CHAPTER 2

Outline and the objectives of thesis work

2.1 Introduction

This chapter describes the main objectives of the thesis work and the plan of the research work presented in the subsequent chapters. As, described in the chapter-1, the drugs available for TB disease are being used since 1950 and no new drugs are developed for the past 30 years [1]. The drugs available to treat TB disease are not completely effective against the persistent bacteria [2]. For these reasons, multidrug therapies are required to treat the TB disease. Long term therapy has led to patient poor-compliance which in turn contributed the emergence of drug-resistant TB [3]. The MDR and XDR TB are often fatal, difficult and expensive to treat. Thus, there is an urgent need for new anti-TB drugs which are effective against the persistant bacteria, which could clear the infection within short duration of time

2.2 Objectives

Few decades ago, developing a new drug was a time consuming and expensive process, but now the computer aided drug design reduced such process. Drug design involves the design of small molecules based on the knowledge of biological target [4]. Usually, ligand design from the small organic molecules is that ligand should bind tightly to its receptor [5]. Design of new drugs also require knowledge about the physical and chemical properties of available drugs. Modern crystallography and the advancement of computer technology allows not only to predict the crystal structure but also the charge density distribution of molecules in the crystal. This defines the most important property like electrostatic potential of the molecule, which receives a considerable attention for the past two
decades, as it plays an important role on understanding the process of molecular recognition including drug-receptor interactions and the evaluation of lattice energy. Experimental charge density approach (ECDA) provides the information about the topological properties of electron density at the bond critical points (bcp's) of molecules. It also quantifies the strength of intermolecular interactions. The pharmacological action of most drug molecules is achieved by the recognition of receptor. Moreover, biological activities are dominated by intermolecular interactions, the strength of such interactions exhibit complementary electronic properties of the involved species, plays a distinctive role in this process. From the high resolution X-ray diffraction experiment at low temperature, the structural and electronic properties of the materials can be determined. Recent technological developments like CCD area detectors [6] coupled with cryosystem and synchrotron radiation [7] enables, to obtain high resolution X-ray data in a reasonable time [8]. The electron clouds around the atoms in molecules primarily deformed due to chemical bonding and secondarily due to the non-bonded interactions such as hydrogen bonds. These effects can be well understood from the aspherical electron density model developed by Hansen and Coppens multipole-formalism [9] where the electron densities of an atom can be represented as three components, the core, the spherical expansion and contraction term (\(C\)) in the valence shell and the valence deformation density in terms of normalized spherical harmonic (\(P_{lm}\)), the radial expansion and contraction parameter of the valence shell (\(C'\)) [9]. As electron density is a physically observable quantity, which is directly related to the chemical [10] and physical [11] properties of the materials. The bond topological and electrostatic properties of the molecule can be determined from the electron density distribution. The experimental electron
density distribution of the molecule can be directly compared with the corresponding theoretical values as theoretical methods provide results with sufficient accuracy due to the developments in theoretical methods. Electron density properties in a crystal can serve as a model for the interactions under biological conditions, since the same types of effects as the ligand-target interactions should exist in the environment. Due to this, ED properties are much better suited to simulate physiological conditions than those from theoretical gas phase molecule [11]. The main objectives of the thesis are to perform charge density analysis of some of the first line and second line anti-TB drugs. The following sections outline the work presented in this thesis.

2.3 Anti-TB drugs

2.3.1 First-line anti-TB drugs

The drugs used to treat drug-susceptible (DS) are called first-line anti-TB drugs. Chemotherapy for TB started in 1940’s. A number of agents have been discovered which are Para-aminosalicylic acid, isoniazid, pyrazinamide, ethambutol and rifampicin; in which, except para-aminosalicylic acid and the other drugs are known as first-line antitubercular drugs (figure 2.1). For drug-susceptible TB, combination of isoniazid, a rifampicin, pyrazinamide and ethambutol should be given for 2 months. This initial phase of treatment is followed by isoniazid and rifampicin for the last 4 months [12]. Thus, the first-line chemotherapy is to give for 6 months for TB infected individuals.

First-line drug names with standard three letters and a single letter abbreviation

Isoniazid – INH – H
Pyrazinamide – PZA – Z
Ethambutol – EMB – E
Rifampicin – RMP- R.

Each drug has its own targets and their mechanism of action differs. Rifampicin and isoniazid are the corner stones of TB chemotheraphy [13].

![Chemical structures of Isoniazid, Pyrazinamide, Ethambutol, and Rifampicin](image)

Figure 2.1: Chemical structure of some of the first-line drugs.

### 2.3.2 Second-line anti-TB drugs

Second line drugs are being used only when the drug resistance strains emerged for the first-line tuberculosis. Usually, second line drugs are useful to treat the MDR tuberculosis and XDR tuberculosis. These drugs are termed as second-line drugs due to the three possible reasons:

(i) it may be less effective than the first-line drugs (e.g., p-aminosalicylic acid)
(ii) it may have toxic side effects (e.g., cycloserine)

(iii) it may be unavailable in many developing countries (e.g. fluoroquinolones)

There are six classes of second-line drugs using for the treatment of TB

Aminoglycosides
Polypeptides
Fluoroquinolones
Thioamides
Cycloserine
Terizidone

The drugs ethionamide and moxifloxacin belongs to the class of second line drugs. Here, the structure and charge density analysis for the ethionamide and moxifloxacin were carried out, which are presented in the thesis. The chemical structure of ethionamide and moxifloxacin is shown in figure 2.2.

