Letters in Applied NanoBioScience
Platinum Open Access Journal (ISSN: 2284-6808)

Article
Volume 10, Issue 3, 2021, 2438 - 2445
https://doi.org/10.33263/LIANBS103.24382445

Application of Chitosan/Fe_3O_4 Nanocomposite as Biosensor

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Received: 25.11.2020; Revised: 4.01.2021; Accepted: 11.01.2021; Published: 19.01.2021

Abstract: Modified chitosan with metal oxides show a potential application in many areas of research. Accordingly, a model molecule of three chitosan units was modified with Fe_3O_4 through the chitosan's O-linkage (Cs). The modified structure is supposed to interact with alanine amino acid as a protein structure model. Density functional theory method at B3LYP level was used with 6-31G(d,P) basis set. At this level, the total dipole moment (TDM), the bandgap energy as the difference between the highest and lowest molecular orbital (HOMO/LUMO bandgap). The molecular electrostatic potential (MESP) is mapped at the same level of theory. HOMO/LUMO for Cs was 2.9217 eV modified for Cs/ Fe_3O_4 to be 0.5919 eV. As far as it interacts with alanine, it became 0.3415 eV, which indicated a sharp decrease in HOMO/LUMO energy. The MESP map of Cs/ Fe_3O_4 indicated that chitosan's reactivity is improved as the electronegativity increased. These results dedicated Cs/ Fe_3O_4 as a sensor for the amino acid alanine.

Keywords: DFT; Protein; Biosensor; Cs/Fe_3O_4.

1. Introduction

Chitosan is described as a biopolymer derived from chitin and a structural polymer for all arthropods' external structures and cephalopods' internal structures. It can be obtained from a shrimp shell by chitin's deacetylation in a basic medium [1]. Due to its non-toxic, biocompatible, and biodegradable properties, chitosan is widely used as biosensors according to its ability to form film [2,3]. The sensor is interpreted as a device that translates biological, physical, or chemical information into a quantifiable signal. One of the electrochemical sensors' characteristics is being very sensitive and selective for different types of analyzes [4]. It is also used for faster and easier analysis than traditional analytical techniques such as chromatography, mass spectrometry (MS), and nuclear magnetic resonance (NMR). The macromolecule chain of chitosan can be easily modified by using nanoparticles and conductive polymers to produce compounds with a high electron transfer rate and a specific surface area to develop new electrochemical sensors [5,6]. Also, due to the presence of free amine and alcohol groups, chitosan is widely used to inhibit biomolecules such as enzymes [7], DNA, and antibodies/antigens for the preparation of electrochemical biosensors [8]. Chitosan is a prominent candidate for preparing Molecularly imprinted polymer (MIPs) due to the presence

of its amine and alcohol groups that can interact with different types of crosslinkers and form specific cavities for many types of analyzes. Molecularly printed chitosan (MICs) is widely applied to develop powerful electrochemical sensors [9,10]. The anti-microbial activity of chitosan has been described and spread widely in recent years for its action as an antagonist to a wide range of microorganisms. It fights many parasites and reduces inflammatory activity [11]. Chitosan is used in many different fields due to its different properties, especially being multi-layered and considered distinct from natural polymers. It is used in applications in economically promising sectors such as food [12], cosmetics [13], pharmaceutical [14], agricultural industries [15], and in developing biosensors and gas sensors [16, 17]. The use of chitosan as a drug packaging material has been studied and developed through the existing research work.

On the other hand, magnetic nanoparticles (NPs) can be divided into metal NPs, metal oxide NPs, and alloy NPs. Minerals NPs include iron, cobalt, and nickel [18]. Metal oxide NPs contained iron oxides (γ-Fe_2O_3 and Fe_3O_4) and ferrite ($CoFe_2O_4$ and $Mn0.6Zn0.4Fe_2O_4$) [19]. The NPs alloys include Fe Co and Fe Pt [20]. MNPs also have an important role in removing and mitigating toxic pollutants, separation of membranes for water treatment and purification. Researchers use MNPs to stop biomolecules' movement (antibodies, proteins, enzymes, etc.) and the simple, fast, cheap, and effective separation of target biomolecules [21-22]. Because MNPs possess a high surface area to volume ratio and a high binding rate to the detector material, the magnetically controllable aggregation and dispersion can result, making pre-concentration, purification, and nucleic acid separation easy [23-24]. MNPs have good dissociation potential, which can bind biomolecules quickly and effectively. The binding is reversible, and the assembly and dispersion can be controlled. Active substances such as bioactive adsorbents or other surface-attached bonds of MNPs can be combined with specific biomolecules, such as enzymes, DNA, and proteins, and separated under the influence of an external magnetic field [25-28]. Biosensors contain functional proteins, nucleic acids, or cellular organelles, or they can be whole living cells. They may be fixed ("immobile") on the surface of a physicochemical transducer that has the ability to translate the specific interactions of a fixed biological entity with its corresponding binding partner (Analytical) The concentration in electrical signals can be measured [29, 30]. It is stated earlier that molecular modeling is used effectively for elucidating molecular structures for biopolymers such as cellulose [31-32], sodium alginate [33], chitosan [34-36], as well as many other systems and molecules [37-39]. The present work is conducted to study the possible application of modified chitosan as a sensor for protein.

