COMPUTATIONAL STUDIES ON THE MECHANISM OF BROMATE OXIDATION OF METHIONINE

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Declaration

I hereby declare that the work in this dissertation titled “Computational studies on the mechanism of Bromate oxidation of Methionine” has been performed by me in the Department of Chemistry under an intensive supervision of Pof. Uzairu Adamu and Dr Hamza Abba. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at any institution.

Abdul HAMIDU

.....	.....	.....
Name of student	**Signature**	**Date**

Certification

This dissertation titled "**COMPUTATIONAL STUDIES ON THE MECHANISM OF BROMATE OXIDATION OF METHIONINE" by Abdul HAMIDU** meets the requirement /regulations governing the award of the Degree of masters of Ahmadu Bello University and it are approved for its contribution to knowledge and literary presentation.

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Abstract

Oxidation of methionine is one of the degradation pathways of proteins. Bromate as a strong oxidizing agent can oxidize methionine to methionine sulphoxide. Computational chemistry was used to investigate the mechanism of oxidation of methionine by bromate at molecular level, using semi-empirical method at Parameterized method 3 and Modified Neglet of Diatomic Overlap level. The proposed mechanism involved seven consecutive steps. The heats of reaction of the computed proposed reaction mechanism were calculated to be -78.71kJ/mol,-3.5kJ/mol at 0^oC and 25^oC, respectively, at Parameterized method 3 level. While the heat of reaction based on Modified Neglect of Diatomic Overlap level of computation are -631.83kJ/mol and -533.82kJ/mol at 0^oC and 25^oC, respectively.The stoichiometry of the reaction was found to be 2:1 bromate to methionine, and the rate limiting step, step 2 involves the reaction between $HBrO_3$ and methionine, which leads to the formation of intermediates that reacted and disproportionate to give the products. The equilibrium and rate constants obtained support the rate determining step.

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I. Mechanism of Oxidation of Methionine by Bromate based on Semi Empirical Method

List of Abbreviations

AM1	The Austin Model 1
AO	Atomic orbital
aug-cc-pVDZ	Augmented Versions of Correlation Consistent-Polarized Valence Double Zeta
BrO_3^-	Bromate anion
cc-pV5Z	Correlation Consistent-Polarized Valence Five Zeta
cc-pVDZ	Correlation Consistent-Polarized Valence Double Zeta
cc-pVQZ	Correlation Consistent-Polarized Valence Quadruple Zeta
cc-pVTZ	Correlation Consistent-Polarized Valence Triple Zeta
CGTO	Contracted Gaussian-Type Orbital
CNDO	Complete neglect of differential overlap
CNS	Central nervous system
C^o	Concentration (taken to be 1)
CPU	Central Processing Unit
CSPI	Centre for science in the public interest
DCP	Dream Calculator Program
DFT	Density Functional Theory
DZ	Double-Zeta basis set
EDSAC	Electronic delay storage automatic calculator
FDA	Food drug administration
GTOs	Gaussian-Type Orbitals
H	Planck's constant (6.626176×10^{-34}Js)
H^+	Hydrogen ion
H_2O	Dihydrogen oxide
H_2O_2	Hydrogen peroxide
HF	Hartree-Fock
HOMO	Highest Occupied Molecular Orbital
HSDB	Harzadous substances data base.
IARC	International agency for research on cancer
IPCS	International programme on chemical safety

IR	Infra-red
K	Rate constant
k(298.15K)	Reaction rate constantat Temperature(298.15K)
k_B	Boltzmann constant (1.380662 X 10^{-23} J/K);
kJ/mol	kilo Joule per mole
LADWP	Los Angeles Department of Water and Power
LCAO	Linear combination of atomic orbitals
LUMO	Lowest Unoccupied Molecular Orbital
MC	Monte Carlo
MD	Molecular Dynamics
MIT	Massachusetts institute of technology
MM	Molecular Mechanics
MNDO	Modified neglect of diatomic overlap
Msr	Methionine sulfoxide residues
MsrA	Peptidyl methionine sulfoxidereductase
PES	Potential Energy Surface
PM3	Parameterization method 3
P_x	Products x
QM	Quantum Mechanics
R	Gas Constant (8.31441 J/Mol. K)
R	Rate
RAM	Random-Access Memory
ROS	reactive oxygen species
$RSCH_3$	Methionine
R_x	Reactants x
SAM	S-Adenosyl-L-Methionine
SE	Semi -Empirical
STOs	Slater-Type Orbitals
SV	Split-Valence
T(298.15K)	Temperature (298.15K)
tBHP	*t*-butyl hydroperoxide

TS_x	Transition state x
TZ	Triple-Zeta basis set
UV	Ultra-Violet
VDZ	Valence Double Zeta
VTZ	Valence Triple-Zeta
WHO	World health organization
$\Delta‡G^o$	Gibbs free energy of activation
ΔH_f	Change in heat of formation

CHAPTER ONE

1.0 INTRODUCTION

1.1 Mechanism of Methionine Oxidation

The oxidation of methionine plays an important role *in vivo*, during biological conditions of oxidative stress, as well as for protein stability *in vitro*. Depending on the nature of the oxidizing species, methionine may undergo a two-electron oxidation to methionine sulfoxide or one-electron oxidation to methionine radical cations. Both reaction mechanisms derive catalytic support from neighboring groups, which stabilize electron-deficient reaction centers. *In vivo*, methionine sulfoxide is subject to reduction by the methionine sulfoxidereductase (Msr) system, suggesting that some methionine sulfoxide residues may only be transiently involved in the deactivation of proteins through reactive oxygen species (ROS). Other methionine sulfoxide residues may accumulate, depending on the accessibility to Msr. Moreover, methionine sulfoxide levels may increase as a result of a lower abundance of active Msr and/or the required cofactors as a consequence of pathologies and biological aging. On the other hand, methionine radical cations will enter predominantly irreversible reaction channels, which ultimately yield carbon-centered and/or peroxyl radicals. These may become starting points for chain reactions of protein oxidation.

1.2 Bromate

Bromate (BrO_3^-) is a negatively charged polyatomic ion containing one bromine and three oxygen atoms. Typical salts of bromate are potassium bromate($KBrO_3$) and sodium bromate. The acid form bromic acid is only stable in water. Many of the bromate salts are possible, but potassium bromate and sodium bromate are the most common (WHO, 2005). Table1.1 below describes these and some other available forms:

1.2.1 Physical Properties of bromates

The bromates of alkali and alkaline earth metals are colourless, odourless crystals which can be in regular or powder form and have negligible vapour pressure. These salts are soluble in water and dissociate in water to the metal and bromate ions (Merk,1983;CDC, 2003; Health Canada,1999). The salts are strong oxidizers. Table 1.1summarizes the properties of some common bromates.

1.2.2 Chemical properties of bromate

Bromate, thermally decompose according to the equation (1.1) and (1.2), depending on the temperature and relative stability of the bromide or oxide of metal.

$2BrO_3^- \rightarrow 2Br^- + 3O_2$.....(1.1)

$4BrO_3^- \rightarrow 2Br_2 + 5O_2 + 2O^{-2}$.....(1.2)

Bromate is less stable than chlorate and iodate thermally. The initial step in the thermal decomposition of alkali metal bromate is the rupture of Br-O bond which occurs only at high temperature (300^{o}C) decomposition of ammonium bromate begins at much lower temperature (-5^{o}C).

Both thermodynamically and kinetically the oxidizing power of the bromate depends on the hydrogen ion concentration. Bromates undergo some redox reactions in alkaline solutions; thus reaction with hydroxylamine or hydrazine (as their sulphates) produces nitrogen. Bromate and halides react in various possible combination, iodides are oxidized quantitatively to iodine and bromides to bromine as shown in equation (1.3) and (1.4)

$BrO_3^- + 5Br^- + 6H^+ \rightarrow 3Br_2 + 3H_2O$.....(1.3)

$BrO_3^- + 6I^- + 6H^+ \rightarrow Br^- + 3I_2 + 3H_2O$.....(1.4)

Chlorides produce bromine and chlorine with bromates. Iodine replaces bromine in bromates:

$I_2 + 2BrO_3^- \rightarrow Br_2 + 2IO_3^-$.....(1.5)

The oxidation of NH_3 by BrO_3^- in perchloric acid solution affords N_2, N_2O, OBr^-, and Br_2; the oxygen in N_2O is derived from the solvent. Bromates form unstable mixtures with combustible or oxidizable substances.

Table 1.1: Physical properties of some common bromates.

Parameter/ properties	Calcium bromate	Potassium bromate	Sodium bromate
Formula	$Ca(BrO_3)_2.H_2O$	$KBrO_3$	$NaBrO_3$
Molecular weight	313.90	167.0	150.9
Colour ,form	Monoclinic crystals	Colourless white granules	Colourlesscrystals,granules or crystalline powder.
Physical state	Solid	Solid	Solid
Melting point	180^oC	350^oC	381^oC
Decomposition point	-	370^oC	381^oC
Density (g/cm^3)	3.33	3.27	3.34
Solubility water	Very soluble	75 g/L at 25^oC	364 g/L at 25 ^oC
Solubility organic solvents.	-	Slightly soluble in alcohols, insoluble in ethers and acetone.	Insoluble in alcohols and ether.

1.2.3Occurrence/production of bromates

Bromate is not commonly found in water, but formed as a byproduct of ozonation disinfection of drinking water and also as a contaminant introduced from treatment of water with concentrated hypochlorite(Haag and Holgne,1993;IPCS,2000; Weinberg *et al*., 2003; WHO,2005;Fawell and Walker,2006). The ozonation of drinking water represents an important potential pathway of bromate formation. In such condition, drinking water is the primary route

of exposure to bromate. In Netherlands, the exposure of bromate to humans to bromate in drinking water relative to other pathways of exposure has been reported as approaching 100%(Van DijkLooijaard and Van Genderen, 2000). The bromate formation is affected by bromide concentration , pH, temperature, carbonate, alkalinity, ultra violet light, disinfectant concentration, and time(mg/L-min) and transferred ozone dose(Amy and associates,2000). Formation of bromate and haloamines after exposure to sunlight had been reported earlier in sea water to which chlorinated waste water had been discharged(Macalady*etal.*,1997). Bromate was recently discovered at relatively high levels two Los Angeles reservoirs used to store water already treated by chlorine(Kemslay,2008). Water officials speculated that sunlight might have interacted with residual chlorine to oxidize bromide to bromate. The bromate level in Silver Lake and Elysian reservoirs were reported as 68 and 106 ppb, respectively. High bromate levels were reported also in two other reservoirs in San Diego County.

No environmental scenario in which bromate enters the ambient air in significant quantities , although if it were present in dusts it could become airborne. Bromate salts have negligibly small vapour pressure and decompose at melting point so that they will not volatilize into the atmosphere (WHO, 2004, 2006).

Bromate only slightly adsorbs to soil and its properties as strong oxidizing agent most likely lead to reactions with organic matter to form the bromide (Br^-) (WHO, 2004, 2006). Bromide similarly would only slightly adsorb to soil or sediment (Health Canada, 1999).

Bromate salts are also produced intentionally, for some commercial uses. Sodium bromate is produced by the introduction of bromine into solution of sodium carbonate. Potassium bromate

is produced by passing bromine into a solution of potassium hydroxide. An industrial electrolytic process is used for large scale production of potassium bromate(IARC,1986;HSDB,1991).

1.2.4Uses of bromates

Both the potassium and sodium salts of bromate are currently in commercial use worldwide. Potassium bromate, the more acutely toxic of the two, is used as a flour or bread dough "improver" or maturing agent (Mack, 1998; Dupuis, 1997; WHO, 2004, 2006). Calcium bromate has also been used for this purpose. The maximum allowable level of potassium bromate in flour is75 ppm (FDA, 2007a), however, as a result of a request from the FDA in 1991, most companies have omitted potassium bromate from their products on a voluntary basis (Dupuis, 1997). Potassium bromate is also used as a chemical component of neutralizer solutions in permanent wave hair care products (Mack, 1988; DeAngelo*et a;* 1998; Health Canada,1999). Potassium bromate is also used in certain types of beer and cheese making (DeAngelo*etal*., 1998; WHO, 2005).

Similarly, sodium bromate is also used in making neutralizer solutions for permanent wave hair straightening products (WEEL, 2007). While the use of the potassium bromate in these hair care preparations has significantly decreased (Mack, 1998), they remain permitted as commercial ingredients, with the maximum limits to their concentration within the cosmetic product (FDA, 2006a).

Bromate appears to be rapidly absorbed from the gastrointestinal tract at least in part unchanged, following oral administration (Fujii*etal*., 1984), but reduced to bromide in the body tissues.*Invitro* studies indicate that liver and kidney tissues degrade bromate to bromide and that glutathione (GSH) or other sulphurydyl containing compound are probably involved in that

degredation (Tanaka *etal.*, 1984). Bromate is mainly excreted in the urine partly as bromate and partly as bromide. Some bromate may also be eliminated in the faeces(Fujii*etal.*, 1984). Acute bromate intoxication in humans is caused by accidental or suicidal ingestion of products containing either 2% potassium bromate or 10% sodium bromate. Severe gastrointestinal irritation (vomiting,pain and dirrheoa), and CNS depression (lethargy,hypotension, hypotoxicity, and loss of reflexes) are the most common acute signs. Anemia from intravascular hemolysis may also occur. These effects are usually reversible. Later sequelae (usually within several days) include marked renal injury and hearing loss. Death from renal failure may ensue if medical intervention is not successful. If support is successful, renal function generally returns after 5-10 days. Hearing loss is usually reversible. Estimated doses in these cases ranged from about 20-1000mg BrO_3^-/Kg.

Bromates are formed in many different ways in municipal drinking water. The most common is the reaction ofozone and bromide is shown in equation (1.6)

$$Br^- + O_3 \rightarrow BrO_3^- \quad \text{.....(1.6)}$$

Electrochemical processes, such as electrolysis of brine without a membrane operating to form hypochlorite, will also produce bromate when bromide ion is present in the brine solution.

Photo activation (sunlight exposure) will encourage liquid or gaseous chlorine to generate bromate in bromide-containing water.

In laboratories, bromates can be synthesized by dissolving Br_2 in a concentrated solution of potassium hydroxide (KOH). The following reactions (equation(1.7) and (1.8) will take place via the intermediate creation of hypobromite.

$$Br_2 + 2OH^- \rightarrow Br^- + BrO^- + H_2O \quad \text{.....(1.7)}$$

$$\mathbf{3BrO^- \rightarrow BrO_3^- + 2Br^-} \quad \text{.....(1.8)}$$

1.2.5 Human health issues

Bromate in drinking water is undesirable because it is a suspected human carcinogen(IARC, 2008;Kurokawa*et al.*, 1990)Its presence in Coca Cola's Dasani bottled water forced a recall of that product in the UK(BBC News,2004)

Bromate usually forms when water-containing bromide is purified using ozone, a method used at filtration plants. Proposals to reduce bromate formation include switching to enclosed atmospheric tank contact systems, lowering the water pH to between 5.9 - 6.3 , and limiting the doses of ozone. (KNBC News, 2007).

1.2.6 Bromate reservoirs, pollution incidence and its effect

On December 14, 2007, it was announced by the Los Angeles Department of Water and Power(LADWP) that the Silver Lake and Elysian reservoirs were going to be drained due to bromate contamination. At the Silver Lake and Elysian reservoirs, a combination of bromide from well water, chlorine and sunlight formed bromate. The decontamination took four months and resulted in the discharge of over 600 million US gallons (2.3×10^6 m^3) of contaminated water(Neemann*et al.*, 2004).

On June 9, 2008 the LADWP began covering the surface of the 10-acre (4 ha), 58-million-US-gallon (0.22×10^6 m^3) open Ivanhoe reservoir with black, plastic balls to block the sunlight which causes the naturally present bromide to react with the chlorine used in treatment. It will require 30 million of the 40 cent balls ($12 million) to cover the Ivanhoe and Elysian reservoirs (Vara-Orta and Francisco, 2008).

Bromate is formed when ozone used to disinfect drinking water reacts with naturally-occurring bromide found in source water. Bromate formation in disinfected drinking water is influenced by factors such as bromide ion concentration, pH of the source water, the amount of ozone and the reaction time used to disinfect the water.

The U.S. Environmental Protection Agency developed a level that it considers protective of non-cancer health effects from long-term exposure, including individuals who may be more susceptible including women of child-bearing age and children. Assuming an adult drinks about two quarts of water a day at the drinking water standard of 10 micrograms per liter, their exposure is about a sixth of that level. The increased lifetime cancer risk from drinking this water everyday poses a moderate risk level of about two in ten thousand. These exposure and

risk estimates are likely to be overestimates since most people would not consume two quarts of water-containing bromate at the standard for their lifetime.

The information on the toxicity of bromate comes from accidental or intentional poisonings in people and from studies on laboratory animals.

Some people who ingested large amounts of bromate had gastrointestinal symptoms such as nausea, vomiting, diarrhea and abdominal pain. Some individuals who ingested high concentrations of bromate also experienced kidney effects, nervous system effects and hearing loss. However, these people were exposed to bromate levels many thousand of times the amount that would come from drinking water at its standard.

