COMPUTATIONAL ANALYSIS ON ADDUCTION OF ALIZARIN WITH BOVINE SERUM ALBUMIN

By

Jemsheena, N.B

(13PPH007)

Thesis Submitted to

Avinashilingam Institute for Home science and Higher Education for Women,

Coimbatore-641 043

In partial fulfilment of the requirements for the Degree of

Master of Science in Physics

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CERTIFIED AS A BONAFIDE RESEARCH

30.3.15

Signature of the Head of the Department

30.3.2015

Signature of the Supervisor

ACKNOWLEDGEMENT

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I owe my sincere thanks to **Lord Almighty** and **My Loveable** Parents without whom I would have been nothing and showering their generous blessings upon me in all endeavors.

I am grateful to (**Thiru) Dr.T.S.K.MeenakshiSundaram**, M.A., M.Phil., Ph.D., Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for providing the facilities to conduct this study.

I extend my thanks to **HON.COL. Dr.(Tmt.) SheelaRamachandran**, M.Sc., P.G.Dip., Ph.D., Vice Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for providing flamboyant help towards the completion of the study.

I record my deep sense of gratitude to**Dr.(Tmt) Venmathi**, Msc,M.PhilRegistrar, Avinashilingam Institute for Home science and Higher education for providing adequate facilities required to carry out the work.

I also wish to express my gratitude to **Dr. (Tmt.) A.Parvathi**, Msc. Dip., Ed., M.Phil., Ph.D., Dean, Faculty of science Avinashilingam Institute for Home science and Higher Education for Women, Coimbatore for timely help renderd throughout the course.

I whole hearty thank **Dr. (Tmt.) J.Shanthi**, M.Sc., M. Phil., PhD, Associate Professor and Head (i/c) of the Department of Physics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for her encouragement and generous help which was of great value.

I am very much indebted to my Guide**, Mrs.S.Anitha**, M.Sc., M.Phil. Assistant Professor , Department of Physics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for her inspiring guidance, meticulous care, motivation, valuable advice, untiring support, timely suggestion, help, encouragement, holding me strong in all the places I faltered.

I also wish to thank **Dr. N.Shanthi**AssistantProfessor,Department of Bioinformatics,Biochemistry,Biotechnology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for their help and providing lab facilities to carry out research work.

I also thank **all the staff members** of the Department of Physics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for being supportive and understanding.

I would thank **all my friends** for their support, understanding and co-operation for the successful completion of the project.

JEMSHEENA N.B.

CONTENT

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INTRODUCTION

CHAPTER 1

INTRODUCTION

1.1SERUM ALBUMIN

Serum Albumin is a type of blood of globular protein. Human Serum Albumin is enclosed by ALB gene [1].Other mammalian forms, such as Bovine Serum Albumin is chemically same. Serum Albumin is produced by the liver, dissolved in blood plasma and most abundant blood protein in mammals. Albumin is essential for maintaining the pressure needed for proper distribution of body fluids between blood vessels and body tissue. Serum Albumin contains eleven distinct binding domains for hydrophobic compounds. One hemin and six long chain fatty acids can bind to Serum Albumin at the same time .Serum Albumin is a common blocking agent because of its binding properties and it can reduce contamination binding interfere in common molecular biology. It is also used as blocking agent in many other clinical applications including diagnostic procedures, medical devices and surgical stents [2].Serum Albumin diagram as shown in the **FIGURE: 1.1**

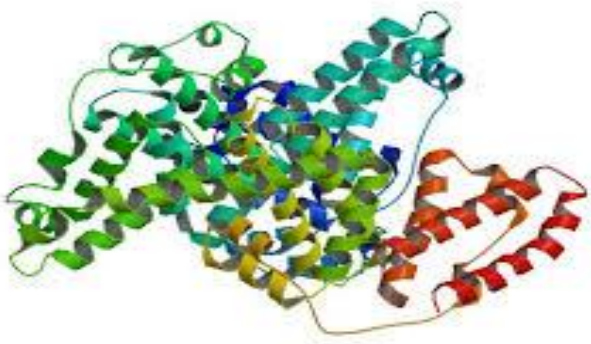

FIGURE: 1.1 SERUM ALBUMINS

1.2 BOVINE SERUM ALBUMIN

Serum albumin is a globular protein produced by liver in mammals and dissolved in bloodPlasmaAlbumin is essential to maintain the oncotic pressure and also regulates. The proper distributionofbody fluids between blood vessels and body tissues.

Albumin architecture is predominately helical and consists of three domains with very similar conformations that create an overall heart-like shape. [3-4] Due to a hydrophobic cleft, albumin binds fatty acids, bilirubin, Hormones and drugs.[4-5] The primary structure of BSA is a sequence of 583 amino acid residues where the secondary structure contains 67% alpha helix with six turns and 17 disulphide linkages.[6-8]. The tertiary structure is formed by three homologous domains I →III, each of which is divided into two sub-domains A and B. [7]. The pair-wise sequence alignment has only one gap over all the residues of the BSA sequence with 75% identity and 87% similarity shared between human and the bovine sequence [7]. Owing to their easy accessibility, high stability, ability to bind various ligand and similarity to HSA, mammalian serum albumins, especially bovine serum albumin (BSA), are utilized in kinetic and affinity drug tests as replacements for HSA, as well as in many biochemical and pharmacological applications [8].

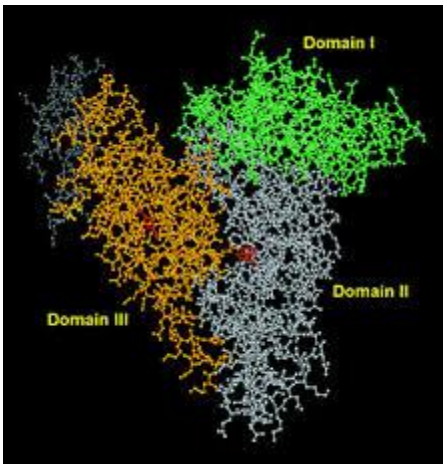

FIGURE: 1.2 BOVINE SERUM ALBUMIN

1.3 AMINO ACIDS

Amino acid is the group of organic compound containing two functional groups, amino and carboxyl. The amino group ($-NH_2$) is basic while the carboxylic group (-COOH) is acidic in nature [9]. Amino acids are regarded as "building block of protein". As many as 300 amino acids occur in nature, of these 3rdfunctional group (side chains) are classified into 20 amino acids as standard and repeatedly found in the structure of proteins [4] **table 1.1.** The general formula and structure of amino acids as shown in **figure 1.3**. Amino acid contains about 16 percentage nitrogen

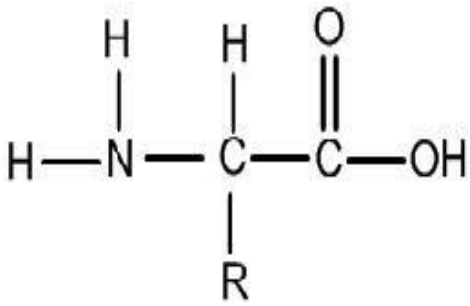

FIGURE 1.3 GENERAL STRUCTURE OFAMINO ACIDS

TABLE:1.1 List of Amino Acid

Amino acids	**Abbreviation**	**Molecular Formula**	**Comments**
GLYCINE	Gly – G	$C_2H_5NO_2$	AliphaticNeutral
ALANINE	Ala – A	$C_3H_7N_1O_2$	Aliphatic Hydrophobic Neutral
VALINE	Val – V	$C_5H_{11}N_1O_2$	AliphaticHydrophobic Neutral
LEUCINE	Leu – L	$C_6H_{13}N_1O_2$	AliphaticHydrophbic Neutral
ISOLEUCINE	Ile – I	$C_6H_{13}N_1O_2$	Aliphatic Hydrophobic Neutral
SERINE	Ser –S	$C_3H_7N_1O_3$	PolarHydrophobic Neutral
THREONINE	Thr –T	$C_4H_9N_1O_3$	PolarHydrophobic Neutral
CYSTEINE	Cys –C	$C_3H_7N_1O_2S_1$	PolarHydrophobic Neutral
METHIONINE	Met – M	$C_5H_{11}N_1O_2S_1$	HydrophobicNeutral
ASPARTIC ACID	Asp –D	$C_4H_7N_1O_4$	PolarHydrophobic charged(-)
ASPARAGINE	Asn –N	$C_5H_8N_2O_3$	PolarHydrophobic Neutral

GLUTAMIC ACID	Glu –E	$C_5H_9N_1O_4$	Polar Hydrophobic Neutral
GLUTAMINE	Gln –Q	$C_5H_{10}N_2O_2$	Polar Hydrophobic Neutral
ARGININE	Arg –R	$C_6H_{14}N_{14}O_2$	Polar Hydrphobic Charged(+)
LYSINE	Lys –K	$C_6H_{14}N_2O_2$	Polar HydrophobicCharged
HISTIDINE	His –H	$C_6H_9N_3O_2$	Aromatic Polar Hydrophobic Charged
PHENYLALANINE	Phe –F	$C_9H_{11}N_1O_2$	Aromatic Hydrophobic Neutra
TYROSINE	Tyr –Y	$C_9H_{11}N_1O_3$	Aromatic polar Hydrophobic
TRYPTOPHAN	Trp –W	$C_{11}H_{12}N_2O_2$	Aromatic Hydrophobic Neutral
PROLINE	Pro –P	$C_5H_9N_1O_2$	Hydrophobic Neutral

1.3.1 STEREOISOMERISUM OF AMINO ACIDS

Stereoisomerisms are isomeric molecule with same molecular formula and sequence of bounded atom, but differ in three - dimensional orientations of atom in space .Two amino acids in which, one is general structure and other will be mirror image isomer or (enantiomer) of the first compound. All the amino acid in protein contains L-form. Bacteria and antibodies contain D- form [10].

1.4 CLASSIFICATION OF AMINO ACIDS

The following are the types of amino acids

1. Non polar amino acid
2. Polar uncharged amino acid
3. Positive charged amino acid
4. Negative charged amino acid

1.4.1 NON POLAR AMINO ACID

Non polar amino acids are alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine and tryptophan. Amino acids, in which R group should be either aliphatic or aromatic. The amino acids are also referred to as to as hydrophobic and have no charge on the R group.

1.4.2 POLAR UNCHARGED AMINO ACID

Polar uncharged amino acids are glycine, serine, cysteine, threonine, tyrosine, asparagine, and glutamine. The amino acids are not only polar but also carry a positive charge and negative charge .Both aliphatic and aromatic compounds are included in this group.

1.4.3 POSITIVE CHARGED AMINO ACID

Three amino acids included in this group namely Lysine, Histidine, and Argine. Histidine is the only amino acid which has proton that dissociate in the neutral pH range [11].

1.4.4 NEGATIVE CHARGED AMINO ACID

Two amino acids namely Aspartic and glutamic acid included in this group namely. At natural pH, second carboxyl group dissociate giving of to these compounds.

1.5 PROTEIN

The word “protein” is derived from the Greek word “protos’’ meaning “primary” or holding the important places. Proteins are more plentiful macromolecule found in the cell [12].They regulate a verity of activities in all known organism, from replication of the genetic code to transporting oxygen, and responsible for regulating the cellular machinery and determining the phenotype of an organism. The proteins are the first recognizable and distinctive expressions of genetic information. The proteins enable them to act as catalyst (enzymes), which control the rate of all biological reaction and serve as the carries of essential substances, act as regulators within the organism (hormones) and to serve as the building block of sub cellular organic structure [13]. The proteins are considered as the unique polymer of amino acid. The overall chemical and structural properties of the protein are determined by amino acids [14]. Proteins have shapes that allow to them to interact selectively with other molecules.

1.6 STRUCTURE OF PROTEINS

Proteins are polymers specially polypeptides –sequences of L-α amino acids.

Proteins are formed from chains of amino acids. The bond between amino acid chains are complex protein structure which divided into four levels of organisms. They are,

a) Primary structure

b) Secondary structure

c) Tertiary structure

d) Quaternary structure

1.6.1 PRIMARY STRUCTURE

Primary structure of protein are the linear chain of amino acids forming the polypeptide of protein (backbone).Primary structure can be specified by the sequence of amino acid and each protein have unique chain of amino acids determined by the genes. The primary structure of protein is the identification elements of amino acids with their quality, quantity, and sequence in the structure shown **figure 1.3.**

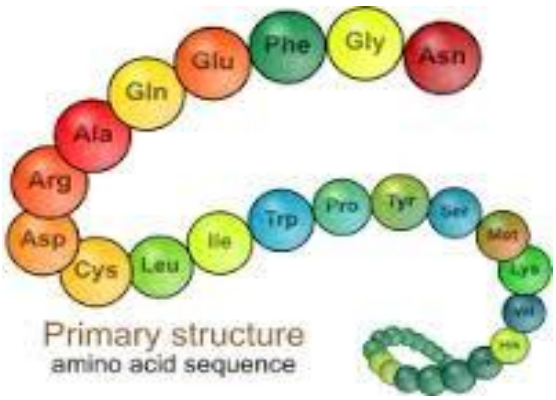

FIGURE: 1.3PRIMARY STRUCTURE

1.6.2 SECONDARY STRUCTURE

There are two common secondary structures in protein such as alpha helix and beta sheets. A common element of more secondary structure is the presence of hydrogen bond. The secondary structure of protein is the conformation of polypeptide chain by twisting or folding amino acids are close to each other in their sequence. There are two type of secondary structure, there are

α -helix

β -sheet

A) α -HELIX

The shape α-helix is a narrow-bore tube with it polypeptide backbone coiled up like a very tight clockwise screw thread and hydrogen bonds which are parallel to helical

axis [15].α-helix structure was proposed by Pauling and Corey in 1951 and found that polypeptide chain with planar peptide bonds from a right-handed helical structure by simple twist, i.e from α-carbon-to –carboxyl and α-carbon-to-nitrogen called as α-helix. The α-helix is a rod like structure and depends on intermolecular hydrogen bonds between the NH and CO groups of the peptide bonds **Figure 1.4.**