Accordingly, three units of chitosan are modified with Fe_3O_4. The interaction is formed through the O-linkage to produce chitosan/ Fe_3O_4 nanocomposite. The studied composite is supposed to interact with alanine as a model molecule for protein. Physical parameters such as total dipole moment and HOMO/LUMO band gap energy were calculated at B3LYP/6-31G(d,P). Furthermore, the molecular electrostatic potential was mapped at the same level of theory.

2. Materials and Methods

2.1. Computational details.

All the studied structures were subjected to calculations with Gaussian09 program [40] using a personal computer at the Spectroscopy Department, National Research Center, Egypt.

All proposed structures are optimized at B3LYP/6-31G(d,P) [41-43] basis set. Total dipole moment (TDM), the difference between highest and lowest molecular orbital (HOMO/LUMO band gap), and molecular electrostatic potential (MESP) are calculated at the same level of theory.

3. Results and Discussion

3.1. Building model molecules.

Chitosan model molecule is indicated in figure 1; chitosan is consisting of three units. As indicated in Figure 2, chitosan interacts with Fe_3O_4 through the O-linkage forming chitosan/Fe_3O_4 composite. The chitosan/Fe_3O_4 composite interacts with protein so that alanine is chosen as a model molecule for protein. Figure 3 presents an interaction between chitosan/Fe_3O_4 composite and alanine. The interaction is supposed through the H of the NH_2. The reactivity of chitosan and its composite are described in terms of total dipole moment (TDM), HOMO/LUMO bandgap energy, and molecular electrostatic potential (MESP). Early that the reactivity of a given chemical and/or biological compound could be correlated with their TDM and HOMO/LUMO values [44-45]. While mapping MESP is an important step to understand the active sites of a given biological molecule [46-47]. Accordingly, the chitosan/Fe3O4 composite's ability to act as a protein sensor is tested with B3LYP/6-31G(d,P) through the studied physical parameters.

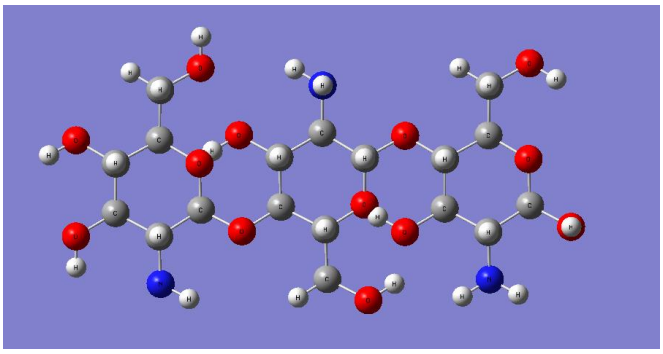


Figure 1. Model molecule for chitosan, which consists of 3 chitosan units.

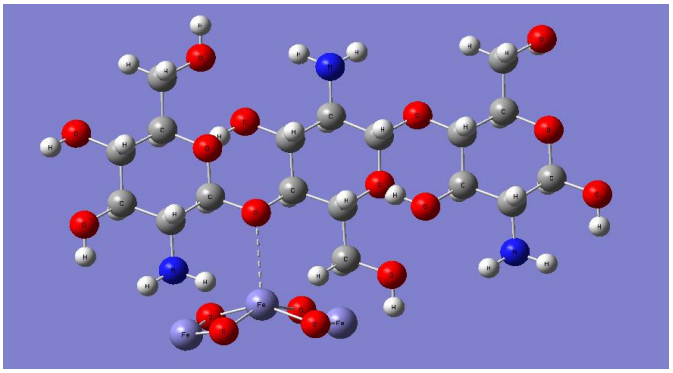


Figure 2. Model molecule for chitosan interacted with Fe_3O_4 through the O-linkage.