Exposure to large amounts of bromate for a long period of time caused kidney effects in laboratory animals. Long-term exposure to high levels of bromate has also caused cancer in rats. Some people may be at greater risk for developing health effects from bromate exposure or have concerns for their pregnancy or nursing infant. Because bromate can cause health effects in kidneys, it is possible that those with pre-existing kidney conditions could be at greater risk. The information on the effects of bromate on reproductive health is limited, but does not indicate a concern at levels near drinking water.

1.2.7Methionine

The formula of methionine is $HO_2CCH(NH_2)CH_2CH_2SCH_3$, and its IUPAC nomenclature is 2-amino-4-(methylthio)butanoic acid.Methionine, an essentialamino acid, is one of the two sulfur-containing amino acids. The side chain is quitehydrophobic and methionine is usually found buried within proteins. Unlike cysteine, the sulfur of methionine is not highly nucleophilic, although it will react with some electrophilic centers. It is generally not a participant in the covalent chemistry that occurs in the active centers of enzymes.

The chemical linkage of the sulfur in methionine is a thiol ether. Compare this terminology with that of the oxygen-containing ethers. The sulfur of methionine, as with that of cysteine, is prone to oxidation. The first step, yielding methionine sulfoxide, can be reversed by standard thiol-containing reducing agents. The second step yields methionine sulfone, and is effectively irreversible. It is thought that oxidation of the sulfur in a specific methionine of the

elastaseinhibitor in human lung tissue by agents in cigarette smoke is one of the causes of smoking-induced emphysema.

Methionine as the free amino acid plays several important roles in metabolism. It can react to form S-Adenosyl-L-Methionine (SAM) which serve as a methyl donor in reaction.(http://www.biology.arizona.edu)

1.2.8Methionine oxidation

Methionine oxidation is a significant form of protein damage caused by endogenous or environmental oxidizing agents (Stadtman,1992).Methionine residues may be oxidized to the sulfoxide form with*t*-butyl hydroperoxide (tBHP) or hydrogen peroxide (H_2O_2) underrelatively mild conditions (Keck,1996) or to the sulfone form withother oxidizing agents under harsher conditions (Lischwe and Sung,1977). Althoughoxidation of methionine residues has no effect on the functionof some polypeptides (Glaser and Li 1974; Keck,1996), in other proteins methionineoxidation severely inhibits biological function (Chu *et al.*, 1993;Teh *et al.*,1987).Since methionine oxidation can alter protein function, it is notsurprising that cells have at least two mechanisms for dealingwith proteins containing oxidized methionine residues. First,it appears likely that oxidized proteins are preferentially degraded*in vivo* (Stadtman,1992). Second, an enzyme termed peptidyl methionine sulfoxidereductase (MsrA), which can reduce methionine sulfoxide residuesin proteins to methionine and thus restore protein function (Moskovitz*et al;*996,1995), has been identified in both prokaryotes and eukaryotes. The equation below give an example of oxidation of methionine by hydrogen peroxide.

$$Methionine(protein) + H_2O_2 \rightarrow Methionine\ Sulfoxide(protein) + H_2O$$

$$Methionine\ Sulfoxide(protein) + NADPH + H^+ \rightarrow Methionine(protein) + NADP^+ + H_2O$$

1.2.9Introduction to computational chemistry

Computational chemistry is a branch of chemistry that uses principles of computerscience to assist in solving chemical problems. It uses the results of theoreticalchemistry, incorporated into efficient computerprograms, to calculate the structures and properties of molecules and solids.

The term theoretical chemistry may be defined as the mathematical description of chemistry. Currently, there are two ways to approach theoretical chemistry problems: computational theoretical chemistry and non-computational theoretical chemistry.

Computational theoretical chemistry is primarily concerned with the numerical computation of molecular electronic structures and molecular interactions and non-computational quantum chemistry deals with the formulation of analytical expressions for the properties of molecules and their reactions.

The term computational chemistry is usually used when a mathematical method is sufficiently well developed that it can be automated for implementation on a computer. Computational chemistry is the application of chemical, mathematical and computing skills to solve interesting chemical problems.

The quantum and classical mechanics as well as statistical physics and thermodynamics are the foundation for most of the computational chemistry theory and computer programs. This is because they model the atoms and molecules with mathematics.

Its necessity arises from the well-known fact that apart from relatively recent results concerning the hydrogenmolecularion, the quantum n-body problem cannot be solved analytically, much less in closed form. While its results normally complement the information obtained by chemical experiments, it can, in some cases, predict hitherto unobserved chemical phenomena. It is widely used in the design of new drugs and materials.

Examples of such properties are structure (i.e. the expected positions of the constituent atoms), absolute and relative (interaction) energies, electroniccharge distributions, dipoles and higher

multipole moments, vibrational frequencies, reactivity or other spectroscopic quantities, and cross sections for collision with other particles.

Computational studies can be carried out to find a starting point for a laboratory synthesis, or to assist in understanding experimental data, such as the position and source of spectroscopic peaks. Computational studies can be used to predict the possibility of so far entirely unknown molecules or to explore reaction mechanisms that are not readily studied by experimental means.
Thus, computational chemistry can assist the experimental chemist or it can challenge him to find entirely new chemical objects.
Several major areas may be distinguished within computational chemistry:
The prediction of the molecular structure of molecules by the use of the simulation of forces, or more accurate quantum chemical methods, to find stationary points on the energy surface as the position of the nuclei is varied.
Storing and searching for data on chemical entities.
Identifying correlations between chemical structuresand properties.
Computational approaches to help in the efficient synthesis of compounds.
Computational approaches to design molecules that interact in specific ways with other molecules (e.g. drug design and catalysis).

The methods employed in computational chemistry cover both static and dynamic situations. In all cases, the computer time and other resources (such as memory and disk space) increase rapidly with the size of the system being studied. That system can be a single molecule, a group of molecules, or a solid. Computational chemistry methods range from highly accurate to very

approximate; highly accurate methods are typically feasible only for small systems. *Ab initio* methods are based entirely on theory from first principles. Other (typically less accurate) methods are called empirical or semi-empirical because they employ experimental results, often from acceptable models of atoms or related molecules, to approximate some elements of the underlying theory.

Both *ab initio* and semi-empirical approaches involve approximations. These range from simplified forms of the first-principles equations that are easier or faster to solve, to approximations limiting the size of the system (for example, periodicboundaryconditions), to fundamental approximations to the underlying equations that are required to achieve any solution to them at all. For example, most *ab initio* calculations make the Born–Oppenheimerapproximation, which greatly simplifies the underlying Schrödingerequation by freezing the nuclei in place during the calculation. In principle, *abinitio*methods eventually converge to the exact solution of the underlying equations as the number of approximations is reduced. In practice, however, it is impossible to eliminate all approximations, and residual error inevitably remains. The goal of computational chemistry is to minimize this residual error while keeping the calculations tractable.

In some cases, the details of electronic structure are less important than the long-time phasespace behavior of molecules. This is the case in conformational studies of proteins and protein-ligand binding thermodynamics. Classical approximations to the potential energy surface are employed, as they are computationally less intensive than electronic calculations, to enable longer simulations of moleculardynamics. Furthermore, cheminformatics uses even more

empirical (and computationally cheaper) methods like machinelearning based on physico-chemical properties. One typical problem in cheminformatics is to predict the binding affinity of drug molecules to a given target.

1.3 The Research Problem

Bromate, as a strong oxidizing agent can oxidize methionine in proteins. Oxidation of methionine damages the protein in human body and pharmaceutical products, which can lead to a lot of health defects.

1.4 Justification of the Study

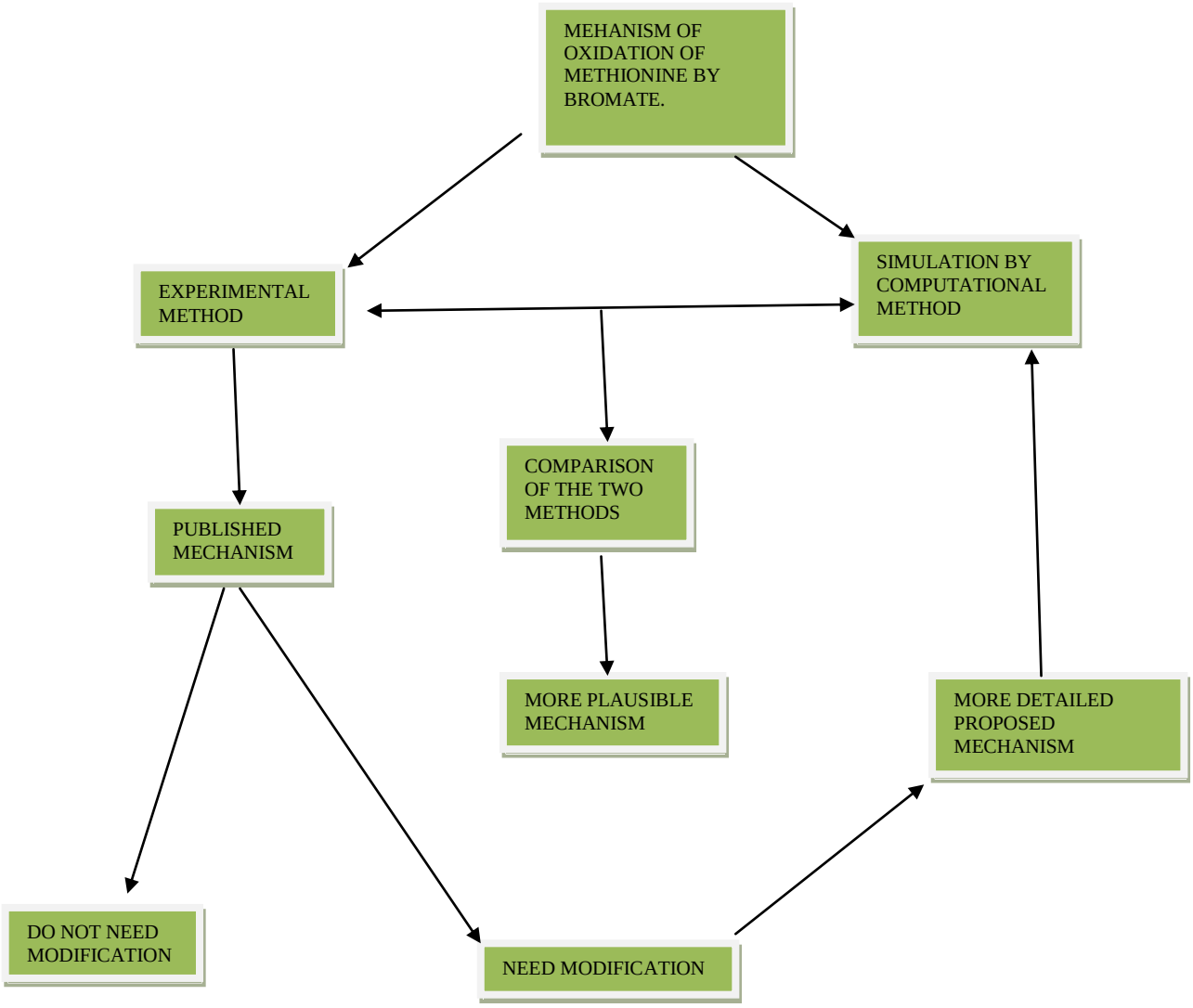
The detailed knowledge and understanding of the mechanism of oxidation of methionine by bromate would be useful in developing better ways of controlling protein oxidation by bromates in the body and pharmaceuticals.The study will provide a more detailed mechanism that can be used for the design of formulation that will prevent and control methionine oxidation which leads to protein damage

1.5 Research Hypothesis

Null hypothesis: All the published mechanisms of oxidation of methionine by bromate that involves initial uniprotonation of bromate are correct.

Alternative hypothesis: The published mechanism of oxidation of methionine by bromate needs modification.

1.6Conceptual Framework



1.7Theoritical Framework

Investigation of mechanism of oxidation of methionine by bromate can be followed through experimental or theoretical technique (computational method).The two may agree with each other to give a more detailed mechanism. If not, comparing the energies of the steps involved in each method will yield a more plausible mechanism. In computational method, a more detailed

mechanism is proposed that has reactants, transition states, intermediates, and products. This can be verified experimentally as well. This leads to the modification of earlier mechanism.

1.8Aim and Objectives of the Research

Aims

The aim of the research is to propose a more detailed and plausible mechanism for the oxidation of methionine by bromate using computational chemistry.

Objectives

1. Optimize the geometries of all the reactants, transition states, intermediates, and products involved in the proposed states.
2. Obtain the thermodynamic parameters of all the species involved.
3. Calculate the enthalpy, entropy, and Gibbs free energy change for all the steps.
4. Plot the potential energy diagrams for PM3 and MNDO computation respectively.
5. Calculate the rate constants for the various steps.
6. Locate the rate determining step from the results.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1Protein Stabilization

Therapeutic proteins, such as insulin, interferon, and -9EPO (erythroprotein), represent an important and rapidly growing class of pharmaceuticals, presently accounting for $35B/yr in revenue worldwide. Proteins are useful as therapeutics because they have a wide range of physiological functions and are extremely potent. Natural proteins in the body, as well as man-made proteins, can often carry out their functions at extremely low concentrations, such as 10M, 10-12M, or even lower. Unfortunately, proteins are also only marginally stable, and are degraded and inactivated rapidly.

In industry, the inherent instability of proteins presents a serious problem, and a disadvantage relative to small molecule therapeutics. To optimally serve patients, it is desirable to store proteins at high purity and for long times, often for up to two years after manufacture. Thus, proteins must not only be removed from their natural cellular environment, but they must also be stable against degradation for unnaturally long periods of time. This is the challenge faced by researchers and practitioners in the area of protein stabilization.

Specific degradation routes that must be addressed include aggregation, deamidation, oxidation, and hydrolysis.

*Aggregation:*Empirically, it has been observed that by adding low molecular weight components, such as salts, sugars, or polyols, to protein solutions, the propensity of the protein to aggregate (as well as degrade by other routes) can often be significantly affected. Unfortunately, because proteins are tremendously diverse in chemistry and structure, additives that work well for a

particular protein generally do not work universally. In addition, current understanding of the mechanisms by which additives confer stability on proteins is limited. Thus, there is often no theoretical guidance to aid selection of optimal additives.

This lack of understanding necessitates that protein stabilization be carried out on a case-by-case basis using heuristic experimental screens. This limits the additive search space and the possible formulation patent protection to those additive combinations which are explicitly tested. In some cases, additives that confer a useful level of stability cannot be identified.

As protein therapeutics branch out into new routes of administration, such as inhalers, implants, and stents, significant new stability challenges are presented. These new routes of administration involve protein-damaging factors such as atomization, elevated temperature, and high protein concentration, all of which can contribute in an unfavorable way to aggregation and other routes of degradation. Thus, there is an ever-increasing need to understand how to control these degradation processes to ensure that the full potential of proteins as therapeutics can be realized. Brian *et al*., (2004), addresses these needs by developing mechanistic understanding of additive function, thereby paving the way for rational design and selection of additives to stabilize proteins against aggregation.

Brian *et al*.,(2004), applied molecular simulation technique to study the mechanism by which arginine, a common refolding buffer additive, deters protein aggregation. They proposed that arginine is a member of a class of antiaggregation additives, which they termed as “neutral crowders,” characterized by their (Baynes and Bemhardt, 2003) negligible effect on the free

energy of isolated protein molecules and (Baynes and Bemhardt, 2004) large size relative to water. With a simplified statistical-mechanical model, they have shown that such additives selectively increase the free energy of protein-protein encounter complexes by being preferentially-excluded from the gap between the protein molecules in such complexes. This free energy effect, which they called "the gap effect," slows protein association reactions (Saito and Kobayashi, 1965). The gap effect is a novel mechanism by which solution additives can affect a broad range of association processes such as aggregation and crystallization.

In accordance with the gap effect model predictions, they showed experimentally that arginine slows the association of model globular proteins (antibodies and antigens) and of folding intermediates and aggregates of carbonic anhydrase II. They predicted that neutral crowders larger than arginine will be superior anti-aggregation additives.

*Oxidation:*It is also well-known that proteins can be degraded through chemical pathways under various stresses encountered in aqueous solution. One of the major chemical degradation pathways is methionine oxidation due to many possible reasons, e.g. the presence of reaction oxygen species (ROS) such as hydrogen peroxide, hydroxyl radicals, superoxide radicals,etc (Ripmeester and Ratcliffe, 1988). The interest of many researchers is in preventing or hindering the oxidation of methionine residues whose chemical modification significantly changes the bioactivity and structure of protein. Brian *et al*.,(2004), focused on hydrogen peroxide-induced oxidation of methionine in which an oxygen atom is added covalently to the reactant methionine sulfur site to form methionine sulfoxide.

