ROLE OF MANGANESE OXIDES IN CHEMICAL EVOLUTION

A THESIS

Submitted in partial fulfilment of the requirements for the award of the degree of

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in

CHEMISTRY

by

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CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled **ROLE OF MANGANESE OXIDES IN CHEMICAL EVOLUTION** in partial fulfilment of the requirements for the award of the Degree of **Doctor of Philosophy** and submitted in the **Department of Chemistry** of the Indian Institute of Technology Roorkee, Roorkee is an authentic record of my own work carried out during a period from **January 2008 to December 2011** under the supervision of **Dr. Kamaluddin**, Professors, Department of Chemistry, Indian Institute of Technology Roorkee, Roorkee.

The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other Institute.

(BRIJ BHUSHAN)

This is to certify that the above statement made by the candidate is correct to the best of my knowledge.

Dated: December 23, 2011

(KAMALUDDIN)
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The Ph.D. Viva-Voce Examination of **Mr. BRIJ BHUSHAN**, Research Scholar, has been held on.....June 15, 2012..... .

Signature of Supervisor 15/6/12 | Chairman, SRC 15-6-2012 | Signature of External Examiner

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ABSTRACT

Through historical contributions by Oparin, Haldane, Miller, Urey, Cairns-Smith etc., the intellectual challenge of the origin of life enigma has unfolded that life originated on Earth through a series of physicochemical processes known as Chemical Evolution. Investigations revealed a predominantly reducing prebiotic atmosphere and the main compounds of the primordial atmosphere were CO_2, N_2, and water vapor. The early ocean where such physicochemical processes have been assumed to have occurred was a prebiotic soup, a rich diversity of organic and inorganic compounds suitable for assembling into more complex precursors of life forms. Several experiments have been conducted to trace out the possible steps of chemical evolution. Experimental results suggest that such chemical evolution processes began with the formation of important biomonomers, such as amino acids and nucleotides, from simple molecules present in the prebiotic environment and their subsequent condensation to biopolymers. Small reactive intermediates are the backbone of prebiotic organic synthesis. These include hydrogen cyanide, formaldehyde, ethylene, cyanoacetylene, acetylene, and such other molecules that combine to form large and more complex precursors with ultimate formation of stable biomolecules. Subsequent reactions would have depended on the balance between atmospheric production rates and the degradation rates of small intermediates, dependent \on the temperature and pH of the early ocean.

The polymerization of the biomonomers relies on the mechanism of concentrating the basic ingredients from vastly diluted early oceans and it was believed that natural minerals like clays would have provided a surface for the adsorption of organic molecules. Like natural minerals, transition metals may have been important as catalysts for the formation of biopolymers during chemical evolution and the origin of life. Catalysts may have been important for the origins of life because they tend to direct the reaction along a few reaction

pathways so that a limited array of products is obtained. Catalysts bind specific types of compounds to their surfaces and then convert them to a limited number of products. Manganese is the 10^{th} most abundant element in the biosphere (~10^{14} kg of suspended and dissolved manganese found in oceans) and is second only to iron in relative terrestrial abundance of the transition metals. On an average, crustal rocks contain about 0.1% by weight of Mn, coordinated with oxygen, and may also exist in the bottom of seas as nodules. The existence of manganese on Mars has also been reported. In the early stages of the Earth's evolution, volcanoes were a major source of such elements which in turn may have been involved in adsorption and catalytic reactions of biomolecules in molecular evolution. The catalytic activity of manganese for many reactions in the presence of nucleotides, mRNA to give oligoribonucleotides is already reported. Manganese exists in various oxidation states on Earth. The microbial oxidation of soluble Mn (II) is an important process for the formation of soluble Mn (III, IV) oxides in natural environments. We proposed that since the redox potential of the primitive Earth's atmosphere was low and the atmosphere was less oxidized, manganese oxides of lower oxidation states were more important for selectively adsorbing and concentrating bio-molecules during chemical evolution.

In the thesis, results of work on the role of manganese oxides towards different aspects of chemical evolution and origin of life have been presented. It has been proposed that adsorption was the first step for the polymerization of biomonomers. That is why the interaction of ribose nucleotides with manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)) has been studied. During the study it has been found that manganese oxides are good adsorbents towards ribose nucleotides. Further in order to investigate catalytic efficiency of manganese oxides these have been used in the

formation of nucleobases from formamide. Manganese oxides were also found suitable for the oligomerization of amino acids under the simulated conditions.

The **First chapter** of the thesis deals with the introduction and literature review of the topic "chemical evolution and origin of life". Various aspects on prebiotic scenario, amino acid synthesis, synthesis of ribose, nucleobases, phosphates and their assembly studies, peptide synthesis, role of various inorganic minerals, clays, metal cyanogen complexes and metal oxides, which have been efficient in concentrating the organic molecules on their surfaces and subsequently catalyzed a class of prebiotic reactions during the course of chemical evolution have been discussed.

The **Second chapter** describes experimental methodology and instrumentation. This chapter presents the method of synthesis of some metal oxides, their characterization and methods of chemical analyses involved. The metal oxides have been synthesized by precipitation method and characterized using X–ray diffraction, FE-SEM and TEM analysis. Experimental conditions and techniques used for the adsorption of ribose nucleotides on metal oxides have been given. Discussions have also been made on methods used for the synthesis of nucleobases from formamide and oligomerization of amino acids in the presence of manganese oxides. Further the conditions for the interaction of aromatic amines with manganese oxides and their oxidation to various compounds have also been discussed.

The **Third chapter** comprises the result of studies on the interaction of ribose nucleotides (5'-AMP, 5'-GMP, 5'-CMP and 5'-UMP) with various manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)). Adsorption trend was found to follow the Langmuir Adsorption Isotherm. Maximum adsorption was found to occur at neutral pH (~7.0), whereas among the ribose nucleotides, 5'-GMP was found to be adsorbed more on Manganosite (MnO) used. Infrared spectral studies

on the adsorption adducts showed that adsorption of ribose nucleotides takes place due to interaction of positively charged surface of metal oxides and negatively charged sites of ribose nucleotides.

The **Fourth chapter** presents the results of studies on the formation of several nucleobases from formamide in the presence of manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)). It was observed that manganosite (MnO) afforded the maximum yield of products. However, the number of products formed in each case was the same. The only difference was in the yields. Possible explanation has been given on the basis of their structural arrangements.

The **Fifth chapter** comprises the result of studies on the oligomerization of amino acids (glycine and alanine) in the presence of manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)) at various experimental conditions such as temperature range 50-120 ^{0}C and for a time span of 35 days. It was observed that all the four manganese oxides catalyzed the oligomerization of amino acids with glycine up to trimer whereas alanine afforded only dimer. Yield of the products was in accordance with their surface area.

The **Sixth chapter** illustrates the results of studies on interaction of aromatic amines (aniline, p-toluidine, p-chloroaniline and p-anisidine) with manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)). Adsorption trend was found to follow Langmuir Adsorption Isotherm. Maximum adsorption was found to occur at neutral pH (~7.0), whereas p-toluidine was found to be adsorbed more on manganosite (MnO). Infrared spectral studies on the adsorption adducts showed that adsorption of ribose nucleotides occurred due to interaction of positively charged surface of manganese oxide and the basicity of amines. During adsorption studies of the aromatic amines on manganese

oxides, it was found that some of the amines were oxidized in alkaline medium (pH~9) and afforded several other products. The results of the above studies clearly support the idea that a specific manganese oxide might have played its important role in chemical evolution for the concentration and polymerization of biomolecules.

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At last I will like to dedicate this thesis to my **mother and father** who, apart from providing me the best available education, have also encouraged me in all my endeavors.

IIT Roorkee

December, 2011 (BRIJ BHUSHAN)

LIST OF PAPERS
PUBLISHED/ACCEPTED/COMMUNICATED

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2. Adsorption of Ribose nucleotides on Varied Mn/O ratio of Manganese Oxides: Implications for Chemical Evolution by **Brij Bhushan**, Uma Shanker, and Kamaluddin, Origin of Life and Evolution of Biosphere **41**:469-482(2011).
3. Oligomerization of Amino Acids Catalyzed by Iron Oxides: Implication in Chemical Evolution and Origin of Life by Uma Shanker, **Brij Bhushan**, G. Bhattacharjee and Kamaluddin, Origin of Life and Evolution of Biosphere (under revision).
4. **Brij Bhushan**, Uma Shanker, and Kamaluddin "Manganese oxides catalysis Prebiotic Nucleobases Synthesis from Formamide". Astrobiology (communicated)
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LIST OF PAPERS PRESENTED AT NATIONAL / INTERNATIONAL CONFERENCES

1. International Workshop on Chemical Evolution and Origin of Life, Department of Chemistry, IIT Roorkee, February 12-15, 2008.
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5. Operation and Maintenance of Sophisticated Instruments like GC-MS/GC, HPLC, CHNS, AOX Analyzer, IIT Roorkee 15-19 Feb,2010 (sponsored by Central Pollution Control Board, Delhi)
6. International Workshop on Chemical Evolution and Origin of Life, Department of Chemistry, IIT Roorkee, March 5-7, 2010.
7. **Brij Bhushan** and Kamaluddin "Adsorption of Ribose Nucleotides on Manganese Oxides with Varied Mn/O Ratio: Implication in Chemical Evolution" First combined international conference (Origins 2011) in Montpellier (France) in July 2011, P-17.

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INTRODUCTION

1.1 Chemical Evolution and Origin of Life

The question of the origin of life on earth is a topic that engages scientists of various disciplines. Geologists have the difficult task of reconstructing the primordial scenario and environment that was present on earth around 4 billion years ago, a time for which most of the direct evidences have been destroyed or have at least undergone strong changes due to influences of plate tectonics, temperature, radioactivity, weather, water, etc., being the reason that the knowledge about the exact conditions at the time during the emergence of life on earth is still a little blurred. Chemists pick up the possible prebiotic conditions proposed by geologists and investigate possible pathways how organic molecules and biomolecules could be formed in such an environment, how they might have interacted, and how this might lead to more complex and finally "living" systems. Biologists usually see biomolecules in a different context, starting from the high complexity of a modern organism and looking for the very essential biological cycles and interactions and then trying to find how something similar but much more simple might have evolved. Astronomers try to achieve conclusions about the origin of our earth and about its physical development by examining other planets, and they also investigate whether our earth was severely affected by extraterrestrial objects like asteroids or meteorites that might have influenced the origin of life or even brought life to Earth from another place in the universe.

Chemistry of living systems is extremely complicated and it seems impossible that even the simplest contemporary cell could have evolved spontaneously. Most probably, evolution went a long way, starting from very simple organic molecules that formed larger biomolecules, which then started to interact with other classes of molecules in the environment. As soon as membrane-like structures able to form closed spheres were available, also less stable molecules and polymers that would otherwise hydrolyze or not even form at all in the harsh primordial environment had the opportunity to participate in

chemical evolution inside these cell-like entities in a protected and more "comfortable" surrounding.

One severe problem in trying to understand such a possible pathway from simple molecules to the first cell is that in contemporary one class of biomolecules is strongly dependent on the others and vice versa. DNA, for example, carries all the information that is needed to build up proteins, but by itself strongly depends on proteins that catalyze all the required reactions. This leads to a kind of hen and egg problem: what came first? An often-encountered answer is that there once was an "RNA world" before the proteins came into play [1,2]. RNA molecules are a little simpler than DNA; they are able to carry genetic information and can also have catalytic properties. From a chemical point of view, concerning possible formation pathways and stability under primordial conditions, however, the existence of unprotected poly-RNA has to be excluded—a salty ocean would have destroyed them within minutes.

Peptides and proteins have major advantages concerning stability under such conditions, and they also possess all the properties needed to build up a kind of simple life form. This leads to the assumption that a primary peptide and protein world was much more likely than an "RNA world" at the starting point of life.

In 1924, A.I. Oparin, a Russian graduate from university of Moscow, was the first to suggest a series of hypothetical steps by which life might have emerged. Experimental verification of these steps in the laboratory would offer a proof of the origin of life. Scientists can now investigate each stage of Oparin's hypothesis. Based upon the present knowledge of biochemistry, one can define a living system as one having the ability to carry out energy transfer reactions, substrate metabolism and information transfer ability and all these characteristic functions being mediated through proteins and nucleic acid in the cell. The most fundamental reactions necessary for life are:

1. Synthesis of Proteins:- The formation of the peptide bond through interaction of the carboxyl function of an amino acid with the amino group of another amino acid, with the liberation of a molecule of water. (Figure 1.1)

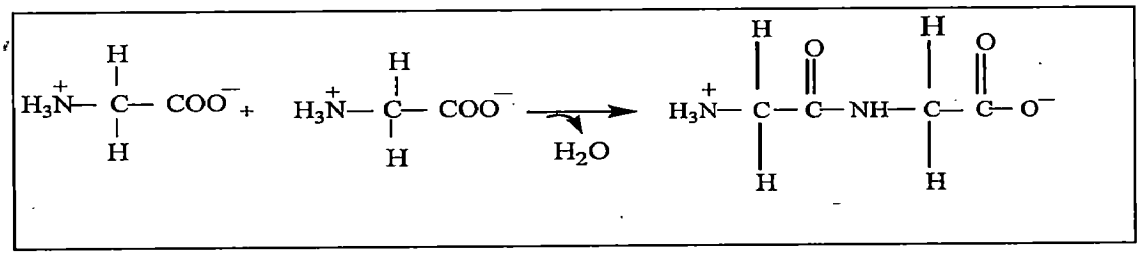


Figure 1.1 Peptide bond formations

2. Nucleic acid synthesis: It is series of small steps:

   (i) Nucleoside synthesis through the reaction of a purine or pyrimidine with ribose or deoxyribose to form nucleoside. (Figure 1.2)

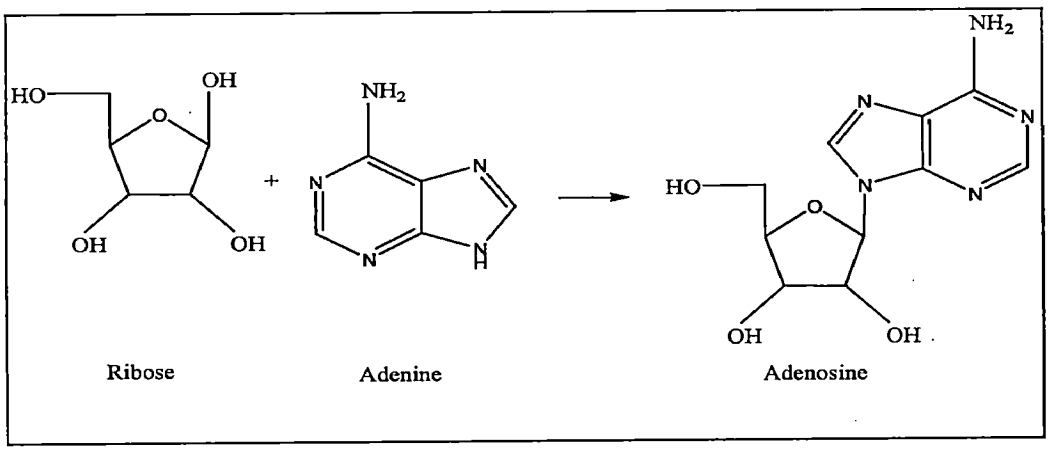


Figure 1.2 Adenosine formation

   (ii) Phosphorylation of nucleoside leading to the synthesis of a nucleotide. (Figure. 1.3)

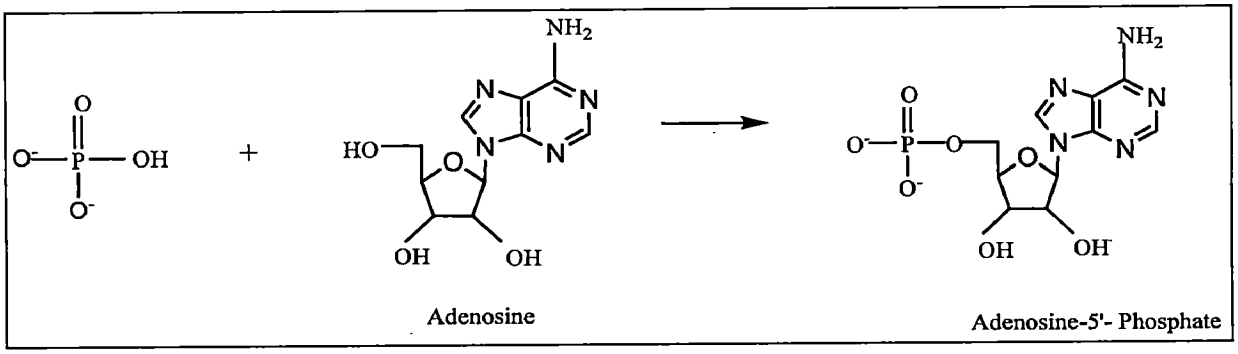
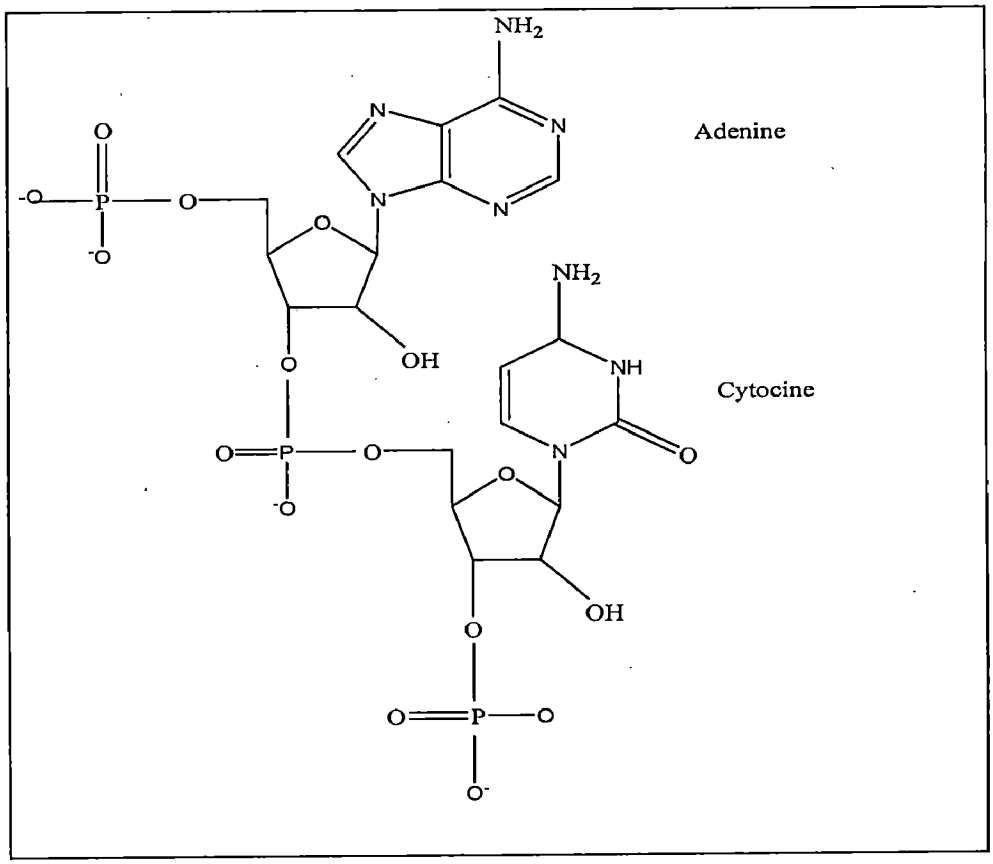


Figure 1.3 Nucleotide formation

(iii) Polymerization of nucleotides through formation of ester bonds between the phosphate residue of one nucleotide and hydroxyl group of the pentose residue. (**Figure. 1.4**)



The building blocks of first living organism must have been abundant on the primitive Earth. A gradual change from simple molecules to more complex organic molecules led to the emergence of life successively, probably through the following events:

1. The synthesis of the small molecules (e.g., amino acids, the monosaccharides, organic bases, etc.)
2. The condensation of these small molecules into biomacromolecules-especially proteins and nucleic acids.
3. The organisation of the macromolecules into a system of increasing complexity; and
4. Finally the emergence of "life".

^{14}C dating of fossils suggested that the oldest living system on the Earth must be around 3.7 billion years, while the Earth itself is 4.6 billion years old. It means that for the first living cell to originate on the Earth took around 0.9 billion years.

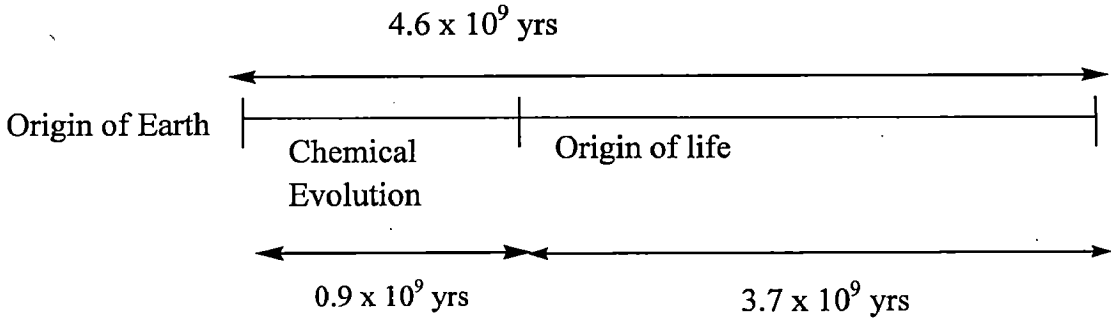


Figure 1.5 Origin of Life

The process of series of chemical reactions that made 'life' to appear on the Earth is known as Chemical Evolution. The ocean received organic matter from the land and the atmosphere, as well as from falling meteorites and comets. Small molecules such as water, carbon dioxide, methane, and hydrogen cyanide were the key precursors for sugars, amino acids, and nucleotides, which in turn are the building blocks of proteins, nucleic acids and carbohydrates, ubiquitous to all living organisms. Development of RNA and DNA molecules was critical, which directed biological processes and preserved life's "operation instructions" for future generation. Nucleic acid helices formed were some of the living threads, however, other polymers derived from planetary processes such as ocean chemistry and volcanic activity. These evolving bundles of polymers from a number of sources, are enough illustrative to explain the origin of life triggered not only by special molecules such as RNA or DNA, but also by the chemical and physical properties of the Earth's primitive environments. Ever since the historical contributions by A. I. Oparin, in the 1920s, the intellectual challenge of the origin of life enigma has unfolded that life originated on Earth through physicochemical processes that can be supposed, comprehended, and simulated. Haldane (1929) and Oparin (1938) proposed that the early ocean was a prebiotic soup, a rich diversity of organic and inorganic compounds suitable

for assembling into more complex precursors of life forms [3, 4]. Miller and Urey verified it by subjecting a mixture of ammonia, methane, hydrogen and water to heat and electric discharge, and obtained hydrogen cyanide; aldehydes and amino acids [5,6]. These precursors can be further converted to nucleotide bases (from HCN and methane) and sugars (from HCHO) [7-12]. In turn, polymerization can generate polypeptides and, notably, polynucleotides [13-17]. The different steps of chemical evolution through, which life has originated on Earth, like from the formation of Earth core and primitive Earth's atmosphere, may be summarized as follows.

The first step involved formation of core and primitive atmosphere. The red–hot (5000–6000 °C) gaseous cloud consisting of innumerable free atoms of several elements (such as hydrogen, oxygen, carbon, nitrogen, sulphur and phosphorous etc.) began to rotate. With rotation, gravitation temperature slowly decreased and then the atoms of cloud segregated into three concentric masses depending upon their atomic weights. The heaviest metallic atoms (Fe, Ni, Cu etc) collected in the centre formed hot lava on the core of Earth. Medium weight atoms (Na, K, Si, I, Mg, Cl, F, S, Al, P, etc.) formed lithosphere at a later stage. The light atomic weight atoms (N, H, O, Ar, C etc.) formed the primitive atmosphere which was highly reducing because of hydrogen atoms were in excess and reactive.

The second step involved the origin of molecules and inorganic compounds. Numerous atoms of primitive atmosphere were spread in the space due to high temperature but as the temperature gradually decreased, these free atoms combined to form molecules of the same elements (H_2, N_2, O_2 etc) as well as of different elements giving rise to simple inorganic compounds. Later on these molecules condensed into solid matter forming hard lithosphere. Combining of H with O and N produced H_2O and NH_3 respectively, which were probably the first inorganic compounds formed on the primitive Earth.

The third step of the hypothesis describes the evolution of organic compounds. The hard lithosphere was studded with actively erupting volcanos as a result of which hot metal lava of the core was poured with gases like CO_2, CO, N_2 and H_2 etc. and thus metallic atoms of lava could form their carbides and nitrides which later formed the crust of the Earth. Hot water vapours reacted with metallic carbides and nitrides to form simple compounds such as CH_4 and NH_3, etc. Both CH_4 and NH_3 under prebiotic environment might have formed HCN. With lowering of temperature, the water vapours condensed on lithosphere as torrential rains, and again turned into vapours as soon as it touched the hot ground. Such cyclic process continued for millions of years. As the Earth cooled down below 100 ^{0}C, the rain water deposition emerged into vast primitive oceans. The oceanic water contained large amount of NH_3, CH_4, HCN, nitrides, carbides and various dissolved gasses and elements which probably formed complex molecules. Methane and other hydrocarbons, ethylene and acetylene on further reaction might have formed aldehydes, ketones, alcohols and organic acids at a later stage. High energies of UV–radiation sunlight, electric discharge of thunderstorms during the rains and heat of volcanic eruptions, might have helped the various organic molecules in the oceanic water to form more and more complex molecules like amino acids, sugars, fatty acids and nitrogenous organic bases etc. At the time of chemical evolution the oceanic water was rich in these organic polymers and formed a soup of organic compounds. The atmosphere was reducing in nature and these complex molecules could not be oxidized and continued react and ultimately polymerized to large linear polymers or macromolecules such as proteins, carbohydrate and fats, which are the main constituents of present day protoplasm.

The fourth step involved formation of the colloids and coacervates. Oceanic macromolecules having full freedom of movement and collisions formed small colloid sphere in the form of insoluble droplets.

The fifth step is likely to be the origin of the first autocatalytic systems. In due course of time nucleic acids (ribo and deoxyribo nucleic acids) gradually evolved in the primitive oceans. Nucleotides formed in the primitive oceanic water by the combination of nitrogenous organic bases with pentose sugars and phosphoric acid. Certain enzymatic proteins combined with nucleic acids to afford nucleoproteins. A self-duplication system resembling the chromosomes of nucleus of present eukaryotic cell was evolved in the primitive oceans. In the structural and functional organization of life, the nucleic acids possess the property of carry out synthesis of various proteins in accordance with their own nucleotide monomers sequence. It was actually the nucleic acids that might have displayed the first signs of self life.

Under simulated conditions as that of primitive atmosphere, researches are trying to trace the possible pathways of chemical evolution. During the last five decades the scientists have carried out numerous experiments in environment similar to the primitive Earth conditions showing the synthesis of various biomonomers from simple starting materials. Some of the significant successful investigations have contributed in understanding the "origin of life".

1.2 Prebiotic Scenario

When our Earth was formed around 4.5 to 4.6 billion years ago, it was a very hot and hostile place due to heavy bombardment by meteors and asteroids during the first several hundred million years. Although scientists have diverging opinions about the environment where the first chemical steps toward life were done, there is a general agreement that liquid water is an essential prerequisite for any possible scenario. There is evidence that 4.3 [18] and even 4.4 [19] billion years (Gyr) ago, a liquid hydrosphere existed, although these first oceans might have been completely evaporated again by subsequent big impact events, and all (or at least the larger) biomolecules possibly formed

would have been destroyed again. Around 4 Gyr ago, the bombardment of the Earth declined and permanent, still rather hot oceans could form, being the most likely places where a chemical evolution could start to form a first form of life. The atmosphere at that time is of crucial importance for this process, as the main source of the building blocks for biomolecules should have been atmospheric components. In a hot surrounding, the lightest gases like hydrogen and helium would have rapidly escaped the weak gravitational field of the Earth. Other compounds like methane and ammonia are very labile under the UV radiation of the sun [20-22] and would, therefore, have been rapidly decomposed in the primordial atmosphere. Geologists also agree that there were only small amounts of free oxygen available before around 2.3 Gyr ago [23-26]. Some oxygen, however, could have been produced when CO_2 and water vapor were exposed to electric discharges and high-energy types of radiation as well as through thermal decomposition of oxidic minerals.

The main compounds of the primordial atmosphere, following the arguments of modern geology, were CO_2, N_2, and water vapor [26-28], which were constantly delivered by volcanic outgassing. This means that the early atmosphere was neutral or possibly due to some traces of oxygen or sulfur dioxide even slightly oxidizing. Some prebiotic chemists, however, still favor a reducing atmosphere, like the one assumed some decades ago, because a reducing atmosphere makes the formation of several organic compounds needed for life more easy. They try to collect arguments that reducing species such as methane, hydrogen, or carbon monoxide might also have been present in larger amounts [29, 30]. Investigations of 3.8 billion-year-old rocks from West Greenland containing carbonate minerals show, however, that they could not have formed or existed in a reducing atmosphere [31, 32]. The temperature after the formation of the first oceans can be assumed to have stayed at an elevated level (~70–100 °C) for a long time, although the activity of the sun was markedly lower 4 Gyr ago. The high concentrations of CO_2 and water vapour in the atmosphere provided a strong greenhouse effect [33], may be also with

the intermediate help of some methane or ammonia [20, 21]. The prebiotic ocean certainly contained large amounts of dissolved inorganic substances, mainly well-soluble salts of Na^+, K^+, $Mg^{2}+$, $Ca^{2}+$ (the salt concentration is assumed to have been as high as today [34]), and an increasing concentration of organic molecules according to Oparin's hypothesis [35]. Due to high concentrations of dissolved CO_2, the prebiotic sea must have been relatively acidic [36, 37].

The earliest evidence for cell-like structures dates back 3.5–3.8 billion years, the later dating being based on findings of what seems to be fossilized cyanobacteria in the Apex Chert formation in the Warrawoona Group in the Eastern Pilbara near Marble Bar, Western Australia [38, 39] and in Swaziland, South Africa. The earlier one considers ^{12}C-enriched carbon deposits in the Isua supracrustal belt in West Greenland [40, 41], a possible sign of biological carbon assimilation. This leaves a time window of a few hundred million years for the emergence of the first living systems

1.3 Amino Acid Synthesis

For more than 50 years, various possibilities for the synthesis of amino acids on the prebiotic Earth have been proposed and experimentally simulated. Diverse atmosphere compositions were exposed to energy sources, and in many cases the synthesis of amino acids resulted. Taking into account the plausibility of such reactions all over the Earth, it seems reasonable to assume that atmospheric processes were the main source of amino acids and other small organic molecules, e.g., sugars. In hydrothermal vent environments, the formation of amino acids is also possible, although under rather specific and most probably not very widespread conditions. Another possibility would be that amino acids came to the Earth from space as ingredients of carbonaceous meteorites, but this assumption only moves the question of the process of amino acid synthesis to another location in our universe.

1.3.1 Gas-phase experiments

Most experiments related to prebiotic amino acid syntheses were performed in the gas phase. Stanley Miller's experiments [42-44], in which it was showed that amino acids can easily form from a model atmosphere containing methane, hydrogen, ammonia, water, and some other simple compounds under the influence of electric discharges and heat, laid the foundation for this type of experiment and also for experimental prebiotic chemistry in general. After a reaction time of a few days, a multitude of organic molecules, such as formaldehyde, cyanide, organic acids, and some amino acids were detected. Miller's experiments were repeated under varying conditions (different composition of the "atmosphere", ultraviolet radiation, and other energy sources), leading also to other precursor molecules such as sugars and nucleic bases in some cases. It was further shown that similar processes, e.g., Fischer–Tropsch-type syntheses (heterogeneous catalytic reactions on Fischer–Tropsch catalysts containing Fe, Co, or Ni), can also lead to intermediates and organic molecules under reducing atmospheric conditions [45]. One problem concerning the formation of organic molecules in such gas-phase reactions is that it works much better with strongly reducing atmospheric compositions comparable to the original Miller experiment. Most of the researchers had to add at least some reducing gases like hydrogen, methane, or carbon monoxide to obtain detectable amounts of organic molecules. Table 1 gives an overview of some of the gas-phase experiments to synthesize amino acids in assumed prebiotic atmospheres. Amino acid formation in a neutral atmosphere as assumed for the primordial Earth by modern geochemistry is much less efficient, however, but recently (2004) we could prove the formation of amino acids from an atmosphere consisting only of CO_2, N_2, and water vapor evaporating from the liquid phase. Electric discharges (60 kV, ~30 mA) between a submerged copper anode under water and a tungsten cathode in the "atmosphere" were the energy source simulating lightning on the prebiotic Earth. Under various conditions (room temperature, 80 °C;

water, sodium chloride solution), several amino acids including glycine, alanine, valine, serine, proline, lysine, and histidine [46, 47] were formed, and although the yields are relatively small in a neutral atmosphere, such reactions should have produced substantial amounts of amino acids on the primordial Earth, continuously occurring almost everywhere on the planet over a long time span.

Table 1.1

Summary of experiments related to gas-phase amino acid syntheses under simulated prebiotic earth conditions

Author	Reactants	Energy Source	Results reported
Miller [42]	CH_4, NH_3, H_2O, H_2	Electric discharge	Simple amino acids, organic compounds
Garrison et al. [48]	CO_2, H_2O	40 MeV helium ions	Formic acid, formaldehyde
Abelson [49]	CO, CO_2, N_2, NH_3, H_2, H_2O	Electric discharges	Simple amino acids, HCN
Bar-Nun et al. [50]	CH4, NH3, H2O	Shock wave	Simple amino acids
Harada, Fox [51]	CH_4, NH_3, H_2O	Thermal energy (900–1200 °C)	14 proteinogenic amino acids
Lawless, Boynton [52]	CH_4, NH_3, H_2O	Thermal energy	Glycine, alanine, aspartic acid, β-alanine, *N*-methyl-β-alanine, β-amino-*n*-butyric acid
Groth, Weyssenhoff [53]	CH_4, NH_3, H_2O	Ultraviolet light	Simple amino acids (low yields)
Sagan, Khare [54]	CH_4, C_2H_6, NH_3, H_2O, H_2S	Ultraviolet light	Simple amino acids (low yields)
Yoshino, Haratsu, Anders [55]	H_2, CO, NH_3, montmorillonite	700 °C	Glycine, alanine, glutamic acid, aspartic acid, histidine, lysine, arginine
Kobayashi et al. [56]	CO, N_2, H_2O	Proton irradiation	Various amino acids
Palm, Calvin [57]	H_2, CH_4, NH_3, H_2O	Electron irradiation	Glycine, alanine, aspartic acid
Miyakawa, Kobayashi, Sawaoka [58]	CO, N_2, H_2, H_2O	High-temperature plasma	Glycine, alanine, aspartic acid
Kobayashi et al. [59]	CO, CO_2, N_2, H_2O	Proton irradiation	Glycine, alanine, aspartic acid, serine, threonine, glutamic acid
Plankensteiner, Reiner, Schranz, Rode [42, 43]	CO_2, N_2, H_2O	Electric discharges	Glycine, alanine, valine, serine, proline, lysine, histidine

Source: Daniel Fitz, Hannes Reiner, and Bernd Michael Rode, Chemical evolution toward the origin of life, Pure Appl. Chem., Vol. 79, No. 12, pp. 2101–2117, 2007

1.3.2 Hydrothermal vents

Hydrothermal vents have increasingly become a focus of interest in geological studies, and some of these investigations also refer to their possible role in the synthesis of biologically relevant molecules [60]. Wächtershäuser and co-workers, and some other groups have postulated that life might have originated in hydrothermal vent environments on the surface of pyrite (FeS_2) [61-64]. Under quite specific conditions, in particular, high partial pressure of carbon monoxide and high temperature, a pendant to the reductive citric acid cycle as a kind of first metabolism is assumed to form pyruvate, which then, but under different and much milder conditions, can further react to amino acids. The occurrence of environments providing such specific conditions may have been more widespread on the primordial Earth than today, but compared to the huge volume of the atmosphere as production site for amino acids, the quantities obtained in the hydrothermal vents would have been rather marginal, even if the atmosphere was of a neutral character, thus providing relatively moderate yields. Other ways of amino acid synthesis in hydrothermal solutions have been proposed, starting from NH_4HCO_3, C_2H_2, H_2, and O_2 [65], however, it is difficult to estimate to what extent these specific conditions could be realized locally on the primitive Earth.

1.3.3 Extraterrestrial origin of amino acids

Additional organic material including amino acids was imported to Earth via asteroids, meteorites, and organic dust particles. Especially, the Murchison meteorite that came down in Australia in 1969 has been very well investigated in this aspect. It contains a wide variety of organic substances, such as amino acids (proteinogenic and nonproteinogenic ones), hydroxy acids, sulfonic acids, phosphonic acids, etc. [66-68]. How such organic molecules can be formed in space was shown, when ice films containing H_2O, CH_3OH, NH_3, and HCN were irradiated with UV light [69]. Amino acids like glycine, alanine, and serine are formed in this scenario that seems highly plausible for

interstellar ice grains. It is difficult to estimate how much organic material delivered from space has contributed to the total pool of biologically important small molecules on the early Earth. Optimistic estimations say that the endogenous production and the exogenous delivery might have been somehow comparable [70, 71], but this strongly depends on the Early atmosphere and on the survival rate of organic molecules in the impact events.

All in all, the availability of amino acids on the primordial Earth does not seem to be a major difficulty on the way to the origin of life, and, therefore, the most crucial question concerning early chemical evolution is the further involvement of these molecules in it, in particular their oligomerization.

1.4 Synthesis of Ribose, Nucleobases and Phosphates and their assembly

In the 19th century, Alexander Michailovich Butlerov discovered the formose reaction, a polymerization reaction of formaldehyde forming a mixture of carbohydrates in aqueous solution catalyzed by calcium hydroxide [72-74]. This condensation reaction provides glycolaldehyde as first product, which is later converted to glyceraldehyde and a variety of tetrose, pentose, and hexose sugars. However, it had not been possible so far to achieve a Butlerov reaction forming ribose, providing a pathway to the sugar component of the nucleotides. This indicates that the ribose concentration in the "primordial soup" was very low and that this sugar was only a minor component of the heterogenous mixture of formed sugars [75, 76]. Furthermore, a high formaldehyde concentration would have been needed in the primordial scenario, and this is highly questionable, as the reactions of other compounds present in the "prebiotic soup" with formaldehyde would rapidly convert it into numerous other products. Besides that, the pH of the primordial ocean was rather acidic than basic due to the high carbon dioxide concentration in the atmosphere [77] and thus unfavourable for the Butlerov condensation. Further, under such reaction conditions, any produced sugars are rapidly decomposed again [76], if they are not protected by complexation with borates [78] or silicates [79]. Polyphosphates and inorganic phosphates

are the most plausible source of phosphate in a prebiotic scenario, but the main problem for the association of phosphate with nucleic bases and ribose—besides the lack of a plausible reaction mechanism under primitive Earth conditions—is the availability of dissolved phosphate in the slightly acidic prebiotic sea: polyphosphates would quickly hydrolyze and phosphate ions precipitate as insoluble compounds of metal ions like Ca^{2+} as indicated by many rocks and minerals consisting of metal phosphates. In the 1950s, Juan Oro showed for the first time a possible way to the formation of relevant nucleobases as building blocks for RNA. Adenine was produced in appreciable yields by refluxing a solution of hydrocyanic acid or ammonium cyanide for six hours [80-84]. Further experiments investigated related reactions by varying the reaction conditions and obtained further nucleobases [85-91]. The major problem with all these reactions is the high starting concentration of HCN or NH_4CN needed, which seems more than questionable on the primitive Earth. Enrichment of nucleobases can be achieved via freezing of aqueous solutions [92], but that is again in contradiction to a hot primordial earth with vigorous volcanism. If all three essential parts—ribose, phosphates, and nucleobases—were formed in adequate amounts under the rough Earth conditions, they would have to be assembled to nucleotides and further polymerize to RNA molecules. Related to the specific composition and complex structure of nucleic acids, the probability that they could have been formed in such a hostile environment is almost zero. The formation of purine ribonucleosides upon heating of their constituents in an otherwise pure solvent has been shown experimentally, but even in this clean environment only a small percentage of the products has biologically relevant linkages [93, 94]. Analogous reactions with pyrimidines have failed completely so far [93]. Furthermore, a chemical process with the correct regioselectivity for the assembly of all the building blocks is almost impossible to imagine in the chemical environment of the prebiotic scenario [95]. Reactions like oligomerization of phosphorimidazolides of nucleobases on montmorillonite [96] work well under laboratory conditions but are very

hard to imagine in a realistic primordial soup. All these aspects and arguments almost exclude the nucleic acids as a candidate for the first step of chemical evolution toward life.

1.4.1 Stability/Carriers of Information /Replicators

Nucleic acids are the present carriers of information and responsible for replication, and they do their job in a very efficient but complicated way with the help of major biochemical (protein-based) machinery. However, at the beginning of life, peptides and proteins could have preceded them, as peptides can also work as information-carrying molecules and can self-replicate, as shown recently [97-99], albeit not as efficiently as nucleic acids. Under the rough conditions of the primitive earth, they would have had many advantages, however. In addition to the previously discussed problems in the formation of nucleic acids under primordial earth conditions, another strong argument against a "RNA world" is the physical and chemical instability of nucleic acids and, in particular, their polymers, which rapidly decompose in hot salty solution. The decomposition of ribose occurs very rapidly at temperatures around 80 °C and at pH values of 7 and higher [100, 101]. Experiments regarding the stability of the four nucleobases in aqueous solutions at elevated temperature and over a wide pH range showed that at 100 °C their half-life time ranges from days to years, the shorter values being associated with the prebiotically more significant lower pH values [102]. Besides these aspects, the presence of salts and metal ions would further increase the decomposition rates [101-103]. As the primitive Earth had not yet formed an ozone layer, high-energetic UV irradiation would efficiently decompose unprotected nucleobases and RNA molecules within a short time [104]. Dissolved ions and several organic compounds in the sea water absorb a large portion of the damaging radiation of wavelengths below 220 nm, thereby protecting most amino acids that absorb in this region. Nucleobases, on the other side, are most sensitive to UV light around 260 nm and, therefore, are much less protected against this additional damaging influence. Information theoretical approaches comparing the error-resistance of

RNA vs. a peptide system also show strong preferences for the second case. Nowadays, replication of RNA or DNA is accompanied by frequent errors that are corrected by a complex enzymatic repair machinery in contemporary organisms. In a pure RNA world, however, such mistakes would quickly lead to catastrophic events such as "selfish RNA", "short circuit", and "population collapse", all of which end in a loss of catalytic activity of the system, as was established by computer simulations of Manfred Eigen's hyper cycles [105] performed by his co-workers [106]. Freeman Dyson showed with his "toy model" [107] that a larger set of building blocks (instead of 4 different nucleobases, one could assume 8–10 amino acids) is much more tolerant against errors and would thus allow the stepwise production of more complex systems. If an error occurs, the "wrong" amino acid might have similar properties as the original one and the newly formed peptide would still be auto catalytically active, not interrupt the evolutionary pathway and possibly even lead to a favourable "mutant".

1.5 Peptide Synthesis

Since peptides and proteins appear the presumably only choice to form polymers in chemical evolution, the consideration of oligomerization of amino acids toward a possible "peptide world" becomes the most essential topic for the evolutionary scenario. While large amounts of liquid water are an important prerequisite for any chemical evolution scenario, the formation of peptides in aqueous solution is a rather unfavourable reaction, both thermodynamically and kinetically, as the condensation of amino acids to peptides requires the removal of water from the reaction partners. In the case of diglycine formation in water at a temperature of 85 °C, the equilibrium constant is 8.41×10^{-3}, and thus less than 0.01 % of glycine would be converted into diglycine in aqueous solution. Considering the principle of Le Chatelier, this condensation reaction can only be carried out in substantial amounts under special conditions. Moreover, the zwitterionic form of the amino acids leads to deactivation of the amino group for a nucleophilic attack at the carboxyl-C

of other amino acids or peptides. To overcome these thermodynamic and kinetic barriers, some possible pathways for peptide formation have been proposed and experimentally investigated.

1.5.1 Melting processes

By heating mixtures of amino acids containing large amounts of acidic or basic amino acids to temperatures of ~180 °C under anhydrous conditions to get a melt without decomposition of the amino acids, polymerization occurs [108-110] and so-called "proteinoids" with high molecular weights were formed. However, these proteinoids contain only marginal amounts of peptide bonds, they are mostly based on ester-like connections [111]. Furthermore, the requirement of an excess of acidic or basic amino acids and the absence of disturbing other molecules or salts is not compatible with any accepted prebiotic scenario nor the typical distribution of amino acid yields in Miller-type experiments. For this reason, melting processes do not seem to be a feasible way to peptides and proteins.

1.5.2 Hydrothermal vent systems

Wächtershäuser and others achieved peptide formation under rather specific conditions that might have occurred in hydrothermal vent environments. In an aqueous slurry of (Ni, Fe)S and in the presence of CO and H_2S or CH_3SH as a catalyst and condensation agent, di- and tripeptides were formed at elevated temperatures at pH values between 7 and 10 [112, 113]. These conditions and problems inevitably connected to them such as high hydrolysis rates and racemisation of the amino acids do not lend this scenario much credibility as a main production site for biologically relevant peptides. Oligomerization of the most simple amino acid glycine can be quite easily achieved, for example, in a simulated submarine hydrothermal system where an aqueous solution of glycine was circulated through a flow reactor in which the fluid was alternately heated to

over 200 °C in a high-pressure chamber and then cooled down again [114]. When some $CuCl_2$ was added, oligomers up to hexaglycine were found.

1.5.3 Condensation reagents

A large number of attempts to form peptides under "possible prebiotic conditions" in solution implied substances that could act as condensation reagents when present in sufficient amounts. Under optimal conditions reagents such as cyanates [115], trimetaphosphate [116], imidazole [117-119], triazole, cyanamides [120, 121], linear and cyclic inorganic polyphosphates [122, 123], ATP [124] and UTP [117] have been shown to yield considerable amounts of peptides from simple amino acids. The existence of these condensation reagents in the required concentrations in the aqueous environment of the primordial earth has never been plausibly argued, however.

Khorana proposed the mechanism of condensation of amino acids using carbodimide as a condensing agent [125]. Yamanaka proposed Trimetaphosphate in condensation of oligoglycines in aqueous solutions [123]. Aminoacetonitrile has also been used as prebiotic condensing agent for dipeptide synthesis from amino acids [126]. Odom have studied oligomerization of nucleotides in presence of kaolinite, ammonium chloride and cyanamide and suggested that cyanamide is only responsible in the condensation processes [127-128]. Halmann also used cyanamide as the condensing agent in formation of diglycine from glycine [129].

1.5.4 Heterogeneous systems

1.5.4.1 Role of clay in peptide formation

Clay minerals such as kaolinite and montmorillonite can catalyze the formation of peptides on their active surface and edges [130-133]. As their reactivity is rather low and the condensation limited to a few simple amino acids, their main role can be seen in subsequent processes, in concentrating peptides and stabilizing them against hydrolysis through adsorption, and in promoting a chain elongation of formed peptides [133]. Pure

silica and alumina have also been investigated [134-138] in this context, showing even more favorable properties than composite natural minerals. Clays are widely distributed on whole Earth surface. Clays are characterized by high surface area, strong affinity for organic compounds, specific distribution of surface charge, and metal ion exchange capacity. Clays are known for their strong adsorption capacity and are good catalysts. Montmorillonite a clay mineral formed by the weathering of volcanic ash may have played a central role in the evolution of life.

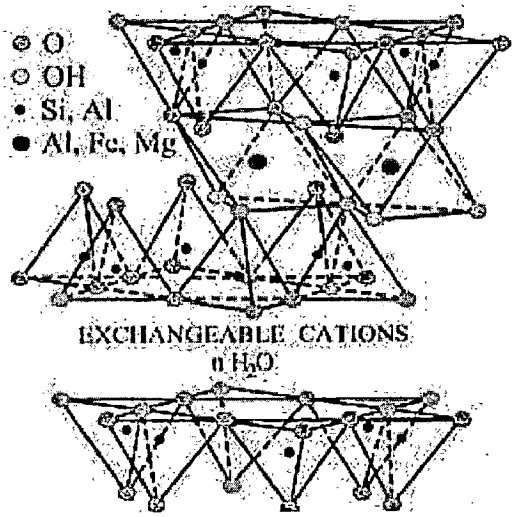


Figure 1.6 Structure of Montmorillonite

Above is the schematic Figure showing the structure of the crystal lattice of montmorillonite. Each layer is made up of two outer silica sheets composed of SiO_4 - tetrahedrons and a central sheet comprised of aluminium cations surrounded in octahedral form by oxide anions of silicate molecules. By introducing trivalent aluminium and iron ions into the central sheet, the interlayer space is provided for the presence of cations which are capable of exchange and can be easily replaced by the other cations of strong binding affinity. Crude clays consist of calcium ions as well and such bentonites are known as calcium bentonite. Because of its structure, montmorillonite tends to adsorb organic compounds to contribute its ability to catalyze a variety of organic reactions, some of these reactions were critical in origin of life. Bernal [139] was the first to propose the role of

clays and minerals in chemical evolution. Bernal proposed that clay minerals could have catalyzed the polymerization of biomonomers from their aqueous solutions and also a number of chemical reactions of prebiotic importance from which life could have emerged on the primitive Earth. The remarkable features of clay are: (i) Their ordered arrangement, (ii) Their high adsorption capacity, (iii) Their shielding capacity against sunlight (U.V. radiation), (iv) Their ability to concentrate organic chemicals and (v) Interestingly their ability to serve as polymerization templates.

Bernal's hypothesis has been verified by many researchers and demonstrated the important role of clay minerals during the course of chemical evolution.

According to the Bernal's hypothesis, clay minerals helped in chemical evolution in the following sequences:

(i) Clay minerals have catalyzed the synthesis of biomonomers from gases of primordial atmosphere.

(ii) Clay minerals adsorbed biomonomers formed on their surfaces providing a highly concentrated system having biomonomers in specific orientation.

(iii) Clay minerals facilitated condensation reactions between adsorbed biomonomers from biopolymers.

The ability of montmorillonite to catalyse a reaction was first observed during cyclization of 3'-nucleotides to 2', 3'-cyclic nucleotide. The yield of the cyclic product was almost doubled, when the reaction was performed in the presence of Zn^{2+}-Montmorillonite [140]. Encouraged by the results, further, investigation on formation of phosphodiester bond between 5'-nucleotides using a carbodimide [141], and then with the phosphorimidazolide of nucleotides using Na^{+} montmorillonite as the catalyst were carried out by Ferris et al. [142-143]. Formation of 6-14 mers, in aqueous pH~8 solutions, with the oligomer length depended on the base present in nucleotides [144-146]. Kinetic studies on the reaction of ImpA revealed that the montmorillonite notably enhanced the rate constant

for oligomer formation by 100-1000 times over that for the hydrolysis of the imidazole-activating group [147]. Changing the reaction conditions and the phosphate-activating group led to the formation of significantly longer oligomers. A 'feeding reaction', where the activated monomer is added daily to a 10 mers nucleic acid primer bound to montmorillonite, resulted in the addition of 30–40 mers to the primer in 12–14 days [148-149]. Changing the phosphate activating group from imidazole to 1-methyladenine resulted in the formation of 40–50 mers of A or U in just 1–3 days without a primer [96]. These investigations were important since they generated longer oligomers with the capability of storing more genetic information as well having enhanced catalytic capability. Ferris et al. had shown that RNA molecules can bind efficiently to clays and that montmorillonite can catalyze the formation of longer molecules (oligomers), thus lending support to the RNA world hypothesis that the life based on RNA preceded over DNA and protein chemistry of present life form [150]. Marco Franchi and Enzo Gallori studied the possibility of development of the RNA world on a clay substrate by investigating the capacity of different RNA molecules adsorbed/bound on clay minerals, montmorillonite (M) and kaolinite (K) and even persisted in the presence of a degrading agent (RNase-A), to interact specifically with complementary RNA strands, and likely to transmit the information contained in their nucleotide sequences [151]. Oligomerization of the 5-phosphorimidazolide of uridine (ImpU) in the presence of montmorillonite clay has also been studied [152].

The effect of exchangeable cations, namely, alkali and alkaline metal, ammonium and aluminium ions, on montmorillonite (M) catalyzed peptide formation was investigated by Juraj Bujdak. Amino acid (AA) dimerization and peptide chain elongation proceed with relatively low yields on macroscopically swelling montmorillonite due to redistribution and insufficient concentration of reactant molecules on the clay surface. Cyclic anhydride (CA) formation, proceeding by monomolecular mechanism, is primarily affected by the

accessibility of catalytic sites for dimer activation. Weaker sorbed exchangeable cations do not block catalytic sites, and thus favour CA formation [153]. Role of metal ions in chemical evolution has been studied for the polymerization of alanine and glycine [154]. Five proteinaceous and six nonproteinceous amino acids were synthesized from a CH_4-N_2 atmosphere when exposed to an electric discharge in the presence of Na^+- montmorillonite in water. Glycine, DL-alanine, DL-α-aminobutyric acid, and sarcosine were the four major amino acids produced [155]. The adsorption of protein and non- protein amino acids by Na^+- montmorillonite at different pH has also been studied. Five pairs of amino acids, ranging from 2-6 carbon atoms were used at concentrations equal to 100% of the clay cation exchange capacity. The following pairs of protein and nonprotein amino acids were used: glycine and sarcosine, α-alanine and β-alanine, α-aminobutyric and λ-aminobutyric acids, valine and norvaline, L-isoleucine and D-alloisoleucine. Adsorption of β and γ-amino acids was three and four-fold greater, respectively, than that of α-amino acids under acidic and neutral conditions [156]. Peptides were formed from aspartic acid, glutamine, glycine, alanine, valine, isoleucine, leucine, phenylalanine, lysine, histidine, and arginine with a kaolinite or montmorillonite catalyst under fluctuating moisture conditions (dehydration followed by hydration) at temperatures below the boiling point of H_2O. The adsorption of ATP and ADP on montmorillonite, kaolinite, and $Al(OH)_3$ was studied as a function of pH, and as a function of the ionic composition of the system. At the three minerals exhibited different adsorption characteristics. Mg^{2+} and Zn^{2+} montmorillonite adsorbed ATP and ADP more than Na^+ montmorillonite [157]. Montmorillonite was also found to efficiently catalyse the condensation of formamide to a large panel of nucleic acid bases and derivatives, including purine, adenine, and hypoxanthine [158].

1.5.4.2 Role of Double Metal Cyanides in Peptide Synthesis

Cyanide has been reported as an important product in almost all the simulated prebiotic experiments and is supposed to be readily available under prebiotic environment. It is assumed that cyanide ions might have formed a number of soluble and insoluble complexes with transition metal ions present in primeval seas. Beck proposed that cyanide ion which was presumably present on the prebiotic environment might have formed stable complexes with transition metals present in the primeval seas [159]. A hypothesis has been proposed that cyano complexes were the probable catalysts in the prebiotic Earth conditions. Orgel [160], have supported the formation of cyano complexes of transition metals, which could have played very important role in chemical evolution. Considering all these concepts a number of metal cyano complexes have been tested for their role in chemical evolution. Interaction studies of nucleotides and amino acids with several of the cyano complexes have been studied by Kamaluddin et al. and Ali et al. [161-164]. Adsorption of adenine, adenosine, and adenosine nucleotides on nickel (II) hexacyanoferrate (II) has been studied by Viladkar et al. [165]. Adsorption of ribose and 2'-deoxyribose-5'-nucleotides on metal ferrocyanides has been studied by Kamaluddin et al. and their role in chemical evolution has been discussed [166].

1.5.4.3 Role of Metal Oxides in Peptide Synthesis

Bernal proposed the role of clays in chemical evolution; search of new possible solid surfaces and their involvement in chemical evolution has also started. It is proposed that all the substances which act as good adsorbents for organic molecules, could have helped in concentrating these organic molecules and subsequently helped in the formation of the first cell.

Egami correlated the concentrations of minor transition metals with their biological behaviour in primeval seas [167]. It is proposed that transition metal ions abundantly present in primeval seas might have formed complexes with simple molecules readily

available to them and had played crucial role in chemical evolution. Kobayashi studied the importance of transition metals in chemical evolution [168-170]. The result strongly supports the heterogeneous condensation hypothesis, yet at the same time gives poor insights into mechanisms of the peptide formation and the role of various surface atoms/groups. The reason due to the complexity of clays and related materials in terms of surface chemistry, like the presence of different active surface sites, from single atoms and surface functional groups to planes, layers, edges etc. In an approach, which can be more respective for elucidating the chemical mechanisms, is "splitting" the clay compound into complex oxide catalysts and into simpler or elementary (when possible) oxide components, first of all silica and alumina. Initially, by studying the catalytic properties independently and then by complemented experiments performed on clays. Such experiments have helped to understand what are the surface active sites or atoms which might be responsible for the catalytic activity. Metal oxides are the important constituents of the Earth crust and also of other planets. Hence, their possibility of catalyzing different important reactions in the course of chemical evolution and origin of life cannot be ruled out. Number of investigations is in progress for understanding the role of metal oxides in chemical evolution. Under mild conditions, alumina is found to catalyze peptide formation. Alumina has also been found as a good catalyst for the peptide formation of alanine. All three forms of alumina, acidic, neutral and basic are used for the formation of $(Ala)_2$, $(Ala)_3$ and cyclic alanine [171]. Adsorption and thermal condensation of glycine on silica has also been studied [172]. The interaction between glycine and alumina has been studied [173]. It is proposed that metal oxides present on primitive Earth could have provided surfaces onto which biomonomers could have been concentrated through the means of selective adsorption. Matrajt and Blanot used synthetic ferrihydrite as amino acid adsorbent and a promoter of peptide bond formation [174]. They studied polymerization of alanine, glycine and norvaline using ferrihydrite as catalyst. Iron (III) hydroxide oxide catalyses the

condensation of DL–glyceraldehydes to ketohexoses. Sorbose, fructose, psicose, tagatose and dendroketose were formed [175]. Activated alumina has been used as an energy source for peptide bond formation [176]. They have used various forms of alumina for alanine dimerization. Highest yield of $(Ala)_2$ was obtained with neutral alumina. Peptide bond formation on the surface of activated alumina has been studied [177]. The reactions of the dipeptides and glycine oligopeptides on activated alumina have yielded longer chain oligomers up to $(Gly)_{11}$. Reverse reactions of polymer degradation to measure the impact of mineral surface catalysis on peptide bonds have also been studied. It was observed that the reactions of aqueous glycine (G), diglycine (GG), diketopiperazine (DKP), and triglycine (GGG) occurred with minerals (calcite, hematite, montmorillonite, pyrite, rutile, or amorphous silica), in the presence of 0.05 M $KHCO_3$ buffer and 0.1 M NaCl as background electrolyte in a thermos tatted oven at 25, 50 or 70^{0}C and pH 8.1. The minerals were not found to have measurable effects on the degradation or elongation of glycine, glycylglycine or diketopiperazine at 70 ^{0}C in solution, while triglycine was degraded [178]. Prebiotic synthesis of purine, adenine, cytosine, thymine and 4(3H)-pyrimidinone from formamide has been found to be catalyzed by inorganic oxides, such as, calcium carbonate, silica, alumina, kaolin, and zeolite with useful application in the origin of life [179]. Titanium dioxide catalysed synthesis of nucleobases and acylonucleosides from formamide has also been established [180].

Table 1.2

The Ten Most Abundant Compounds in the Earth's Crust

Compound	Formula	Abundance Percent/Weight	Abundance Parts per million by weight
Silicon dioxide	SiO_2	42.86%	428,600
Magnesium oxide	MgO	35.07	350,700
Ferrous oxide	FeO	8.97	89,700
Aluminium oxide	Al_2O_3	6.99	69,900
Calcium oxide	CaO	4.37	43,700
Sodium oxide	Na_2O	0.45	4,500
Ferric oxide	Fe_2O_3	0.36	3,600
Titanium dioxide	TiO_2	0.33	3,300
Chromic oxide	Cr_2O_3	0.18	1,800
Manganese dioxide	MnO_2	0.14	1,400

Source: Exploring Chemical Elements and their Compounds; David L. Heiserman, 1992

1.6 Problem Statement

Extensive literature survey revealed that the inorganic minerals present on the primitive Earth might have played a crucial role in the numerous processes of chemical evolution leading to the formation of biomonomers from simple molecules and then their condensation for the formation of biopolymers. There are ample research papers and reviews concerned with the role of clays [140- 159] and metal cyano complexes as adsorbent and catalyst in the course of chemical evolution [160- 167]. Metal oxides such as silica, alumina are the important constituents of Earth and other planets such as Mars, it is assumed that they might have catalyzed many important reactions in chemical evolution. Exploration on role of metal oxides in chemical evolution is still in progress. Metal oxides are good adsorbents and efficient catalysts. Hence it is thought that they have played an important role in chemical evolution. In the present work the role of manganese oxides in adsorbing and catalyzing important reactions of chemical evolution has been studied.

1.7 Organization of the Work

To investigate the role of metal oxides as prebiotic catalyst, metal oxides such as manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)) have been synthesized and characterized using X–ray diffraction, TGA/ DTA, IR spectral studies, and Scanning Electron Microscopy (SEM), Transmission electron microscopy (TEM). The surface area of all the metal oxides has been found out. Adsorption studies have been carried out using UV, IR, and FE-SEM analysis and it was observed that the manganese oxides are good adsorbents for ribose nucleotides. Manganese oxide (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)) catalyzed formation of nucleobases from formamide has also been studied. Catalytic property of manganese oxides has been observed in oligomerization of amino acids (glycine and alanine). Adsorption and further oxidation of aromatic amines have also been studied in the presence of manganese oxides. Studies involved in this thesis were carried out under the following steps:

1. Adsorption of ribose nucleotides (5'-AMP, 5'-GMP, 5'-CMP and 5'-UMP) on manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)).
2. Formation of nucleobases (purine, cytocine, adenine, thymine, 4(3H)-pyrimidinone etc) from formamide in the presence of manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)).
3. Manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)) catalyzed oligomerization of amino acids (glycine and alanine).
4. Adsorption and oxidation of aromatic amines (aniline, p-chloroaniline, p-anisidine and p-toluidine) on manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)).