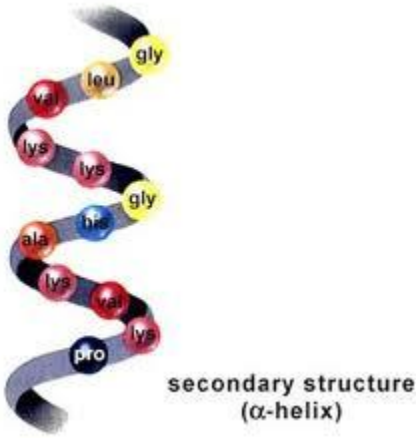

FIGURE: 1.4 α –Helix

B) β –SHEET

The β –sheet is the second form of regular secondary structure in protein. It is less common than α –helix. β -sheets consist of beta strands connected laterally by at least two or three backbone hydrogen bond, forming generally twisted, pleated sheet. A beta strand is a stretch of polypeptide chain typically 3to 10 amino acids long with backbone in an extended conformation [16].The formation of β-sheets depends on intermolecular hydrogen bonding through intermolecular hydrogen bonds are present. β –SHEET as shown in the **figure: 1.5**

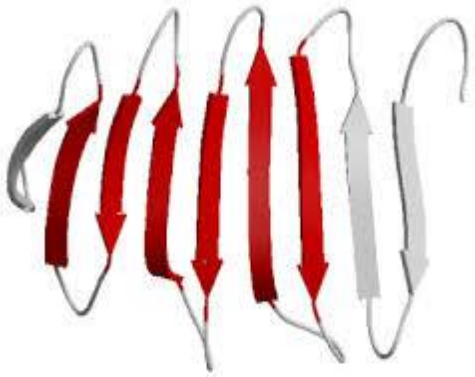

FIGURE: 1.5 β –SHEET

1.6.3 TERTIARY STRUCTURE

Three dimensional arrangement of protein structure referred as tertiary structures which are compact in structure with hydrophobic side chains held interior, while hydrophobic on the surface of protein structure.Tertiary structure will have a single backbone with one or more protein secondary structure. Amino acid chain interaction and bonds of side chain within a specific protein decide its tertiary structure [17]. This structure involves the folding of the helices of globular proteins **Figure 1.6.**

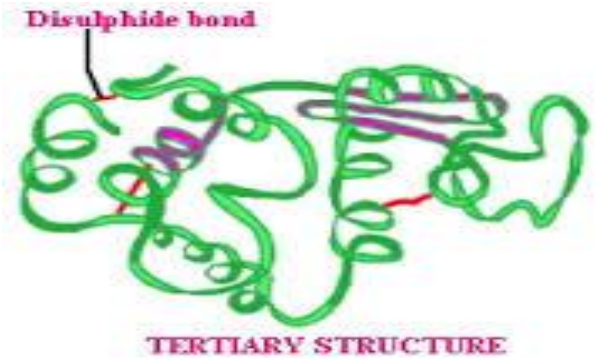

FIGURE: 1.6 TERTIARY STRUCTURE

1.6.4QUATENEAY STRUCTURE

A four degree of difficulty in protein structure has been recognized to be great value in many proteins as shown in **figure 1.7.** Some protein consists of 2 or more interacting peptide chains are called subunit. Quaternary structure is the special arrangement of subunits [18].

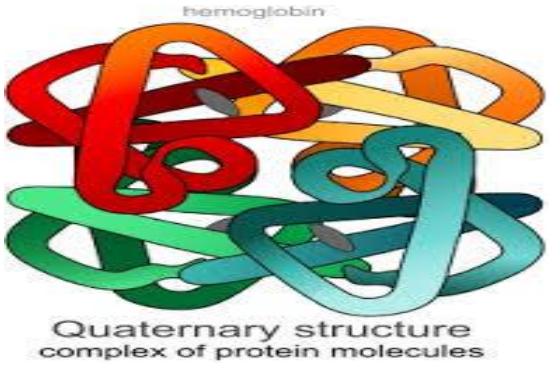

FIGURE: 1.7 QUATERNARY STRUCTURE

1.7 TYPES OF MOLECULAR BONDS

1. Covalent bond
2. Ionic bond
3. Hydrogen bond

1.7.1 COVALENT BOND

Large interatomic forces are created by the columbic effect produced by positive and negative charged ion. Covalent bonds are the strongest bond in protein structure [19]. Spin of two electrons must be oriented in different direction. Covalent bond is more stable .Examples of covalent bond, N, O, C and Clect,[20].Covalent bond involves mutual sharing of the pair of electron between two atoms **figure1.8.**

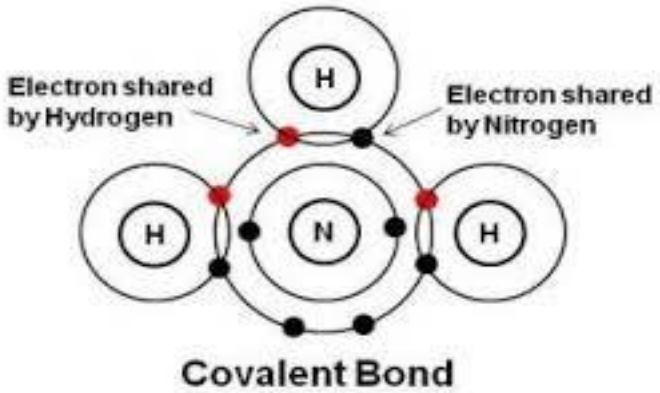

FIGURE: 1.8COVALENT BOND

1.7.2 IONIC BOND

Ionic bond is the complete transfer of the valence electron between two atoms. Moleculesconsist of charged ion with opposite charge is called ionic. Ionic bonds are formed in amino acids using electrostatic method. The principle of Electrostatic method is, two different charges attract each other and same charges repel each other [21]

PROPERTIES OF IONIC BOND

- Ionic bonds are very strong, a lot of energy needed to break the bond.
- Ionic bond is electro covalent bond, type of bond formed from the electrostatic attraction between opposite charged ion in a chemical compound.
- Boiling point of ionic bond is high.
- Ionic bonds no definite shape.
- Ionic bond occure between one metal and one non-metal.
- Ionic bonds are crystalline solid.

1.7.3 HYDROGEN BOND

Hydrogen bond is covalently bounded to a strong electronegative and small sized atom (O_2, N), the shared electron pair between the hydrogen atom and strongly electronegative atom lies much more nearer to the electronegative atom. This results in the development of partial ionic character in the covalent bond, with a fractional positive charge on the hydrogen atom and a fractional negative charge on the electronegative atom. The attractive force that binds hydrogen atom of one molecule with electronegative atom of another molecule of the same substance, called hydrogen bond or Hydrogen Bridge because hydrogen bond is usually denoted by a dotted line as shown in **figure 1.9 [**15**]** and important in many biological molecules such as DNA, where it helps to control the possible paring between the two stands of the molecule and in certain Ferro electric crystals [15].

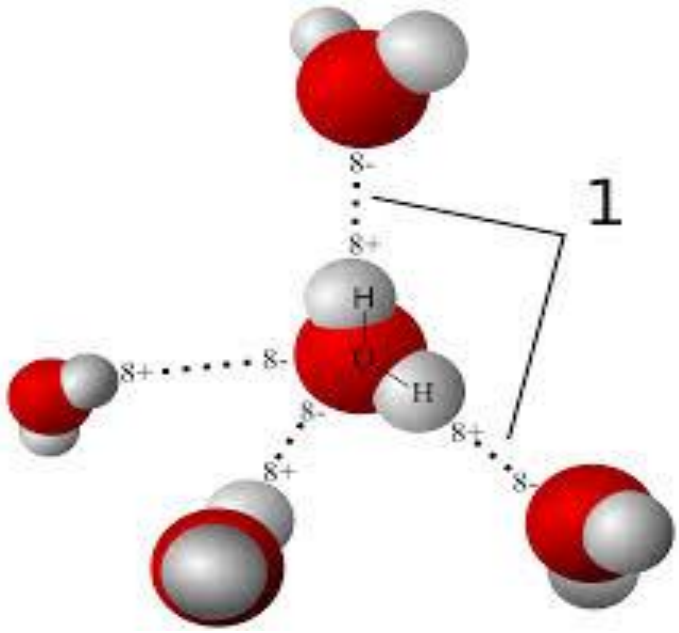

FIGURE: 1.9. HYDROGEN BOND

1.8 ALIZARIN (1, 2 dihydroxy-9, 10-anthraquione)

A Dihydroxy anthraquinone is an isomeric organic compound with formula $C_{14}H_8O_4$, derived from 9, 10-anthraquinone by replacing two hydrogen atoms by hydroxyl groups. Hydroxyanthrequinones are a class of molecules which have attracted wide interest from both applied and fundamental point of view [21].The dihydroxy-9, 10-anthraquinone functional group occurs widely in natural products, and had important feature of the anthracycline anti-tumour antibiotics.

1,2-Dihydroxy-9,10-anthraquinone (Alizarin) is among the most important natural and synthetic compounds, which has found wide application as analytical reagent and indicator, biologically active agent and medicine, dye and intermediate product in the synthesis of dyes, chemical agent for data recording and storage material, ect[22].Alizarin is the core moiety of adriamycin, an important antitumor drug and has numerous applications owing to their interesting photo activity [23].The structure of Alizarin is shown in the **Figure: 1.10.**

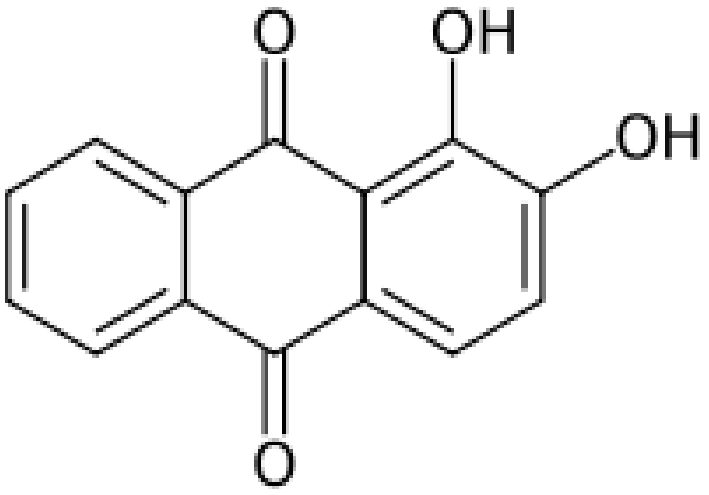

FIGURE: 1.10 ALIZARIN

1.9 OBJECTIVE OF THE PRESENT WORK

- ✓ To dock the ligand Alizarin with Bovine Serum Albumin.
- ✓ To optimize the Docking structure of Alizarin with Bovine Serum Albumin by ONIOM method.
- ✓ To study the energy and geometric parameters.
- ✓ To analyze the spectrum of this Docked structure.

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REVIEW OF LITERATURE

CHAPTER 2
REVIEW OF LITERATURE

2.1 INTRODUCTION

Review of literature is an important part of project. Purpose of this chapter carried out on topic to the structure and Interaction of alizarin with bovine serum albumin was briefly recorded in this chapter. In these literature, geometrical parameters and its interaction are studied by using varies theoretical method.

1. **V Sasirekha et al (2011)** examined the interactions between alizarin and bovine serum albumins (BSA) were studied by florescence and optical absorption techniques under physiological conditions. The results show that the alizarin can strongly bind to BSA molecules. The binding constant and number of binding site of a alizarin with BSA were estimated. Synchronous fluorometric determination of Alizarin bound to BSA. In their study, they have also investigated the effect of energy transfer between Alizarin and BSA by spectrofluorimetry.

2. Arumugam Selva Sharma et al (2014) investigation the interaction of a biologically active photodynamic therapeutic agent Toluidine blue O (TBO) with Human Serum Albumin and Bovine Serum Albumin was studied using absorption, emission, circular dichroism spectroscopy and molecular docking experiments. The emission titration experiment between HAS/BSA and TBO revealed the existence of strong interactions between TBO and the proteins. The site competitive experiment of HAS and BSA showed primary binding site of TBO is located in site 1 of HAS/BSA involving hydrophobic, hydrogen bonding and electrostatic interaction. To ascertain the results of site competitive experiments..Molecular docking was utilized to characterize the binding models of TBO-HAS/BSA complexes. From the molecular docking studies, free energy calculations were undertaken to examine the energy contributions and the role of various amino acid residues of HAS/BSA in TBO binding. The existence of Forester Resonance Energy transfer between the ligand and protein was utilized to calculate the donor-acceptor distance of TBO and protein. The TBO induced conformational changes of HAS/BSA was established using synchronous emission, three dimensional emission and circular dichroism studies.

3. Jie-hua Shi et al (2013) analyse the intermolecular interaction between cyaniding-3-glucside and Bovine Serum Albumin was investigated using fluorescence. Circular dichroism and molecular docking methods. The experimental results revealed that the fluorescence quenching of BSA AT 338nm by Cy-3-G resulted from the formation of Cy-3-G-BSA complex. The number of binding sites (n) for Cy-3-G to BSA, Cy-3-G is closer to the Try residue than the Try residue than the Trp residue; the secondary structure of BSA almost not change, the binding process of Cy-3-G with BSA is spontaneous, and Cy-3-G can be inserted into the hydrophobic cavity of BSA (site2) in the binding process of Cy-3-G with BSA. Moreover, based on the sign and magnitude of the enthalpy and entropy changes and the molecular docking results, it can be suggested that the main interaction forces of Cy-3-G with BSA are Van der Waals and hydrogen bonding interactions.

4. Narla.S.N et al (2014) they have been reported BSA with boronic acid (BA) conjugates as lectin mimetic and their glycol-capturing capacity. The BSA-BA conjugates were synthesized by amidation of carboxylic groups in BSA with amino phenyl boronic acid in the presence of EDC, and were characterized by Alizarin Red S assay and SDS-PAGE gel. The BSA-BA conjucates were immobilized onto maleimide-functionalized silica beads and their sugar capturing capacity and specificity were confirmed by ARS displacement assay. Further Plasmon resonance (SPR) analysis of the glycol-capturing activity of the BSA-BA based lectinmimetics for glycomics and biosensor research and applications.