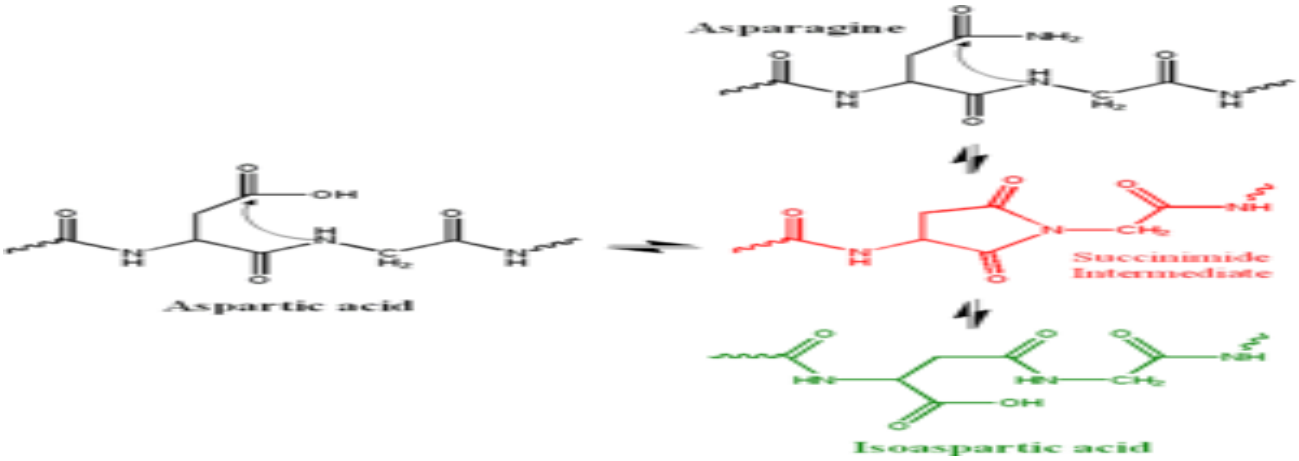
The oxidation mechanism has been the focus of many studies since 1968, such as the SN^2 type

displacement reaction mechanism (Anderson *et al*., 2004), or recently proposed acid mediated mechanism or with both acid-catalyzed and protonated water mediated mechanism (Bazant and Trout, 2001). But these mechanisms cannot explain all of the available experimental data such as the reaction activation energy and the pH dependence of the oxidation rate. Thus, we tried to resolve this discrepancy by using molecular computational methods. First, we used*ab initio* quantum chemistry calculation to determine the reaction pathway in gas phase with 2 or 3 water molecules present around the sulfur site of a single methionine. Theyproposed a more reasonable reaction mechanism where 2 or 3 water molecules stabilize the transition complex via specific interactions including formation of hydrogen bonds with H_2O_2 but not proton transfer as previously assumed in literature. It has been investigated that the appropriate reaction coordinate leading to oxidation of methionine is the separation of O-O bond together with the formation of S-O bond. According to them the mechanism meets all available experimental data (Lo *et al*., 2004).

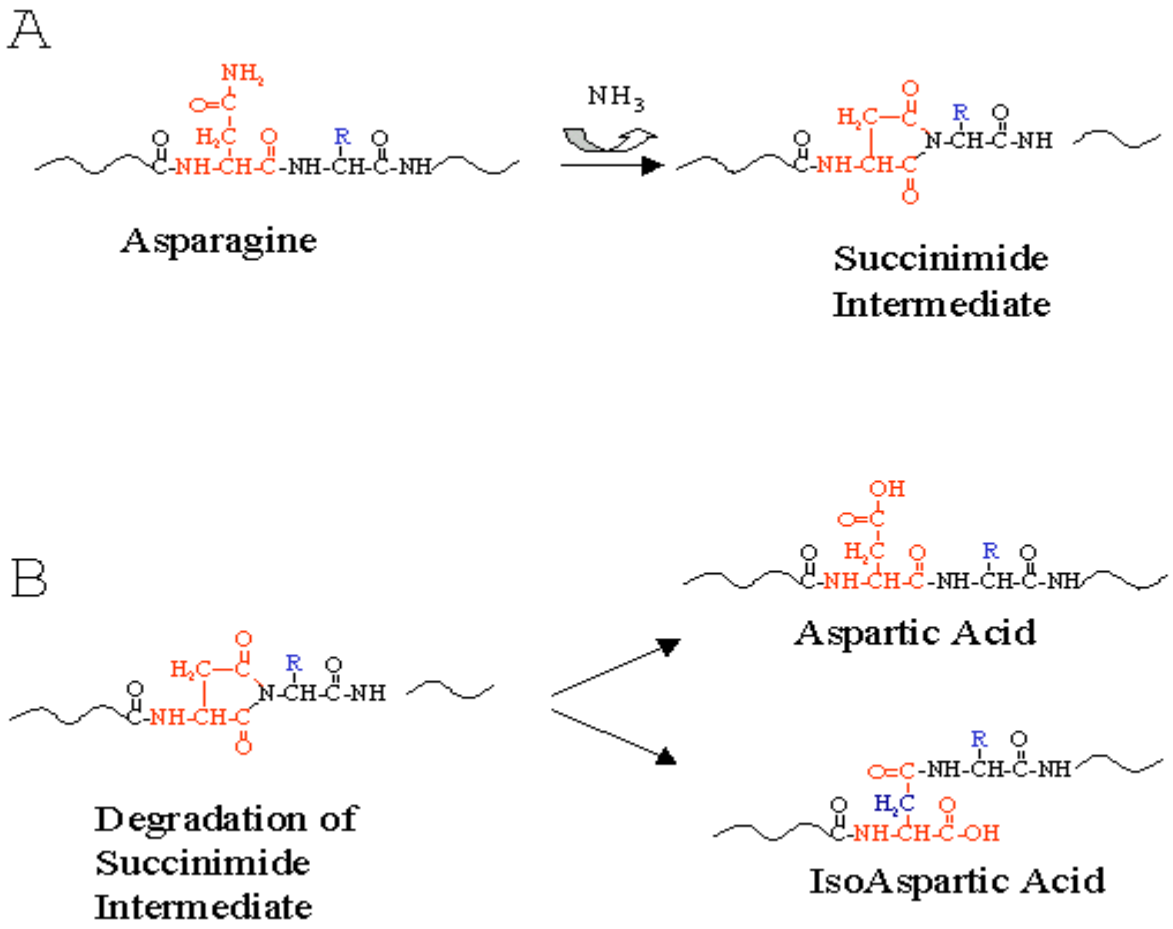
Chu *et al*., 2004, developed a computational method to address the oxidation of the amino acid methionine by peroxides in aqueous formulations of proteins which is a critical issue in the development of therapeutic products. The oxidation must be controlled so that therapeutic proteins can maintain their activity. In addition, oxidized therapeutics are undesirable due to their possible immunogenetic effects. An understanding of the mechanism and the factors that influence the reactivity of different methionine sites toward oxidation is therefore important. In the studies, a mechanism by which peroxides oxidize the sulfur atom of methionine is developed. The rate-limiting step was found to be the breaking of the O-O bond of H_2O_2 and the formation of the S-O bond during which significant charge separation is developed.

Deamidation: Deamidation is a chemical reaction in which an amidefunctionalgroup is removed from an organiccompound. In biochemistry, the reaction is important in the degradation of proteins because it damages the amide-containing sidechains of the aminoacidsasparagine and glutamine.(Clark, 1987).

In the biochemical deamidation reaction, the side chain of an asparagine attacks the following peptide group (in black at top right of Figure), forming a symmetric succinimide intermediate (in red). The symmetry of the intermediate results in two products of its hydrolysis, either aspartate (in black at left) or in isoaspartate, which is a betaaminoacid (in green at bottom right). This process is considered a deamidation because the amide in the asparagine side chain is replaced by a carboxylate group. However, a similar reaction can occur in aspartate side chains, yielding a partial conversion to isoaspartate.Deamidation reactions have been conjectured to be one of the factors that limit the useful lifetime of proteins.Deamidation proceeds much more quickly if the susceptible amino acid is followed by a small, flexible residue such as glycine whose low steric hindrance leaves the peptide group open for attack. Deamidation reactions also proceed much more quickly at elevated pH (>10) and temperature.(Stephensen and Clark, 1989)



Deamidation reaction of Asn-Gly (top right) to Asp-Gly (at left) or iso(Asp)-Gly (in green at bottom right).



2.2Potassium Bromate and its Toxicological Effect

Potassium bromate is a flour improver that acts as a maturing agent (Vadlamani and Seib, 1999). It has been in use as a food additive for the past 80 years. It acts principally in the late dough stage giving strength to the dough during the late proofing and early baking (Vadlamani and Seib, 1999). In laymen's term, potassium bromate prevents dough from falling. This property has been manipulated by many Nigerian bakers in profit making. Over time, it has been discovered that potassium bromate is toxic and is a possible carcinogen in man (Kurokawa*et al*., 1986). This led to the proposal for its ban in the United States. Potassium bromate has been banned in several countries including the United Kingdom in 1990, Nigeria in

1993 and Canada in 1994. However, since the ban, not too many Nigerian bakers have complied and stopped its use even as the National Agency for Food and Drug Administration and Control banned its use.

Toxicological studies have convincingly shown that potassium bromate affects the nutritional quality of bread as the main vitamins available in bread are degraded (Sai*et al*., 1992). Carcinogenic and mutagenic effects of potassium bromate have been reported in experimental animals (Kurokawa*et al*., 1987). Lethal oral doses of bromate in humans have been estimated to be between 154 and 385 mg/kg body weight while serious poisoning results at doses of 46–92 mg/kg body weight (Mark, 1988). Oral doses of 185– 385 mg/kg body weight results in irreversible toxic effects like renal failure and deafness in humans while lower doses are associated with vomiting, diarrhea, nausea and abdominal pain (Mark, 1988).

Potassium bromate is extremely irritating and injurious to tissues especially those of the central nervous system and kidneys. The pathologic findings include kidney damage and haemolysis (Robert and William, 1996). Bromate was first found to cause tumour in rats in 1982. Subsequent studies on rats and mice confirmed that it causes tumour of the kidney, thyroid and other organs (CSPI, 1999). It is known that potassium bromate induces oxidative stress in tissues (Sai*et al*., 1991; Watanabe *et al.*, 1992; Parsons and Chipman, 1992, 2000). Indeed, oxidative damage appears to be the basis of bromate-induced carcinogenesis (Chipman*et al*., 2006). Several cases of accidental poisoning in children resulting from ingestion of bromate solution and sugar contaminated with bromate were reported as the source of an outbreak of mild poisoning in New Zealand (Paul, 1966).

2.3Causes of Protein Damage

With the advent of recombinant DNA technology, protein pharmaceuticals, including functional regulators and supplements, enzyme activators and inhibitors, antibodies and various vaccines have become indispensable in combating human diseases. In developing a protein as a therapeutic product, one of the most difficult steps is to deal with the physical and chemical instabilities such as aggregation, deamidation, and oxidation(Cleland & Langer,1994; Shahrokh,1997; Wang,1999; Meyer *et al*; 2002).If physical and/or chemical degradation pathways modify a protein molecule during storage, the biological and immunogenicity of the molecule can be changed as well. Therefore, chemical and physical instabilities need to be prevented in any formulation of therapeutic proteins (Cleland *et al*;1993). The objective of protein stabilization is thus to design a formulation to prevent physical and chemical instabilities so that an acceptable shelf life(>2 years) can be achieved for a therapeutic protein product. During storage, various degradation pathways can proceed at different rates depending on the physico-chemical properties of the formulation (temperature, pH, ionic strength, additives,etc) and the protein molecule (primary sequence tertiary structures, unfolding, melting temperatures).The complexity of protein systems makes the design of formulation a difficult challenge, and is addressed currently empirically.

Oxidation is one of the major chemical instabilities of protein pharmaceuticals and the side chains of methionine (Met); cysteine(Cys), histidine(His), tryptophan(Trp) and tyrosine(Tyr) residues are potential sites for protein oxidative modification (Li *et al*; 1995). The thioethergroup of methionine makes it one of the amino acids that ismost liable to oxidation in aqueous solution(Wang, 1999, Li *etal*., 1995). Potential reactive oxygen species involves singlet

oxygen ($^1O_2$), superoxide radical,($^{\cdot}O_2^-$), peroxides, (ROOH), and hydroxyl radical($^{\cdot}OH$).

Of the residues susceptible to oxidation, methionine is the most important, since the majority of the protein oxidation identified thus far are related to modification of methionine (Clelan*det al*.,1993). The covalent addition of an oxygen atom to the sulphur atom of methionine changes the chemical properties of proteins and generally leads to the loss of biological function. Examples of proteins that are destabilized by oxidation of methionine include Human α-1proteinase inhibitor (Matheson et al., 1998) calmodulin (Levine *etal*;1996., Gao*et al.*, 1998), human Parathoid hormone (Nabidi*et al.*,1998), Antithrombin(Patten *etal.*,1999), Glutamine synthetase(Levine, 1983), α-1 antitrypsin (Griffiths and Cooney, 2002), and Granulocyte colony-stimulating factor (Lu *et al*., 1999).

2.4.1 History of computational chemistry

Building on the founding discoveries and theories in the history of quantum mechanics, the first theoretical calculations in chemistry were those of Walter Heitler and Fritz London in 1927. The books that were influential in the early development of computational quantum chemistry include Linus Pauling and E. Bright Wilson's 1935 *Introduction to Quantum Mechanics – with Applications to Chemistry*, Eyring, Walter and Kimball's 1944 *Quantum Chemistry*, Heitler's 1945 *Elementary Wave Mechanics – with Applications to Quantum Chemistry*, and later Coulson's 1952 textbook *Valence*, each of which served as primary references for chemists in the decades to follow.

With the development of efficient computer technology in the 1940s, the solutions of elaborate wave equations for complex atomic systems began to be a realizable objective. In the early

1950s, the first semi-empirical atomic orbital calculations were carried out. Theoretical chemists became extensive users of the early digital computers. A very detailed account of such use in the United Kingdom is given by Smith and Sutcliffe.(Smith, 1997) The first *ab initio*Hartree–Fock calculations on diatomic molecules were carried out in 1956 at MIT, using a basis set of Slater orbitals. For diatomic molecules, a systematic study using a minimum basis set and the first calculation with a larger basis set were published by Ransiland Nesbet respectively in 1960 (Schaefer, 1972). The first polyatomic calculations using Gaussian orbitals were carried out in the late 1950s. The first configurationinteraction calculations were carried out in Cambridge on the EDSAC computer in the 1950s using Gaussian orbitals by Boys and coworkers(Boys, 1956). By 1971, when a bibliography of *ab initio* calculations was published(Richards,1971), the largest molecules included were naphthalene and azulene(Preuss , 1968; Bruenker, 1969). Abstracts of many earlier developments in *ab initio* theory have been published by Schaefer(Schaefer, 1984).

In 1964, Hückel method calculations (using a simple linear combination of atomic orbitals (LCAO) method for the determination of electron energies of molecular orbitals of π electrons in conjugated hydrocarbon systems) of molecules ranging in complexity from butadiene and benzene to ovalene were generated on computers at Berkeley and Oxford(Streitweiser, 1965). These empirical methods were replaced in the 1960s by semi-empirical methods such as CNDO(Pople, 1970).

In the early 1970s, efficient *ab initio* computer programs such as ATMOL, GAUSSIAN, IBMOL, and POLYAYTOM, began to be used to speed up *ab initio* calculations of molecular

orbitals. Of these four programs, only GAUSSIAN, now massively expanded, is still in use, but many other programs are now in use. At the same time, the methods of molecular mechanics, such as MM2, were developed, primarily by Norman Allinger (Allinger,1977).

One of the first mentions of the term "computational chemistry" can be found in the 1970 book *Computers and Their Role in the Physical Sciences* by Sidney Fernbach and Abraham Haskell Taub, where they state "It seems, therefore, that 'computational chemistry' can finally be more and more of a reality."(Fernbach, 1970). During the 1970s, widely different methods began to be seen as part of a new emerging discipline of *computational chemistry.* The *Journal of Computational Chemistry* was first published in 1980.

2.4.2 Methods of computational chemistry.

A single molecular formula can represent a number of molecular isomers. Each isomer is a local minimum on the energy surface (called the potential energy surface) created from the total energy (i.e., the electronic energy, plus the repulsion energy between the nuclei) as a function of the coordinates of all the nuclei. A stationary point is a geometry such that the derivative of the energy with respect to all displacements of the nuclei is zero. A local (energy) minimum is a stationary point where all such displacements lead to an increase in energy. The local minimum that is lowest is called the global minimum and corresponds to the most stable isomer. If there is one particular coordinate change that leads to a decrease in the total energy in both directions, the stationary point is a transition structure and the coordinate is the reaction coordinate. This process of determining stationary points is called geometry optimization.

The determination of molecular structure by geometry optimization became routine only after efficient methods for calculating the first derivatives of the energy with respect to all atomic coordinates became available. Evaluation of the related second derivatives allows the prediction of vibrational frequencies if harmonic motion is estimated. More importantly, it allows for the characterization of stationary points. The frequencies are related to the eigenvalues of the Hessian matrix, which contains second derivatives. If the eigenvalues are all positive, then the frequencies are all real and the stationary point is a local minimum. If one eigenvalue is negative (i.e., an imaginary frequency), then the stationary point is a transition structure. If more than one eigenvalue is negative, then the stationary point is a more complex one, and is usually of little interest. When one of these is found, it is necessary to move the search away from it if the experimenter is looking solely for local minima and transition structures.