References:

1. Orgel, L. E. "Prebiotic Chemistry and the Origin of the RNA World". Crit. Rev. Biochem. Mol. Biol., **39**, 99 (2004).
2. Joyce, G. F. "The antiquity of RNA-based evolution". Nature, **418**, 214 (2002).
3. Haldane, J. B. S. "The origin of life". Rationalist Annual, **148**, 3 (1929).
4. Oparin, A. I. "The origin of life". MacMillan, New York, (1938).
5. Miller, S. L. "A Production of Amino Acids under Possible Primitive Earth Condition". Science, **117**, 528 (1953).
6. Miller, S. L. and Urey, H. C. "Organic Compound Synthesis on the Primitive Earth". Science, **130**, 245(1959).
7. Oró, J. and Kimball, A. P. "Synthesis of purines under possible primitive Earth conditions". Arch. Biochem. Biophy., **94**, 217 (1961).
8. Sanchez, R. A., Ferris, J. P., and Orgel, L. E. "Studies in prebiotic synthesis. II Synthesis of purine precursors and amino acids from aqueous hydrogen cyanide". J. Mol. Biol., **30**, 223 (1967).
9. Sanchez, R. A. and Orgel, L. E. "Studies in prebiotic synthesis. V. Synthesis and photoanomerization of pyrimidine nucleosides". J. Mol. Biol., **47**, 531 (1970).
10. Fuller, W. D., Sanchez, R. A. and Orgel, L. E. "Studies in prebiotic synthesis. VII Solid-State Synthesis of Purine Nucleosides". J. Mol. Biol., **1**, 249 (1972).
11. Ferris, J. P. and Hagan, Jr. "The adsorption and reaction of adenine nucleotides on montmorillonite". Orig. Life. Evol. Biosph., **17**, 69 (1986).
12. Shapiro, R. "The prebiotic role of adenine: a critical analysis". Orig. Life Evol. Biosph., **25**, 83(1995).
13. Fox, S. W. & Harada, K. "Thermal copolymerization of amino acids to a product resembling protein". Science, **128**, 1214 (1958).

14. Schramm, G., Grotsch, W. and Pollman, W. "Nonenzymatic synthesis of polysaccharides, nucleosides, nucleic acids and the origin of self reproducing systems". Angew. Chem. (Int. English. Edn), **1**, 1 (1962).

15. Naylor, R. and Gilham, P. T. "Studies on Some Interactions and Reactions of Oligonucleotides in Aqueous Solution". Biochemistry, **5**, 2722 (1966).

16. Von Kiedrowski, G., Wlotzka, B. and Helbing, J. "Sequence dependence of template-directed syntheses of hexadeoxynucleotide derivatives with 3k-5k pyrophosphate linkages". Angew. Chem. (Int. English. Edn), **28**, 1235 (1989).

17. Luther, A., Brandsch, R. and von Kiedrowski, G. "Surface-promoted replication and exponential amplification of DNA analogues". Nature, **396**, 245 (1998).

18. Mojzsis, S. J., Harrison, T. M., and Pidgeon. R. T. "Oxygen-isotope evidence from ancient zircons for liquid water at the Earth's surface 4,300 Myr ago". Nature, **409**, 178 (2001).

19. Wilde, S. A., Valley, J. W., Peck, W. H., and Graham, C. M. "Evidence from detrital zircons for the existence of continental crust and oceans on the Earth 4.4 Gyr ago". Nature, **409**, 175 (2001).

20. Sagan, C., and Chyba, C. "The Early Faint Sun Paradox: Organic Shielding of Ultraviolet-Labile Greenhouse Gases". Science, **276**, 1217 (1997).

21. Kasting, J. F. "Warming Early Earth and Mars". Science, **276**, 1213 (1997).

22. Ogura, K. Migita, C. T., Yamada, T. "Photolysis of CH_4---NH_3---H_2O mixture: formation of methylamine and ethylenediamine". J. Photochem. Photobiol., A **49**, 53 (1989).

23. Wiechert, U. H. "Earth's Early Atmosphere". Science, **298**, 2341 (2002).

24. Holland, H. D. "When did the Earth's Atmosphere become Oxic? A Reply". Geochem. News, **100**, 20 (1999).

25. Kasting, J. F. and Siefert, J. L. "Life and the Evolution of Earth's Atmosphere". Science, **296**, 1066 (2002).

26. Levine, J., Augustsson, T. and Natarajan, M. "The prebiological paleoatmosphere: Stability and composition". Orig. Life Evol. Biosph., **12**, 245 (1982).

27. Delano, J. W. "Redox History of the Earth's Interior since ~3900 Ma: Implications for Prebiotic Molecules". Orig. Life Evol. Biosph., **31**, 311 (2001).

28. Holland, H. D. The Chemical Evolution of the Atmosphere and Oceans, pp. 29, Princeton, University Press, Princeton (1984).

29. Tian, F., Toon, O. B., Pavlov, A. A. and De Sterck, H. "A Hydrogen-Rich Early Earth Atmosphere". Science, **308**, 1014 (2005).

30. Miyakawa, S., Yamanashi, H., Kobayashi, K., Cleaves, H. J., and Miller, S. L. "Prebiotic synthesis from CO atmospheres: Implications for the origins of life". Proc. Natl. Acad. Sci.USA, **99**, 14628 (2002).

31. Nutman, A. P., McGregor, V. R., Friend, C. R. L. and Bennett, V. C., The Itsaq, P. D. "Gneiss Complex of southern West Greenland; the world's most extensive record of early crustal evolution (3900-3600 Ma)". Kinny. Precambr. Res., **78**, 1 (1996).

32. Holland, H. D. The Chemical Evolution of the Atmosphere and Oceans, pp. 129, Princeton, University Press, Princeton (1984).

33. Nisbet, E. G. and Sleep, N. H. "Review Article The habitat and nature of early life". Nature, **409**, 1083 (2001).

34. Rubey, W. W. The Origin and Evolution of Atmospheres and Oceans, John Wiley, New York (1964).

35. Oparin, A. I. The Origin of Life on Earth, Oliver & Boyd, Edinburgh (1957).

36. Owen, T., Cess, R. D. and Ramanathan V. "Enhanced CO_2 greenhouse to compensate for reduced solar luminosity on early Earth". Nature, **277**, 640 (1979).

37. Kasting, J. F. and Ackerman, T. P. "Climatic consequences of very high carbon dioxide levels in the earth's early atmosphere". Science **234**, 1383 (1986).

38. Schopf, J. W. "Microfossils of the Early Archean Apex Chert: New Evidence of the Antiquity of Life". Science, **260**, 640 (1993).

39. Hofmann, H. J., Grey, K., Hickman, A. H. and Thorpe, R. "Origin of 3.45 Ga coniform stromatolites in Warrawoona Group, Western Australia". Bull. Geol. Soc. Am., **111**, 1256 (1999).

40. Mojzsis, S. J., Arrhenius, G., McKeegan, K. D., Harrison, T. M., Nutman, A. P. and Friend, C. R. L. "Evidence for life on Earth before 3,800 million years ago". Nature, **384**, 55 (1996).

41. Hayes, J. M. "The earliest memories of life on Earth". Nature, **384**, 21 (1996).

42. Miller, S. L. "A Production of Amino Acids Under Possible Primitive Earth Conditions". Science, **117**, 528 (1953).

43. Miller S. L. "Production of Some Organic Compounds under Possible Primitive Earth Conditions[1]". J. Am. Chem. Soc., **77**, 2351 (1955).

44. Bada, J. L. and Lazcano, A. "Prebiotic Soup--Revisiting the Miller Experiment". Science, **300**, 745 (2003).

45. Miller, S. L., Urey, H. C. and Oro, J. Origin of organic compounds on the primitive earth and in meteorites". J. Mol. Evol., **9**, 59 (1976).

46. Plankensteiner, K., Reiner, H., Schranz, B. and Rode, B. M. "Prebiotic Formation of Amino Acids in a Neutral Atmosphere by Electric Discharge". Angew. Chem., Int. Ed., **43**, 1886 (2004).

47. Plankensteiner, K., Reiner, H. and Rode, B. M. "Amino acids on the rampant primordial Earth: Electric discharges and the hot salty ocean". Mol. Divers., **10**, 3 (2005).

48. Garrison, W. M., Morrison, D. C., Hamilton, J. G., Benson, A. A. and Calvin, M. "Reduction of Carbon Dioxide in Aqueous Solutions by Ionizing Radiation". Science, **114**, 416 (1951).

49. Abelson, P. H. "Paleobiochemistry, Inorganic Synthesis of Amino Acids". Carnegie Inst. Wash. Yearbook, **55**, 171 (1956).

50. Bar-Nun, A., Bar-Nun, N., Bauer, S. H. and Sagan, C. "Shock Synthesis of Amino Acids in Simulated Primitive Environments". Science, **168**, 470 (1970).

51. Harada, K. and Fox, S. W. "Thermal Synthesis of Natural Amino-Acids from a Postulated Primitive Terrestrial Atmosphere". Nature, **201**, 335 (1964).

52. Lawless, J. G. and Boynton, C. G. "Thermal Synthesis of Amino Acids from a Simulated Primitive Atmosphere". Nature, **243**, 405 (1973).

53. Groth, W. and Weyssenhoff, H. V. "Photochemische Bildung von Aminosäuren aus Mischungen einfacher Gase". Naturwissenschaften, **44**, 510 (1957).

54. Sagan, C. and Khare, B. N. "Long-Wavelength Ultraviolet Photoproduction of Amino Acids on the Primitive Earth". Science, **173**, 417 (1971).

55. Yoshino, D., Haratsu, R. and Anders, E. "Origin of organic matter in early solar system—III. Amino acids: Catalytic synthesis". Geochim. Cosmochim. Acta, **35**, 927 (1971).

56. Kobayashi, K., Tsuchiya, M., Oshima, T. and Yanagawa, H. "Abiotic synthesis of amino acids and imidazole by proton irradiation of simulated primitive earth atmospheres". Orig. Life Evol. Biosph., **20**, 99 (1990).

57. Palm, C. and Calvin, M. "Primordial Organic Chemistry. I. Compounds Resulting from Electron Irradiation of $C^{14}H_4$". J. Am. Chem. Soc., **84**, 2115 (1962).

58. Miyakawa, S., Kobayashi, K. and Sawaoka, A. B. "Amino acid synthesis from $CO\text{-}N_2$ and $CO\text{-}N_2\text{-}H_2$ gas mixtures via complex organic compounds". Adv. Space Res., **24**, 465 (1999).

59. Kobayashi, K., Masuda, H., Ushio, K., Ohashi, A., Yamanashi, H., Kaneko, T., Takahashi, J., Hosokawa, T., Hashimoto, H. and Saito, T. "Formation of bioorganic compounds in simulated planetary atmospheres by high energy particles or photons". Adv. Space Res., **27**, 207 (2001).

60. Russell, M. J., Hall, A. J., Boyce, A. J. and Fallick, A. E. ">On Hydrothermal Convection Systems and the Emergence of Life". Econ. Geol., **100**, 419 (2005).

61. Wächtershäuser, G. "Evolution of the first metabolic cycles". Proc. Natl. Acad. Sci. USA, **87**, 200 (1990).

62. Wächtershäuser, G. "Life as We Don't Know It". Science, **289**, 1307 (2000).

63. Cody, G. D., Boctor, N. Z., Filley, T. R., Hazen, R. M., Scott, J. H., Sharma, A. and Yoder Jr, H. S. "Primordial Carbonylated Iron-Sulfur Compounds and the Synthesis of Pyruvate". Science, **289**, 1337 (2000).

64. Huber, C. and Wächtershäuser, G. "Activated Acetic Acid by Carbon Fixation on (Fe,Ni)S Under Primordial Conditions". Science, **276**, 245 (1997).

65. Marshall, W. L. "Hydrothermal synthesis of amino acids". Geochim. Cosmochim. Acta, **58**, 2099 (1994).

66. Kvenvolden, K., Lawless, J., Pering, K., Peterson, E., Flores, J., Ponnamperuma, C., Kaplan, I. R. and Moore, C. "Evidence for Extraterrestrial Amino-acids and Hydrocarbons in the Murchison Meteorite". Nature, **228**, 923 (1970).

67. Kvenvolden, K. A., Lawless, J. G. and Ponnamperuma, C. "Nonprotein Amino Acids in the Murchison Meteorite". Proc. Natl. Acad. Sci. USA, **68**, 486 (1971).

68. Krishnamurthy, R. V., Epstein, S., Cronin, J. R., Pizzarello, S. and Yuen, G. U. "Isotopic and molecular analyses of hydrocarbons and monocarboxylic acids of the Murchison meteorite". Geochim. Cosmochim.Acta, **56**, 4045 (1992).

69. Bernstein, M. P., Dworkin, J. P., Sandford, S. A., Cooper, G. W. and Allamandola, L. J. "Racemic amino acids from the ultraviolet photolysis of interstellar ice analogues", Nature, **416**, 401(2002).

70. Whittet, D. C. B. "Is extraterrestrial organic matter relevant to the origin of life on earth?". Orig. Life Evol. Biosph., **27**, 249 (1997).

71. Pierazzo, E. and Chyba, C. F. "Amino acid survival in large cometary impacts". Meteoritics Planet. Sci., **34**, 909 (1999).

72. Butlerov. A. M. and Acad, C. R. "Formation synthétique d'une substance sucreé". Sci., **53**, 145 (1861).

73. Mizuno, T. and Weiss, A. H. "Synthesis and utilization of formose sugars". Adv. Carbohydr. Chem. Biochem., **29**, 173 (1974).

74. Breslow, R. "On the mechanism of the formose reaction". Tetrahedron Lett., **21**, 22 (1959).

75. Decker, P., Schweer, H., Pohlmann, R. and Chromatogr, J. "Bioids: X. Identification of formose sugars, presumable prebiotic metabolites, using capillary gas chromatography/gas chromatography—mass spectrometry of *n*-butoxime trifluoroacetates on OV-225". J. Chromatogr., A, **244**, 281 (1982).

76. Shapiro, R. "Prebiotic ribose synthesis: A critical analysis". Orig. Life Evol. Biosph., **18**, 71 (1988).

77. Ohmoto, H., Watanabe, Y. and Kumazawa, K. "Evidence from massive siderite beds for a CO_2-rich atmosphere before ~ 1.8 billion years ago". Nature, **429**, 395 (2004).

78. Ricardo, A., Carrigan, M. A., Olcott, A. N. and Benner, S. A. "Borate Minerals Stabilize Ribose". Science, **303**, 196 (2004).

79. Lambert, J. B., Lu, G., Singer, S. R. and Kolb, V. M. "Silicate Complexes of Sugars in Aqueous Solution". J. Am. Chem. Soc., **126**, 9611 (2004).

80. Oro, J. "Comets and the formation of biochemical compounds on the primitive Earth". Nature, **190**, 289 (1961).

81. Oro, J. "Mechanism of Synthesis of Adenine from Hydrogen Cyanide under Possible Primitive Earth Conditions". Nature, **191**, 1193 (1961).

82. Oro, J. "Synthesis of adenine from ammonium cyanide". Biochem. Biophys. Res. Commun., **2**, 407 (1960).

83. Oro, J. and Kimball, A. P. "Synthesis of purines under possible primitive earth conditions. I. Adenine from hydrogen cyanide". Arch. Biochem. Biophys., **94**, 217 (1961).

84. Oro, J. and Kimball, A. P. "Synthesis of purines under possible primitive earth conditions II. Purine intermediates from hydrogen cyanide". Arch. Biochem. Biophys., **96**, 293 (1962).

85. Joyce, G. F. "RNA evolution and the origins of life". Nature, **338**, 217 (1989).

86. Ferris, J. P. and Orgel, L. E. "Aminomalononitrile and 4-Amino-5-cyanoimidazole in Hydrogen Cyanide Polymerization and Adenine Synthesis". J. Am. Chem. Soc., **87**, 4976 (1965).

87. Ferris, J. P. and Orgel, L. E. "An Unusual Photochemical Rearrangement in the Synthesis of Adenine from Hydrogen Cyanide". J. Am. Chem. Soc., **88**, 1074 (1966).

88. Ferris, J. P., Donner, D. B. and Lotz, W. "Chemical evolution. IX. Mechanism of the oligomerization of hydrogen cyanide and its possible role in the origins of life". J. Am. Chem. Soc., **94**, 6968 (1972).

89. Ferris, J. P. and Hagan Jr, W. J. "HCN and chemical evolution: The possible role of cyano compounds in prebiotic synthesis". Tetrahedron, **40**, 1093 (1984).

90. Levy, M., Miller, S. L. and Oro J. "Production of Guanine from NH_4CN Polymerizations". J. Mol. Evol., **49**, 165 (1999).

91. Ferris, J. P. "Prebiotic Synthesis on Minerals: Bridging the Prebiotic and RNA Worlds". Biol. Bull., **196**, 311 (1999).

92. Orgel, L. E. "Prebiotic Adenine Revisited: Eutectics and Photochemistry". Orig. Life Evol. Biosph., **34**, 361 (2004).

93. Gibson, L. J. "Did Life Begin in an "RNA World?". Origins, **20**, 45 (1993).

94. Joyce, G. F., Schwartz, A. W., Miller, S. L. and Orgel, L. E. "The case for an ancestral genetic system involving simple analogues of the nucleotides". Proc. Natl. Acad. Sci. USA, **84**, 4398 (1987).

95. Eschenmoser, A. "Chemical Etiology of Nucleic Acid Structure". Science, **284**, 2118 (1999).

96. Ferris, J. P. "Montmorillonite Catalysis of 30–50 Mer Oligonucleotides: Laboratory Demonstration of Potential Steps in the Origin of the RNA World". Orig. Life Evol. Biosph., **32**, 311 (2002).

97. Isaac, R. and Chmielewski, J. "Approaching Exponential Growth with a Self-Replicating Peptide", J. Am. Chem. Soc., **124**, 6808 (2002).

98. Lee, D. E., Granja, J. R., Martinez, J. A., Severin, K. and Ghadiri, M. R. "A self-replicating peptide". Nature, **382**, 525 (1996).

99. Yao, S., Ghosh, I., Zutshi, R. and Chmielewski, J. "Selective amplification by auto- and cross-catalysis in a replicating peptide system". Nature, **396**, 447 (1998).

100. Larralde, R., Robertson, M. P. and Miller, S. L. "Rates of decomposition of ribose and other sugars: implications for chemical evolution". Proc. Natl. Acad. Sci. USA, **92**, 8158 (1995).

101. Lazcano, A. and Miller, S. L. "The Origin and Early Evolution of Life: Prebiotic Chemistry, the Pre-RNA World, and Time". Cell, **85**, 793 (1996).

102. Levy, M. and Miller, S. L. "The stability of the RNA bases: Implications for the origin of life". Proc. Natl. Acad. Sci. USA, **95**, 7933 (1998).

103. Shapiro, R. "The prebiotic role of adenine: A critical analysis". Orig. Life Evol. Biosph., **25**, 83 (1995).

104. Cleaves, H. J. and Miller, S. L. "Oceanic protection of prebiotic organic compounds from UV radiation". Proc. Natl. Acad. Sci. USA, **95**, 7260 (1998).

105. Eigen, M. and Schuster, P. "A principle of natural self-organization". Naturwissenschaften, **64**, 541 (1977).

106. Niesert, U., Harnasch, D. and Bresch, C. "Origin of life between scylla and charybdis". J. Mol. Evol., **17**, 348 (1981).

107. Dyson, F. "Origins of Life". pp. 48, Cambridge University Press, Cambridge (1999).

108. Fox, S. W. and Harada, K. "Thermal Copolymerization of Amino Acids to a Product Resembling Protein". Science, **128**, 1214 (1958).

109. Harada, K., Fox, S. W. "The Thermal Condensation of Glutamic Acid and Glycine to Linear Peptides", J. Am. Chem. Soc., **80**, 2694 (1958).

110. Fox, S. W. and Harada, K. "The Thermal Copolymerization of Amino Acids Common to Protein". J. Am. Chem. Soc., **82**, 3745 (1960).

111. Andini, S., Benedetti, E., Ferrara, L., Paolillo, L., Temussi, P. A. "NMR studies of prebiotic polypeptides", Orig. Life Evol. Biosph., **6**, 147 (1975).

112. Huber, C. and Wächtershäuser, G. "Peptides by Activation of Amino Acids with CO on (Ni, Fe) S Surfaces: Implications for the Origin of Life". Science, **281**, 670 (1998).

113. Huber, C., Eisenreich, W., Hecht, S. and Wächtershäuser, G. "A Possible Primordial Peptide Cycle". Science, **301**, 938 (2003).

114. Imai, E., Honda, H., Hatori, K., Brack, A. and Matsuno, K. "Elongation of Oligopeptides in a Simulated Submarine Hydrothermal System". Science, **283**, 831 (1999).

115. Flores, J. J. and Leckie, J. O. "Peptide Formation mediated by Cyanate". Nature, **244**, 435 (1973).

116. Chung, N., Lohrmann, R., Orgel, L. E. and Rabinowitz, J. "The mechanism of the trimetaphosphate-induced peptide synthesis". Tetrahedron, **27**, 1205 (1971).

117. Weber, L. A., Caroon, J. M., Warden, J. T., Lemmon, R. M. and Calvin, M. "Simultaneous peptide and oligonucleotide formation in mixtures of amino acid, nucleoside triphosphate, imidazole, and magnesium ion". Biosystems, **8**, 277 (1977).

118. Sawai, H. and Orgel, L. E. "Prebiotic peptide-formation in the solid state III. Condensation Reactions of Glycine in Solid State Mixtures Containing Inorganic Polyphosphates". J. Mol. Evol., **6**, 185 (1975).

119. Sawai, H., Lohrmann, R. and Orgel, L. E. "Prebiotic peptide-formation in the solid state II. Reaction of Glycine with Adenosine 5'-Triphosphate and P_1, P_2-Diadenosine-Pyrophosphate". J. Mol. Evol., **6**, 165 (1975).

120. Steinman, G., Lemmon, R. M. and Calvin, M. "Cyanamide: a possible key compound in chemical evolution". Proc. Natl. Acad. Sci. USA, **52**, 27 (1964).

121. Steinman, G. and Cole, M. N. "Synthesis of biologically pertinent peptides under possible primordial conditions". Proc. Natl. Acad. Sci. USA, **58**, 735 (1967).

122. Rabinowitz, J., Flores, J., Krebsbach, R. and Rogers, G. "Peptide Formation in the Presence of Linear or Cyclic Polyphosphates". Nature, **224**, 795 (1969).

123. Yamanaka, J., Inomata, K. and Yamagata, Y. "Condensation of oligoglycines with trimeta- and tetrametaphosphate in aqueous solutions". Orig. Life Evol. Biosph., **18**, 165 (1988).

124. Rishpon, J., O'Hara, P. J., Lahav, N. and Lawless, J. G. "Interaction between ATP, metal ions, glycine, and several minerals". J. Mol. Evol., **18**, 179 (1982).

125. Khorana, H. G., "The use of dicyclohexylcarodiimide in the synthesis of peptides". Chem. Ind., **23**, 1087 (1955).

126. Kamaluddin, Yanagawa, H. and Egami, F. "Possible role of aminoacetonitrile as condensing agent in chemical evolution". Ind. J. Biochem. Biophy., **18**, 215 (1981).

127. Odom, D., Lahav, N. and Chang, S. "Prebiotic nucleotide oligomerization in a fluctuating environment: Effect of kaolinite and cynamide". J. Mol. Evol., **12**, 259 (1979).

128. Odom, D., Rao, M., Oró, J. and Lawless, J. "Association of nucleotides with homoionic clays". J. Mol. Evol., **12**, 365 (1979).

129. Halmann, M., "Cyanamide–induced condensation reactions of glycine". Arch. Biochem. Biophys., **128**, 808 (1968).

130. Lahav, N., White, D. and Chang, S. "Peptide formation in the prebiotic era: thermal condensation of glycine in fluctuating clay environments". Science, **201**, 67 (1978).

131. Flores, J. J. and Bonner, W. A. "On the asymmetric polymerization of aspartic acid enantiomers by kaolin". J. Mol. Evol., **3**, 49 (1974).

132. Bujdak, J., Faybikova, K., Eder, A. H., Yongyai, Y. and Rode, B. M. "Peptide chain elongation: A possible role of montmorillonite in prebiotic synthesis of protein precursors". Orig. Life Evol. Biosph., **25**, 431(1995).

133. Bujdak, J., Slosiarikove, H., Texler, N., Schwendinger, M. G., Rode B. M. "On the possible role of montmorillonites in prebiotic peptide formation". Monatsh. Chem., **125**, 1033 (1994).

134. Basiuk, V. A., Gromovoy, T. Y., Golovaty, V. G. and Glukhoy, A. M. "Mechanisms of amino acid polycondensation on silica and alumina surfaces". Orig. Life Evol. Biosph., **20**, 483(1990).

135. Bujdak, J. and Rode, B. M. "Silica, Alumina, and Clay-Catalyzed Alanine Peptide Bond Formation". J. Mol. Evol., **45**, 457 (1997).

136. Bujdak, J. and Rode B. M. "Silica, Alumina and Clay Catalyzed Peptide Bond Formation: Enhanced Efficiency of Alumina Catalyst". Orig. Life Evol. Biosph., **29**, 451 (1999).

137. Bujdak, J. and Rode, B. M. "Activated alumina as an energy source for peptide bond formation: Consequences for mineral-mediated prebiotic processes". Amino Acids, **21**, 281 (2001).

138. Bujdak, J. and Rode, B. M. "Alumina catalyzed reactions of amino acids". J. Therm. Anal. Calorim., **73**, 797 (2003).

139. Bernal, J. D. "The physical bases of Life". Routledge and Kegan Paul, London, **80** (1951).

140. Ferris, J. P., Huang, C. H. and Hagan, Jr, W. J. "Clays as prototypical enzymes for the prebiological formation of phosphate esters". Orig. Life Evol. Biosph., **17**, 173 (1986).

141. Ferris, J. P., Ertem, G. and Agarwal, V. K. "Mineral catalysis of the formation of dimers of 5'-AMP in aqueous solution: the possible role of montmorillonite clays in the prebiotic synthesis of RNA". Orig. Life Evol. Biosph., **19**, 165 (1989).

142. Kebbekus, R. B. S. "Thesis", Rensselaer Polytechnic Institute, Troy, NY I2180-3590, U&A (1988).

143. Ferris, J. P. and Ertem, G. "Oligomerization reactions of ribonucleotides: the reaction of the 5'-phosphorimidazolide of adenosine with diadenosine pyrophosphate on montmorillonite and other minerals". Orig. Life Evol. Biosph., **23**, 229 (1993b).

144. Ferris, J. P. and Ertem, G. "Montmorillonite catalysis of RNA oligomer formation in aqueous solution. A model for the prebiotic formation of RNA". J. Am. Chem. Soc., **115**, 12270 (1993a).

145. Ding, Z. P, Kawamura, K., and Ferris, J. P. "Oligomerization of uridine phosphorimidazolide on montmorillonite: a model for the prebiotic synthesis of RNA on minerals". Orig. Life Evol. Biosph. **26**, 151(1996).

146. Kawamura, K. and Ferris, J. P. "Clay catalysis of oligonucleotide formation: kinetics of the reaction of the 5'-phosphorimidazolides of nucleotides with the non-basic heterocycles uracil and hypoxanthine". Orig. Life Evol. Biosph., **29**, 563 (1999).

147. Kawamura, K. and Ferris, J. P. "Kinetic and mechanistic analysis of dinucleotide and oligonucleotide formation from the 5'-phosphorimidazolide of adenosine on Na+ montmorillonite". J. Am. Chem. Soc., **116**, 7564 (1994).

148. Ferris, J. P., Hill, Jr A. R., Liu, R. and Orgel, L. E. "Synthesis of long prebiotic oligomers on mineral surfaces". Nature, **381**, 59 (1996).

149. Huang, W, and Ferris, J. P. "Synthesis of 35- 40 mers of RNA oligomers from unblocked monomers: A simple approach to the RNA world". Chemical Communications, **2003**, 1458 (2003).

150. Ferris, J. P. "Montmorillonite-catalysed formation of RNA oligomers: the possible role of catalysis in the origins of life". Phil. Trans. R. Soc., **361**, 1777 (2006).

151. Franchi, M. and Gallori, E. "A surface-mediated origin of the RNA world: Biogenic activities of clay-adsorbed RNA molecules". Gene, **346**, 205 (2005).

152. Ferris, J. P., Joshi, P. C., Wang, K. J. Miyakawa, S. and Huang, W. "Catalysis in prebiotic chemistry: Application to the synthesis of RNA oligomers". Adv. Space Res., **33**, 100 (2004).

153. Bujdak, J. Son Le, H. and Rode, B. M. "Montmorillonite catalyzed peptide bond formation: The effect of exchangeable cations". Journal of Inorganic Biochemistry, **63**, 199 (1996).

154. Lawless, J. G. and Levi, N. "The role of metal ions in chemical evolution: Polymerization of alanine and glycine in a cation-exchanged clay environment". J. Mol. Evol., **13**, 281 (1979).

155. Akira, S., Neal, B. and Ponnamperuma, C. "Synthesis of amino acids under primitive Earth conditions in the presence of clay". Origin Life, Proc. ISSOL Meet, **2nd** (1978).

156. Friebele, E., Shimoyama, A. and Ponnamperuma, C. "Adsorption of protein and nonprotein amino acids on a clay mineral: A possible role of selection in chemical evolution". J. Mol. Evol., **16**, 269 (1980).

157. Lavrent'ev, G. A., Rakitin, L. Yu., Strigunkova, T. F. and Egorov, I. A. "Modelling of the synthesis of peptide-like compounds in the lithosphere of the primitive Earth". Biochem, **261**, 1263 (1981).

158. Saladino, R, Crestini, C, Ciambecchini, U., Ciciriello, F., Costanzo, G., and Di Mauro, E. "Synthesis and degradation of nucleobases and nucleic acids by formamide in the presence of montmorillonite", Chembiochem, **5**, 1558 (2004).

159. Beck, M. T. and in Sigel, H. (ed.) "The possible role of transition metal complexes in chemical evolution". Marcel Dekker, New York Metal ions in biological systems, **7**, 1 (1978)

160. Orgel, L. E. "Sedimentary minerals under reducing conditions". Origins of Life and Evol. Biochem., 369 (1974).

161. Kamaluddin and Ali, S. R. "Studies on copper chromicyanide as prebiotic catalyst". Astrobiology, **7**, 153 (2004).

162. Ali, S. R., Alam, T. and Kamaluddin "Interaction of tryptophan and phenylalanine with metal ferrocyanides and its relevance in chemical evolution". Astrobiology, **4**, 420 (2004).

163. Ali, S. R., and Kamaluddin "Interaction of aromatic amino acids with metal hexacyanochromate (III) complexes: A possible role in chemical evolution". Bull. Chem. Soc. Jpn., **79**, 1541 (2006).

164. Ali, S. R. and Kamaluddin "The interaction of ribonucleotides with metal hexacyanochromate (III) and the relevance to chemical evolution". Bull. Chem. Soc. Jpn., **77**, 1681 (2004).

165. Viladkar, S., Agarwal, S., Rachana and Kamaluddin "Adsorption of adenine, adenosine, and adenosine nucleotides on nickel (II) hexacyanoferrate (II)". Bull. Chem. Soc. Jpn., **69,** 95 (1996).

166. Kamaluddin, Nath, M. and Sharma, A. "Role of metal ferrocyanides in chemical evolution. Adsorption of ribose and 2'-deoxyribose 5'-nucleotides on metal ferrocyanides". Orig. Life Evol. Biosph., **24**, 469 (1994).

167. Egami, F. "Minor elements and evolution". J. Mol. Evol, **4**, 113 (1974).

168. Kobayashi, K. and Ponnamperuma, C. "Trace elements in chemical evolution I", Orig. Life, **15**, 55 (1985).

169. Kobayashi, K. and Ponnamperuma, C. "Trace elements in chemical evolution II". Orig. Life, **16**, 67 (1985).

170. Kobayashi, K. and Haraguchi, H. "Metalloenzymes in sea water–their significance in geochemistry and chemical evolution". Nihon Kaisui Gakkaishi, **42**, 168 (1988).

171. Basiuk, V. A. and Sainz-Rojas, J. "Catalysis of peptide formation by inorganic oxides: High efficiency of alumina under mild conditions on the Earth–like planets". Adv. Space Res., **27**, 225 (2001).

172. Meng, M., Stievano, L. and Lambert, J. F. "Adsorption and thermal condensation mechanisms of amino acids on oxide supports, glycine on silica". Langmuir, **20**, 923 (2004).

173. Tzvetkov, G., Koller, G., Zubavichus, Y., Fuchs, O., Casu, M. B., Heske, C., Umbach, E., Grunze, M., Ramsey, M. G. and Netzer, F. P. "Bonding and structure of glycine on ordered Al_2O_3 film surfaces". Langmuir, **20**, 10551 (2004).

174. Matrajt, G. and Blanot, D. "Properties of synthetic ferrihydrite as an amino acid adsorbent and a promoter of peptide bond formation". Amino Acids, **26**, 153 (2004).

175. Weber, A. L. "Prebiotic sugar synthesis: Hexose and hydroxyl acid synthesis from glyceraldehydes catalyzed by Iron (III) hydroxide oxide". J. Mol. Evol., **35**, 1 (1992).

176. Bujdak, J. and Rode, B. M. "Activated alumina as energy source for peptide bond formation: Consequences for mineral-mediated prebiotic processes". Amino acids, **21**, 281(2001).

177. Bujdak, J. and Rode, B. M. "Peptide bond formation on the surface of activated alumina: Peptide chain elongation". Catalysis letters, **91**, 149 (2003).

178. Bowman, M. K., Ohara, S., Sverjensky, D. A., Hazen, R. M., and James, H. C. "Catalytic peptide hydrolysis by mineral surface: Implications for prebiotic chemistry". Geochimica et Cosmochimica Acta, **74**, 5852 (2010).

179. Saladino, R., Crestini, C., Costanzo, G., Negri, R. and Di Mauro, E. "A possible prebiotic synthesis of purine, adenine, cytosine, and 4(3H)-pyrimidinone from formamide: Implications for the origin of life". Bioorganic and Medicinal Chemistry, **9**, 1249 (2001).

180. Saladino, R., Ciambecchini, U., Crestini, C., Costanzo, G., Negri, R., and Di Mauro, E. "One-pot TiO_2-catalyzed synthesis of nucleic bases and acylonucleosides from formamide: Implications for the origin of life". Chembiochem, **4**, 514 (2003).

CHAPTER-2

EXPERIMENTAL

2.1 Chemicals Used

The chemicals used in the present work in the thesis are as follows:

Manganese acetate (Merck), Ammonium oxalate, liquid ammonia (Rankem), 5'-AMP (Sigma), 5'-GMP (Sigma), 5'-CMP(Sigma), 5'-UMP(Sigma), hydrochloric acid (Merck), sodium hydroxide (Merck), formamide (Sigma-Aldrich), orthophosphoric acid (Merck), potassium dihydrogen phosphate (Merck), cytosine (Sigma), 4(3H)-pyrimidinone (Aldrich), adenine (Sigma), purine (Aldrich), L–alanine (Fluka), Sodium hexane sulphonate (Rankem,, HPLC grade), L-alanyl-L-alanine (Fluka), glycine (Fluka), glycylglycine (Fluka), glycylglycylglycine (Fluka), aniline (Merck), p-toluidine (Merck), p-chloroaniline (Merck), p-anisidine (Merck), potassium hydroxide (Merck). All other chemicals used were of analytical grade. Millipore water was used throughout the studies.

2.2 Synthesis of Metal Oxides

2.2.1 Synthesis of Manganese Oxides (MnO, Mn_2O_3 and Mn_3O_4):

Manganese oxides were prepared from manganese oxalate through a reported method. First, manganese oxalate was synthesized from two micro-emulsions (I and II) as described below. In the second step, the manganese oxalate particles were subjected to thermal decomposition to yield manganese oxides. MnO, Mn_2O_3 and Mn_3O_4 were obtained by thermal decomposition of manganese oxalate in vacuum (12 h), air (6 h), and nitrogen (8h), respectively. Micro-emulsion **I** is composed of cetyltrimethylammonium bromide (CTAB) as the surfactant, n-butanol as the co-surfactant, isooctane as the hydrocarbon phase and 0.1 M manganese acetate solution as the aqueous phase. Micro-emulsion **II** is comprised of the same constituents as above except for having 0.1 M ammonium oxalate instead of manganese acetate as the aqueous phase. The weight fractions of various constituents in these micro-emulsions are as follows: 16.76% CTAB, 13.90% n-butanol, 59.29% isooctane and 10.05% aqueous phase. These two micro-emulsions were mixed slowly and stirred overnight on a magnetic stirrer, and the resulting precipitate was

separated from the apolar solvent and surfactant by centrifuging and washing it with 1:1 mixture of methanol and chloroform. The precipitate was dried in an oven at 120 ^{0}C for 1 h. The precursors were heated under different conditions to obtain the oxides. The precursor on heating in air at 450 ^{0}C for 6 h leads to the formation of Mn_2O_3. Under nitrogen at 500 ^{0}C (8 h) Mn_3O_4 was obtained. MnO was obtained by decomposing manganese oxalate in a sealed quartz tube at a pressure ~10^{-5} torr. The sealed tube was heated slowly to 500 ^{0}C at the rate of 50 ^{0}C h^{-1}. It was then annealed for 12 h and then cooled at the rate of 25 ^{0}C h^{-1}.

2.2.2 Synthesis of Manganese dioxide (MnO_2):

Manganese dioxide (MnO_2) was prepared as follows: Manganese acetate ($MnAc_2 \cdot 4H_2O$) and citric acid ($C_6H_8O_7 \cdot H_2O$) in a mol ratio 1:2 were dissolved in distilled water (500ml) in a beaker. The solution was adjusted to pH ~6 by addition of ammonia. Then, the solution was heated to 80 ^{0}C with a magnetic stirring and kept at this temperature for several hours until a wet gel was obtained. The wet gel was dried at 110 ^{0}C in a drying box, and a dried gel was obtained. Then the dried gel was calcined at 380 ^{0}C for 12 h in a muffle furnace. The calcined product was treated with a 2M aqueous H_2SO_4 solution (50ml) for 2 h at 80 ^{0}C with magnetic stirring in order to increase the degree of oxidation of the product. After acid-treatment, the product was rinsed with distilled water and filtered, then dried at 105 ^{0}C, and finally the brownish black manganese dioxide material was obtained.

Synthesized manganese oxides were ground and sieved through a 100 mesh sieve and were subjected to spectral (1600 series FTIR, Perkin-Elmer and XRD (PW1410/20, Philips Hall) and adsorption studies.

2.3 Characterization of Metal Oxides

Metal oxides were characterized by powdered X–ray diffraction analysis, FE-SEM, TEM, surface area measurement and IR spectral studies.

2.3.1 Powder X–Ray Diffraction Studies

Synthesized metal oxides were analyzed by powder X-ray diffraction (XRD) using a Bruker AXS D8 powder diffractometer. The X-ray diffraction analysis was performed under the following conditions.

Target metal = Cu

λ = 1.5418 A°

Current = 45 mA

Scanning = 5° – 90°

Chart speed = 1 cm min^{-1}

Voltage = 40 KV

G. M. speed = 2^0 min^{-1}

Range = 2 KC δ^{-1}

The X- ray diffraction spectrograms were used to calculate relative intensities and value of θ for prominent peaks. The values of θ were employed for calculation of interplaner spacing (d) values using the following equation:

$$\lambda = 2d \sin\theta$$

$$d = \lambda / (2 \sin\theta)$$

The relative intensities and d values were used to check the purity of the synthesized oxides by comparing them with the reported values.

Figures 2.1-2.5 represent the XRD spectra of manganese oxalate (Mn $(C_2O_4).2H_2O$), manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)) respectively, While, Tables 2.3-2.7 represent the d spacing corresponding to manganese oxalate (Mn $(C_2O_4).2H_2O$), manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)).

2.3.2 Field Emission Electron Microscopy (FE-SEM)

Field emission electron microscope (FE-SEM) images were recorded using a FEI Quanta 200F microscope operating at an accelerating voltage of 20 kV. FE-SEM images indicate that the particles of manganese oxides are agglomerated. Manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)) show needle like morphology and irregular shaped particles (Figure 2.6).

2.3.3 Transmission Electron Microscopy (TEM)

In order to study the inner surface morphology, and to get information about agglomerated particles i. e. whether they contain homogeneously dispersed small particles with well defined grain boundaries, Transmission electron microscopy (TEM) images were recorded using a FEI TECNAI G2 microscope operating at 200 kV. It can be noticed that the mean size obtained from TEM was smaller than that obtained from SEM because of the higher resolution power of TEM (Figure 2.7). The selected area electron diffraction pattern for all the forms of manganese oxides are shown in Figure 2.7. The presence of well defined spots in the concentric rings in diffraction pattern indicates crystalline nature of samples. It can be concluded from TEM images that all manganese oxides are good crystalline form with larger particle size (50-200 nm) of spherical rod shape while, all the particle are of different size and shape. Some have rod like while other have simple spherical shape of size 20-200 nm range.

2.3.4 Fourier Transform-Infra Red Spectroscopy (FT-IR)

The infrared spectra of samples were recorded in KBr discs on a Perkin-Elmer FTIR spectrophotometer (Model Perkin-Elmer-1600 Series) USA. Figure 2.8 represents IR spectra of manganese oxides: (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)). Figures 2.9 represent IR spectra of standard ribose nucleotides: AMP, GMP, CMP and UMP respectively. Figures 2.10 represent IR spectra of

standard aromatic amines: p-toluidine, p-anisidine, aniline and p-chloroaniline respectively.

2.3.5 Surface Area Measurement

The specific surface area of individual metal oxide was determined using BET method. The method involved physical adsorption of N_2 at its boiling temperature. The equation for finding out surface area by BET method is as follows:

$$\frac{P}{V_a(Po-P)} = \frac{1}{V_m C} + \frac{C-1}{V_m C} \times \frac{P}{Po}$$

Where,

P = Adsorption equilibrium pressure

P_O = Saturated vapor pressure of adsorbate

Va = Volume of adsorbate corresponding to pressure P

V_m = Volume of adsorbate required for a mono layer coverage

C = A constant relating to the heat of adsorption

According to the BET method, a plot of P/V_a (P_o-P) against P/P_o yields a straight line when $P/P_o < 0.3$. From the slope and intercept of the straight line Vm can be calculated by the following equation.

$$Surface Area\,(m^2/g) = \frac{Vm \times N}{22414 \times w} \times Am$$

Where

V_m = Monolayer volume in ml at STP

N = Avogadro number

W = Weight of the powdered sample (g)

A_m = Cross Sectional area of adsorbate molecule

($0.162\ nm^2$ for N_2)

Surface area values of manganese oxides are given in Table 2.1

2.3.6 Magnetic Susceptibility Measurement

The magnetic susceptibility measurements of all the synthesized metal oxides were carried out on Vibrating Sample Magnetometer (Model 155, Princeton Applied Research). Magnetic moment for metal oxides was calculated using the formula

$$\mu eff = 2.84 \times \sqrt{RTM / Hw}$$

$$\mu eff = \sqrt{n(n+2)}$$

Where, μeff = Magnetic moment (BM)

R = Observed magnetic moment (e.m.u.)

H = Applied magnetic field (gause)

w = Weight of sample subjected to analysis

n = number of unpaired electrons present in complexes

Magnetic moment was used to calculate the number of unpaired electrons present in respective metal oxide. The values of μeff (cal) were obtained by the formula $\mu eff = \sqrt{n(n+2)}$. Number of unpaired electrons, n, and hence magnetic properties of the samples were determined. Number of unpaired electrons n and μeff (obs) for the metal oxides are shown in Table 2.2. In the case of manganese oxide magnetic measurements were carried out at temperatures ranging from 300 K to 100 K to determine the temperature of Morin transition.

We have carried out detailed magnetic studies of manganese oxalate nanorods as well as the oxides obtained from them. The high temperature susceptibility yields a Weiss temperature H = 236 K and an effective moment of 5.8 mB, consistent with Mn^{2+} expected for manganese oxalate. A sharp magnetic transition is observed near 15 K. This temperature is somewhat higher than the reported Neel temperature TN of manganese oxalate (2.6 K). However, previous studies have also found a peak in the susceptibility at a temperature much above TN. This has been attributed to the presence of linear spin chains

in the system. Magnetization of Mn_3O_4 nanoparticles (100 nm) are shown paramagnetic molar susceptibility yields H = 2240 K and an effective moment of 4.7 mB for x values taken between 200 and 300 K. The reduction in the moment could be in part due to the lack of nonlinearity of the susceptibility in the Curie plot. At low temperature, there is the expected ferrimagnetic transition at 43 K. Ferrimagnetism may also lead to the reduction of the moment due to competition between the spin sub lattices. In MnO nanoparticles (28 nm) the high temperature susceptibility yields H = 2400 K and an effective moment of 5.9 mB. The effective moment is somewhat less than that expected for Mn^{2+}. Bulk MnO has an antiferromagnetic transition at 118 K, but there is no indication of any magnetic transition in the neighborhood of TN, just a deviation from linearity in the Curie plot. However, there is a weak transition near 45 K. Close inspection of the ac susceptibility in the temperature range in the neighborhood of the anomaly suggests that it may be the result of Mn_3O_4 impurity. The presence of 1% Mn_3O_4 impurity would be sufficient to explain the observed signal. Since Mn_3O_4 is ferrimagnetic, its magnetization is much larger than that of an antiferromagnet.

The magnetization of a-Mn_2O_3 nanoparticles is shown paramagnetic susceptibility gives H = 2160 K and an effective moment of 5.0 mB, consistent with Mn^{3+} (effective moment 4.9 mB). At low temperatures, it shows two transitions, a broad one just above 80 K and the other near 40 K. The observation of two transitions in this compound has been reported in the literature for bulk Mn_2O_3, but again this magnetic anomaly could be attributed to about a 1% impurity of Mn_3O_4.

Nearly spherical grains of size 28 nm of MnO displayed weak antiferromagnetism. Mn_2O_3 (50 nm) exhibited an antiferromagnetic transition at about 80 K. Both MnO and Mn_2O_3 nanoparticles showed weak magnetic transitions near 45 K, indicative of a small amount of Mn_3O_4 impurity. The 100 nm sized spherical particles Mn_3O_4 were stabilized in a tetragonal structure and showed a ferrimagnetic transition at 43 K. The magnetic property

of the composites has been considered by measuring the magnetic susceptibility. The magnetic moment (lB) of as received MnO2 has been measured and is equal 4.12 B.M., which is almost equals 4.9 B.M. This confirmed that the Mn^{4+} ion in MnO_2 has three unpaired electrons (paramagnetic properties).

2.4 Estimation of Adsorbate Concentrations

Electronic spectra of ribose nucleotides were recorded using Shimadzu UV–1601 spectrophotometer and have shown characteristic $\lambda_{max.}$ values of ribose nucleotides at 259nm, 254nm, 278nm, and 262nm, for 5'-AMP, 5'-GMP, 5'-CMP and 5'-UMP, respectively. The concentration of ribose nucleotides namely, 5'-AMP, 5'-GMP, 5'-CMP and 5'-UMP was estimated spectrophotometrically which involved the measurement of nucleotides absorbance at the wavelength of maximum absorption (λ_{max}). Standard curves were prepared by plotting absorbance versus concentration (Table.2.8). The standard curves for ribose nucleotides are shown in Figures 2.11-2.14.

2.5 Adsorption Studies

2.5.1 Adsorption of Ribose Nucleotides

Adsorption of all the four ribose nucleotides on metal oxides was studied over wide concentration range. Ribose nucleotide solution 5 ml (5×10^{-5} M - 60×10^{-5} M) was added to the test tube having 25 mg of adsorbent each time. The required pH was adjusted by adding very small amount of concentrated HCl or NaOH solutions. The suspensions were shaken, then allowed to equilibrate and centrifuged at 5000 rpm on a Centrifuging machine (Model–220V 50~1ϕ Ac manufactured by REMI motors, Mumbai, India) and the supernatant liquid was decanted. The concentrations of nucleotides left over after adsorption i.e. equilibrium concentration was then determined spectrophotometrically. The amount of nucleotides adsorbed on metal oxides was calculated from the difference between their initial concentration and equilibrium concentration.

2.5.2 Determination of Adsorption Isotherms

The equilibrium concentration of adsorbate and amount (X_e) adsorbed per gram of adsorbent were used to plot the adsorption isotherms. The amount of adsorbate adsorbed on one gram of adsorbent has been calculated using the formula:

$$Xe = \frac{(Ci - Ceq) \times v \times M \times 1000}{w} mg/mg$$

Where

X_e = Amount (mg) of adsorbate adsorbed on one gram of adsorbent

Ci = Initial concentration of adsorbate

C_{eq} = equilibrium concentration of adsorbate

V = Volume of adsorbate solution

M = Molecular weight of adsorbate

w = Amount (mg) of adsorbent used

2.5.3 Determination of Percent Binding

The percent binding of aromatic acids and ribosenucleotides on metal oxides under study was determined at the saturation point of adsorption isotherms. The percent binding was calculated using the optical densities (O.D.) of adsorbate before and after adsorption as shown below:

$$\% \text{ Binding} = \left(\frac{\text{O.D. of adsorbate before adsorption - O.D. of adsorbate after adsorption}}{\text{O.D. of adsorbate before adsorption}} \right) \times 100$$

2.6 Formation of Nucleobases from Formamide in the Presence of manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2))

The reaction was carried out by taking formamide (5.7g, 5ml, 0.12 mol) in the presence of 50 mg of selected catalyst manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2) in the temperature range 100-160 ^{0}C for 12-96 h. A blank experiment (absence of manganese oxides) was also performed under similar reaction

conditions. The reaction mixture was centrifuged and filtered through 0.2 μm filter paper and then the mixture was divided into two parts, one part was used for HPLC analysis and the other for ESI-MS analysis. It was observed that the product gradually appeared after 12h and concentration of the products became constant after 48h thus the optimum time for the formation of the products is approximately 48 hrs. Low yields of the products were observed below 160 °C. Our main attention was mainly focused on the formation and identification of purine and pyrimidine derivatives at 160 °C where relatively high yield was observed. The main identified products were purine, thymine, 9-(hydroxyacetyl) purine, 4(3H)-pyrimidinone, cytosine and adenine. Only purine formed in the blank experiment.

2.6.1 HPLC Analysis

All the solutions obtained from the reaction system were analyzed with an Agilent 1100 series LC system using an Agilent Hypersil (ODS 5 μm/ 200 × 2.2 cm) column. The mobile phase used was a buffer solution of (KH_2PO_4 + H_3PO_4) of pH~4.05; with a flow rate of 0.75 ml/minute under isocratic condition at 260 nm.

The HPLC chromatograms showed a number of peaks including purines and pyrimidines in all the reaction mixtures. The products identified were purine, thymine, 9-(hydroxyacetyl) purine, cytosine, 4(3H)-pyrimidinone and adenine, which were confirmed by co-injection with authentic samples. It was noted that all the four manganese oxides used produced nearly the same products, with different % yield. Manganosite (MnO) has shown the highest yield of purine, thymine, 9-(hydroxyacetyl) purine, 4(3H)-pyrimidinone, cytosine and adenine from formamide. Hausmannite (Mn_3O_4) also showed a higher yield of purine compared to bixbyite (Mn_2O_3) but other products were in lesser yield. Pyrolusite (MnO_2) afforded the lowest yield of the products in comparison to other manganese oxides used. The yield obtained was measured in mg of product formed per gram of formamide with the help of standard curve shown in Figure 2.15-2.18.

2.6.2 Electro spray Ionization–Mass Spectrometry Analysis

A Bruker Esquire 4000 (Bruker Daltonics Data Analysis 3.3, Germany) ion trap mass spectrometer interfaced to electro spray ionization (ESI) source was used for mass analysis of nucleobases formed from formamide in the presence of manganese oxides. Ionization of analytes was carried out using the following setting of ESI: nebulizer gas flow 10 psi, dry gas 5 L min^{-1}, dry temperature 300 ^{0}C, capillary voltage 4000 V. Calibration of m/z was performed using ES-tuning mix. The ESI-MS/MS experiments of the products were also performed under the same conditions using positive ionization mode.

2.7 Oligomerization of Glycine and L–Alanine in the presence of manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2))

Each of the catalysts, manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)) were impregnated (0.1 g) with aqueous solution of amino acids: glycine, L-alanine and β-alanine (0.1 ml, 0.1M) separately. The suspension was dried at 90 ^{0}C for 3h and used to study possible formation of peptides. The samples were heated at three different temperatures of 50 ^{0}C, 90 ^{0}C and 120 ^{0}C for 1-35 days. The reaction was monitored after every seven days. No fluctuating drying/wetting conditions were simulated. Control experiments of amino acids without catalyst were also performed. After heating the samples, peptide condensation products obtained were washed with 1ml of 0.1M calcium chloride solution to leach out adsorbed amino acids and related reaction products. The supernatant liquid of the reaction was filtered and divided into two parts; one part of the filtrate was used for HPLC analysis while other for ESI-MS analysis.

2.7.1 HPLC Analysis:

All the solutions obtained from the reaction system were analyzed with HPLC (Waters 2489, binary system) equipped with a column of Waters (Spherisorp 5 μm ODS2 4.6mm × 250 mm). UV detection was performed at 200 nm wavelength. The mobile phase compositions were 10 mM sodium hexane sulphonate acidified with phosphoric acid to pH~2.5 (solvent A) and acetonitrile of HPLC grade (solvent B) with a flow rate of 1 ml/min. The reaction products were identified by retention time and co-injection method. Yields of the products were determined comparing peak area of products to the standards Figures 2.19-2.21.

2.7.2 ESI-MS Analysis:

A Bruker Esquire 4000 (Bruker Daltonics Data Analysis 3.3, Germany) ion trap mass spectrometer interfaced to electrospray ionization (ESI) source was used for mass analysis for the formation of peptides in the presence of manganese oxides. Ionization of analytes was carried out using the following setting of ESI: nebulizer gas flow 10 psi, dry gas 5 L min^{-1}, dry temperature 300 ^{0}C, capillary voltage 4000 V. Calibration of m/z was performed using ES-tuning mix. The ESI-MS/MS experiments of the products were also performed under the same conditions using positive ionization mode.

2.8 Adsorption and Oxidation of Aromatic Amines in the Presence of manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2))

2.8.1 Estimation of Amines Concentrations

The concentration of aromatic amines was estimated spectrophotometrically which involved the measurement of amine at the wavelength of maximum adsorption (λ_{max}). Electronic spectra of ribose nucleotides were recorded using Shimadzu UV–1601 spectrophotometer which showed characteristic λ_{max} values of amines at 280nm, 295nm, 290nm and 286nm, for aromatic amines namely aniline, p-anisidine, p-chloroaniline, p-

toluidine respectively. Standard curves were prepared by plotting absorbance versus concentration (Table.2.9). The standard curves for various amines are shown in Figures 2.22-2.25.

2.8.2 Adsorption of Aromatic Amines

Preliminary experiments were initially carried out to find out the suitable conditions for the adsorption of amines. The adsorption of aniline, p-toluidine, p-anisidine and p-chloroaniline on manganese oxides as a function of amine concentration (2.0×10^{-5} M to 1.4×10^{-4} M) was studied by adding 5 ml of amine solution to 0.1 g of manganese oxides each time. The amine-metal oxides suspensions were shaken initially for half an hour to equilibrate at temperature of 30 ^{0}C. The suspensions were centrifuged after 24 hr, supematant liquid decanted off, and leftover amine concentration in it was determined spectrophotometritally. For adsorption study, aniline and p-toluidine suspension was kept for 24 hr and p-chloroaniline suspension was kept for 72 hr due to poor adsorption. The amount of amine adsorbed has been calculated as the difference between amine concentration before and after adsorption. The concentration of amine at equilibrium and the amount adsorbed were used to obtain the adsorption isotherm.

2.8.3 Determination of Adsorption Isotherms

The equilibrium concentration of adsorbate and the amount (X_e) adsorbed per gram adsorbent was used to study the adsorption isotherms. The amount of adsorbate which adsorbed on one gram of adsorbent has been calculated using the formula:

$$X_e = \frac{(Ci - Ceq)\times V \times M \times 1000}{w}$$

Where

X_e = Amount (mg) of adsorbate adsorbed on one gram of adsorbent

C_i = Initial concentration of adsorbate

C_{eq} = equilibrium concentration of adsorbate

V = Volume of adsorbate solution

M = Molecular weight of adsorbate

w = Amount (mg) of adsorbent used

2.8.4 Determination of Percent Binding

The percent binding of aromatic amines on metal oxides under study was determined at the saturation point of adsorption isotherms. The percent binding was calculated using the optical densities (O.D.) of adsorbate before and after adsorption as shown below:

$$\%\,\text{Binding} = \left(\frac{\text{O.D of adsorbate before adsorption} - \text{O.D of adsorbate after adsorption}}{\text{O.D of adsorbate before adsorption}} \right) \times 100$$

2.8.5 Analysis of Reaction Products by GC-MS

In alkaline medium (pH>8) brown-red colored products of oxidized amine were deposited on the manganese oxide (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)) surfaces within 24 h. These colored products were extracted and concentrated in benzene for GC–MS analysis. Products thus formed were analyzed on a GC-MS (Perkin Elmer–Claurus-500) with FID detector equipped with a capillary column (ELITE-1 with stationary phase 100% dimethyl polysiloxane column length 30 m, diameter 0.32 mm). The conditions for GC were as follows: Injector temperature, 250 ^{0}C; transfer line temperature, 250 ^{0}C. The capillary column temperature was programmed as follows: 80 ^{0}C for 2 min; from 80 ^{0}C to 260 ^{0}C at 10 ^{0}C min^{-1}, held at 250 ^{0}C for 15 min. Helium was used as a carrier gas with a flow rate of 2 ml min^{-1}. The mass spectrometer conditions were ion source 250 ^{0}C and ionization energy 40 eV.