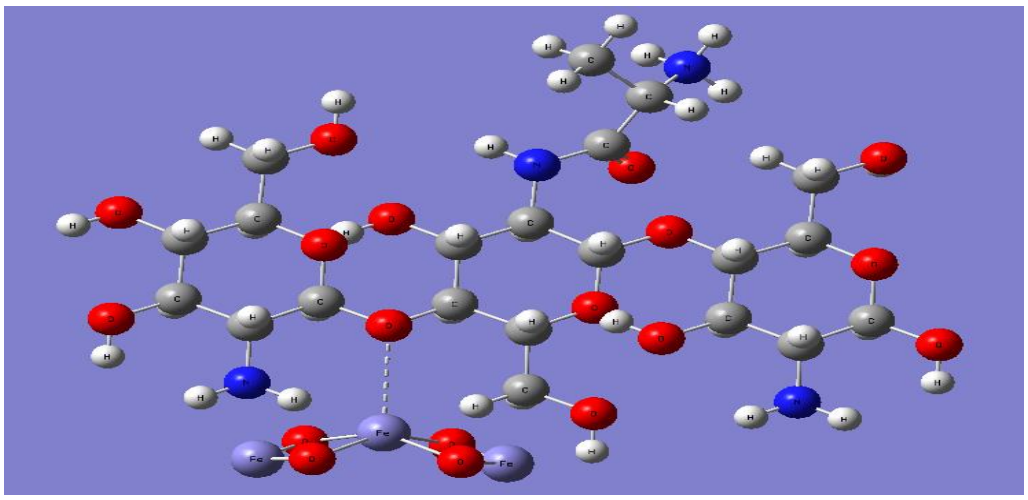


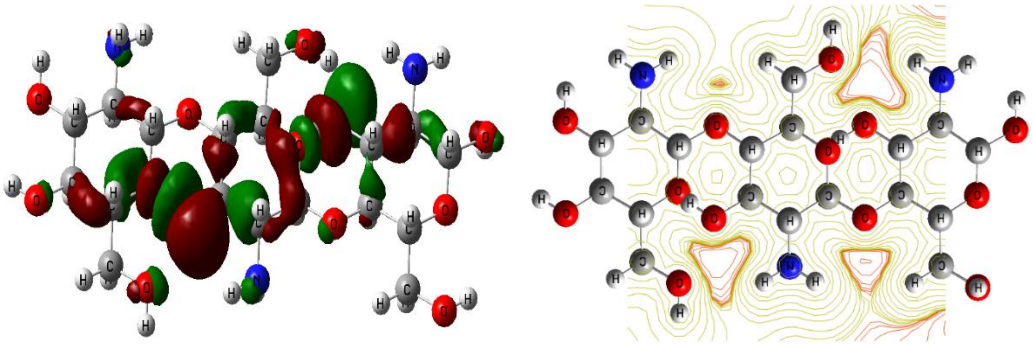
Figure 3. Model molecule for chitosan /Fe_3O_4 interacting with amino acid alanine through the H of the NH_2.

Table 1 presents the calculated TDM as Debye and HOMO/LUMO energy (eV) for chitosan, chitosan/Fe_3O_4 composite, and chitosan/Fe_3O_4 composite/ alanine. As indicated in the table, the TDM of chitosan is influenced strongly through the addition of Fe_3O_4 and Fe_3O_4 /alanine. Where TDM decreased from 15.2472 Debye to 8.6087 Debye for chitosan/Fe_3O_4 composite and to 11.1364 Debye for chitosan/Fe_3O_4 composite/alanine. Meanwhile, HOMO/LUMO bandgap of chitosan decreased from 2.9217 eV to 2.9217 and 0.3415 eV for chitosan/Fe_3O_4 composite and chitosan/Fe_3O_4 composite/alanine, respectively. Figures 4-a, 5-a, and 6-a present the distribution of electrons within the orbitals (i.e., HOMO/LUMO bandgap energy) for chitosan, chitosan/Fe_3O_4 composite, and chitosan/Fe_3O_4 composite/alanine, respectively. These changes in the TDM and bandgap energy indicated that chitosan's reactivity is improved due to interaction with Fe_3O_4. The chitosan/Fe_3O_4 composite can be used as a sensor for alanine amino acid.

Table 1. B3LYP/6-31G(d,P) calculated total dipole moment as (Debye) and HOMO/LUMO bandgap energy as (eV) for the studied structures.