The total energy is determined by approximate solutions of the time-dependent Schrödinger equation, usually with no relativistic terms included, and by making use of the Born–Oppenheimer approximation, which allows for the separation of electronic and nuclear motions, thereby simplifying the Schrödinger equation. This leads to the evaluation of the total energy as a sum of the electronic energy at fixed nuclei positions and the repulsion energy of the nuclei. A notable exception is certain approaches called direct quantum chemistry, which treat electrons and nuclei on a common footing. Density functional methods and semi-empirical methods are variants on the major theme. For very large systems, the relative total energies can be compared using molecular mechanics. The ways of determining the total energy to predict molecular structures are:

2.4.2.1 ***Ab initio* methods**

Ab-initio, (Latin for "from scratch") a group of methods in which molecular structures can be calculated using nothing but the Schrödinger equation, the values of the fundamental constants and the atomic numbers of the atoms present.

The programs used in computational chemistry are based on many different quantum-chemical methods that solve the molecular Schrödinger equation associated with the molecular Hamiltonian. Methods that do not include any empirical or semi-empirical parameters in their equations – being derived directly from theoretical principles, with no inclusion of experimental data – are called *ab initio methods*. This does not imply that the solution is an exact one; they are all approximate quantum mechanical calculations. It means that a particular approximation is rigorously defined on first principles (quantum theory) and then solved within an error margin that is qualitatively known beforehand. If numerical iterative methods have to be employed, the aim is to iterate until full machine accuracy is obtained (the best that is possible with a finite word length on the computer, and within the mathematical and/or physical approximations made).

The simplest type of *ab initio* electronic structure calculations is the Hartree–Fock (HF) scheme, an extension of molecular orbital theory, in which the correlated electron–electron repulsion is not specifically taken into account; only its average effect is included in the calculation. As the basis set size is increased, the energy and wave function tend towards a limit called the Hartree–Fock limit. Many types of calculations (known as post-Hartree–Fock methods) begin with a Hartree–Fock calculation and subsequently correct for electron–electron repulsion, referred to

also as electroniccorrelation. As these methods are pushed to the limit, they approach the exact solution of the non-relativistic Schrödinger equation. In order to obtain exact agreement with experiment, it is necessary to include relativistic and spinorbit terms, both of which are only really important for heavy atoms. In all of these approaches, in addition to the choice of method, it is necessary to choose a basisset. This is a set of functions, usually centered on the different atoms in the molecule, which are used to expand the molecular orbitals with the LCAO. *Ab initio* methods need to define a level of theory (the method) and a basis set.

The Hartree–Fock wave function is a single configuration or determinant. In some cases, particularly for bond breaking processes, this is quite inadequate, and several configurations need to be used. Here, the coefficients of the configurations and the coefficients of the basis functions are optimized together.

The total molecular energy can be evaluated as a function of the molecular geometry; in other words, the potential energy surface. Such a surface can be used for reaction dynamics. The stationary points of the surface lead to predictions of different isomers and the transition structures for conversion between isomers, but these can be determined without a full knowledge of the complete surface.

A particularly important objective, called computational thermochemistry, is to calculate thermochemical quantities such as the enthalpy of formation to chemical accuracy. Chemical accuracy is the accuracy required to make realistic chemical predictions and is generally considered to be 1 kcal/mol or 4 kJ/mol. To reach that accuracy in an economic way, it is

necessary to use a series of post-Hartree–Fock methods and combine the results. These methods are called quantum chemistry composite methods.

2.4.2.2 Density functional methods

Density functional theory (DFT) methods are often considered to be *ab initio methods* for determining the molecular electronic structure, even though many of the most common functionals use parameters derived from empirical data, or from more complex calculations. In DFT, the total energy is expressed in terms of the total one-electrondensity rather than the wave function. In this type of calculation, there is an approximate Hamiltonian and an approximate expression for the total electron density. DFT methods can be very accurate for little computational cost. Some methods combine the density functional exchange with the Hartree–Fock exchange term and are known as hybridfunctional methods.

2.4.2.3 Semi-empirical and empirical methods

Semi-empirical techniques use approximations from empirical (experimental) data to provide the input into the mathematical models.

Semi-empirical quantum chemistry methods are based on the Hartree–Fock formalism, but make many approximations and obtain some parameters from empirical data. They are very important in computational chemistry for treating large molecules where the full Hartree–Fock method without the approximations is too expensive. The use of empirical parameters appears to allow some inclusion of correlation effects into the methods.

Semi-empirical methods follow what are often called empirical methods, where the two-electron part of the Hamiltonian is not explicitly included. For π-electron systems, this was the

Hückelmethod proposed by ErichHückel, and for all valence electron systems, the extended Hückel method proposed by RoaldHoffmann.

2.4.2.4Molecular mechanics

Molecular mechanics uses classical physics and empirical or semi-empirical (predetermined) force fields to explain and interpret the behavior of atoms and molecules.

In many cases, large molecular systems can be modeled successfully while avoiding quantum mechanical calculations entirely. Molecularmechanics simulations, for example, use a single classical expression for the energy of a compound, for instance the harmonicoscillator. All constants appearing in the equations must be obtained beforehand from experimental data or *ab initio* calculations.

The database of compounds used for parameterization, i.e., the resulting set of parameters and functions are called the forcefield, which is crucial to the success of molecular mechanics calculations. A force field parameterized against a specific class of molecules, for instance proteins, would be expected to only have any relevance when describing other molecules of the same class.

These methods can be applied to proteins and other large biological molecules, and allow studies of the approach and interaction (docking) of potential drug molecules (e.g.Smith, 1997; Schaefer, 1972).

2.4.2.5 Methods for solids

Computational chemical methods can be applied to solid state physics problems. The electronic structure of a crystal is in general described by a band structure, which defines the energies of electron orbitals for each point in the Brillouin zone. *Ab initio* and semi-empirical calculations yield orbital energies; and therefore, they can be applied to band structure calculations. Since it is time-consuming to calculate the energy for a molecule, it is even more time-consuming to calculate them for the entire list of points in the Brillouin zone.

2.4.2.6 Chemical dynamics

Once the electronic and nuclear variables are separated (within the Born–Oppenheimer representation), in the time-dependent approach, the wavepacket corresponding to the nuclear degreesoffreedom is propagated via the time evolutionoperator (physics) associated to the time-dependent Schrödingerequation (for the full molecularHamiltonian). In the complementary energy-dependent approach, the time-independent Schrödinger equation is solved using the scatteringtheory formalism. The potential representing the interatomic interaction is given by the potentialenergysurfaces. In general, the potentialenergysurfaces are coupled via the vibroniccoupling terms.

The most popular methods for propagating the wavepacket associated to the moleculargeometry are:

thesplit operator technique,

theChebyshev (real) polynomial,

themulti-configuration time-dependent Hartree method (MCTDH),

thesemi-classical method.

2.4.2.7 Molecular dynamics

Molecular dynamics consists of examining the time-dependent behavior of a molecule, such as vibrational motion or Brownian motion. This is most often done within a classical mechanical description similar to a molecular mechanics calculation. The application of molecular dynamics to solvent/solute systems allows the computation of properties such as diffusion coefficients or radial distribution functions for use in statistical mechanical treatments. Usually the scheme of a solvent/solute calculation is that a number of molecules (perhaps 1000) are given some initial position and velocity. New positions are calculated small time later based on this movement and this process is iterated for thousands of steps in order to bring the system to equilibrium and give a good statistical description of the radial distribution function. In order to analyze the vibrations of a single molecule, many dynamics steps are done, then the data is Fourier transformed into the frequency domain. A given peak can be chosen and transformed back to the time domain, in order to see what the motion at that frequency looks like.

The words *exact* and *perfect* do not appear in computational chemistry, as very few aspects of chemistry can be computed exactly. However, almost every aspect of chemistry can be described in a qualitative or approximate quantitative computational scheme.

Molecules consist of nuclei and electrons, so the methods of quantummechanics apply. Computational chemists often attempt to solve the non-relativistic Schrödingerequation, with

relativistic corrections added, although some progress has been made in solving the fully relativistic Diracequation. In principle, it is possible to solve the Schrödinger equation in either its time-dependent or time-independent form, as appropriate for the problem in hand; in practice, this is not possible except for very small systems. Therefore, a great number of approximate methods strive to achieve the best trade-off between accuracy and computational cost.

Accuracy can always be improved with greater computational cost. Significant errors can present themselves in *ab initio* models comprising many electrons, due to the computational expense of full relativistic-inclusive methods. This complicates the study of molecules interacting with high atomic mass unit atoms, such as transitional metals and their catalytic properties. Present algorithms in computational chemistry can routinely calculate the properties of molecules that contain up to about 40 electrons with sufficient accuracy. Errors for energies can be less than a few kJ/mol. For geometries, bond lengths can be predicted within a few picometres and bond angles within 0.5 degrees. The treatment of larger molecules that contain a few dozen electrons is computationally tractable by approximate methods such as density functional theory (DFT).

There is some dispute within the field whether or not the latter methods are sufficient to describe complex chemical reactions, such as those in biochemistry. Large molecules can be studied by semi-empirical approximate methods. Even larger molecules are treated by classical mechanics methods that employ what are called molecular mechanics. In QM/MM methods,

small portions of large complexes are treated quantum mechanically (QM), and the remainder is treated approximately (MM).

2.4.3 Fields of application

In theoretical chemistry, chemists, physicists and mathematicians develop algorithms and computer programs to predict atomic and molecular properties and reaction paths for chemical reactions. Computational chemists, in contrast, may simply apply existing computer programs and methodologies to specific chemical questions. It uses computers to generate information such as properties of molecules or simulated experimental results. Very few aspects of chemistry can be computed exactly, but almost every aspect of chemistry has been described in a qualitative or approximate quantitative computational scheme. The biggest mistake that computational chemists can make is to assume that any computed number is exact. However, just as not all spectra are perfectly resolved, often a qualitative or approximate computation can give useful insight into chemistry if you understand what it tells you and what it doesn't.

Computational chemistry has become a useful way to investigate materials that are too difficult to find or too expensive to purchase. It also helps chemists make predictions before running the actual experiments so that they can be better prepared for making observations.

Using computational chemistry software you can, in particular, perform: electronic structure determinations, geometry optimizations, frequency calculations, definition of transition structures and reaction paths, protein calculations, i.e. docking, electron and charge distributions calculations, calculations of potential energy surfaces (PES), calculations of rate constants for chemical reactions (kinetics), thermodynamic calculations- heat of reactions, energy of

activation, etc, calculation of many other molecular and bulk physical and chemical properties. Table 2.1 summarizes the features, advantages, disadvantages and suitability of the most important numerical techniques these are*ab-initio*, semi-empirical and molecular mechanics.

Table 2.1: Computational methods

Method type	Features	Advantages	Disadvantages	Best for
Molecular Mechanics	Uses classical physics Relies on force-field with embedded empirical parameters Computationally least intensive - fast and useful with limited computer resources Can be used for molecules as large as enzymes	Relies on potentials that have to be somehow supplied Sometimes inaccurate because the supplied potentials are used beyond their proven range of validity	Particular force field, applicable only for a limited class of molecules Does not calculate electronic properties Requires experimental data (or data from *ab initio* calculations)	Large systems (~1000 of atoms) Systems or processes with no breaking or forming of bonds
Semi empirical	Uses quantum physics Uses experimentally derived empirical parameters Uses many approximation	Less demanding computationally than *ab initio* methods Capable of calculating transition states and excited states	Requires experimental data (or data from *ab initio*) for parameters Less rigorous than *ab initio*) methods Computationally expensive	Medium-sized systems (hundreds of atoms) Systems involving electronic

Ab Initio	Uses quantum physics Mathematically rigorous, no empirical parameters Uses approximation extensively	Useful for a broad range of systems does not depend on experimental data Capable of calculating transition states and excited states	Small systems (tens of atoms) Systems involving electronic transition Molecules without available experimental data Systems requiring rigorous accuracy

2.5Basis Set

A basis set in theoretical and computational chemistryis a set of functions (called basis functions) which are combined inlinear combinations(generally as part of a quantum chemical calculation) to create molecular orbitals. For convenience these functions are typicallyatomic orbitals centered on atoms, but can theoretically be any function; plane waves are frequently used in materials calculations.

2.5.1 Introduction to basis set

In modern computational chemistry,quantum chemicalcalculations are typically performed using a finite set of basis functions. In these cases, the system in question are represented as vectors, the components of which correspond to coefficients in a linear combination of the basis functions in the basis set used. The operatorsare then represented asmatrices, (rank two tensors), in this

finite basis. B*asis function* and *atomic orbital* are sometimes used interchangeably, although it should be noted that these basis functions are usually not actually the exact atomic orbitals, even for the corresponding hydrogen-like atoms, due to approximations and simplifications of their analytic formulas. If the finite basis is expanded towards an infinite complete set of functions, calculations using such a basis set are said to approach the basis set limit (Roman,2010).

When molecular calculations are performed, it is common to use a basis composed of a finite number of atomic orbitals, centered at each atomic nucleus within the molecule (linear combination of atomic orbitals). These atomic orbitals are well described with Slater-type orbitals(STOs), as STOs decay exponentially with distance from the nuclei, accurately describing the long-range overlap between atoms, and reach a maximum at zero, well describing the charge and spin at the nucleus. STOs are computationally difficult and it was later realized by Frank Boys that these Slater-type orbitals could in turn be approximated as linear combinations of Gaussian orbitals instead. Because it is easier to calculate overlap and other integrals with Gaussian basis functions, this led to huge computational savings.

Today, there are hundreds of basis sets composed of Gaussian-type orbitals (GTOs). The smallest of these are called *minimal basis sets*, and they are typically composed of the minimum number of basis functions required to represent all of the electrons on each atom. The largest of these can contain literally dozens to hundreds of basis functions on each atom.

A minimum basis set is one in which, on each atom in the molecule, a single basis function is used for each orbital in aHartree–Fockcalculation on the free atom. However, for atoms such as lithium, basis functions of p type are added to the basis functions corresponding to the 1s and 2s

orbitals$a^2 + b^2 = c^2$ of the free atom. For example, each atom in the second period of the periodic system (Li - Ne) would have a basis set of five functions (two s functions and three p functions) (Errol, 2000).

The most common addition to minimal basis sets is probably the addition ofpolarization functions, denoted (in the names of basis sets developed by Pople) by an asterisk,*. Two asterisks, **, indicate that polarization functions are also added to light atoms (hydrogen and helium). These are auxiliary functions with one additional node. For example, the only basis function located on a hydrogen atom in a minimal basis set would be a function approximating the 1s atomic orbital. When polarization is added to this basis set, a p-function is also added to the basis set. This adds some additional needed flexibility within the basis set, effectively allowing molecular orbitals involving the hydrogen atoms to be more asymmetric about the hydrogen nucleus. This is an important result when considering accurate representations of bonding between atoms, because the very presence of the bonded atom makes the energetic environment of the electrons spherically asymmetric. Similarly, d-type functions can be added to a basis set with valence p orbitals, and f-functions to a basis set with d-type orbitals, and so on. Another, more precise, notation indicates exactly which and how many functions are added to the basis set, such as (d, p).

Another common addition to basis sets is the addition of diffuse functions, denoted in Pople-type sets by a plus sign, +, and in Dunning-type sets by "aug" (from "augmented"). Two plus signs indicate that diffuse functions are also added to light atoms (hydrogen and helium). These are very shallow Gaussian basis functions, which more accurately represent the "tail" portion of the

atomic orbitals, which are distant from the atomic nuclei. These additional basis functions can be important when considering anions and other large, "soft" molecular systems.

2.5.2 Minimal basis sets

The most common minimal basis set is STO-nG, where n is an integer. This *n* value represents the number of Gaussian primitive functions comprising a single basis function. In these basis sets, the same number of Gaussian primitives comprises core and valence orbitals. Minimal basis sets typically give rough results that are insufficient for research-quality publication, but are much cheaper than their larger counterparts. Commonly used minimal basis sets of this type are: STO-3G, STO-4G, STO-6G, STO-3G* - Polarized version of STO-3G.There are several other minimum basis sets that have been used, such as the MidiX basis sets.

2.5.3Split-valence basis sets

During most molecular bonding, it is the valence electrons which principally take part in the bonding. In recognition of this fact, it is common to represent valence orbitals by more than one basis function (each of which can, in turn, be composed of a fixed linear combination of primitive Gaussian functions). Basis sets in which there are multiple basis functions corresponding to each valence atomic orbital are called valence double, triple, quadruple-*zeta*, and so on, basis sets (zeta, ζ, was commonly used to represent the exponent of an STO basis function (Davidson and Feller, 1986). Since the different orbitals of the split have different spatial extents, the combination allows the electron density to adjust its spatial extent appropriate to the particular molecular environment. Minimum basis sets are fixed and are unable to adjust to different molecular environments.

2.5.4 Pople basis sets

The notation for the *split-valence* basis sets arising from the group of John Pople is typically *X-YZg* (Dtchfield*et al*., 1971). In this case, *X* represents the number of primitive Gaussians comprising each core atomic orbital basis function.*Y* and *Z* indicate that the valence orbitals are composed of two basis functions each, the first one composed of a linear combination of *Y* primitive Gaussian functions, the other composed of a linear combination of *Z* primitive Gaussian functions. In this case, the presence of two numbers after the hyphens implies that this basis set is a *split-valence double-zeta* basis set. Split-valence triple- and quadruple-zeta basis sets are also used, denoted as *X-YZWg*, *X-YZWVg*, etc. Here is a list of commonly used split-valence basis sets of this type:3-21G, 3-21G* - Polarized, 3-21+G - Diffuse functions, 3-21+G* - With polarization *and* diffuse functions, 4-21G, 4-31G, 6-21G, 6-31G, 6-31G*, 6-31+G*, 6-31G(3df, 3pd), 6-311G, 6-311G*, 6-311+G*.