Table 2.1

Table 2.2 Surface area of manganese oxides

Metal oxides	Surface area values(m^2/g)
Manganosite (MnO)	238.89
Bixbyite (Mn_2O_3)	226.56
Hausmannite (Mn_3O_4)	218.35
Pyrolusite (MnO_2)	214.57

Table 2.2

Magnetic susceptibility data of Manganese oxides

Metal Oxide	$\mu_{eff\,(cal)}$ (B.M.)	$\mu_{eff\,(obs)}$ (B.M.)
Manganosite (MnO)	5.9	4.9
Bixbyite (Mn_2O_3)	4.9	5.0
Hausmannite (Mn_3O_4)	4.9	4.7
Pyrolusite (MnO_2)	3.9	4.1

Table 2.3

d spacing corresponds to manganese oxalate ($Mn(C_2O_4).2H_2O$)

d/Å (reported)	d/Å (observed)	h	k	l
5.65	5.65	1	0	1
4.59	4.60	1	0	2
3.66	3.68	1	0	3
3.62	3.61	0	1	3
3.13	3.18	2	0	0
3.04	3.05	0	2	0
2.836	2.852	2	0	2
1.982	1.994	3	1	0
1.777	1.789	1	3	3

Table 2.4

d spacing corresponds to Manganosite (MnO)

d/Å (reported)	d/Å (observed)	h	k	l
2.5449	2.5675	1	1	1
2.204	2.2067	2	0	0
1.5584	1.5718	2	2	0
1.3290	1.3494	3	1	1
1.2724	1.2817	2	2	2
1.102	1.098	4	0	0

Table 2.5

d spacing corresponds to Bixbyite (Mn_2O_3)

d/Å (reported)	d/Å (observed)	h	k	l
2.7164	2.7197	2	2	2
2.5149	2.5041	1	2	3
2.3525	2.3552	4	0	0
2.2179	2.1975	3	3	0
1.8454	1.8351	4	3	1
1.7180	1.6958	5	2	1
1.6634	1.6643	4	4	0
1.4186	1.4191	6	2	2
1.1762	1.1854	8	0	0

Table 2.6

Table 2.5 d spacing corresponds to Hausmannite (Mn_3O_4)

d/Å (reported)	d/Å (observed)	h	k	l
4.9203	4.9244	1	0	1
3.0854	3.0867	1	1	2
2.8825	2.8809	2	0	0
2.7624	2.7666	1	0	3
2.4871	2.4864	2	1	1
2.4601	2.4598	2	0	2
2.3605	2.3641	0	0	4
2.0382	2.0368	2	2	0
1.7945	1.7974	1	0	5
1.5764	1.5755	3	2	1
1.5427	1.5424	2	2	4
1.4412	1.4394	4	0	0

Table 2.7
d-spacing corresponds to Pyrolusite (MnO_2)

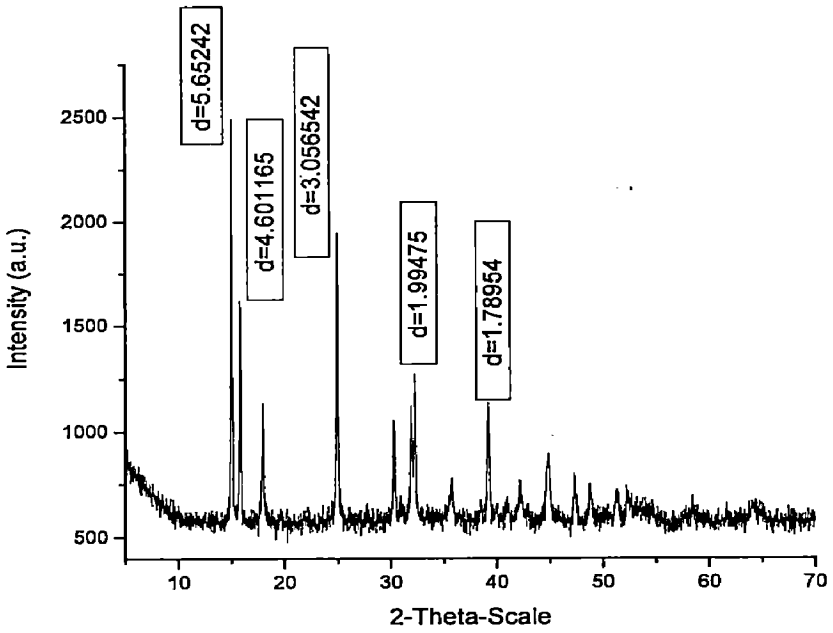
d/Å (reported)	d/Å (observed)	h	k	l
4.9203	4.9276	1	1	0
3.0854	3.0887	1	0	1
2.8825	2.8772	2	0	0
2.7624	2.7665	2	1	1
2.4871	2.4865	2	2	0
2.3605	2.3654	3	0	1
2.0382	2.0372	1	1	2
1.7945	1.7972	2	0	2
1.5764	1.5750	3	2	1
1.5427	1.5428	4	0	0

Table 2.8
Concentration versus absorbance data for standard curve of ribose nucleotides

Concentration ($Mx10^5$)	Absorbance			
	5'-AMP λ_{max} = 259 nm	5'-GMP λ_{max} = 254 nm	5'-CMP λ_{max} = 278 nm	5'-UMP λ_{max} = 262 nm
0	0	0	0	0
8	1.01	0.86	0.77	0.60
12	1.49	1.40	1.11	0.88
16	1.97	1.81	1.57	1.23
20	2.39	2.29	2.01	1.54
24	2.75	2.68	2.52	1.76
28	3.34	3.17	2.82	2.06

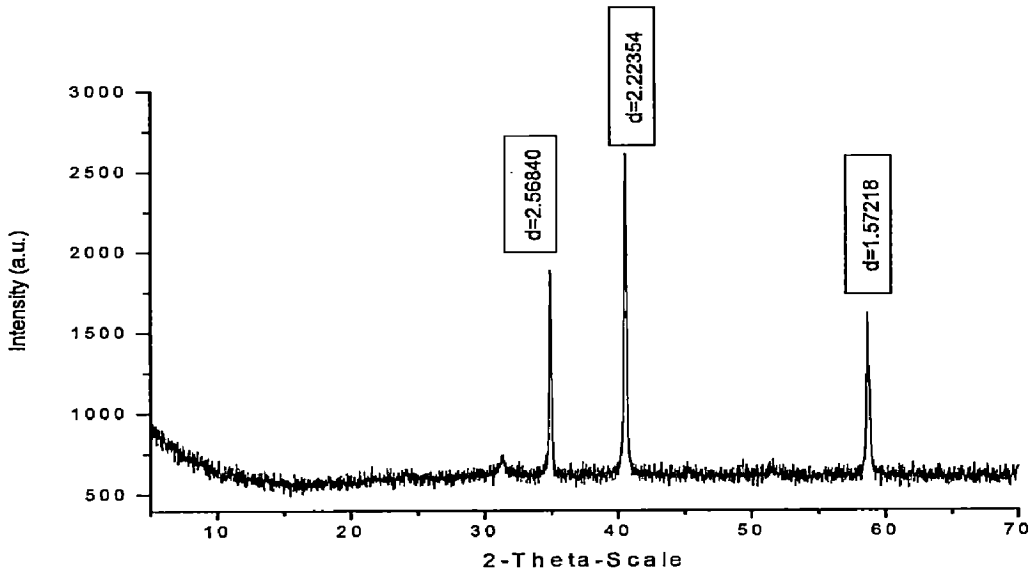
Table: 2.9
Concentration versus absorbance data for standard curve of amines

Concentration ($Mx10^4$)	Absorbance			
	Aniline λ_{max} = 280 nm	**p-chloroaniline** λ_{max} = 290nm	**p-anisidine** λ_{max}= 295nm	**p-toluidine** λ_{max} = 286 nm
0	0	0	0	0
1	0.13	0.204	0.213	0.263
2	0.28	0.44	0.44	0.47
4	0.54	0.891	0.94	0.92
6	0.821	1.372	1.45	1.35
8	1.083	1.824	1.95	1.76
10	1.385	2.272	2.45	2.18
12	1.662	2.772	2.95	2.62
15	2.12	3.452	3.74	3.214



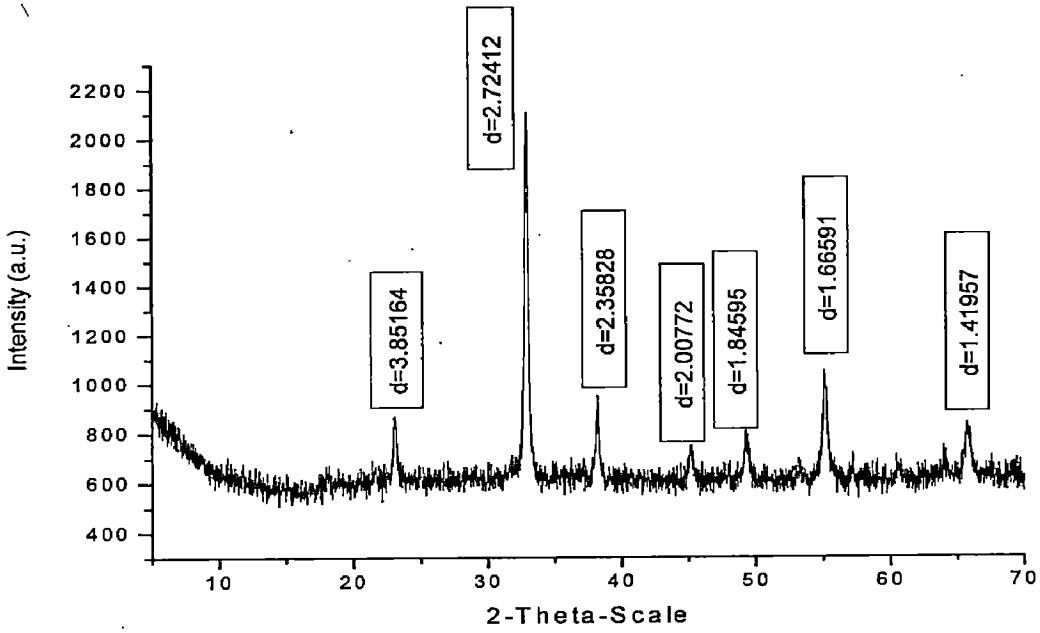
X- Ray diffraction pattern of manganese oxalate ($Mn(C_2O_4).2H_2O$)

Figure 2.1



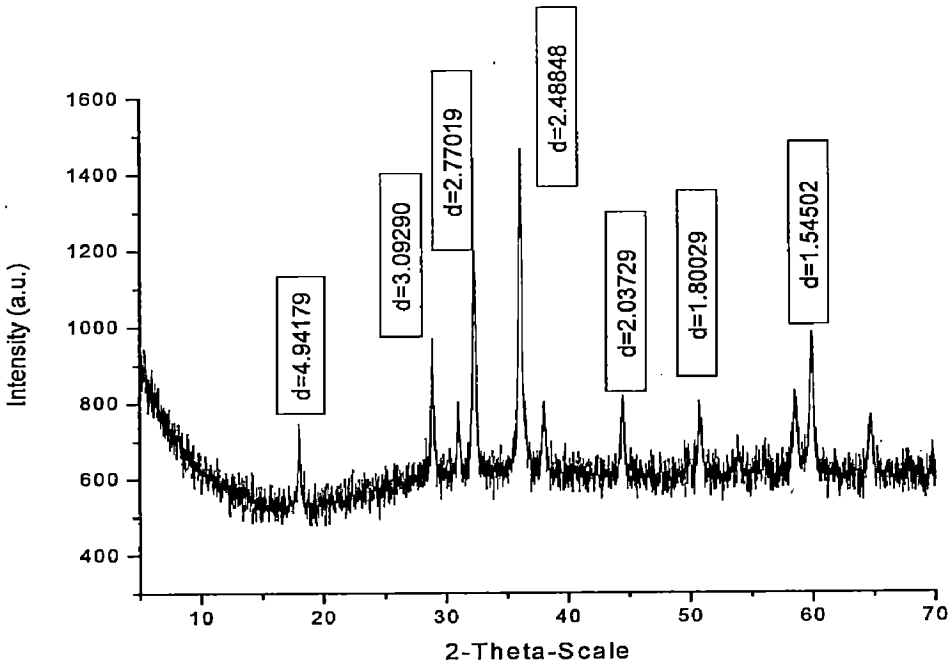
XRD spectra of Manganosite (MnO)

Figure 2.2



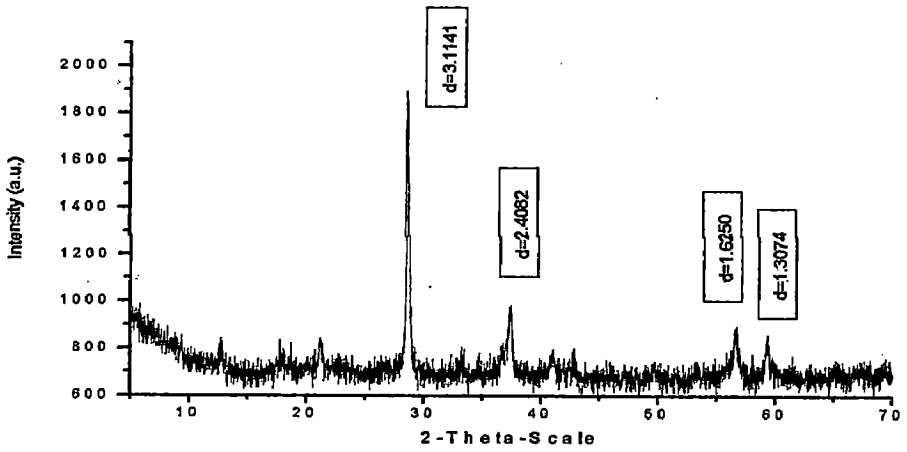
XRD spectra of Hausmannite (Mn_3O_4)

Figure 2.3



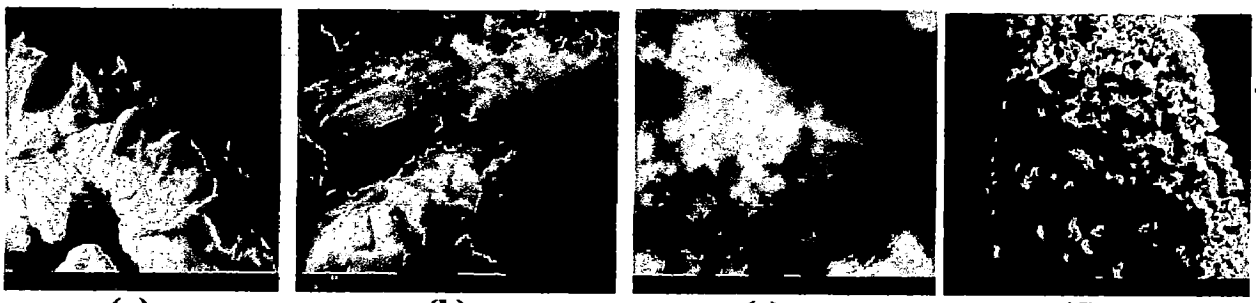
XRD spectra of Bixbyite (Mn_2O_3)

Figure 2.4



XRD spectra of Pyrolusite (MnO_2)

Figure 2.5



(a) (b) (c) (d)

FE-SEM images of manganese oxides (a): MnO,

(b): Mn_2O_3, (c): Mn_3O_4, (d): MnO_2

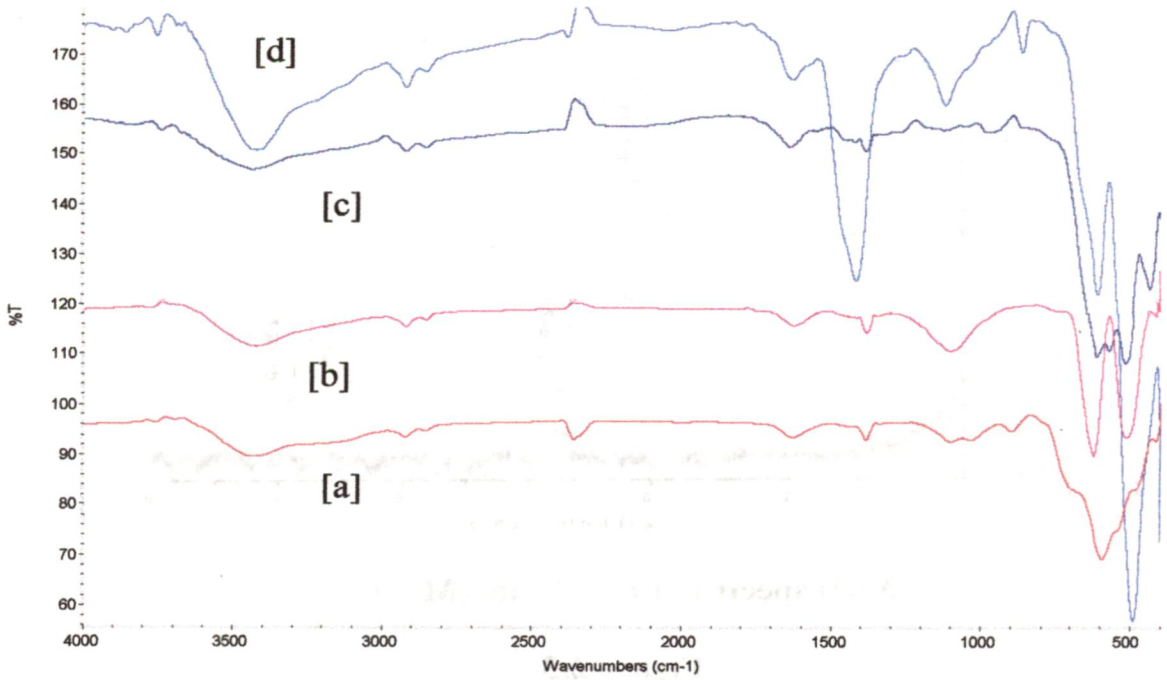
Figure 2.6

(a) (b) (c) (d)

TEM Images of Manganese Oxides (a): MnO,

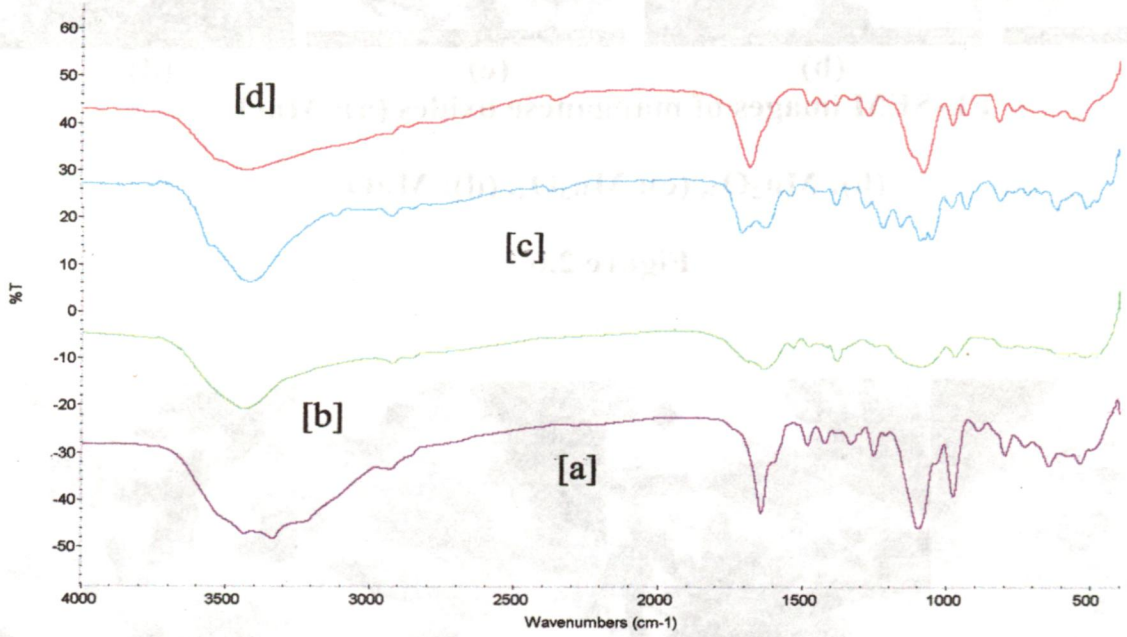
(b): Mn_3O_4, (c): Mn_2O_3 (d): MnO_2

Figure 2.7



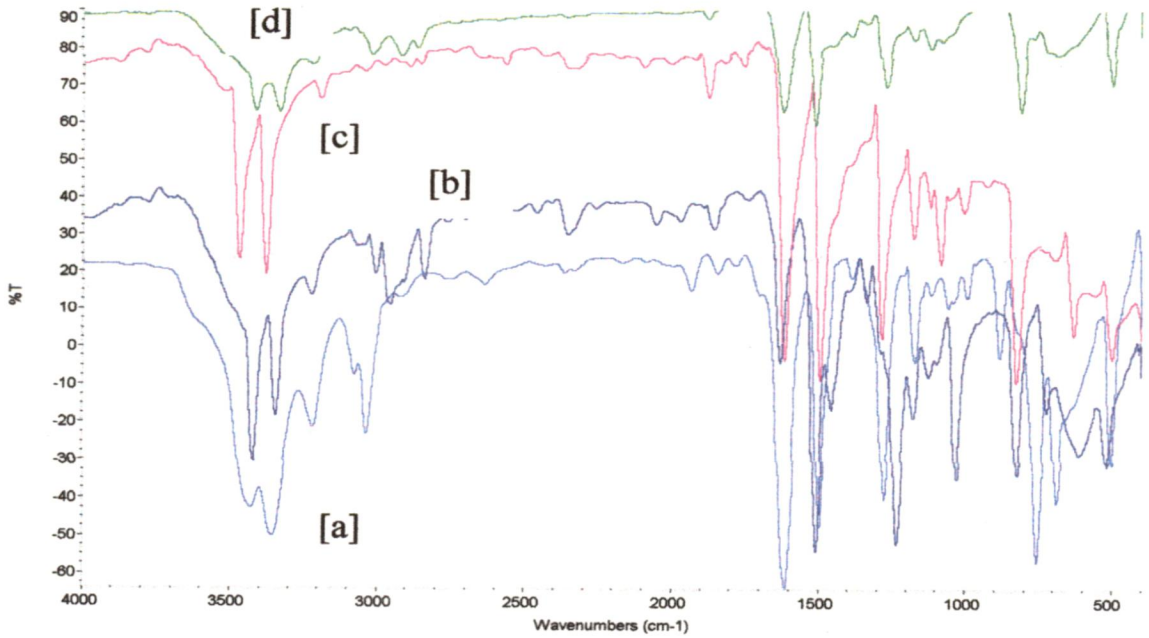
IR spectra of (a): manganosite (MnO), (b): bixbyite (Mn_2O_3), (c): hausmannite (Mn_3O_4), (d): pyrolusite (MnO_2)

Figure 2.8



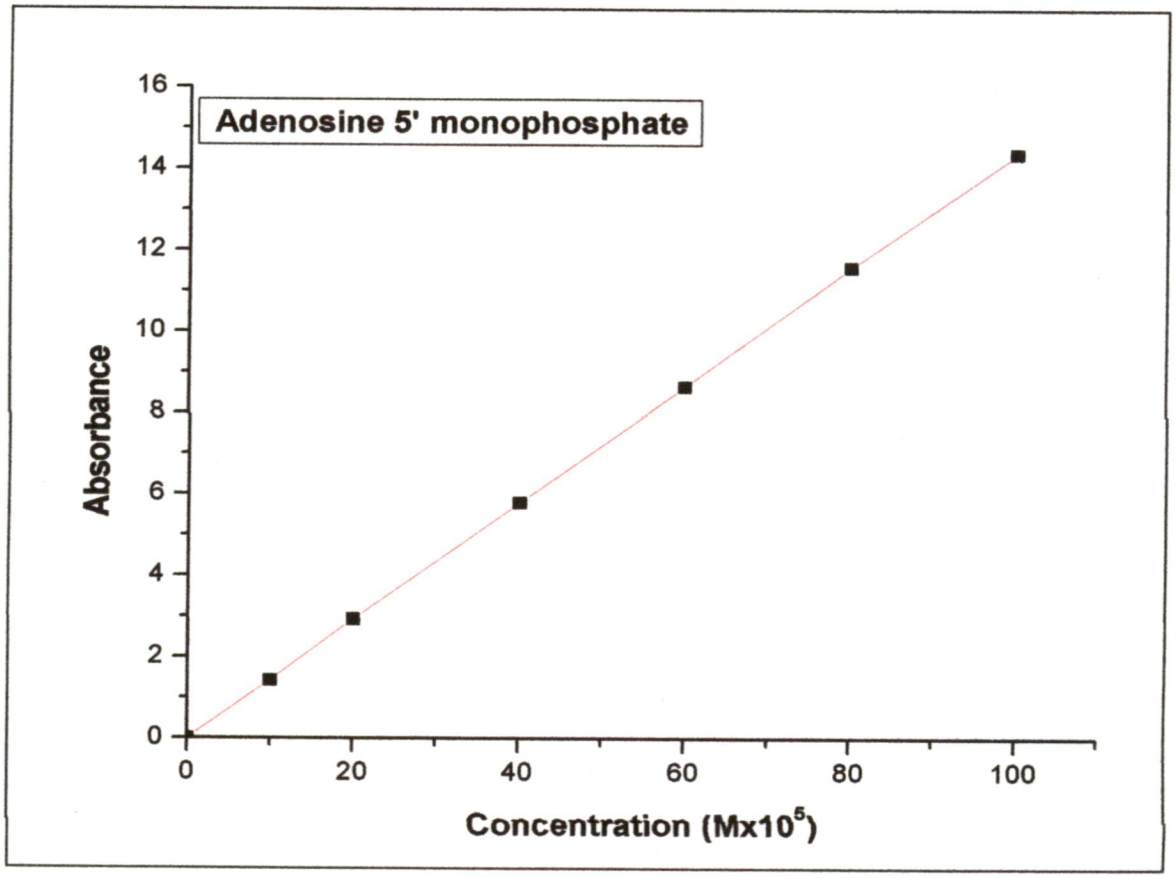
IR spectra of (a): AMP, (b): GMP, (c): CMP, (d): UMP

Figure 2.9



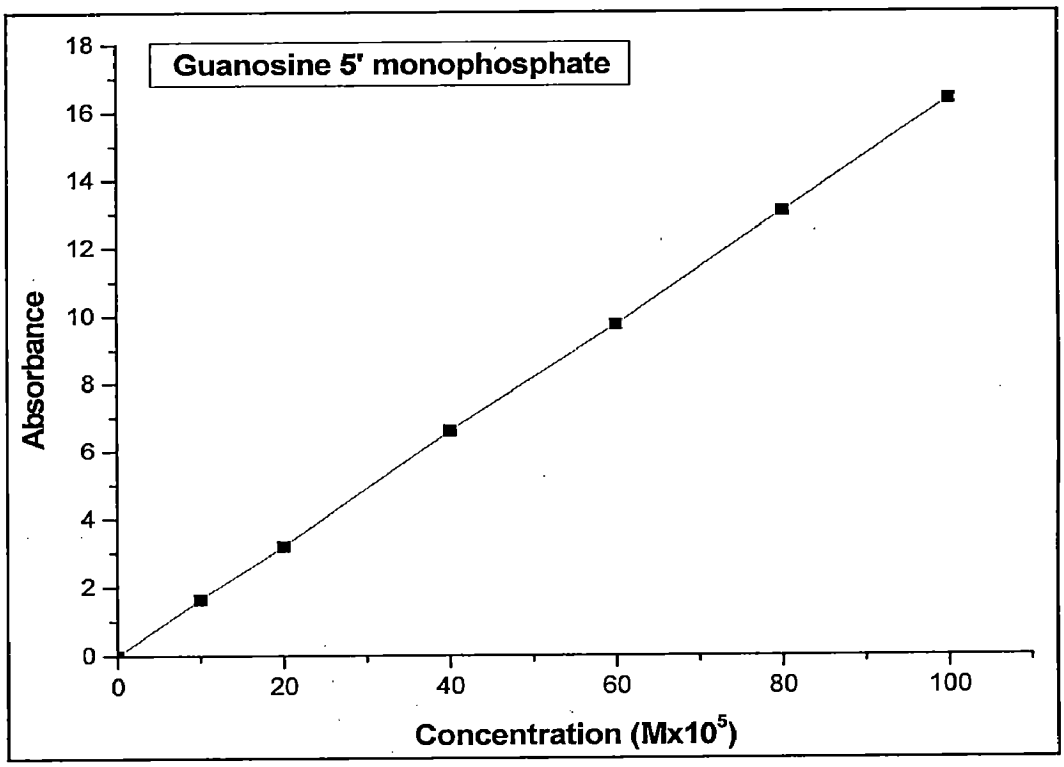
IR spectra of (a): aniline, (b): p-chloroaniline, (c): p-anisidine, (d): p-toluidine

Figure 2.10



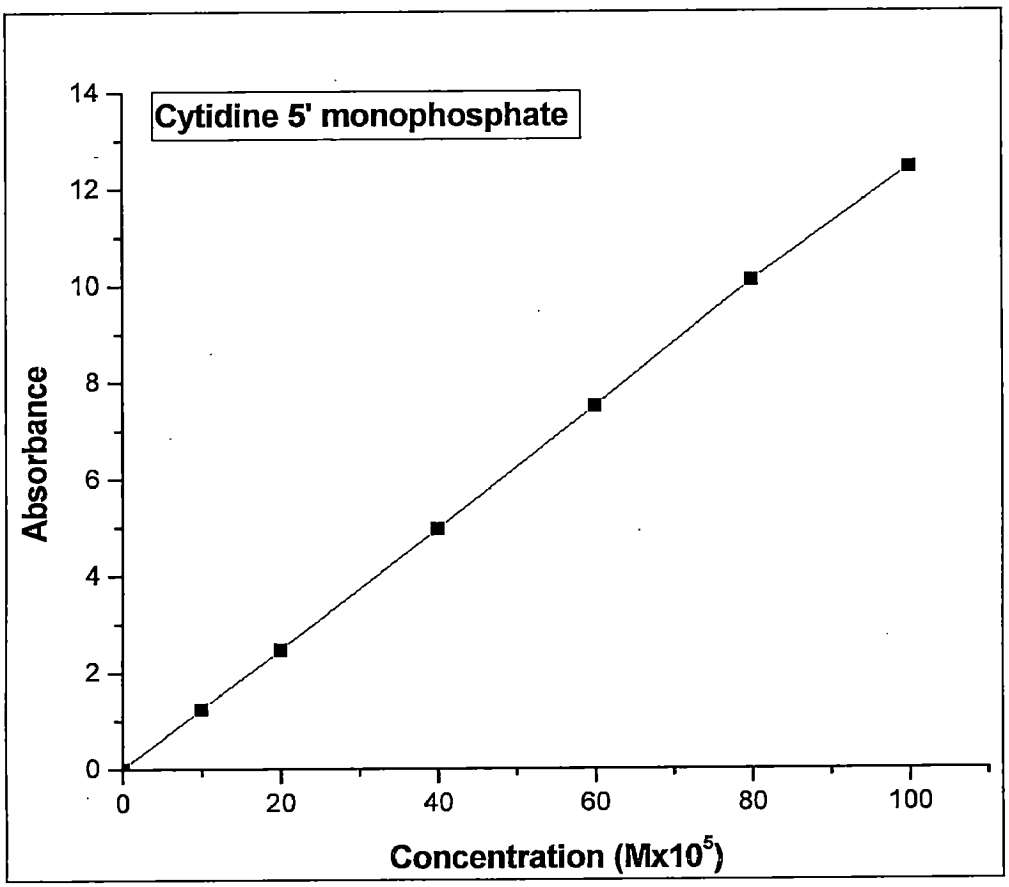
Standard curve of 5'-AMP

Figure 2.11



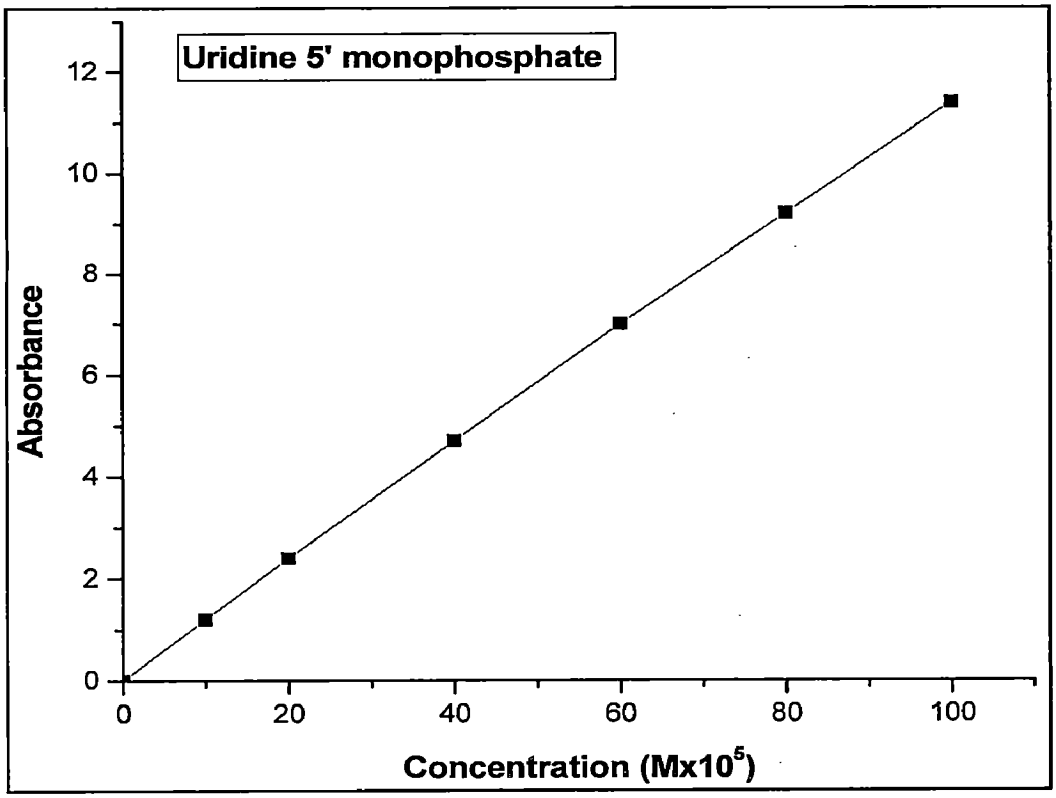
Standard curve of 5'-GMP

Figure 2.12



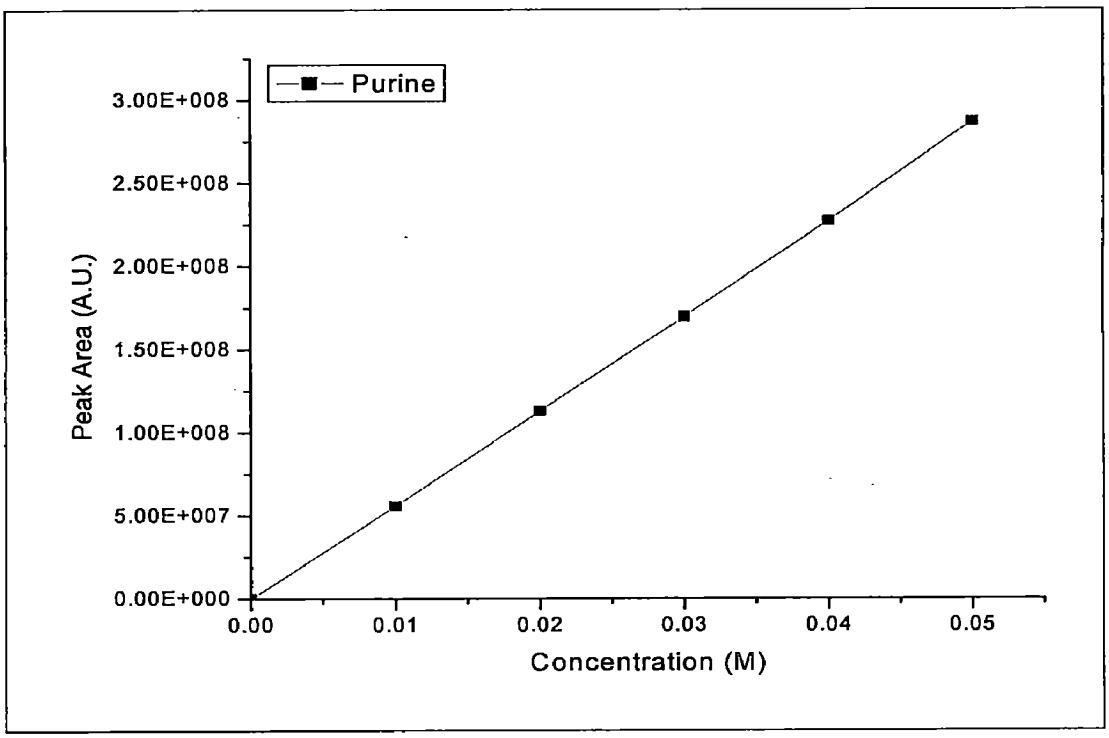
Standard curve of 5'-CMP

Figure 2.13



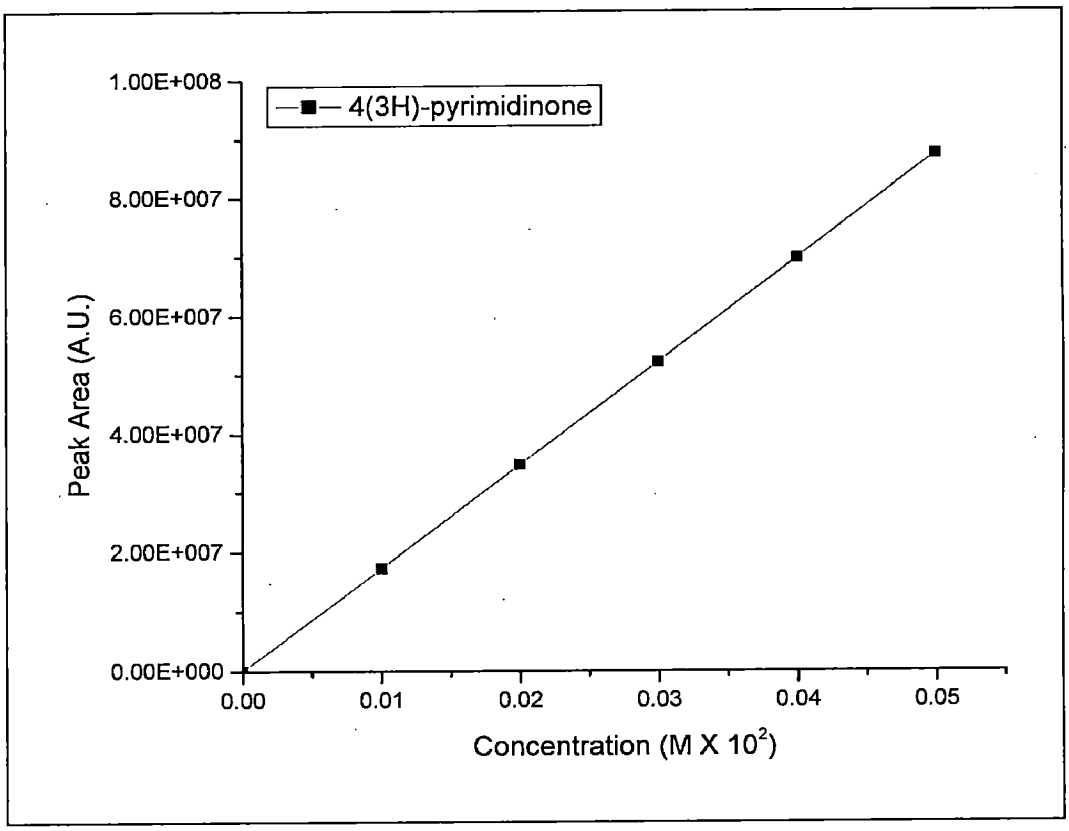
Standard curve of 5'-UMP

Figure 2.14



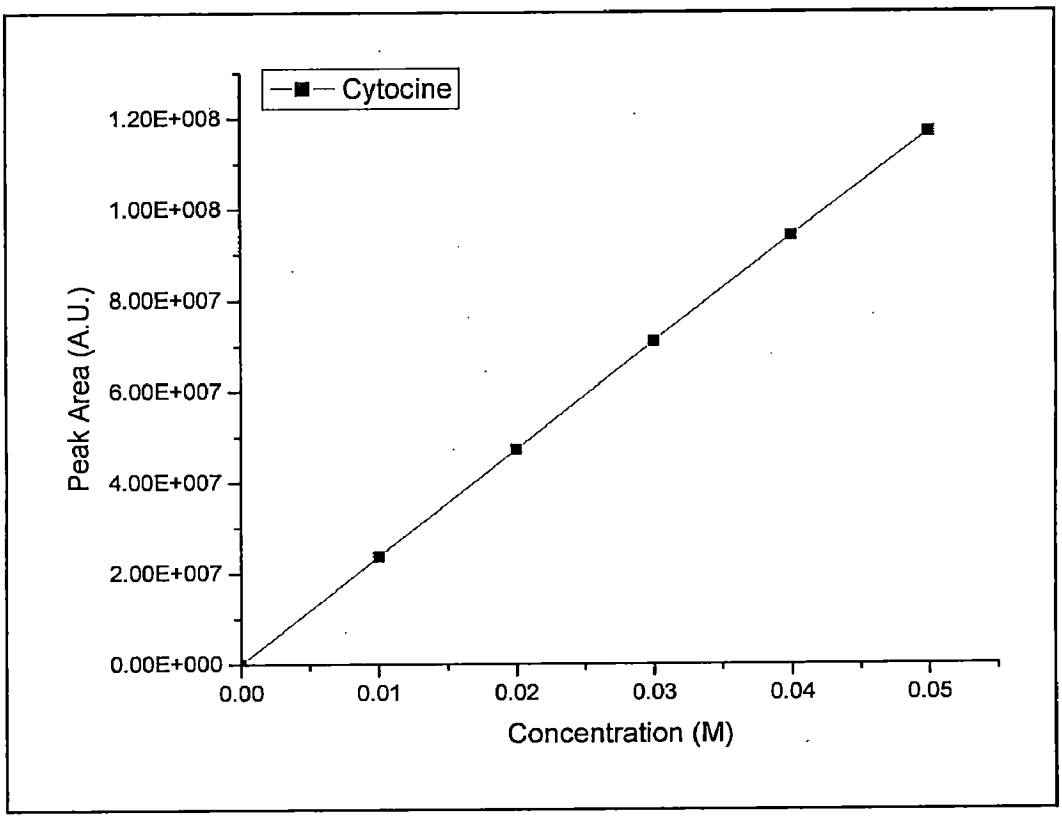
Standard curve of purine

Figure 2.15



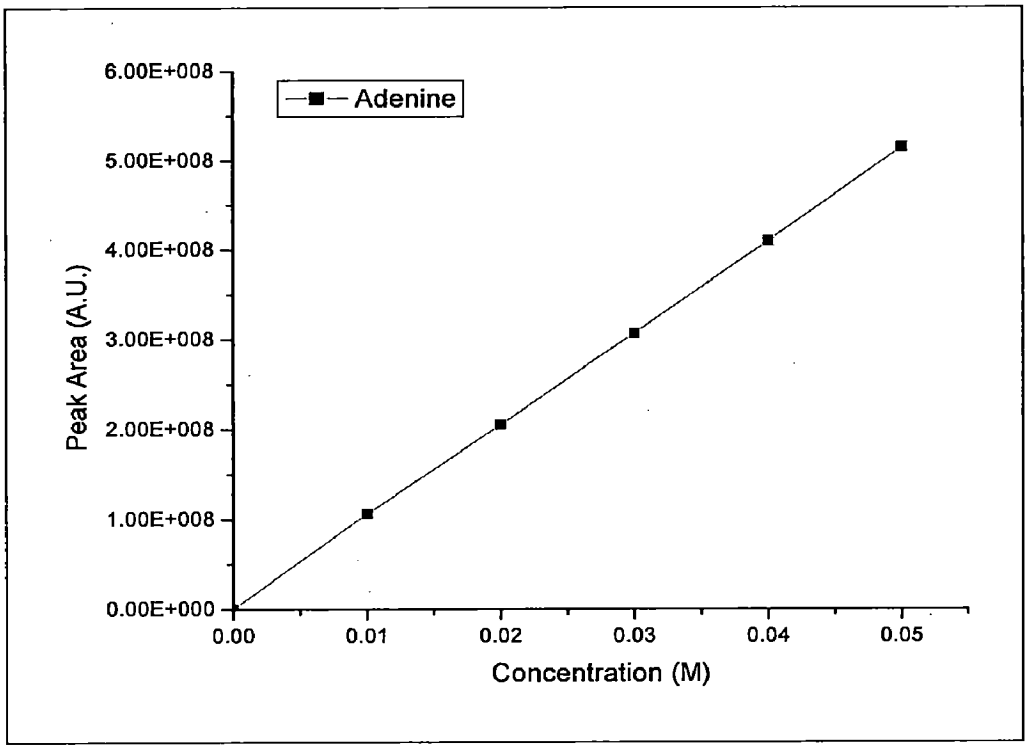
Standard curve of standard 4(3H)-pyrimidinone

Figure 2.16

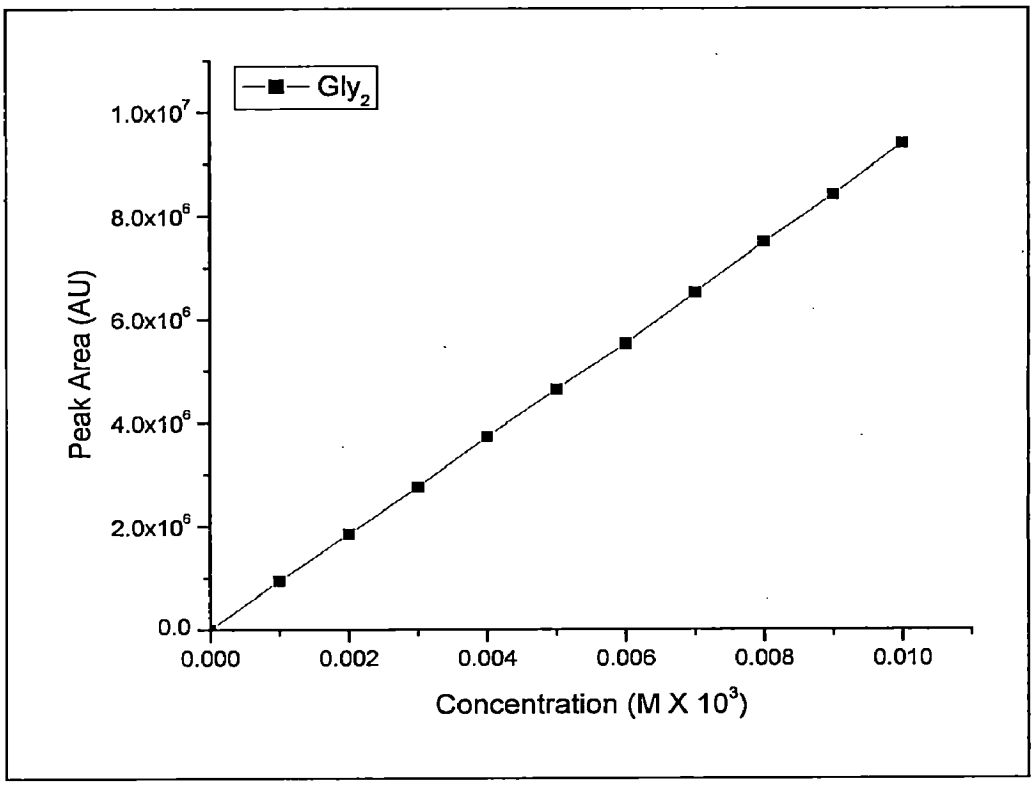


Standard curve for standard cytosine

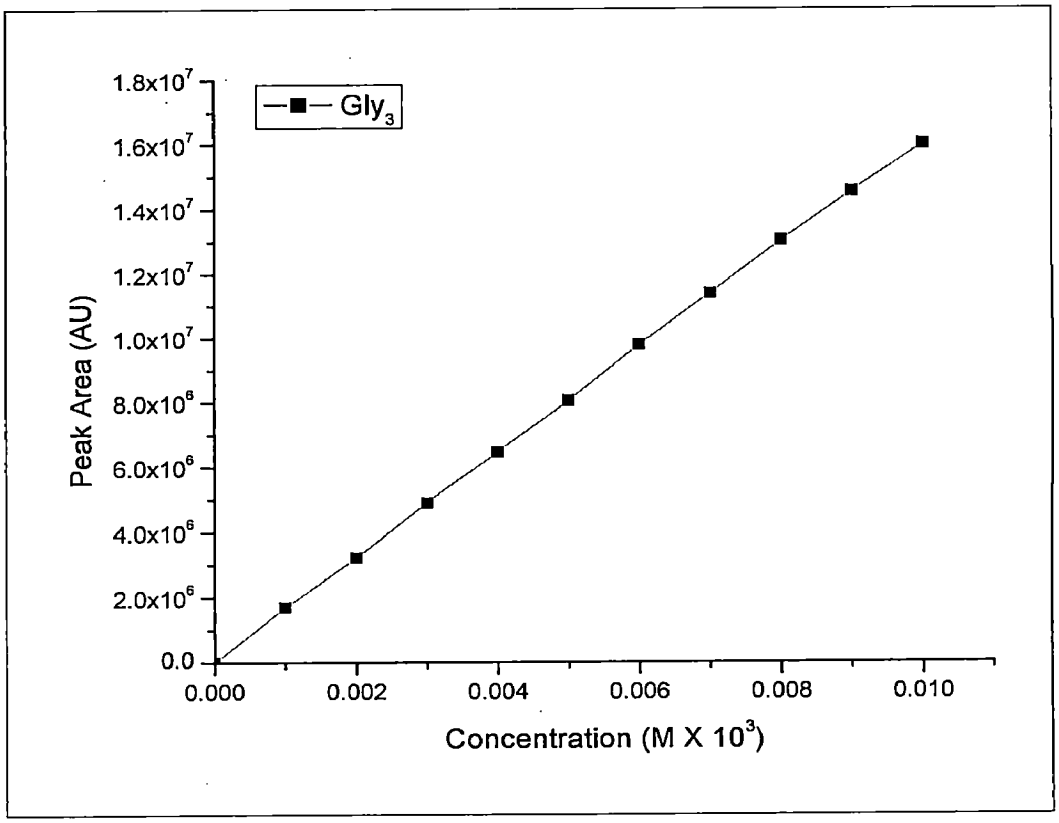
Figure 2.17



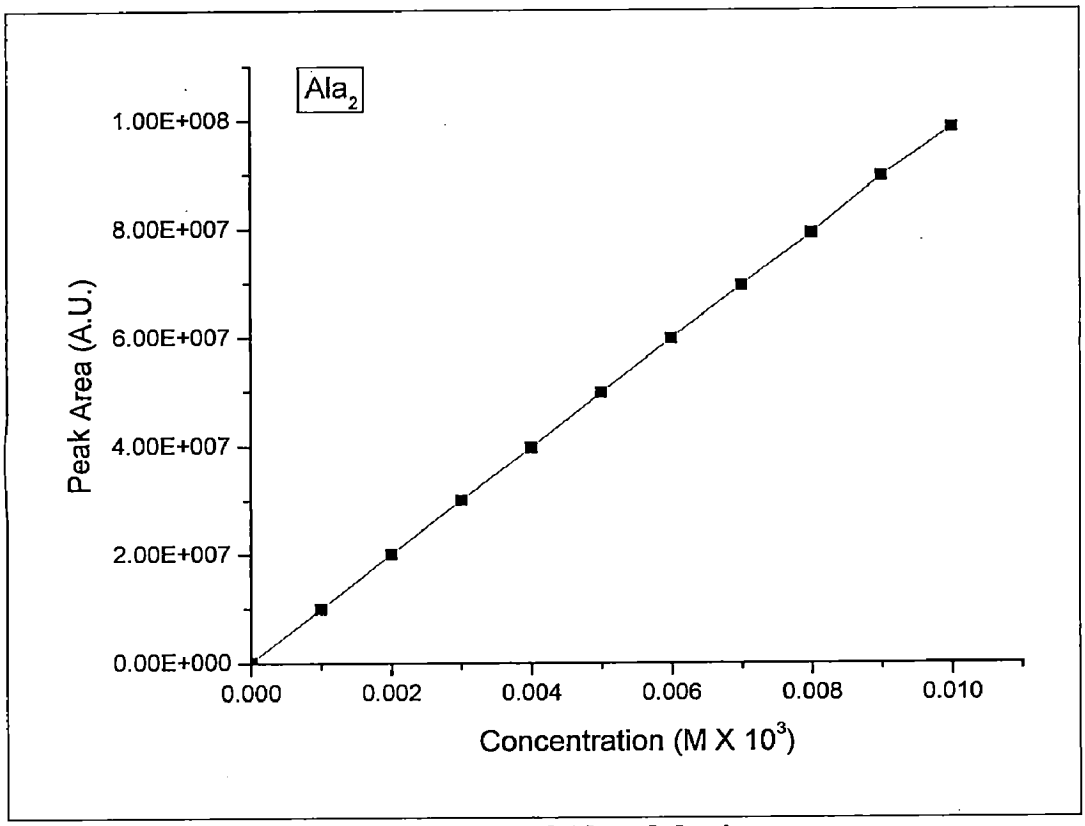
Standard curve of adenine
Figure 2.18



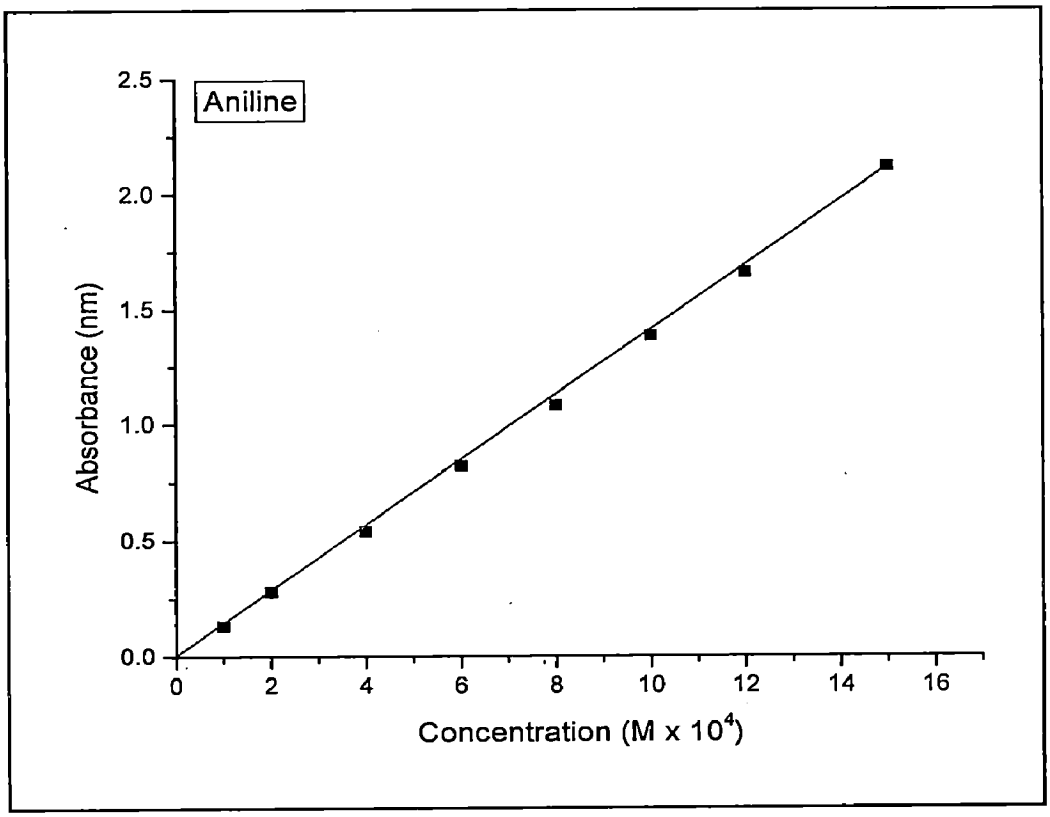
Standard curve of Glycylglycine
Figure 2.19



Standard curve of Glycylglycylglycine
Figure 2.20

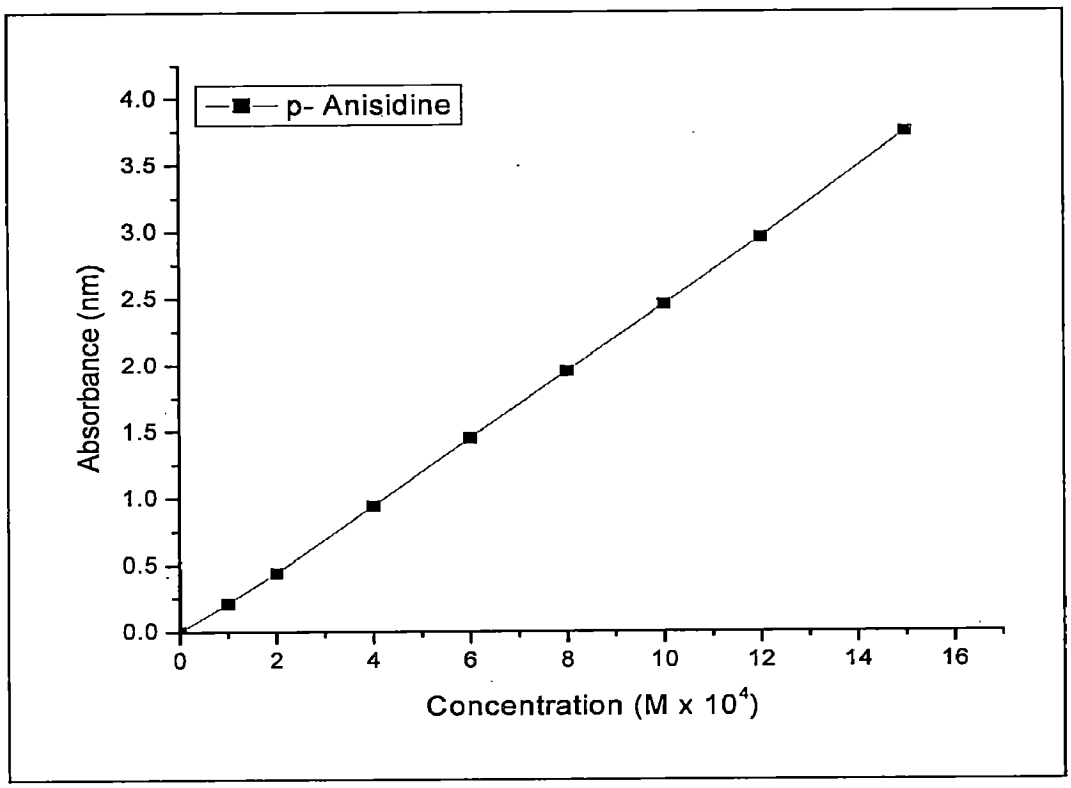


Standard curve of Alanylalanine
Figure 2.21



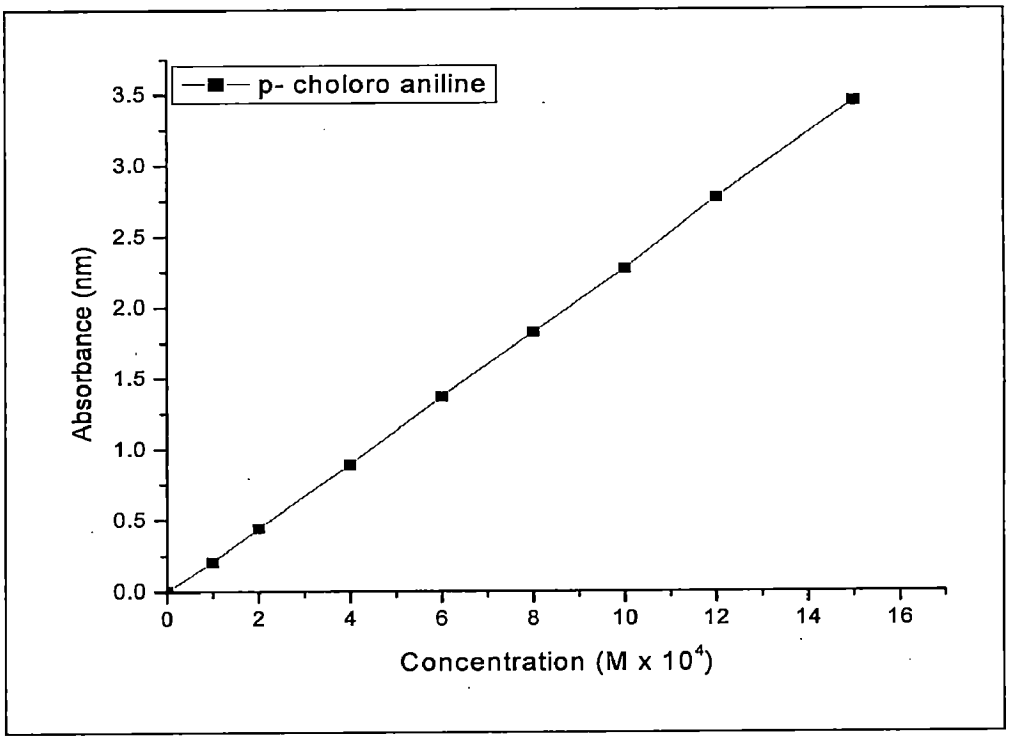
Standard curve of aniline

Figure 2.22



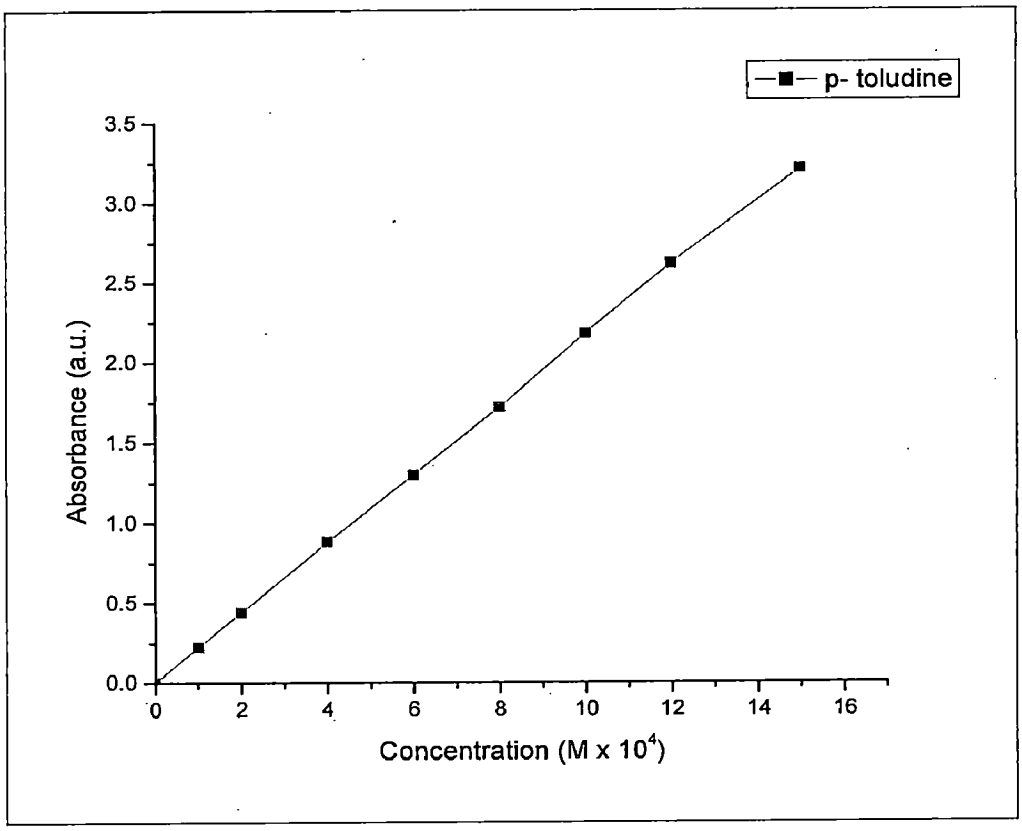
Standard curve of p-anisidine

Figure 2.23



Standard curve of p-chloroaniline

Figure 2.24



Standard curve of p-toluidine

Figure 2.25

CHAPTER-3

INTERACTION OF RIBOSE NUCELOTIDES WITH MANGANESE OXIDES

3.1 Introduction

Though life that exists today is based on DNA genomes and protein enzymes, there is convincing evidence that hypothesizes the origin of life based on RNA [1]. This primordial era referred to as the "RNA world" [2] hypothesizes that all modern organisms are descendants of a prebiotic self-replicating moiety, which utilized RNA as both genetic as well as catalytic material [3]. This hypothesis is now an almost universally held view [4-6]; but could this RNA World have stood at the ultimate origin of life? This is currently still an open question. Yet while there are still substantial problems, there are now good leads for simple, spontaneous processes on the early Earth for both the synthesis of nucleotides and their concentration to oligonucleotides.

Further study on the origin of life addressed questions regarding the sources of small organic molecules that composed the first self-replicating system and the mechanism of evolution of cell-like enclosure from an abiotic supply of biomonomers [7-8]. Numerous chemical and biochemical studies pertaining to the chemical evolution and origin of life, performed under simulated prebiotic conditions, have appeared owing to various pioneering work in this field [9-13] but such aggregation of biological precursors into prebiotic biopolymers was puzzling to many. It was Bernal who proposed that adsorption of organic molecules onto mineral surface like clays would have catalytic relevance to the emergence of prototypical polymeric templates [14]. Similarly Ferris showed that clays could catalyze activated nucleotide polymerization reactions [15-19]. Smith studied the potential role of natural minerals on which aggregation and entrapment of nucleobases might have occurred thereby, reinforcing the potential role of natural minerals acting as scaffolds and aiding in the catalysis of reactions necessary for the evolution of early life [20].

Like natural minerals, transition metals may have been important as catalysts for the formation of biopolymers during chemical evolution and the origin of life [21-23].

Catalysts may have been important for the origins of life because they tend to direct the reaction along a few reaction pathways so that a limited array of products is obtained. Catalysts bind specific types of compounds to their surfaces and then convert them to a limited number of products. Manganese is the 10^{th} most abundant element in the biosphere ($\sim 10^{14}$ kg of suspended and dissolved manganese found in oceans) and is second only to iron in relative terrestrial abundance of the transition metals. On average, crustal rocks contain about 0.1% by weight of Mn [24], coordinated with oxygen, and may also exist in the bottom of seas as nodules. The existence of manganese on Mars has also been reported [25]. In the early stages of the Earth's evolution, volcanoes were a major source of such elements which in turn may have been involved in adsorption and catalytic reactions of biomolecules in molecular evolution. The catalytic activity of manganese for many reactions in the presence of nucleotides, mRNA to give oligoribonucleotides is already reported [26]. Visscher and Schwartz have concluded that the synthesis of pyrophosphate-linked oligomers from the bis-phosphoimidazolides of deoxyadenosine and deoxyguanosine, as well as from acyclic analogs of these nucleosides, is catalyzed much more effectively by Mn (II) [27].

Manganese exists in various oxidation states on Earth. The microbial oxidation of soluble Mn (II) is an important process for the formation of soluble Mn (III, IV) oxides in natural environments [28]. Abiotic oxidation of Mn (II) under aerobic condition at neutral pH proceeds only at a limited rate [29-30]. Microorganisms are responsible for much of the Mn-oxide as they greatly accelerate its formation rate [28] and produce oxidation states of ≥ 3.4 compared to the abiotic laboratory synthesis value of 3.1 [29].

We proposed that since the redox potential of the primitive Earth's atmosphere was low and the atmosphere was less oxidized, manganese oxides of lower oxidation states were more important for selectively adsorbing and concentrating bio-molecules during

chemical evolution. In the present work, we studied the interaction of ribose nucleotides namely, 5'-GMP, 5'-AMP, 5'-CMP and 5'-UMP with manganese oxides of varied Mn/O ratio (MnO, Mn_3O_4, Mn_2O_3 and MnO_2). Ribose nucleotides have negatively charged phosphate groups, lone pair containing nitrogen atoms, and aromatic rings with π electron clouds. So interaction could have taken place with the negatively charged moieties of nucleotide and the positively charged surface of manganese oxides [31-32]. It was observed through a series of adsorption studies that manganese oxides of lower oxidation state are adsorbents for ribonucleotides and thus may have played a role in chemical evolution.

3.2 Evaluation of Parameters Suitable for Adsorption Studies of Ribose Nucleotides on Manganese Oxides

The parameters such as concentration range of adsorbate, particle size of adsorbent, contact time to attain the equilibrium for adsorbate and adsorbent, quantity of adsorbent, pH and the temperature were optimized to study the adsorption of ribose nucleotides on manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)).

The adsorption of ribose nucleotides (5'-AMP, 5'-GMP, 5'-CMP and 5'-UMP) on manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)) was studied as a function concentration of adsorbates at neutral pH. The concentration range of all the four ribose nucleotides was ~ (05×10^{-5} M – 60×10^{-5} M). The manganese oxide was of 200 mesh size and 25 mg of the oxide was used for the adsorption studies. The equilibrium ranging time from 1 hr to 48 hr was studied to determine the equilibrium time. The 24 hr time was found to be suitable to achieve the equilibrium indicating maximum adsorption.

