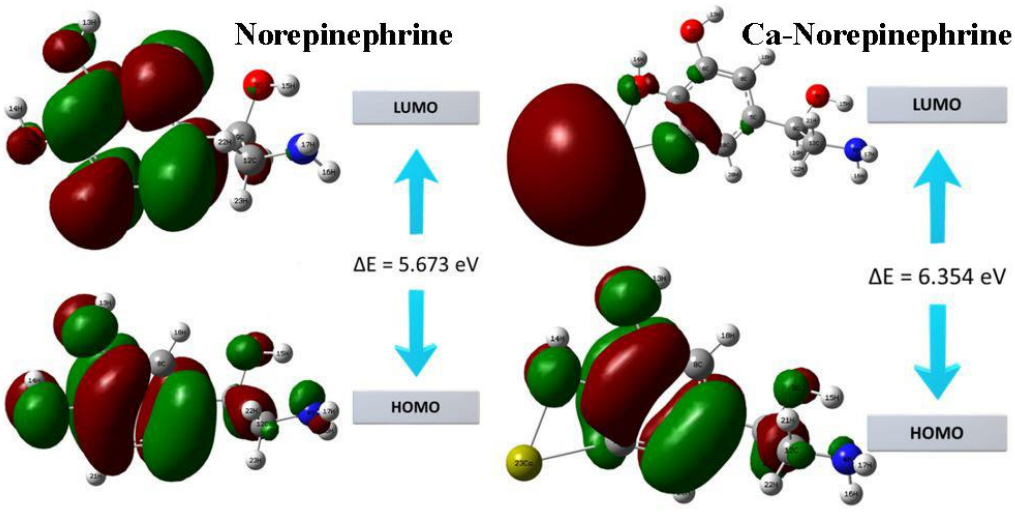


Figure 4. HOMO-LUMO structure with the energy level diagram of norepinephrine and Ca doped norepinephrine

3.3. Vibrational Spectroscopic Analysis Spectrum

The FT-IR spectrum for the investigated molecule between 3500 cm^{-1}-0 cm^{-1} wave number is shown in Figure 5. Spectral and vibrational frequency similarity indicates complex similarity. According to the principles of vibrational spectroscopy, the vibrational frequency of a bond increases as the bond strength increases and the mass of the bond atom decreases. When the absorption peaks for the norepinephrine molecule were analyzed, vibrations were observed between 3008 cm^{-1}-1672 cm^{-1}. The transmittance is between 42% and 45%.

When Ca is doped into the molecule, spectra in the range of 4500 cm^{-1}-0 cm^{-1} are obtained for FT-IR analysis (Figure 5). The energy range is between 3563 cm^{-1}-1908 cm^{-1} wave number. Transmittance is between 82% and 46%. The reason for the increase in the energy range in the Ca doped molecule is that calcium changes the vibration, electron affinity, translation and rotation of the structure due to the binding energy of calcium. An increase in peak intensity was observed as a result of interactions between Ca and the norotransmitter molecule due to complex formation. Relatively shallowly and large wave number peak shifts, on the other hand, imply the production of norepinephrine and Ca doped complexes.