The 6-31G* basis set (defined for the atoms H through Zn) is a 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.

2.5.5 Correlation-consistent basis sets

Some of the most widely used basis sets are those developed by Dunning and coworkers, (Dunning, 1989), since they are designed to converge systematically to the complete-basis-set (CBS) limit using empirical extrapolation techniques. For first- and second-row atoms, the basis sets are cc-pVNZ where N=D,T,Q,5,6,... (D=double, T=triples, etc.). The 'cc-p', stands for ‘correlation-consistent polarized’ and the ‘V’ indicate they are valence-only basis sets. They

include successively larger shells of polarization (correlating) functions (*d, f, g,* etc.). More recently, these 'correlation-consistent polarized' basis sets have become widely used and are the current state of the art for correlated orpost-Hartree–Fock calculations. Examples of these are:
cc-pVDZ - Double-zeta, cc-pVTZ - Triple-zeta, cc-pVQZ - Quadruple-zeta, cc-pV5Z - Quintuple-zeta, etc.aug-cc-pVDZ, etc. - Augmented versions of the preceding basis sets with added diffuse functions.

For period-3 atoms (Al-Ar), additional functions are necessary; these are the cc-pV(N+d)Z basis sets. Even larger atoms may employ *pseudo*potential basis sets, cc-pVNZ-PP, or relativistic-contracted Douglas-Kroll basis sets, cc-pVNZ-DK.
These basis sets can be augmented with core functions for geometric and nuclear property calculations, and with diffuse functions for electronic excited-state calculations, electric field property calculations, and long-range interactions, such as van der Waals forces. A recipe for constructing additional augmented functions exists; as many as five augmented functions have been used in second hyperpolarizability calculations in the literature. Because of the rigorous construction of these basis sets, extrapolation can be done for almost any energetic property, although care must be taken when extrapolating energy differences as the individual energy components may converge at different rates.

H-He	**Li-Ne**	**Na-Ar**
cc-pVDZ [2*s*1*p*] → 5 func.	[3*s*2*p*1*d*] → 14 func.	[4*s*3*p*1*d*] → 18 func.
cc-pVTZ [3*s*2*p*1*d*] → 14 func.	[4*s*3*p*2*d*1*f*] → 30 func.	[5*s*4*p*2*d*1*f*] → 34 func.
cc-pVQZ [4*s*3*p*2*d*1*f*] → 30 func.	[5*s*4*p*3*d*2*f*1*g*] → 55 func.	[6*s*5*p*3*d*2*f*1*g*] → 59 func.

To understand how to get the number of functions take the cc-pVDZ basis set for H, There are two *s* (L = 0) orbitals and one *p* (L = 1) orbital that has 3 components along the *z*-axis (m_L = -

1,0,1) corresponding to p_x, p_y and p_z. Thus, five spatial orbitals in total. Note that each orbital can hold two electrons of opposite spin.

For example, Ar [1s, 2s, 2p, 3s, 3p] has 3 s orbitals (L=0) and 2 sets of p orbitals (L=1). Using cc-pVDZ, orbitals are [1s, 2s, 2p, 3s, 3s', 3p, 3p', 3d'] (where ' represents the added in polarisation orbitals), with 4 s orbitals, 3 sets of p orbitals and 1 set of d orbitals.

2.5.6Other split-valence basis sets

Some other split-valence (SV) basis sets are :SV(P), SVP, DZV - Valence double-zeta, TZV - Valence triple-zeta, TZVPP - Valence triple-zeta plus polarization, QZVPP - Valence quadruple-zeta plus polarization.

2.5.7 Plane-wave basis sets

In addition to localized basis sets, plane-wave basis sets can also be used in quantum-chemical simulations. Typically, a finite number of plane-wave functions are used, below a specific cutoff energy which is chosen for a certain calculation. These basis sets are popular in calculations involving periodic boundary conditions. Certain integrals and operations are much easier to code and carry out with plane-wave basis functions than with their localized counterparts.

In practice, plane-wave basis sets are often used in combination with an 'effective core potential' or *pseudo*potential, so that the plane waves are only used to describe the valence charge density. This is because core electrons tend to be concentrated very close to the atomic nuclei, resulting in large wavefunction and density gradients near the nuclei which are not easily described by a plane-wave basis set unless a very high energy cutoff, and therefore small wavelength, is used.

This combined method of a plane-wave basis set with a core*pseudo*potential is often abbreviated sas a *PSPW* calculation.

Furthermore, as all functions in the basis are mutually orthogonal and are not associated with any particular atom, plane-wave basis sets do not exhibit basis-set superposition error. However, they are less well suited to gas-phase calculations. Using Fast Fourier Transforms, one can work with plane-wave basis sets in reciprocal space in which not only the aforementioned integrals, such as the kinetic energy, but also derivatives are computationally less demanding to be carried out. Another important advantage of a plane-wave basis is that it is guaranteed to converge in a *smooth, monotonic manner* to the target wavefunction, while there is only a guarantee of monotonic convergence for all Gaussian-type basis sets when used in variational calculations. (An exception to the latter point is the correlation consistent basis sets). The properties of the Fourier Transform allow a vector representing the gradient of the total energy with respect to the plane-wave coefficients to be calculated with a computational effort that scales as NPW*ln(NPW) where NPW is the number of plane-waves. When this property is combined with separable pseudopotentials of the Kleinman-Bylander type and pre-conditioned conjugate gradient solution techniques, the dynamic smulation of periodic problems containing hundreds of atoms becomes possible.

2.5.8 Real-space basis sets

On the same principle as the plane waves but in real space, there are basis sets whose functions are centered on a uniform mesh in real space. This is the case for the finite difference, the functionssinc orwavelets. In the case of the latter, it is possible to have an adaptive mesh closer to the nucleus using the scaling properties of wavelets. These methods use functions that are localized which allow the development of order N methods.

2.6 Interpreting Molecular Wave Functions

The atoms in molecules model developed by Richard Bader was developed in order to effectively link the quantum mechanical picture of a molecule, as an electronic wavefunction, to chemically useful older models such as the theory of Lewis pairs and the valence bond model. Bader has demonstrated that these empirically useful models are connected with the topology of the quantum charge density. This method improves on the use of Mulliken population analysis.

2.7 Software Packages

There are many self-sufficient softwarepackages used by computational chemists. Some include many methods covering a wide range, while others concentrating on a very specific range or even a single method. Some common computer softwareused for computational chemistry includes: *Gaussian W03*(ab-initio, semi-empirical, molecular mechanics calculations) *Hyperchem 7.5* (*ab-initio*, semi-empirical, molecular mechanics & dynamics calculations).Spartan (*ab-initio*, semi-empirical, molecular mechanics and dynamics calculations).Data visualization is the process of displaying information in any sort of pictorial or graphical representation. A number of computer programs are now available to apply a colorization scheme to data or work with three dimensional representations (*Gaussview, MolDen,gOpenMol*). The programs used in some of the softwares

are:

aBiomolecularmodelling programs: proteins, nucleicacid.

bMolecularmechanics programs.

cQuantum chemistry and solid state physics software supporting several methods.

dMoleculardesignsoftware

eSemi-empirical programs.

fValence bond programs

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

The following are the materials used in carrying out the computational research work:

A. System Softwares

i. Microsoft windows XP professional version 2002 SP3 computer system, with Intel (R) Pentium(R) Dual CPU, E2200@2.20 GHz 219 GHz, 3.24GB of RAM.

ii. Microsoft windows 7 ultimate version 2008 SP1 computer system, with Intel (R) Pentium (R) Dual CPU, E2200@2.20 GHz 219 GHz, 4.00 GB of RAM.

iii. Microsoft windows 8 professional version 2012 SP1 computer system, AMD E-450 APU with Radeon (tm) HD Graphics 1.65 GHz, 4.00 GB of RAM.

B. Application Softwares

i. ChemBioOffice 2008 software.

ii. Spartan ’08 V1.1.0 program package.

iii. Spartan ’14 V1.1.0 program package.

iv. DreamCalc DCP 4.9.0 professional calculator program package .

v. Design Science MathType 6.9 program.

vi. GraphPad Prism 5.

C Reactants

Methionine, formula = $C_5H_{11}NO_2S$, molar mass = 149.21 g/mol.

Hydrogen trioxobromate acid, formula=$HBrO_3$, molar mass = 128.91g/mol.

3.2Methods

The Spartan ’08 v1.1.0 semi-empirical, PM3 and MNDO methods were used on Microsoft windows XP professional version 2002 SP3 computer system, with Intel (R) Pentium (R) Dual CPU,E2200@2.20 GHz 219 GHz, 3.24GB of RAM for the computational study of the oxidation of methionine by bromate.

The Spartan‘14v1.1.0 semi empirical (PM3 and MNDO) methods were used on Microsoft windows7 ultimate version 2008 SP1 computer system, computer system, with Intel (R) Pentium (R) Dual CPU,E2200@2.20 GHz 219 GHz, 3.24GB of RAM for the computational study of oxidation of methionine by bromate..

The Spartan‘14v1.1.0 semi empirical (PM3 and MNDO) and density functional theory (DFT) methods were used on Microsoft windows 8 professional version 2012 SP1 computer system, AMD E-450 APU with Radeon (tm) HD Graphics 1.65 GHz, 4.00 GB of RAM for the computational study of the oxidation of methionine by bromate.

The starting geometries for all the species were first optimized using PM3, method in the Spartan Global calculations environment work space of the various Spartan packages as listed above. The geometries calculations included in the reaction of all the species involved were fully optimized using the semi-empirical, PM3 method (Hehre*et al.*, 1986; Izadyara, 2004). To check the influence of the levels, PM3, MNDO of semi-empirical method were employed to optimize the geometries of the reactants, intermediates, transition states and products (Stewart, 1989; 2007; Zhang and Hase, 2010).There were marked differences between PM3 and MNDO results. It thus indicated that the geometrical parameters were sensitive to the sizes of the computational levels (Bingham, *et al., 1975*; Dewar and Thiel, 1977; Dewar, *et al.,* 1985). The starting geometries for

all the species were optimized in the Spartan Global calculations environment work space using PM3 method. The optimized geometries of the reactants, intermediates, transition states and products were confirmed in terms of vibrational analysis (Schlegel, 1986; Kalkanis and Shields, 1991; Kahn and Bruice, 2000). The transition state for each step was located and confirmed by animating the vibration corresponding to the reaction coordinate by selecting the imaginary frequency at the top of the list of frequencies on the IR tab. No arbitrary assumptions were imposed on finding the most likely geometries for the transition state in each case.

3.2.1 Details of the computational procedures

The computational method used for calculating geometries in this work is termed a "cascade mesthod" because of its use of molecular mechanics to remove strain energies followed by semi-empirical methods (Hehre, 1995). The method has been described elsewhere (Hehre, 1995; Hehre*et al.* 1998). The attractiveness of the method lies in its ability to make calculations less computationally taxing by relegating initial geometry calculations to less computationally intensive (and possibly more inaccurate) methods. The initial calculations, which may be initialized in geometry far from that of equilibrium, are performed by those methods requiring less computational effort, allowing equilibrium geometries to be "honed in on" in later stages, leaving the refining to the more accurate and computationally intensive theories

Details of how the computation tasks were carried out are given the subsequent sub-sections.

3.2.2 Geometry optimization of reactants, activated complexes, intermediates and products using semi-empirical methods

To optimize the structure of the bromate ion, first the Spartan software package was launched. On the Spartan user interface the file menu was selected. On the file menu "New build" was selected to launch the (organic, inorganic, peptide, nucleotide or substituent) tool kit for building

molecules, depending on the molecule to be constructed. Bromine with the appropriate geometry that constitute the bromate ion to be built was selected and clicked on, then oxygen with a free valence attached was selected and added to the free valences of bromine already selected and displayed on theSpartan window. The two other oxygen atoms are added in the same way. Two of the three single bonds formed between bromine and oxygen were converted to double bonds by clicking on the bonds once. Next, the minimization option was clicked to remove any strain in the molecule.

After the previous steps, the "set up" menu on the open window was selected and on the drop down menu "calculation" was clicked followed by "Equilibrium Geometry" at "Ground state". On the same interface," Semi-empirical" at the desired level of computation was selected (usually, current or MMFF). "Total charge" of the molecule together with the calculated "multiplicity" of the molecule was also selected. "IR'', ''Orbitals & Energies'', "Thermodynamic" "Vibrational Modes" and "Charges & bond Orders" tabs were selected. "Global Calculations" was also selected before submission. The file was then saved with the appropriate name.

The calculation was then started by clicking "Ok" on the calculation windows. After the calculation has completed, the "Display" menu was selected to see the output. On the output menu, properties, orbital energies, surfaces, spectra, Formula, spreadsheet, plots, similarities or reactions were selected, depending on the feature wanted.

The methionine molecule was constructed by clicking on the peptide menu, where methionine was selected from its dialogue box. Next, the minimization option was clicked to remove any strain in the molecule.

After the construction of the methionine molecule on Spartan window, the "set up" menu on the open window was selected and on the drop down menu "calculation" was clicked followed by "Equilibrium Geometry" at "Ground state". On the same interface," Semi-empirical" at the desired level of computation was selected (usually, current or MMFF). "Total charge" of the specie (bromate ion or methionine) together with the calculated "multiplicity" of the specie (bromate ion or methionine) was also selected. "IR'', ''Orbitals & Energies'', "Thermodynamic" "Vibrational Modes" and "Charges & bond Orders" tabs were selected. "Global Calculations" were selected before submission. The file was then saved with the appropriate name. The calculation was then started by clicking "Ok" on the calculation windows. After the calculation has completed, the "Display" menu was selected to see the output. On the output menu, properties, orbital energies, surfaces, spectra, Formula, spreadsheet, plots, similarities or reactions were selected, depending on the feature wanted.

After successful completion of optimization process or transition state search calculation, the "Display" menu was selected and on the Display" menu, "Molecule properties" was selected. On the "Molecule properties" menu, the following was displayed: Formula, CAS, Energy, Energy (aq), Solvation E, E HOMO, E LUMO, Heat, T1 Heat, Weight, Dipole Moment, Pt. Group, Tautomers and Conformers.

When “thermodynamics” was selected on the Molecule properties dialogue box, the calculated thermo chemical properties, i.e. H^o, S^o, G^o, ZPE and C_v are displayed. The values of these data were then converted to the desired units for further use in the discussion of results.

Same procedure was followed for the calculation based on MNDO level. The results are shown in Table 4.1 and 4.2 for PM3 and MNDO respectively.

3.3 Calculation of HOMO and LUMO of Optimized Species

After the optimization process, the HOMO and LUMO in each case of the bromate ion and the methionine molecule were calculated by selecting “Surfaces” on the display menu. On the surface menu, “Add” was clicked on, then HOMO, LUMO, were selected. The HOMO or the LUMO was displayed whenever one of them was selected depending on the specie under consideration. The results of the HUMO and LUMO calculations are presented table 4.3.

3.3.1 Determination of chemical reactivity of optimized species: mapping of HOMO and LUMO densities of optimized molecules.

Optimized structures of the species that would collide to form the transition states or activated complex were copied and pasted on a new Spartan window. The two would be oriented such that the HOMO of one would be facing the LUMO of the other with the right orientations. Next, on the “search” menu “Transition State” was selected to activate the colliding molecules. To form new bond, the “Shift” key is held down, followed by a click on the atom bearing the HOMO, then the atom bearing the LUMO and back. To break a bond, the bond to be broken is clicked on followed by clicking on the centre that would accept the electrons. These operations would result in a curved arrow being drawn on the reactant structure, extending from an atom, or a centre of a bond, or the centre of a dotted line that has been drawn between atoms that were to be broken or

bonded. After all reaction arrows have been properly designated, the button at the bottom right of the screen would be clicked to replace the reactants with a guess of the transition state structure. Next, from the "set up" menu, "calculation" was then selected, followed by "Transition State Geometry" and then either "Semi-empirical" or "Density Functional " as the case may be, followed by the chosen level or basis set of interest. The file was saved with a chosen name. Before submission, all the parameters to be calculated ("IR'', ''Orbitals & Energies'', "Thermodynamic" "Vibrational Modes" and "Charges & bond Orders" tabs) were selected. "Global Calculations" would be selected as done in the optimization process discussed above (section 3.1 for semi-empirical calculations).

When the job has completed, "Spectra" was selected from the display menu and the IR tab clicked to display the IR spectrum of the activated complex. Presence of only one imaginary frequency confirmed that the transition state was found. In cases were there is no one imaginary frequency the whole processes were repeated until the transition state was found.

3.4 Construction of Potential Energy Surface Diagrams

To construct the potential energy surface diagrams for a system, H^o for reactants, transition state and products for each proposed elementary step were tabulated and exported into Micro Soft Excel sheet or the GraphPad Prism 5 Excel sheet for plotting the diagrams. This was achieved by copying the appropriate values for H^o and pasting them into columns for reactants (R), transition states (TS) and products (P). The values reactants (R) and products (p) are usually, the sum of the reactant molecules and the sum of the product molecules, respectively, for each elementary

step. The values in the three columns were then used to plot the potential energy surface diagrams. The diagrams are presented in figure 4.5 and 4.6 for the PM3 and MNDO respectively.