3.3 Results and Discussion

3.3.1 Interaction of Ribose Nucleotides with Manganese Oxides

Adsorption of 5'-AMP, 5'-GMP, 5'-CMP and 5'-UMP on manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)) were studied as a function of concentration at neutral pH~7. As it is well established as per our previous observation that maximum adsorption occurs at neutral pH [21-23]. The results obtained are shown in Table 3.3.1-3.3.16 and Figures 3.3.1-3.3.4 which indicate the amount (mg) of ribose nucleotides adsorbed per gm of manganese oxide at neutral pH.

It was observed that Purine nucleotides showed higher adsorption than pyrimidine nucleotides. The order of adsorption is as follow:

5'-GMP>5'-AMP> 5'-CMP > 5'-UMP

After optimizing conditions, adsorption of nucleotides on a fixed amount of manganese oxide (25mg) was studied by varying concentrations of nucleotides (05×10^{-5} M to 60×10^{-5} M), at neutral pH at room temperature.

The adsorption of ribose nucleotides followed Langmuir adsorption isotherm as given below.

$$\frac{Ce}{X_e} = \frac{Ce}{X_m} + \frac{1}{X_m} k_L$$

where,

C_e = equilibrium concentration of nucleotide (mole/liter)

X_e = amount (in mg) of nucleotide adsorbed per gram weight of adsorbent (mg)

X_m = amount of nucleotide adsorbed at saturation (mg/g)

k_L = Langmuir adsorption constant (l/mg).

The adsorption data were obtained at neutral pH (~7.0) and over a wide concentration range of adsorbate (05×10^{-5} M to 60×10^{-5} M). A plot of Ce vs. X_e was

sigmoid in nature. From the data of the curve at saturation, the percent binding was calculated and are listed in Table 3.3.17. The adsorption data were found to follow Langmuir adsorption isotherm.

The initial portion of the isotherm represents a linear relationship between the amount adsorbed and the equilibrium concentration of the ribonucleotides. At a higher concentration range, the isotherm showed a saturation phenomenon indicating no further adsorption. A typical graph of C_e/X_e vs C_e is a straight line Figures 3.3.5-3.3.8. K_L and X_m values were determined and are shown in Table 3.3.18. In general, manganese oxide; manganosite (MnO) was found to have extremely high affinity in its oxide for ribose nucleotides as the extent of surface covered ($\theta = \frac{k_L Ce}{1 + k_L Ce}$) was determined to be about 100% for all the four nucleotides.

The nature of interaction between the ribonucleotide and the oxide plays a vital role in understanding the mechanism underlying the higher adsorption affinity on certain oxides of manganese [33-37]. In a manganese oxide/water system, $Me\text{-}OH_2^+$ and $Me\text{-}O^{-1}$, hydroxyl groups on the solid surface are the most important sites for surface interactions. These groups can act as acids or bases, depending on the pH of the solution. In the present study, according to the literature [38-41], the zero points of charge P_{ZPC} of MnO, Mn_2O_3, Mn_3O_4 and MnO_2 are >10, >10, 7.7 and 7.3 respectively. At neutral pH ($pH < P_{ZPC}$), the hydroxyl groups on the manganese oxide surface exist in the acidic form and the adsorbent surface is positively charged. Electrostatic interactions of positively charged surface of manganese oxides (MnO and other manganese oxides) with nucleotides may take place through negatively charged atoms in the phosphate groups, via the π electrons of the aromatic ring or rings, and/or via the lone pair of electrons on N and O atoms. Such explanations are verified by the infrared spectral studies conducted.

The nature of the interaction between ribonucleotides and the manganese oxides is mainly electrostatic, which was further elucidated by means of infrared absorption study which showed shifting of particular metal oxide-phosphate peaks [42-45]. Electrostatic interaction of phosphate groups with positively charged groups and hydroxyl sites at the surface of magnetite was postulated by Daou et al. Antelo et al. similarly stressed the importance of pH and ionic strength while explaining the higher adsorption of phosphate to goethite in acidic medium. Higher adsorption of phosphate by manganosite (MnO) at acidic pH was also explained by Chitrakar et al. and Li and Stanforth in lines of electrostatic forces of attraction as the primary mode of interaction. The shift in wavelengths of characteristic frequencies of ribonucleotides, manganese oxide in nucleotide-manganese adducts (Figure 3.3.9 (a), (b) and (c), respectively) indicates interactions between the ribonucleotides and manganese, which also confirmed by (Tables 3.3.20-3.3.21). It appears that the binding may also be mediated by either N-1 or N-7 for 5'GMP and 5'AMP, and N-3 for 5'CMP and 5'UMP. The adsorption is presumably related to the involvement of N-1, N-3 and N-7 of the base residues, lone pair of nitrogen atom, and to the dissociation of the two available protons of the phosphate groups. A shift towards lower wavelength of N-H bending from1647cm^{-1} to 1629 cm^{-1} shows the involvement of the NH_2 groups present on the base of 5'GMP ribonucleotides in the interaction of ribonucleotides with manganese. A strong band at 1084 cm^{-1} in 5'GMP corresponding to C-O-P vibration has been shifted to 1102 cm^{-1}. Thus interaction likely takes place through amino and phosphate groups of ribonucleotides with positively charged manganese oxide surfaces [23].

The presence of significant change in the typical infrared frequencies of N-H and C-O-P suggests that the adsorption of ribonucleotides is a surface phenomenon occurring on the surface of metal oxides. Ribonucleotides interact through their purine or

pyrimidines residue and phosphate group with the positively charged surface of the mineral. Greater adsorption of 5'-GMP and 5'-AMP may be due to the higher number of π electrons in their aromatic ring system.

Manganese oxides with different oxidation states like 2, 3 and 4 exist [46-49] with decreasing average volume per Mn ion (A^3) in each form of the oxide [45]. The prebiotic condition existing in the bottom of sea was essentially reducing [50]. This is suitable for the adsorption of ribonucleotides on the surface of the Mn (II) and Mn (III) which changed slowly to the more stable state of manganese oxide, Mn (IV) with the increasingly oxidizing nature of the primitive Earth's atmosphere [51]. The oxic conditions prevailing at bottom of seas were clarified by thallium isotope studies carried out by Nielson [52] and further reported by Sleep and Bird [53].

Field Emission Scanning Electron Microscopic studies of the GMP, manganese oxide and GMP–manganese oxide adduct was also studied and is shown in Figure 3.2.10. FE-SEM photographs of manganese oxide-nucleotide adduct shows that surface of manganese oxide becomes smooth and colour of surface of manganese oxide changes to that of GMP. EDX pattern also supports the adsorption as percentage of C, N, P and O increases in the EDX pattern of adduct.

Among the manganese oxides used, Manganosite (MnO) showed maximum affinity towards ribose nucleotides while Pyrolusite (MnO_2) the least, which may be due to available surface area. The order of affinity towards ribose nucleotides observed:

Manganosite (MnO)> Hausmannite (Mn_3O_4)>Bixbyite (Mn_2O_3)>Pyrolusite (MnO_2)

3.4 Conclusion

Manganese oxides may have played a role in adsorbing and concentrating molecules in the primeval soup during chemical evolution. The results of the present study on the adsorption of ribonucleotides on manganese oxides support the hypothesis by

demonstrating a high degree of adsorption and strong affinity of ribose nucleotides towards manganese oxides. All of the manganese oxides synthesized from manganese oxalate were nanosized, their surface morphology was irregular and porous, and all had more or less similarly high specific surface areas. All these observations are favorable and conducive to higher adsorption of ribose nucleotides. Our studies otherwise revealed that nucleotides have a greater binding affinity on lower oxidation state manganese oxides (MnO and Mn_3O_4). Adsorption was higher for 5'-GMP and 5'-AMP compared to 5'-CMP and 5'-UMP. Our studies also revealed that electrostatic interactions are likely the main contributing factors for the observed adsorption on manganese (II) and (III) oxides. The higher electrostatic interaction between the positive metal-oxide surface and the negative part of the ribonucleotide could have been conducive for adsorption and preservation of these biomolecules as well as for promoting further polymerization reactions. Adsorption process may have protected these biomolecules from degradation and helped for further polymerization reactions.

Table 3.3.1

Adsorption of 5'-GMP on Manganosite (MnO)
Temperature = $25^{O}C$
Amount of adsorbent used = 25mg

S. No.	Initial Conc.(C_i) 5'-GMP ($Mx10^5$)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of 5'-GMP ($Mx10^5$)	$C_i - C_{eq}$ ($Mx10^5$)	Amount (Xe) of 5'-GMP adsorbed ($mg\ g^{-1}$)	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	5	0.62	0.09	1.01	3.99	1.16	3.16
3	10	1.44	0.17	1.81	8.19	2.38	2.75
4	20	2.92	0.38	3.84	16.16	4.69	2.97
5	30	4.32	0.67	6.66	23.34	6.78	3.57
6	40	5.54	1.16	11.42	28.58	8.30	4.99
7	50	7.11	2.18	21.32	28.68	8.33	9.29
8	60	8.46	3.20	31.22	28.78	8.36	13.56

Table 3.3.2

Adsorption of 5'-GMP on Hausmannite (Mn_3O_4)
Temperature = $25^{O}C$
Amount of adsorbent used = 25mg

S. No.	Initial Conc.(C_i) 5'-GMP ($Mx10^5$)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of 5'-GMP ($Mx10^5$)	$C_i - C_{eq}$ ($Mx10^5$)	Amount (Xe) of 5'-GMP adsorbed (mg g^{-1})	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	5	0.62	0.10	1.10	3.90	1.13	3.21
3	10	1.44	0.21	2.19	7.81	2.27	3.31
4	20	2.92	0.42	4.23	15.77	4.58	3.36
5	30	4.32	0.85	8.41	21.59	6.27	4.87
6	40	5.54	1.84	18.02	21.98	6.39	10.25
7	50	7.11	2.86	27.92	22.08	6.41	15.81
8	60	8.46	3.84	37.44	22.56	6.56	20.74

Table 3.3.3

Adsorption of 5'-GMP on Bixbyite (Mn_2O_3)
Temperature = $25^{O}C$
Amount of adsorbent used = 25mg

S. No.	Initial Conc.(C_i) 5'-GMP ($Mx10^5$)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of 5'-GMP ($Mx10^5$)	$C_i - C_{eq}$ ($Mx10^5$)	Amount (Xe) of 5'-GMP adsorbed (mg g^{-1})	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	5	0.62	0.04	0.50	4.50	1.31	1.40
3	10	1.44	0.13	1.40	8.60	2.50	2.03
4	20	2.92	0.63	6.27	13.73	3.99	5.71
5	30	4.32	1.51	14.82	15.18	4.41	12.20
6	40	5.54	2.47	24.14	15.86	4.61	19.02
7	50	7.11	3.44	33.55	16.45	4.78	25.50
8	60	8.46	4.44	43.26	16.74	4.86	32.31

Table 3.3.4

Adsorption of 5'-GMP on Pyrolusite (MnO_2)

Temperature = $25^{O}C$
Amount of adsorbent used = 25mg

S. No.	Initial Conc.(C_i) 5'-GMP ($Mx10^5$)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_e) of 5'-GMP ($Mx10^5$)	$C_i - C_{eq}$ ($Mx10^5$)	Amount (Xe) of 5'-GMP adsorbed (mg g^{-1})	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	5	0.62	0.24	2.49	2.51	0.73	12.36
3	10	1.44	0.62	6.17	3.83	1.11	20.18
4	20	2.92	1.65	16.15	3.85	1.12	52.86
5	30	4.32	2.65	26.13	3.87	1.12	78.60
6	40	5.54	3.71	36.17	3.89	1.13	118.21
7	50	7.11	4.73	46.08	3.92	1.14	146.84
8	60	8.46	5.76	56.08	3.92	1.14	178.71

Table 3.3.5

Adsorption of 5'-AMP on Manganosite (MnO)
Temperature = 25^{O}C
Amount of adsorbent used = 25mg

S. No.	Initial Conc.(C_i) 5'-AMP (Mx10^5)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of 5'-AMP (Mx10^5)	$C_i - C_{eq}$ (Mx10^5)	Amount (Xe) of 5'-AMP adsorbed (mg g^{-1})	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	5	0.54	0.06	0.70	4.30	1.19	2.03
3	10	1.11	0.16	1.75	8.25	2.29	2.65
4	20	2.17	0.42	4.19	15.81	4.39	3.32
5	30	3.24	0.76	7.53	22.47	6.24	4.19
6	40	4.32	1.57	15.40	24.60	6.83	7.82
7	50	5.52	2.55	24.91	25.09	6.97	12.41
8	60	6.49	3.56	34.72	25.28	7.02	17.17

Table 3.3.6
Adsorption of 5'-AMP on Hausmannite (Mn_3O_4)
Temperature = $25^{O}C$
Amount of adsorbent used = 25mg

S. No.	Initial Conc.(C_i) 5'-AMP ($Mx10^5$)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of 5'-AMP ($Mx10^5$)	$C_i - C_{eq}$ ($Mx10^5$)	Amount (Xe) of 5'-AMP adsorbed (mg g^{-1})	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	5	0.54	0.05	0.66	4.34	1.21	1.90
3	10	1.11	0.14	1.51	8.49	2.36	2.23
4	20	2.17	0.48	4.82	15.18	4.22	3.96
5	30	3.24	1.04	10.25	19.75	5.49	6.49
6	40	4.32	2.01	19.67	20.33	5.65	12.09
7	50	5.52	3.02	29.48	20.52	5.70	17.95
8	60	6.49	4.04	39.38	20.62	5.73	23.87

Table 3.3.7

Adsorption of 5'-AMP on Bixbyite (Mn_2O_3)
Temperature = $25^{O}C$
Amount of adsorbent used = 25mg

S. No.	Initial Conc.(C_i) 5'-AMP ($Mx10^5$)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of 5'-AMP ($Mx10^5$)	$C_i - C_{eq}$ ($Mx10^5$)	Amount (Xe) of 5'-AMP adsorbed (mg g^{-1})	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	5	0.54	0.04	0.53	4.47	1.24	1.49
3	10	1.11	0.20	2.10	7.90	2.20	3.32
4	20	2.17	0.79	7.83	12.17	3.38	8.03
5	30	3.24	1.59	15.59	14.41	4.00	13.53
6	40	4.32	2.60	25.40	14.60	4.06	21.74
7	50	5.52	3.62	35.30	14.70	4.08	30.02
8	60	6.49	4.63	45.11	14.89	4.14	37.86

Table 3.3.8

Adsorption of 5'-AMP on Pyrolusite (MnO_2)
Temperature = 25^0C
Amount of adsorbent used = 25mg

S. No.	Initial Conc.(C_i) 5'-AMP (Mx10^5)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_e) of 5'-AMP (Mx10^5)	$C_i - C_{eq}$ (Mx10^5)	Amount (Xe) of 5'-AMP adsorbed (mg g^{-1})	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	5	0.54	0.11	1.22	3.78	1.05	4.05
3	10	1.11	0.47	4.72	5.28	1.47	11.17
4	20	2.17	1.50	14.72	5.28	1.47	34.83
5	30	3.24	2.51	24.52	5.48	1.52	55.98
6	40	4.32	3.53	34.43	5.57	1.55	77.22
7	50	5.52	4.54	44.23	5.77	1.60	95.88
8	60	6.49	5.54	53.94	6.06	1.68	111.30

Table 3.3.9

Adsorption of 5'-CMP on Manganosite (MnO)
Temperature = 25^{O}C
Amount of adsorbent used = 25mg

S. No.	Initial Conc.(C_i) 5'-CMP ($Mx10^5$)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of 5'-CMP ($Mx10^5$)	$C_i - C_{eq}$ ($Mx10^5$)	Amount (Xe) of 5'-CMP adsorbed (mg g^{-1})	C_e/X_e (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	5	0.46	0.05	0.65	4.35	1.12	1.87
3	10	0.89	0.16	1.69	8.31	2.15	2.54
4	20	1.56	0.40	4.04	15.96	4.13	3.16
5	30	2.37	0.72	7.15	22.85	5.91	3.91
6	40	3.24	1.54	15.11	24.89	6.44	7.59
7	50	3.97	2.56	25.01	24.99	6.46	12.51
8	60	4.86	3.56	34.72	25.28	6.54	17.17

Table 3.3.10
Adsorption of 5'-CMP on Hausmannite (Mn_3O_4)
Temperature = $25^{O}C$
Amount of adsorbent used = 25mg

S. No.	Initial Conc.(C_i) 5'-CMP ($Mx10^5$)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of 5'-CMP ($Mx10^5$)	$C_i - C_{eq}$ ($Mx10^5$)	Amount (Xe) of 5'-CMP adsorbed (mg g^{-1})	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	5	0.46	0.05	0.63	4.37	1.13	1.81
3	10	0.89	0.13	1.42	8.58	2.22	2.06
4	20	1.56	0.45	4.52	15.48	4.00	3.65
5	30	2.37	1.14	11.22	18.78	4.85	7.47
6	40	3.24	2.04	19.96	20.04	5.18	12.45
7	50	3.97	3.02	29.48	20.52	5.31	17.95
8	60	4.86	4.03	39.28	20.72	5.36	23.70

Table 3.3.11

Adsorption of 5'-CMP on Bixbyite (Mn_2O_3)
Temperature = $25^{O}C$
Amount of adsorbent used = 25mg

S. No.	Initial Conc.(C_i) 5'-GMP ($Mx10^5$)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of 5'-GMP ($Mx10^5$)	$C_i - C_{eq}$ ($Mx10^5$)	Amount (Xe) of 5'-GMP adsorbed ($mg\ g^{-1}$)	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	5	0.46	0.04	0.55	4.45	1.15	1.56
3	10	0.89	0.19	2.00	8.00	2.07	3.13
4	20	1.56	0.71	7.05	12.95	3.35	6.80
5	30	2.37	1.59	15.59	14.41	3.73	13.53
6	40	3.24	2.58	25.20	14.80	3.83	21.29
7	50	3.97	3.59	35.01	14.99	3.88	29.19
8	60	4.86	4.57	44.52	15.48	4.00	35.96

Table 3.3.12

Adsorption of 5'-CMP on Pyrolusite (MnO_2)
Temperature = $25^{O}C$
Amount of adsorbent used = 25mg

S. No.	Initial Conc.(C_i) 5'-CMP ($Mx10^5$)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_e) of 5'-CMP ($Mx10^5$)	$C_i - C_{eq}$ ($Mx10^5$)	Amount (Xe) of 5'-CMP adsorbed ($mg\ g^{-1}$)	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	5	0.46	0.03	0.49	4.51	1.17	1.34
3	10	0.89	0.16	1.71	8.29	2.14	2.58
4	20	1.56	0.85	8.41	11.59	3.00	9.07
5	30	2.37	1.85	18.12	11.88	3.07	19.06
6	40	3.24	2.85	27.83	12.17	3.15	28.57
7	50	3.97	3.86	37.63	12.37	3.20	38.03
8	60	4.86	4.86	47.34	12.66	3.27	46.74

Table 3.3.13

Adsorption of 5'-UMP on Manganosite (MnO)
Temperature = 25^{O}C
Amount of adsorbent used = 25mg

S. No.	Initial Conc.(C_i) 5'-UMP ($Mx10^5$)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of 5'-UMP ($Mx10^5$)	$C_i - C_{eq}$ ($Mx10^5$)	Amount (Xe) of 5'-UMP adsorbed (mg g^{-1})	C_e/X_e (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	5	0.42	0.04	0.50	4.50	1.17	1.40
3	10	0.74	0.10	1.11	8.89	2.31	1.56
4	20	1.52	0.26	2.68	17.32	4.49	1.93
5	30	2.26	0.91	8.99	21.01	5.45	5.35
6	40	2.96	1.87	18.31	21.69	5.63	10.55
7	50	3.82	2.89	28.21	21.79	5.65	16.19
8	60	4.57	3.89	37.92	22.08	5.73	21.47

Table 3.3.14

Adsorption of 5'-UMP on Hausmannite (Mn_3O_4)
Temperature = $25^{O}C$
Amount of adsorbent used = 25mg

S. No.	Initial Conc.(C_i) 5'-UMP ($Mx10^5$)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of 5'-UMP ($Mx10^5$)	$C_i - C_{eq}$ ($Mx10^5$)	Amount (Xe) of 5'-UMP adsorbed (mg g^{-1})	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	5	0.42	0.03	0.47	4.53	1.18	1.28
3	10	0.74	0.09	1.07	8.93	2.32	1.49
4	20	1.52	0.46	4.62	15.38	3.99	3.76
5	30	2.26	1.16	11.42	18.58	4.82	7.68
6	40	2.96	2.15	21.03	18.97	4.92	13.86
7	50	3.82	3.17	30.93	19.07	4.95	20.28
8	60	4.57	4.18	40.74	19.26	5.00	26.44

Table 3.3.15

Adsorption of 5'-UMP on Bixbyite (Mn_2O_3)
Temperature = $25^{O}C$
Amount of adsorbent used = 25mg

S. No.	Initial Conc.(C_i) 5'-UMP ($Mx10^5$)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of 5'-UMP ($Mx10^5$)	$C_i - C_{eq}$ ($Mx10^5$)	Amount (Xe) of 5'-UMP adsorbed (mg g^{-1})	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	5	0.42	0.04	0.51	4.49	1.16	1.43
3	10	0.74	0.14	1.53	8.47	2.20	2.26
4	20	1.52	0.72	7.15	12.85	3.33	6.95
5	30	2.26	1.62	15.88	14.12	3.66	14.06
6	40	2.96	2.63	25.69	14.31	3.71	22.44
7	50	3.82	3.65	35.59	14.41	3.74	30.88
8	60	4.57	4.66	45.40	14.60	3.79	38.86

Table 3.3.16

Adsorption of 5'-UMP on Pyrolusite (MnO_2)
Temperature = $25^{O}C$
Amount of adsorbent used = 25mg

S. No.	Initial Conc.(C_i) 5'-UMP ($Mx10^5$)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_e) of 5'-UMP ($Mx10^5$)	$C_i - C_{eq}$ ($Mx10^5$)	Amount (Xe) of 5'-UMP adsorbed (mg g^{-1})	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	5	0.42	0.02	0.36	4.64	1.20	0.97
3	10	0.74	0.12	1.32	8.68	2.25	1.90
4	20	1.52	0.89	8.80	11.20	2.91	9.81
5	30	2.26	1.90	18.60	11.40	2.96	20.40
6	40	2.96	2.89	28.21	11.79	3.06	29.92
7	50	3.82	3.87	37.73	12.27	3.18	38.43
8	60	4.57	4.87	47.44	12.56	3.26	47.20

Table 3.3.17

Percent binding of nucleotides on manganese oxides

Nucleotide	Percent binding			
	Manganosite (MnO)	Hausmannite (Mn_3O_4)	Bixbyite (Mn_2O_3)	Pyrolusite (MnO_2)
5'-AMP	63.69	53.52	39.87	18.36
5'-GMP	79.06	66.79	55.42	33.03
5'-CMP	52.18	37.06	20.39	12.06
5'-UMP	36.91	27.46	11.27	2.50

$$\%\,\text{Binding}=\left(\frac{\text{O.D.of nucleotide before adsorption- O.D.of nucleotide after adsorption}}{\text{O.D of nucleotide before adsorption}}\right)\times 100$$

Table 3.3.18

Langmuir constants for adsorption of nucleotides on manganese oxide

Nucleotide	(Manganosite (MnO)		Hausmannite (Mn_3O_4)		Bixbyite (Mn_2O_3)		Pyrolusite (MnO_2)	
	k_L (l mg^{-1})	X_m (mg/g)	k_L (l mg^{-1})	X_m (mg/g)	k_L (l mg^{-1})	X_m (mg/g)	k_L (l mg^{-1})	X_m (mg/g)
5'-AMP	11.69	8.20	1.95	1.75	1.66	1.24	1.50	0.48
5'-GMP	18.05	10.10	3.75	2.02	1.51	1.37	0.60	0.32
5'-CMP	9.49	7.25	2.12	1.75	1.72	1.27	1.05	1.02
5'-UMP	4.61	6.06	1.45	1.59	1.14	1.20	1.09	1.01

Table 3.3.19

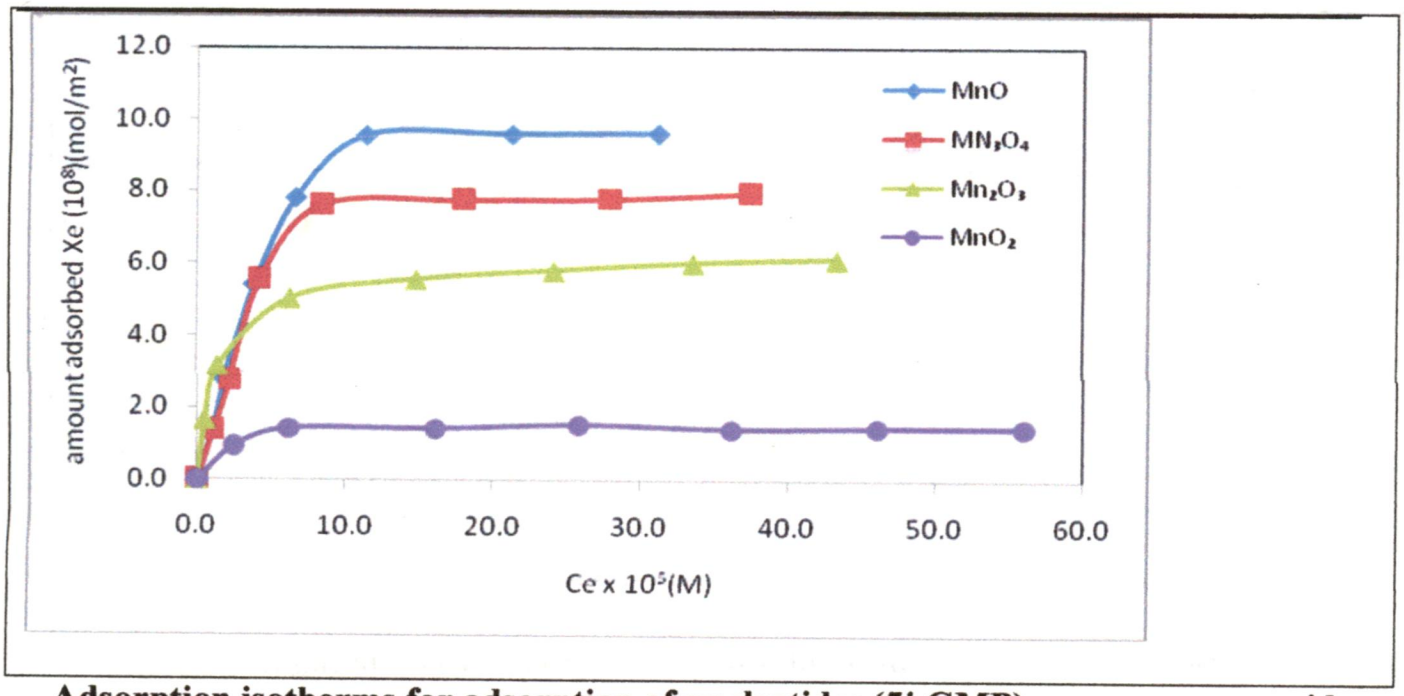
Typical Infrared frequencies (cm^{-1}) of ribose nucleotides before and after adsorption on manganosite (MnO)

Ribonucleotides	δ_{NH}	$\nu_{P=O}$
5'-AMP	1647	1084
	(1617)	(1126)
5'-GMP	1605	1083
	(1594)	(1121)
5'-CMP	1539	1091
	(1532)	(1102)
5'-UMP	1473	1082
	(1468)	(1085)

Table 3.3.20

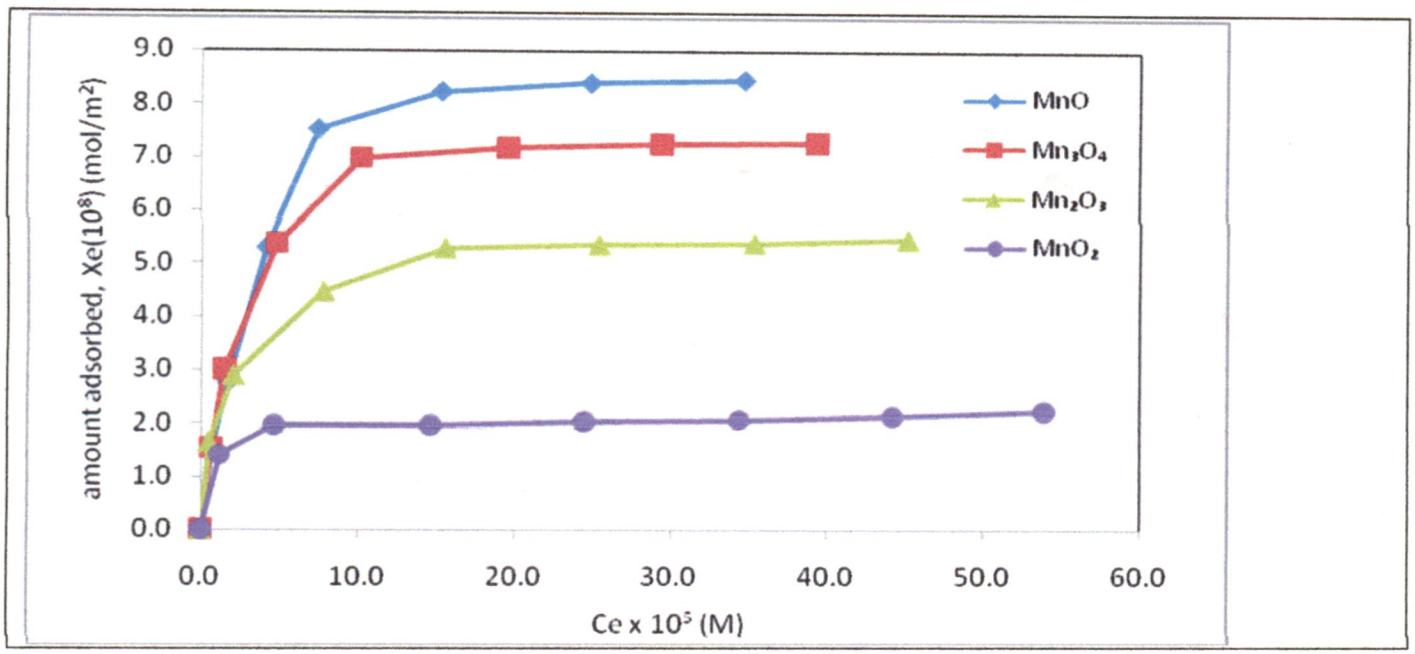
Typical infrared spectral frequencies (cm^{-1}) of ribonucleotides before and after adsorption on manganosite (MnO)

Ribonucleotides	ν_{NH_2}		δ_{NH}	$\nu_{P=O}$
5'-AMP	3221	3343	1647	1084
	(3395)*	(3462)	(1635)	(1121)
5'-GMP	3143	3433	1605	1083
	(3263)	(3478)	(1594)	(1099)
5'-CMP	3122	3334	1539	1091
	(3275)	(3384)	(1532)	(1102)
5'-UMP			1473	1082
			(1468)	(1085)



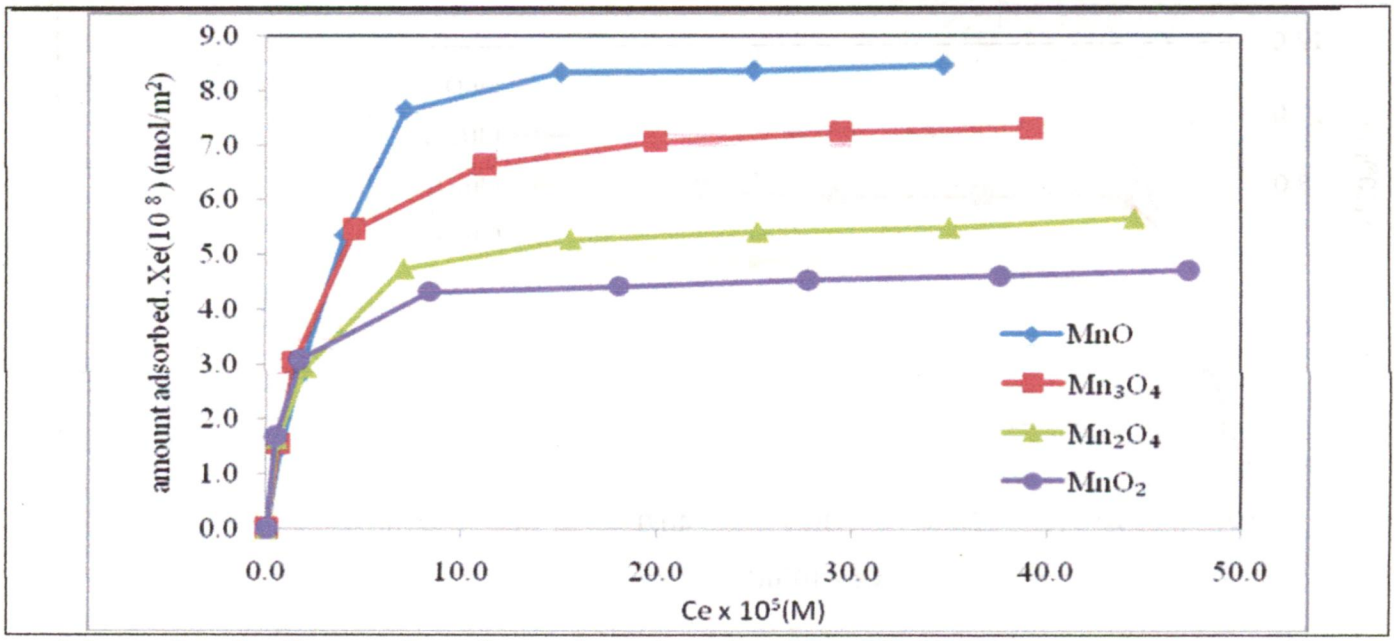
Adsorption isotherms for adsorption of nucleotides (5′-GMP) on manganese oxides

Figure 3.3.1



Adsorption isotherms for adsorption of nucleotides (5′-AMP) on manganese oxides

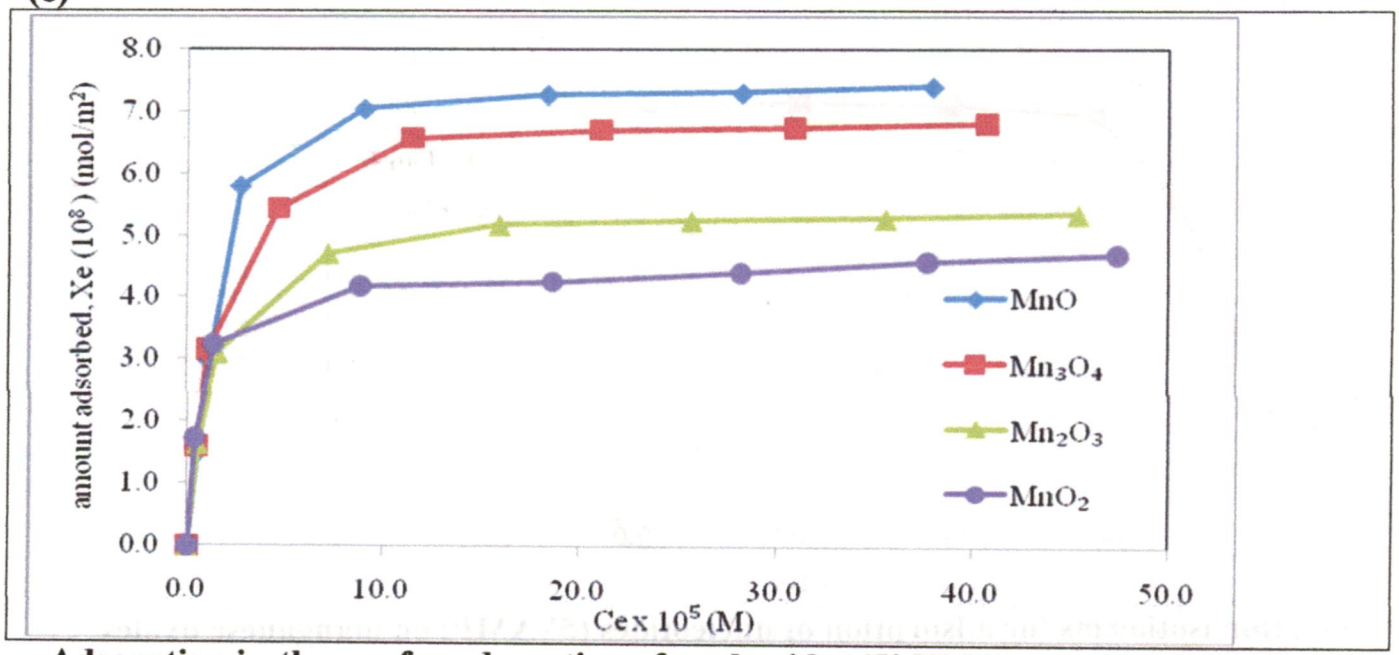
Figure 3.3.2



Adsorption isotherms for adsorption of nucleotides (5′-CMP) on manganese oxides

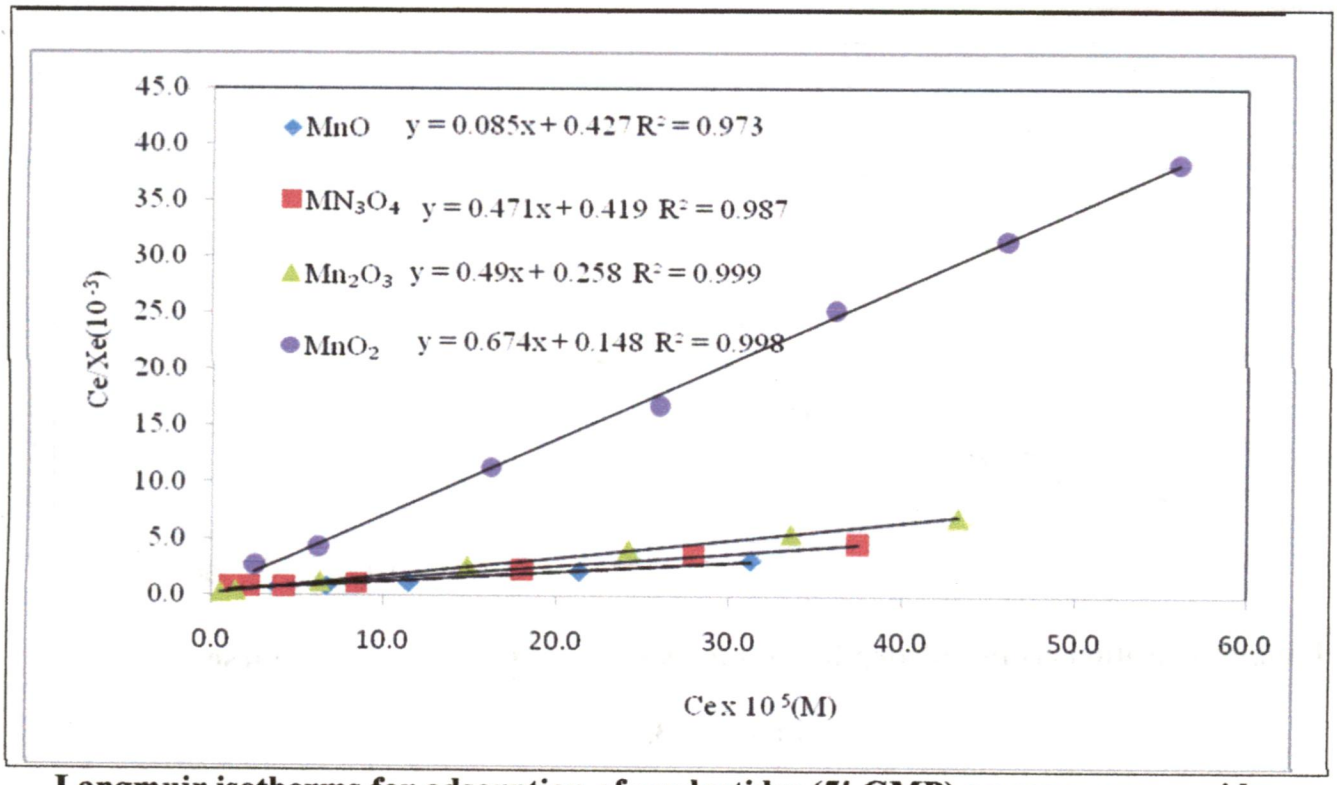
Figure 3.3.3

(c)



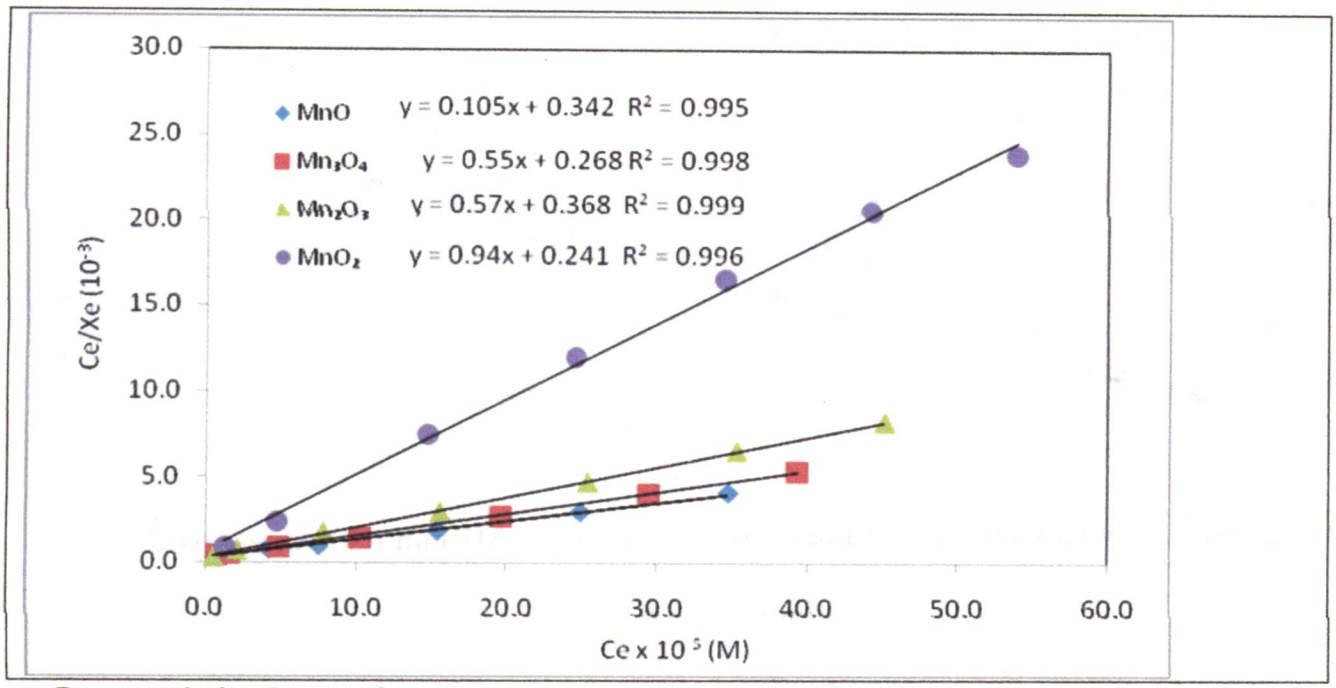
Adsorption isotherms for adsorption of nucleotides (5′-UMP) on manganese oxides

Figure 3.3.4



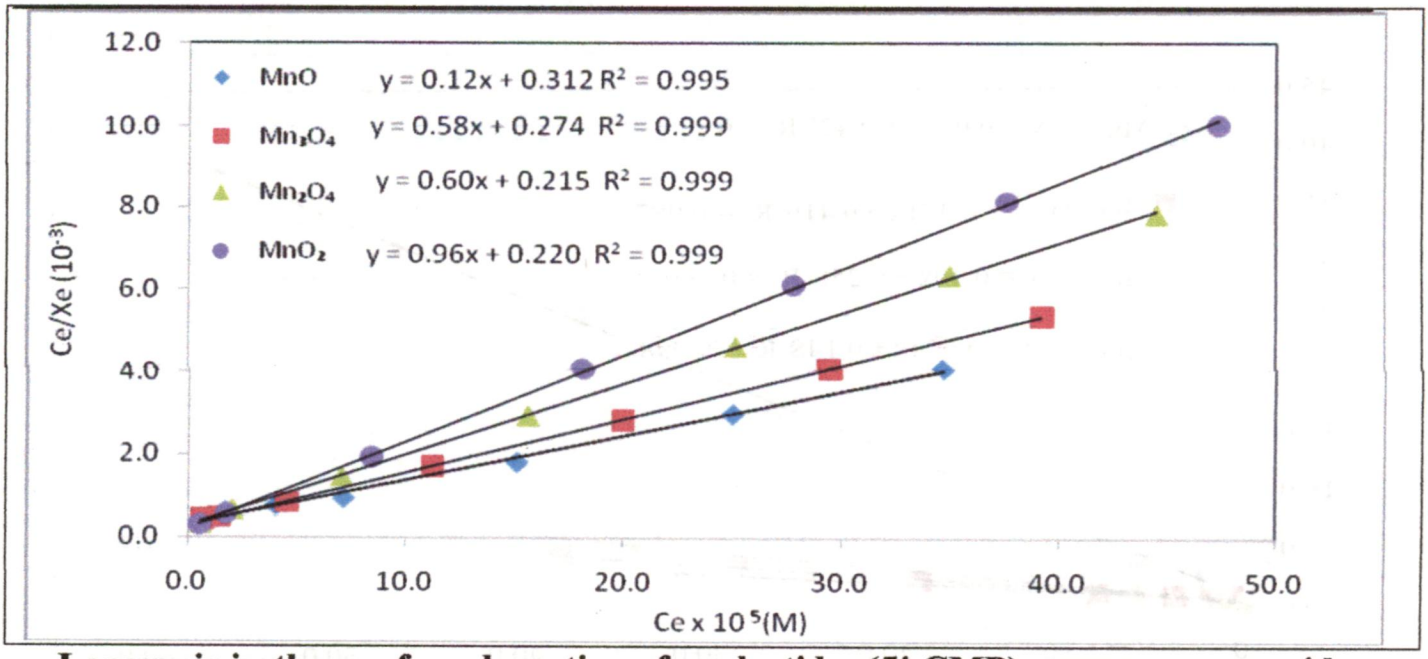
Langmuir isotherms for adsorption of nucleotides (5′-GMP) on manganese oxides

Figure 3.3.5



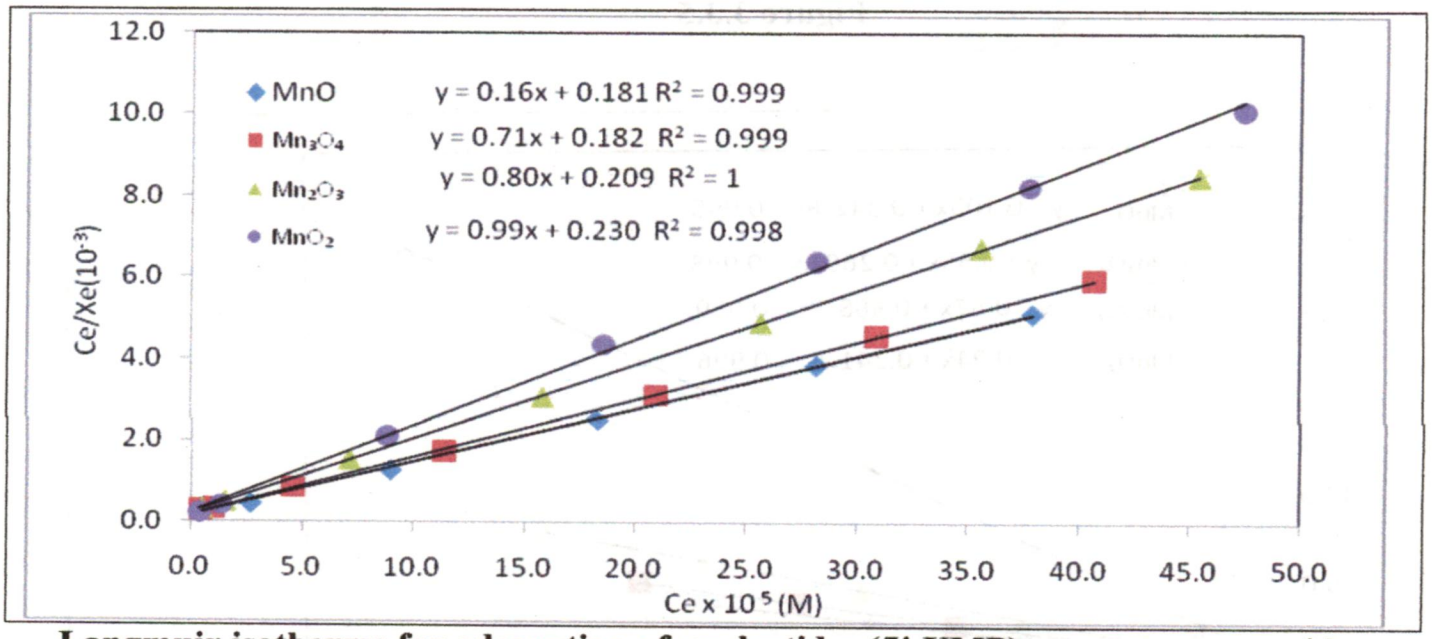
Langmuir isotherms for adsorption of nucleotides (5′-AMP) on manganese oxides

Figure 3.3.6



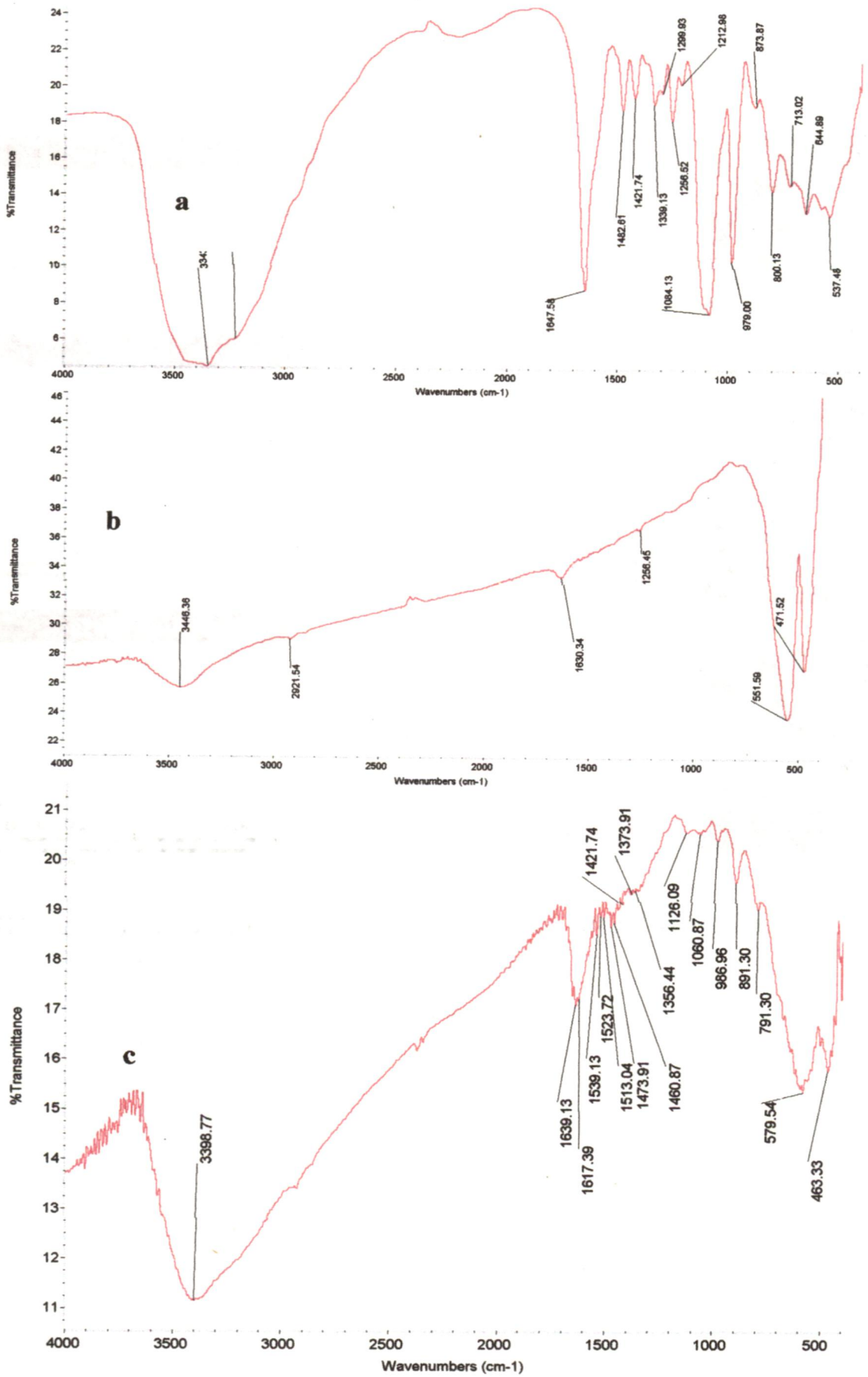
Langmuir isotherms for adsorption of nucleotides (5′-CMP) on manganese oxides

Figure 3.3.7



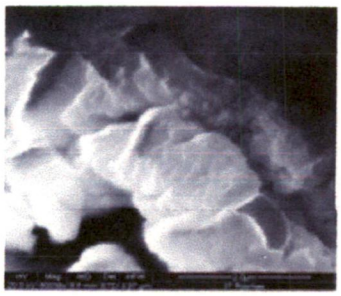
Langmuir isotherms for adsorption of nucleotides (5′-UMP) on manganese oxides

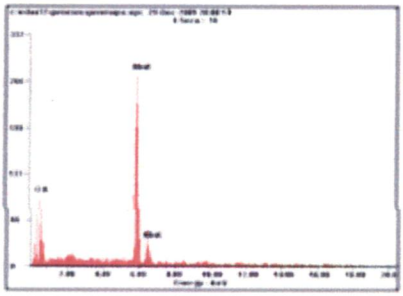
Figure 3.3.8



Representative IR spectra of AMP (a) MnO (b) and AMP-MnO adduct (c)

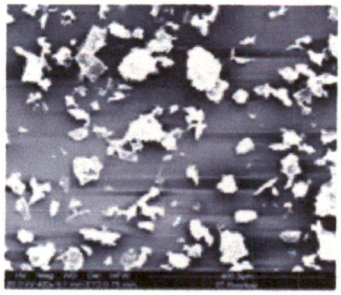
Figure 3.3.9

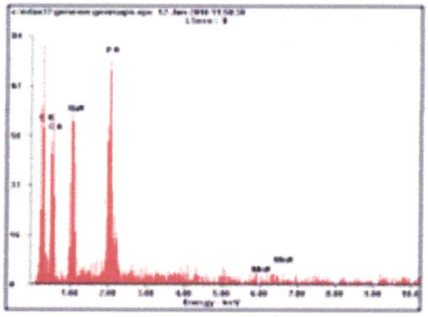




Element	Wt%	At%
OK	13.63	35.14
MnK	86.37	64.86
Matrix	Correction	ZAF

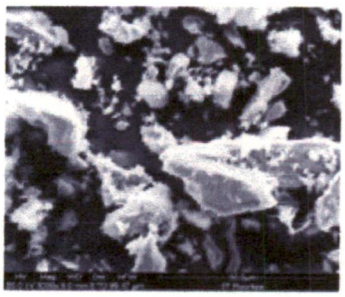
(a)

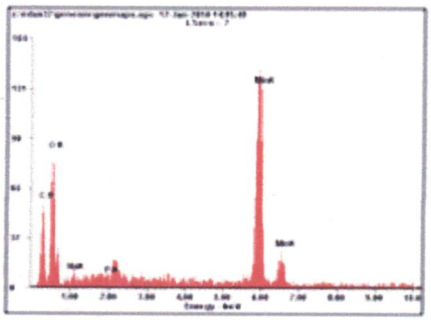




Element	Wt%	At%
CK	53.29	64.95
OK	25.30	23.14
NaK	11.75	07.48
PK	09.00	04.25
MnK	00.66	00.17
Matrix	Correction	ZAF

(b)





Element	Wt%	At%
CK	26.12	49.02
OK	19.54	27.53
NaK	01.48	01.45
PK	00.94	00.69
MnK	51.92	21.31
Matrix	Correction	ZAF

(c)

Representative FE-SEM photographs of MnO (a), GMP (b) and GMP-MnO adduct (c)

Fig.3.3.10

References:

1. Joyce, G. F., and Orgel, L. E. "Prospects for understanding the origin of the RNA world". In The RNA World, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 49 (1999).
2. Gilbert, W. "Origin of life: The RNA world". Nature, **319**, 618(1986).
3. Srivatsan, S. G. "Modeling prebiotic catalysis with nucleic acid-like polymers and its implications for the proposed RNA world". Pure Appl. Chem., **76**, 2085 (2004).
4. Joyce, G. F. "The antiquity of RNA based evolution", Nature, **418**, 214 (2002).
5. Orgel, L. E. "Prebiotic Chemistry and the Origin of the RNA World". Crit. Rev. Biochem. Mol. Biol., **39**, 99 (2004).
6. Gestland, R. F., Cech, T. R. and Atkins, J. F. "The RNA World". Cold Spring Harbour, NY, USA: Cold Spring Harbour Laboratory Press (2006).
7. Lazcano, A. and Miller, S. L. "The Stepwise Evolution of Early Life Driven by Energy Conservation". J. Mol. Evol., **49**, 424(1999).
8. Luisi, P. L., Rasi, P.S. and Mavelli, F. "A possible route to prebiotic vesicle reproduction". Artif. Life, **10**, 297 (2004).
9. Cairns-Smith, A. G. "Genetic Takeover: And the Mineral Origins of Life". Cambridge University Press, New York (1982).
10. Orgel, L. E. "The origin of life—a review of facts and speculations Trends Biochem". Sci., **23**, 491 (1998).
11. Miller, S. L. "Peptide nucleic acids and prebiotic chemistry". Nat. Struct. Biol., **4**, 167 (1997).
12. Ferris, J. P. "Catalysis and prebiotic RNA synthesis". Origins. Orig. Life Evol. Biosphere, **5**, 307 (1993).

13. Schwartz, A. W. and Orgel, L. E. "Template-directed synthesis of novel, nucleic acid-like structures". Science, **228**, 585 (1995).

14. Bernal, J. D. "The Physical Basis of Life". Routledge and Kegan Paul, London (1951).

15. Ertem, G. and Ferris, J. P. "Template-directed synthesis using the heterogeneous templates produced by montmorillonite catalysis: A possible bridge between the prebiotic and RNA worlds". J. Am. Chem. Soc., **119**, 7197 (1997).

16. Ferris, J. P., Ertem, G. and Agarwal, V. K. "The adsorption of nucleotides and polynucleotides on montmorillonite clay". Orig. Life Evo. Biosph., **19**, 153 (1989).

17. Ferris, J. P. and Ertem, G. "Oligomerization of ribonucleotides on montmorillonite: reactions of the 5 –phosphorimidazolide of adenosine". Sci., **257**, 1387 (1992).

18. Ferris, J. P. and Hagan, W. J. "Adsorption and reaction of adenine nucleotide on montmorillonite". Orig. Life Evo. Biosph., **17**, 69 (1986).

19. Ferris, J. P., Kamaluddin and Ertem, G. "Oligomerization reactions of deoxyribonucleotides on montmorillonite clay: the effect of mononucleotide structure, phosphate activation and montmorillonite composition on phosphodiester bond formation". Orig. Life Evo. Biosph., **20**, 279 (1990).

20. Smith, J. V. "Biochemical evolution. I. Polymerization on internal, organophilic silica surfaces of dealuminated zeolites and feldspars". Proc. Natl. Acad. Sci., USA **95**, 3370 (1998).

21. Arora, A. K. and Kamaluddin "Interaction of ribose nucleotides with zinc oxide and relevance in chemical evolution". Colloids surf. A: Physicochemical and Engineering Aspect., **298**, 186 (2007).

22. Arora, A. K., Tomar, V., Aarti, Venkateswararao, K. T. and Kamaluddin "Hematite–Water system on Mars and its possible role in chemical evolution". Int. J. Astrobiol., **6**, 267 (2007).

23. Arora, A. K. and Kamaluddin "Role of Metal Oxides in Chemical Evolution: Interaction of Ribose Nucleotides with Alumina". Astrobiol., **9**, 165 (2009).

24. Turekian, K. K. and Wedepohl, K. L. "Distribution of the Elements in Some Major Units of the Earth's Crust". Geol. Soc. Am. Bull., **72**, 275(1961).

25. Heiserman David, L. "Exploring Chemical Elements and their Compounds". Blue Ridge Summit, PA Libraries Australia (1992).

26. Hroacki, S. and Hiromichi, W. "Synthesis and reaction of nucleoside 5-diphosphate imidazolide: A nonenzymatic capping agent for 5-monophosphorylated oligoribonucleotides in aqueous solution". J. Org. Chem., **64**, 5836 (1999).

27. Visscher, J. and Schwartz, A. W. "Manganese-Catalyzed Oligomerizations of Nucleotide Analogs". J. Mol. Evol., **29**, 284 (1989).

28. Nealson, K. H., Tebo, B. M. and Rosson, R. A. "Occurrence and Mechanisms of Microbial Oxidation of Manganese". Adv. Appl .of Microbiology., **33**, 279 (1988).

29. Tebo, B. M., Ghiorse, W.C., Van Waasbergen, L. G., Siering, P. L. and Caspi, R. "Bacterially mediated mineral formation: insights into manganese (II) oxidation from molecular genetics and biochemical studies". Rev. Mineral., **35**, 225(1997).

30. Tebo, B. M., Bargar, J. R. and Clement, B. "Manganese bioxides: properties and mechanisms of formation". Annu. Rev. Earth. Pl. Sc., **32**, 287 (2004).

31. Yao, W. and Millero, F. J. "Adsorption of phosphate on manganese dioxide in seawater". Envir. Sci. Technol., **30**, 536 (1996).

32. Daou, T. J., Begin-Colin, S. and Greneche, J. M. "Phosphate Adsorption Properties of Magnetite-Based Nanoparticles". Chem. Mater., **19**, 4494 (2007).

33. Stumm, W., Huang, C. P. and Jenkins, S. R. "Specific chemical interaction affecting the stability of dispersed systems". Croat. Chem. Acta., **42**, 223 (1970).

34. Parks, G. A. and De Brum, P. L. "The zero point of charge of oxides". J. Phys.Chem., **66**, 967 (1962).

35. De Bruyn, P. L. and Agar, G. E. "In Froth Flotation". (D. W. Fuerstenau, Ed.). AIME, New York (1962).

36. Bepuee, Y. G. and De Brvy, P. L. "Adsorption at the rutile-solution interface: I. Thermodynamic and Experimental Study". J. Colloid. Interface Sci., **27**, 305 (1968).

37. Atkinson, R. J., Posner, A. M. and Quirk, J. P. "Adsorption of potential determining ions at the ferric oxide-aqueous electrolyte interface". J. Phys. Chem., **71**, 550 (1967).