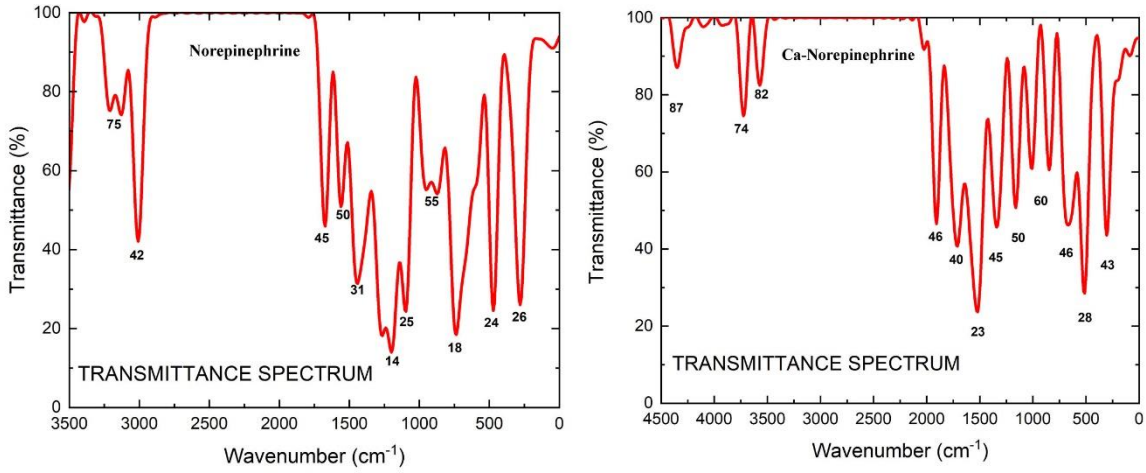
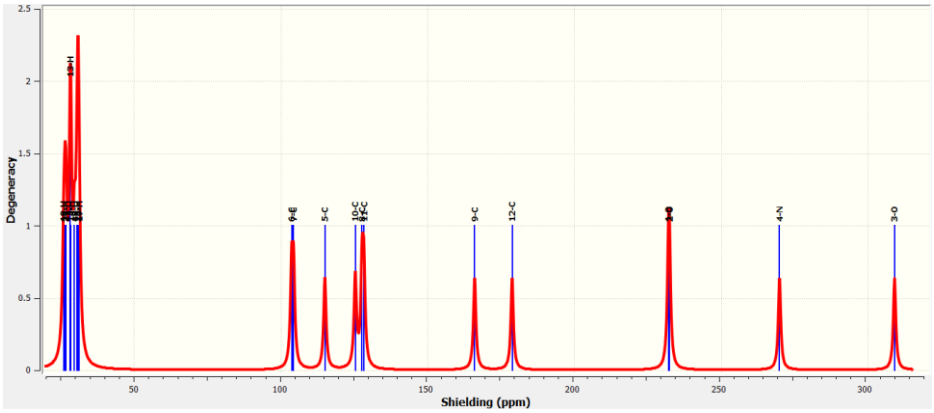


Figure 5. Vibrational Spectroscopic Analysis Spectrum of Molecules

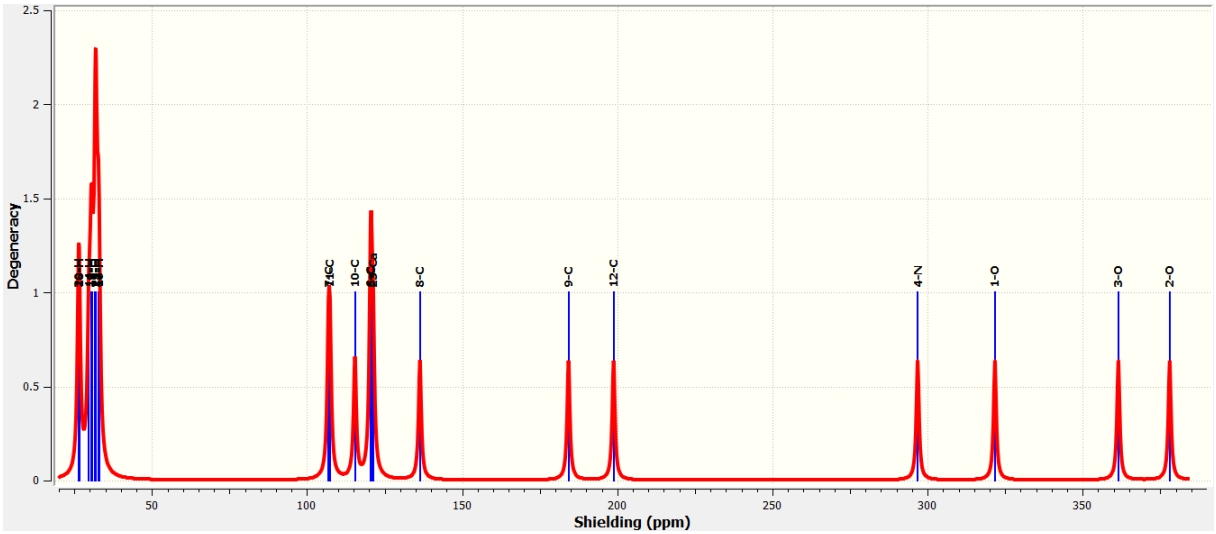
3.4. Nuclear Magnetic Resonance Spectroscopy