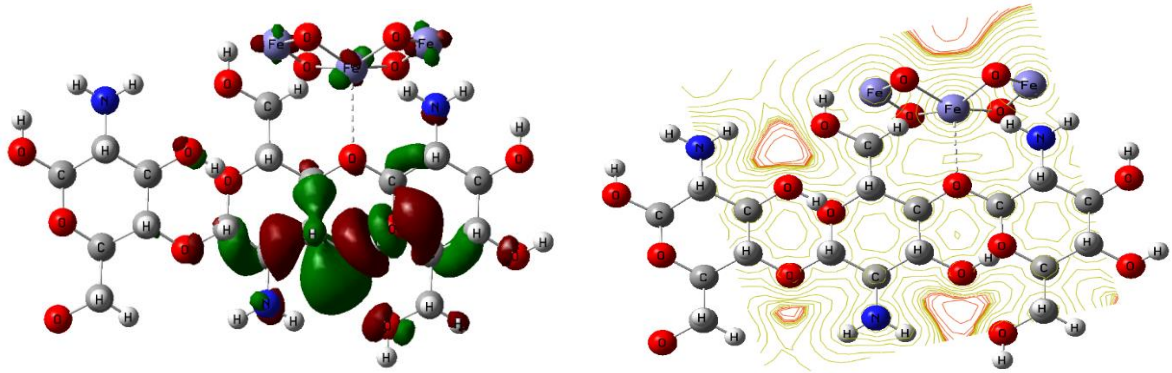
Structure	TDM (Debye)	ΔE (eV)
Cs	15.2472	2.9217
Cs/ Fe_3O_4	8.6087	0.5919
Cs/ Fe_3O_4 /Alanine	11.1364	0.3415

Additionally, molecular electrostatic potential (MESP) is calculated for all structures as presented in figure 4-b, 5-b, and 6-b for chitosan, chitosan/Fe_3O_4 composite, and chitosan/Fe_3O_4 composite/alanine, respectively. MESP is considered a powerful method for describing the distribution of electrons within the studied structures. Where it defines the active sides present in the molecule. Usually, MESP is indicated by a color mapping ranging from red, orange, yellow, green, and blue, where the red color refers to the most active site present, while the yellow and blue refers to the neutral and electron- positive side. Increasing the intensity of the red color indicates that the electronegativity of the studied structure increased, i.e., it becomes more reactive as presented in figures 4-b, 5-b, and 6-b its clear that the red color increased within the proposed structure of chitosan as a result of interaction with Fe_3O_4 and alanine, respectively.



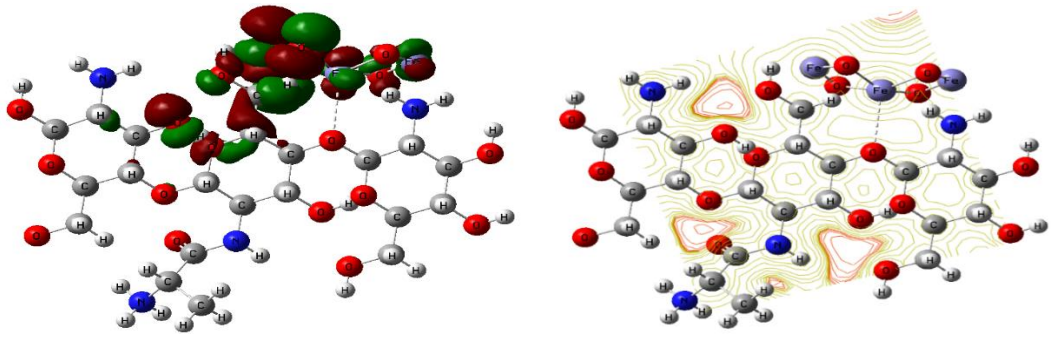
a- **b-**

Figure 4. B3LYP/6-31G(d,P) calculated: a-HOMO/LUMO band gap energy, b- Molecular electrostatic potential for chitosan model molecule that consists of 3 chitosan units,



a- b-

Figure 5. B3LYP/6-31G(d,P) calculated: a-HOMO/LUMO band gap energy, b- Molecular electrostatic potential for chitosan interacted with Fe_3O_4 through the O-linkage.



a- b-

Figure 6. B3LYP/6-31G(d,P) calculated: a-HOMO/LUMO band gap energy, b- Molecular electrostatic potential for chitosan /Fe_3O_4 interacting with amino acid alanine through the H of the NH_2.

4. Conclusions

Chitosan is modified with Fe_3O_4 forming nanocomposite, possibly applied as a biosensor for protein. The application of chitosan/ Fe_3O_4 as a sensor for alanine as a model molecule for protein is studied at B3LYP/6-31G(d,P). Such sensitivity is studied in terms of TDM, HOMO/LUMO energy gap, and MESP. Based on the obtained results, it is concluded that chitosan/ Fe_3O_4 can be used as a biosensor for alanine amino acid where HOMO/LUMO band gap energy decreased sharply and reached 0.3415 eV upon interaction with alanine. Additionally, MESP results indicated that the reactivity of chitosan is improved as the electronegativity increased.

Funding

This research received no external funding.

Acknowledgments

This research has no acknowledgment.

Conflicts of Interest

The authors declare no conflict of interest.

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