38. Kosmulski, M. "pH-Dependent Surface Charging and Points of Zero Charge". J. Colloid Interface Sci., **253**, 77 (2002).

39. Kosmulski, M. "pH-Dependent Surface Charging and Points of Zero Charge II. Update". J. Colloid Interface Sci., **275**, 214 (2004).

40. Kosmulski, M. "pH-Dependent Surface Charging and Points of Zero Charge III. Update". J. Colloid Interface Sci., **298**, 730 (2006).

41. Healy, T. W., Herring, A. P. and Fuerstenau, D. W. "The effect of crystal structure on the surface properties of a series of manganese dioxides". J. Colloid. Interface Sci., **21,** 435 (1966).

42. Daou, T. J., Begin-Colin, S. and Greneche, J. M. "Phosphate Adsorption Properties of Magnetite-Based Nanoparticles". Chem. Mater., **19**, 4494 (2007).

43. Antelo, J., Avena, M., Fiol, S., Lopez, R. and Arce, F. "Effects of pH and ionic strength on the adsorption of phosphate and arsenate at the goethite–water interface". J. Colloid Interface Sci., **285,** 476 (2005).

44. Chitrakar, R., Tezuka, S., Sonoda, A., Sakane, K., Ooi, K. and Hirotsu, T. "Phosphate adsorption on synthetic goethite and akaganeite". J. Colloid Interface Sci., **298**, 602 (2006).

45. Li, L. and Stanforth, R. "Distinguishing Adsorption and Surface Precipitation of Phosphate on Goethite (α-FeOOH)". J. Colloid Interface Sci., **230,** 12 (2000).

46. Bish, D. L. and Post, J. E., "Rietveld refinement of the todorokite structure". Am. Mineral. **74**, 861(1988).

47. Burns, R. G., Burns, V. M. and Stockman, H. W. "The todorokitebuserite problem: further considerations". Am. Mineral., 70, 205(1985).

48. De Guzman, R. N., Shen, Y.F., Shaw, B. R., Suib, S. L. and O'young C. L. "Role of Cyclic Voltammetry in Characterizing Solids: Natural and Synthetic Manganese Oxide Octahedral Molecular Sieves". Chem. Mater., **5,** 1395 (1993).

49. Shen, Y. F., Zerger, R. P., Suib McCurdy, L., Potter, D. I. and O'Young, C. L. "Octahedral molecular sieves: preparation, characterization and applications". J. Chem. Chem. Commun., **17**, 1213 (1992).

50. Wachtershauser, G. "Before enzymes and templates: theory of surface metabolism Microbiol". Rev., **52**, 452 (1988).

51. Jortner, J. "Conditions for the emergence of life on the early Earth: summary and reflections". Phil. Trans. R. Soc., B. **361**, 1877 (2006).

52. Nielsen, S. G., Rehkamper, M., Norman, M. D., Halliday, A. N. and Harrison, D. "Thallium isotopic evidence for ferromanganese sediments in the mantle source of Hawaiian basalts". Nature, 439, 314 (2006).

53. Sleep, N. H. and Bird, D. K. "Evolutionary ecology during the rise of dioxygen in the Earth's atmosphere". Phil. Trans. R. Soc., B **363**, 2651 (2008).

CHAPTER-4

FORMATION OF NUCLEOBASES FROM FORMAMIDE IN THE PRESENCE OF MANGANESE OXIDES

4.1 Introduction

Formation of small reactive intermediates serve as the backbone in prebiotic organic synthesis, such as hydrogen cyanide, formaldehyde, formamide, ethylene, cyanoacetylene, acetylene and many more molecules which probably combine to form large and more complex precursors that ultimately form stable biomolecules. Most of these reactive intermediates might have been produced relatively by slower rate; hence their concentrations were lower in the primitive ocean. Subsequent reactions were dependent on many factors, such as the balance between atmospheric production rates and rain-out rate of small reactive intermediates, degradation rates, temperature and pH of early ocean. HCN has long been regarded as a key intermediate in the synthesis of nitrogen containing biomolecules on the primitive Earth [1-2].

The prebiotic origin of ribonucleic acid (RNA) is of great importance due to the hypothesis that the earliest form of life used RNA or proto-RNA for information storage and catalysis [3]. For these reasons, and the fact that adenine can be synthesized by a variety of model prebiotic reactions, it is widely speculated that nucleobases were likely part of the very first proto-RNA polymers. These simpler molecules then went on to form RNA or a molecule similar to RNA (proto-RNA) and eventually gave rise to the RNA world. The hypothesis that RNA preceded the emergence of proteins and that the first self-replicating system was made of RNA or proto-RNA known as the "RNA World Hypothesis". Due to the unlikely formation of RNA directly from the "prebiotic soup", many chemists believe that RNA was not the first self–replicating molecule to form, but was instead preceded by simpler molecules.

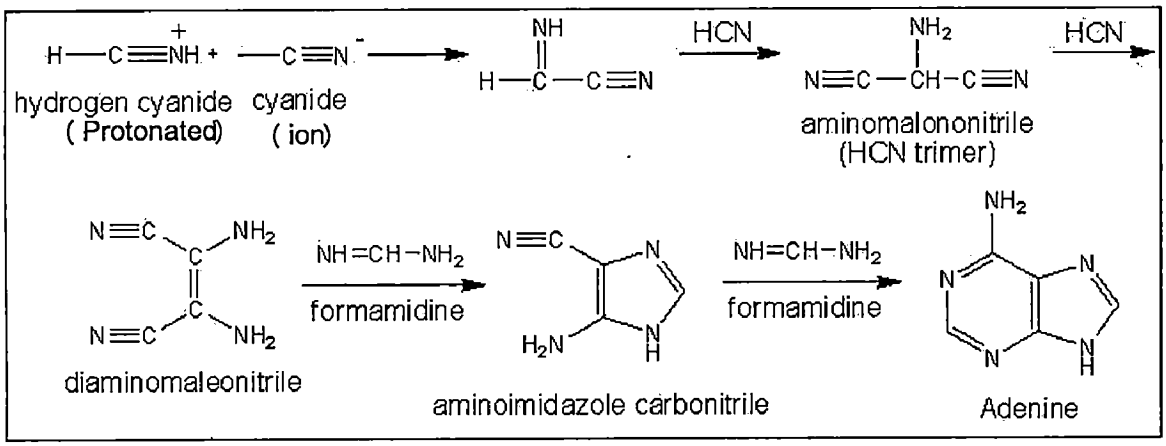
The robustness of nucleic acid base pairing in molecular recognition suggests that even the earliest genetic polymers of life may have used a similar mechanism for information storage and transfer, perhaps even with the same canonical nucleobases that are used in

contemporary life. It has also been suggested that the first genetic polymers would have assembled from pre existing building blocks (i.e. nucleobases, sugars, phosphate) through condensation-dehydration reactions [4-7]. Thus, the abiotic synthesis of the RNA nucleobases and related molecules is of great interest for understanding the chemical origin of life.

Deoxyribonucleic acid (DNA), the cell's repository for genetic information, and ribonucleic acid (RNA), the chemical intermediate between DNA and proteins, encode their information of purine (adenine and guanine) and pyrimidine bases (cytosine, thymine and uracil). In DNA and RNA, each base is bonded to a sugar, and the sugars are linked by phosphate to form information-carrying polymers. Although there are currently five main nucleic acid bases many alternative bases might have been present in the primitive ocean. Steven Benner proposed that early life on earth may have started with an expanded set of nucleobases, some of which have been lost or replaced in due course of time due to Darwinian natural selection [8].

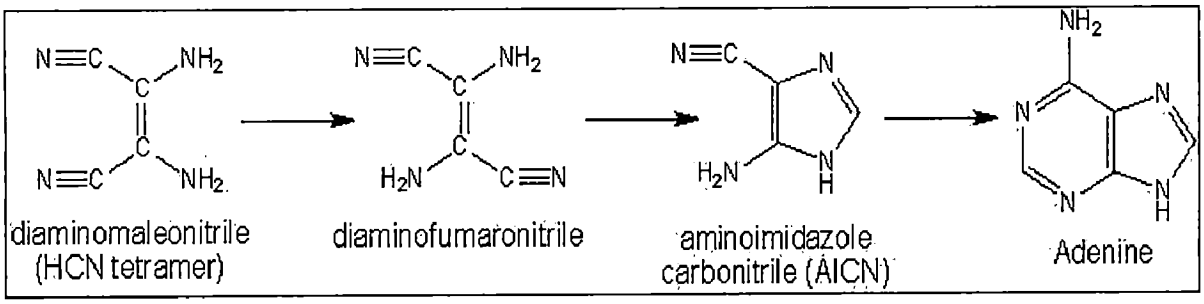
Historic synthesis of adenine from ammonium cyanide followed by synthesis of amino acids, purine and purine intermediates from HCN demonstrated the importance of hydrogen cyanide [9]. The mechanism for the formation of purines has been postulated extensively with a variety of different reactants including HCN, formamide and formamidine [10-15].

The most studied mechanism is the formation of adenine from HCN, the proposed pathway for this reaction is shown below.



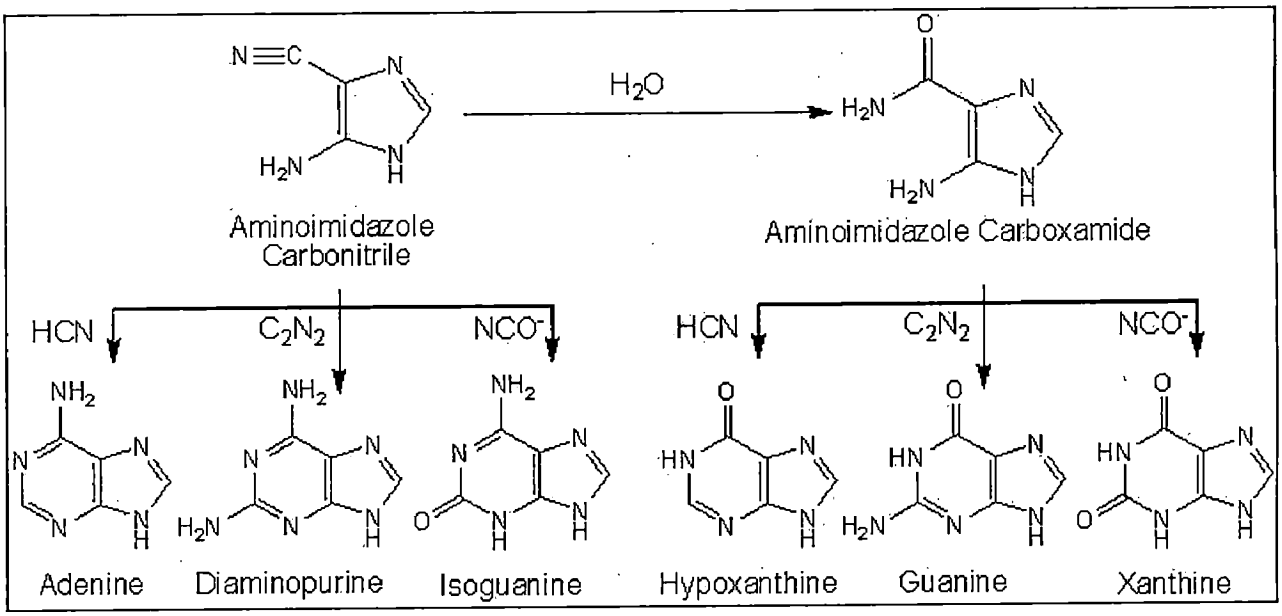
Putative mechanisms for the formation of adenine from hydrogen cyanide

The limiting reaction in this mechanism is the step in which the HCN tetramer (diaminomaleonitrile) combines with formamidine to form aminoimidazole carbonitrile. Orgel and Ferris (1966) showed that this step can be bypassed via a two-photon photochemical rearrangement of diaminomaleonitrile [16]. This process has been shown to proceed readily to give high yields of aminoimidizole carbonitrile from diaminofumaronitrile. Once the aminoimidizole carbonitrile has formed it is readily converted to adenine as follows.



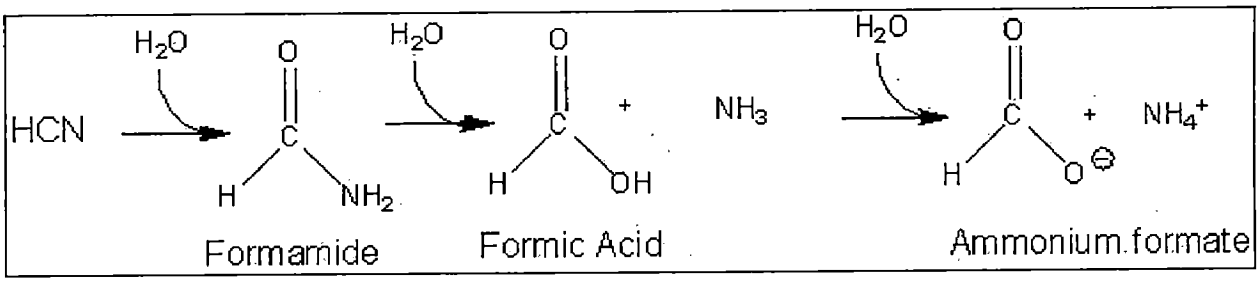
Putative pathway for the formation of adenine from diaminomaleonitrile

Along with the production of adenine, other purines could have been generated from aminoimidizole carbonitrile, including diaminopurine and isoguanine. Aminoimidazole carbonitrile (AICN) is easily converted to aminoimidazole carboxamide (AICA) that can then lead to the formation of hypoxanthine, guanine, and xanthine as given below [17-18].



Putative pathways to the formation of purines from AICN

A linkage between hydrogen cyanide chemistry and formamide chemistry is made due to the fact that formamide is the hydrolysis product of hydrogen cyanide as shown below:



Formation of ammonium formate and formamide from hydrogen cyanide

Formamide ($HCONH_2$) being a simple amide containing one carbon atom has received great attention. It also displays a high dielectric constant value and boiling point without an azeotropic effect and can be concentrated from dilute solutions simply by water evaporation [19]. With HCN chemistry, two main issues remain unresolved: (i) the thermodynamic instability of HCN under hydrolytic conditions and (ii) the narrow panel of nucleobases, limited only to purines that can be formed via HCN condensation processes. HCN chemistry in homogenous solution first requires dissolution in water. After the dissolution process,

polymerization and hydrolysis of HCN compete, and the resulting concentrations are determined. Hydrolysis to formamide predominates in dilute solutions while polymerization takes over at higher concentrations. Since HCN is more volatile than water it cannot be concentrated by simple evaporation at pH lower than its pK_a 9.2 at 25 ^{0}C. This suggests that eutectic freezing is a valid means for HCN to reach a sufficient concentration for polymerization.

While eutectic freezing is a valid pathway for the formation of the purines from HCN, it is assumed that the eutectic freezing of formamide will not lead to the formation of purines. However, the above assumption does not take into consideration that (i) formamide can be formed from prebiotic compounds diffused over the early Earth other than HCN and (ii) formamide is a liquid over a wide range of temperature and pressure values, with a boiling point of 210 ^{0}C and with very limited azeotropic effects [20].

Formamide, unlike HCN, can be very easily envisioned as a prebiotic molecule using the drying lagoon model. The drying lagoon model implies that the formamide would become concentrated if the water were to dry because of the high boiling point of formamide compared to water and the fact that water and formamide do not form an azeotrope. Formamide can be very easily concentrated by evaporating off any lower boiling point compounds leaving behind the pure formamide.

Saladino, Di Mauro and co-workers have described formamide thermochemistry in detail, cataloguing the variety of potentially prebiotic compounds generated when neat formamide is heated in the presence of montmorillonite and other clays, phosphate minerals, cosmic dust analogues, and iron-sulphur minerals [21-24]. They have identified a variety of purines and pyrimidines among mineral-catalyzed formamide reaction products, as well as

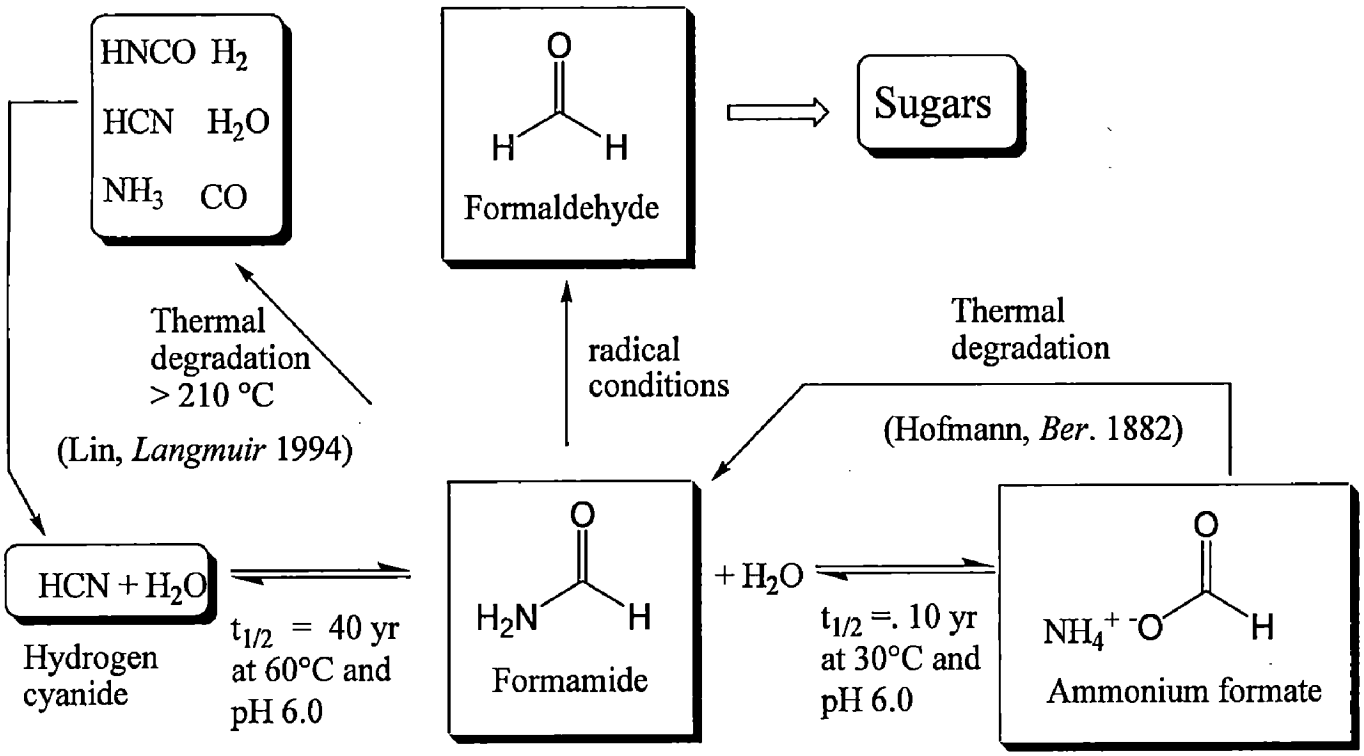
acylonucleosides, some simple amino acids, and other small molecules of prebiotic importance, such as carbodimide and urea.

Interestingly formamide was also detected in gaseous phase in the interstellar medium [25], in long period comet Hale-Bopp [26] and tentatively in the solid phase of grains around the young stellar object W33A [27]. The selectivity and reactivity of formamide can be finely tuned by the presence of minerals and metal oxides. These compounds are heterogeneous catalysts for the condensation of formamide to biomolecules, or alternatively, they can control the degradation kinetics of formamide to other compounds which are useful intermediates for prebiotic syntheses [28]. Moreover, minerals are able to preserve newly synthesized biomolecules from chemical and photochemical degradations [29-30].

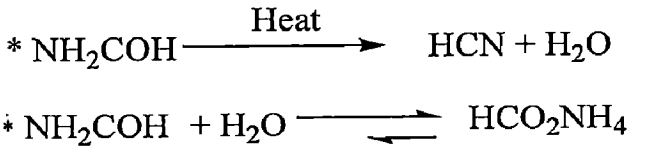
Early earth conditions where prebiotic formamide formed, it is most likely that the reaction of formamide occurred with clays. Possible reactions could have been brought on by the fact that suspended components of clays were in the formamide dilute solutions, the clays could have provided the surface on which the formamide reactions occurred.

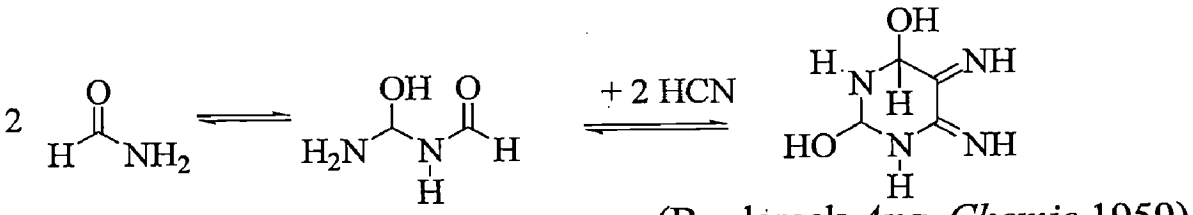
Metal oxides characterized by photo reactivity, such as titanium dioxide (TiO_2) served as efficient catalysts for the synthesis of different nucleobases from formamide [31]. Synthesis of monomers, building blocks of protein and nucleotides from formamide using different metal oxides and minerals as catalysts has extensively been studied [32]. Minerals can catalyze in situ decomposition of formamide to other chemicals that are potentially candidates for the construction of both purine and pyrimidine scaffolds, such as ammonia and HCN [21]. Recently, the role of clays in the prebiotic synthesis of amino sugar derivatives starting from a mixture of formamide and formaldehyde has also been reported. Since amino sugars are the key intermediates in the synthesis of complex nucleic acid derivatives, this simple procedure

opened up a novel pathway for the formation of nucleosides under plausible primordial conditions [33].



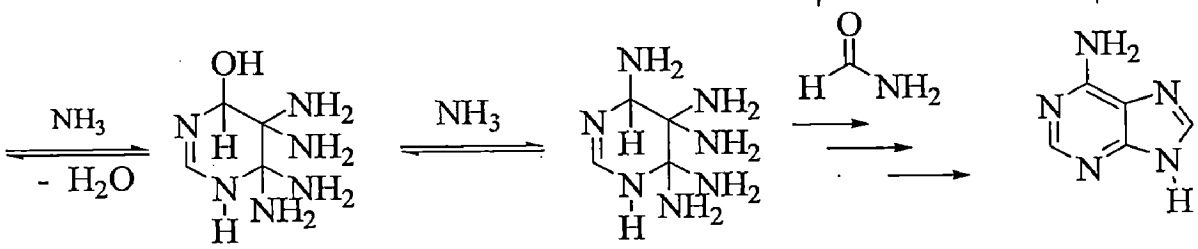
Basic formamide chemistry (Saladino et al., 2007) [36]



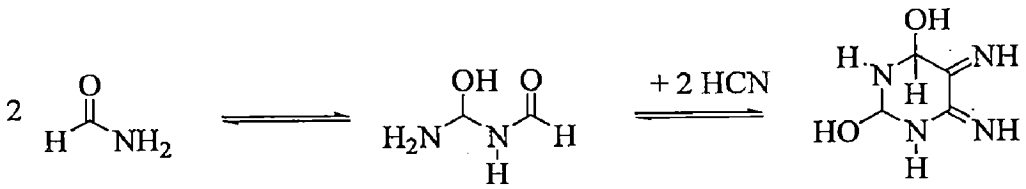


formamide dimer

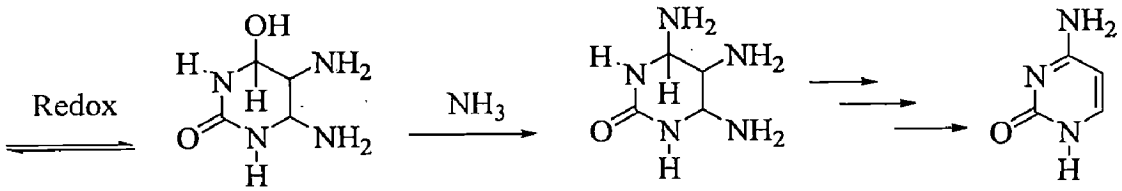
(Bredereck *Ang. Chemie* 1959)



Mechanism of formation of purine nucleobases:



formamide dimer



Cytosine

Mechanism of formation of pyrimidine nucleobases

A main aim of the present study is to explore more about the formation of nucleobases from formamide using manganese oxides in different forms (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)) with varying Mn/O ratio. Different

forms iron oxides having in the same oxidation state could also affect the yield of the products due to their regioselectivity were being studied in previous work [34].

The upper limit temperature 180 °C for the synthesis of nucleobases from formamide in our experiment was set as the boiling point of formamide is 211 °C. The temperature at which formamide undergoes appreciable thermal decomposition is reported as 180–190 °C [35]. Thus the most favorable set of conditions for the synthesis of nucleobases from formamide would be (i) high concentration of formamide, (ii) the presence of catalytic system and (iii) a temperature range 100-180 °C [23]. The reaction performed by taking neat formamide (5.7g, 5ml, 0.12 mol) at temperature range 100-160 °C for 12-96 h in the presence of 50 mg of selected catalysts (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)). Blank experiments were also performed under similar conditions. The reaction mixture was then centrifuged and filtered using 0.2 μm filter paper and then this mixture was divided into two parts, one part was used for HPLC analysis and the other for ESI-MS analysis. Attention was focused on the formation and identification of purine and pyrimidine derivatives in the cases where of the products formed were in higher yield only. The main identified products were purine, 9-(hydroxyacetyl) purine, 4(3H)-pyrimidinone, cytosine, thymine and adenine. Only purine was formed in the blank experiment.

4.2 Results and Discussion

Studies on the formation of nucleobases from formamide showed that in the absence of a catalyst only purine was formed, while in the presence of manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2) formamide afforded a number of various nucleobases namely, adenine, cytosine, purine, 9-(hydroxyacetyl) purine, thymine and 4(3H)-pyrimidinone in good yields (Table 4.1).

The identification of the products were carried out by HPLC and further confirmed by electro spray ionization mass spectrometry (ESI-MS) technique. Figure 4.1 represents the HPLC chromatograms of standards (purine, 4(3H)-pyrimidinone, cytosine, thymine and adenine).The yields were recorded in mg of product formed per gram of formamide. The HPLC results (Figure 4.2) showed number of peaks including purines and pyrimidines after analysis of all the reaction mixtures. The products identified were purine, 9-(hydroxyacetyl) purine, cytosine, 4(3H)-pyrimidinone, thymine and adenine, which were confirmed by co-injection with authentic samples. Prepared solutions of at least five different concentrations of each standard compound were used to construct standard calibration curves. Spiking of reaction products with standard compounds was also performed to confirm retention time. It was observed that all the forms of manganese oxides used produced nearly the same products, with different % yield. Manganosite (MnO) was found to produce the highest yield of purine, 9-(hydroxyacetyl) purine, 4(3H)-pyrimidinone, cytosine, thymine and adenine from formamide. Hausmannite (Mn_3O_4) also produced a higher yield of purine compared to manganosite (MnO) but other products in lesser yield. Bixbyite (Mn_2O_3) and pyrolusite (MnO_2) afforded the lowest yield of the products in comparison to other manganese oxides used. It was observed that formation of product gradually started after 12h and yield of the products became constant after 48h hence it was concluded that 48h was the optimum time for the formation of the products (Figure 4.2). Low yield of the products were observed below 150 °C. Time dependent analysis of product formation (formamide heated with manganosite (MnO)) is shown in Figure 4.3.

The mass spectra of the reaction mixtures obtained were characterized by $[M + nH]^+$ ions. Analysis of the reaction solutions by ESI-MS showed formation of cytosine $[M+ H]^+$ m/z 112, 4(3H)-pyrimidinone $[M+ H]^+$ m/z 97, purine $[M+ H]^+$ m/z 121, 9-(hydroxyacetyl)

purine $[M+H]^+$ 179, adenine $[M+H]^+$ m/z 136, thymine $[M+H]^+$ m/z 127 and the adenine $[M+3H]^+$ m/z 139. Figure 4.4 illustrates the ESI MS analysis results of the products formed by heating formamide with manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)). As shown in Figure 4.4 the peaks at m/z 97, 112, 121, 179, 136, 127 and 139 etc were identified as protonated 4(3H)-pyrimidinone, cytosine, purine, 9-(hydroxyacetyl) purine, thymine and adenine, respectively and their mass fragmentations also shown Figure . Plots shown in Figure 4.5 belong to the MS/MS spectra of products formed.

The results suggested that manganese oxides are efficient catalysts for the formation of nucleobases from formamide. The significance of the above study is that manganosite (MnO) and hausmannite (Mn_3O_4) afford almost the same yield of purine while hausmannite (Mn_3O_4) affords low yield under the same condition of the experiment. The surface area of manganosite (MnO) is 238.89 m^2/g whereas hausmannite (Mn_3O_4), bixbyite (Mn_2O_3) and pyrolusite (MnO_2) have 226.56 m^2/g, 218.35 m^2/g and 214.57 m^2/g respectively. Since the surface area of manganosite (MnO) is not much larger than that of hausmannite (Mn_3O_4), therefore it seems to be reasonable that the yield of the products could not be explained only on the basis of surface area of the catalysts but some other factors such as structural shape, surface acidity etc are also important. In view of these results it is proposed that manganese oxides present on the primitive Earth could have played a significant role in the synthesis of various biologically important compounds. These catalysts might have increased the thermal stability of nucleobases through molecular recognition processes or by simply isolating them from the external environmental condition.

It is assumed that minerals having metals in reduced form might have been more active during the course of chemical evolution. From present study it may be concluded that different forms of manganese oxides having lower Mn/O ratio have higher yields of product. But all the

forms of manganese oxides are also equally important for the formation of various biologically important molecules such as nucleobases from the precursors like formamide.

The present work shows the importance of manganese oxide ((manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)) as prebiotic catalysts in chemical evolution and origin of life which might have played a significant role in the synthesis of small molecules like nucleobases and also in condensation of these small moieties into larger molecules which are ultimately responsible for the emergence of life on the Earth. Above study also supports the hypothesis that formamide is a probable precursor for the formation of purine and pyrimidine bases during the course of chemical evolution and origin of life.

4.3 Conclusion:

The present work shows the importance of manganese oxide as prebiotic catalysts in chemical evolution and origin of life which might have played a significant role in the synthesis of small molecules like nucleobases and also in condensation of these small moieties into larger molecules which are ultimately responsible for the emergence of life on the earth. The present study also supports the hypothesis that formamide is a probable precursor for the formation of purine and pyrimidine bases during the course of chemical evolution and origin of life. It is also confirmed that minerals having metals in reduced form might have been more active during the course of chemical evolution due to the presence of reduced atmosphere at previous Earth condition. From our experimental results it may be concluded that different forms of manganese oxides having lower Mn/O ratio have more reactive in reaction involving in the biological system.

Table 4.1

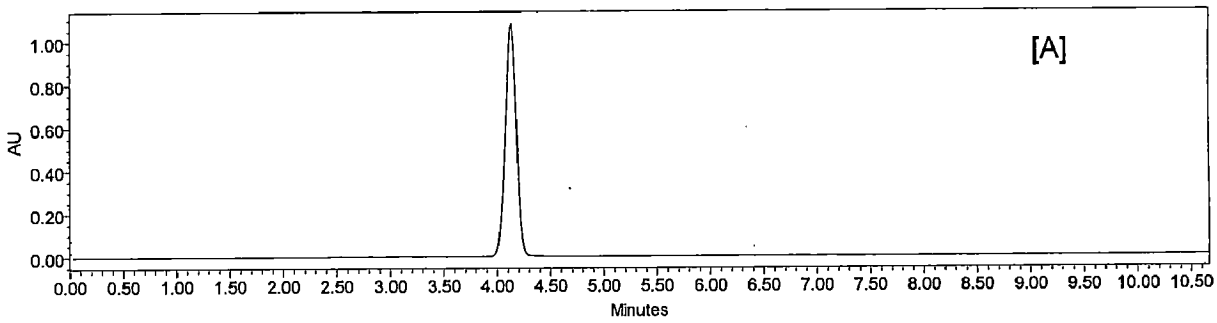
Condensation of formamide at 160^{0}C in the presence of manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)) for 48 h

Catalysts[a]	Yield[b] of products[c] formed (mg/g of formamide)					
	Purine	4(3H) - Pyrimidinone	Cytosine	Adenine	9-(hydroxyacetyl) purine	Thymine
No catalyst	25.625	-	-	-	-	
Manganosite (MnO)	51.244	17.973	6.434	4.664	2.886	3.545
Hausmannite (Mn_3O_4)	48.453	16.614	4.129	4.346	2.695	3.511
Bixbyite (Mn_2O_3)	36.821	9.571	3.782	4.397	1.875	2.974
Pyrolusite (MnO_2)	34.873	7.892	2.982	4.112	2.542	2.481

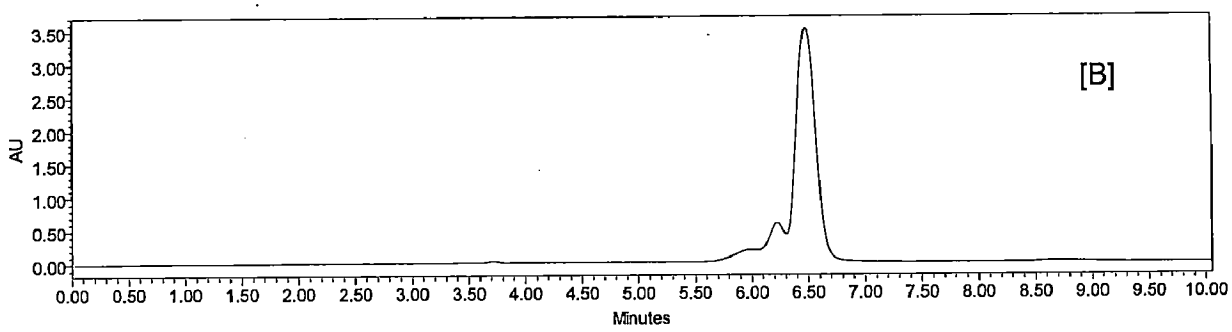
[a] Reactions were performed in the presence of 50 mg of catalyst.

[b] Quantitative evaluation was performed by HPLC (*Agilent 1100 series LC system)* equipped with Agilent Hypersil (ODS 5 µm/ 200 × 2.2 cm) column. Mobile phase used was a buffer solution of (KH_2PO_4 + H_3PO_4) of pH ~ 4.05; with a flow rate of 0.75 ml/minute under isocratic condition and detection of products formed was done by measuring absorbance at 260 nm. Because of the uncertainty of the number of formamide molecules involved in the synthesis of the recovered products the yield was calculated as mg of the product formed per gram of formamide. The yields of products were calculated by comparing peak area to the standards.

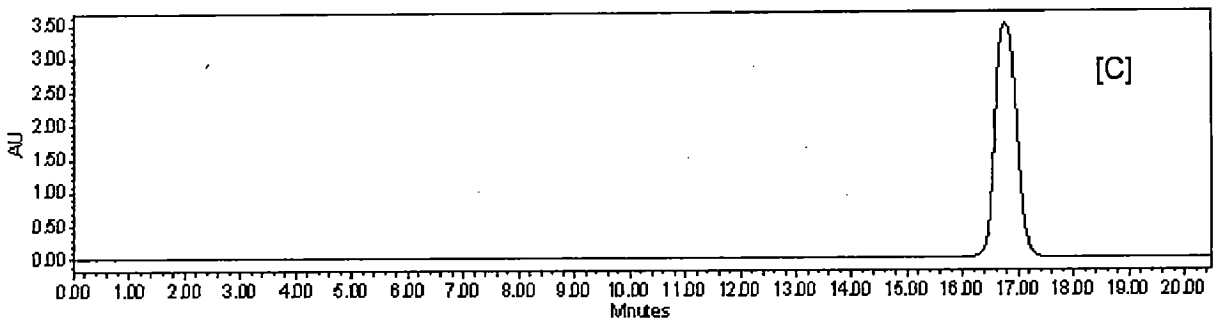
[c] Products were identified by co-injection analysis with authentic samples.



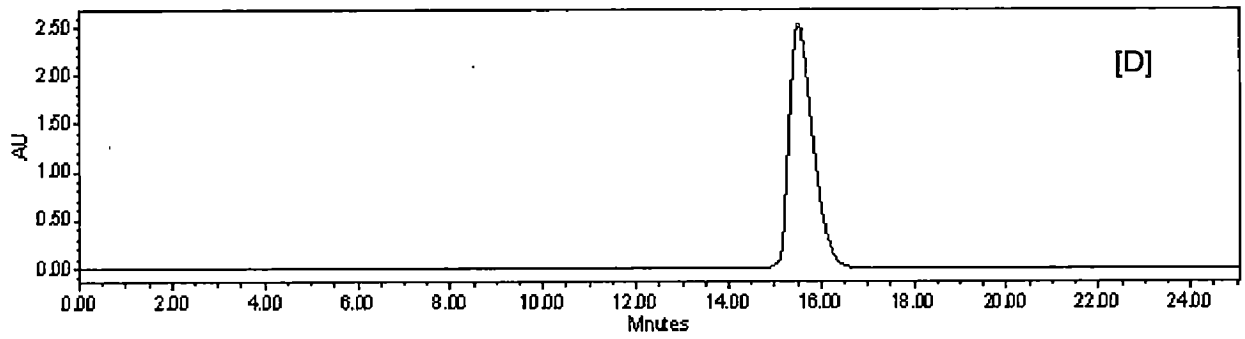
Cytosine



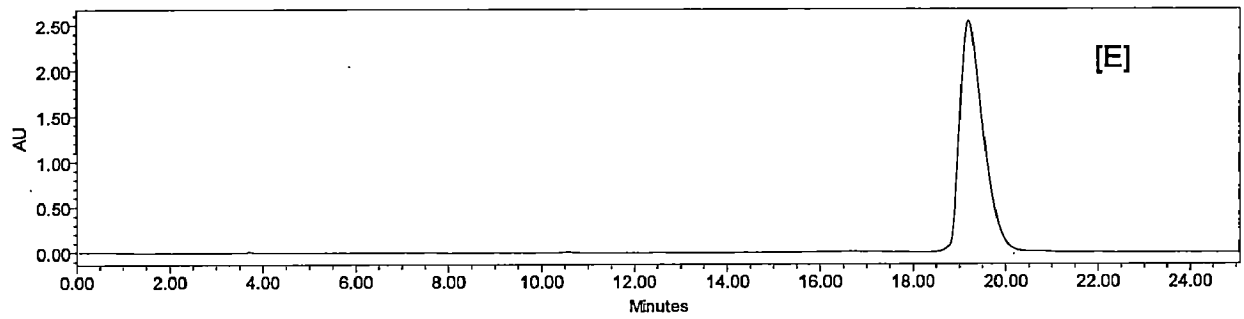
4(3H)-pyrimidinone



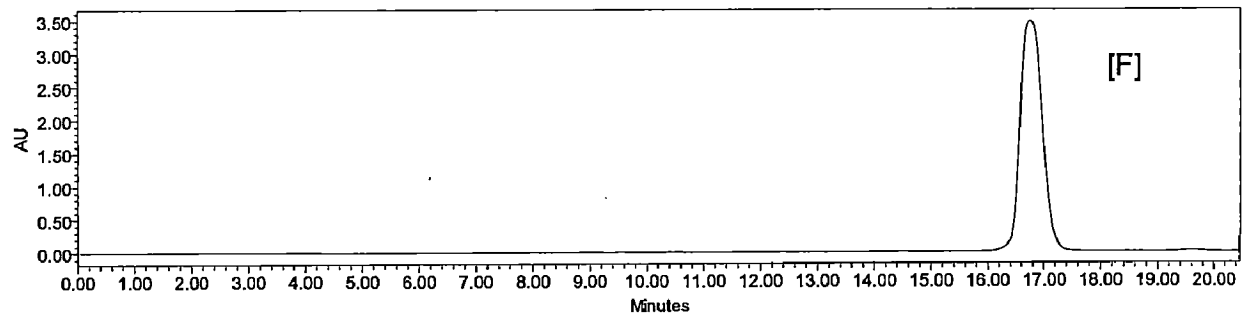
9-(hydroxyacetyle) purine



Adenine



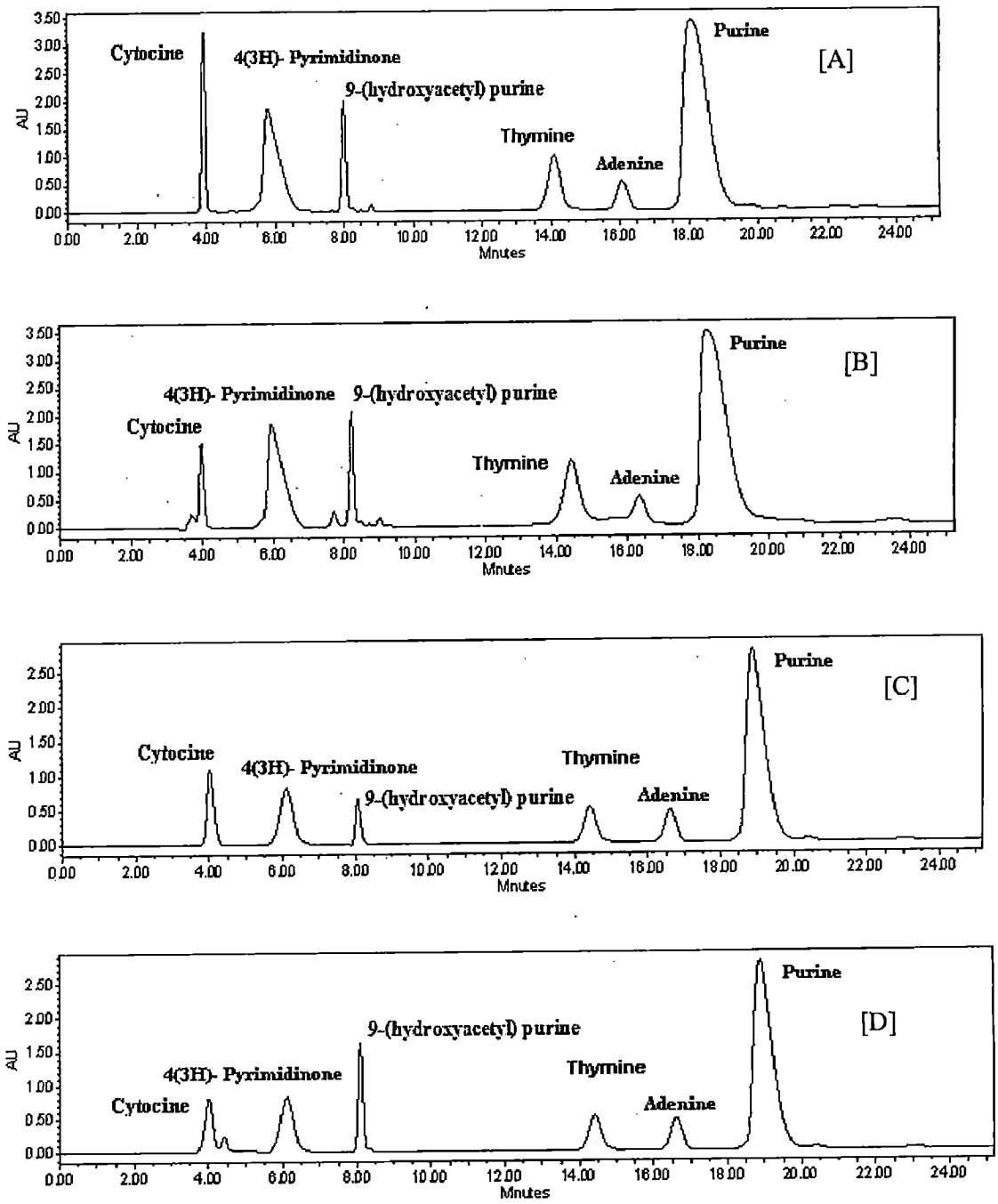
Purine



Thymine

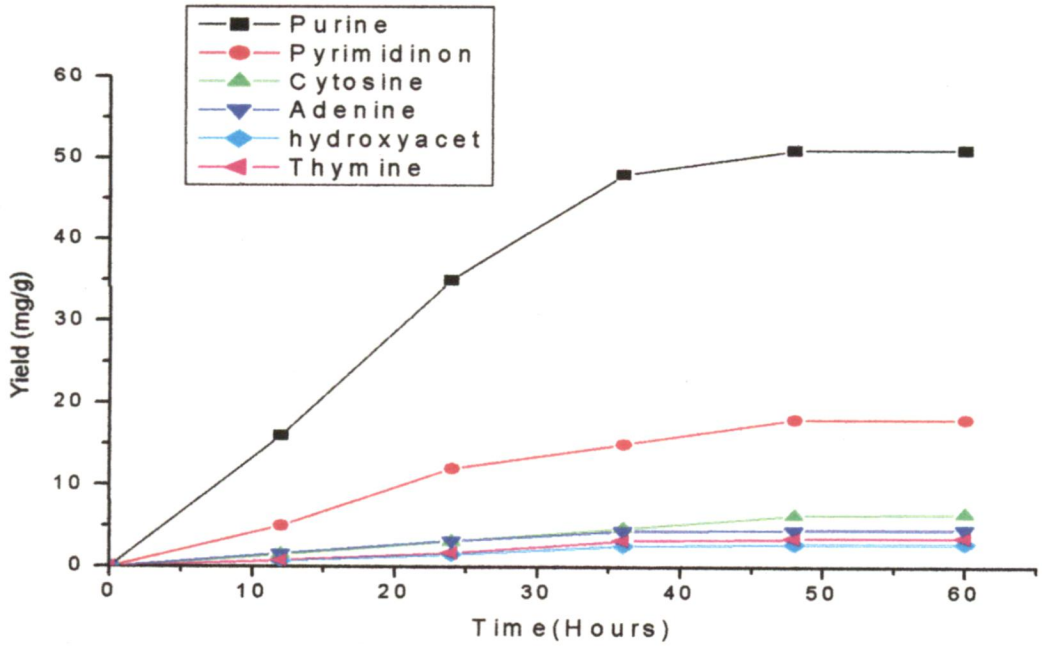
HPLC chromatograms of cytocine[A], 4(3H)-pyrimidinone [B], 9-(hydroxyacetyle) purine [C], adenine [D], purine [E] and thymine

Figure 4.1



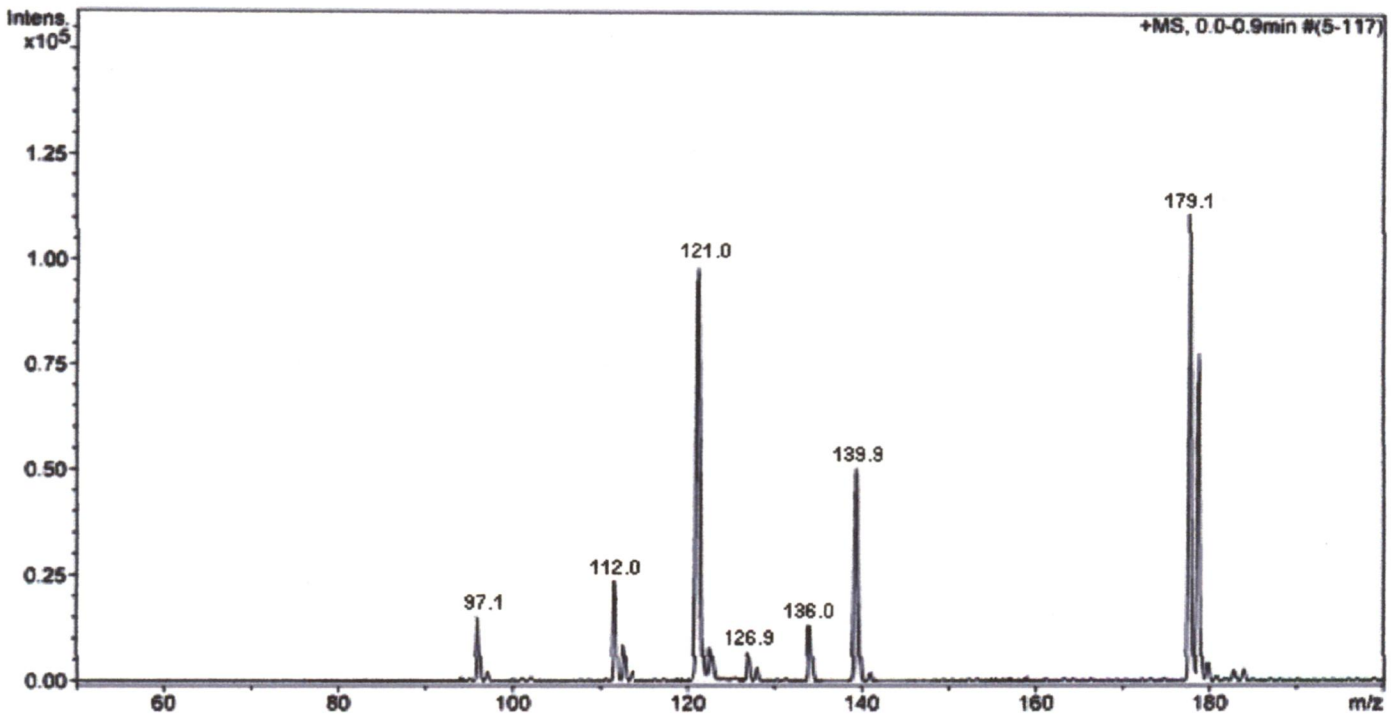
HPLC chromatograms of products obtained when: formamide was heated in the presence of manganese oxides at 160 °C for 48 h: [A] manganosite (MnO), [B] hausmannite (Mn_3O_4), [C] hausmannite (Mn_3O_4) and [D] pyrolusite (MnO_2)

Figure 4.2



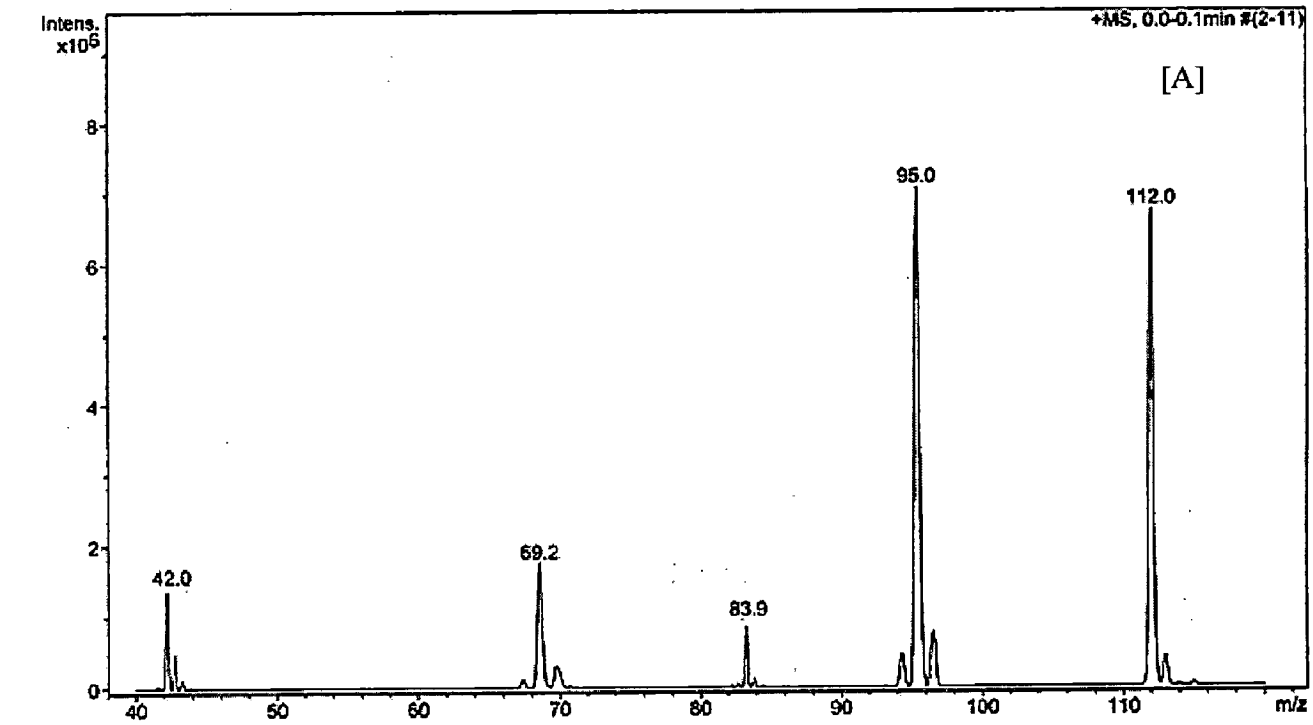
Time dependent analysis of product formation (formamide heated with manganosite (MnO))

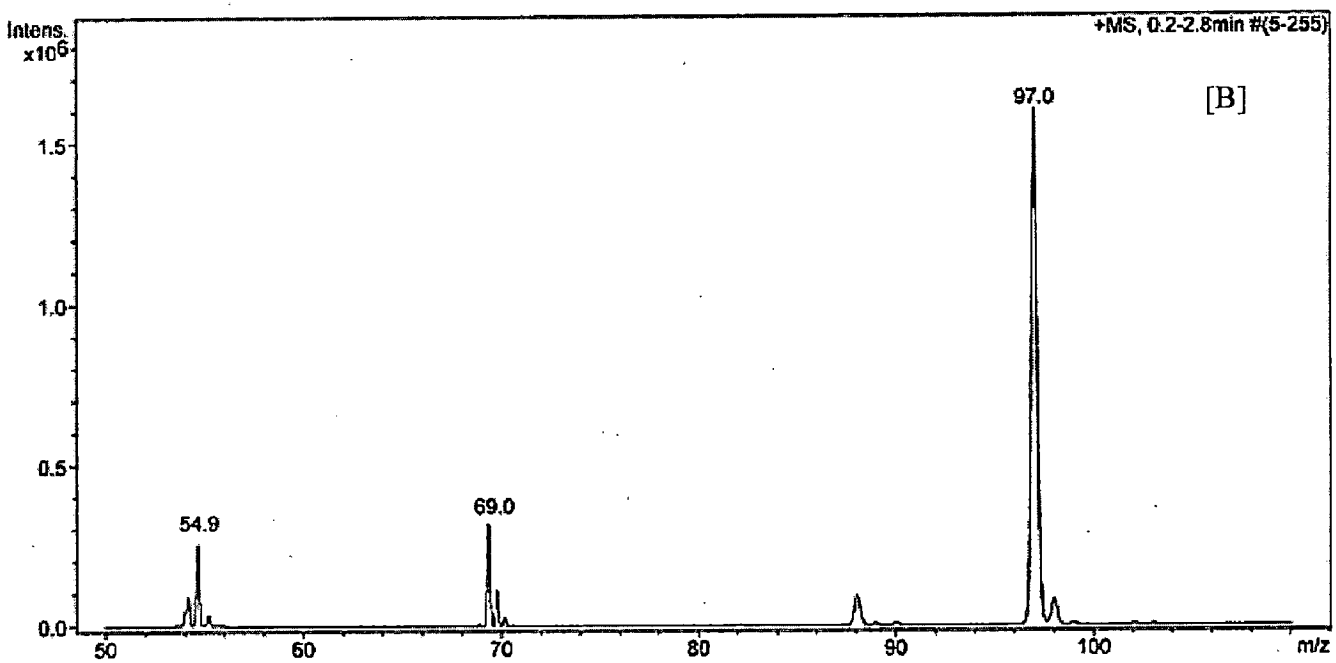
Figure 4.3

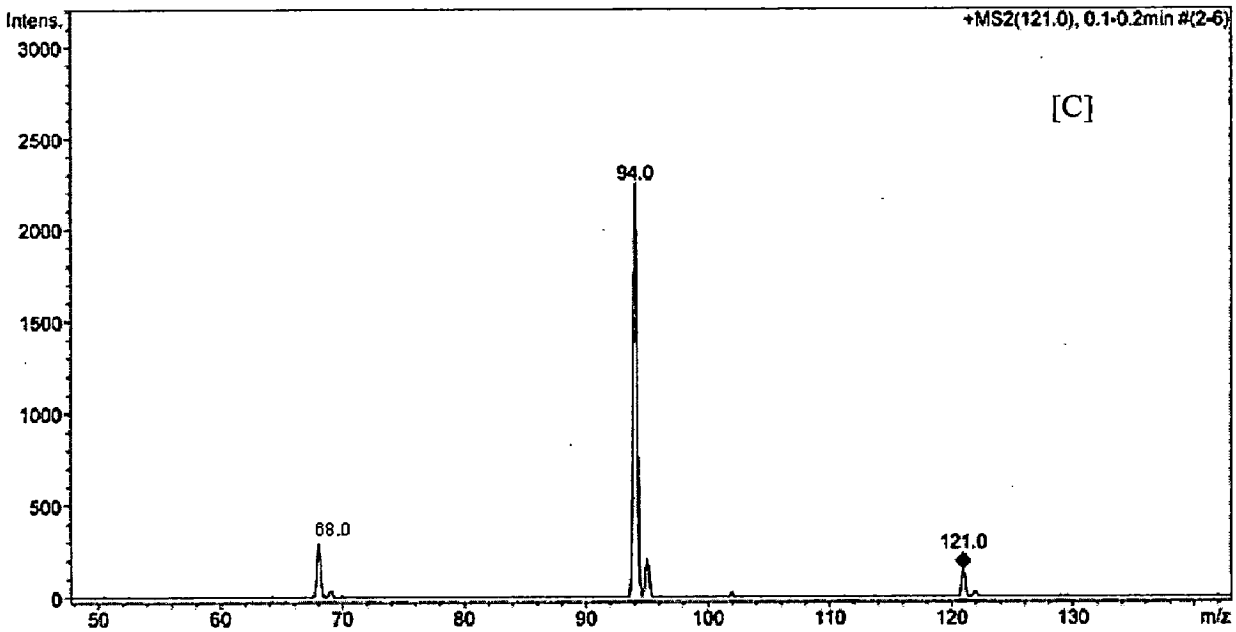


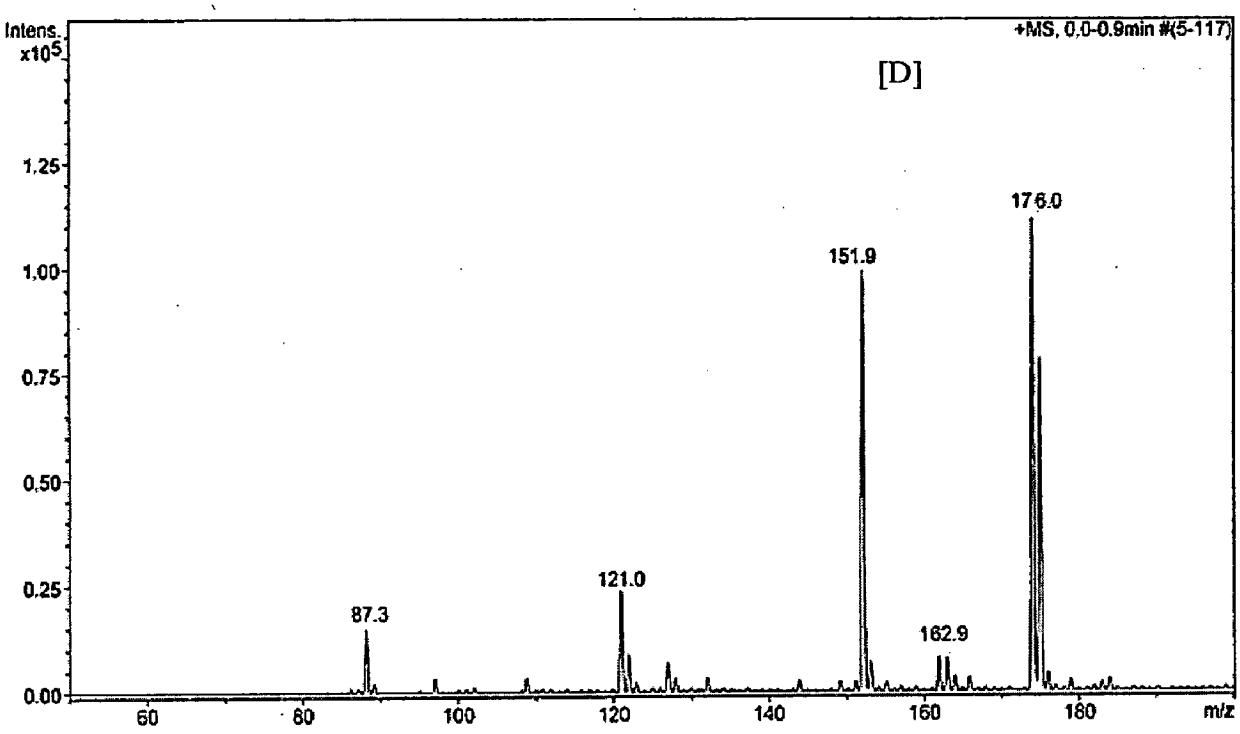
ESI-MS spectra resulting from formamide heated at 160 °C for 48 hours in the presence of manganosite (MnO)

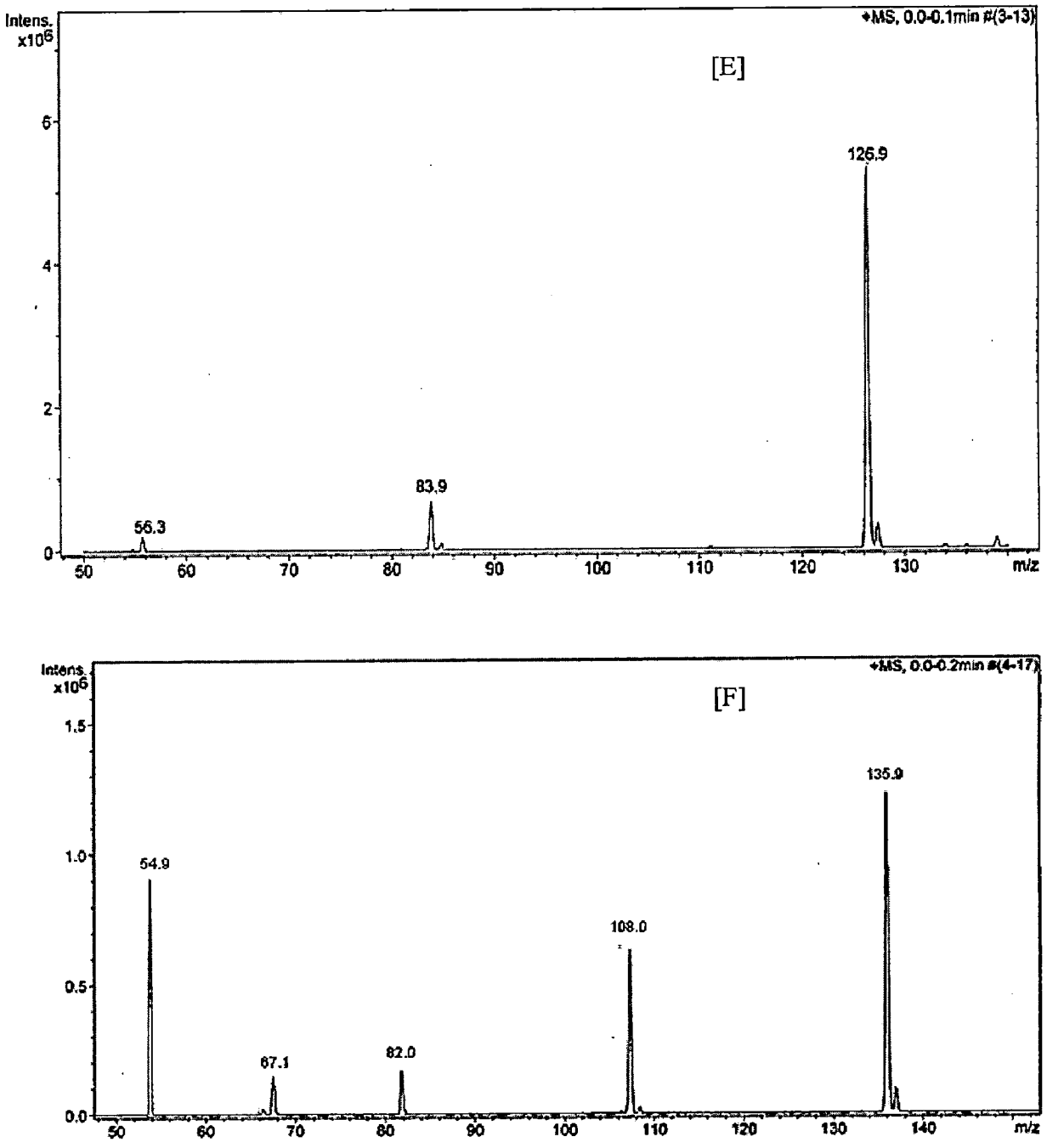
Figure 4.4


Intens.
x10^6
+MS, 0.0-0.1min #(2-11)
[A]
95.0
112.0
69.2
42.0
83.9
m/z


Intens.
x10^6
+MS, 0.2-2.8min #(5-255)
[B]
97.0
69.0
54.9
m/z


Intens.
+MS2(121.0), 0.1-0.2min #(2-6)
[C]
94.0
68.0
121.0
m/z


Intens.
x10^5
+MS, 0.0-0.9min #(5-117)
[D]
176.0
151.9
121.0
87.3
162.9
m/z



Mass spectra resulting after MS/MS of cytocine[A], 4(3H)-pyrimidinone [B], 9-(hydroxyacetyle) purine [C], adenine [D], thymine[E] and purine [F]

Figure 4.5

References:

1. Abelson, P. H. "Chemical events on the primitive Earth". Proc. Natl. Acad. Sci., **55**, 1365 (1966).
2. Miller, S. L. "Production of some organic compounds under possible primitive Earth conditions". Journal of the American Chemical Society, **77**, 2351 (1955).
3. Gesteland, R. F., Cech, T. R., and Atkins, J. F. "The RNA World". **3**rd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 309 (2006).
4. Battersby, T. R., Albalos, M. and Friesenhahn, M. J. "An unusual mode of DNA duplex association: Watson-Crick interaction of all-purine deoxyribonucleic Acids". Chemistry and Biology, **14**, 525 (2007).
5. Crick, F. H. C. "The Origin of the genetic code". Journal of Molecular Biology, **38**, 367 (1968).
6. Heuberger, B. D. and Switzer, C. "An alternative nucleobases code: characterization of purine-purine DNA double helices bearing guanineisoguanine and diaminopurine 7-deaza-xanthine base pairs". Chem. Bio. chem., **9**, 2779 (2008).
7. Tso, P. O. P., Melvin, I. S. and Olson, A. C. "Interaction and Association of Bases and Nucleosides in Aqueous Solutions". J. Am. Chem. Soc., **85**, 1289 (1963).
8. Benner, S. A. "Understanding Nucleic Acids Using Synthetic Chemistry". Accounts of Chemical Research, **37**, 784 (2004).
9. Oró, J. "Mechanism of synthesis of adenine from hydrogen cyanide under possible primitive Earth conditions". Nature, **191**, 1193 (1961).
10. Ehrenfreund, P., Irvine, W., Becker, L., Blank, J., Brucato, J. R., Colangeli, L., Derenne, S., Despois, D., Dutrey, A., Fraaije, H., Lazcano, A., Owen, T., and

Robert, F. "Astrophysical and astrochemical insights into the origin of life". Intl. Space Sci, I. I. T. Reports on Progress in Physics, **65**, 1427 (2002).