3.5 Heat of Reaction, Entropy Change, and Gibb's Free Energy Change.

The enthalpies of reaction were calculated by using the heats of formation at standard temperature of 298.15K and pressure of 1 atmosphere obtained from Spartan software package as described in section 3.1. The calculations were done by taking the appropriate sums and differences as given in equation (3.1).

$$\Delta_r H^o \ \ 298.15K \ = \sum_{products} n_{prod} \Delta_f H^o_{prod} \ \ 298.15K \ - \sum_{reac\,tants} n_{react} \Delta_f H^o_{react} \ \ 298.15K$$

……. (3.1)

(Ochterski, 2000;McQuaid and Rice, 2006; McQuaid*et al*., 2004; 2002)

Where n_{prod} and n_{react} are the stoichiometric coefficient of the products and reactants, respectively, and $\Delta_r H^o(298.15K)$, $\Delta_f H^o_{prod}(298.15K)$ and $\Delta_f H^o_{react}(298.15K)$ are the standard heat reaction, standard heat formation of products and standard heat formation of reactants, respectively, at the specified standard temperature of 298.15K. The result is shown in table 4.3

In instances where there were no convergences or completion of calculation during optimization, some of the thermodynamic parameters such as ΔG^oandΔS^o would not be calculated by default by the Spartan software package. In such instances therefore, these parameters can be calculated by using the Eyring equation (Engel and Reid, 2006; Mee, 1971) as given in equation (3.2).

$$\ln K = -\frac{\Delta G^o}{RT} = \frac{\Delta S^o}{R} - \frac{\Delta H^o}{RT}$$

……. (3.2)

Alternatively, the entropy of reaction and free energy of reaction can be evaluated from equations (3.3) and (3.40, respectively (McLauchlan, 2004; Sousa *et al*.2013).

$$\Delta_r S^o(298.15K) = \sum_{prod} n_{prod} S^o_{prod}(298.15K) - \sum_{react} n_{react} S^o_{react}(298.15K) \text{ .... (3.3)}$$

$$\Delta_r G^o = \Delta_r H^o - T\Delta_r S^o \quad \text{..... (3.4)}$$

Activation energies (E_a) of the various elementary steps were calculated according to equation (3.5) (McQuaid and Rice, 2006; McQuaid*et al*., 2004; 2002).

$$E_a(298.15K) = \sum_{trans} n_{trans} \Delta_f H^o_{trans}(298.15K) - \sum_{react} n_{trans} \Delta_f H^o_{react}(298.15K)$$

…….. (3.5)

Where $n_{trans} = 1$ and n_{react} are the stoichiometric coefficient of the transition state and reactants, respectively, while $\Delta_f H^o_{trans}(298.15K)$ and $\Delta_f H^o_{react}(298.15K)$ are standard heat formation oftransition state and standard heat formation of reactants, respectively, at the specified standard temperature of 298.15K.

For DFT calculations $\Delta_f H^o_{trans}(298.15K)$ and $\Delta_f H^o_{react}(298.15K)$ are usually written as $E_e(trans)$ and $E_e(react)$,(McQuaid and Rice, 2006; McQuaid et al. 2004; 2002), which are total electronic energies of formation of transition state and reactants, respectively. In this case, equation (3.5) can be written as equation (3.6) below.

$$E_a(298.15K) = \sum_{trans} n_{trans} E_{e\ (trans)}(28.15K) - \sum_{react} n_{trans} E_{e\ (react)}(298.15K) \ldots$$

(3.6)

The results are shown on table 4.5 and 4.6 for PM3 and MNDO respectively.

3.6Calculation of Activation Parameters, Equilibrium and Rate Constants.

The activation parameters were also calculated by the difference between the energy of the transition state and the reactant as follows:

$\Delta^{\#}H = H_{TS} - H_R$ …….. (3.7)

$\Delta^{\#}S = S_{TS} - S_R$ ……. (3.8)

$\Delta^{\#}G = G_{TS} - G_R$ …… (3.9)

Where H_{TS} is the energy of the transition state , and H_R sum of the enthalpy of the reactant. And other parameters are calculated in the same way.

The equilibrium constants were calculated using equation 3.91:

$$LogK = \frac{\Delta G^o}{-2.303RT} \quad \text{…….. (3.91)}$$

Where K= equilibrium constant; ΔG^O=Gibbs free energy change; R= ideal gas constant (8.31441J/mol.K); T= temperature (298.15K).

The rate constant calculations were computed according to equation (3.92) (McLauchlan, 2004Ochterski, 2000; McQuaid and Rice, 2006).

$$k\ T = \frac{k_B T}{hc^o} e^{-\Delta \ddagger G^o/RT} \quad \textbf{…….. (3.92)}$$

Where k(T) = reaction rate constant at temperature (298.15K); k_B= Boltzmann constant (1.380662 X 10^{-23} J/K); T = temperature (298.15K); h = Planck's constant (6.626176 X 10^{-34}Js); C^o = concentration (taken to be 1); $\Delta \ddagger G^o$ = Gibbs free energy of activation (kJ/mol); R = gas constant (8.31441 J/mol. K).

The activation parameters, equilibrium constant, and rate constants are shown in the table in table 4.5 and 4.6 for the PM3 and MNDO respectively:

3.7 Post Computation Processing

After the computation of all reactants, intermediates, transition states and products of every system, all structures were sketched using ChemBioOffice 2008 software. All equations used in this work were created with Design Science MathType 6.9 program while all calculations of the heat of formations, heat of reactions, thermodynamic parameters, activation parameters and rate constants were calculated using DreamCalc DCP 4.9.0 professional calculator program package

CHAPTER FOUR

4.0 RESULTS

4.1 The Optimized Structures:

The 3D geometries of all the optimized structures, reactants, transition states, and products are shown in the figure 4.1 , where BrO_3^-= bromate anion, TS1 = $H^+…BrO_3^-$,hydrogen bromate transition state, $HBrO_3$ = hydrogen bromate, $RSCH_3$ = methionine,TS2 = $BrO_2..HO..SRCH_3$, hydrogen bromate /methionine transition state, RCH_3S^+OH = hydroxy methionine cation, BrO_2^- = bromite anion, TS3 = $BrO_2^-…H^+$, hydrogen bromite transition state, $HBrO_2$ = hydrogen bromite, TS4 = $RCH3S^+…OH…HBrO_2$, hydrogen bromite/hydroxymethionine transition state, RCH_3S^+-O-H_2BrO_2 = hydrogen bromite/ hydroxymethionine intermediate, TS5 =$RCH_3SO..H..HBrO_2$, transition state from the previous intermediate which disproportionate to form methionine sulphoxide, RCH_3SO = methionine sulphoxide, TS6 = $BrO_2..H-O..H..BrO_2$, a transition state from the reaction of hydrogen bromite and hydrogen bromate, Br_2O_4 = bromine dioxide dimer, H_2O = water, TS7 = O-O..Br-Br…O-O, transition state of the decomposition of the dimer, Br_2O_4 to give Br_2, bromine and O_2, oxygen. While H^+ = hydrogen ion.

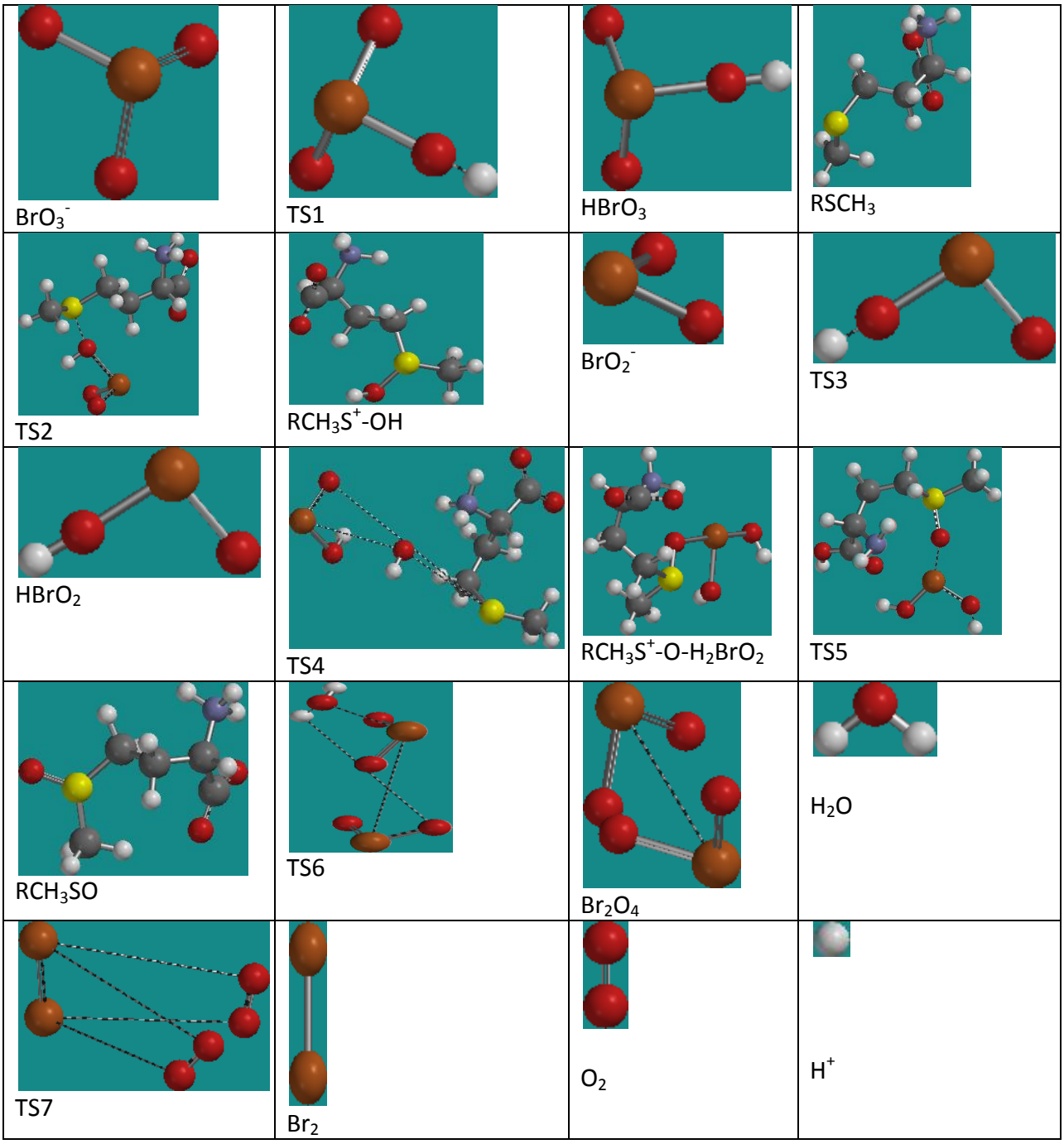
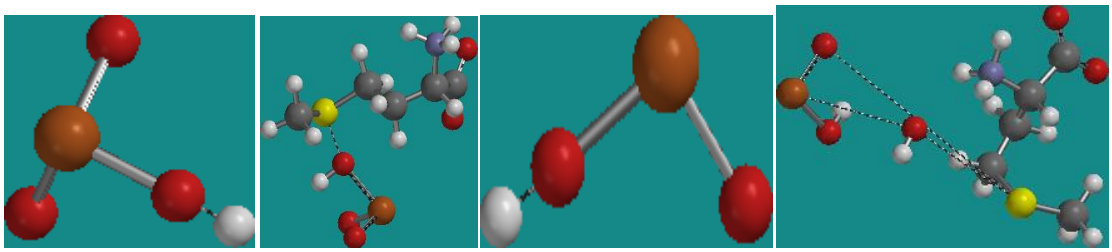
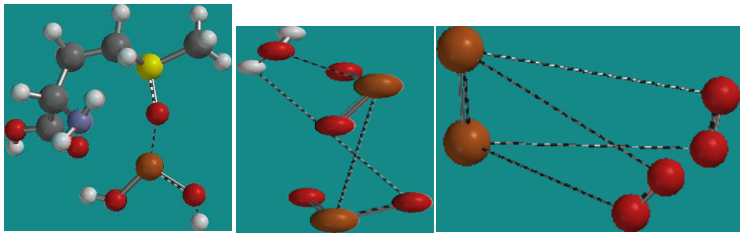


Figure 4.1:The 3D structures of the reactants transition states and the products.



TS1 TS2 TS3 TS4



TS5 TS6 TS7

Figure 4.2: The transition states in the proposed reaction mechanism

4.2 Results of the Calculation of Heat of Formation and the Thermodynamic Parameters of the Species.

Table4.2a: The heat of formation and the thermodynamic parameters of the species based on PM3 level of computation.

Species	Energy (kJ/mol)	H^o (kJ/mol)	S^o (J/mol K)	G^o (kJ/mol)
BrO_3-	-165.67	-127.81	296.57	-216.23
TS1(H^+…BrO_3^-)	540.94	608.42	297.19	519.82
$HBrO_{3\ (P1)}$	45.47	111.45	296.69	22.99
RSCH3	-413.89	46.78	448.16	-86.84
TS2 (BrO_2..HO..$SRCH_3$)	1481.13	2004.7	567.96	1835.37
RCH_3S^+-OH	117.93	618.65	480.92	475.27
BrO_2^-	-49.89	-26.21	273.17	-107.65
TS3 (BrO_2^-…H^+)	134.77	183.77	276.24	101.41
$HBrO_2$	-2.54	54.84	282.37	-29.35
TS4 ($RCH3S^+$…OH…$HBrO_2$)	1047.34	1606.47	575.86	1434.77
RCH_3S-O-H_2BrO_2	-134.08	433.56	607.74	252.37
RCH_3SO	-532.26	-60.85	462.92	-198.87
TS5(RCH_3SO..H..$HBrO_2$)	1063.44	1631.24	562.62	1463.5
H_2O	-223.54	-156.22	188.24	-212.34
BrO_2..BrO_2	254.33	310.65	366.14	201.48
TS7 (O-O..Br-Br…O-O)	282.35	335.37	315.24	241.39
Br_2	20.61	33.52	245.88	-39.79
O_2	76.92	99.37	195.27	41.15

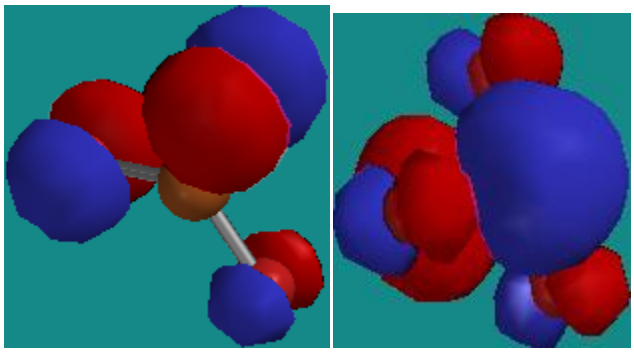
Species	**Energy (kJ/mol)**	**H^o (kJ/mol)**	**S^o (J/mol K)**	**G^o (kJ/mol)**
R1 ($BrO_3^- + H^+$)	-165.67	-127.81	296.57	-216.23
P1 ($HBrO_3$)	45.47	111.45	296.69	22.99
R2 ($HBrO_3 + RSCH_3$)	-368.42	158.23	744.85	-63.85
P2($HOS^+RCH_3+BrO_2^-$)	68.04	592.44	754.09	367.62
R3 ($BrO_2^- + H^+$)	-49.89	-26.21	273.17	-107.65
P3 ($HBrO_2$)	-2.54	54.84	282.37	-29.35
R4 ($HOS^+RCH_3+HBrO_2$)	115.39	673.49	763.29	445.92
P4(RCH_3S-O-H_2BrO_2)	-134.08	433.56	607.74	252.37
R5(RCH_3S-O-H_2BrO_2)	-134.08	433.56	607.74	252.37
P5 ($HBrO_2 + RCH_3SO + H^+$)	-534.8	-6.01	745.29	-228.22
R6 ($HBrO_3 + HBrO_2$)	42.93	166.29	579.06	-6.36
P6 ($Br_2O_4+H_2O$)	30.79	154.43	554.38	-10.86
R7 (Br_2O_4)	254.33	310.65	366.14	201.48
P7 ($Br_2 + 2O_2$)	97.53	132.89	441.15	80.94

Table 4.2b:The heat of formation and the thermodynamic parameters of the species based on MNDO level of computation.

