This chapter explains the investigation of electrocatalysis of [5,10,15,20-tetrakis(4-methoxyphenyl)porphyrinato]Mn(III)chloride (TMOPPMn(III)Cl) towards the oxidation of trimethoprim (TMP) by computational methods. DFT calculations were performed at the B3LYP level to detect the site of oxidation as well as to survey the role of manganese porphyrin in the facile oxidation of TMP. Atoms in molecule (AIM) analysis and natural bond orbital (NBO) analysis also substantiate the utility of TMOPPMn(III)Cl in TMP oxidation by confirming the co-ordination of trimethoprim to manganese porphyrin on removal of proton.

8.1 Introduction

The search for accurate electronic wave functions of polyatomic molecules uses mainly the molecular orbital method. The presence of several nuclei causes greater computational difficulties than for diatomic molecules. Molecular quantum mechanical methods are classified as either ab initio or semiempirical. Semiempirical methods use a simpler Hamiltonian than the correct molecular Hamiltonian and use parameters whose values are adjusted to fit experimental data or the results of ab initio calculations. In contrast, ab
initio calculation uses the correct Hamiltonian and does not use experimental data other than the values of the fundamental physical constants. An ab initio self consistent field calculation uses the approximation of taking $\Psi$ as an antisymmetrized product of one electron spin orbitals and uses a finite and hence incomplete basis set.\textsuperscript{241,242}

A basis set is a set of functions used to create the molecular orbitals which are expanded as a linear combination of such functions with the weights or coefficients to be determined. Usually these functions are atomic orbitals in that they are centered on atoms. Otherwise the functions are centered on bonds or lone pairs. In modern computational chemistry, quantum chemical calculations are typically performed within a finite set of basis functions. The wave functions under consideration are all represented as vectors, the components of which correspond to coefficients in a linear combination of the basis functions in the basis set used. The operators are then represented as matrices. The basis functions are usually not exactly the exact atomic orbitals. Initially these atomic orbitals were typically Slater orbitals which correspond to a set of functions which decayed exponentially with distance from the nuclei, later it was realized by Franck boys that these slater type orbitals could be approximated as linear combinations of Gaussian orbitals instead.\textsuperscript{241,243}

Minimal basis sets are composed of minimum number of basis functions required to represent all the electrons on each atom. Eg: STO-3G, STO-4G, STO-3G*, STO-6G.

The minimal basis sets suffer from several deficiencies. The atoms at the end of a period of the periodic table are described using the same number of basis functions as the atoms at the beginning of the period. A minimal basis set only contains one contraction per atomic orbital and as the radial exponents
are not allowed to vary during the calculation, the functions cannot expand or contract in size in accordance with the molecular environment. The third drawback is that a minimal basis set cannot describe non spherical aspects of the electronic distribution.

These problems with minimal basis sets can be addressed if more than one function is used for each orbital. A basis set which doubles the number of functions in the minimal basis set is called a double zeta basis set. An alternative to the double zeta basis approach is to double the number of functions used to describe the valence electrons but to keep a single function for the inner shells and is called split basis set. Three Gaussian functions are used to describe the core orbitals. The valence electrons are also represented by three Gaussians, the contracted part by two Gaussians and the diffuse part by one Gaussian. Eg: 3-21 G, 4-21 G, 6-21 G, 6-31 G.

The use of split valence basis set can help to surmount the problems with non isotropic charge distribution but not completely. This problem can be overcome by introducing polarization functions into the basis set. The polarization functions have a higher angular quantum number and hence correspond to p orbitals for hydrogen and d orbitals for the first and second row elements. Eg: 6-31G*. The inability to deal with species such as anions and molecules containing lone pairs which have a significant amount of electron density away from the nuclear centres can be overcome by adding highly diffuse functions to the basis set. Eg: 3-21+G. Diffuse functions can also be included for hydrogen as well as for heavy atoms. Eg: 6-31+G(d).