11. Ferris, J. P. and Orgel, L. E. "Aminomalenonitrile and 4-Amino-5-cyanoimidazole in Hydrogen Cyanide Polymerization and Adenine Synthesis". J. Am. Chem. Soc., **87**, 4976 (1965).
12. Levy, M., Miller, S. L., and Oró, J. "Production of guanine from NH_4CN polymerizations", J. Mol. Evol., **49**, 165 (1999).
13. Ferris, J. P. and Orgel, L. E. "An Unusual Photochemical Rearrangement in the Synthesis of Adenine from Hydrogen Cyanide". J. Am. Chem. Soc., **88**, 1074 (1966).
14. Joyce, G. F. "RNA evolution and the origin of life". Nature, **338**, 217 (1989).
15. Fuller, W. D., Orgel, L. E., and Sanchez, R. A. "Studies in prebiotic synthesis. VII Solid-State Synthesis of Purine Nucleosides". J. Mol. Evol., **1**, 249 (1972).
16. Ferris, J. P. and Orgel, L. E. "Studies in Prebiotic Synthesis. I. Aminomalenonitrile and 4-Amino-5-cyanoimidazole". J. Am. Chem. Soc., **88**, 3829(1966).
17. Schopf, J. W. "Life's Origin: The Beginnings of Biological Evolution". University of California Press: Berkeley, 113 (2002).
18. Zubay, G. and Mui, T. "Prebiotic synthesis of nucleotides". Orig. Life Evol. Biosph. **31**, 87(2001).
19. In ECT Kirk-Othmer Encyclopaedia of Chemical Technology Wiley-Interscience: **11**, 258(1978).
20. Saladino, R., Crestini, C., Ciambecchini, U., Ciciriello, F., Costanzo, G., and Di Mauro, E. "Synthesis and degradation of Nucleobases and nucleic acids by

formamide in the presence of montmorillonite". Chem. Bio. Chem., **5,** 1558 (2004).

21. Saladino, R. Crestini, C. Ciciriello, F. Costanzo, G. and Di Mauro, E. "Formamide Chemistry and the Origin of Informational Polymers". Chemistry and Biodiversity Helvetica Chimica Acta, **4**, 694 (2007).
22. Saladino, R., Crestini, C., Costanzo, G., Negri, R. and Di Mauro, E. "Possible prebiotic synthesis of purine, adenine, cytosine and 4(3H)-pyrimidinone from formamide: Implications for the origin of life". Bioorganic and Medicinal Chemistry, **9**, 1249 (2001).
23. Saladino, R., Crestini, C., Neri, R.., Brucato, J. R., Colangeli, L., Ciciriello, F., Di Mauro E., and Costanzo, G. "Synthesis and degradation of nucleic Acid components by formamide and cosmic dust analogues". Chem. Bio. Chem., **6,** 1368 (2005).
24. Sanchez, R. A., Ferris, J. P., and Orgel, L. E. "Conditions for purine synthesis: Did prebiotic synthesis occur at low temperatures". Science, **153,** 72 (1966).
25. Crovisier, J. "The molecular complexity of comets in Astrobiology: Future perspectives". Kluwer Eds. Chapter, **8,** 79 (2004).
26. Bockelee, M. D., Lis, D. C. and Wink, J. E. "Cometary and Interstellar Material". Astron Astrophysics., **353,** 1101 (2000).
27. Schutte, W. A., Boogert, A. C. A., Tielens, A. G. G. M., Whittet, D. C. B., Gerakines, P. A., Chair, J. E. Ehrenfreund, P., Greenberg, J. M., Deshoeck, Van E. F. and Degraauw, T. H. "Weak ice absorption features at 7.24 and 7.41:μm in the spectrum of the obscured young object W33 A". Astron, Astrophysics. **343,** 906 (1999).

28. Saladino, R., Negri, V., Crestini, C., Costanzo, G., Graciotti, M., and Di Mauro, E. "Synthesis and Degradation of Nucleic Acid Components by Formamide and Iron Sulphur Minerals". J. Am. Chem. Soc., **130**, 15512 (2008).

29. Gallori, E. Biondi, E., and Branciamore, S. "Looking for the primordial genetic honeycomb". Orig. Life Evol. Biosph., **36**, 493 (2006).

30. Scappini, F., Casadei, F., Zambone, R., Franchi, M., Gallori, E. and Monti, S. "Protective effect of clay minerals on adsorbed nucleic acid against UV radiation: possible role in the origin of life". Int. J. Astrobiol., **3**, 17 (2004).

31. Saladino, R., Ciambecchini, U., Crestini, C., Costanzo, G., Negri, R., and Di Mauro, E. "One-pot TiO_2-catalyzed synthesis of nucleic bases and acylonucleosides from formamide: Implications for the origin of life". Chem. Bio. Chem, **4,** 514 (2003).

32. Saladino, R., Crestini, C., Costanzo, G., and Di Mauro, E. "Advances in the prebiotic synthesis of nucleic acids bases: Implications for the origin of life". Current Organic Chemistry, **8**, 1425 (2004).

33. Saladino, R., Neri, V., and Crestini, C. "Role of clays in the prebiotic synthesis of sugar derivatives from formamide". Philosophical Magazine **90**, 2329 (2010).

34. Uma Shanker, Brij Bhushan, G. Bhattacharjee and Kamaluddin "Formation of Nucleobases from Formamide in Presence of Iron Oxides: Implication in Chemical Evolution and Origin of Life". Astrobiology, **11**, 225 (2011).

35. Kirkpatrick, E.C. (to E.I. Dupont de Nemours & Co. Inc) U.S. Patent, **2**, 346, 425 (1944).

36. Costanzo, G., Saladino, R., Crestini, C., Ciciriello, F., and Di Mauro E. "Formamide as the main building block in the origin of nucleic acids". BMC Evolutionary Biology, **7**, 1 (2007).

CHAPTER-5

OLIGOMERIZATION OF AMINO ACIDS CATALYZED BY MANGANESE OXIDES

5.1 Introduction:

Life probably emerged through chemical evolution [1-2], initially involving combinations of simple molecules present on the primitive Earth into organic compounds (e.g. amino acid, and nucleotides) [3]. These simple precursors produced eventually more complicated biopolymers [4-6]. The first biopolymers might have been condensed phases of peptides at the surface of minerals or other substances [7]. Peptide-like polymers seem to be among the first macromolecules, which might have played a key role in chemical processes leading to emergence of the first living cell [8]. On the basis of theoretical and experimental considerations, primitive oceans have been proposed as the probable starting place of life on Earth. One of the considerations strongly supporting early oceans as primeval locale for chemical evolution was that the organic compounds once formed and settled in the ocean were protected from degradation by strong ultraviolet light on the primitive Earth. In addition to the above consideration, the hypothesis of chemical evolution in the primeval seas is more favorable because the seas might have provided a wealth of catalytic surfaces, source of transportation and mixing of intermediate products [9]. The interaction of wide variety of organic molecules such as amino acids, peptides, nucleic acid bases, nucleotides, among each other on the clay and clay minerals have been investigated in detail [10-13]. Correlation of the concentration of minor transition metals with their biological behavior in primeval seas has also been established [14]. It has been proposed that transition metal ions abundantly present in primeval seas might have formed complexes with simple molecules readily available to them and played crucial roles in chemical evolution. Studies on the importance of transition metals in chemical evolution have also been reported [15].

Amino acid condensation catalyzed by inorganic oxide surfaces is a widely recognized scenario for the prebiotic peptide formation on the planets of the Earth-like group [16-26]. All the works reported in these lines have involved different kind of Clays. The results obtained strongly support the heterogeneous condensation hypothesis, but at the same time gave poor insight into chemical mechanism of the peptide formation and the role of different surface. This was probably due to the complexity involved in minerals surface chemistry, i.e. presence of different active surface sites, surface functional groups to planes, layers, edges, etc. [27]. For a better understanding, oxides of silica and alumina were introduced for catalytic activity in the amino acid condensation [28-29]. Metal oxides constitute an important component of the Earth crust and other planets and their role in catalyzing different important reactions in the course of chemical evolution and origin of life cannot be ruled out. Under mild conditions, alumina has been reported to catalyze peptide bond formation of alanine. All the three forms of alumina, acidic, neutral and basic are useful for the formation of Ala_2, Ala_3 and cyclic Ala_2 [30]. Activated alumina has been used as energy source for peptide bond formation [31]. Highest yield of Ala_2 was obtained with neutral alumina. Alumina-catalyzed peptide bond formation up to trimmers and tetramers has also been reported. Use of activated alumina afforded longer chain oligomers of glycine up to $(Gly)_{11}$ [32]. Synthetic ferrihydrite was found to act as amino acid adsorbent and promoter for peptide bond formation [33]. Condensation of DL – glyceraldehydes to ketohexoses in the presence of iron (III) oxide hydroxide has also been reported [34].

In an effort for studies on peptide bond formation, the catalytic activity of manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite

(MnO_2)) having different surface area and surface acidity has been studied. In the present work study on oligomerization of glycine, alanine and β-alanine in the presence of manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)) in the temperature range 50-120 °C has been carried out.

5.2 Results and Discussion:

In the control experiments with glycine only, formation of a trace of Cyclic $(Gly)_2$ and $(Gly)_2$ was observed after 35 days, however, formation of a peptide in the blank experiment of alanine was not at all observed, in accordance with the previous observations [35]. Tables 5.1-5.2 shows list the yields of products obtained by heating glycine and alanine in the presence of manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)) at temperatures 50 °C, 80 °C and 120 °C for 35 days. Representative HPLC chromatograms are shown in Figures. 5.1-5.3. The reaction products were identified by retention times and co-injection method. The overall yield of products as the function of time and temperature are given by Figures 5.4-5.11. Yields of the products were determined comparing peak area of products to the standards.

Formation of peptides up to trimer was observed with glycine only whereas alanine afforded only its dimer. Glycine in the presence of manganosite (MnO) afforded formation of $(Gly)_3$ (16.7%) along with $(Gly)_2$ (33.2%) and Cyclic$(Gly)_2$ (9.4%) while alanine afforded $(Ala)_2$ (12.75%) and Cyclic(Ala_2) (4.7 %) after 35 days of heating at 90 °C. The results on formation of peptide bond at 50 °C suggest that peptide formation can also occur at lower temperatures and do not require the presence of localized heat sources, such as volcanoes and hydrothermal vents. Thus, Manganese is the 10^{th} most abundant element in the biosphere (~10^{14} kg of suspended and dissolved manganese found in oceans) and is

second only to iron in relative terrestrial abundance of the transition metals. On average, crustal rocks contain about 0.1% by weight of Mn [36], coordinated with oxygen, and may also exist in the bottom of seas as nodules; abiotic peptide synthesis might be a highly feasible process at a very short astronomical time scale. All the oxides afforded formation of cyclic anhydride along with dimer of amino acid. Up to trimer of glycine was found in the presence of manganese oxide.

The formation of diketopiperazine was favored at higher temperature, 120 °C (Table 5.1) with all the oxides because that the amount of adsorbed water (i.e. the thickness of hydrate layer at the oxides surface) was relatively low as compared to ambient temperatures that shift equilibrium of dehydration reactions. Under such conditions, the cyclization of $(gly)_2$ and $(ala)_2$ into diketopiperazine is much more favorable than peptide chain elongation. Thus it would be quite natural to detect $(gly)_3$, at lower temperature 90 °C, though the overall reaction rate decreases.

ESI-MS spectra of standards have also been given in Figures 5.12-5.13. Figure 5.14 represent the ESI MS spectra of products obtained when glycine and alanine were heated at 90 °C for 28 days in the presence of manganosite (MnO). In the MS spectra of glycine, mass 76.1 corresponds to $[Gly+H]^+$, 115 for $[CycGly_2+H]^+$, 132.9 for $[Gly_2+H]^+$ and 189.9 for $[Gly_3+H]^+$. The ms spectra of alanine mass 90.1 corresponds to $[Ala + H]^+$, 115 for $[CycAla_2 + H]^+$ and 160.9 for $[ala_2 + H]^+$. The results of oligomerization of simple amino acids support the view of Holm et al. about β-$FeOOH.Cl_n$ as an interesting candidate as prebiotic replication matrices [37]. The Red Sea and other sea floor are proposed as spreading centers and possess most of the characteristics that are necessary for prebiotic formation of organic substances [38]. Under such conditions catalytic polymerization of

prebiotic molecules after sorption of the monomer organic molecules like amino acids the crystalline host structures may have the vital role.

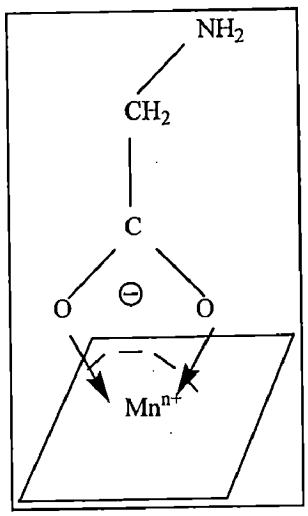
It is important to note that among the all forms of manganese oxides, (manganosite (MnO) and are the most efficient, as it produced longer oligomers of amino acids as well as high yield of both glycine and alanine. While bixbyite (Mn_2O_3) and pyrolusite (MnO_2) produced glycine and alanine oligomers in comparatively low yields. The observed yield of the products with three iron oxides studied followed the trend;

Manganosite (MnO)> hausmannite (Mn_3O_4)> bixbyite (Mn_2O_3)> pyrolusite (MnO_2)

Catalytic efficiency of manganese oxides can be explained on the basis of surface area and surface acidity of the catalyst used. The observed results clearly reflect the role of surface area of the catalysts having potent chemical functional groups. The most reasonable explanation for the formation of oligopeptides at the surface of manganese oxides is the specific surface area and surface acidity i.e. surface hydroxyl groups. The observed trend is in conformity with the decreasing surface area of manganese oxides. As could be observed from the data of surface area that manganosite (MnO), having maximum surface area (238.89 m^2/g) is the most effective compared to hausmannite (Mn_3O_4) (226.56 m^2/g), bixbyite (Mn_2O_3) (218.35 m^2/g) and pyrolusite (MnO_2) (214.57m^2/g). Besides surface area, surface acidity of manganese oxides might also be responsible for the higher yield. In a manganese oxide/water system, Me-OH_2^+ and Me-O^{-1}, hydroxyl groups on the solid surface are the most important sites for surface interactions. These groups can act as acids or bases, depending on the pH of the solution the zero points of charge P_{ZPC} of MnO, Mn_2O_3, Mn_3O_4 and MnO_2 are >10, >10, 7.7 and 7.3 respectively. At neutral pH (pH < P_{ZPC}), the hydroxyl groups on the manganese oxide surface exist in the acidic form and the adsorbent surface is

positively charged. Electrostatic interactions of positively charged surface of manganese oxides (MnO and other manganese oxides) with amino acids may take place through negatively charged atoms present in amino acids by carboxylic and amino groups, via the nonpaired electrons of the oxygen and nitrogen. Probable Mechanism for the peptide bond formation on a manganese oxide surface has also been proposed (Figure. 5.15).

In the presence of β-alanine, formation of oligopeptides also occurs under the similar conditions. Alanine and β-alanine both afforded dimer. After 35 days yield almost remained constant. Formation of oligopeptides was supposed to take place by the interaction between Lewis acid Mn^{n+} centers with negatively charged oxygen of amino acids as shown in figure given below:



5.3 Conclusion

1. Manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)) are able to catalyze the reaction of peptide bond formation in glycine and alanine without applying drying/wetting conditions.
2. Formation of peptide bond was observed even at 50 °C after 7 days of heating. High temperature favored formation of diketopiperazine derivatives.

3. Glycine on manganosite (MnO) produced Cyclic $(Gly)_2$, $(Gly)_2$ and $(Gly)_3$, and with alanine, Cyclic $(Ala)_2$ and $(Ala)_2$. Hausmannite (Mn_3O_4) also produced the same products but in lesser yield, while bixbyite (Mn_2O_3) and pyrolusite (MnO_2) produced cyclic anhydride of glycine and alanine with a trace amount of dimer.
4. It may be concluded that during the course of chemical evolution the role of each of these oxides was very significant in catalyzing polymerization of biomonomers.

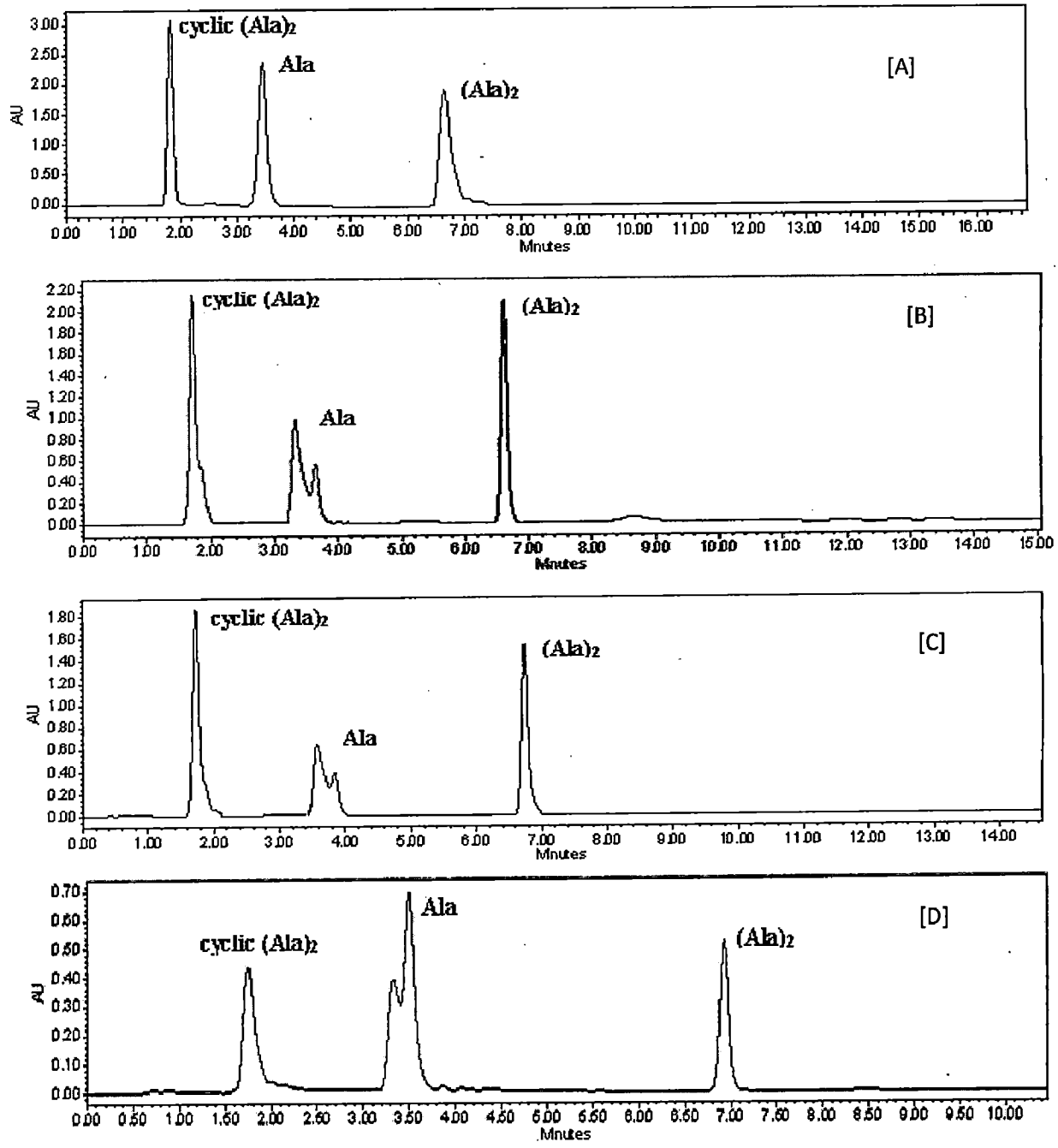
Catalyst[a]	**% yield[b] of the products[c] formed when glycine were heated at 50^0, 90^0 and 120^0 C for 35 days**								
	$Cyc(Gly)_2$			**$(Gly)_2$**			**$(Gly)_3$**		
	50^0	**90^0**	**120^0**	**50^0**	**90^0**	**120^0**	**50^0**	**90^0**	**120^0**
Manganosite (MnO)	0.34	6.41	24.90	1.28	22.74	19.81	6.56	10.87	4.88
Hausmannite (Mn_3O_4)	0.26	2.63	13.20	0.92	09.28	7.66	4.85	5.34	3.14
Bixbyite (Mn_2O_3)	0.18	2.18	9.87	0.54	06.29	4.34	3.84	2.55	2.71
Pyrolusite (MnO_2)	0.09	1.44	7.21	0.39	4.52	3.82	2.46	2.04	1.35

Table 5.1: Yield of the products formed by heating glycine in the presence of manganese oxides

[a] Reactions were performed in the presence of 100 mg of manganese oxide.
[b] Quantitative evaluation was performed by HPLC (Waters 2489, binary system) equipped with a column of Waters (Spherisorp 5 μm ODS2 4.6mm × 250 mm). UV detection was performed at 200 nm wavelength. The mobile phase compositions were 10 mM sodium hexane sulphonate acidified with phosphoric acid to pH~2.5 (solvent A) and acetonitrile of HPLC grade (solvent B), with a flow rate of 1 ml/min. The yields of products were calculated by comparing peak area with the standards.
[c] Products were identified by co-injection analysis with authentic samples.

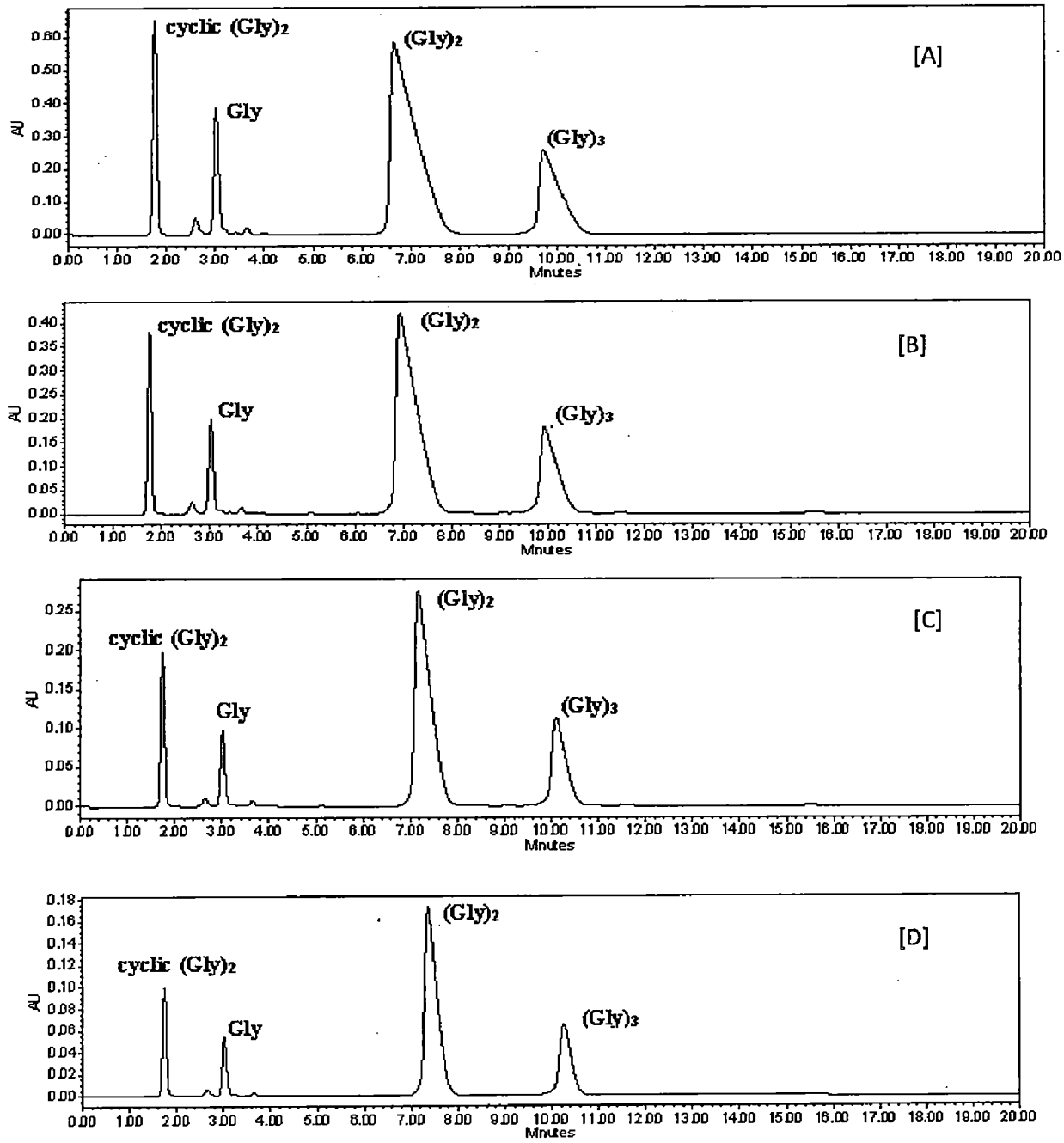
Catalyst[a]	% yield[b] of the products[c] formed when glycine and alanine were heated at 50°, 90° and 120°C for 35 days											
	Cyc(Ala)$_2$			(Ala)$_2$			Cyc(β-Ala)$_2$			(β-Ala)$_2$		
	50°	90°	120°	50°	90°	120°	50°	90°	120°	50°	90°	120°
Manganosite (MnO)	0.19	4.36	16.17	1.24	20.22	11.47	0.14	3.47	14.23	0.96	12.82	10.05
Hausmannite (Mn_3O_4)	0.15	2.23	12.18	0.62	14.67	7.87	0.12	2.12	11.25	0.54	10.02	5.96
Bixbyite (Mn_2O_3)	0.08	1.14	10.26	0.46	9.92	4.25	0.06	0.89	8.84	0.37	7.28	2.44
Pyrolusite (MnO_2)	0.06	2.84	11.45	0.24	7.04	2.38	0.03	0.56	6.48	0.21	4.45	1.71

Table 5.2: Yield of the products formed by heating Alanine and β-Alanine in the presence of manganese oxide



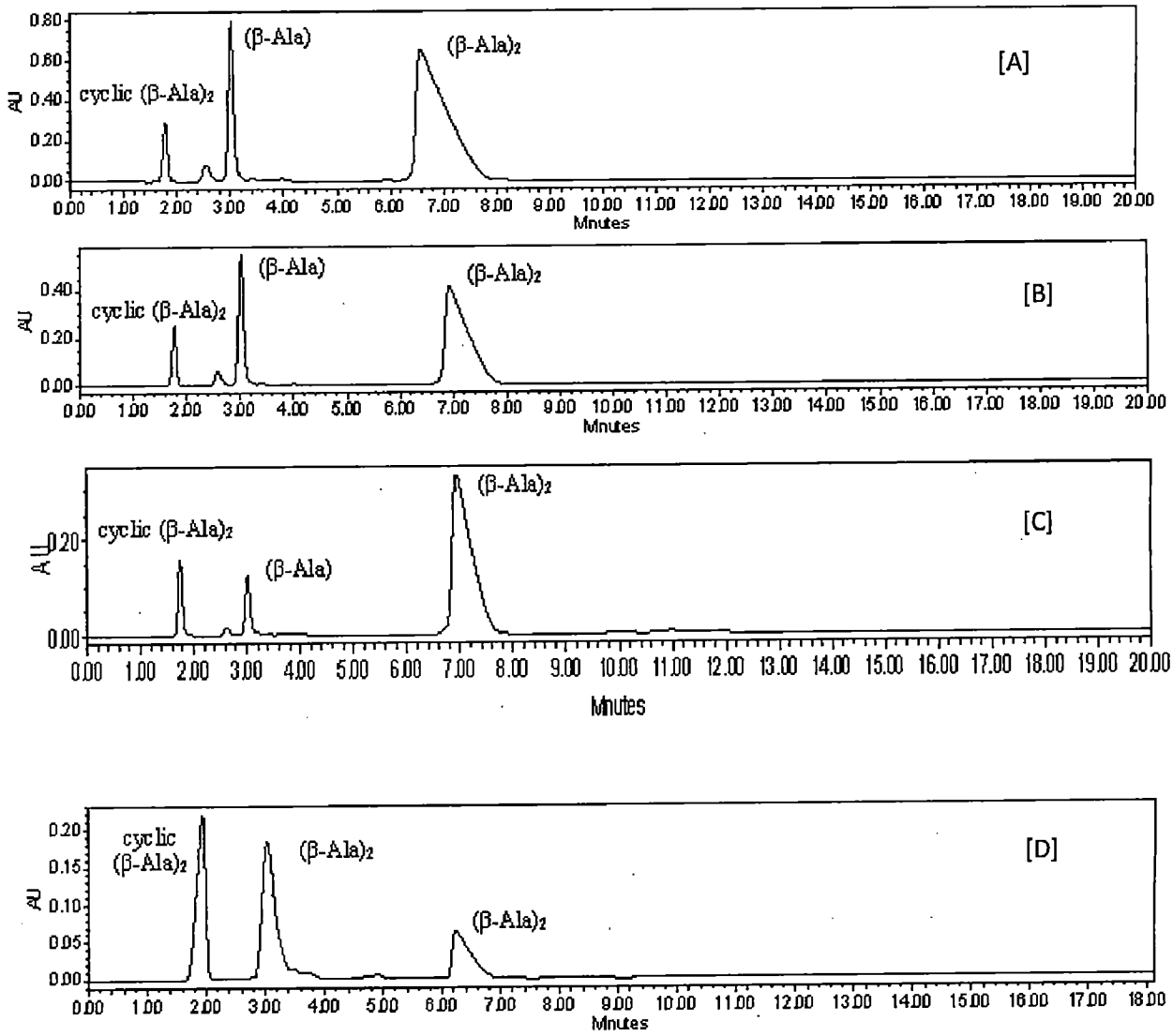
HPLC chromatogram of products formed when glycine was heated at 90 ^{0}C for 35 days in the presence of [A] manganosite (MnO), [B] hausmannite (Mn_3O_4), [C] bixbyite (Mn_2O_3)and [D] pyrolusite (MnO_2)

Figure 5.1



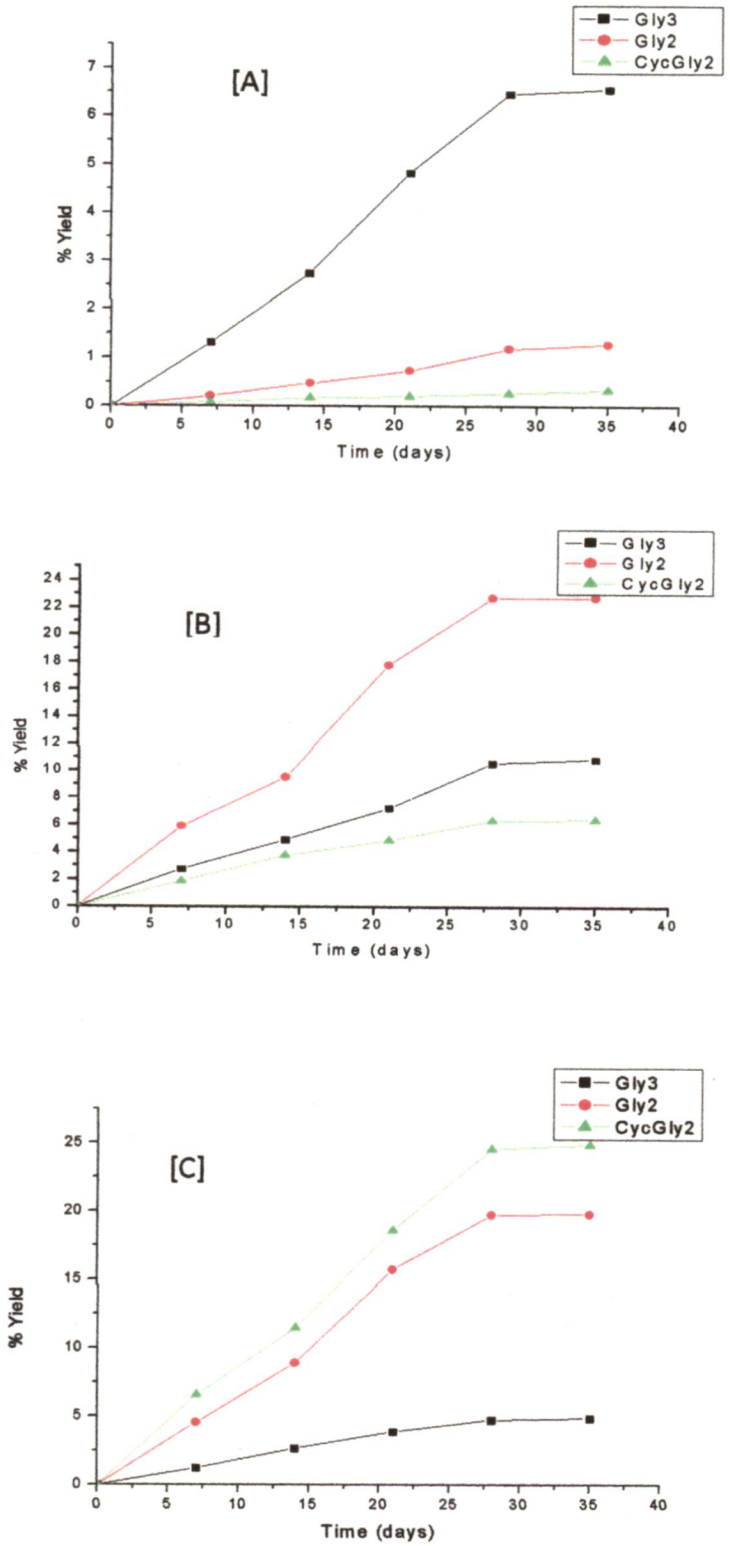
HPLC chromatogram of products formed when alanine was heated at 90 ^{0}C for 35 days in the presence of [A] manganosite (MnO), [B] hausmannite (Mn_3O_4), [C] bixbyite (Mn_2O_3)and [D] pyrolusite (MnO_2)

Figure 5.2



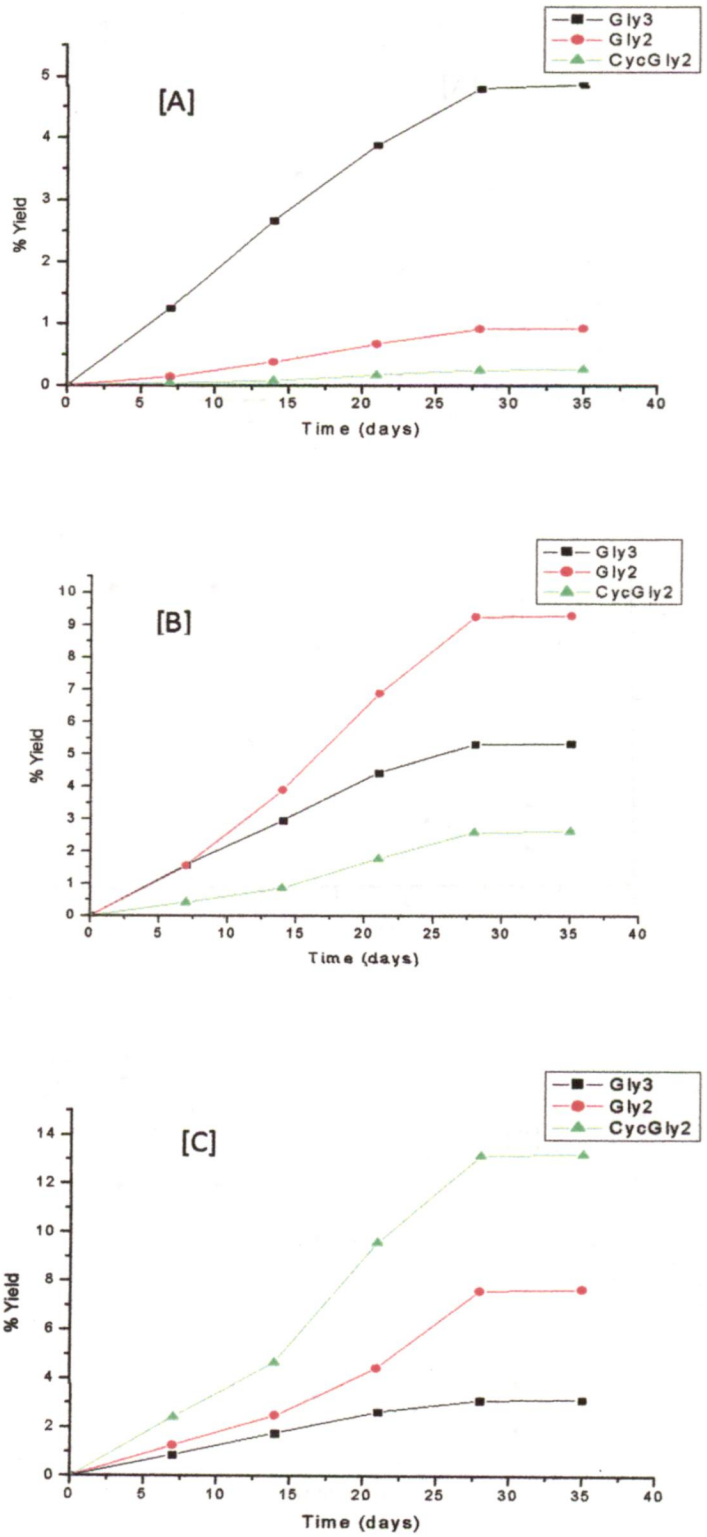
HPLC chromatogram of products formed when β-alanine was heated at 90 ^{0}C for 35 days in the presence of [A] manganosite (MnO), [B] hausmannite (Mn_3O_4), [C] bixbyite (Mn_2O_3)and [D] pyrolusite (MnO_2)

Figure 5.3



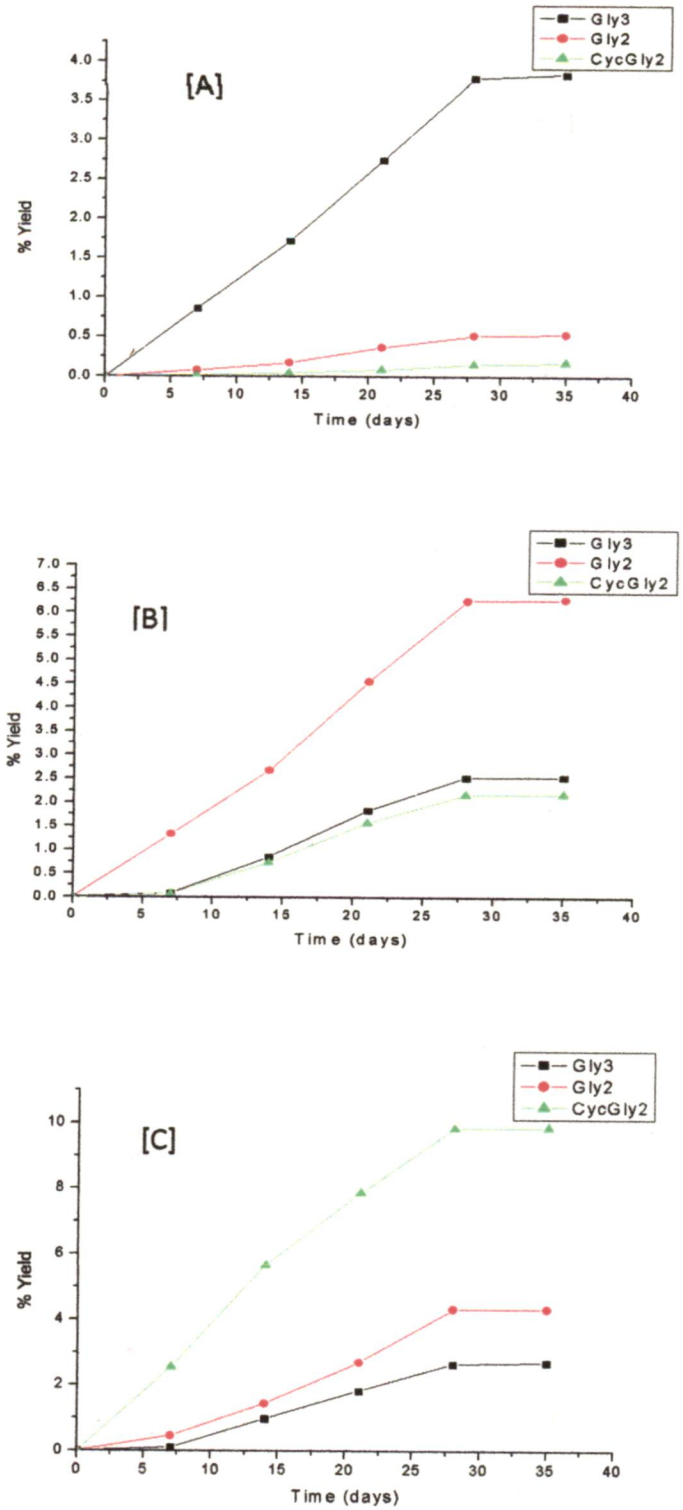
Manganosite (MnO) catalyzed formation of DKP and oligomers of glycine at [A] 50 °C, [B] 90 °C and [C] 120 °C.

Figure 5.4



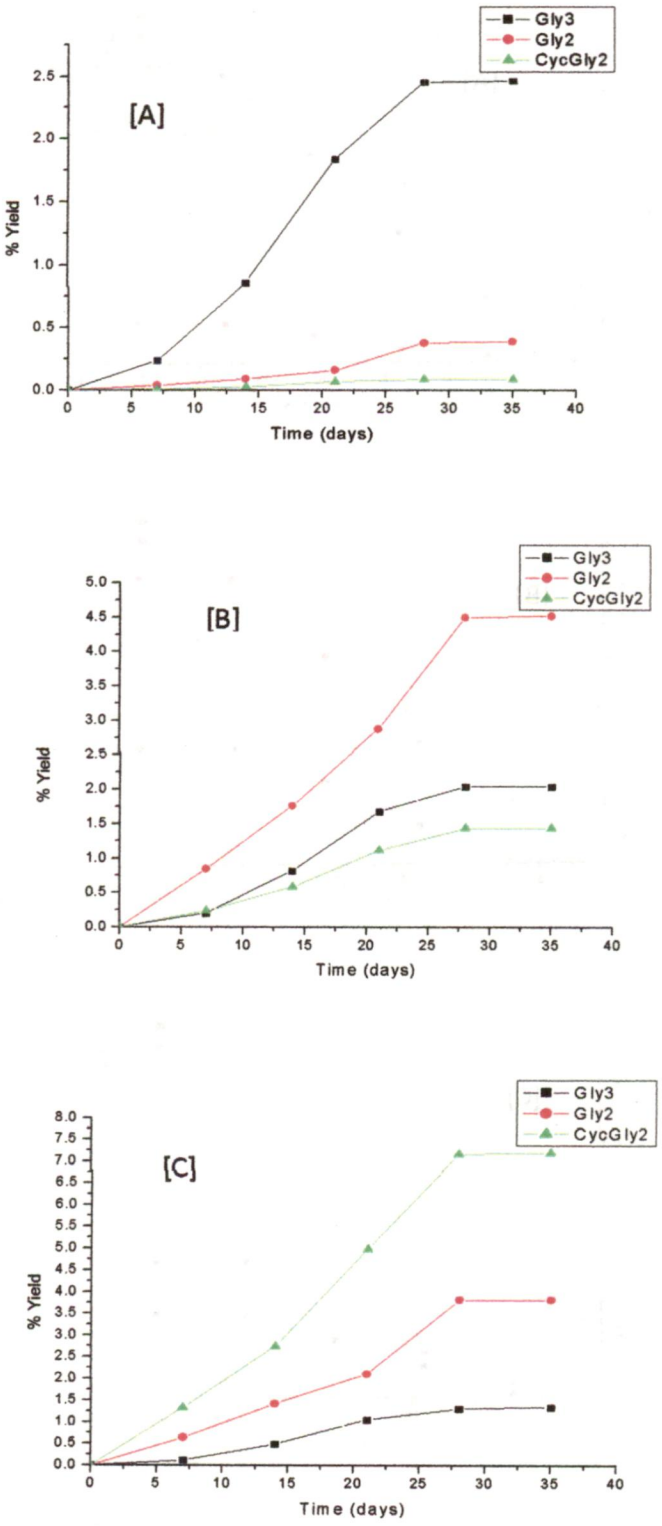
Hausmannite (Mn_3O_4) catalyzed formation of DKP and oligomers of glycine at [A] 50 °C, [B] 90 °C and [C] 120 °C.

Figure 5.5



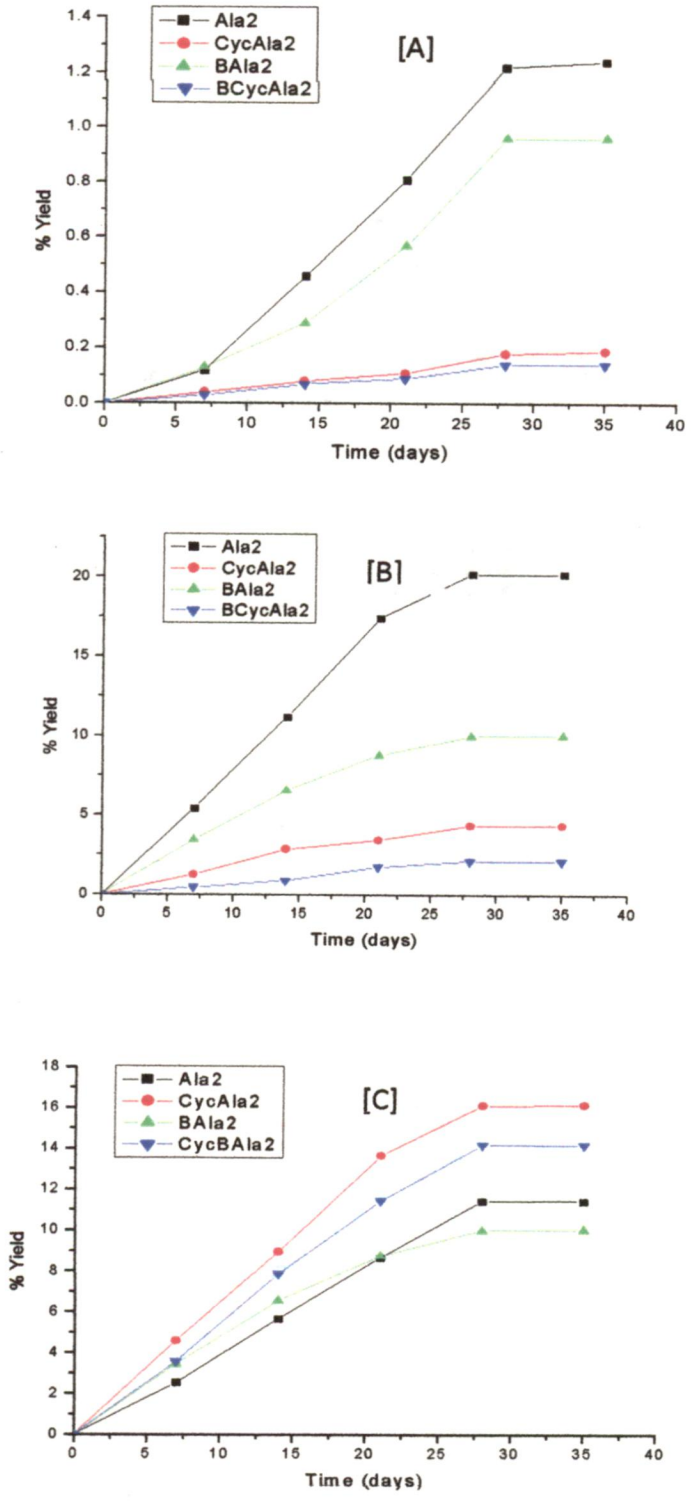
Bixbyite (Mn_2O_3) catalyzed formation of DKP and oligomers of glycine at [A] 50 °C, [B] 90 °C and [C] 120 °C.

Figure 5.6



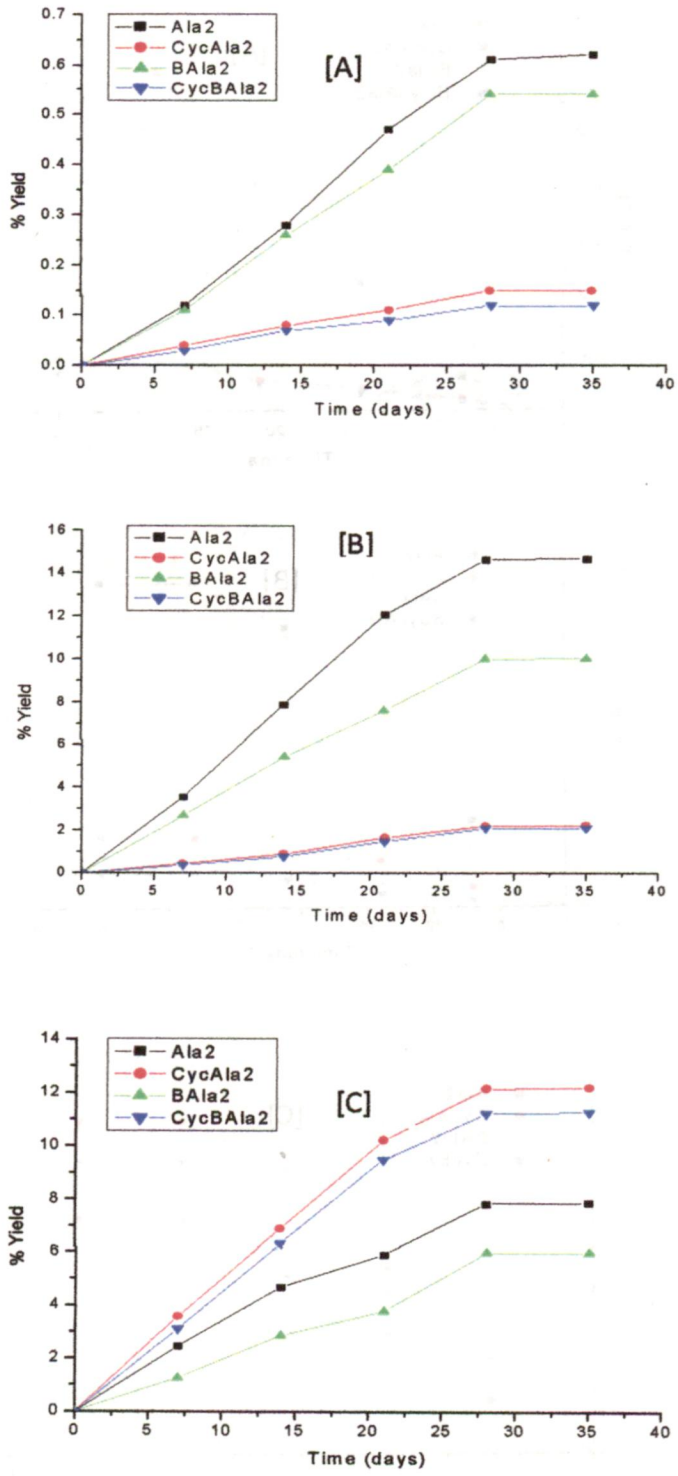
Pyrolusite (MnO_2) catalyzed formation of DKP and oligomers of glycine at [A] 50 °C, [B] 90 °C and [C] 120 °C.

Figure 5.7



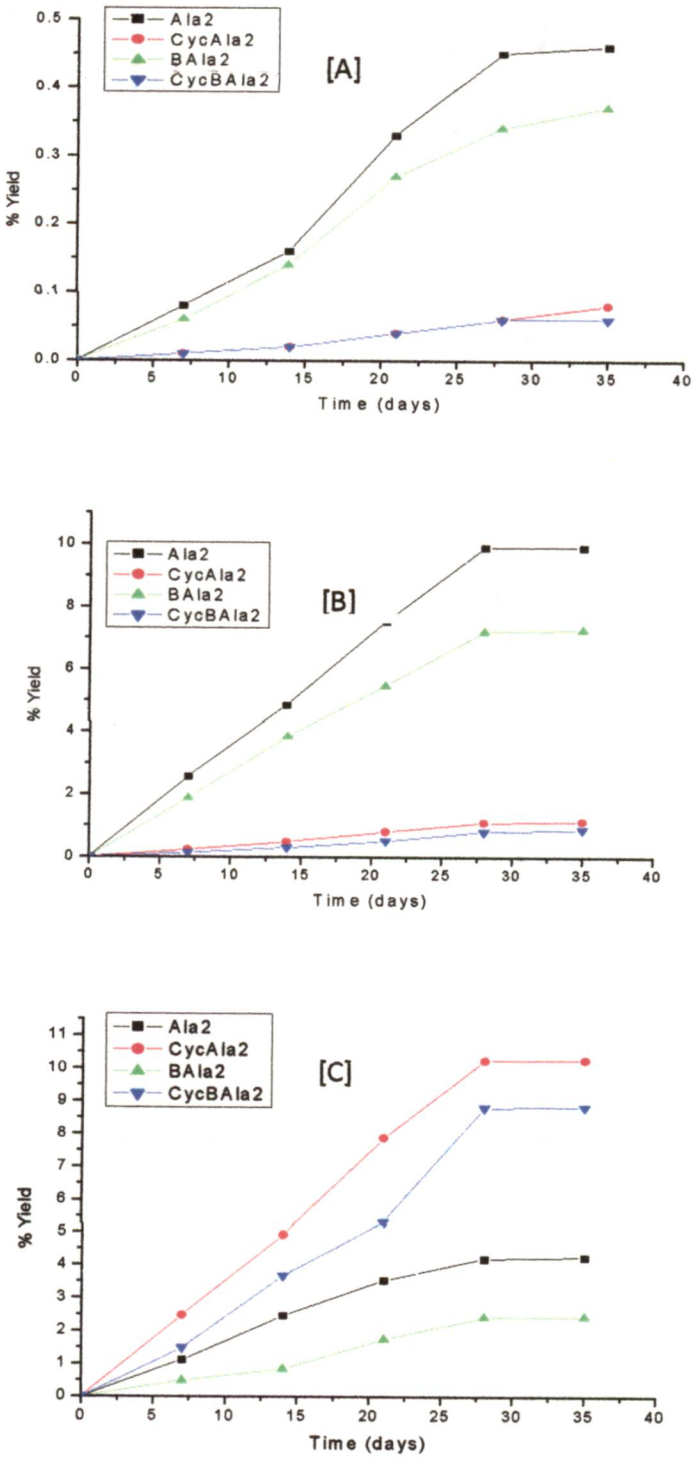
Manganosite (MnO) catalyzed formation of DKP and oligomers of alanine and β-alanine at [A] 50 °C, [B] 90 °C and [C] 120 °C.

Figure 5.8



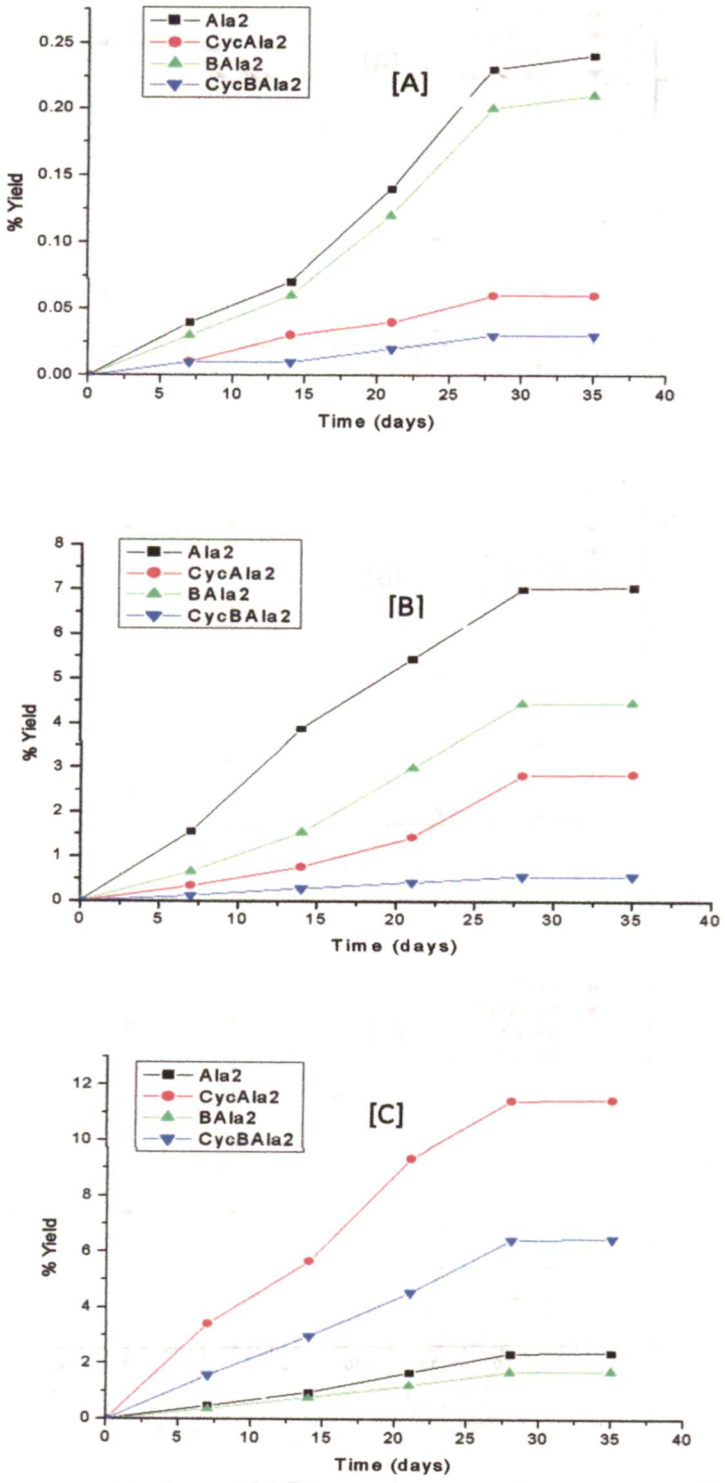
Hausmannite (Mn_3O_4) catalyzed formation of DKP and oligomers of alanine and β-alanine at [A] 50 °C, [B] 90 °C and [C] 120 °C.