Due to the fact that hydrogen-containing groups in a molecule can be detected along with the groups adjacent to this group, NMR calculations play an important role in obtaining information about the structure to be examined. In addition, NMR is a very useful method to explain the relationship between molecular structure and electronic properties [14]. By analyzing the behavior of nuclei in a magnetic field, characteristic resonance signals of the nucleus are obtained and it is possible to obtain information about the skeletal structure of the molecule. In this study, NMR chemical shift calculations for molecular structure determination of the pure and doped complexes were performed using Gauge-Including Atomic Orbitals (GIAO) using the DFT method SDD basis set and HF approach LanL2MB basis set, respectively. NMR spectra of both molecules are shown in Fig. 6. The calculated

chemical shift values of the analysed molecules are shown in Table 2. The values given in the table show the results for the positions at which the atoms that make up the molecule shield. When the table and graph are viewed, the sudden peaking in the graph shows where the characteristic feature of the structure located. Norepinephrine shows an sudden peaking of atoms H18, H21, H20, H14, H15, H19, H13, H23, H22, H17, H16. Ca doped norepinephrine shows H20, H18, H14, H13, H19, H15, H21, H22, H17, H16 atoms. Both norepinephrine and Ca-doped norepinephrine have common atoms. But H23 is not present in Ca-doped norepinephrine. The reason is that H23 is replaced by a Ca atom.



(a)



(b)

Figure 6. NMR spectrum of **(a)** Norepinefrin **(b)** Ca-norepinephrine

Table 2. *Chemical shifts of norepinephrine and Ca-norepinephrine*

Norepinephrine		Ca-Norepinephrine	
Method	Shielding (ppm)	Method	Shielding (ppm)
18-H	25.0966	20-H	26.3567
21-H	25.3305	18-H	26.558
20-H	25.9606	14-H	29.6746
14-H	26.8605	13-H	30.3761
15-H	28.1527	19-H	30.7421
19-H	28.5211	15-H	31.7158
13-H	28.9583	21-H	31.8593
23-H	29.903	22-H	32.0086
22-H	30.5651	17-H	32.6892
17-H	31.9338	16-H	32.9837
16-H	32.547	7-C	106.9171
7-C	44.743	11-C	107.4007
6-C	45.6936	10-C	115.4294
5-C	56.1802	6-C	120.4617
10-C	69.0409	5-C	120.6685
11-C	72.5559	23-Ca	121.2953
8-C	78.2376	8-C	136.3823
9-C	113.8041	9-C	184.2692
12-C	136.8239	12-C	198.8041
2-O	217.5328	4-N	296.8148
1-O	222.3375	1-O	321.7163
4-N	234.662	3-O	361.499
3-O	268.2187	2-O	378.042

3.5. UV–Visible analysis

The wavelengths and energy of the light absorbed by the molecule are displayed in the UV absorption spectrum. Electronic absorption spectra in the UV-visible range have been obtained to characterize the compounds. Spectroscopy of UV-visible absorption searches for absorption between 200 nm-800 nm. Absorptions in this range are typically observed for compounds with double bonds or triple bonds (conjugation). At wavelengths less than 200 nm, conjugated aliphatic molecules are absorbed. The air substantially absorbs UV light below this wavelength, which is extremely hazardous for living things [15, 16]. The compounds' UV-visible absorption spectra have been shown in Figure 7. The wavelength of the UV absorption spectrum for norepinephrine is between 100 nm and 400 nm. It peaked at 185 nm, is at a wavelength lower than 200 nm and is dangerous. The wavelength for norepinephrine is between 500 nm and 1000 nm. It is seen to peak at 676 nm. The absorption in the 200 to 800 nm range is between and is molecule containing double bonds or triple bonds (i.e. conjugation).