5. Baosheng Liu et al (2012) investigating the interaction between Avelox and Bovine Serum Albumin was investigated at different temperatures by fluorescence spectroscopy. Results shows that Avelox could quench the intrinsic florescence of BSA strongly, and quenching mechanism was a static quenching process with Forester spectroscopy energy transfer. The electrostatic force played an important role on the conjugation reaction between BSA and Avelox. The order of magnitude of binding constant was 10^4; the number of binding site (n) in the binary system was approximately equal to 1. The binding distance (r) was less than 3nm and the primary binding site for Avelox was located in sub domain IIA of BSA .Synchronous fluorescence spectra clearly revealed that the microenvironment of amino acid residues and the conformation of BSA were changed during the binding reaction. In

addition, the effect of some antibiotics on the binding constant of Avelox with BSA was also studied.

6. Yan-Jun Hu et al (2007) analyzed the interaction between methylene blue (MB) and BSA was investigated by the fluorescence and VU-vis absorbance spectroscopy. In the mechanism discussion, it was provide that the fluorescence quenching of BSA by MB is mainly a result of the formation of MB-BSA complex and electrostatic interactions play an important role to stabilize the complex. The Stem-Volmer quenching constant K and corresponding thermodynamic parameters H, G and S were calculated. The distance between donor (BSA) and acceptor (MB) was obtained according to fluorescence energy transfer. The effect of MB on the conformation of BSA has been analyzed by means of UV-vis absorbance spectra and synchronous fluorescence spectroscopy.

7. Ying Li et al (2007) investigating the binding of rehein with HAS has been studied in detail by spectroscopic method including circular dichroism, Fourier transformation infrared spectra, fluorescence spectra. The binding parameters for the reaction have been calculated according to Scat chard equation at different temperatures. The plots indicated that the binding of HAS to rehein at 303,310 and 318 k is characterized by one binding site with the affinity constant K at $(4.93\pm0.16)\times10^5$, $(4.02\pm0.16)\times10^5$ and $(2.69\pm0.16)\times10^5 M^{-1}$, respectively .The secondary structure compositions of free HAS and its rehein complexes were estimated by the FT-IR spectra. FT-IR and curve-fitting results of amide I band are in good agreement with the analyses of CD spectra. Molecular Modeling method was used to calculate the interaction mode between the drug and HSA.

8. PrateekPandya et al (2014) they have been investigated the Vinblastine; a cytotoxic alkaloid is used extensively against various cancers types and the crystal structure of its tubulin complex is already known. Multitarget affinity of vinblastine has been investigated and the nature of binding with biological receptors namely, duplex DNA and HAS has been compared to the binding characteristics of its known complex wit natural high affinity receptor tubulin using molecular docking and QM/MM calculations. VLB is found to interact with DNA as well as HAS protein, through, with weaker affinity as compared to tubulin. Analysis of various docked

complexes revealed that the H-bonds and cation-pi bonds do not have significant contribution to the binding interactions and despite its large size, VLB remains in relaxed in the conformation and fits in the hydrophobic regions on the receptors.

9. MuzaffarUl Hassan et al (2014) analyze the effect of dodecyl betainte or DBG on the structure and function of bovine serum albumin by using fluorescence, time resolved fluorescence, circular dichroism and dynamic light scattering techniques. The Stern-Volmer quenching constants KSV and the corresponding thermodynamic parameters H, G and S have been estimated by the fluorescence quenching method. The results indicated that DBG binds spontaneously with BSA through hydrophobic interaction. Time resolved fluorescence data show that the quenching follows the static mechanism pathway. It can be seen from far UV CD spectra that the α -helical network of BSA is disrupted and its content increases from 71% to79% at lower concentrations which again decreases to 38% at higher concentration. DLS measurements suggested that hydrodynamic radius decreases in the presence of 30-40µM, of DBG while it increases when the concentration of DBG was 70 and 100 µM. The molecular docking study indicated that DBG is embedded into sub domain IIA of BSA and binds with the R-914, R-195 and R-217 residues by hydrogen bonding and by hydrophobic interaction.

[10] S.Bakkialakshmi et al (**2011)** have investigated interaction of anticancer drugs (1) Uracil (U), (2) 5-Fluorouracil (5FU) and (3) 5-Chlorouracil (5ClU), with bovine serum albumin at two levels of temperature was studied by the fluorescence of quenching method .UV-vis, time –resolved fluorescence, Fourier transform infra red spectroscopy, proton nuclear magnetic resonance and scanning electron microscope analyze were also made. Binding constants (Ka) and binding sites (n) at various levels of temperature were calculated. The binding sites were found to be equal to one for all the three quenchers at two different temperature levels .Thermo dynamical parameters $\Delta H, \Delta G, and\, \Delta S$ have been calculated and were presented in tables. Change in FTIR absorption intensity shows strong binding of anticancer drugs to BSA. Change in chemical shifts of NMR and fluorescence lifetimes of the drugs indicate the presence of interaction and binding of BSA to anticancer drugs.1 H NMR spectra and SEM photographs also conform this binding.

[11] Hongliang Xu et al (2013) they have been investigated the interaction of eupatorin and BSA using ultraviolet-visible absorption, fluorescence, synchronous fluorescence, circular dichroism spectroscopies, and molecular modeling at pHvalue is 7.4.Results of UV-vis and fluorescence spectroscopies illustrated that BSA fluorescence was quenched eupatorin via a static quenching mechanism. Thermodynamic parameters revealed that hydrophobic and electrostatic interactions played major roles in the interaction. Moreover, the efficiency of energy transfer, and the distance between BSA and acceptor eupatorin, were calculated. The effects of eupatorin on the BSA conformation were analyzed using the UV-vis, CD, and synchronous fluorescence and finally the binging of eupatorin to BSA was modeled using the molecular docking method.

[12] Zhuang J et al (1998) were studied the interaction of alizarin red S with bovine serum albumin by UV spectra in pHvalue is 4.35 buffer solution. The binding number (n=8) and binding constant (Kc =1.5×105) of the probe and protein were obtained. The main sort of binding force was investigated. It was investigated. It was found that the red shift of isosbetic point of the complex was 28 nm and the sodium chloride concentration had significant effect on the binding reaction. The “phase Distribution Model” is the proper description of the interaction between Alizarin S and the protein.

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METHODOLOGY

CHAPTER 3
METHODOLOGY

3.1 INTRODUCTION

Computational chemistry is a branch of chemistry that uses computer simulation to assist in solving chemical problems. It uses methods of theoretical chemistry, incorporated into efficient computer programs to calculate the structures and properties of molecules and solids. Its necessity arises from the fact that apart from relatively resent results concerning the hydrogen molecular ion-the quantum many body problems cannot be solved analytically, much information less in closed form. While computational results normally complement the obtained by chemical experiments. It is widely used in the design of new drugs and materials. This method range from highly accurate to very approximate; highly accurate methods are typically feasible only for small system. Ab initio methods are based entirely on quantum mechanics and other methods are called empirical or semi-empirical because they employ additional empirical parameters [1].

3.2 DOCKING

Molecular Docking is the computer aided prediction of the bound geometry of two or more molecules. Molecules may be docked manually with the aid of computer graphics or automatically by using computer algorithm. Molecular docking is a key tool in structural molecular biology and computer assisted drug design. The goal of ligand-protein docking is to predict the predominant binding mode of ligand with a protein of know three-dimensional structure. Successfully docking methods search high-dimensional space effectively and use a scoring function that correctly ranks candidate dockings .Docking can be used perform virtual screening on large libraries of compounds, rank the results, and purpose structural hypotheses of how the ligands inhibit the target, which is invaluable in lead optimization [2].

Molecular Docking, docking is a method which predicts the preferred orientation of one molecule to second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules.

Receptor: To “receiving” molecule most commonly a protein or other biopolymer.

Ligand: The complementary partner molecule which binds to the receptor. Ligand is most often small molecules but could also be another biopolymer.

Docking: Computational simulation of a candidate ligand binding to a receptor.

Scoring: The process of evaluating a particular pose by counting the number of favorable inter molecular interaction such as Hydrogen bonds and hydrophobic contacts.

Ranking: The processes of classifying which ligand are most likely to interact favorably to a particular receptor based on the predicted free energy of binding.

Scoring function are first approximate mathematical methods used to predict the strength of non covalent interaction between two molecules after they have been docked. Most commonly one of the molecules is a small organic compound such as a drug protein receptor. Scoring functions have also been developed to predict the strength of other types of intermolecular interaction.

3.3 COMPUTATIONAL CHEMISTRY

Computational chemistry is a branch of chemistry that uses the results of theoretical chemistry incorporated into efficient computer programs to calculate the structure and solid, applying these programs to real chemical problems. Examples of such properties are structure, energy and interaction energy, charges, charges, vibrational frequencies, reactivity or other particles. Computational chemistry is usually used when a mathematical method is sufficient well developed and automated for implementation on a computer [3].

3.4 QUANTUM CHEMISTRY

Quantum chemistry is a branch of chemistry whose primary focus is the application of quantum mechanics in physical models and experimental chemical system. It involves heavy interplay of experimental and theoretical methods. Experimental quantum chemists give data on spectroscopy, through which information regarding the quantization of energy on a molecular scale can be obtained. Common methods are infra-red (IR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy. Theoretical quantum chemistry, the working of which also tends to fall under the category of computational chemistry, seeks to calculate the predictions of quantum theory on atoms and molecules. It also have discrete energies; as this task, these calculations are performed using computers rather than by analytical.

In these ways, quantum chemist investigate chemical phenomena

- In reactions, quantum chemistry studies the ground state of individual atoms and molecules, the excited states, and the transition states that occur during chemical reactions.
- On the calculations: quantum chemical studies use also semi-empirical and other methods based on quantum mechanical principles, and deal with time dependent problems. Many quantum chemical studies assume the nuclei are at rest (Born –Oppenheimer Approximation). Many calculations involve iterative methods that include self-consistent field methods. Major goals of quantum chemistry include increasing accuracy of the results for small molecular system, and increased the size of large molecules that can be processed, which is limited by scaling considerations-the computation time increases as a power of the number of atoms.

3.5 THEORITICAL METHOD

In short**,** a theoretical model or method is a way to model a system using a specific set of approximations. These approximations are combined with a calculation algorithm and are applied to atomic orbital, defined by the basis set, in order to compute molecular orbital and energy. In general, the methods can be separated into 4 types

- Ab initio
- Semi Empirical Method
- The Density functional theory
- The Molecular mechanics

3.5.1 AB INITIO METHOD

An *Ab initio* is a Latin for "From the beginning" and indicates a calculation based on fundamental principles. Methods that do not include any semi empirical parameters in their equations being derived directly from theoretical principles, with no inclusion of experimental data are called *Ab initio*method [4].

This type of computation is based only on theoretical principles, using no experimental data. The numerous methods have the same basic approach, but difference in the mathematical approximations used.

Ab initio electronic structure methods have the advantage that they can be made to converge to the exact solution, when all approximations are sufficiently small in magnitude and when the finite set of basic functions tends towards the limit of a complete set. Conformation interaction, where all possible configurations are

included, tends to the exact non-relativististic solution of the electronic Schrodinger equation. Ab initio calculations provide data on bond angles, and bond lengths, molecular conformation and internal rotation barriers, for ground and excited stated of molecules. They are very useful for calculating accurate thermo chemistry, ionization energy, oscillator strength dipole moments and excitation energies. The accuracy of the calculation and the magnitude of the system are limited ultimately by computer speed and capacity [5].

Ab initio methods often take larger amounts of computer time, memory, and disk space, though, with modern advances in computer science and technology such considerations are becoming less of an issue. It calculates energy and orbital of the molecule.

3.5.2 HATREE-FOCK METHOD

Hatree-Fock is the basic *ab initio* model. It uses the approximation that Columbic electron-electron repulsion can be averaged, instead of considering explicit repulsion interactions. There are two ways to compute molecular orbital using HF: UHF (unrestricted) or RHF (restricted).UHF uses a separate orbital for each electron, even if they are paired (used for ions, excited states, radicals, ect). RHF uses the same orbital spatial function for electrons in the same pair (good for species with paired electrons, no spin contamination).The major drawback of HF method is the exclusion of electron correlation. The following models start with an HF and then correct for electron repulsion.

In computational physics and computational chemistry, the Hartree -Fock (HF) method is an approximate method for the determination of the ground state wave function and excited state energy of a quantum many-body system. Hartree -Fock is the best single-determinant wave function, Multi-determinant methods are computationally much more involved than the HF model, but results systematically approach the exact solution of Schrodinger equation [6].

On solving the time independent Schrodinger equation

$$H\psi = E\psi \qquad (1)$$

3.5.3 SEMI EMPERICAL METHOD

Semi-empirical quantum chemistry methods are based on the HF formalism, but make many approximations and obtain some parameters from empirical data. Semi empirical calculations are much faster than their ab initio counterparts, mostly due to the use of the zero differential overlap approximation. It is very important in computational chemistry for treating large molecules where the full Hartree-Fock method without the approximations is too expensive. Semi empirical methods follow empirical methods follow empirical methods where the two electron part of the Hamiltonian is not explicitly included.

Semi empirical molecular quantum-mechanical methods use a simpler Hamiltonian than the correct molecular Hamiltonian and use parameters whose value are adjusted to fit experimental data or the results of ab initio calculations; an example is Huckel molecular orbital treatment of conjugated hydrocarbons, which uses a one-electron Hamiltonian and takes the bond integrals as adjustable parameters rather than quantities to be calculated theoretically. Semi empirical quantum chemistry methods are based on the Hartree- Fock formalism, but make many approximations and obtain some parameters from empirical data [7].

$$F_{\mu\nu} = \langle \mu \mid F \mid \mu \rangle = \langle \mu \mid F \mid \mu \rangle + \sum \lambda, \rho P\lambda\rho \left[(\mu\nu \mid \lambda\sigma) - {}^{1}/_{2} (\mu\nu \mid \lambda\sigma) \right] \quad (2)$$

In which P is the density matrix, given as

$$P\lambda\sigma = 2\sum_{i}^{occ} Ci\lambda Ci\sigma \quad (3)$$

To simplify matters drastically, the Zero Differential Overlap (ZDO) approximation assume:

$$\Phi \mu(r) \; \varphi \nu(r) = 0 \text{ for } \mu \neq \nu$$

This implies that

$$S_{\mu\nu} = \langle \mu \mid \nu \rangle = \delta_{\mu\nu} \quad (4)$$

$$S_{\mu\nu} = 0 \text{ if } \mu \neq \nu, \; \delta_{\mu\nu} = 1 \quad (5)$$

This can be justified when the atomic basis orbital are orthonormalized.