Figure 5.9



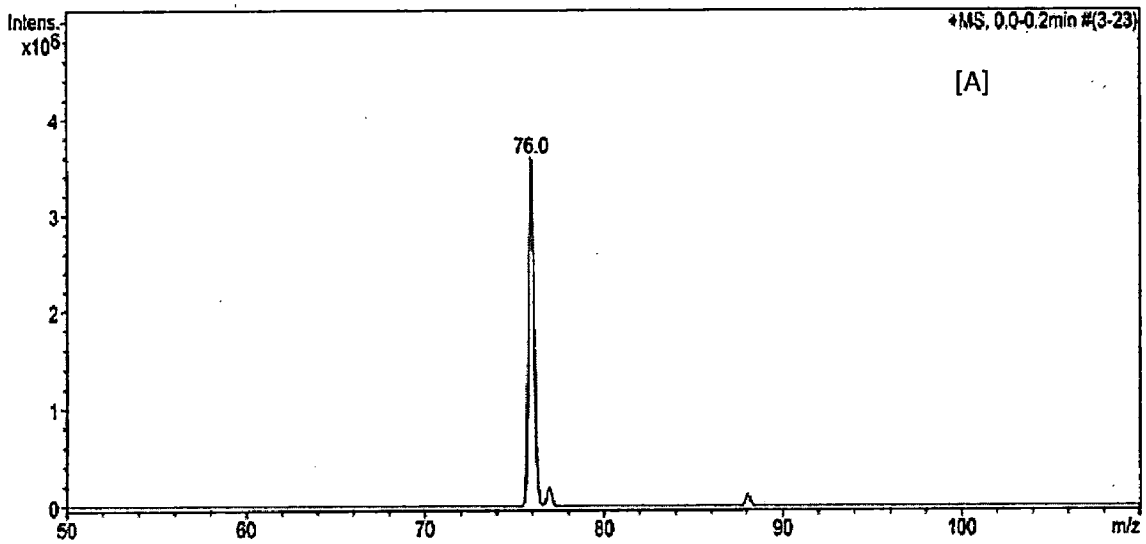
Bixbyite (Mn_2O_3) catalyzed formation of DKP and oligomers of alanine and β-alanine at [A] 50 °C, [B] 90 °C and [C] 120 °C.

Figure 5.10



Pyrolusite (MnO_2) catalyzed formation of DKP and oligomers of alanine and β-alanine at [A] 50 °C, [B] 90 °C and [C] 120 °C.

Figure 5.11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x10^6
+MS, 0.0-0.2min #(3-23)
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2
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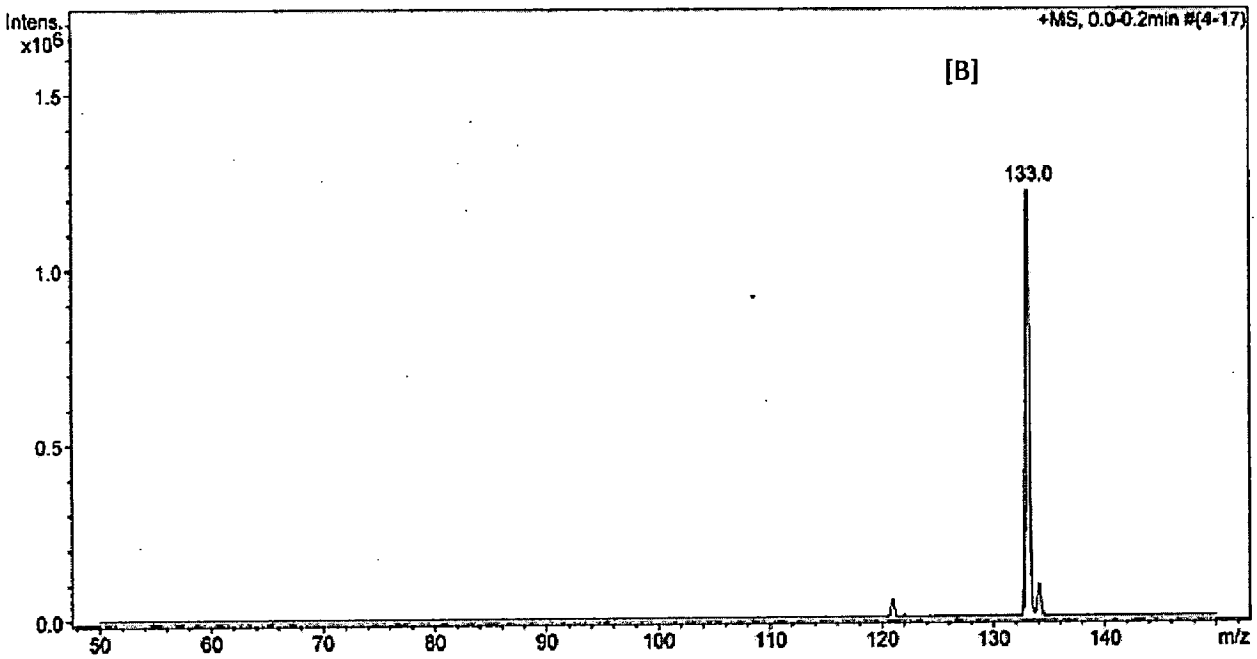
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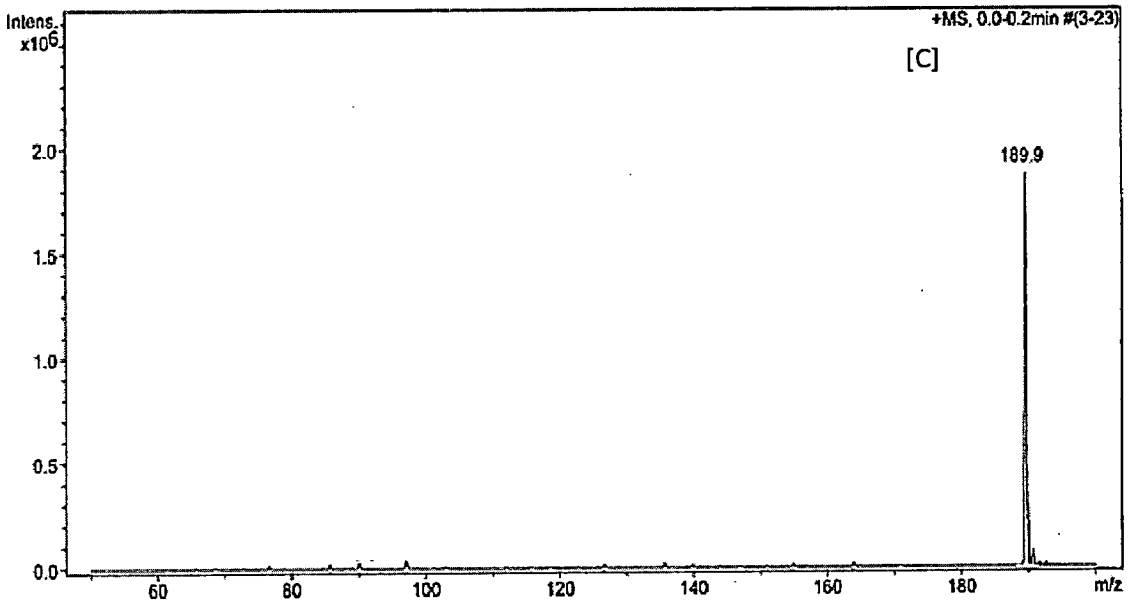
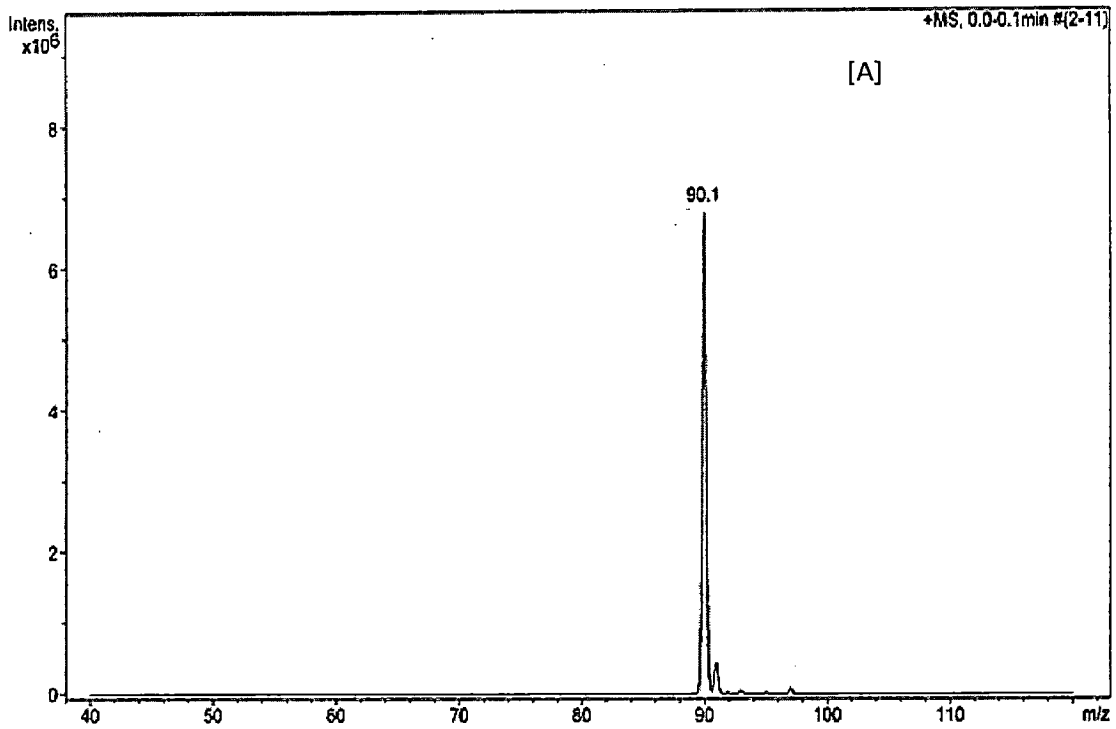
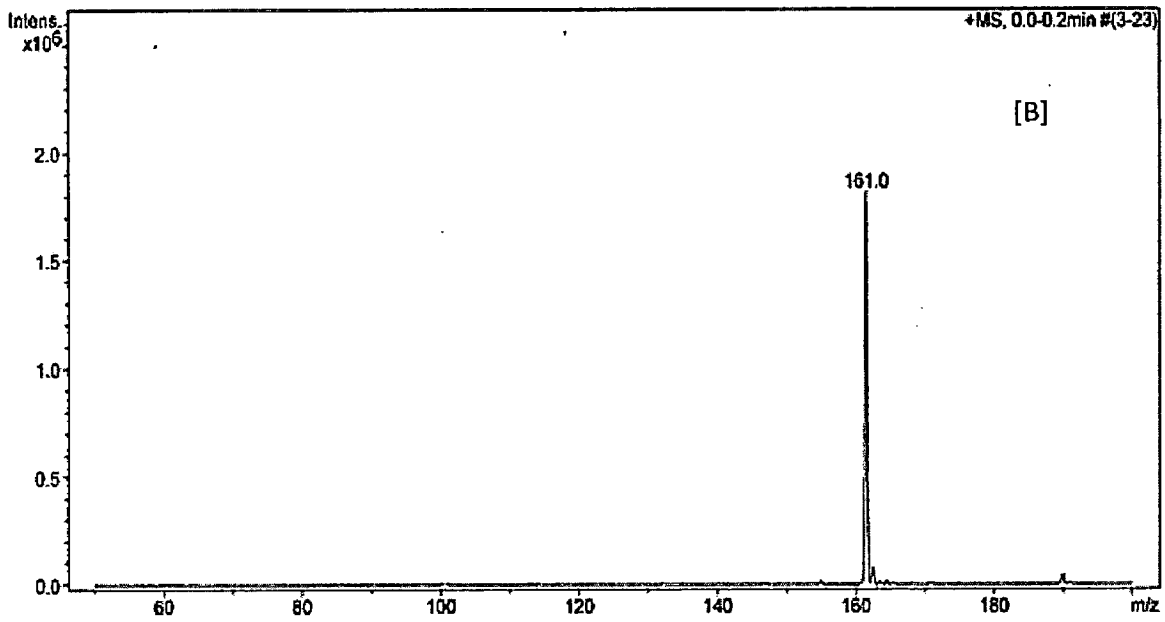
m/z



ESI MS spectra of [A] Glycine, [B] Glycylglycine, [C] Glycylglycylglycine

Figure 5.12

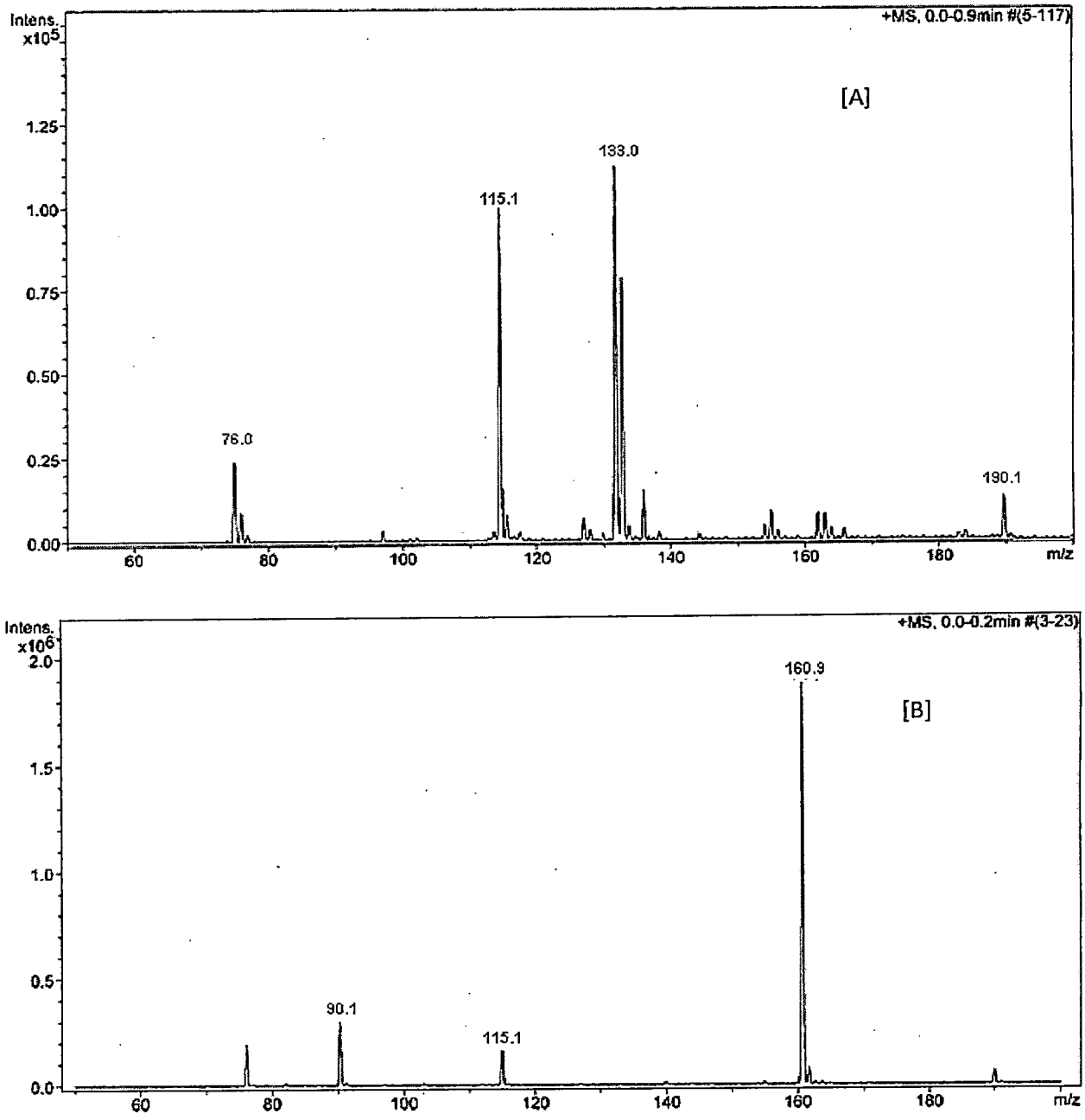




E

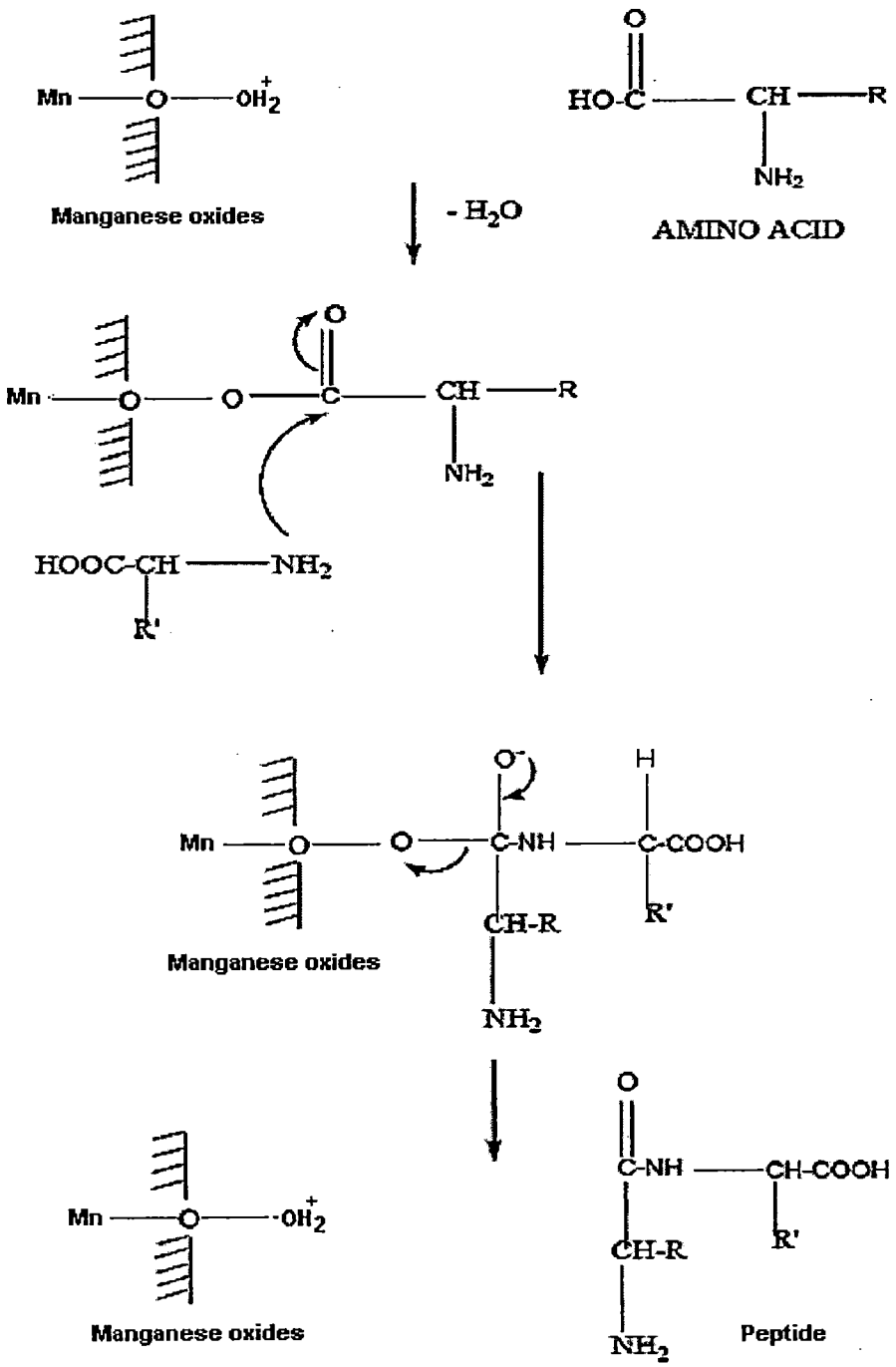
ESI MS spectra [A] Alanine and [B] Alanylalanine

Figure 5.13



ESI MS spectra of products formed when glycine [A] and alanine [B] was heated for 35 days at 90 ^{0}C in the presence of manganosite (MnO)

Figure 5.14



Mechanism for the peptide bond formation on manganese oxides surface

Figure 5.15

References:

1. Cohen, P. "Let there be life". New Sci., **2037**, 22 (1996).
2. Dose, K. "The origin of life: more questions than answers". Interdiscipl. Sci. Rev., **13**, 348 (1988).
3. Miller, S. L. "A Production of Amino Acids Under Possible Primitive Earth Conditions". Science, **117,** 528 (1953).
4. Rode, B. M. "Peptides and the origin of life". Peptides, **20,** 773(1999).
5. Getz, W. M. "Polycondensation and peptide chain-length distribution under prebiotic conditions". Bio Systems, **24,** 177 (1990).
6. Voigt, C. A., Kauffman, S. and Wang, Z. G. "Rational evolutionary design: the theory of *in vitro* protein evolution". Adv. Protein Chem., **55,** 79 (2000).
7. Kunin, V. "A System of Two Polymerases – A Model for the Origin of Life". Orig. Life Evol. Biosph., **30,** 459 (2000).
8. Brack, A. and Barbier, B. "Chemical activity of simple basic peptides". Orig. Life Evol. Biosph., **20,** 139 (1990).
9. Egami, F. "Minor elements and evolution". J. Mol. Evol., **4**, 113 (1974).
10. Greenland, D. J., Laby, R. H. and Quirk, J. P. "Adsorption of glycine and its di-, tri-, and tetra-peptides by montmorillonite". Trans. Faraday Soc., **58**, 829 (1962).
11. Greenland, D. J., Laby, R. H. and Quirk, J. P. "Adsorption of amino acids and peptides by montmorillonite and illite. Part-1 cation exchange and proton transfer". Trans. Faraday Soc., **61**, 2013 (1965a).

12. Greenland, D. J., Laby, R. H. and Quirk, J. P. "Adsorption of amino-acids and peptides by montmorillonite and illite. Part- 2 Physical adsorption". Trans. Faraday Soc., **61**, 2024 (1965b).

13. Graf, G. and Laganly, G. "Interaction of clay minerals with adenosine-5-phosphate". Clays. Clay Miner., **28**, 12 (1980).

14. Kobayashi, K. and Ponnamperuma, C. "Trace elements in chemical evolution I". Orig. Life. 15, 41(1985).

15. Kobayashi, K. and Ponnamperuma, C. "Trace elements in chemical evolution. II: Synthesis of amino acids under simulated primitive earth conditions in the presence of trace elements". Orig. Life, 16, 57 (1985).

16. Bujdak, J. and Rode, B. M. "The Effect of Smectite Composition on the Catalysis of Peptide Bond Formation". J. Mol. Evol., **43**, 326 (1996).

17. Bujdak, J. and Rode, B. M. "Silica, Alumina, and Clay-Catalyzed Alanine Peptide Bond Formation", J. Mol. Evol., **45**, 457 (1997a).

18. Bujdak, J. and Rode, B. M. "Glycine Oligomerization on Silica and Alumina". React. Kineto Catal. Lett., 62, 281 (1997b).

19. Bujdak, J. and Rode, B. M. "The Effect of Clay Structure on Peptide Bond Formation Catalysis". J. Mol. Catal., A **144,** 129 (1999a).

20. Bujdak, J. and Rode, B. M. Silica, "Alumina and Clay Catalyzed Peptide Bond Formation: Enhanced Efficiency of Alumina Catalyst". Orig. Life Evol. Biosph., 29, 451 (1999b).

21. Lahav, N. "Minerals and the Origins of Life: Hypotheses and Experiments in Heterogeneous Chemistry". Heterogen. Chem. Rev., **1**, 159 (1994).

22. Lahav, N., White, D. and Chang, S. "Peptide Formation in the Prebiotic Era: Thermal Condensation of Glycine in Fluctuating Clay Environments". Science, **201**, 67 (1978).

23. Porter, T. L., Eastman, M. P., Hagerman, M. E., Price, L. B., and Shand, R. F. "Site-Specific Prebiotic Oligomerization Reactions of Glycine on the Surface of Hectorite". J. Mol. Evol., **47**, 373 (1998).

24. Rode, B. M., Son, H. L. Suwannachot, Y. and Bujdak, J. "The Combination of Salt-Induced Peptide Formation Reaction and Clay Catalysis: A Way to Higher Peptides under Primitive Earth Conditions". Origins Life Evol. Biosphere, **29**, 273 (1999).

25. Smith, J. V. "Biochemical Evolution. I. Polymerization on Internal, Organophilic Silica Surfaces of Dealuminated Zeolites and Feldspars". Proc. Natl. Acad. Sci. USA, **95**, 3370 (1998).

26. Zamaraev, K. I., Romannikov, V. N., Salganik, R. I., Wlasoff, W. A., and Khramtsov, V. V. "Modelling of the Prebiotic Synthesis of Oligopeptides: Silicate Catalysts Help to Overcome the Critical Stage". Origins Life Evol. Biosphere, **27**, 325 (1997).

27. Basiuk, V. A. and Sainz-Rojas, J. "Catalysis of Peptide Formation by Inorganic Oxides: High Efficiency of Alumina under Mild Conditions on the Earth-Like Planets". Adv. Space Res., **27**, 225 (2001).

28. Basiuk, V. A., Gromovoy, T. Yu., Golovaty, V. G. and Glukhoy, A. M. "Mechanisms of Amino Acid Polycondensation on Silica and Alumina Surfaces". Origins Life Evol. Biosphere, **20**, 483 (1991).

29. Gromovoy, T. Yu., Basiuk, V. A., and Chuiko, A. A. "Growth of Peptide Chains on Silica in Absence of Amino Acid Access from Without". Origins Life Evol. Biosphere, **21**, 119 (1991).

30. Bujdak, J. and Rode, B. M. "Activated alumina as energy source for peptide bond formation: Consequences for mineral-mediated prebiotic processes". Amino acids, **21**, 281(2001).

31. Bujdak, J. and Rode B. M. "Prefential amino acid sequences in alumina-catalyzed peptide bond formation". Journal of Inorganic Chemistry, **90**, 1 (2002).

32. Bujdak, J. and Rode, B. M. "Peptide bond formation on the surface of activated alumina: Peptide chain elongation". Catalysis letters, **91**, 149 (2003).

33. Matrajit, G. and Blanot, D. "Properties of synthetic ferrihydrite as an amino acid adsorbent and a promoter of peptide bond formation". Amino acids, **26**, 153 (2004).

34. Weber, A. L. "Prebiotic sugar synthesis: Hexose and hydroxyl acid synthesis from glyceraldehydes catalyzed by Iron (III) hydroxide oxide". Journal of Molecular Evolution, **35**, 1(1992).

35. Martra, G. "Lewis acid and base sites at the surface of microcrystalline TiO_2 anatase: relationships between surface morphology and chemical behaviour". Applied Catalysis A: **200**, 275(2000).

36. Turekian, K. K. and Wedepohl, K. L. "Distribution of the Elements in Some Major Units of the Earth's Crust". Geol. Soc. Am. Bull., **72**, 275 (1961).

37. Holm, N. G., Dowler, M. J, and Wadsten T., Arrhenius G. "β-FeOOH.Cln (akaganéite) and $Fe_{1-x}O$ (wüstite) hot brine from the Atlantis II Deep (Red Sea) and uptake of amino acids by synthetic β-FeOOH.Cl_n". Geochim Cosmochem Acta, **47**, 1465(1983).

38. Ingrmanson, D. E. and Dowler, M. J. "Chemical evolution and the evolution of the Earth's crust". Origins of Life **8**, 221 (1977).

CHAPTER-6

INTERACTION AND OXIDATION OF AROMATIC AMINES WITH MANGANESE OXIDES

6. 1 Introduction:

The basic platform for all the prebiotic reaction producing polymeric substances from which life has emerged is clays and clay minerals. So clay minerals might have concentrated biomolecules and catalyzed their Oligomerization during the course of chemical evolution [1]. The interaction of a wide variety of organic molecules such as amino acids, peptides [2-4], nucleic acid bases [5], nucleotides [6-8] on the clays and clay minerals have been investigated in detail. The adsorption of organic molecules on clay surfaces take place mainly through cation exchange, ion dipole, coordination, hydrogen bonding and interaction of aromatic amines by clays have also been studied in detail by a number of workers [9-11].

Amines are one of the important classes of organic compounds which are widely distributed in nature in the form of amino acids, alkaloids and vitamins. The presence of amino acids containing aromatic ring on the primitive Earth has been proposed by Friedmann and Miller [12]. One could, reasonably postulate the presence of aromatic amines on the primitive Earth environment. During the last few years, experimental studies on the catalytic role of cyano complexes and their possible role in chemical evolution have been carried out. A number of metal hexacyanoferrate (II) have been prepared and their interaction properties towards amino acids [13-14], nucleotides [15-17], aromatic amines [18-21] and amino pyridines [22-24] have been studied. It has been established by the above study that metal hexacyanoferrate (II) are highly efficient in adsorbing amines in neutral as well as alkaline media (pH~9) to bring about oxidation of the amines. It has been proposed that transition metal ions abundantly present in primeval seas might have formed complexes with simple molecules readily available to them and played crucial roles in chemical evolution. Studies on the importance of transition metals in chemical evolution have also been reported [25-27]. Manganese oxides with different Mn/O ratio also found significantly good adsorbent for ribose nucleotide in source of chemical evolution [28].

In order to further investigate the importance of manganese oxides during the course of chemical evolution in catalyzing different reactions, aromatic amines were interacted with manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2). Spectral studies have indicated that surface acidity of manganese oxides is responsible for interaction.

6.2 Adsorption of Aromatic Amines on Manganese Oxides:

6.2.1 Evaluation of parameters necessary for investigating the adsorption equilibrium:

The optimum conditions which set up for investigating the adsorption equilibrium for the amine and metal oxides are

I. Concentration of adsorbate

II. Particle size of adsorbent

III. Equilibrium time for adsorbate and adsorbent

IV. Quantity of adsorbent

V. Effect of pH and effect of temperature

For determining the adsorption isotherm of amines on manganese oxides a moderate concentration range of amines (2×10^{-5} to 1.4×10^{-4}) has been chosen in order to get absorbance of amines in the suitable range of the absorbance scale on the spectrophotometer.

Manganese oxides of various particle sizes have been tested and it was found that particle size of 20-100 mesh was the most suitable one. In order to fix up the equilibrium time and quantity of adsorbent for optimum adsorption, experiments were performed varying the time of contact (30 minute to 24 hour) and the amount of metal oxides at a fixed adsorbate concentration (5×10^{-4}M). It was observed that the maximum adsorption took place when the amount used was 50 mg per 10 ml of adsorbent solution for aniline, p-toluidine, p-anisidine and p-chloroaniline for 24 hour. Adsorption of p-chloroaniline on a

metal oxide was found to be a slow process and equilibrium was found to establish after 72 hours.

6.3 Effect of pH

Adsorption of amine was studied over wide pH range (4-9) on all metal oxides. Buffer used to maintain pH were acetate (pH 4 to 7) and Borax (pH~9). It was found that adsorption of aromatic amines on manganese oxide was negligible in acidic and basic media. A neutral pH range (6.8-7.12) was found to be suitable for maximum adsorption.

Electronic spectra of aniline, p-anisidine, p-toluidine and p-chloroaniline were recorded on a UV spectrophotometer (Model UV-188-240V, Shimadzu Corporation Kyoto, Japan). The characteristic λ_{max} values for amines (aniline, p-chloroaniline, p-toluidine and p-anisidine are 280 nm, 290 nm, 286 nm and 295 nm, respectively. Infrared spectra of adsorbates, adsorbent, and adsorption adducts were recorded on a Perkin-Elmer 1600 FTIR spectrophotometer using KBr pellets. A series of 50 ml glass test tubes were employed and each tube was filled with 10 ml of amine solution of varying concentration. Manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2) (100 mg) was added to each tube. The pH of the solution was adjusted to the desired value using acetate or borax buffers. Species of these buffers did not get adsorbed onto the manganese oxide surface. This was verified by conductivity measurements as there was no change in the inflection point of buffers with and without manganese oxides. The tubes were shaken with a spinix votex shaker initially for 1 h and then allowed to equilibrate at 25 ^{0}C with intermittent shaking at fixed time intervals. The equilibrium was attained within 6 h. The equilibrium time and concentration range were, however, decided after a good deal of preliminary investigations. The concentration of aniline, p-chloroaniline, p-toluidine and p-anisidine was measured spectrophotometrically at 280 nm, 290 nm, 286 nm and 295 nm, respectively. The amount of amine adsorbed was calculated from the difference between the amine concentration before and after adsorption, all the results are shown in Table

6.3.1-6.3.16. The equilibrium concentration of amine and the amount adsorbed were fitted in the adsorption isotherm (Figures 6.3.1-6.3.4). However, in alkaline medium (pH>8) brown-red colored products were deposited on the Hausmannite (Mn_3O_4) surfaces within 24 h. These colored products were concentrated for GC–MS analysis by extraction in benzene. Analysis of the reaction products was performed on a Perkin Elmer gas chromatograph coupled directly to a Perkin Elmer mass spectrometer system. Separation was performed on a fused silica capillary column (Elite-5 model) with a composition 5% diphenyl and 95% dimethyl polysiloxane. The conditions for GC were as follows: Injector temperature, 280 ^{0}C; transfer line temperature, 250 ^{0}C. The capillary column temperature was programmed as follows: 80 ^{0}C for 2 min; from 80 to 260 ^{0}C at 10 ^{0}C min^{-1}, held at 250 ^{0}C for 15 min. Helium was used as a carrier gas with a flow rate of 2 ml min^{-1}. The mass spectrometer conditions were ion source 250 ^{0}C and ionization energy 40 eV.

6.4 Results and Discussion

A neutral pH range (~7.0) was selected for preliminary adsorption studies of aromatic amines on manganese oxides (manganosite (MnO), bixbyite (Mn_2O_3), hausmannite (Mn_3O_4) and pyrolusite (MnO_2)). A remarkable change in the amount of adsorbate was observed by varying the pH of the solution. Subsequent studies were performed at pH~7.0 which was optimum for the maximum adsorption of all the amines. The neutral pH is physiologically significant as most of the reactions in living systems take place in neutral media. Since amines are basic with lone pair of electrons on nitrogen and also assisted by π-electron clouds on aromatic rings, easily interact with positively charged surface of manganese oxides. It was observed that at lower pH range aniline showed greater uptake as compared to p-anisidine. This can be explained on the basis of the fact that in acidic media p-anisidine has a greater tendency for salt formation and hence a lesser tendency to interact with manganese oxides. On the other hand, aniline has a lesser tendency in comparison with p-anisidine for salt formation resulting in a greater tendency

to interact with manganese oxides. At a neutral pH, p-anisidine has more electrons available to interact with both the manganese oxide and thus there is a drastic increase in uptake. Adsorption isotherms of aniline, p-chloroaniline, p-toluidine and p-anisidine in the present case show that adsorption is fast in both the cases and the isotherms are regular, positive, and concave to the concentration axis. A typical graph of Ce/Xe vs Ce is a straight line (Figures 6.4.1-6.4.4). Adsorption data can be represented through a Langmuir adsorption isotherm which assumes the formation of a monolayer of solute molecules on the surface of the adsorbent and given by

$$\frac{Ceq}{X_e} = \frac{Ceq}{X_m} + \frac{1}{X_m} k_L$$

where,

C_{eq} = equilibrium concentration of solute (mole/liter)

X_e = amount of solute adsorbed per gram weight of adsorbent (mg)

X_m = the moles of solute required per gram weight of goethite and akaganeite for the formation of complete monolayer on the surface

k_L = Langmuir adsorption constant (l/mg)

The percent uptake of p-anisidine, p-chloroaniline, p-toluidine and aniline is represented by (Table 6.4.17). The values of X_m and K_L were also calculated (Table 6.4.18). X_m values also indicate that anisidine adsorption is more in comparison to that of other amines. It is observed from percent binding that p-anisidine is strongly adsorbed on manganese oxides in comparison to aniline. The observed adsorption trend is also related to the basicities of the amines. This indicates that p-anisidine is a stronger base than other amines which reflects the greater availability of electrons for the interaction.

The infrared spectra of manganese oxides before and after adsorption were recorded and analyzed. The results are given in Figures 6.4.5-6.4.8. The change in characteristic frequencies of manganese oxides after adsorption was not remarkable. The

pronounced change in the characteristic frequencies of amino group indicates that the interaction occurred through the manganese oxides with an amino group.

Field Emission Scanning Electron Microscopic studies of the amine and manganese oxide was also performed. Figure 6.4.5 represents FE-SEM of anisidine, manganese oxide and p-anisidine–manganese oxide adduct. FE-SEM photographs of manganese oxide-nucleotide adduct shows that surface of manganese oxide becomes smooth and colour of surface of manganese oxide changes to that of amine. EDX pattern also supports the adsorption as percentage of C, N, and O increases in the EDX pattern of adduct.

Further it was also observed that in alkaline medium pH >8 all the amines reacted with hausmannite (Mn_3O_4) to give the colored products.

6.5 Oxidation of Aromatic Amines in the Presence of Manganese Oxides

In alkaline medium (pH>8) brown-red colored products were deposited on the hausmannite (Mn_3O_4) surfaces within 24 h. These colored products were concentrated for GC–MS analysis by extraction in benzene. Analysis of the reaction products was performed on a Perkin Elmer gas chromatograph coupled directly to a Perkin Elmer mass spectrometer system. Separation was performed on a fused silica capillary column (Elite-5 model) with composition 5% diphenyl and 95% dimethyl polysiloxane. The conditions for GC were as follows: injector temperature, 280 ^{0}C; transfer line temperature, 250 ^{0}C. The capillary column temperature was programmed as follows: 80 ^{0}C for 2 min; from 80 to 260 ^{0}C at 10 ^{0}C min^{-1}, held at 250 ^{0}C for 15 min. Helium was used as a carrier gas with a flow rate of 2 ml min^{-1}. The mass spectrometer conditions were ion source 250 ^{0}C and ionization energy 40 eV.

The analysis of products extracted in benzene was performed with gas chromatography interfaced with mass spectrometer.

6.5.1 Reaction of aniline with hausmannite (Mn_3O_4)

Mass spectra of the reaction products corresponding to peaks at retention time (R_t) 3.31, 7.29, 16.62 and 25.51 min are shown in Figures 6.5.1-6.5.3. The formation of benzoquinone (R_t 7.29 min) is clearly evidenced by the peaks corresponding to *m/z* 108, 82, and 54, in accordance with its fragmentation pattern determined by electron bombardment. However, GC–MS study of the peak with R_t 16.62 min showed the formation of azobenzene confirmed by a peak at *m/z* 182. Some other mass peaks in the fragmentation pattern of the product at *m/z* 105, 77, and 51 were also observed. R_t 25.51 peak corresponds to tetramer of aniline, confirmed by their fragmentation pattern. A well-known mechanism is proposed for the formation of benzoquinone, tetramer of aniline and azobenzene (Figures 6.5.7-6.5.8).

6.5.2 Reaction of anisidine with hausmannite (Mn_3O_4)

In the case of anisidine the chromatogram showed two major peaks with R_t 3.81 and 15.72 min corresponding to anisidine and its dimer, respectively. Mass analysis of the product with R_t 15.72 min showed major peaks at *m/z* 242, 135, and 107 which correspond to fragmentation of anisidine dimer (Figure 6.5.4). A possible mechanism for the formation of dimer of anisidine may be proposed as shown in Figure 6.5.9.

6.5.3 Reaction of p-chloroaniline with hausmannite (Mn_3O_4)

In the case of p-chloroaniline the chromatogram showed two major peaks with R_t 3.87 and 30.72 min. Peak with retention time (R_t) 3.87,corresponding to p-chloroaniline whereas the second peak at retention time (R_t) 30.72 min corresponds to its dimer. Mass analysis of the product with R_t 30.72 min showed major peaks at *m/z* 139, 111, 92 and 77 which correspond to fragmentation of p-chloroaniline dimer (Figure 6.5.5). A possible mechanism for the formation of dimer of p-chloroaniline may be proposed as shown in Figure 6.5.10.

6.5.4 Reaction of p-toluidine with hausmannite (Mn_3O_4)

The oxidation products of p-toluidine gave well defined and well separated peaks in gas chromatogram with R_t 3.25 and 27.59 min. Mass spectra of the peak with R_t 27.59 min showed molar mass 317 (100 %), which corresponds to trimer of p-toluidine Figure 6.5.6. Some high fragments observed were 300, 226, 211, 107, 91 and 77. Above got fragmentation masse patterns resembled with possible fragmentation pattern of a trimer. Peak with R_t 3.25 min corresponds to the starting material, i.e. p-toluidine. Possible mechanism for the formation of dimer of p-toluidine was also proposed (Figure 6.5.11).

6.6 Conclusion

In view of present studies it can be suggested that manganese oxides once formed in the prebiotic environment, could have interacted with important biomolecules in order to protect them from degradation and have catalyzed their oligomerization. The implications of this research in prebiotic chemistry are of significance because it has shown experimentally that lower metal oxygen ratio provide a good adsorption site that could have played an important role in early stages of chemical evolution. The present research has shown that manganese oxides Manganosite (MnO), Bixbyite (Mn_2O_3), Hausmannite (Mn_3O_4) and Pyrolusite (MnO_2) to be reasonable and plausible candidate in studies involving adsorption and condensation of biologically important molecules.

Table 6.4.1

Adsorption of aniline on Manganosite (MnO)

Temperature = 25^0 C

Amount of adsorbent used = 50 mg

S. No.	Initial Conc.(C_i) of aniline ($M \times 10^5$)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of aniline ($M \times 10^5$)	$C_i - C_{eq}$ ($Mx10^5$)	Amount (Xe) of aniline adsorbed (mg g^{-1})	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	2	0.26	0.03	0.13	1.87	0.18	0.85
3	4	0.52	0.06	0.37	3.63	0.36	1.27
4	6	0.81	0.10	0.69	5.31	0.52	1.62
5	8	1.01	0.17	1.25	6.75	0.67	2.31
6	10	1.22	0.23	1.73	8.27	0.82	2.61
7	12	1.54	0.36	2.77	9.23	0.91	3.75
8	14	1.76	0.54	4.21	9.79	0.96	5.37

Table 6.4.2

Adsorption of p-anisidine on Hausmannite (Mn_3O_4)

Temperature = 25^0 C

Amount of adsorbent used = 50 mg

S. No.	Initial Conc.(C_i) of p-anisidine (M x 10^5)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of p-anisidine (M x 10^5)	$C_i - C_{eq}$ (Mx10^5)	Amount (Xe) of p-anisidine adsorbed (mg g^{-1})	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	2	0.26	0.04	0.21	1.79	0.18	1.45
3	4	0.52	0.08	0.53	3.47	0.34	1.90
4	6	0.81	0.12	0.85	5.15	0.51	2.06
5	8	1.01	0.19	1.41	6.59	0.65	2.67
6	10	1.22	0.26	1.97	8.03	0.79	3.06
7	12	1.54	0.41	3.17	8.83	0.87	4.48
8	14	1.76	0.57	4.45	9.55	0.94	5.82

Table 6.4.3

Adsorption of p-anisidine on Bixbyite (Mn_2O_3)

Temperature = 25^0 C

Amount of adsorbent used = 50 mg

S. No.	Initial Conc.(C_i) of p-anisidine (M x 10^5)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of p-anisidine (M x 10^5)	$C_i - C_{eq}$ (Mx10^5)	Amount (Xe) of p-anisidine adsorbed (mg g^{-1})	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	2	0.26	0.04	0.21	1.79	0.18	1.45
3	4	0.52	0.08	0.53	3.47	0.34	1.90
4	6	0.81	0.14	1.01	4.99	0.49	2.52
5	8	1.01	0.22	1.65	6.35	0.63	3.24
6	10	1.22	0.29	2.21	7.79	0.77	3.54
7	12	1.54	0.45	3.49	8.51	0.84	5.12
8	14	1.76	0.63	4.93	9.07	0.89	6.79

Table 6.4.4

Adsorption of p-anisidine on Pyrolusite (MnO_2)

Temperature = 25^0 C

Amount of adsorbent used = 50 mg

S. No.	Initial Conc.(C_i) of p-anisidine (M x 10^5)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of p-anisidine (M x 10^5)	$C_i - C_{eq}$ (Mx10^5)	Amount (Xe) of p-anisidine adsorbed (mg g^{-1})	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	2	0.26	0.04	0.21	1.79	0.18	1.45
3	4	0.52	0.09	0.61	3.39	0.33	2.24
4	6	0.81	0.15	1.09	4.91	0.48	2.77
5	8	1.01	0.24	1.81	6.19	0.61	3.65
6	10	1.22	0.35	2.69	7.31	0.72	4.60
7	12	1.54	0.51	3.97	8.03	0.79	6.18
8	14	1.76	0.70	5.49	8.51	0.84	8.06

Table 6.4.5

Adsorption of p-toluidine on Manganosite (MnO)

Temperature = 25^0 C

Amount of adsorbent used = 50 mg

S. No.	Initial Conc.(C_i) of p-toluidine (M x 10^5)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of p-toluidine (M x 10^5)	$C_i - C_{eq}$ (Mx10^5)	Amount (Xe) of p-toluidine adsorbed (mg g^{-1})	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	2	0.23	0.05	0.23	1.77	0.15	1.63
3	4	0.49	0.08	0.50	3.50	0.30	1.77
4	6	0.74	0.12	0.85	5.15	0.44	2.06
5	8	0.95	0.17	1.29	6.71	0.58	2.41
6	10	1.20	0.26	2.09	7.91	0.68	3.30
7	12	1.37	0.39	3.24	8.76	0.75	4.62
8	14	1.58	0.55	4.65	9.35	0.80	6.23

Table 6.4.6

Adsorption of p-toluidine on Hausmannite (Mn_3O_4)

Temperature = 25^0 C

Amount of adsorbent used = 50 mg

S. No.	Initial Conc.(C_i) of p-toluidine ($M \times 10^5$)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of p-toluidine ($M \times 10^5$)	$C_i - C_{eq}$ ($Mx10^5$)	Amount (Xe) of p-toluidine adsorbed (mg g^{-1})	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	2	0.23	0.05	0.23	1.77	0.15	1.63
3	4	0.49	0.08	0.50	3.50	0.30	1.77
4	6	0.74	0.14	1.03	4.97	0.43	2.58
5	8	0.95	0.21	1.65	6.35	0.54	3.24
6	10	1.20	0.32	2.62	7.38	0.63	4.44
7	12	1.37	0.44	3.68	8.32	0.71	5.53
8	14	1.58	0.61	5.19	8.81	0.76	7.35

Table 6.4.7

Adsorption of p-toluidine on Bixbyite (Mn_2O_3)

Temperature = 25^0 C

Amount of adsorbent used = 50 mg

S. No.	Initial Conc.(C_i) of p-toluidine ($M \times 10^5$)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of p-toluidine ($M \times 10^5$)	$C_i - C_{eq}$ ($Mx10^5$)	Amount (Xe) of p-toluidine adsorbed ($mg\ g^{-1}$)	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	2	0.23	0.05	0.23	1.77	0.15	1.63
3	4	0.49	0.09	0.58	3.42	0.29	2.14
4	6	0.74	0.16	1.20	4.80	0.41	3.14
5	8	0.95	0.26	2.09	5.91	0.51	4.42
6	10	1.20	0.37	3.06	6.94	0.59	5.52
7	12	1.37	0.52	4.39	7.61	0.65	7.21
8	14	1.58	0.69	5.89	8.11	0.69	9.09

Table 6.4.8

Adsorption of p-toluidine on Pyrolusite (MnO_2)

Temperature = 25^0 C

Amount of adsorbent used = 50 mg

S. No.	Initial Conc.(C_i) of p-toluidine (M x 10^5)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of p-toluidine (M x 10^5)	$C_i - C_{eq}$ (Mx10^5)	Amount (Xe) of p-toluidine adsorbed (mg g^{-1})	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	2	0.23	0.05	0.23	1.77	0.15	1.63
3	4	0.49	0.11	0.76	3.24	0.28	2.94
4	6	0.74	0.18	1.38	4.62	0.40	3.74
5	8	0.95	0.28	2.27	5.73	0.49	4.94
6	10	1.20	0.40	3.33	6.67	0.57	6.23
7	12	1.37	0.57	4.83	7.17	0.61	8.43
8	14	1.58	0.74	6.34	7.66	0.66	10.33

Table 6.4.9
Adsorption of aniline on Manganosite (MnO)
Temperature = 25^0 C
Amount of adsorbent used = 50 mg

S. No.	Initial Conc.(C_i) of aniline ($M \times 10^5$)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of aniline ($M \times 10^5$)	$C_i - C_{eq}$ ($Mx10^5$)	Amount (Xe) of aniline adsorbed ($mg\ g^{-1}$)	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	2	0.31	0.07	0.22	1.78	0.13	1.54
3	4	0.53	0.11	0.52	3.48	0.26	1.88
4	6	0.86	0.16	0.90	5.10	0.38	2.21
5	8	1.20	0.25	1.58	6.42	0.48	3.08
6	10	1.42	0.36	2.42	7.58	0.56	3.98
7	12	1.63	0.49	3.40	8.60	0.64	4.94
8	14	1.81	0.68	4.84	9.16	0.68	6.61

Table 6.4.10

Adsorption of aniline on Hausmannite (Mn_3O_4)

Temperature = 25^0 C

Amount of adsorbent used = 50 mg

S. No.	Initial Conc.(C_i) of aniline ($M \times 10^5$)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of aniline ($M \times 10^5$)	$C_i - C_{eq}$ ($Mx10^5$)	Amount (Xe) of aniline adsorbed ($mg\ g^{-1}$)	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	2	0.31	0.07	0.22	1.78	0.13	1.54
3	4	0.53	0.12	0.60	3.40	0.25	2.20
4	6	0.86	0.18	1.05	4.95	0.37	2.66
5	8	1.20	0.29	1.89	6.11	0.46	3.86
6	10	1.42	0.43	2.95	7.05	0.53	5.22
7	12	1.63	0.59	4.16	7.84	0.58	6.63
8	14	1.81	0.81	5.83	8.17	0.61	8.91

Table 6.4.11

Adsorption of aniline on Bixbyite (Mn_2O_3)

Temperature = 25^0 C

Amount of adsorbent used = 50 mg

S. No.	Initial Conc.(C_i) of aniline (M x 10^5)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of aniline (M x 10^5)	$C_i - C_{eq}$ (Mx10^5)	Amount (Xe) of aniline adsorbed (mg g^{-1})	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	2	0.31	0.07	0.22	1.78	0.13	1.54
3	4	0.53	0.12	0.60	3.40	0.25	2.20
4	6	0.86	0.19	1.13	4.87	0.36	2.90
5	8	1.20	0.33	2.19	5.81	0.43	4.71
6	10	1.42	0.48	3.33	6.67	0.50	6.23
7	12	1.63	0.67	4.77	7.23	0.54	8.23
8	14	1.81	0.89	6.43	7.57	0.56	10.62

Table 6.4.12

Adsorption of aniline on Pyrolusite (MnO_2)

Temperature = 25^0 C

Amount of adsorbent used = 50 mg

S. No.	Initial Conc. (C_i) of aniline (M x 10^5)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of aniline (M x 10^5)	$C_i - C_{eq}$ (Mx10^5)	Amount (Xe) of aniline adsorbed (mg g^{-1})	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	2	0.31	0.07	0.22	1.78	0.13	1.54
3	4	0.53	0.13	0.67	3.33	0.25	2.53
4	6	0.86	0.24	1.51	4.49	0.33	4.19
5	8	1.20	0.38	2.57	5.43	0.40	5.91
6	10	1.42	0.55	3.86	6.14	0.46	7.85
7	12	1.63	0.73	5.22	6.78	0.51	9.62
8	14	1.81	0.98	7.11	6.89	0.51	12.91

Table 6.4.13

Adsorption of p-chloroaniline on Manganosite (MnO)

Temperature = 25^0 C

Amount of adsorbent used = 50 mg

S. No.	Initial Conc.(C_i) of p-chloroaniline (M x 10^5)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of p-chloroaniline (M x 10^5)	$C_i - C_{eq}$ (Mx10^5)	Amount (Xe) of p-chloroaniline adsorbed (mg g^{-1})	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	2	0.31	0.05	0.22	1.78	0.18	1.54
3	4	0.58	0.10	0.57	3.43	0.35	2.10
4	6	0.88	0.16	1.00	5.00	0.51	2.50
5	8	1.18	0.28	1.85	6.15	0.63	3.76
6	10	1.42	0.41	2.77	7.23	0.74	4.80
7	12	1.73	0.62	4.26	7.74	0.79	6.89
8	14	1.98	0.85	5.89	8.11	0.83	9.09

Table 6.4.14

Adsorption of p-chloroaniline on Hausmannite (Mn_3O_4)

Temperature = 25^0 C

Amount of adsorbent used = 50 mg

S. No.	Initial Conc.(C_i) of p-chloroaniline ($M \times 10^5$)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of p-chloroaniline ($M \times 10^5$)	$C_i - C_{eq}$ ($Mx10^5$)	Amount (Xe) of p-chloroaniline adsorbed ($mg\ g^{-1}$)	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	2	0.31	0.06	0.29	1.71	0.17	2.13
3	4	0.58	0.14	0.86	3.14	0.32	3.41
4	6	0.88	0.26	1.71	4.29	0.44	4.98
5	8	1.18	0.47	3.20	4.80	0.49	8.33
6	10	1.42	0.71	4.90	5.10	0.52	12.01
7	12	1.73	0.93	6.46	5.54	0.57	14.58
8	14	1.98	1.23	8.59	5.41	0.55	19.84

Table 6.4.15

Adsorption of p-chloroaniline on Bixbyite (Mn_2O_3)

Temperature = 25^0 C

Amount of adsorbent used = 50 mg

S. No.	Initial Conc.(C_i) of p-chloroaniline (M x 10^5)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of p-chloroaniline (M x 10^5)	$C_i - C_{eq}$ (Mx10^5)	Amount (Xe) of p-chloroaniline adsorbed (mg g^{-1})	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	2	0.31	0.07	0.36	1.64	0.17	2.76
3	4	0.58	0.18	1.14	2.86	0.29	4.99
4	6	0.88	0.39	2.63	3.37	0.34	9.76
5	8	1.18	0.62	4.29	3.71	0.38	14.46
6	10	1.42	0.88	6.11	3.89	0.40	19.60
7	12	1.73	1.15	8.02	3.98	0.41	25.20
8	14	1.98	1.45	10.15	3.85	0.39	32.94

Table 6.4.16

Adsorption of p-chloroaniline on Pyrolusite (MnO_2)

Temperature = 25^0 C

Amount of adsorbent used = 50 mg

S. No.	Initial Conc.(C_i) of p-chloroaniline (M x 10^5)	Absorbance before adsorption	Absorbance after adsorption	Equilibrium conc.(C_{eq}) of p-chloroaniline (M x 10^5)	$C_i - C_{eq}$ (Mx10^5)	Amount (Xe) of p-chloroaniline adsorbed (mg g^{-1})	Ce/Xe (10^2) (Mole/L)
1	0	0	0.00	0.00	0.00	0.00	0
2	2	0.31	0.08	0.43	1.57	0.16	3.45
3	4	0.58	0.21	1.35	2.65	0.27	6.40
4	6	0.88	0.44	2.99	3.01	0.31	12.38
5	8	1.18	0.70	4.83	3.17	0.32	19.04
6	10	1.42	0.97	6.74	3.26	0.33	25.90
7	12	1.73	1.22	8.52	3.48	0.36	30.58
8	14	1.98	1.52	10.65	3.35	0.34	39.67

Table 6.4.17

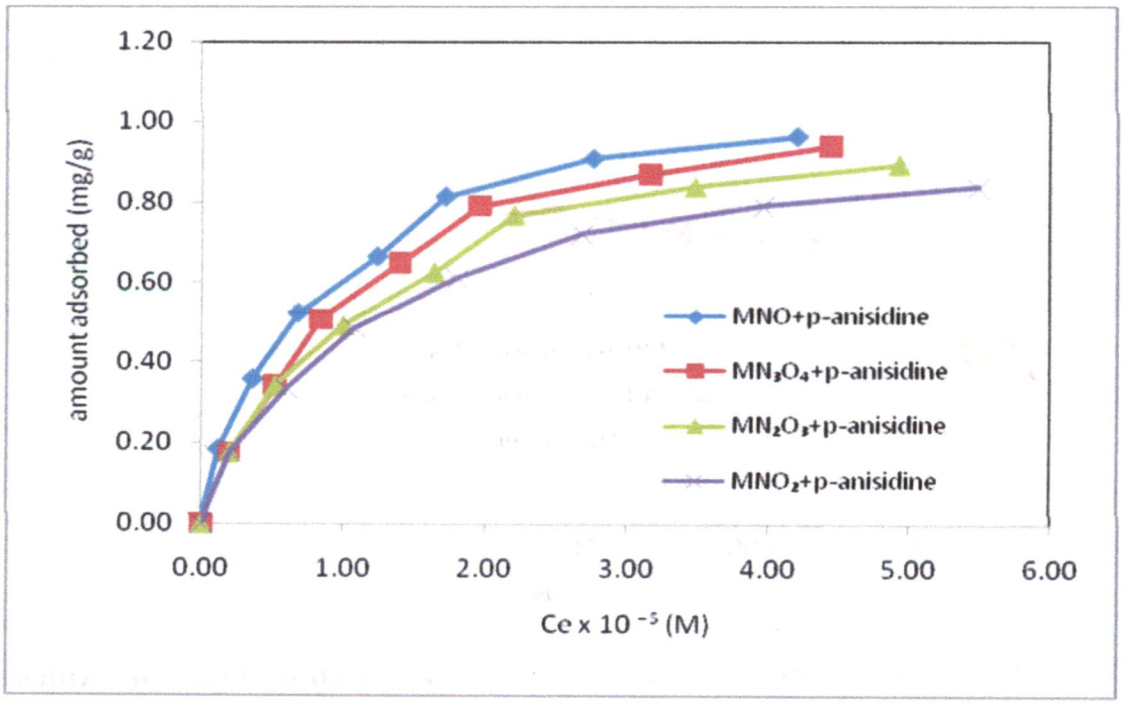
Percent uptake of amines on manganese oxides

Nucleotide	Percent binding			
	Manganosite (MnO)	Hausmannite (Mn_3O_4)	Bixbyite (Mn_2O_3)	Pyrolusite (MnO_2)
p-Anisidine	81.15	78.69	76.23	71.31
p-Toluidine	75.83	73.33	69.17	67.50
Aniline	73.24	69.72	64.79	61.27
p-Chloroaniline	71.13	50.00	38.03	31.69

Table 6.4.18

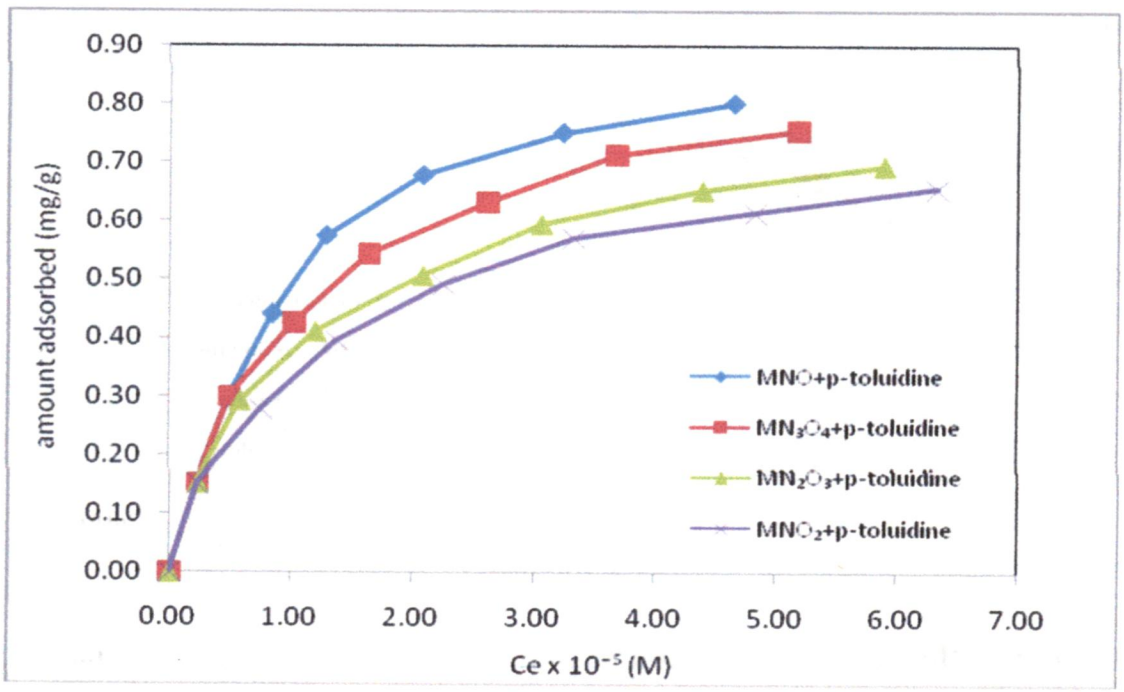
Langmuir constants for adsorption of amines on manganese oxide

Nucleotide	(Manganosite (MnO)		Hausmannite (Mn_3O_4)		Bixbyite (Mn_2O_3)		Pyrolusite (MnO_2)	
	k_L (l mg^{-1})	X_m (mg/g)	k_L (l mg^{-1})	X_m (mg/g)	k_L (l mg^{-1})	X_m (mg/g)	k_L (l mg^{-1})	X_m (mg/g)
p-Anisidine	1.39	1.23	1.27	1.01	1.15	0.89	1.13	0.82
p-Toluidine	1.36	1.06	1.22	0.89	1.12	0.76	1.05	0.71
Aniline	1.14	0.86	1.04	0.77	0.95	0.69	0.86	0.62
p-Chloroaniline	1.03	0.78	0.72	0.48	0.55	0.33	0.48	0.28



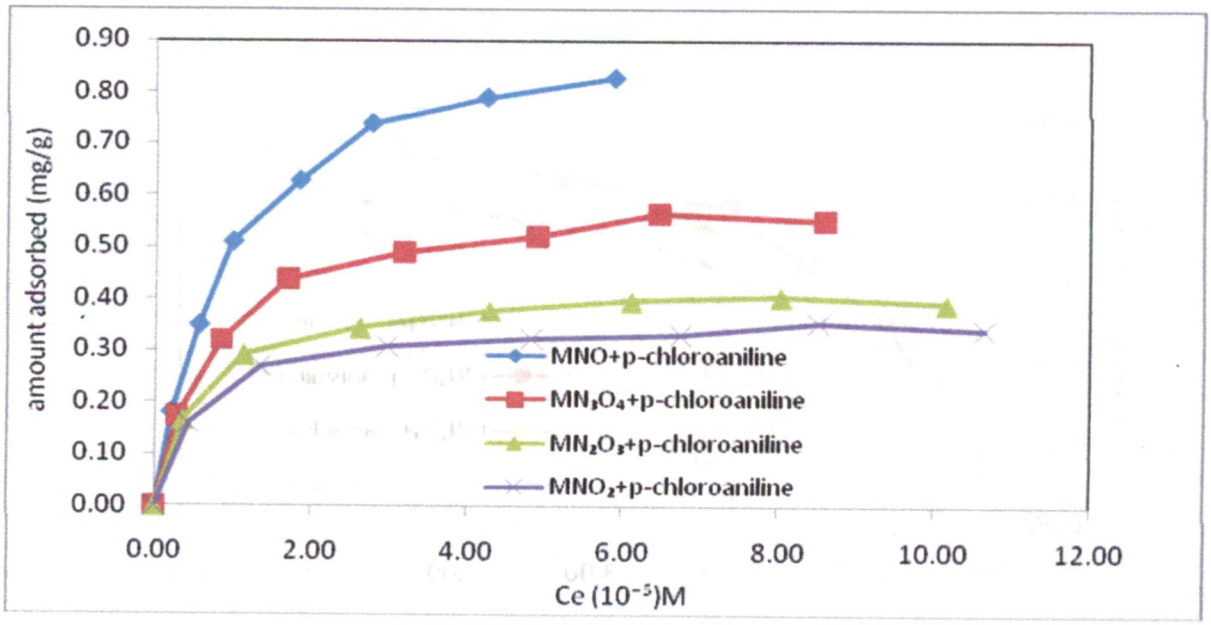
Adsorption isotherm for adsorption of aniline on manganese oxides