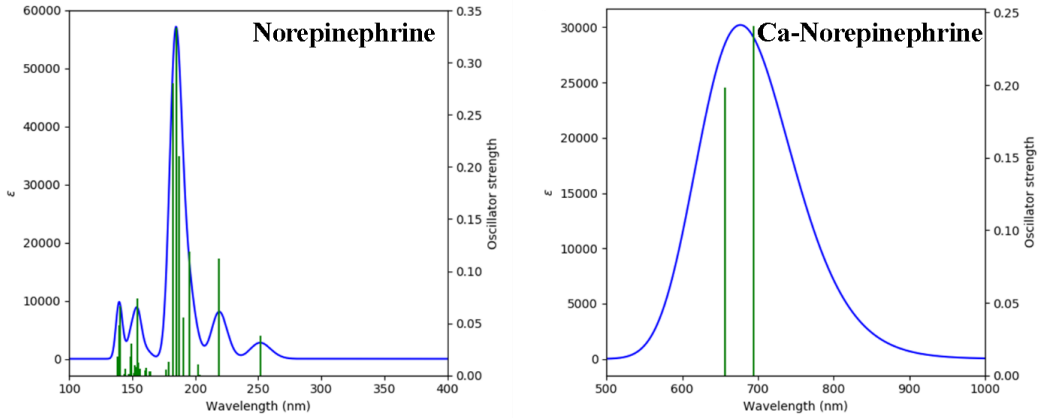


Figure 7. UV-Visible absorption of norepinephrine and Ca-norepinephrine

3.6. Density of States (DOS)

The GaussSum program was used to calculate the Density of States (DOS), which displays the appropriate DOS in Figure 8 and provides the molecular orbital contributions of the various basic components for the compound's whole system [17]. The blue and green lines in the DOS spectrum indicate the HOMO and LUMO levels. The boundary trajectories spatial redistribution is a three-dimensional depiction of the local density of states that graphically displays the molecule's electron density [18]. The DOS spectrum can be used to confirm the variation of the HOMO-LUMO range [19]. The number of places that are accessible at a specific energy position is calculated using this spectrum. On the energy axis of the graph, the initial lines from -20 eV to 10 eV are known as occupied, filled and donor orbitals, and from -5 eV to 0 eV as virtual, unfilled and acceptor orbitals. The high density of DOS at a given energy level suggests that a large number of places are open for occupancy. The absence of any states that the system could occupy is shown by the zero density of DOS.

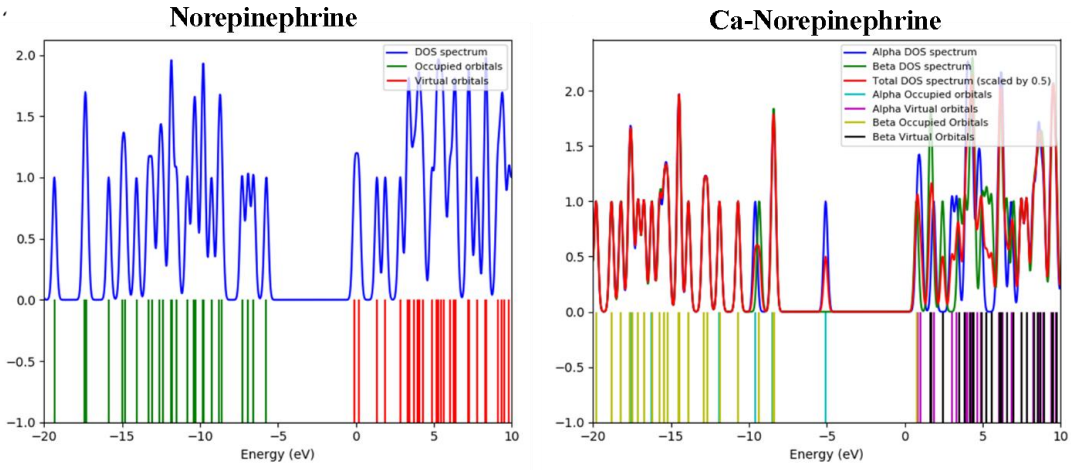


Figure 8. The density of states (DOS) with the contribution of norepinephrine and Ca-norepinephrine

4. Conclusion and Discussion

ΔE (HOMO-LUMO) value increased when Ca-norepinephrine complex formed. This means that the compound generated is highly chemically stable. It suggests that electrons have a reduced ability for transferring to higher energy levels. Because of it will make the interaction of the reactants difficult, the molecule is a hard molecule in terms of chemical reaction. In the binding of the Ca atom to the norepinephrine molecule, NMR did not exhibit a distinguishing feature in the elucidation of the Ca atom. As a result of NMR, Ca behaves like a C atom. The fact that H atoms have only 1 proton and 1 electron spin has no effect as a distinguishing feature. C and O atoms close to the Ca atom change more effectively than others. Frequencies shift towards higher frequencies.

Conflict of Interest

The authors report no conflict of interest relevant to this article.

Research and Publication Ethics Statement

The authors declare that this study complies with research and publication ethics

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