The use of semi empirical parameters appears to allow some inclusion of electron correlation effects into the methods. Within the framework of Hartree - Fock calculations, some pieces of information are approximated or completely omitted. Semi empirical methods are parameterized, and results are fitted by a set of

parameters, and agree with experimental data, but sometimes to agree with ab initio results.

As a result of the ZDO approximation many two electrons integrals vanish: [8]

$$(\mu\nu \mid \lambda\sigma) = \delta\mu\nu\delta_{\lambda\sigma}(\mu\mu \mid \lambda\lambda) \qquad (6)$$

3.5.4 DENSITY FUNCTIONAL THEORY

Density Functional Theory (DFT) is a computational quantum mechanical modeling method used in physics, chemistry, and material science to investigate the electronic structure of many body systems, in particular atoms, molecules, and the condensed phases. With this theory, the properties of a many-electron system can be determined by using functional, i.e. functions of another function, which in this case are the especially dependent electron density. Hence the name density functional theory comes from the use of functional of the electron density. DFT is a among the most popular and versatile methods available in condensed- matter physics, computational physics, and computational chemistry.

Time - independent Schrodinger equation.

$$\hat{H}=[\hat{T}+\hat{V}+\hat{U}]\psi=\left[\sum_{i}^{N}\left(-\frac{\hbar^2}{2mi}\nabla_i^2\right)+\sum_{i}^{N}v(\vec{r}_i)+\sum_{i}^{N}U(\vec{r}_{i,}\vec{r}_j)\right]=\text{E}\,\psi \qquad (7)$$

Where, for the N-electron system, $\hat{H}$ the Hamiltonian, $\hat{E}$is the total energy, $\hat{T}$is the kinetic energy, $\hat{V}$is the potential energy from the external field due to positively charged nuclei, and $\hat{U}$is the electron-electron interaction energy. The operators $\hat{T}$and $\hat{U}$are called universal operators as they are the same for any N-electron system, while $\hat{V}$is system dependent. This complicated interaction term$\hat{U}$.

Here DFT provides an appealing alternative, being much more versatile as it provides a way to systematically map the many-body problem, with$\hat{U}$, onto a single-body problem without$\hat{U}$. In DFT the key variable is the particle density n ($\vec{r}$), which for a normalized ψ is given by

$$\text{n}(\vec{r}) = \text{N}\int d^3\text{r}_2 \ldots\ldots\int d^3\text{r}_{\text{N}}\,\psi^*(\vec{r},\vec{r}_{2,\ldots\ldots\ldots,}\vec{r}_{\text{N}})\,\psi((\vec{r},\vec{r}_{2,\ldots\ldots\ldots,}\vec{r}_{\text{N}}). \qquad (8)$$

This relation can be reversed, i.e., for a given ground-state density n_o(r) is possible, in principle, to calculate the corresponding ground-state wave function ψ_0 ($\vec{r}_1\ldots\ldots,\vec{r}_{N)}$.In other words, ψ is a unique functional of n_o,

$$\Psi_0=\psi[n_o] \qquad (9)$$

and consequently the ground-state expectation value of an observable $\hat{O}$is also a functional of n_0

$$O[n_o] = \langle\ \psi\ [\ n_0]\ |\hat{O}\ |\psi\ [n_0]\rangle \quad (10)$$

In particular, the ground-state energy is a functional of n_0

$$E_0 = E\ [n_0] = \langle\ [n_0]\ |\hat{T} + \hat{V} + \hat{U}\ |\psi\ [n_0]\rangle \quad (11)$$

Where the contribution of the external potential $\langle\ \psi[n_0]\ |\hat{V}\ |\psi[n_o]\rangle$ can be written explicitly in terms of the ground-state density$n_{o.}$

$$V[n_o] = \int V(\vec{r})n_o(\vec{r})d^3r \quad (12)$$

More generally, the contribution of the external potential$\langle\psi\ |\hat{V}\ |\ \psi\rangle$ can be written explicitly in terms of the density n.

$$V(n) = \int V(\vec{r})n(\vec{r})d^3r \quad (13)$$

The functional $T(n)$and $U(n)$ are called universal functional, while $V(n)$ is called a non-universal functional, as it depends on the system under study. Having specified a system, i.e., having specified$\hat{V}$, one then has to minimize the functional.

$$E[n] = T(n) + U(n) + \int V(\vec{r})n(\vec{r})d^3r \quad (14)$$

With respect to $n(\vec{r})$, assuming one has got reliable expressions for $T(n) and U(n)$.A successful minimization of the energy functional will yield the ground-state density n_o and thus all other ground state observable.

The variation problems of minimizing the energy functional E[n]can be solved by applying the Lagrangian method of undetermined multipliers. First, one considers an energy functional that doesn't explicitly have an electron-electron interaction energy term,

$$E_S[n] = \langle\psi_s[n]\ |\hat{T} + \hat{V}_S\ |\ \psi_S[n\rangle] \quad (15)$$

Where $\hat{T}$denotes the kinetic energy operator and $\hat{V}_S$ is an external effective potential in which the particles are moving, so that

$$n_s(\vec{r}) \overset{def}{=} n(\vec{r}) \quad (16)$$

$$\left[\left[-\frac{\hbar^2}{2m}\nabla^2 + V_S(\vec{r})\ \right]\phi_i(\vec{r}) = \varepsilon_i\phi_i(\vec{r})\right. \quad (17)$$

Which yields the orbital ϕ_i that reproduce the density n ($\vec{r}$) of the original many-body system.

$$n(\vec{r}) \overset{def}{=} n_s(\vec{r}) = \sum_i^N |\phi_i(\vec{r})|^2 \tag{18}$$

The effective single-particle potential can be written in more detail as

$$V_S(\vec{r}) = V(\vec{r}) + \int \frac{e^2 n_s(\vec{r}^1)}{|\vec{r} - \vec{r}^1|} d^3 r^1 + V_{XC}[n_s(\vec{r})] \tag{19}$$

Where, the second term denotes the so-called Hartree term describing the electron-electron Coulomb repulsion, while the last term V_{XC} is called the exchange-correlation potential. Here, V_{XC} includes all the many particle interactions. Since the Hartree term and V_{XC} depend on $n(\vec{r})$, which depend on the ϕ_i , which in turn depend on V_S, the problem of solving the Kohn-Sham equation has to be done in a self-consistent way. Usually one starts with an initial guess for $n(\vec{r})$then calculates the corresponding V_S and solves the Kohn-Sham equations for them ϕ_i . From these one calculates a new density and start again. This procedure is then repeated until convergence is reached. A non-iterative approximate formulation called Harris functional DFT is an alternative approach to this [9].

3.6 QM/MM Methods and ONIOM

Quantum mechanical (QM) methods based on the Schrodinger equation (e.g. ab initio, semi empirical, and DFT methods) are extremely useful and powerful, the scaling and the limitation of computational resources becomes a practical issue when modeling large systems. Certain biological molecules would take too long to model with QM methods since they contain thousands and thousands of atoms.Molecular mechanical (MM) methods have been developed to model the atoms based on classical mechanics. MM methods calculate the potential energy of the system through the use of force fields. Many MM force fields have been developed and parameterized and it has general form,

$$E = E_{\text{bonded}} + E_{\text{non-bonded}}$$

Where E_{bonded} is the summation of the covalent contributions such as bond length, angle, and dihedral terms and $E_{\text{non-bonded}}$ is the summation of the non-covalent

contributions of electrostatics and van der Waals terms. E, is the total potential energy of the system. While MM methods are significantly faster than QM methods, MM methods are not able to compute various chemical reactions such as changes in chemical bonding and quantum effects such as electronic excitation. Therefore, combining the best of both worlds (accuracy of QM and speed of MM) was developed called the hybrid QM/MM models.

3.6.1Hybrid QM/MM method:

The basic approach of the hybrid QM/MM methods is to partition the system into layers. The majority of the system is modeled with faster methods such as MM, while the smaller portion of interest is modeled with more accurate methods such as QM. a mathematical formula, the energy calculated from hybrid QM/MM approaches can be generalized as

$$E=E_{MM}+E_{QM}+E_{QM/MM}$$

The E_{MM} and E_{QM} terms are simple to conceptualize and understand, because it is merely splitting the system into two layers where each is calculated using a different method. However, the boundary. Treatment term ($E_{QM/MM}$) is where the formulation varies depending on the QM/MM implementation. The necessity of this term is due to the fact that even though we are partitioning the system, the regions are not independent of one another. Therefore, $E_{QM/MM}$ describes the energy of interaction of the two layers, which includes Van der Waals, electrostatic, and other interactions used by the force field.

3.6.2 ONIOM Approach

ONIOM called "Our own N-layered Integrated molecular Orbital and molecular Mechanics". The basis of energy subtraction methods are used and calculate the energy with formula of

$$E= E_{M\,M,\,real}+E_{QM,\,model}-E_{MM,\,model}$$

Where real refers to the whole system, i.e. the water molecules, enzyme, substrate, and catalytic residues and model refers to only the substrate and catalytic residues. Gaussian allows keyword ONIOM to do two-or three-layer ONIOM calculations [10]

3.7 BASIS SET

A **basis set** is a set of functions (called basis functions) that describes the shape of atomic orbital (AOs), and combined in linear combinations to create molecular orbital (MO).

Both ab initio and DFT methods sue sets of mathematical functions to represent the atomic orbital. These are called basis set. These mathematical functions are themselves made from combination of simple mathematical functions called Primitives. Increasing the number of primitive functions and including contributions from valence orbital imposes less restriction on the exact molecular orbitals, but correspondingly increasing the combinational cost. The molecular orbitals are approximated as linear combination of the basic functions.

In the ab initio methods, a Gaussian-type atomic function is used as the basis function, which as the general form:

$$g\left(\alpha, r^1\right) = cx^n yz^1 e^{-\alpha r^2} \quad (20)$$

Where vector r^1 is the position of the electron, which is composed of coordinates x, y and z and β is a constant that determines the size of the function. The Gaussian function $e^{-\alpha r^2}$ is multiplied powers of x, y, and z and it is normalized by constant c, so that:

$$\int g^2 = 1 \quad (21)$$

Linear combination of the primitive Gaussian functions in the equation (2) is used to form the basis functions. A set of basis sets has been devised to increase the comparability between researchers and to simplify the nomenclature when describing the model chemistry: STO-3G, 3-21G, 6-21G, 4-31G, 6-31G, 6-31+G(d,p),6-311G,6-311++G(d, p)…….in the order of increasingly large basis sets.

3.7.1 STO BASIS SET

Atomic orbital are used for high accuracy in calculations and it can be a linear combination of several Slater types of functions. The most common basis set is STO-3G, where n is an integer. This n value can be represents the number of Gaussian primitive functions comprise core and valence orbital. Commonly used minimal basis sets of this type are:

- STO-3G
- STO-4G
- STO-6G
- STO-3G*

3.7.2 6-31G BASIS SET

This split valence basis set, where the core orbital are a calculation of six PGTOs. The inner part of the valance orbital is a concentration of three PGTOs and the outer part of the valance represented by one PGTO. In terms of the concentrated basis function, it contains the same number as 3-21G, but the representation of each function is better since more PGTOs are used [11].

The 6-31G* basis set is valence double-zeta polarized basis set that adds to the 6-31G set six d-type Cartesian-Gaussian polarization functions on each of the atoms Li through Ca and ten f-type Cartesian Gaussian polarization functions on each of the atoms Sc through Zn[12].

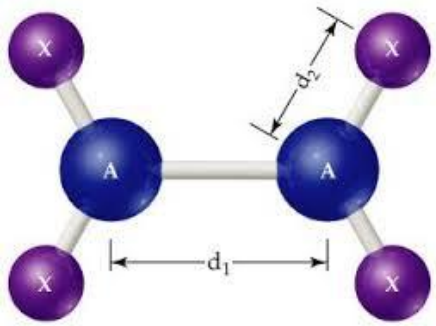
3.8. GEOMETRICAL PARAMETERS

3.8.1 BOND LENGTH

In molecular geometry, **bond length** or **bond distance** is the average distance between nuclei of two bonded atoms in a molecule. It is transferable property of a bond between atoms of fixed types, relatively independent of the rest of the rest of the molecule.

Bond length is related to bond order, when more electrons participate in bond formation dissociation energy, as (all other things being equal) a stronger bond will be shorter. In a bond between two identical atoms half the bond distance is equal to the covalent radius.

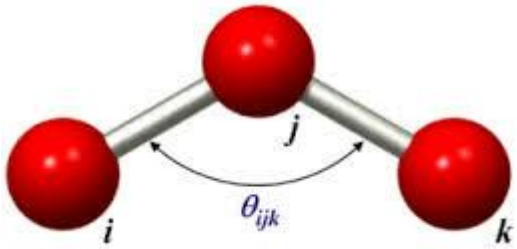
Bond length can be measured by x-ray analysis of crystals, diffraction of x-ray spectroscopy. In the case of single bond, the bond length is the sum of the covalent bond radii of two bonded atoms. A double is shorter than a single bond and triple bond is shorter still.



BOND LENGTH

3.8.2 BOND ANGLE

The average angle between the orbital of the central atom containing the bonding electron pairs in the molecule is known as bond angle between the atoms. The unit of bond angle is either degree or minute or second. This gives an idea about the distribution of orbital's around the central atom in a molecule. Therefore bond angle determines the shape of a molecule [13].