8.1.1 Density functional theory

Density Functional theory (DFT) is an approach to the electronic structure of atoms and molecules which has enjoyed a dramatic surge of
interest since the late 1980's and 1990's. In Hartree Fock (HF) theory, the multi electron wave function is expressed as a Slater determinant which is constructed from a set of N single electron wave functions. DFT also considers single electron functions. Whereas HF theory does calculate the full N electron wave function, DFT only attempts to calculate the total electronic energy and the overall electronic distribution. The idea of DFT is that there is a relationship between the total electronic energy and the overall electronic density. Hoherberg and Kohn showed that the ground state energy and other properties of a system were defined by the electron density. In DFT, the energy functional is written as a sum of two terms

$$E[\rho(r)] = \int V_{\text{ext}}(r) \rho(r) \, dr + \int F[\rho(r)]$$

(8.1)

The first term arises from the interaction of the electrons with an external potential $V_{\text{ext}}$ typically due to the Coulomb interaction with the nuclei. $F[\rho(r)]$ is the sum of kinetic energy of the electrons and the contribution from interelectronic interactions. The minimum value in the energy corresponds to the exact ground state electron density so enabling a variational approach to be used. The best solution corresponds to the minimum of energy and an incorrect density gives an energy above the true energy.

In order to minimize the energy, a Lagrangian multiplier, ($-\mu$) is introduced leading to the following equation:

$$\frac{\delta}{\delta \rho(r)} \left[ E[\rho(r)] - \mu \int \rho(r) \, dr \right] = 0$$

(8.2)

$$\left( \frac{\delta E[\rho(r)]}{\delta \rho(r)} \right)_{V_{\text{ext}}} = \mu$$

(8.3)
Equation (8.3) is the DFT equivalent of the Schrodinger equation.

Kohn and Sham suggested a practical way to solve the Hohnberg-Kohn theorem for a set of interacting electrons. The difficulty with equation (8.1) is that we do not know what the function \( F[\rho(r)] \) is.

\( F[\rho(r)] \) should be approximated as the sum of three terms.

\[
F[\rho(r)] = E_{KE}[\rho(r)] + E_{HI}[\rho(r)] + E_{XC}[\rho(r)]
\]

where \( E_{KE}[\rho(r)] \) is the kinetic energy, \( E_{HI}[\rho(r)] \) is the electron electron Coulombic energy and \( E_{XC}[\rho(r)] \) contains contributions from exchange and correlation.

Adding the electron-nuclear interaction leads to the full expression for the energy of an \( N \) electron system within the Kohn-Sham scheme:

\[
E[\rho(r)] = \sum_{i=1}^{N} \int \psi_i(r) \left( -\frac{\nabla^2}{2} \right) \psi_i(r) \, dr + \frac{1}{2} \iint \frac{\rho(r_1)\rho(r_2)}{|r_1 - r_2|} \, dr_1 \, dr_2 + E_{XC}[\rho(r)]
\]

\[-\sum_{A=1}^{M} \int \frac{Z_A}{|r - R_A|} \rho(r) \, dr\]

where \( E_{XC}[\rho(r)] \) is the exchange correlation functional, which thus not only contains contributions due to exchange and correlation but also a contribution due to the difference between the true kinetic energy of the system and \( E_{KE}[\rho(r)] \).

The density \( \rho(r) \) of the system is written as the sum of the square moduli of a set of one electron orthonormal orbitals \(^{243,245}\)

\[
\rho(r) = \sum_{i=1}^{N} |\psi_i(r)|^2
\]
Correlation effects are incorporated from the beginning in DFT. The exchange correlation energy is written as a sum of the exact exchange term together with the correlation component from the local density approximation. Hybrid functional are a class of approximations to the exchange-correlation energy functional in DFT that incorporate a portion of exact exchange from HF theory with exchange and correlation from other sources (ab initio or empirical). The exact exchange energy functional is expressed in terms of the Kohn-Sham orbitals rather than the density, so is termed an implicit density functional. One of the most commonly used versions is B3LYP, which stands for Becke, 3-parameter, Lee-Yang-Parr.\textsuperscript{246}