![Chemical structure of ethionamide and moxifloxacin](image)

**Figure 2.2:** Chemical structure of some second line drugs.

### 2.4 Docking and charge density analysis

The Part-I of the thesis (chapter 3, 4, 5 and 6) reports the detailed charge density analysis of first-line drug molecules [isoniazid, pyrazinamide, ethambutol
and rifampicin]; in which, chapters 3 and 4 deals with the experimental charge density analysis of nicotinamide analogs [14], isoniazid [15] and pyrazinamide [16]; these are the important anti-TB drugs being used for TB disease. Experimental charge density analysis provides information about the topological properties of electron density, such as electron density, Laplacian of electron density, bond ellipticity and the electrostatic properties such as atomic charges, dipole moment and electrostatic potential of both molecules. A single point energy calculation also carried out for the isoniazid molecule using Gaussian03 software [17] and the theoretical results were compared with the experimental values. For pyrazinamide, periodic calculations were performed with CRystal09 [18], in which the molecular geometry obtained from the experimental multipole refinement was used as an input; in this quantum chemical periodic calculation the B3LYP [19, 20] method was employed with 6-31G** basis set [21].

The chapter 5 describes the docking analysis of ethambutol [22] with antigen85C to understand about its interaction with the amino acid residues present in the active site of the antigen85C. Further, to understand the conformational geometry and the bond topological and the electrostatic properties a single point energy DFT calculation was performed coupled with the theory of atoms in molecules for the molecule lifted from the active site. On the other hand, a gas phase minimum energy DFT calculation (full optimization) and further a charge density analysis also performed to understand its geometry and the charge distribution in gas phase. The comparison between the structural conformation, bond topological and the electrostatic properties of active site and gas phase structure of ethambutol allows to understand the molecular flexibility and the
charge density redistribution and the variation of electrostatic properties of the molecule when it present in the active site.

The Chapter 6 explains about the topological and the electrostatic properties of rifampicin [23] molecule. A single point energy DFT calculation was carried out for the rifampicin molecule lifted from the active site of RNA polymerase. The topological properties of electron density were performed to understand the nature of chemical bonds present in the rifampicin molecule. The charge and ESP are correlated with the interactions formed between rifampicin and aminoacid residues present in the active site of RNA polymerase. The charge density study reveals the nature of charge density distribution and the electrostatic properties of molecule in the active site.

The part-II of the thesis (chapter 7,8) deals with the two second-line drug molecules (ethionamide, moxifloxacin). Further, a theoretical charge density analysis was carried on an antibacterial drug (chapter 9) namely ethyl 2-[(4-hydroxy-phenyl)-hydrazono]-3-oxobutanoate. The chapter 7 describes about the experimental charge density analysis of ethionamide [24-26] to understand its structure at electronic level. The bond topological properties of electron density of ethionamide have been calculated from high resolution X-ray diffraction data. The experimental topological properties were compared with the corresponding theoretical values predicted from DFT calculations (B3LYP/6-311G**). The electrostatic properties such as atomic charges, dipole moment and the electrostatic potential were calculated. The electrostatic potential surface of the molecule emphasizes the reactive regions of the molecule. A model interaction surface of ethionamide in the active site displays, how the molecule interact with the neighbouring aminoacids in the active site.
The chapter 8 deals with the theoretical charge density analysis of second line anti-tubercular drug moxifloxacin [27]. The charge density analysis of this molecule was carried out using high level quantum chemical calculation. The molecule was optimized using HF and B3LYP level of theories with 6-311G** basis set. Thus obtained wave function file was used to calculate the bond topological and electrostatic properties of the molecule. The electron density, Laplacian of electron density and bond ellipticity of the molecule have been calculated. These electron density parameters were calculated to understand the charge accumulation in the bonds, the bond charge concentration/depletion and the shape of electron density of the bonds. The electrostatic potential map has been plotted, which reveals the high electropositive and negative regions in the molecule.

Chapter 9 of the thesis describes the docking and charge density analysis of namely ethyl 2-[(4-hydroxy-phenyl)-hydrazono]-3-oxobutanoate [28] molecule. A docking analysis of this molecule with the Inha enzyme was performed to confirm whether it binds in the active site of Inha. Similar to ethambutol a single point energy DFT calculation and the charge density analysis were carried out for the molecule lifted from the active site. A gas phase calculation also performed for the above mentioned molecule. The structural and bond topological properties of the molecule calculated for the gas phase and the active site were compared.

The final chapter 10 gives the summary and the general conclusion about the first line and second line drug molecules.
2.5 References

References


Yu, T. Merigan, S. Barriere (Eds), Williams & Wilkins, Baltimore.