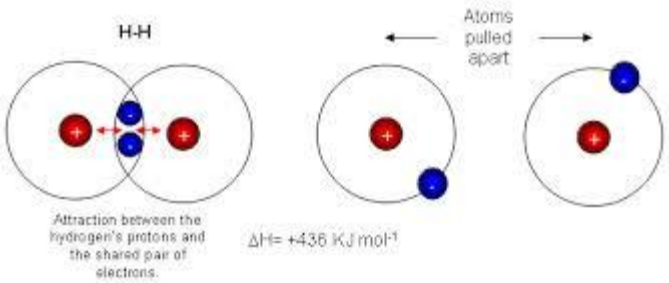
BOND ANGLE

3.8.3 BOND ENERGY

Bond energy (E) is the measure of bond strength in a chemical bond, It is the heat required to break one mole of molecules into their individual atoms [9].Bond energy (E) should not be confused with bond-dissociation energy. It is a roughly transferable property, and enthalpy of formulation can typically be roughly approximated by simply adding tabulated values for bond energies for all bonds in a molecule, with an error of sometimes just a few percent.

Bond strength (energy) can be directly related to the bond length/ bond distance. Therefore, we can use the metallic radius, ionic radius, or covalent radius of each atom in the molecule to determine the bond strength.

The bond order is the number of electron pairs shared between two atoms in the formation of the bond. Bond order for C=C and O=O is 2.The amount of energy required to break a bond is called bond dissociation energy or bond energy. Bond energy is a measure of the strength of a chemical bond. The larger the bond energy, the stronger the bond



BOND ENERGY

3.8.4 DIPOLE MOMENT

The separation of charges due to the electron displacement in a molecule is measured by dipole moment, and is given by the product of electronic charge and the distance between positive and negative centers in the dipole.

$$\mu = e * d$$

The dipole moment is expressed in terms of the unit-cm and is denoted by D, a dipole moment is the measure of polarity and asymmetry of the molecule and hence it measurement gives valuable information about the structure and shape of the molecule. The dipole moment of a molecule of given structure is given by the vector sum of the individual bond moments.

3.8.5 CHEMICAL POTENTIAL

It is measure of the escaping tendency of an electron cloud and constant, through all space for ground state of the system. If a plot of E Vs N for any system is drawn then μ is simple the instantaneous slope of such a curve and designated as

$$\mu = \left(\frac{\partial E}{\partial N} \right) V(r)$$

Where, E is the energy, n is the number of electron and V is the potential due to the fixed nuclei. The definition of μ is

$$\mu = \frac{\left(-I + A \right)}{2}$$

Where, I is the ionization potential ($-E_{HOMO}$) and A is the electron affinity $\left(-E_{LUMO} \right)$.

According to Koopmans's theorem the ionization potential is simply the orbital energy of the (HOMO), with change is sign. For spin paired molecules, the electron affinity is the negative of the orbital energy of the (LUMO).

I= $\left(-E_{HOMO} \right)$,(HOMO) –highest occupied molecular orbital

A= $\left(-E_{LUMO}\right)$, (LUMO) – lowest unoccupied molecular orbital.

3.8.6 CHEMICAL HARDNESS (ŋ)

The chemical hardness of an atom or a molecule is defined as the second derivative of the energy E with respect to the number of electrons n at constant external potential and temperature

$$2\eta = \left(\frac{\partial^2 E}{\partial V^2} \right) V(r)$$

To obtain the other approximation to η it is useful to correlate χ and η with molecular orbital theory. Fortunately, it turns out that they are completely compatible from Koopmans theorem

$$\text{I} = (-E_{HOMO})$$

$$\text{A} = (-E_{LUMO})$$

$$\eta = (-E_{LUMO} - E_{HOMO}) / 2$$

The gap between the HOMO and LUMO is equal to 2η. The hardness of an atom or a molecule is the reciprocal of the respective softness (s) i.e. $\eta = 1/2S$.

3.8.7 THERMO DYNAMICAL PARAMETERS

The enthalpy change (ΔH_0) was regarded as a constant when the temperature changed little, then enthalpy change (ΔH_0) and entropy change (ΔS_0) can be obtained from Van't Hoff equation:

$$\ln K = -\Delta H/RT + \Delta S/R$$

$$\Delta G_0 = \Delta H_0 - T\Delta S_0 = -RT\ln K,$$

Where R was the gas constant and $\Delta G0$ was the standard free energy change.

The thermodynamic parameters of Gibb's free energy change, ΔG°, enthalpy change, ΔH°, and entropy change, ΔS°, for the adsorption processes are calculated using the following equations:

$$\Delta G_o = -RT \text{ l n } K_a$$

3.9 COMPUTATIONAL DETAILS OF THE PRESENT STUDY

- In the present study interaction of alizarin with bovine serum albumin were diameter 4 is optimized using ONIOM (B3LYP/6-31+G (d, p)/ UFF) level of theory.
- All the calculations were performed using Gaussian 03 program.

- The bond length, bond angle, bond energy, dipole moment, molecular vibrations parameter, polarizability and Hyperpolarizability have been calculated using ONIOM (B3LYP/6-31+G(d,p)/UFF) level of theory
- The vibrational frequency calculation has been performed using ONIOM (B3LYP/6-31+G (d, p)/UFF) level of theory.
- The HOMO and LUMO energies were calculated by using ONIOM (B3LYP/6-31+G (d, p) /UFF) level of theory

3.10. REFERENCE

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RESULTS AND DISCUSSION

CHAPTER 4

RESULTS AND DISCUSSION

4.1 INTRODCTION

Serum albumins (SA) contribute to colloid osmotic blood pressure and the maintenance of blood pH. They also play a dominant role in drug disposition and efficacy. Most drugs are transported as a complex with SA, which makes SA an important part of drug metabolism [1-2]. Bovine serum albumin (BSA) is the most abundant protein plasma contributing to osmotic blood pressure which plays important roles in the transport, distribution and metabolism of many exogenous ligand, including fatty acids, amino acids, drugs and pharmaceuticals. Being the major binding protein for drugs and other physiological substances, it is considered as a model protein for studying Drug-protein interaction in vitro. Alizarin (AZ) is an important antitumor drug, which has found wide application as analytical reagent and indicator, biologically active agent and medicine, dye and intermediate product in the synthesis of dyes, chemical agent for data recording and storage materials, etc [3]. Protein–drug interaction is the hot point in the fields of medicine, chemistry and biology. The information regarding the nature of binding with biomolecules, especially with proteins, nucleic acids, etc. is essential in order to understand the mechanism of their interactions and to identify similar possible ligand of potential medicinal importance.

Molecular docking is a fast and reliable tool for the study of inter-molecular interactions in the systems of biological and therapeutic significance. Although, the QM–MM approach, that combines the accuracy of quantum mechanics and speed of molecular mechanics, was introduced as early as 1970s by Warship and Levitt [4] to obtain more realistic information about bio-molecular interactions, most of the early stages of drug discovery process still involve the use of the molecular mechanics based docking programs to obtain dependable prediction towards the efficacy of the pharmaceutically important molecules [5-6]. In the present investigation, an attempt has been made to identify the nature of binding interactions of Alizarin (AZ) with biological receptors of Bovine serum albumin (BSA) using molecular docking program Schrodinger software-maestro and applying QM–MM based ONIOM methodology in Gaussian 09. The binding characteristics of AZ–BSA complex and their molecular geometry, spectral studies are analyzed. Thermo

dynamical properties, polarizability and Hyperpolarizability values of active site of AZ-BSA have been computed using QM/MM methods.

4.2. RESULT AND DISCUSSION

4.2.1 DOCKING STUDY

The amino acid sequence of Bovine Serum Albumin had been taken from NCBI database. PDB file of BSA is taken from PDB ID: 3VO3, water molecules are removed from the complex. This sequence is aligned against Protein Data Bank deposited sequences using BLAST. Alizarin was docked on to BSA protein molecule using the Glide in Maestro of Schrodinger software. The further exploration was carried out with smaller grid map of 10x10x10 points centered at 38.001, 19.138 and 58.284. Three active sites are predicted for AZ-BSA by site map in Maestro. For our theoretical investigation, we have chosen active site II with high active site score is -5.048. The system sizes is reduced by removing the residues located outside the radius of 4Å is shown in the **figure 4.1**

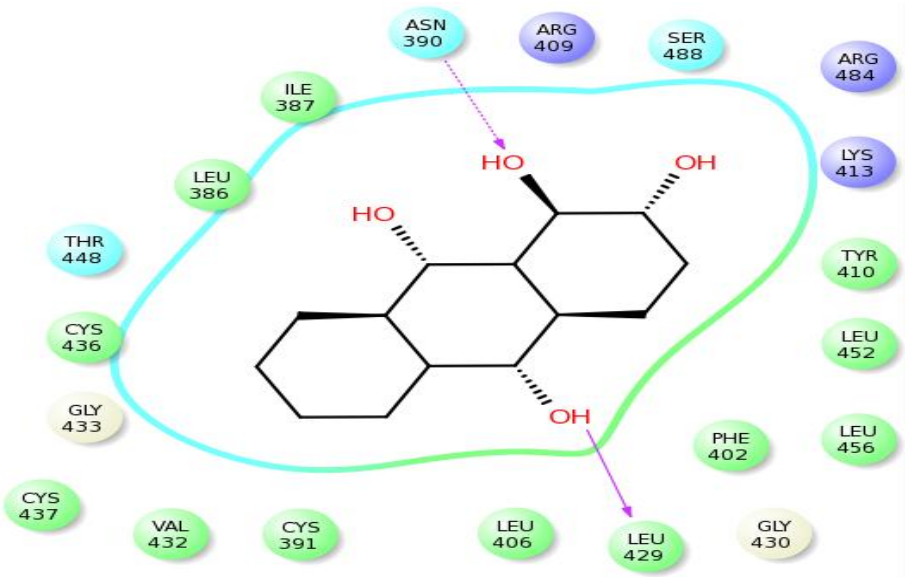

FIGURE: 4.1 LIGAND INTERACTION DIAGRAM OF ACTIVE SITE II OF ALIZARIN WITH BSA

4.2.2 Binding site and binding mode

The analysis of docking results shows that AZ binds with the receptor's sub-domain I-A. The amino acid residue involve in the binding of active site II of AZ with BSA are ASN 390, LEU 429 is shown in the fig 4.1. Binding energy of AZ-BSA (active site II) is 20.711 Kcal/mol. Docking score of above said active site is -7.428.

Based on the docking score, glide energy and hydrogen bond interaction, the best conformation docking score is analyzed. Glide energy, docking score and hydrogen

bond interaction of the ligand with the residues are given in **TABLE: 4.1 .**Docking results showed that the ligand shade good binding affinity in active site II [6]. The ligand(AZ) has shown good interaction with the residues ASN 390 and LEU 429.ASN 390 had bond of N-H-O with bond length 2.06A° and for LEU 429 ,N-H-O by bond length 2.32 A°,Hydrogen interaction diagram as shown in **FIGURE:4.2**

TABLE: 4.1DOCKING SCORE, GLIDE ENERGY AND GRID BOX VALUE OF BINDING SITE OF BSA WITH ALIZARIN.

1	Docking score	-7.428
2	Glide energy	-20.711
3	Glide ligand efficiency	-0.413
4	Grid box X cent	38.001
5	Grid box Y cent	19.138
6	Grid box Z cent	58.284

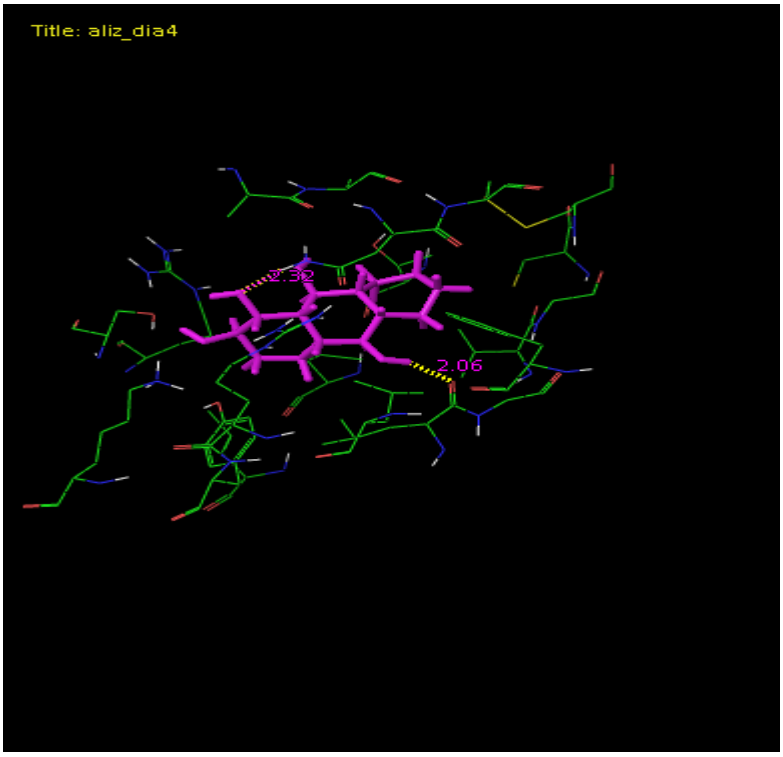

FIGURE: 4 .2 HYDROGEN INTERACTION DIAGRAM OF ACTIVE SITE II OF ALIZARIN WITH BSA USING ONIOM (B3LYP/6-31+G (D, P)/UFF) LEVEL OF THEORY.

4.3 ONIOM-QM CALCULATION

Two layers ONIOM calculation was carried out using Gaussian-09[7].The calculation was performed using best binding docked pose of active site II of Alizarin with BSA domain 1A.For this purpose, the Alizarin molecule was treated in high (QM) layer while BSA was treated with low (MM) layer. The idea was to further optimize the Alizarin pose resulting from docking using Quantum mechanics while keeping within the influence of BSA. The calculation was done for QM layer at DFT level with ONIOM(B3LYP/6-31+G (d, p)/UFF) basis set and treating the BSA in Molecular mechanics layer using UFF force field [8] is show in the **figure4.3**.Energy for active site II of AZ-BSA system have been calculated using ONIOM method is -848.940846995 a.u by the formula,

$$E\,(AZ+BSA)_{ONIOM}=E_1(BSA)_{MM}+E_2(AZ)_{QM}$$

CALCULATED ENERGY OF ACTIVE SITE OF ALIZARIN WITH BSA AT ONIOM (B3LYP/6-31+G (d,p)/UFF) LEVEL OF THEORY.