The popular B3LYP (Becke, three-parameter, Lee-Yang-Parr)\textsuperscript{247,248} exchange-correlation functional is:

\[
E_{xc}^{B3LYP} = (1 - a_0)E_x^{LSDA} + a_0E_x^{HF} + a_x\Delta E_x^{B88} + a_C E_C^{LYP} + (1 - a_C)E_C^{VWN}
\]

where \(E_x^{LSDA}\) is the exchange energy under the local spin density approximation, \(E_x^{HF}\) is the exact exchange from Hartree Fock theory, \(\Delta E_x^{B88}\) is the Becke 88 exchange functional,\textsuperscript{249} \(E_C^{LYP}\) is the correlation functional of Lee, Yang and Parr,\textsuperscript{250} \(E_C^{VWN}\) is the Vosko, Wilk and Nusair local-density approximation to the correlation functional.\textsuperscript{251} \(a_0 = 0.20\) \(a_x = 0.72\) and \(a_C = 0.81\) are the three empirical parameters determined by fitting the predicted values to a set of atomization energies, ionization potentials, proton affinities and total atomic energies\textsuperscript{252} and are generalized gradient approximations.

Density functional methods using gradient corrected functionals can give results for a wide variety of properties that are competitive with and in some cases superior to ab initio calculations using correlation like in MP2. Calculations have been carried out only for the species involved in the reaction, omitting
Computation studies of the Electrooxidation of Trimethoprim

the electrode. Barth et al. have shown by ab initio calculations and scanning tunneling microscopy (STM) observations of tetrapyridyl and Fe(II)-tetrafluoride-porphyrin molecules on the Ag(111) surface that the molecular structure remains largely unaffected by the adsorption on the silver surface.253 Many studies have been carried out in which DFT has been used as a tool to investigate mechanisms of reactions occurring on surfaces, without considering the electrode or the surface on which these reactions occur.254 D'Souza et al. also showed that the HOMO-LUMO energy gaps obtained for porphyrin systems by DFT calculations follow the trends of the electrochemical results. Their studies revealed good agreement between the experimental results and computational predictions.255-257 However, it is important to note that these in vacuo calculations provide a qualitative idea about the oxidation mechanism, and a quantitative estimation will require an explicit consideration for the presence of the electrode and solvation effects.

The AIM approach relies on an analysis of the topological properties of the charge density, $\rho(r)$ and its quality depends on the computational level chosen. Both the gradient and the Laplacian of the charge density, $\nabla \rho$ and $\nabla^2 \rho$ respectively, can be analyzed and provide complementary information on bonds. The critical points of $\nabla \rho$ give information about the existence of bonds, while the sign of $\nabla^2 \rho$ at that point reflects the kind of interaction. Nuclei attract the charge density so that maxima of $\nabla \rho$ are found there. A bond corresponds to a saddle point (the bond critical point), where $\nabla \rho$ becomes zero, a maximum only in one plane of space and is found joining two trajectories of maximum $r$ along the space, toward the nuclei.258-264 NBO analysis is based on a method for optimally transforming a given wave function into localized form. In NBO analysis, the input atomic orbital basis set is transformed via natural atomic
orbitals (NAOs) and natural hybrid orbitals (NHOs) into natural bond orbitals (NBOs). The NBOs obtained in this fashion correspond to the widely used Lewis picture, in which two-center bonds and lone pairs are localized.\textsuperscript{265}