Species	Energy (kJ/mol)	H^o (kJ/mol)	S^o (J/mol K)	G^o (kJ/mol)
BrO_3-	348.34	372.32	292.72	285.04
TS1(H^+…BrO_3^-)	321.51	388.09	291.77	301.09
$HBrO_{3\ (P1)}$	306.99	370.31	291.3	283.46
RSCH3	-387.65	104.18	443.2	-27.96
TS2 (BrO_2..HO..$SRCH_3$)	1527.27	2085.84	519.62	1930.91
RCH_3S^+-OH	155.74	691.07	452.58	556.14
BrO_2^-	-44.39	-19.69	265.56	-98.87
TS3 (BrO_2^-…H^+)	140.69	189.84	272.49	108.6
$HBrO_2$	26.86	85.09	286.67	-0.38
TS4 ($RCH3S^+$…OH…$HBrO_2$)	983.14	1580.05	639.91	1389.26
RCH_3S-O-H_2BrO_2	60.31	662.6	597.18	484.55
RCH_3SO	-494.91	10.78	457.8	-125.71
TS5 (RCH_3S-O..H..$HBrO_2$)	1488.02	2051.98	745.79	1829.62
TS6 (BrO_2..H-O..H..BrO_2)	413.29	530.2	394.13	412.69
H_2O	-254.97	-184.69	188.06	-240.76
BrO_2..BrO_2	321.47	380.28	379.91	267.01
TS7 (O-O..Br-Br…O-O)	418.88	476.09	346.55	372.77
Br_2	27.91	41.05	243.42	-31.52
O2	50.83	75.54	194.77	17.47

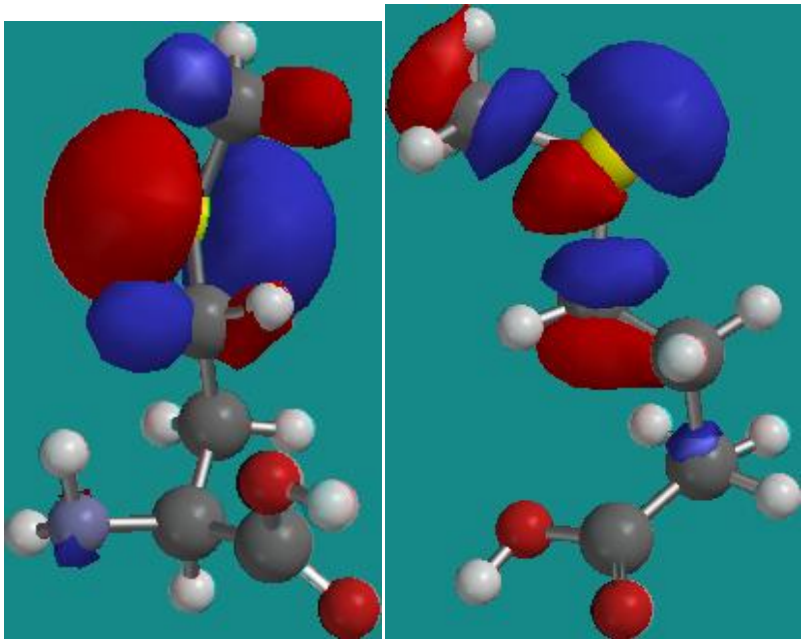
Species	Energy (kJ/mol)	H^o (kJ/mol)	S^o (J/mol K)	G^o (kJ/mol)
R1 ($BrO_3^- + H^+$)	348.34	372.32	292.72	285.04
P1 ($HBrO_3$)	306.99	370.31	291.3	283.46
R2 ($HBrO_3 + RSCH_3$)	-80.66	474.49	734.5	255.5
P2 ($HOS^+RCH_3 + BrO_2^-$)	111.35	671.38	718.14	457.27
R3 ($BrO_2^- + H^+$)	-44.39	-19.69	265.56	-98.87
P3 ($HBrO_2$)	26.86	85.09	286.67	-0.38
R4 (HOS^+RCH_3+$HBrO_2$)	182.6	776.16	739.25	555.76
P4 (RCH_3S-O-H_2BrO_2)	60.31	662.6	597.18	484.55
R5 (RCH_3S-O-H_2BrO_2)	60.31	662.6	597.18	484.55
P5 ($HBrO_2 + RCH_3SO + H^+$)	-468.05	95.87	744.47	-126.09
R6 ($HBrO_3 + HBrO_2$)	333.85	455.4	577.97	283.08
P6 (Br_2O_4+H_2O)	66.5	195.59	567.97	26.25
R7 (Br_2O_4)	321.47	380.28	379.91	267.01
P7 ($Br_2 + 2O_2$)	78.74	116.59	438.19	-14.05

4.3 Results ofCalculation of HOMO and LUMO



HOMOofBrO$_3^-$ anion LUMO of BrO$_3^-$ anion

E HOMO (kJ/mol) = -641.79E LUMO (kJ/mol) =346.78



HOMO of methionine LUMO of methionine

E HOMO (kJ/mol) = -881.07E LUMO (kJ/mol) = 7.47

Figure 4.3: HOMO and LUMO of the reactants.

Table 4.3: HOMO and LUMO Energies of the Reactants.

Specie	HOMO energy(kJ/mol)	LUMO energy(kJ/mol)
Methionine	-881.07	7.47
Bromate anion	-641.79	346.78

4.4:Graphs of Heat of Formation Versus Progress of Reaction Based on PM3.

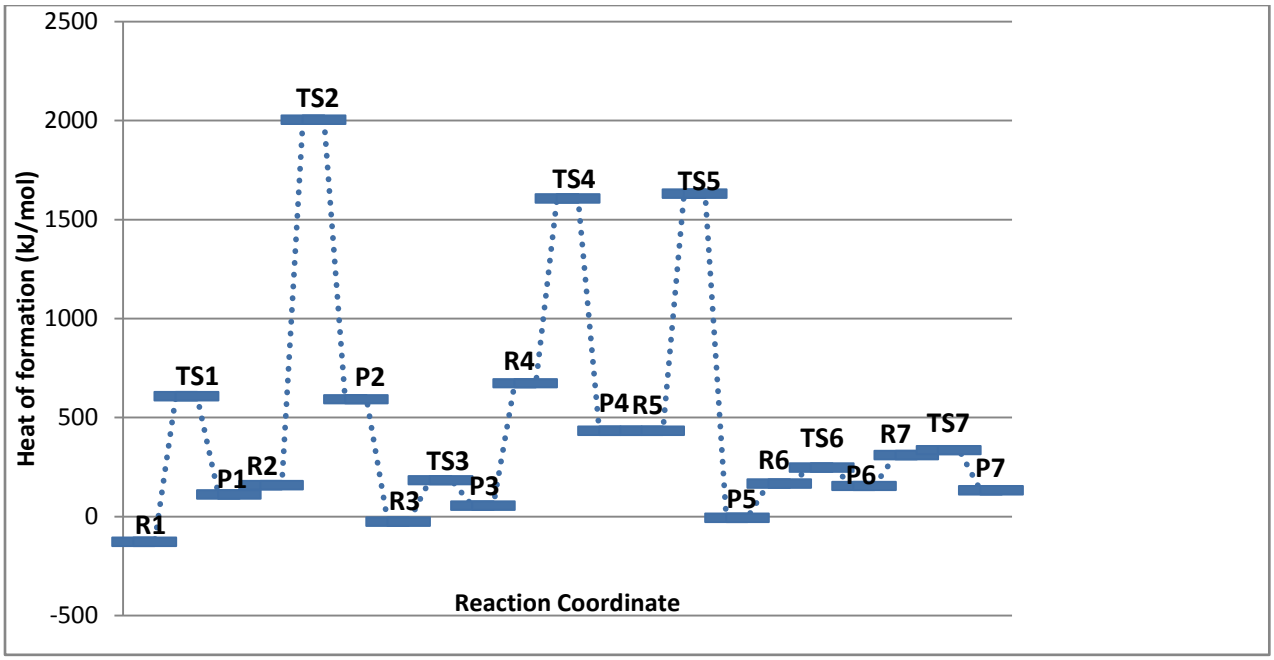


Figure 4.4 : Graph of heat of formation of the species versus progress of reaction based on PM3 method.

Keys: R1= BrO_3^- + H^+, TS1= H^+…BrO_3^-, P1= $HBrO_3$, R2= $HBrO_3$ + $RSCH_3$, TS2= BrO_2..HO..$SRCH_3$,P2= HOS^+RCH_3 + BrO_2, R3= BrO_2^- + H^+, TS3= BrO_2^-…H^+, P3= $HBrO_2$, R4= HOS^+RCH_3+$HBrO_2$, TS4= RCH_3S^+…OH…$HBrO_2$, P4= RCH_3S-O-H_2BrO_2, R5= RCH_3S-O-H_2BrO_2, TS5= RCH_3S-O..H..$HBrO_2$, P5= $HBrO_2$ + RCH_3SO + H^+, R6= $HBrO_3$ + $HBrO_2$, TS6= BrO_2..H-O..H..BrO_2, P6= Br_2O_4 +H_2O, R7= Br_2O_4, TS7=O-O..Br-Br…O-O, and P7= Br_2 + $2O_2$

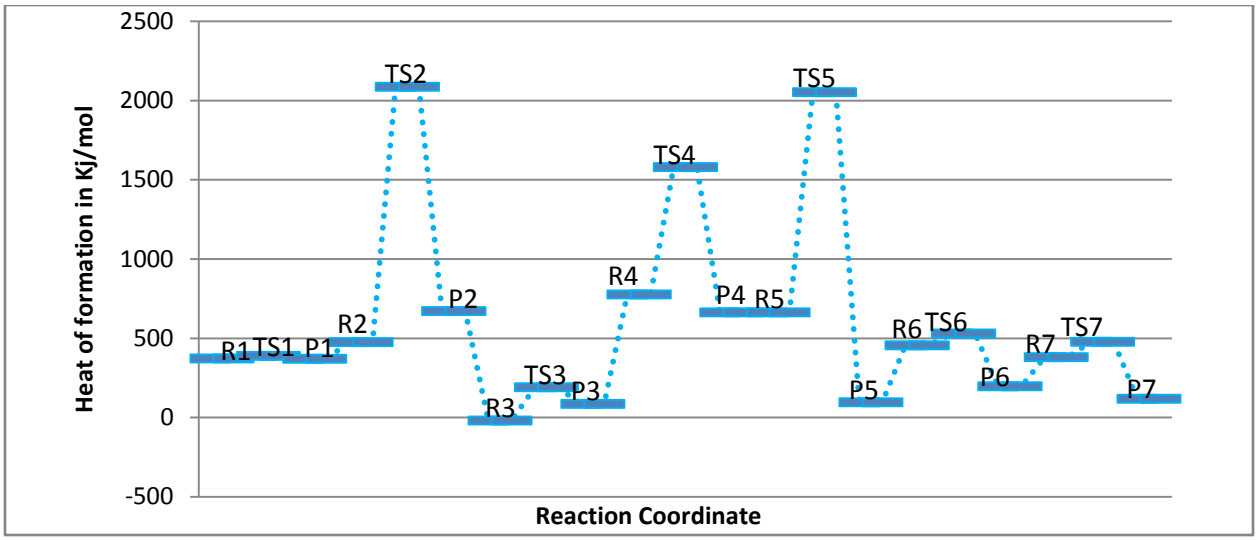


Figure 4.5: Graph of heat of formation of the species versus progress of reaction based on MNDO level.

Keys: R1= BrO_3^- + H^+, TS1= H^+…BrO_3^-, P1= $HBrO_3$, R2= $HBrO_3$ + $RSCH_3$, TS2= BrO_2..HO..$SRCH_3$,P2= HOS^+RCH_3 + BrO_2, R3= BrO_2^- + H^+, TS3= BrO_2^-…H^+, P3= $HBrO_2$, R4= HOS^+RCH_3+$HBrO_2$, TS4= RCH_3S^+…OH…$HBrO_2$, P4= RCH_3S-O-H_2BrO_2, R5= RCH_3S-O-H_2BrO_2, TS5= RCH_3S-O..H..$HBrO_2$, P5= $HBrO_2$ + RCH_3SO + H^+, R6= $HBrO_3$ + $HBrO_2$, TS6= BrO_2..H-O..H..BrO_2, P6= Br_2O_4 +H_2O, R7= Br_2O_4, TS7=O-O..Br-Br…O-O, and P7= Br_2 + $2O_2$

4.5 Results of the Calculation of Heat of Reaction, Enthalpy, Entropy, and Gibb's Free Energy Change.

Table 4.5a: Heat of reactions at different level of computation (from overall equation of reaction.

Method/level	**PM3**	**MNDO**
Heat of reaction at 0^0C in (kJ/mol)	-78.7	-631.83
Heat of reaction at 25^0C in (kJ/mol)	-3.5	-533.82

Table4.5b: The Heat of reaction, enthalpy, entropy, and Gibb's free energy change base on PM3 computation.

Reaction step	ΔE (kJ/mol) 0^oC	ΔH^o (kJ/mol) 25^oC	ΔS^o (J/mol)	ΔG^o (kJ/mol)
R1→P1	211.14	239.26	0.12	239.2
R2→P2	436.46	434.21	9.24	431.5
R3→P3	47.35	81.05	9.2	78.3
R4→P4	-249.5	-239.93	-156	-194
R5→P5	-400.7	-439.57	137.6	-481
R6→P6	-12.14	-11.86	-24.7	-4.5
R7→P7	-156.8	-177.76	75.01	-200

Table 4.5c :Heat of reaction, enthalpy, entropy, and Gibb's free energy change based on MNDO level of computation.

Reaction step	ΔE (kJ/mol) 0^oC	ΔH^o (kJ/mol) 25^oC	ΔS^o (J/mol)	ΔG^o (kJ/mol)
R1→P1	-41.35	-2.01	-1.42	-1.58
R2→P2	192.01	196.9	-16.4	201.8
R3→P3	71.25	104.8	21.11	98.49
R4→P4	-122.29	-116	-142	-71.2
R5→P5	-528.36	-567	-147.3	-611
R6→P6	-267.35	-260	-10	-257
R7→P7	-242.73	-264	58.28	-281

4.6 Result of Calculation of Activation Parameters, Equilibrium and Rate Constants.

Table4.6a: The activation parameters equilibrium and rate constant for various steps based on PM3 level.

Reaction steps	**$\Delta^{\#}E$ kJ/mol 0^oC**	**$\Delta^{\#}H$ kJ/mol 25^oC**	**$\Delta^{\#}S$ J/mol**	**$\Delta^{\#}G$ kJ/mol**	**Equilibrium constants**	**Rate constants s^{-1}**
R1→P1	706.6	736.2	0.62	736.05	0.908	$6.95x\ 10^{-117}$
R2→P2	1850	1846	-177	1899.22	0.84	$1.16x10^{-320}$
R3→P3	184.7	210	3.07	209.06	0.969	$1.47x10^{-24}$
R4→P4	932	933	-187	988.85	1.081	$3.57x10^{-161}$
R5→P5	1198	1198	-45.1	1211.13	1.214	$4.08x10^{-200}$
R6→P6	88.37	81.31	-178.9	134.65	1.002	$1.59x10^{-11}$
R7→P7	28.02	24.72	-50.9	39.91	1.084	$6.33x10^{5}$

Table 4.6b: The activation parameters equilibrium and rate constant for various steps based on MNDO level.

Reaction steps	$\Delta^{\#}E$ kJ/mol 0^oC	$\Delta^{\#}H$ kJ/mol 25^oC	$\Delta^{\#}S$ J/mol	$\Delta^{\#}G$ kJ/mol	Equilibrium constants	Rate constants s^{-1}
R1→P1	-26.83	15.77	-0.95	16.05	1.001	$9.58x10^{9}$
R2→P2	1607.93	1611.35	-214.88	1675.41	0.922	$1.9x10^{-281}$
R3→P3	185.08	209.53	6.93	207.47	0.961	$2.79x10^{-24}$
R4→P4	800.54	803.89	-99.34	833.5	1.029	$5.9x10^{-134}$
R5→P5	1427.71	1389.38	148.61	1345.07	1.28	$1.4x10^{-223}$
R6→P6	79.44	74.8	-183.84	129.61	1.109	$1.22x10^{-10}$
R7→P7	97.41	359.5	-91.64	386.82	1.12	$3.8x10^{-31}$

CHAPTER FIVE

5.0 DISCUSSION

5.1 Optimized Geometry of Reactants, Activated Complexes, Intermediates and Products.

From the results of calculation of heat of formation, and other thermodynamic parameters ie entropy, Gibb's free energy of the species based on PM3 level of computation, Table 4.2a,it is evident that all the transition states from TS1 to TS7 correspond to saddle points of their respective steps in the mechanism. The second transition state, TS2 (BrO_2..HO..$SRCH_3$) has the highest energy,E, enthalpy, H^o, and Gibb's free energy, G^o, therefore is the determining factor for rate of reaction. Similarly, results of the calculation based on the MNDO level, Table 4.2b, display all the transition states as saddle points of their respective steps, and TS2 has the highest E, H^o, and G^o. But these values are higher than the corresponding values of PM3 computation.This suggest that PM3 is the better method for the study of the system.

5.2 Calculated HOMO and LUMO of the Reacting Species

The result of HOMO and LUMO calculation presented in Table 4.3 show that, the HOMO of the bromate anion is spread over the three oxygen atoms, while the LUMO is spread over all the atoms of the ion, but more concentrated on the bromine atom. The HOMO ofthe methionineis spread over sulphur, the two carbon atoms adjacent to the sulphur and nitrogen atom. While the LUMO is located on the sulphur atom, the two carbon atoms adjacent to the sulphur, and the chiral carbon.

The energy of the HOMO of bromate anion is higher than that of the methionine, and the energy of the LUMO of methionine is lower than the energy of the bromate anion, which facilitate movement of electrons from the oxygen of the bromate to the sulphur of the methionine.

5.3 Constructed Graphs of Heat of Formation Versus Progress of Reaction

The construction of Graphs of heat of form versus progress of reaction Figure 4.4 for the PM3 and Figure 4.5 for MNDO, shows that TS2 has the highest peak or saddle point which confirm that step two is the rate determining step.