ONIOM energy	-848.94084(a.u)
Model energy-low	0.162522735(a.u)
Model energy-high	-848.89678(a.u)
Real energy	0.12029307(a.u)

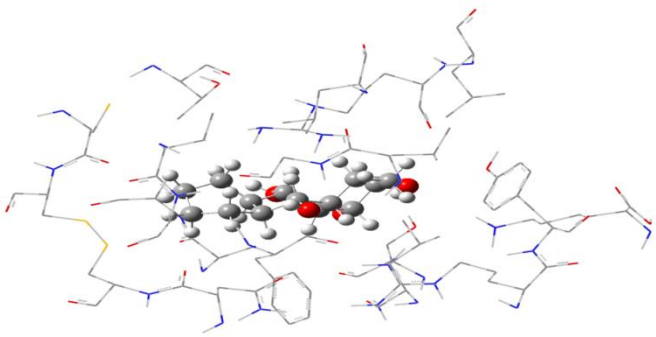

FIGURE: 4.3 THE OPTIMIZED STRUCTURE OF ALIZARIN WITH BSA AT ONIOM (B3LYP/6-31+G (d,p)/UFF)LEVEL OF THEORY.

Bond length, Bond angle of active site II of AZ-BSA using ONIOM (B3LYP/6-31+G (d, p)/UFF) level of theory are tabulated and shown in the **Table: 4.2, 4.3.**

TABLE: 4.2 BOND LENGTHS (IN A^0) OFALIZARIN WITH BOVINE SERUM ALBUMIN OPTIMIZED AT ONIOM (B3LYP/6-31+G (d, p)/UFF) LEVELS OF THEORY

PARAMETER	ONIOM (B3LYP/6-31+G (d,p)/UFF)	PARAMETER	ONIOM (B3LYP/6-31+G (d,p)/UFF)
N_1-H_1	1.04623	C_{21}-C_{19}	1.39731
N_1-C_1	1.45809	C_{22}-C_{21}	1.39739
C_1-C_3	1.51941	O_7=C_{24}	1.22035
C_1-C_2	1.49875	C_{23}-N_7	1.45780
C_2=O_1	1.25963	C_{23}-C_{25}	1.52448
C_2-N_2	1.36903	C_{24}-C_{23}	1.49491
N_2-H_2	1.04575	N_7-H_8	1.04670
N_2-C_4	1.45830	C_{25}-C_{26}	1.51885
N_3-H_3	1.04610	C_{26}-C_{28}	1.51654
C_4-C_6	1.51635	C_{26}-C_{27}	1.51100
C_4-C_5	1.49046	H_9-N_8	1.04606
C_5=O_2	1.21962	N_8-C_{29}	1.45866
H_4-N_4	1.0440	N_8-H_9	1.04606
N_4-H_5	1.04450	N_9-H_{10}	1.04525
N_4-C_{10}	1.34950	N_9-C_{34}	1.36064
N_5-H_6	1.04582	C_{29}-C_{30}	1.50022
N_5-C_{11}	1.45918	C_{29}-C_{31}	1.52220
N_6-H_7	1.04628	C_{29}-N_8	1.45866
C_7-N_3	1.46002	C_{29}-C_{30}	1.50022
C_7-C_8	1.49820	C_{30}=O_8	1.25999
C_8=O_3	1.26012	C_{30}-N_{12}	1.36883
C_8-N_5	1.35745	C_{31}-C_{32}	1.51940
C_9-C_7	1.52576	C_{31}-C_{29}	1.52220
C_{10}=O_4	1.25886	C_{32}-C_{33}	1.51932
C_{10}-C_9	1.49324	C_{32}-C_{31}	1.51940
C_{11}-C_{12}	1.49373	C_{33}-N_9	1.45807

C_{11}-C_{13}	1.52413	C_{33}-C_{32}	1.51932
C_{12}=O_{5}	1.22044	C_{34}-N_{10}	1.35702
C_{13}-S_{1}	1.81824	C_{34}-N_{11}	1.35467
C_{14}-N_{6}	1.45650	N_{10}-H_{11}	1.04307
C_{14}-C_{16}	1.52264	N_{11}-H_{13}	1.04431
O_{6}=C_{15}	1.21928	N_{11}-H_{14}	1.04442
C_{15}-C_{14}	1.49448	H_{12}-N_{10}	1.04332
C_{16}-C_{17}	1.49075	N_{12}-H_{15}	1.04487
C_{17}-C_{18}	1.40050	N_{12}-C_{35}	1.45915
C_{18}-C_{20}	1.39803	C_{35}-C_{37}	1.52763
C_{19}-C_{17}	1.39795	C_{35}-C_{36}	1.49359
C_{20}-C_{21}	1.39781	C_{49}-C_{50}	1.49818
C_{35}-C_{36}	1.49359	C_{49}-N_{15}	1.45979
C_{36}=O_{6}	1.22042	C_{49}-C_{51}	1.52456
C_{37}-C_{38}	1.49302	C_{50}=O_{12}	1.26073
C_{38}-C_{39}	1.40005	C_{50}-N_{16}	1.35707
C_{39}-C_{41}	1.39682	C_{51}-C_{52}	1.51938
C_{40}-C_{38}	1.39835	C_{52}-C_{53}	1.51665
C_{41}-C_{43}	1.39787	C_{52}-C_{53}	1.50983
C_{42}-C_{40}	1.39756	C_{55}-C_{56}	1.48989
C_{43}-C_{42}	1.40270	C_{56}=O_{13}	1.21906
C_{43}-O_{10}	1.39427	C_{57}-N_{17}	1.46092
C_{44}-N_{13}	1.46063	C_{57}-C_{58}	1.50240
C_{44}-C_{109}	1.49934	C_{57}-C_{59}	1.53448
C_{44}-C_{45}	1.52271	C_{58}=O_{14}	1.26011
O_{10}-H_{16}	1.01393	C_{58}-N_{18}	1.36858
N_{13}-H_{17}	1.04689	C_{59}-C_{60}	1.51661
C_{45}-C_{46}	1.51587	C_{59}-C_{61}	1.51848
C_{46}-C_{47}	1.51259	C_{62}-C_{63}	1.48996
C_{47}-C_{48}	1.51819	C_{63}=O_{15}	1.21939
C_{48}-N_{14}	1.45616	C_{64}-C_{65}	1.49848
N_{14}-H_{20}	1.04413	C_{64}-N_{19}	1.4980

N_{14}-H_{19}	1.04580	C_{65}=O_{16}	1.26011
N_{15}-H_{21}	1.04620	C_{65}-N_{20}	1.36894
N_{16}-H_{22}	1.04483	C_{67}-C_{68}	1.49378
N_{16}-C_{55}	1.45582	C_{67}-C_{69}	1.52328
N_{17}-H_{23}	1.04545	C_{68}=O_{17}	1.22044
N_{18}-H_{24}	1.04453	C_{69}-S_{3}	1.81843
N_{18}-C_{62}	1.45580	C_{70}-N_{21}	1.45590
N_{19}-H_{25}	1.04620	C_{70}-C_{71}	1.49415
N_{20}-H_{26}	1.04577	C_{70}-C_{72}	1.53362
N_{20}-C_{67}	1.45863	C_{71}-O_{18}	1.22006
N_{21}-H_{27}	1.04482	C_{72}-C_{73}	1.52163
N_{22}-H_{29}	1.04689	C_{72}-O_{19}	1.41504
N_{23}-H_{30}	1.04693	O_{19}-H_{28}	0.9927
N_{24}-H_{31}	1.04705	C_{74}-N_{22}	1.45817
N_{25}-H_{32}	1.04639	C_{74}-C_{75}	1.45817
N_{25}-C_{91}	1.46375	C_{74}-C_{76}	1.52584
N_{26}-H_{33}	1.04535	C_{75}=O_{20}	1.22044
N_{26}-H_{34}	1.04462	C_{76}-C_{77}	1.51846
N_{27}-H_{36}	1.04468	C_{77}-C_{78}	1.51475
N_{27}-H_{35}	1.04484	C_{77}-C_{79}	1.51224
N_{28}-H_{37}	1.04691	C_{80}-C_{81}	1.49211
N_{28}-H_{46}	0.97029	C_{80}-C_{82}	1.52299
N_{27}-H_{36}	1.04468	C_{77}-C_{79}	1.51475
C_{81}-O_{21}	1.21957	C_{97}-C_{98}	1.09824
C_{82}-C_{83}	1.51900	C_{98}-C_{104}	1.54603
C_{83}-C_{84}	1.51119	C_{98}-H_{52}	1.09692
C_{83}-C_{85}	1.51650	C_{99}-H_{53}	1.09968
C_{86}-N_{24}	1.45681	C_{99}-O_{26}	1.41418
C_{86}-C_{88}	1.52391	C_{100}-H_{54}	1.10211
C_{87}=O_{22}	1.21950	C_{100}-O_{27}	1.43528
C_{88}-C_{89}	1.52309	C_{100}-C_{98}	1.53414
C_{89}-C_{90}	1.52168	C_{101}-O_{25}	1.42848

C_{90}-N_{25}	1.45671	O_{25}-H_{45}	0.97157
C_{91}-N_{27}	1.46208	C_{101}-H_{55}	1.09824
C_{91}-C_{26}	1.46199	C_{101}-C_{105}	1.53151
C_{92}-C_{93}	1.49236	C_{102}-H_{39}	1.09841
C_{92}-N_{28}	1.45783	C_{102}-H_{56}	1.09454
C_{92}-C_{94}	1.51968	C_{102}-C_{96}	1.54266
C_{92}-C_{93}	1.49236	C_{103}-H_{40}	1.09695
C_{93}=O_{23}	1.21931	C_{103}-H_{57}	1.09722
C_{94}-O_{24}	1.39440	C_{103}-C_{107}	1.54422
O_{24}-H_{38}	0.99009	C_{104}-H_{58}	1.10070
C_{95}-C_{96}	1.55746	C_{104}-H_{41}	1.09403
C_{95}-H_{49}	1.09658	C_{105}-H_{59}	1.10269
C_{95}-C_{101}	1.54716	C_{105}-O_{28}	1.42275
C_{96}-H_{50}	1.09510	C_{105}-C_{106}	1.52277
C_{96}-C_{100}	1.54467	C_{106}-H_{42}	1.09387
C_{97}-C_{99}	1.54537	C_{106}-H_{60}	1.09687
C_{97}-H_{51}	1.09710	C_{106}-C_{102}	1.53037
C_{107}-H_{61}	1.09545	O_{26}-H_{47}	0.96510
C_{107}-H_{43}	1.54332	C_{108}-H_{63}	1.09322
C_{107}-C_{108}	1.54332	C_{108}-H_{44}	1.09687

TABLE: 4.3 BOND ANGLE (DEG) OF ALIZARIN WITH BOVINE SERUM ALBUMIN OPTIMIZED AT ONIOM (B3LYP/6-31+G (d,p)/UFF) THEORY

PARAMETER	**ONIOM (B3LYP/6-31+G (d,p)/UFF)**	**PARAMETER**	**ONIOM (B3LYP/6-31+G (d,p)/UFF)**
N_1-C_1-C_3	33.687	C_{11}-C_{12}=O_5	33.060
C_3-C_1-C_2	111.697	C_{11}-C_{13}-S_1	110.984
C_1-C_2=O_1	33.688	C_{12}-C_{11}-C_{13}	35.437
C_1-C_2-N_2	27.946	C_{14}-C_{15}=O_6	120.125
C_1-N_1-H_1	109.372	C_{14}-C_{16}-C_{17}	111.039

C_2-N_2-H_2	119.878	C_{14}-N_6-H_7	109.034
C_2-N_2-C_4	121.295	C_{16}-C_{17}-C_{19}	119.837
H_2-N_2-C_4	25.025	C_{16}-C_{17}-C_{18}	120.288
N_2-C_4-C_6	110.058	C_{16}-C_{14}-C_{15}	34.464
N_2-C_4-C_5	109.992	C_{17}-C_{18}-C_{20}	120.081
C_4-C_5-O_2	119.956	C_{17}-C_{19}-C_{21}	119.984
C_5-C_4-C_6	109.637	C_{18}-C_{20}-C_{22}	119.967
C_7-C_8=O_3	33.612	C_{19}-C_{21}-C_{22}	120.169
C_7-C_8- N_5	121.000	C_{21}-C_{22}-C_{20}	30.035
C_7-N_3- H_3	109.415	C_{23}-C_{24}-O_7	120.560
C_8-N_5- H_3	30.413	C_{23}-C_{25}-C_{26}	111.030
C_8-N_5- C_{11}	30.413	C_{24}-C_{23}-N_7	34.446
C_9-C_7- C_{11}	111.373	C_{24}-C_{23}-C_{25}	35.090
C_9-C_7- C_8	110.998	C_{25}-C_{26}-C_{27}	109.023
C_9-C_{10}- O_4	119.839	C_{28}-C_{26}-C_{27}	35.660
C_9-C_{10}- N_4	120.139	C_{29}-C_{30}-N_{12}	30.986
C_{10}-N_4- H_4	120.587	C_{29}-C_{31}-C_{32}	109.953
H_4-N_4-H_5	118.732	C_{30}-C_{29}-C_{31}	34.488
H_6-N_5-C_{11}	25.105	C_{30}-N_{12}-H_{15}	119.796
N_5-C_{11}-C_{13}	110.164	C_{31}-C_{32}-C_{33}	110.613
N_5-C_{11}-C_{12}	35.690	C_{31}-C_{29}-N_8	33.650
N_6-C_{14}-C_{15}	110.164	C_{32}-C_{33}-N_9	110.503
N_9-C_{34}-N_{10}	121.058	C_{33}-N_9-H_{10}	117.464
H_{10}-N_9-C_{34}	25.831	C_{34}-N_{10}-H_{12}	32.967
N_{11}-C_{34}-N_{10}	30.251	C_{34}-N_{11}-H_{13}	121.042
H_{11}-N_{10}-H_{12}	31.434	C_{34}-N_{11}-H_{14}	121.031