8.2 Computational method

Quantum chemical calculations have been performed using the Gaussian 03 suite of programs.\textsuperscript{266} All the geometries were fully optimized by density functional theory (DFT) using the Becke, three-parameter, Lee-Yang-Parr (B3LYP) hybrid functional theory.\textsuperscript{250,267} 6-31+G(d) basis set\textsuperscript{268,269} was used to optimize all the structures involved in the uncatalyzed reaction of trimethoprim at bare glassy carbon electrode (GCE). For the optimization of the geometries involved in the catalyzed reaction on TMOPPMn(III)Cl/GCE, split basis set method with 6-31+G(d) for the main group elements and compact effective potentials split valence set (CEP-31G)\textsuperscript{270,271} for manganese was utilized. These geometries were also optimized using Los Alamos 19 electron shape consistent relativistic ECP (LANL2DZ) basis set\textsuperscript{272,273} to carry out natural bond orbital (NBO) and atoms-in-molecules (AIM) analysis. [Mn(III) porphyrin]Cl is chosen as a model for TMOPPMn(III)Cl in the computational investigation of the catalyzed reaction. All minimum energy structures have been characterized through frequency calculations to ensure that there are no saddle points corresponding to imaginary frequencies.

8.3 Results and Discussion

Voltammetric behavior of trimethoprim investigated at bare GCE and at TMOPPMn(III)Cl/GCE demonstrates the electrocatalytic ability of TMOPPMn(III)Cl. The details of the voltammetric experiment of the oxidation of trimethoprim on TMOPPMn(III)Cl/GCE is already described in Chapter 3. Figure 3.4 shows the differential pulse voltammograms of $10^{-3}$ M trimethoprim
in phosphate buffer solution at bare GCE (curve a) and TMOPPMn(III)Cl/GCE (curve b). Obviously, the peak potential shifts towards a more negative potential compared to that of a bare GCE. The electrochemical studies of $10^{-3}$ M of TMP carried out in the pH range 3-10 shows that the peak current is highest at pH 5, which can be seen from Figure 3.5. However, the anodic potential decreases with increase in pH value as can be seen from Figure 8.1. The number of electrons involved in the electrochemical reaction of trimethoprim was calculated to be 3.8. Based on this experimental finding, the proposed possible mechanism is shown in Scheme 3.1.

The optimized structure of trimethoprim is shown in Figure 8.2. There are two amino groups, one at position 4 of the pyrimidine ring and the other at position 2 of the stable pyrimidine system.\textsuperscript{132} Deprotonation of NH\textsubscript{2} group attached to either C4 or C2 of trimethoprim can occur and thus both of these possibilities were investigated computationally. The internal energy change ($\Delta E$), enthalpy change ($\Delta H$) and free energy change ($\Delta G$) of the deprotonation of NH\textsubscript{2} attached to C4 are -35.9, -30.8 and -31.4 kcal/mol respectively, while that attached to C2 are -26.4, -27.3 and -28.3 kcal/mol respectively. Clearly, deprotonation of the former is more facile than that of latter. Thus for further calculations, we have considered the removal of proton from the C4 amino group. The detailed mechanism\textsuperscript{132, 274-276} is outlined in Scheme 8.1. The first step involved in the conversion of trimethoprim (I) to nitroso derivative (IX) is the deprotonation of NH\textsubscript{2} group at the 4\textsuperscript{th} position to give trimethoprim anion (II). The formation of (II) from (I) is an exothermic process ($\Delta H = -30.8$ kcal/mol). The distance between C4 and N8 in (I) and (II) are 1.38 and 1.32 Å respectively as shown in Figure 8.3.