5.4 Calculated Heat of Reaction, Enthalpy, Entropy, and Gibb's Free Energy Change.

Based on the calculation result in Table 4.5a, the overall heat of reaction(calculated from overall equation) obtained using PM3is -78.71kJ/mol and,-3.5kJ/mol at 0^oC and 25^oC, respectively. While the heat of reaction based on MNDO level of computation is -631.83kJ/mol and -533.82kJ/mol at 0^oC and 25^oC respectively.

Table 4.5b and 4.5c shows that, in the beginning of the reaction, the bromate anion became uniprotonated and the energies associated with this step are ΔE = 211.1kJ/mol and ΔG^o = 239.2 kJ/mol at 298.15K for the PM3 computation. While the MNDO computation yields ΔE =-41.35kJ/mol and ΔG^o = -1.58kJ/mol. The energy of the first step of the proposed mechanism is lower than that reported in the published mechanism. Step 2 involves the reaction between the $HBrO_3$ and methionine which result in the formation of intermediates RCH3S-OH and BrO2-. The Gibbs free energy change and energy change for this reaction are 431.5 and 436.5 kJ/mol respectively. According to (Anthony and Bell,2007), the rate limiting step is the one that has the highest ΔE and ΔG^o, therefore step 2 which involves reaction between HBrO3 and methionine is

the rate limiting step based on the two level of computation used.This equation has the highest ΔE and ΔG^o therefore is the rate limiting step (Anthony and Bell, 2007) as shown in Table 4.5b and 4.5c.Bromous acid is formed in step3 from the reaction of bromite with hydrogen ion.The ΔE and ΔG^o are 47.35 and 78.3kJ/mol, respectively. Thus the first three steps are endothermic while the last four steps are exothermic Table 4.5b and 4.5c.Step 5 being highly exothermic based on the PM3 computation.

Kinetically, step2 equation (5) has the lowest rate constant, therefore in close agreement with the rate determining step calculated thermodynamically. The step also has the lowest equilibrium constant of 0.84. This conforms to most of the experimentally determined rate determining steps, where reaction of methionine with other species used to be the slowest step.

5.5Rate Law

The rate equation was derived as follows:

$$BrO_3^- + H^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} HBrO_3 \quad \text{.....(5.21)}$$

$$HBrO_3 + RSCH_3 \xrightarrow[slow]{k_2} HOS^+RSCH_3 + BrO_2^- \quad \text{.....(5.22)}$$

$$BrO_2^- + H^+ \xrightarrow{k_3} HBrO_2 \quad \text{.....(5.23)}$$

$$HOS^+RCH_3 + HBrO_2 \xrightarrow{k_4} RCH_3S-O-H_2BrO_2 \quad \text{.....(5.24)}$$

$$RSCH_3S-O-H_2BrO_2 \xrightarrow{k_5} HBrO_2 + RCH_3SO + H^+ \quad \text{.....(5.25)}$$

$$HBrO_3 + HBrO_2 \xrightarrow{k_6} Br_2O_4 + H_2O \quad \text{.....(5.26)}$$

$$Br_2O_4 \xrightarrow{k_7} Br_2 + 2O_2$$ ……(5.27)

Rate = $\frac{-d\ RSCH_3}{dt} = k_2\ HBrO_3\ \ RSCH_3$ … ……(5.28)

But , $\frac{d[HBrO_3]}{dt} = k_1[BrO_3^-][H^+]$…… ……...(5.29)

Therefore, $[HBrO_3] = k_1[BrO_3^-][H^+]$……(5.30)

Substituting eqn (5.30) into equation (5.28):

Therefore, Rate = $k_2 k[RSCH_3][BrO_3^-][H^+]$

Based on the rate law above, the reaction is first order in both [BrO_3^-] and $[RSCH_3]$, which agrees with what Idris *et al*., 2010 reported, but first order in [H^+] instead of second order.

From the table of activation parameters Table 4.6 and rate constants k_1 andk_2 are 6.95×10^{-117} and 1.16×10^{-320}, and respectively, for PM3 ,and 9.58×10^{9} and 1.9×10^{-281} and respectively for MNDO computation. These values were used to calculate the k the rate constant for the reaction, these are $8.062\times10^{-437}s^{-1}$ and 18.202×10^{-272} s^{-1} for PM3 and MNDO respectively. Qualitatively this shows that PM3 yield a better result than the MNDO for thestudy.

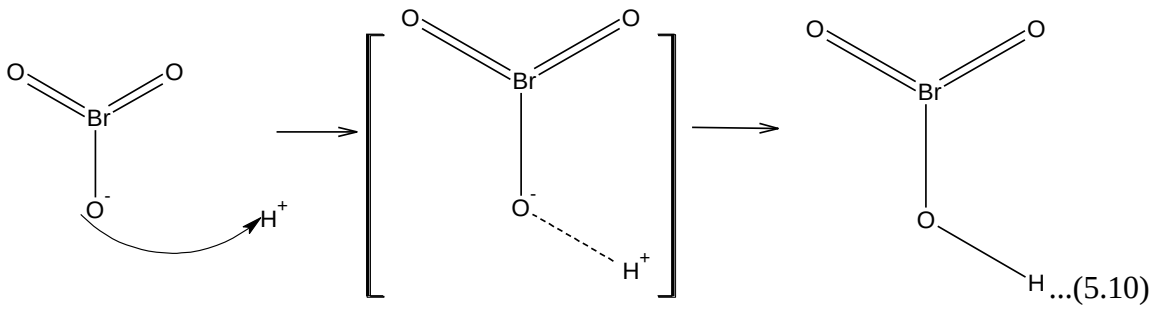
All the results discussed aboveconfirm that our proposed mechanism, stated below is more plausible than that reported by Idris*et al*., 2010 i.e published mechanism stated on page 82.

5.6Proposed Mechanism of the Reaction.

The proposed mechanism of the reaction computed was divided into steps as follows:

Scheme 1

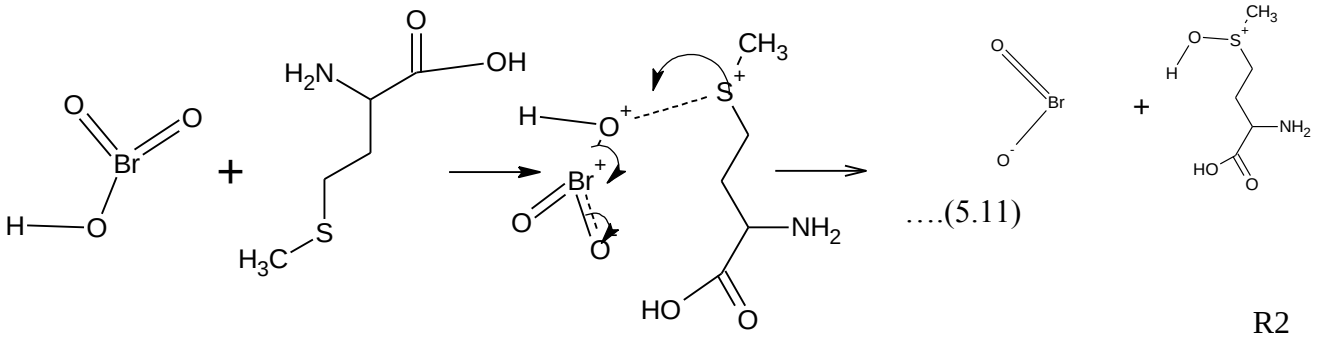
Step 1 of proposed mechanism:This involved protonation of the bromate ion i.e.



...(5.10)

R1 TS1 P1

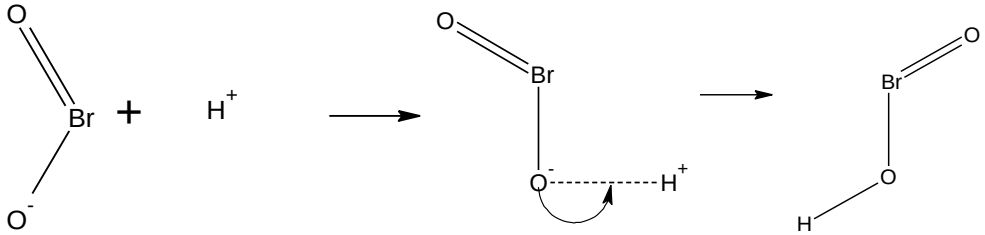
Step 2 of proposed mechanism: In this step, $HBrO_3$ react with the methionine to produce hydroxymethionine and bromite ion via a transition state TS2.



….(5.11)

TS2 P2 R2

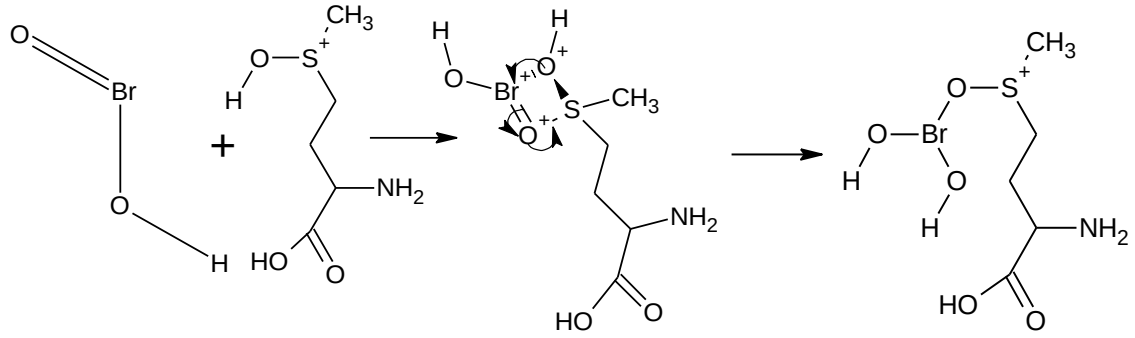
Step 3 of proposed mechanism: This step involves protonation of the bromite ion to $HBrO_2$.



……(5.12)

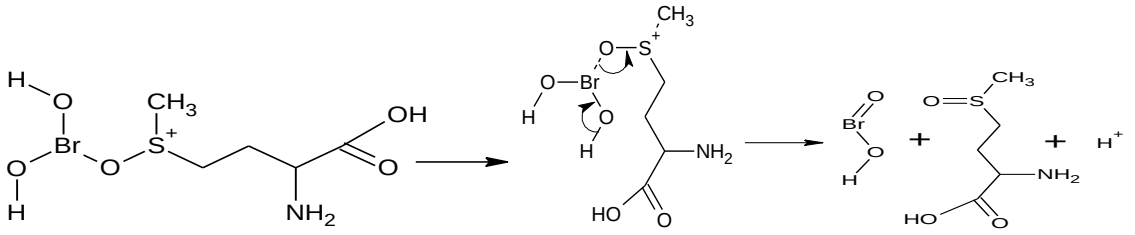
R3 TS3 P3

*Step 4 of proposed mechanism:*Reaction between $RSCH_3OH$ and $HBrO_2$ to give and intermediate $RCH_3S\text{-}O\text{-}H_2BrO_2$.



R4 TS4 P4 ..(5.13)

Step 5 of proposed mechanism: Decomposition of the intermediate, $RCH_3S\text{-}O\text{-}H_2BrO_2$(P4)to give methionine sulphoxide and $HBrO_2$.



R5 TS5 P5 …….(5.14)

Step 6 of proposed mechanism: Reaction between $HBrO_3$ generated in step 4 and $HBrO_2$ generated in step 8.

… (5.15)

Step 7 of proposed mechanism: Formation of the dimer, Br_2O_4, which disproportionate to give the final products, Br_2 and O_2

(5.16)The overall chemical equation for the reaction is therefore as given in equation 5.17,

$$2BrO_3^- + 2H^+ + RSCH_3 \longrightarrow RCH_3SO + Br_2 + 2O_2 + H_2O \text{……... (5.17)}$$

Where R stands for $HOOCCH(NH_2)CH_2CH_2$.

The proposed mechanism can be summarized as follows:

$$\underset{R1}{BrO_3^- + H^+} \xrightarrow{} \underset{TS1}{H^+\ldots BrO_3^-} \xrightarrow{} \underset{P1}{HBrO_3} \text{.....(5.10)}$$

$$\underset{R2}{HBrO_3 + RSCH_3} \xrightarrow{} \underset{TS2}{BrO_2..HO..SRCH_3} \xrightarrow{} \underset{P2}{HOS^+RCH_3 + BrO_2} \text{(5.11)}$$

$$\underset{R3}{BrO_2^- + H^+} \longrightarrow \underset{TS3}{BrO_2^-\ldots H^+} \longrightarrow \underset{P3}{HBrO_2} \text{ …….(5.12)}$$

$$HOS^+RCH_3 + HBrO_2 \rightarrow RCH_3S^+\ldots OH\ldots HBrO_2 \rightarrow RCH_3S\text{-}O\text{-}H_2BrO_2 \text{ …(5.13)}$$

$$\underset{R5}{RCH_3S\text{-}O\text{-}H_2BrO_2} \rightarrow \underset{TS5}{RCH_3S\text{-}O..H..HBrO_2} \rightarrow \underset{P5}{HBrO_2 + RCH_3SO + H^+} \text{ ……. (5.14)}$$

$$\underset{R6}{HBrO_3 + HBrO_2} \xrightarrow{} \underset{TS6}{BrO_2..H\text{-}O..H..BrO_2} \xrightarrow{} \underset{P6}{Br_2O_4 + H_2O} \text{ …(5.15)}$$

$$\underset{R7}{Br_2O_4} \xrightarrow{} \underset{TS7}{O\text{-}O..Br\text{-}Br\ldots O\text{-}O} \xrightarrow{} \underset{P7}{Br_2 + 2O_2} \text{ ……(5.16)}$$

Keys: R1= $BrO_3^- + H^+$, TS1= $H^+\ldots BrO_3^-$, P1= $HBrO_3$, R2= $HBrO_3 + RSCH_3$, TS2= $BrO_2..HO..SRCH_3$,P2= $HOS^+RCH_3 + BrO_2$, R3= $BrO_2^- + H^+$, TS3= $BrO_2^-\ldots H^+$, P3= $HBrO_2$, R4= $HOS^+RCH_3+HBrO_2$, TS4= $RCH_3S^+\ldots OH\ldots HBrO_2$, P4= $RCH_3S\text{-}O\text{-}H_2BrO_2$, R5= $RCH_3S\text{-}O\text{-}H_2BrO_2$, TS5= $RCH_3S\text{-}O..H..HBrO_2$, P5= $HBrO_2 + RCH_3SO + H^+$, R6= $HBrO_3 + HBrO_2$, TS6= $BrO_2..H\text{-}O..H..BrO_2$, P6= $Br_2O_4 + H_2O$, R7= Br_2O_4, TS7=O-O..Br-Br…O-O, and P7= $Br_2 + 2O_2$

The overall chemical equation for the reaction is:

$2BrO_3^- + 2H^+ + RSCH_3 \longrightarrow RCH_3SO + Br_2 + 2O_2 + H_2O$ ……....(5.17)

5.7The Published Mechanism

Idris et al, in their studies on the kinetics and mechanism of oxidation of methionine by bromate, proposed the following mechanism as shown in scheme 1:

Scheme 1

$\boldsymbol{BrO_3^- + H^+ \rightleftharpoons H_2BrO_3^+}$ ……..(5.18)

$\boldsymbol{H_2BrO_3^+ + R^+SCH_3 \longrightarrow RSCH_3O + H_2BrO_2^+}$ ……..(5.19)

$H_2BrO_2^+ + BrO_3^- \rightleftharpoons Br_2 + O_2 + H_2O$ ……...(5.20)

CHAPTER SIX

6.0 SUMMARY CONCLUSION AND RECOMMENDATION

6.1 Summary

The mechanism of oxidation of methionine by bromate was investigated using computational method. Semi-empirical method (at PM3 and MNDO levels) were used for the study. The geometry of all the species involved in the proposed mechanism were optimised using Spartan software. The HOMO and LUMO of reactants were mapped out to know the direction of flow of electrons between them. The thermodynamic parameters obtained were used for the calculation enthalpy change, entropy change, Gibbs free energy change, activation parameters, equilibrium and rate constants using various equations. The potential energy surface diagrams were drawn for the two levels of theory. The rate determining step for the mechanismfor the reaction was located. The energies of the steps in the published mechanism were compared with the steps of our own mechanism to know the most plausible one.

6.2 Conclusion

The study proposed a more detailed mechanism of oxidation of methionine by bromate. The mechanism involve seven consecutive steps which shows how molecules interact with each other at molecular level.The reaction proceeds via initial uniprotonation of the bromate ion, and terminate at the reaction between $HBrO_3$ and $HBrO_2$ to give bromine and oxygen.The rate limiting step is a reaction between neutral molecules step 2. Therefore, the proposed mechanism is more plausible than the published one.

6.3Recommendation

With the development of more efficient and high speed computers and software the mechanism should be studied using higher methods of computation .The interaction between bromate and other sulphurydyl containing amino acid should be carried out both experimentally and theoretically. The reaction of methionine with other halates(chlorate, iodate) should also be studied as they also have oxidizing ability. Agents should be developed that can control the effect of bromate oxidation of methionine..

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