H_{14}-N_{11}-H_{13}	31.035	C_{35}-C_{36}=O_9	120.468
N_{12}-C_{35}-C_{36}	109.443	C_{35}-C_{37}-C_{38}	112.277
N_{13}-C_{44}-C_{45}	35.483	C_{37}-C_{38}-C_{40}	119.473
N_{13}-C_{44}-C_{109}	111.373	C_{37}-C_{38}-C_{39}	120.721
N_{15}-C_{49}-C_{51}	35.158	C_{38}-C_{39}-C_{41}	120.119
N_{15}-C_{49}-C_{50}	111.211	C_{38}-C_{40}-C_{42}	120.141
N_{16}-C_{50}=O_{12}	31.033	C_{39}-C_{38}-C_{40}	30.115
N_{16}-C_{55}-C_{56}	109.657	C_{39}-C_{41}-C_{43}	120.282
H_{19}-N_{14}-H_{20}	35.336	C_{40}-C_{42}-C_{43}	120.150
H_{19}-N_{14}-H_{18}	35.820	C_{42}-C_{43}-O_{10}	29.350
H_{20}-N_{14}-H_{14}	35.373	C_{43}-C_{42}-O_{41}	30.157
O_{10}-C_{43}-C_{41}	30.295	C_{58}-C_{57}-N_{17}	35.375
C_{43}-O_{10}-H_{16}	112.744	C_{59}-C_{57}-N_{17}	112.032
C_{44}-N_{13}-H_{17}	109.138	C_{60}-C_{59}-C_{57}	110.113
C_{44}-C_{45}-C_{46}	110.564	C_{60}-C_{59}-C_{61}	35.375
C_{45}-C_{46}-C_{47}	109.090	N_{18}-C_{58}=O_{14}	31.178
C_{46}-C_{47}-C_{48}	109.870	H_{24}-N_{18}-C_{58}	120.109
C_{47}-C_{48}-N_{14}	110.600	H_{24}-N_{18}-C_{62}	36.417
C_{48}-N_{14}-H_{18}	109.792	N_{18}-C_{62}-C_{63}	110.035
C_{48}-N_{14}-H_{20}	108.256	C_{62}-C_{63}=O_{15}	119.945
C_{48}-N_{14}-H_{19}	109.943	C_{64}-N_{19}-H_{25}	109.378
C_{49}-C_{50}=O_{12}	33.683	C_{64}-C_{65}=O_{16}	33.586
C_{49}-N_{15}=H_{21}	109.327	C_{64}-C_{65}-N_{20}	28.482
C_{50}-C_{49}-C_{51}	111.485	C_{64}-C_{66}-S_2	38.053

H_{22}-N_{16}-C_{50}	120.276	C_{65}-N_{20}-H_{26}	119.905
H_{22}-N_{16}-C_{55}	36.454	C_{65}-C_{64}-N_{19}	34.026
C_{55}-C_{56}=O_{13}	120.103	C_{66}-C_{64}-C_{65}	34.784
C_{57}-N_{17}=H_{23}	109.231	C_{66}-C_{64}-N_{19}	33.480
C_{57}-C_{58}=O_{14}	120.974	O_{16}=C_{65}-N_{20}	28.482
C_{58}-N_{18}-C_{62}	28.258	C_{67}-C_{68}=O_{17}	120.472
N_{20}-C_{67}-C_{69}	34.063	C_{70}-N_{21}-H_{27}	107.453
N_{20}-C_{67}-C_{68}	35.663	C_{70}-C_{72}-O_{19}	56.737
N_{21}-C_{70}-C_{71}	109.430	C_{72}-O_{19}-H_{28}	107.556
N_{21}-C_{70}-C_{72}	111.405	C_{72}-C_{70}-C_{71}	34.049
H_{26}-N_{20}-C_{67}	118.705	C_{73}-C_{72}-C_{70}	34.252
S_{1}-C_{70}-C_{71}	34.049	C_{73}-C_{72}-O_{19}	35.734
N_{22}-C_{74}-C_{75}	35.555	C_{73}-C_{72}-C_{70}	34.252
N_{23}-C_{80}-C_{81}	35.371	C_{74}-N_{22}-H_{29}	109.106
N_{23}-C_{80}-C_{82}	110.758	C_{74}-C_{75}=O_{20}	120.533
O_{18}=C_{71}-C_{70}	120.534	C_{76}-C_{74}-N_{22}	35.450
H_{30}-N_{23}-C_{80}	109.109	C_{77}-C_{76}-C_{74}	34.249
N_{25}-C_{90}-C_{89}	33.934	C_{78}-C_{77}-C_{79}	35.522
N_{25}-C_{91}-N_{26}	35.068	C_{78}-C_{77}-C_{76}	34.962
N_{27}-C_{91}-N_{26}	35.081	C_{79}-C_{77}-C_{76}	35.341
H_{32}-N_{25}-C_{91}	42.458	C_{80}-C_{81}=O_{21}	120.036
H_{33}-N_{26}-C_{91}	107.440	C_{82}-C_{80}-C_{81}	34.686
H_{33}-N_{26}-H_{34}	37.384	C_{83}-C_{82}-C_{80}	34.475
N_{28}-C_{92}-C_{94}	110.632	C_{84}-C_{83}-C_{82}	109.054

N_{28}-C_{92}-C_{93}	35.326	C_{84}-C_{83}-C_{85}	35.547
H_{35}-N_{27}-C_{91}	28.839	C_{85}-C_{83}-C_{82}	110.179
H_{36}-N_{27}-C_{91}	106.910	C_{86}-N_{24}-H_{31}	109.068
H_{36}-N_{27}-H_{35}	105.458	C_{87}-C_{86}-N_{24}	35.475
H_{37}-N_{28}-C_{92}	109.114	C_{88}-C_{86}-C_{87}	111.079
C_{28}-C_{92}-C_{94}	34.861	C_{88}-C_{86}-N_{24}	34.008

4.4 CHEMICAL POTENTIAL, CHEMICAL HARDNESS AND CHEMICAL SOFTNESS

The chemical potential, chemical hardness and chemical softness are important tools to study the stability of the molecular system, which have been calculated at ONIOM (B3LYP/6-31+G (d, p)/UFF) level of theory for Alizarin with Bovine Serum Albumin are given in the **TABLE 4.4.**The chemical potential , chemical hardness and chemical softness of Alizarin with Bovine Serum Albumin is -0.38947 ,0.13556 and 3.68840 respectively at ONIOM (B3LYP/6-31+G (d, p)/UFF) level of theory.

Table: 4.4GEOMETRIC PARAMETERS OF ALIZARIN WITH BOVINE SERUM ALBUMIN at ONIOM (B3LYP/6-31+G (d, p) /UFF) LEVELS OF THEORY.

PARAMETERS	B3LYP/6-31+G (d,p)
Energy (Hartree)	1.769874
Chemical potential (μ)	-0.38947
Chemical hardness(ŋ)	0.13556
Chemical softness(s)	3.68840

4.5 THERMO DYNAMICAL PARAMETERS

Several thermo dynamical parameters have been calculated by using ONIOM (B3LYP/6-31+G (d, p)/UFF) level of theory are given in **Table 4.5.** Scale factors have been recommended [9]for an accurate prediction determining the zero-point vibrational energies for ONIOM calculation. The total energy of the molecule is the sum of the translational, rotational, vibrational and electronic energies, Zero point vibrational energy, entropy, Dipole moment are tabulated.

TABLE: 4.5 THEORETICALLY COMPUTED ENERGIES (kcal/mol.), ZERO-POINT VIBRATIONAL ENERGIES (Kcal/mol), ROTATIONBAL CONSTANT (GHz),AND DIPOLE MOMENT (Debye) at ONIOM (B3LYP/6-31+G (d,p)/UFF) level of theory.

Parameters	**ONIOM (B3LYP/6-31+G (d, p) /UFF)**
Total energy	1110.612 (kcal/mol)
Heat capacity	617.053 (kcal/mol Kelvin)
Zero point vibration energy	1.586126 9 (Hartree/particle)
Entropy	989.352(cal/mol Kelvin)
Rotational constant(GHz) A B C	 0.0090784 0.005414 0.0049095
Rotational energy	0.889
Translational	0.889
Vibrational	1108.835
Dipole moment	6.6190 (Debye)
Enthalpy	1.770818

4.7 HOMO AND LUMO ANALYSIS

Highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) are very important parameters in quantum chemistry to determine the interaction of molecule with other species; they are termed frontier orbitals. HOMO can be through the outermost orbital containing electrons tends to give these electrons such as an electron donor. On the other hand, LUMO can be through the innermost

orbital containing free places to accept electron. The HOMO and LUMO are the most important orbitals in a molecule. TheEigen values of HOMO and LUMO and their energy gap reflects the biological activity of a molecule. A molecule having a small frontier orbitals gap is more polarizable andis generally associatedwith a high chemical reactivity and low kinetic stability. When the energyof theenergy of theHOMO is directly related to the ionization potential, LUMOenergy is directly related to the electron affinity. Energy difference between HOMO and LUMO orbital is called as energy gap that is an important stability for structures.

Electron distribution of HOMO and LUMO with the energy value is presentedat ONIOM (B3LYP/6-31+G/6-31+G (d,p)/UFF)respectively. The HOMO and LUMO energy gap of alizarin with bovine serum albumin is calculated at ONIOM (B3LYP/6-31+G(d,p)/6-3/UFF)level of theory reveals that the energy gap reflects the chemical activity of the molecule. The energy gap of HOMO and LUMO explains the eventual charge transfer interaction within a molecule. The lowest unoccupied molecular orbitals are also localized mainly in carboxylic group [10].

The energy of the highest occupied molecular orbital (HOMO) is -0.25797 eV and lowest unoccupied molecular orbital is (LUMO) is 0.01315 eV in B3LYP/6-31+G level. Energy gap is -0.27112.

4.8 VIBRATIONS

The vibration of modes is two types:

1. Stretching vibration
2. Bending vibration

4.8.1 STRETCHING VIBRATION

In this type of vibration, the atoms move essentially along the bond axis. So that the bond length increases or decreases at higher energy and occur at higher frequency .Stretching vibration is of two types

SYMMETRIC VIBRATION: In this type of stretching with respect to a particular atom, other two atoms in a molecule move in the same.

ASYMMETRIC VIBRATION: In this type of stretching one atom moves away from the central atom, while the other atom moves towards the central atom.

4.8.2BENDING VIBRATION

This vibrations may consists of a change in bond angle between bonds with a common atom the movement of a group of atoms with respect to the remainder of the molecule without movement of the atoms in a group with respect to one another.

These are of four types.

a) Scissoring : In scissoring, the two atoms concerned to a atom move towards and away from each other with deformation of the valency angle(in plane bending).

b) Rocking: In rocking the, the structural units swings back and forth in the plane of molecule (in plane bending)

c) Wagging: In wagging, the structural unit swings back and forth out of the plane of molecule. (Out of plane bending).

d) Twisting: In twisting, the structural unit rotates about the bond which joints it to the remainder of the molecule. (Out of plane molecule)[11].

The occurrence of different modes of vibrations of DL-Valinium dehydrogen phosphate at both HF/3-21G and B3LYP/3-2IG levels of theory are listed in the table

4.9 VIBRATIONAL ASSIGNMENTS

Alizarin with BSA is completely anonlinear molecule and it has (3N-6) degree of freedom. Since the molecule Alizarin with BSA 63 atoms, it is observed with 170 mode of vibration which includes stretching vibrations such as symmetric stretching, asymmetric stretching and bending vibrations like scissoring, rocking, wagging and twisting. The vibrational frequencies of Alizarin with BSA are calculated at ONIOM (B3LYP/6-31+G (d,p)/UFF) and their assignments are given in the **table4.4.**The IR and Raman spectrum are plotted in the graph. The graph plotted between frequency in X-axis and Raman intensity in Y-axis is shown in **figure: 4.4.** And the graph plotted between frequency in the X-axis and IR intensity in the Y-axis is shown in **figure: 4.5.**

The strongest peak of IR vibration spectrum is at3068.94cm^{-1} corresponds to CCC ring bending of Alizarin.The next highest peak is at1083.0385cm^{-1} also corresponds to CCC ring bending of Alizarin. In Raman spectrum, the strongest absorption for Alizarin with BSA is at 3062cm^{-1} corresponds to N-H stretching and the next highest peak is at 3084cm^{-1} corresponds to C-H stretching.

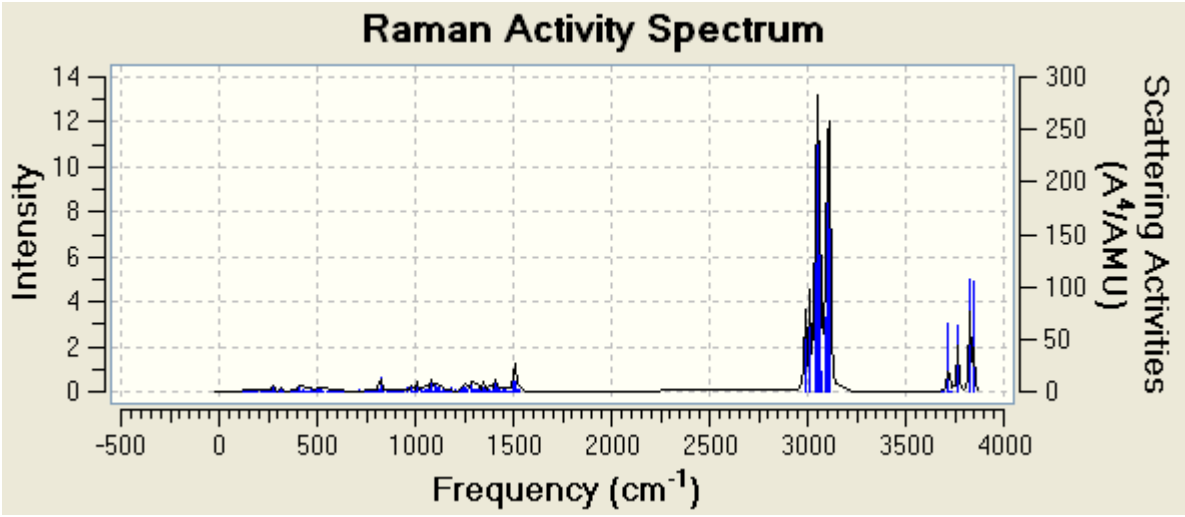

FIGURE:4.4A GRAPH BETWEEN WAVE NUMBER (vs) RAMAN INTENSITY FOR ALIZARIN WITH BOVINE SERUM ALBUMIN AT ONIOM(B3LYP/6-31+G (d, p)/UFF) LEVEL OF THEORY.