Mn(III)porphyrin]Cl−–trimethoprim was optimized with 0, 1 and 2 spin states to analyze their relative stabilities. The geometry with spin state 2
(multiplicity=5) was found to have lowest energy and hence is the most stable one. Hence all the further studies were carried out using [Mn(III)porphyrin] Cl···trimethoprim with spin state 2. The optimized structure of [Mn(III) porphyrin]Cl···trimethoprim with spin state 2 is shown in Figure 8.4. As for the uncatalyzed reaction, the deprotonation of NH₂ of trimethoprim attached to C4 and C2 for the [Mn(III)porphyrin]Cl catalyzed reaction was also investigated. ∆E, ∆H and ∆G corresponding to the deprotonation of NH₂ attached to C4 are found to be -48.3, -48.4 and -48.4 kcal/mol respectively, while that attached to C2 are -43.4, -45.0 and -42.2 respectively. The values of thermodynamic properties suggest that deprotonation at 4ᵗʰ position is more favorable than that at 2ⁿᵈ position. Compared to the uncatalyzed reaction, the coordination of [Mn(III) porphyrin]Cl with trimethoprim anion increases the exothermicity of the deprotonation. This suggests that [Mn(III)porphyrin]Cl···trimethoprim (A) gets further stabilized by deprotonation. It is interesting to note that the distance between N₄₆ and Mn is 3.64 Å in (A), whereas in [Mn(III)porphyrin]Cl···trimethoprim anion (B), it is 2.23 Å as shown in Figure 8.5.

Trimethoprim anion gets stabilized by the interaction of Mn(III) of the metalloporphyrin with N of the NH₂ group at the 4ᵗʰ position of trimethoprim which carries the reaction forward. Selected bond lengths of structures (A) and (B) are summarized in Table 8.1. HOMO-LUMO for (A) and (B) shown in Figure 8.6 reveals that HOMO - LUMO of (B) is interacting, whereas that of (A) is not. Thus, on deprotonation, trimethoprim anion co-ordinates to [Mn(III)porphyrin]Cl thus facilitating further oxidation.

The plot of the gradient of the charge density ρ in [Mn(III) porphyrin]Cl···trimethoprim and [Mn(III)porphyrin]Cl···trimethoprim anion is depicted in Figure 8.7 (A) and (B) respectively. The most striking feature of the plots is the absence of critical points between N₄₆ and Mn in [Mn(III)]
porphyrin]Cl...trimethoprim, whereas in [Mn(III)porphyrin]Cl...trimethoprim anion, there exists a bond critical point between these atoms, with charge density, \( \nabla \rho = 0.0814 \) and Laplacian of charge density, \( \nabla^2 \rho = -0.0786 \). The value of \( \nabla \rho \) shows that there is substantial charge density in between N46 and Mn bond. The negative Laplacian for the bond critical points suggests stabilization through intermolecular interactions.

NBO analysis points out a bond order of 0.02 and 0.45 between N46 and Mn in (A) and (B) respectively. This is a further evidence for the fact that trimethoprim coordinates with the Mn of the [Mn(III)porphyrin]Cl only when it is converted to its anionic form on deprotonation.

8.4 Conclusions

Electrochemical experiments and computational methods show that TMOPPMn(III)Cl acts as an electrocatalyst for the oxidation of trimethoprim. From the voltammetric oxidation of trimethoprim investigated at bare and TMOPPMn(III)Cl modified GCE, it is inferred that the anodic potential required for the oxidation of trimethoprim gets lowered on the TMOPPMn(III)Cl/GCE compared to that on bare GCE. Quantum mechanical calculations explain the role of manganese porphyrin in the easy oxidation of trimethoprim. The calculations show that the oxidation of amino group attached to C4 of trimethoprim is easier than that attached to C2. In comparison to the uncatalyzed, the catalyzed deprotonation, which is the preliminary step in the oxidative mechanism, is energetically more favorable. AIM analysis shows the existence of a bond critical point between manganese and the nitrogen of the trimethoprim anion. NBO analysis further supports the interaction between trimethoprim anion and manganese porphyrin. A bond order of 0.45 is calculated between N46 of trimethoprim anion and manganese
of metalloporphyrin. It is thus deduced that trimethoprim anion interacts with the manganese of the metalloporphyrin. The facile oxidation of trimethoprim in the presence of TMOPPMn(III)Cl can be attributed to the interaction between the metal ion and deprotonated trimethoprim, which assists the transfer of electron in the first step of the reaction. Thus the computational calculations shed light on the role of manganese porphyrin in the easy oxidation of trimethoprim.