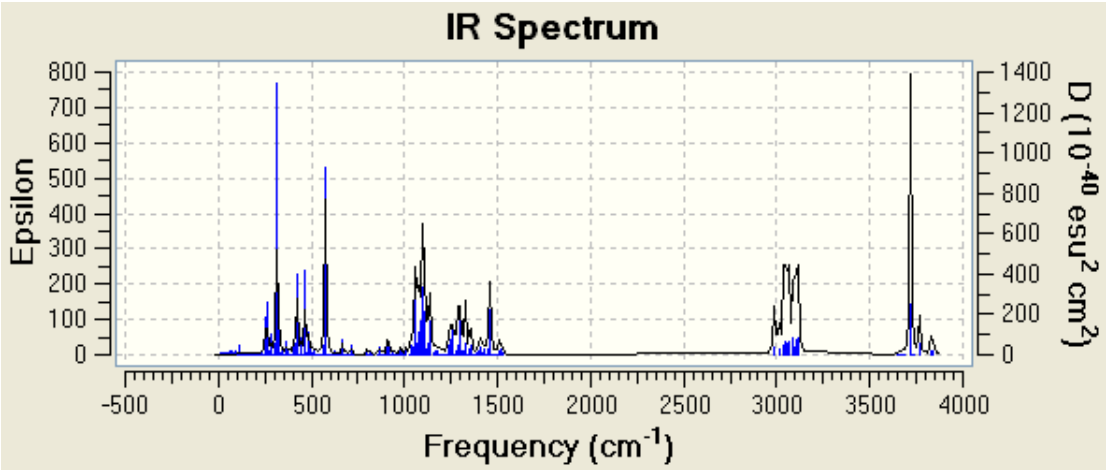

FIGURE: 4.5 A GRAPH BETWEEN WAVE NUMBER (vs) IR INTENSITY FOR ALIZARIN WITH BOVINE SERUM ALBUMIN AT ONIOM (B3LYP/6-31+G (d, p)/UFF) LEVEL OF THEORY

4.9 UV -VISIBLE SPECTRUM

The optimized geometry in the ground state used to obtain framework of TD-DFT. TD-DFT methods are computationally more expensive than semi-empirical methods but allow easily studies of medium size molecules [12]. The theoretical UV –Vis

spectra of the studied molecule are given in the **Figure: 4.6.**The theoretical maximum absorption bands is computed at 210.45nm for TD-DFT /6-31+G (d, p)/UFF) level of theory.

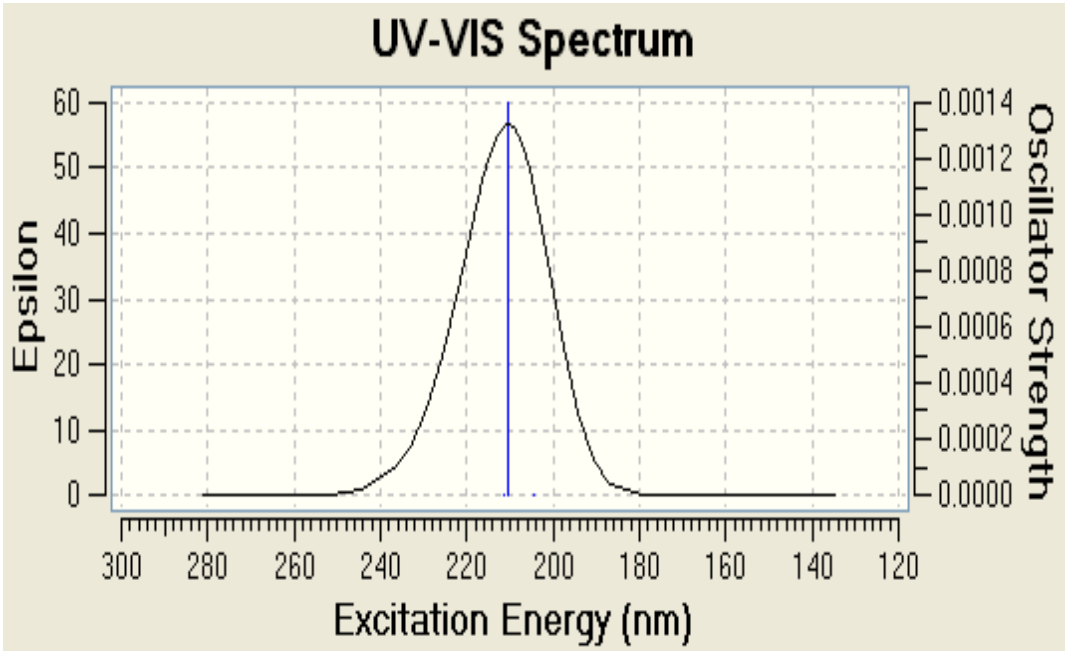

FIGURE: 4.6A GRAPH BETWEEN WAVE NUMBER (vs) UV-Vis INTENSITY FOR ALIZARIN WITH BOVINE SERUM ALBUMIN AT ONIOM (B3LYP/6-31+G (d, p)/UFF) LEVEL OF THEORY.

4.10 VCD AND IR SPECTRUM

VCD Spectrum of Alizarin with BSA at ONIOM is shown in the **figure: 4.7.**Structural spacing of IR Spectra with the stereo-sensitivity of circular dichroism. By comparing VCD spectrum with IR spectrum, Peaks observed in IR are in correspondence with peaks of VCD spectrum. A weak positive peak of IR corresponds to the strongest negative peak of VCD at frequency of 3100cm^{-1}. IR frequency of 3700cm^{-1} corresponds to negative peak in VCD spectrum. SimilarlyVCD spectrum is used as powerful tool to study the chiral optical properties of drug binding with protein molecule.

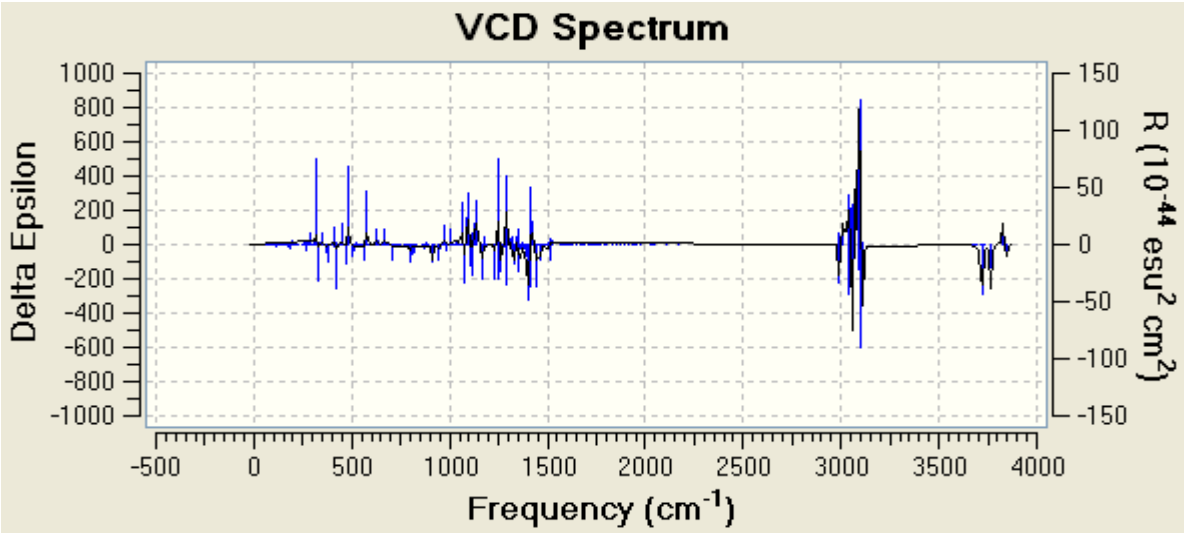

FIGURE: 4.7 A GRAPH BETWEEN WAVE NUMBER (vs) VCD INTENSITY FOR ALIZARIN WITH BOVINE SERUM ALBUMIN AT ONIOM (DFT TD/6-31+G (d, p)/UFF) LEVEL OF THEORY

Table: 4.5 IR AND RAMAN ACTIVITY OF THE VIBRATION OF ALIZARIN WITH BOVINE SERUM ALBUMIN AT ONIOM (B3LYP/6-31+G (d, p) /UFF) THEORY..

Frequency cm^{-1}	Assignments	Raman activity	IR activity
3843.2238	H_5-N_4-H_4 Symmetric stretching	144.8248	20.3362
3826.4032	H_{14}-N_{11}-H_{13} Symmetric stretching	30.2969	20.661
3765.8616	C_5-C_4-C_6 Asymmetric stretching	252.736	48.1453
3719.0581	H_{33}-N_{20}-H_{34} Rocking stretching	37.6408	0
3114.8242	O_{23}=C_{93}-C_{92} Symmetric stretching	154.1678	0.0001
3111.7864	H_{14}-N_{11}-H_{13} Scissoring	19.100	0.001
3101.509	H_{35}-N_{27}-H_{36} Scissoring	57.9117	230.4841
3100.6821	C_{33}-N_9-H_{10} Asymmetric stretching	69.7928	0
3090.0934	H_{14}-C_{11}-H_{13} Scissoring	40.5067	0.0001
3086.774	C_{64}-C_{66}-S_2 Rocking	207.8064	0
3068.9403	C_{73}-C_{72}-C_{70} Scissoring	187.8949	0
3062.9586	H_3-N_{26}-H_{34} Wagging	126.8065	0.0068
3054.2928	C_{73}-C_{72}-C_{70} Scissoring	65.8311	24.622

4.10 HYPERPOLARIZABILITY

Polarizability α , Hyperpolarizability β and electric dipole moment μ of the Alizarin with BSA are calculated by finite field method using B3LYP/6-31+G (d, p) in DFT theory. To calculate the dipole moment and Polarizability for the isolated molecule, the orgine of the Cartesian coordinate system x, y, z= (000).

$$E = E^0 + \mu_\alpha F_\alpha - 1/2\,\alpha_{\alpha\beta} F_\alpha F_\beta - 1/6\,\beta_\alpha \beta_Y F_\alpha F_\beta F_Y .$$

E^0 is the energy of the unperturbed molecules; F_α is the field at the origin $\mu_\alpha, \alpha_{\alpha\beta}, \beta_{\alpha\beta y}$ is the component of dipole moment, Polarizability and the first Polarizability, respectively .The total dipole moment μ.The mean of the Polarizability α_0 an isotropy of the Polarizability $\Delta\alpha$ and the mean of the Hyperpolarizability β_0 using the x, y, z component.

$$\mu = (\mu_x^{\ 2} + \mu_y^2 + \mu_x^2)^{1/2}$$

$$\alpha_0 = \alpha_{xx} + \alpha_{yy} + \alpha_{zz} / 3$$

$$\alpha = (\sqrt{2})^{-1/2} \left[(\alpha_{xx} - \alpha_{yy})^2 + (\alpha_{yy} - \alpha_{zz})^2 + (\alpha_{zz} + \alpha_{xx})^2 + 6\alpha_{xz}^{\ 2} + 6\alpha_{xy}^{\ 2} + 6\alpha_{yz}^{\ 2} \right]^{1/2}$$

$$\beta_0 = (\beta_x^2 + \beta_y^2 + \beta_z^2)\,1/2$$

$$\beta_x = \beta_{xxx} + \beta_{xyy} + \beta_{xyz}$$

$$\beta_y = \beta_{yyy} + \beta_{xxy} + \beta_{yzz}$$

$$\beta_z = \beta_{zzz} + \beta_{xxz} + \beta_{yyz}$$

The Hyperpolarizability of Alizarin with BSA calculated by DFT/6-31+G (d, p) level of theory is 839.415353× 10^{-30}esu [12].

TABLE:4.6 DIPOLE MOMENT(Debye), POLARIZABILITY (a.u), DIPOLE MOMENTS COMPOUNT β AND β TOTAL VALUE OF ALIZARIN WITH BSA ATONIOM(B3LYP/6-31+G(d,p)/UFF) AND ONIOM (DFT/6-31+G(d,p)/UFFLEVEL OF THEORY.

DIPOLEMOMENT ONIOM (B3LYP/6-31+G(d,p) /UFF) in a.u	
μ_x	7.8139
μ_y	-3.1552

μ_z	1.8035
μ	8.6177
Quadruplemoment(B3LYP.6-31+G (d, p) /UFF) in a.u	
α_{xx}	-124.0371
α_{xy}	10.2622
α_{yy}	-125.8964
α_{xz}	12.6404
α_{yz}	-3.2758
α_{zz}	-122.9132
α	740.151750 $\times 10^{-30}$esu.
Octapolemoment(B3LYP/6-31+G(d,p)/UFF) in a.u	
β_{xxx}	155.9946
β_{xxy}	2.2448
β_{yyy}	31.9502
β_{xxz}	169.5843
β_{xyz}	12.2571
β_{yyz}	149.6623
β_{xzz}	12.9750
β_{yzz}	37.4523
β_{zzz}	481.8414
β_{xyy}	72.0455
β_0	839.415353$\times$ 10^{-30}esu

4.11. REFERENCE

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SUMMARY AND CONCLUSION

CHAPTER 5

SUMMARY AND CONCLUSION

Ligand of Alizarin and Bovine serum albumin was docked using Glide/Maestro of Schrodinger USA. The best binding site and hydrogen bond of the interaction was predicted. Active site of these Ligand-Protein interaction was optimized using ONIOM (B3LYP/6-31+G(d,p)/UFF)in Gaussian 09 package. Geometrical parameters and other thermo dynamical parameter were calculated at same level of theory. Spectroscopic analysis like IR, Raman, VCD, are computed using ONIOM (B3LYP/6-31+G(d,p)/UFF).UV-Visible spectra are also observed using ONIOM(TD DFT B3LYP/6-31+G(d,p)/UFF) level of theory. In active site BSA with Alizarin, score and energy is low, binding of ligand in this domain is good. This binding study is of paramount importance in understanding chemico-biological interaction for drug design.