Table 8.1. Selected bond lengths of $[\text{Mn(III)porphyrin}]\text{Cl}^\cdots\text{trimethoprim}$ and $[\text{Mn(III)porphyrin}]\text{Cl}^\cdots\text{trimethoprim anion}$ computed at B3LYP/6-31+G(d), CEP-31G level of theory.

<table>
<thead>
<tr>
<th>Species</th>
<th>$[\text{Mn(III)porphyrin}]\text{Cl}^\cdots\text{trimethoprim}$</th>
<th>$[\text{Mn(III)porphyrin}]\text{Cl}^\cdots\text{trimethoprim anion}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>N46 - Mn</td>
<td>3.64</td>
<td>2.23</td>
</tr>
<tr>
<td>C42 – N46</td>
<td>1.38</td>
<td>1.33</td>
</tr>
<tr>
<td>Mn - Cl</td>
<td>2.35</td>
<td>2.58</td>
</tr>
</tbody>
</table>

The bond lengths are in Angstroms.
Figure 8.1. Variation of anodic peak potential of $10^{-3}$ M of Trimethoprim with pH

Figure 8.2. Optimised structure of Trimethoprim
Figure 8.3. Deprotonation of Trimethoprim

Figure 8.4. Optimised structure of [Mn(III)porphyrin]Cl
Figure 8.5. Deprotonation in [Mn(III)porphyrin]Cl...trimethoprim
Figure 8.6. HOMO-LUMO of (A) $[\text{Mn(III)porphyrin} \cdot \text{Cl} \cdots \text{trimethoprim}]$ and (B) $[\text{Mn(III)porphyrin} \cdot \text{Cl} \cdots \text{trimethoprim anion}]$
Figure 8.7. Plots of the gradient of charge density in (A) [Mn(III)porphyrin] Cl···trimethoprim and (B) [Mn(III)porphyrin]Cl···trimethoprim anion
Scheme 8.1. Detailed mechanism of oxidation of TMP
This chapter presents the objectives of the investigation and the summary of the work done. Future outlook is also described. As part of the present investigation glassy carbon electrode was modified using metalloporphyrin and dyes. The modified electrodes were subsequently used for the determination of various pharmaceuticals.

9.1 Objectives of the work

The prime objectives of the work include:

1) Chemical modification of glassy carbon electrode (GCE).

2) Surface studies of the bare and modified electrode by examining the morphology of the surfaces and calculating the effective surface area.

3) Applying the modified electrode for the voltammetric determination of pharmaceuticals.

4) Comparing the voltammetric response of the drug at bare GCE and modified GCE.
5) Optimisation of electrochemical parameters for the determination of different drugs.

6) Applying the modified electrode for the voltammetric determination of drug in real samples.

7) Computational study of the interaction of the manganese porphyrin with trimethoprim as part of the theoretical investigation of the role of electrode modifier in facilitating the electrochemical reaction of the drug.

9.2 Summary of the work done

Various modified electrodes prepared and the respective drugs they sense are listed below:

- [5,10,15,20-tetrakis(4-methoxyphenyl)porphyrinato] manganese(III)chloride modified GCE Trimethoprim & Ambroxol
- Poly(malachite green) modified GCE Sulfamethoxazole
- Poly(methyl red) modified GCE Domperidone
- Poly(erythrose black T) modified GCE Tinidazole

9.3 Future outlook

Electrochemical sensors are used extensively either as a whole or an integral part of a chemical and biomedical sensing element. The fundamentals of this sensing technique are well established and the critical issue is the applicability of the technique to a complex environment such as in blood or other biological fluids. There lies exciting challenge of designing a biosensor using voltammetric and amperometric principles. Further, computational
studies can predict the role of a modifier in catalysing an electrochemical reaction and suggest a possible mechanism for this. Simulation techniques in computational chemistry can provide more accurate results by mimicking an electrode and the real electrochemical situation.