Figure 6.3.1



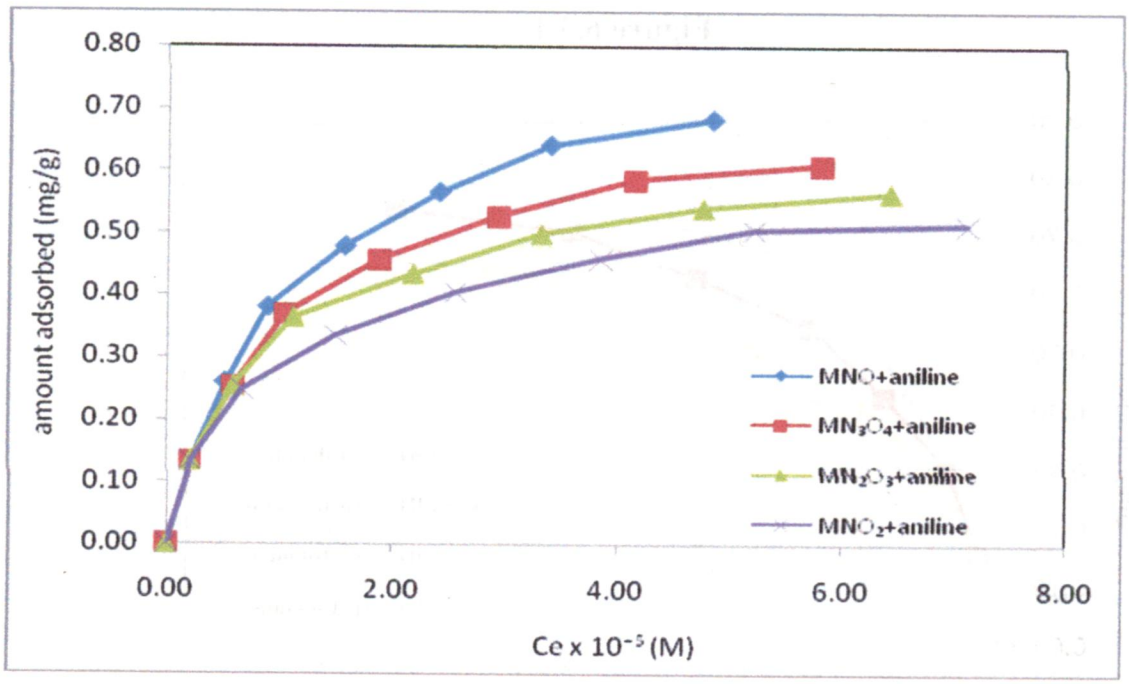
Adsorption isotherm for adsorption of p-toluidine on manganese oxides

Figure 6.3.2



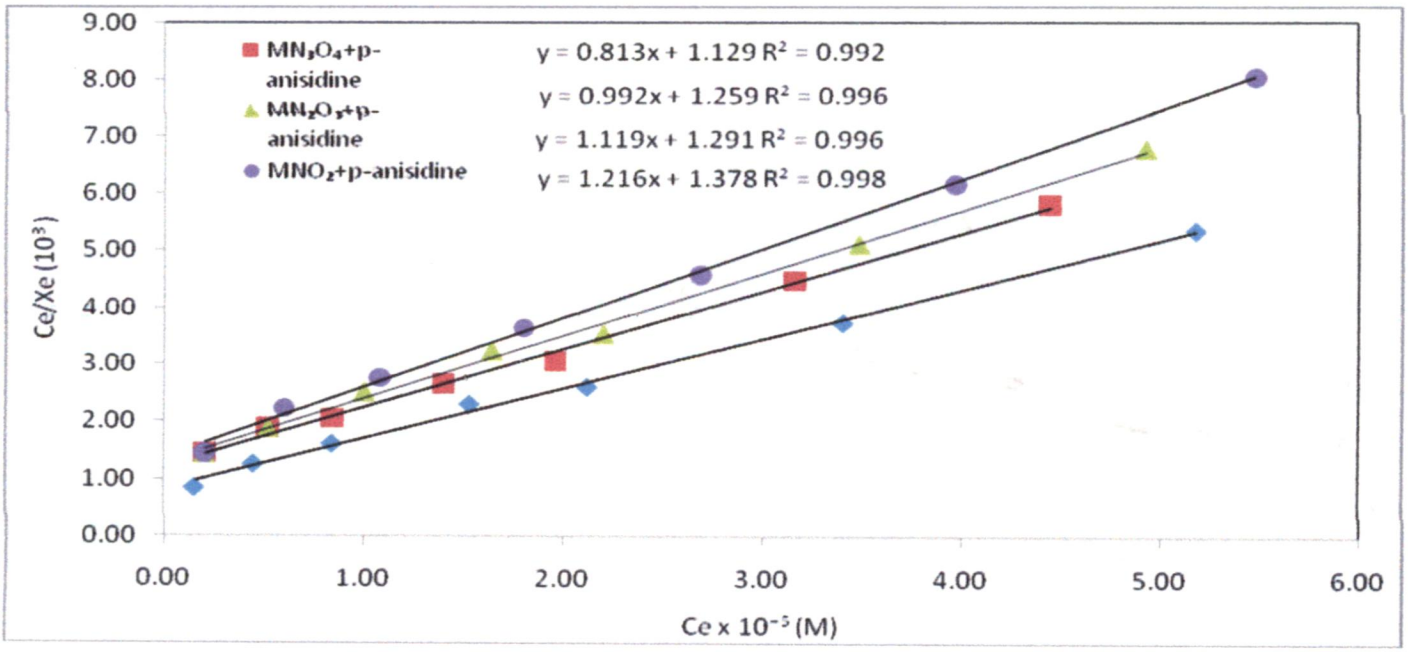
Adsorption isotherm for adsorption of p-chloroaniline on manganese oxides

Figure 6.3.3



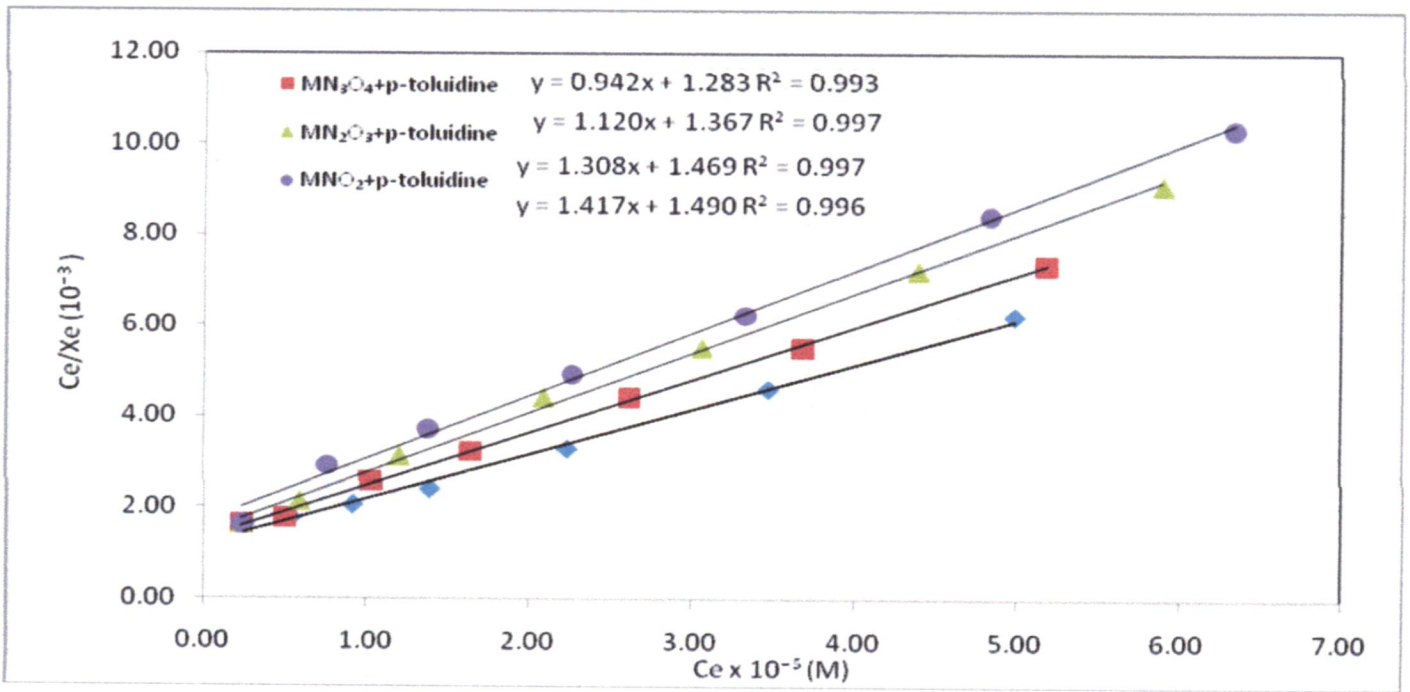
Adsorption isotherm for adsorption of p-anisidine on manganese oxides

Figure 6.3.4



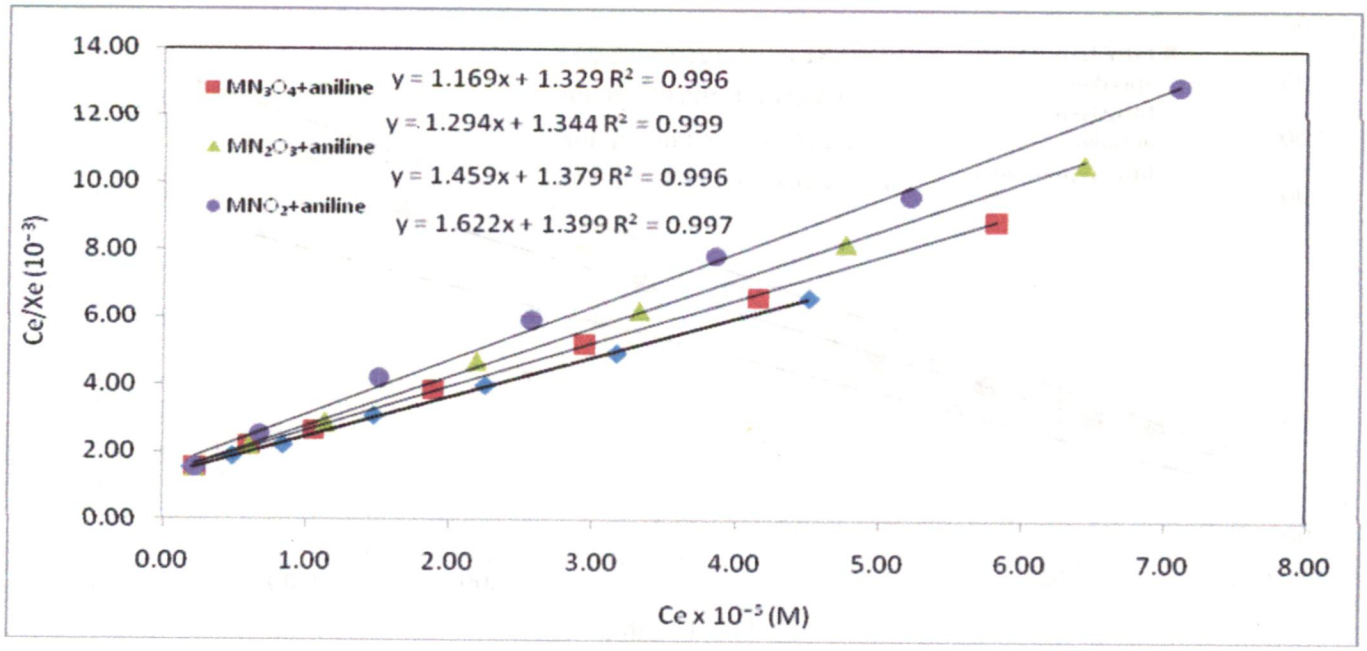
Langmuir isotherms for adsorption of aniline on manganese oxides

Figure 6.4.1



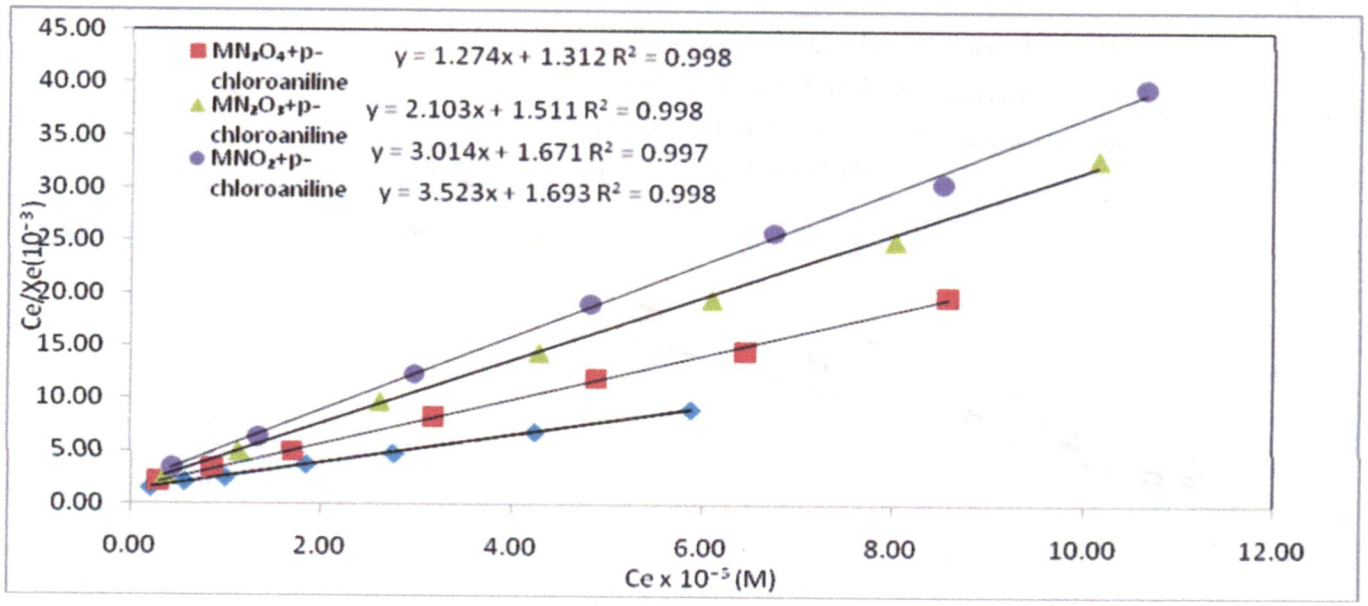
Langmuir isotherms for adsorption of aniline on manganese oxides

Figure 6.4.2



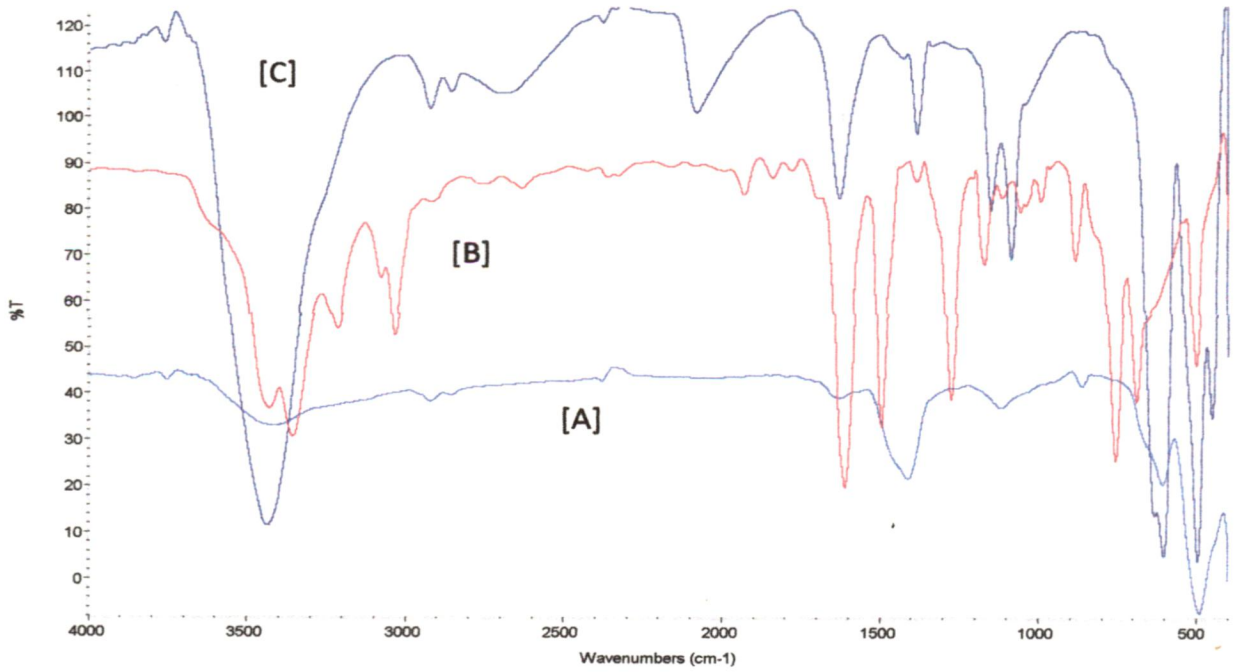
Langmuir isotherms for adsorption of aniline on manganese oxides

Figure 6.4.3



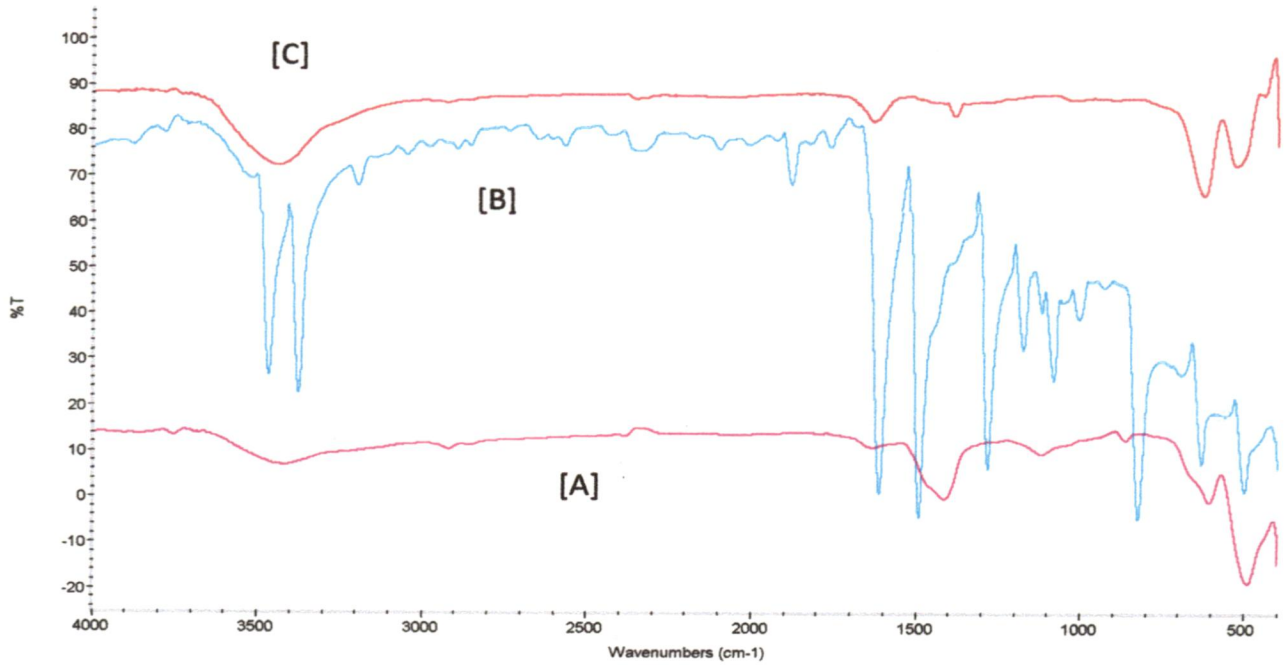
Langmuir isotherms for adsorption of aniline on manganese oxides

Figure 6.4.4



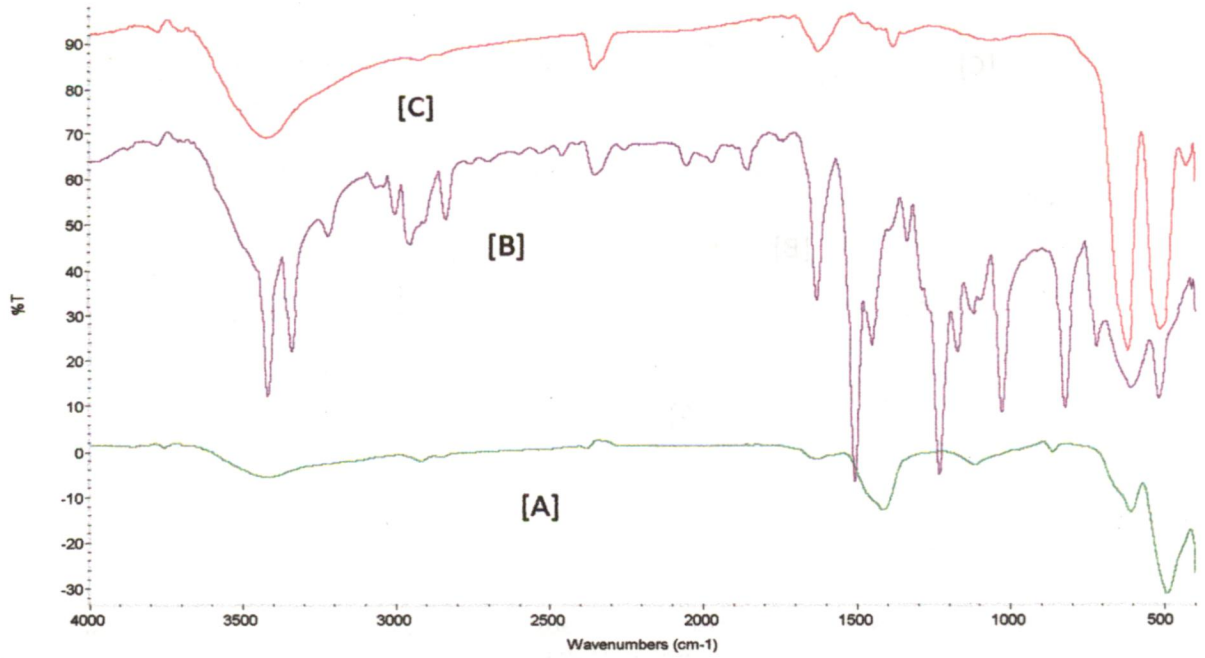
Representative IR spectra of MnO (A) aniline (B) and aniline-MnO adduct (C)

Figure 6.4.5



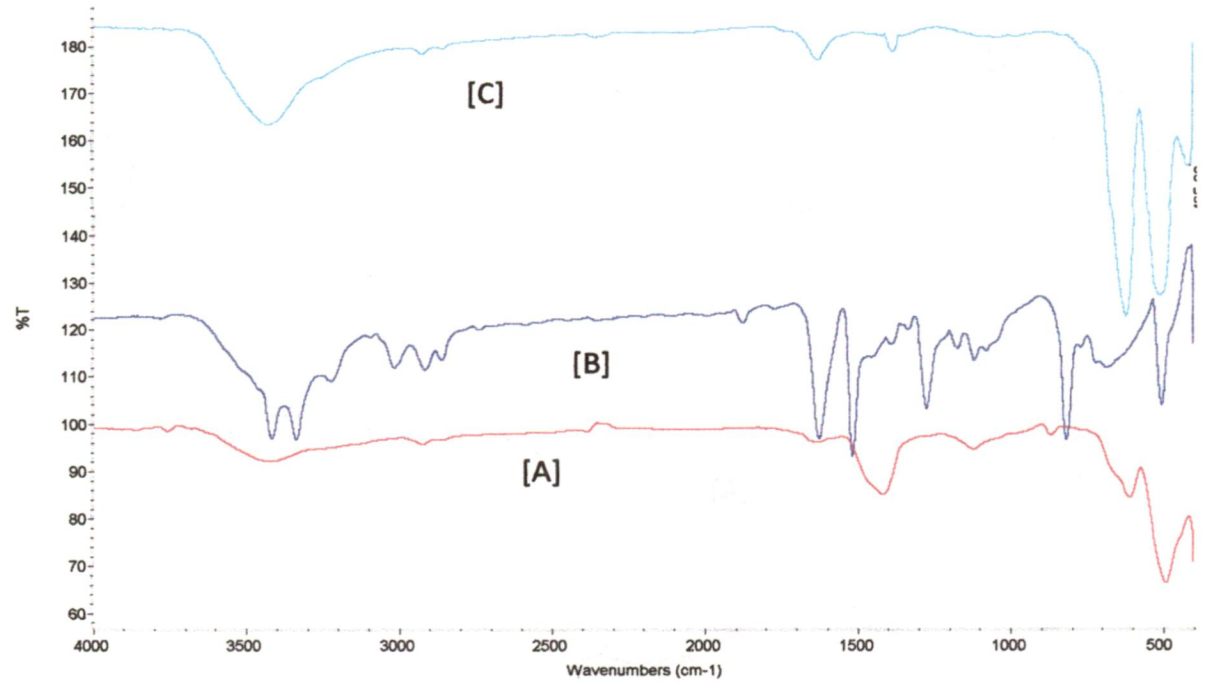
Representative IR spectra of MnO (A) p-anisidine (B) and p-anisidine-MnO adduct (C)

Figure 6.4.6



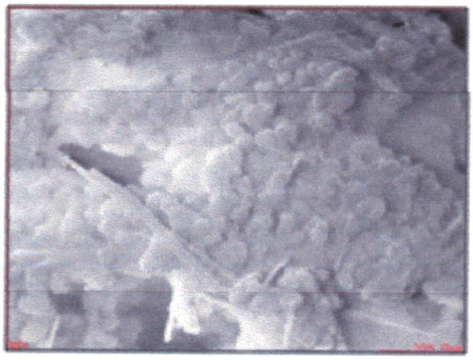
Representative IR spectra of MnO (A) p-chloroaniline (B) and p-chloroaniline-MnO adduct (C)

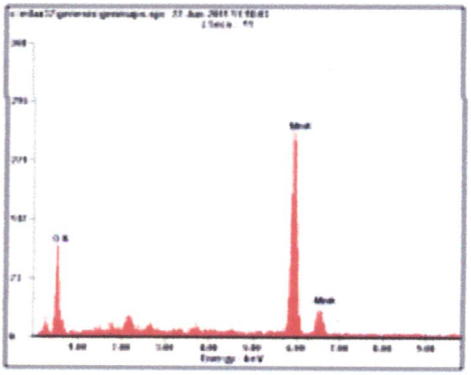
Figure 6.4.7



Representative IR spectra of MnO (A) p-toluidine (B) and p-toluidine-MnO adduct (C)

Figure 6.4.8





Element	Wt%	At%
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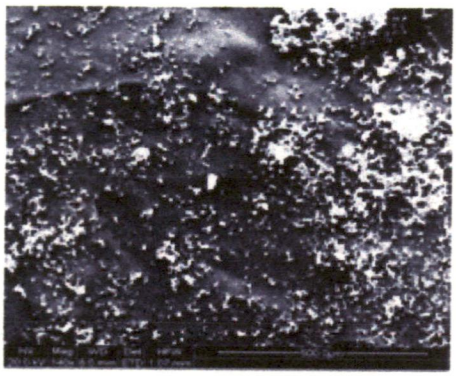
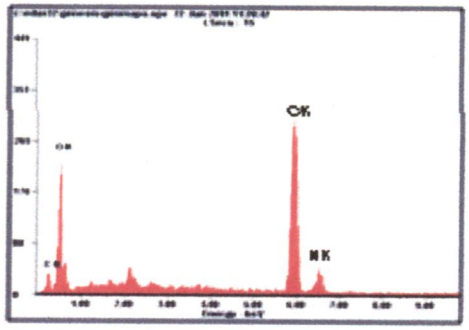
MnK	64.63	32.29
Matrix	Correction	ZAF

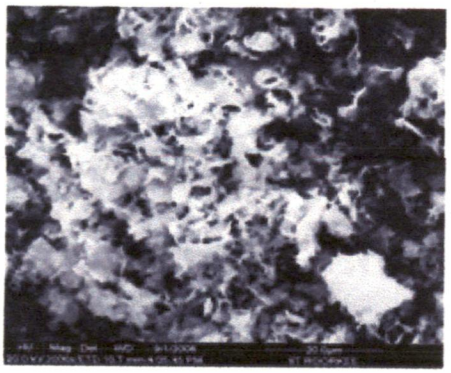
[A]

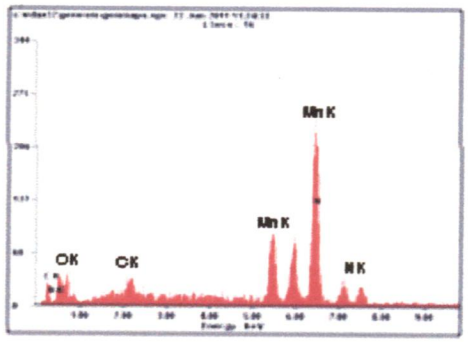




Element	Wt%	At%
NK	21.38	28.29
OK	22.98	39.42
CK	64.63	32.29
Matrix	Correction	ZAF

[B]

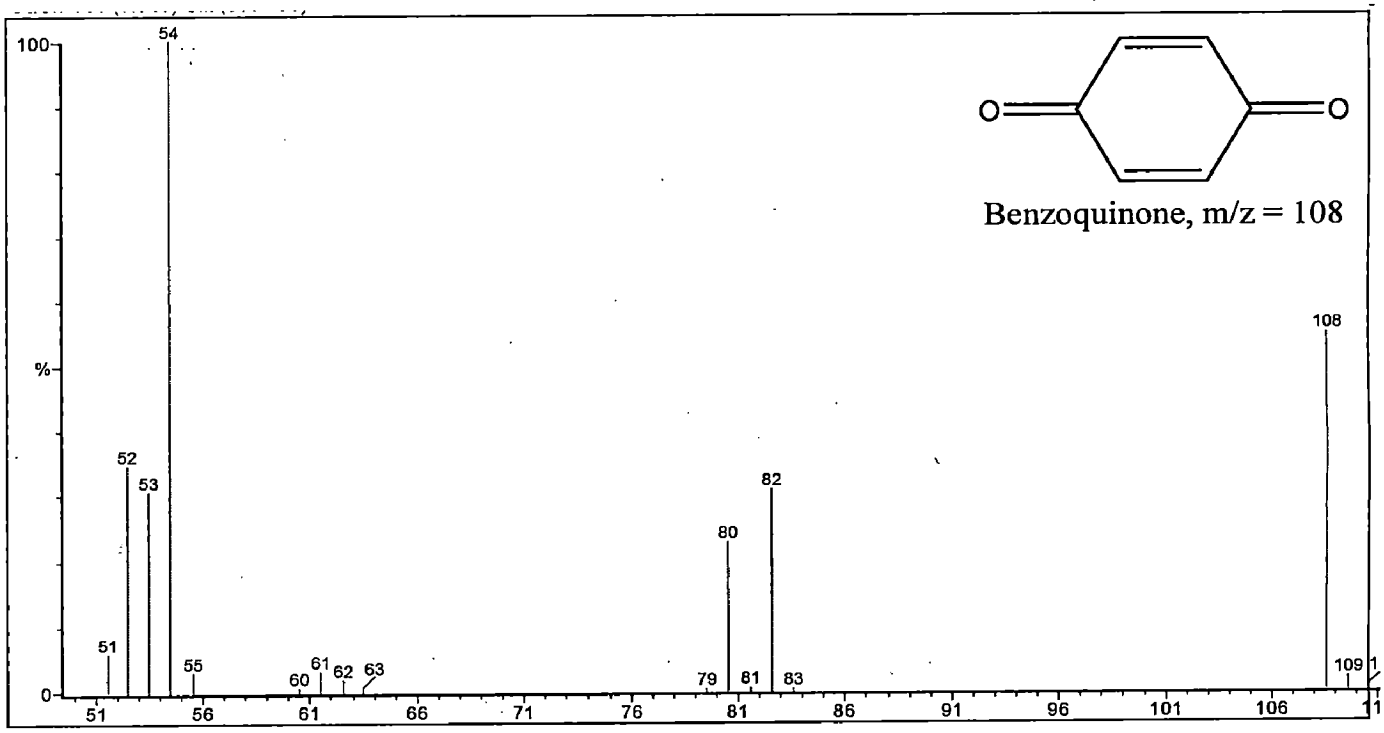




Element	Wt%	At%
CK	30.13	42.92
NK	9.54	19.42
OK	11.63	18.43
MnK	50.33	33.91
Matrix	Correction	ZAF

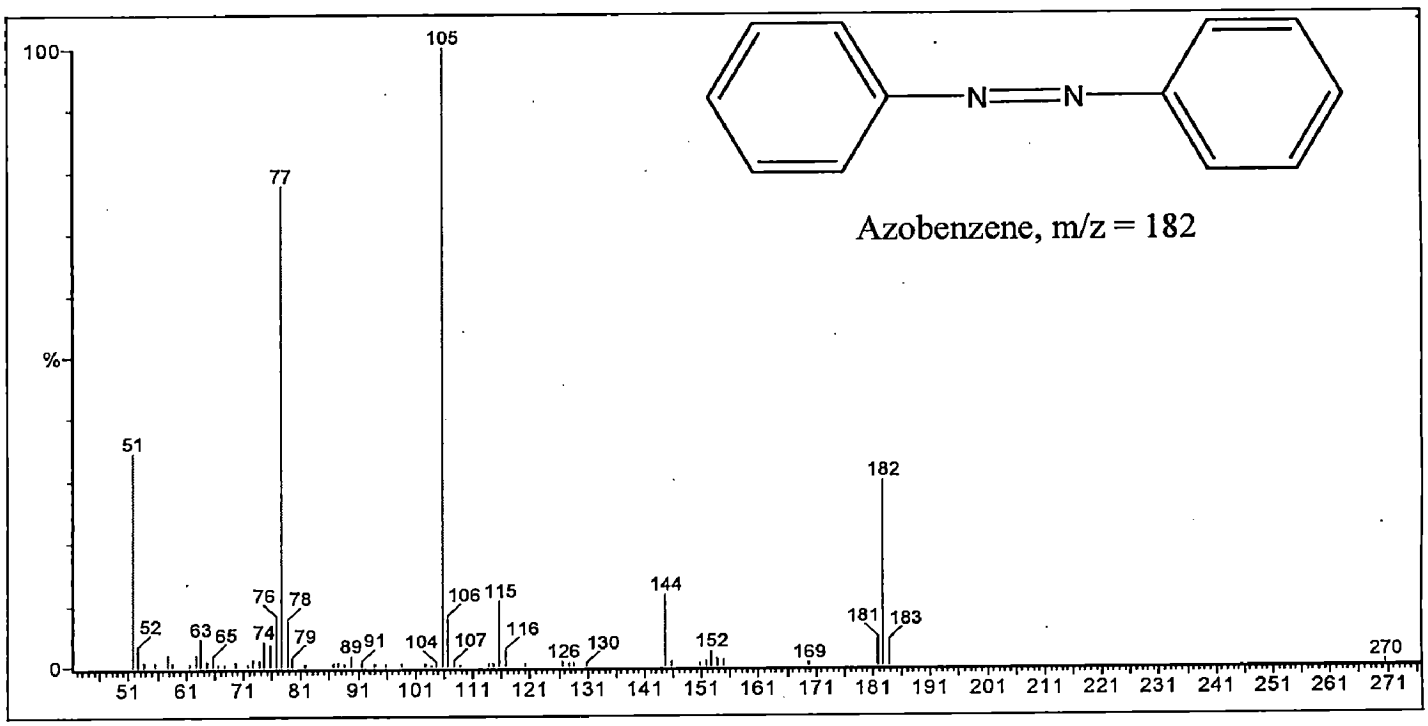
[C]

Representative FE-SEM photographs of MnO (a), p-anisidine (b) and MnO-p-anisidine adduct (c)
Figure 6.5



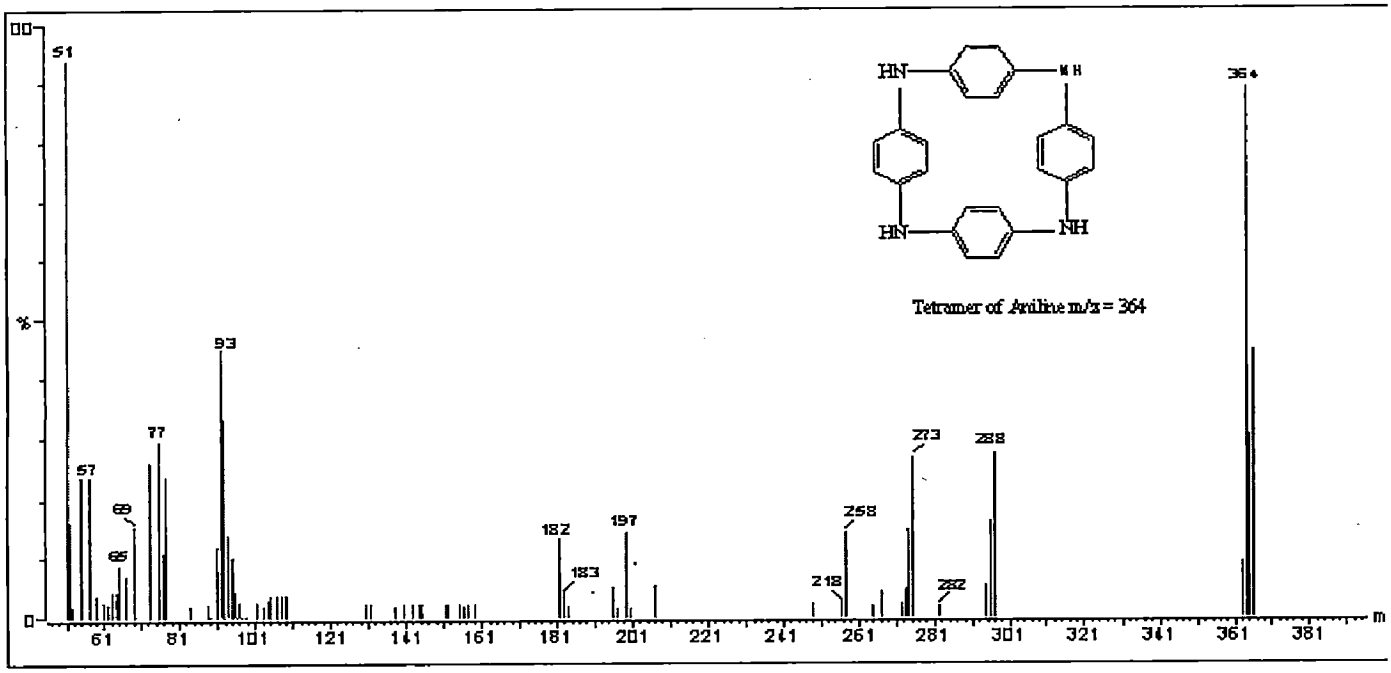
Mass spectrum of benzoquinone

Figure 6.5.1



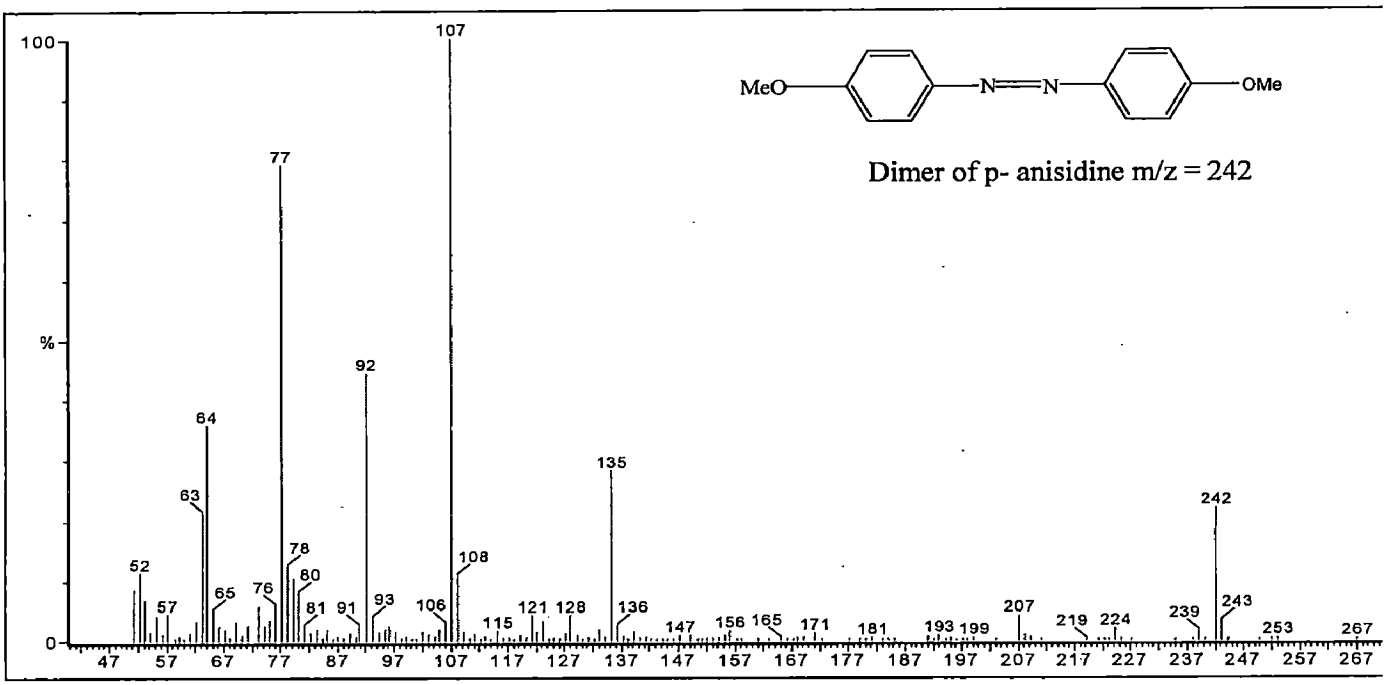
Mass spectrum of azobenzene

Figure 6.5.2



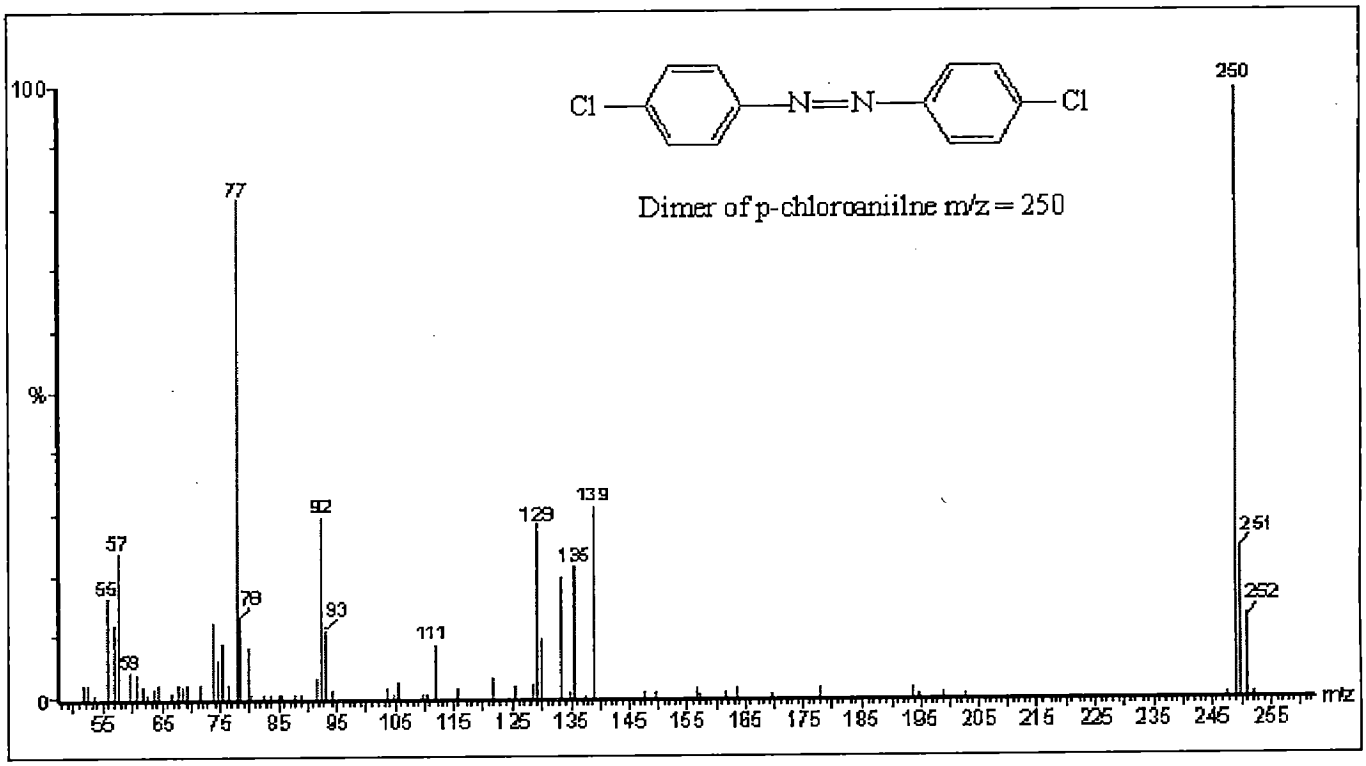
Mass spectrum of Tetramer of aniline

Figure 6.5.3



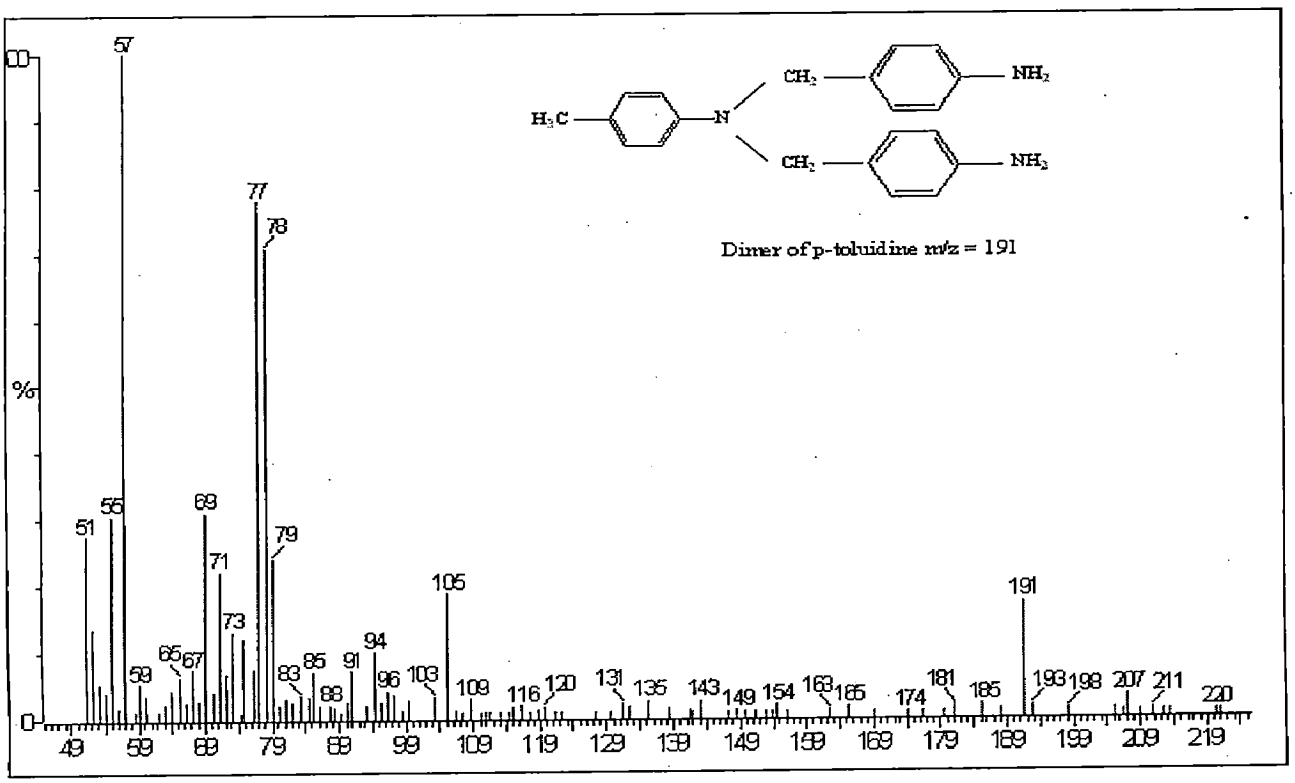
Mass spectrum of dimer of p-anisidine

Figure 6.5.4



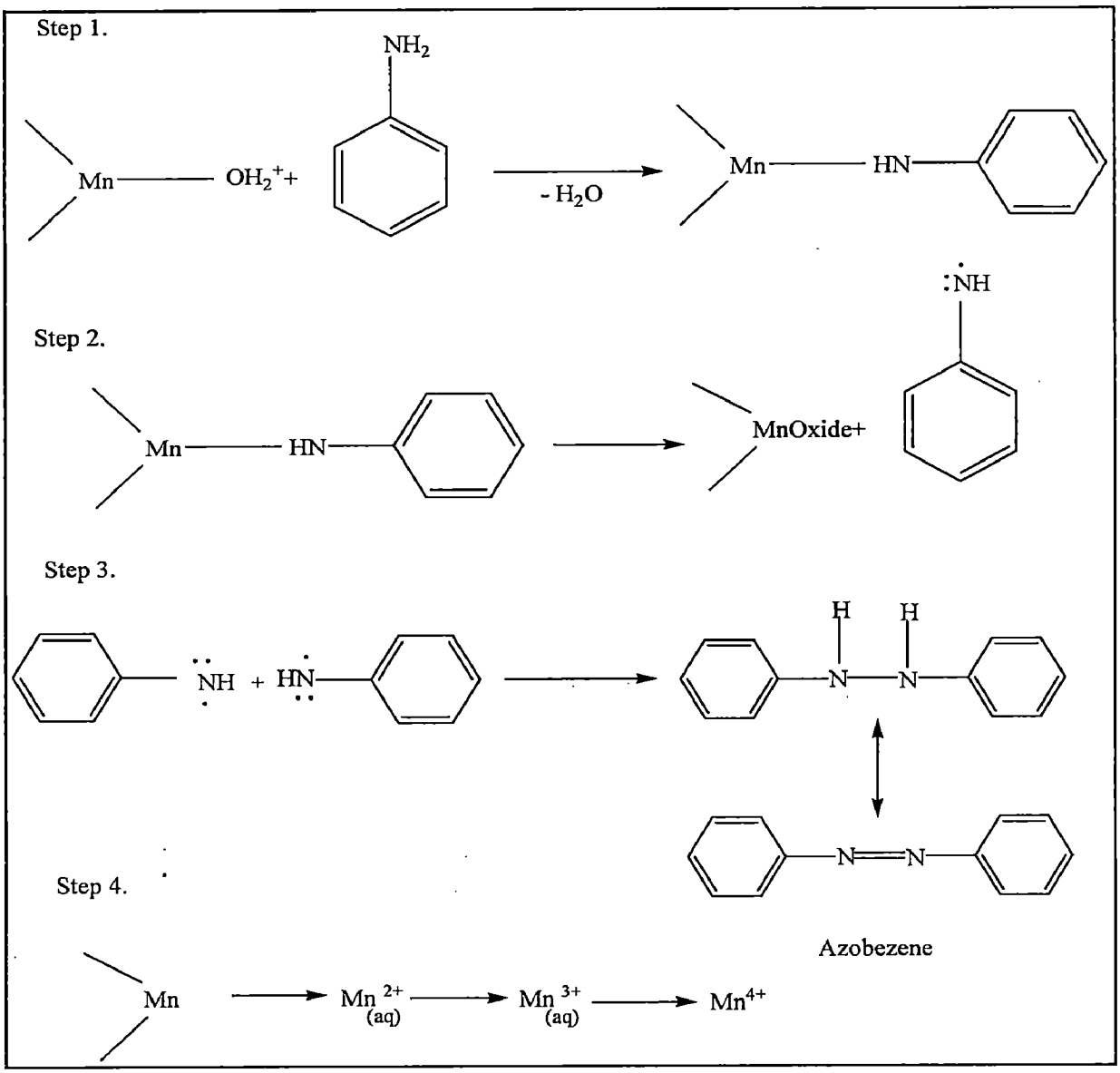
Mass spectrum of dimer of p-chloroaniline

Figure 6.5.5



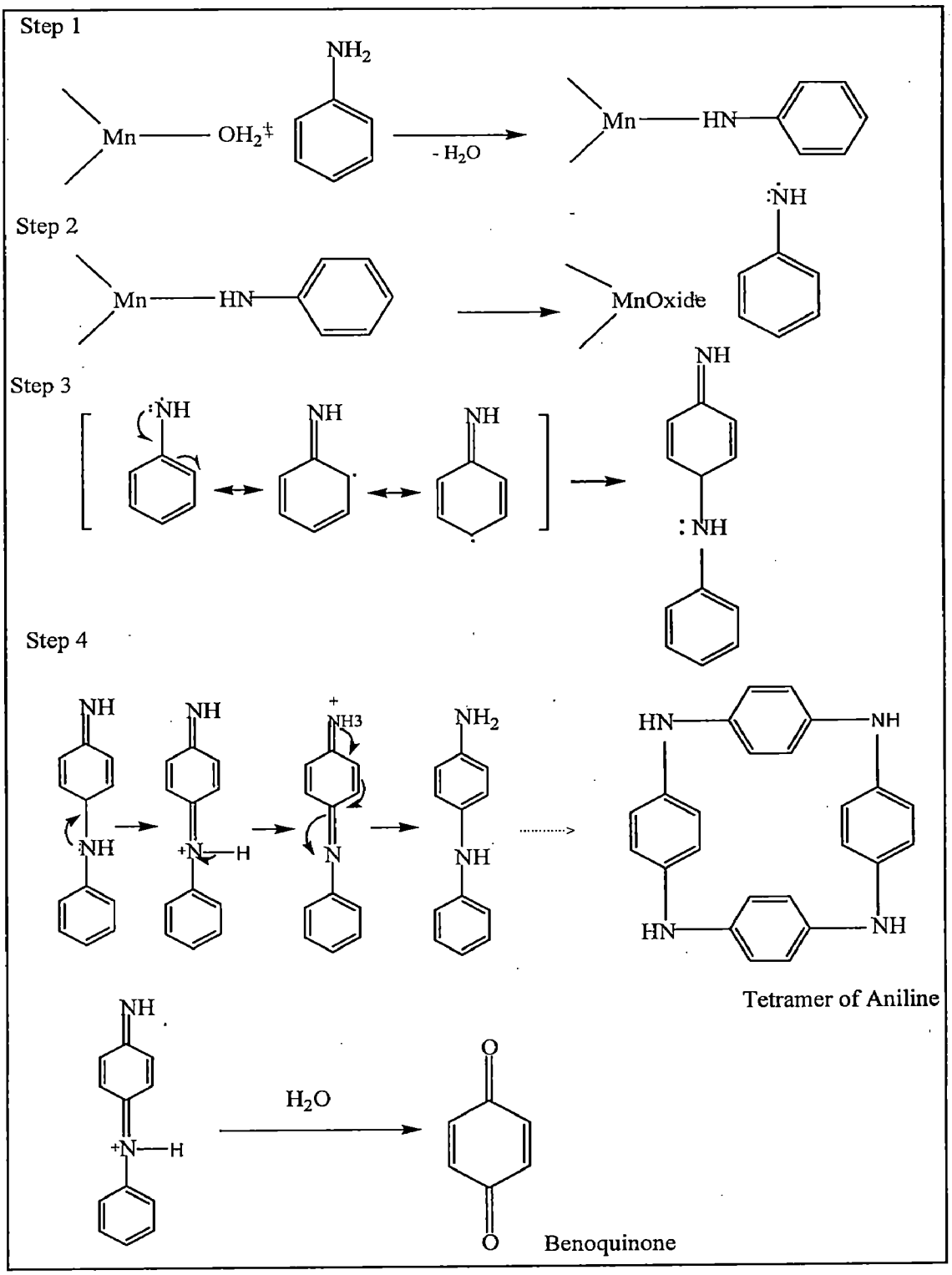
Mass spectrum of dimer of p-toluidine

Figure 6.5.6



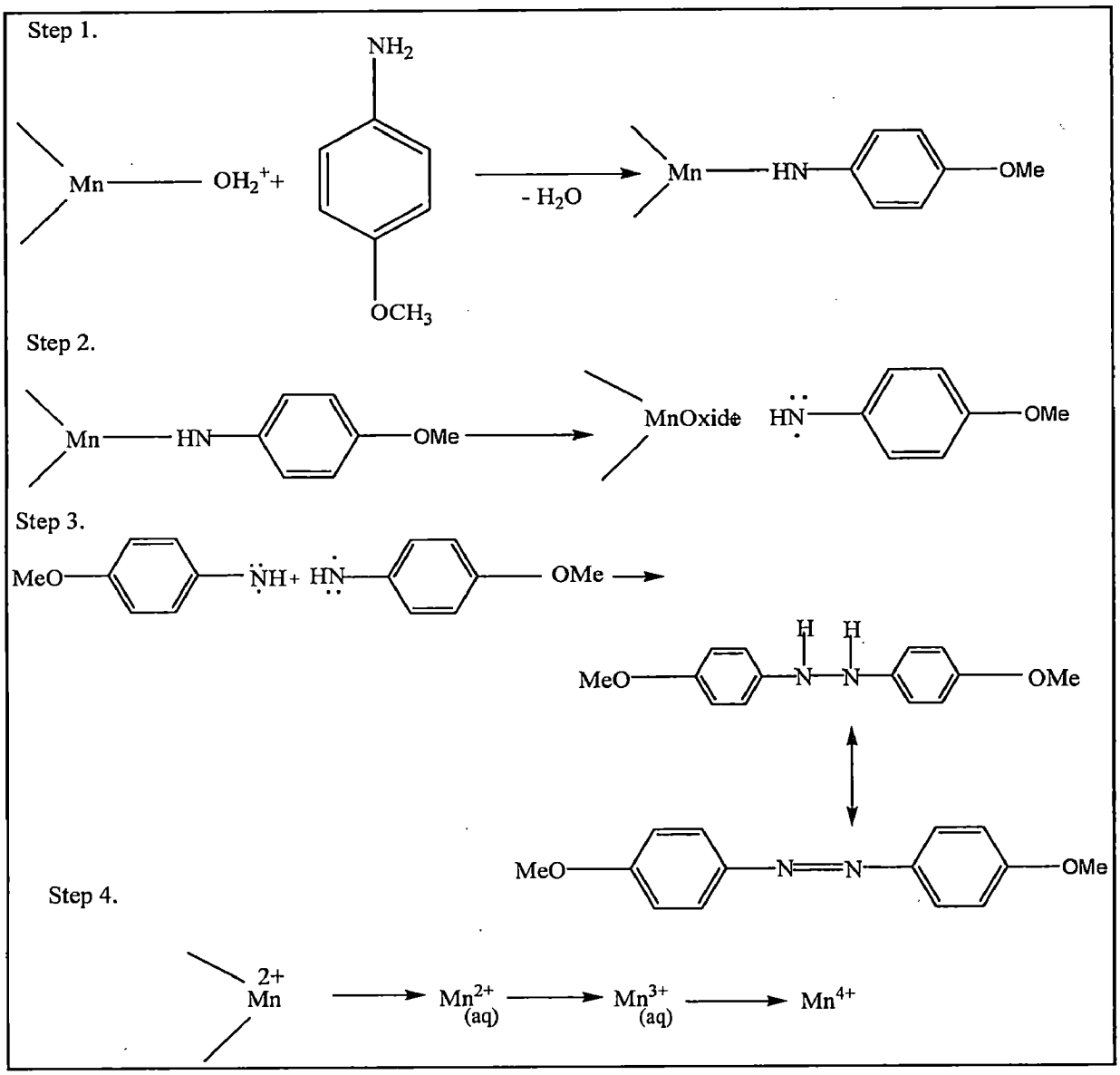
Proposed mechanism for the formation of azobenzene

Figure 6.5.7



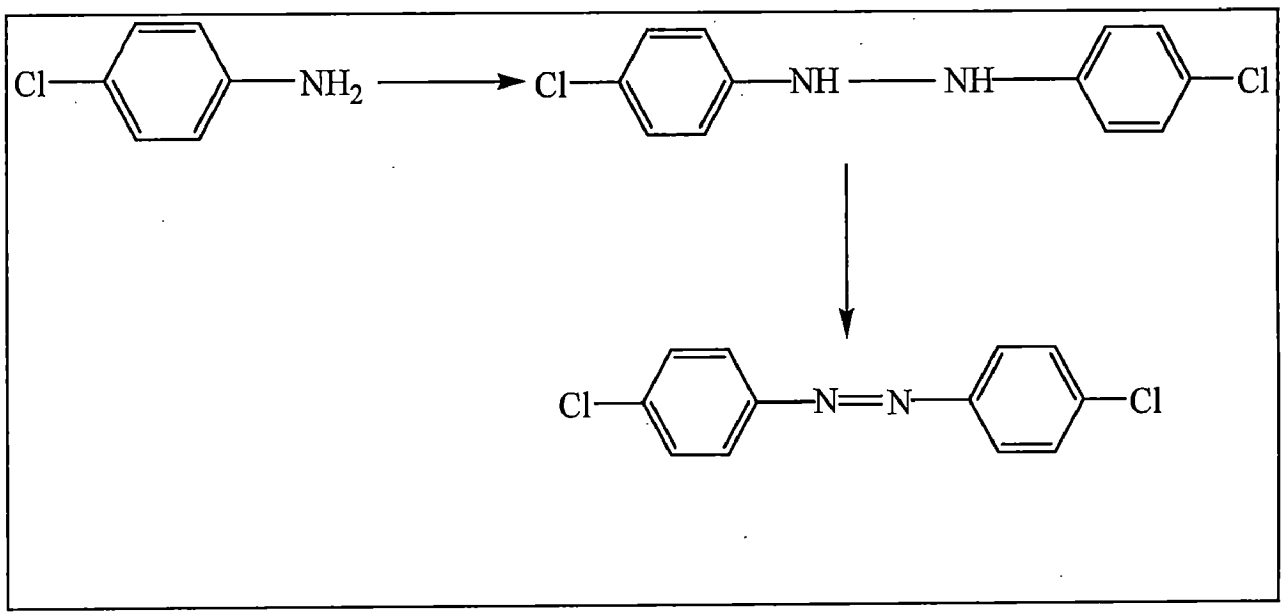
Proposed mechanism for the formation of tetramer of aniline and benzoquinone

Figure 6.5.8



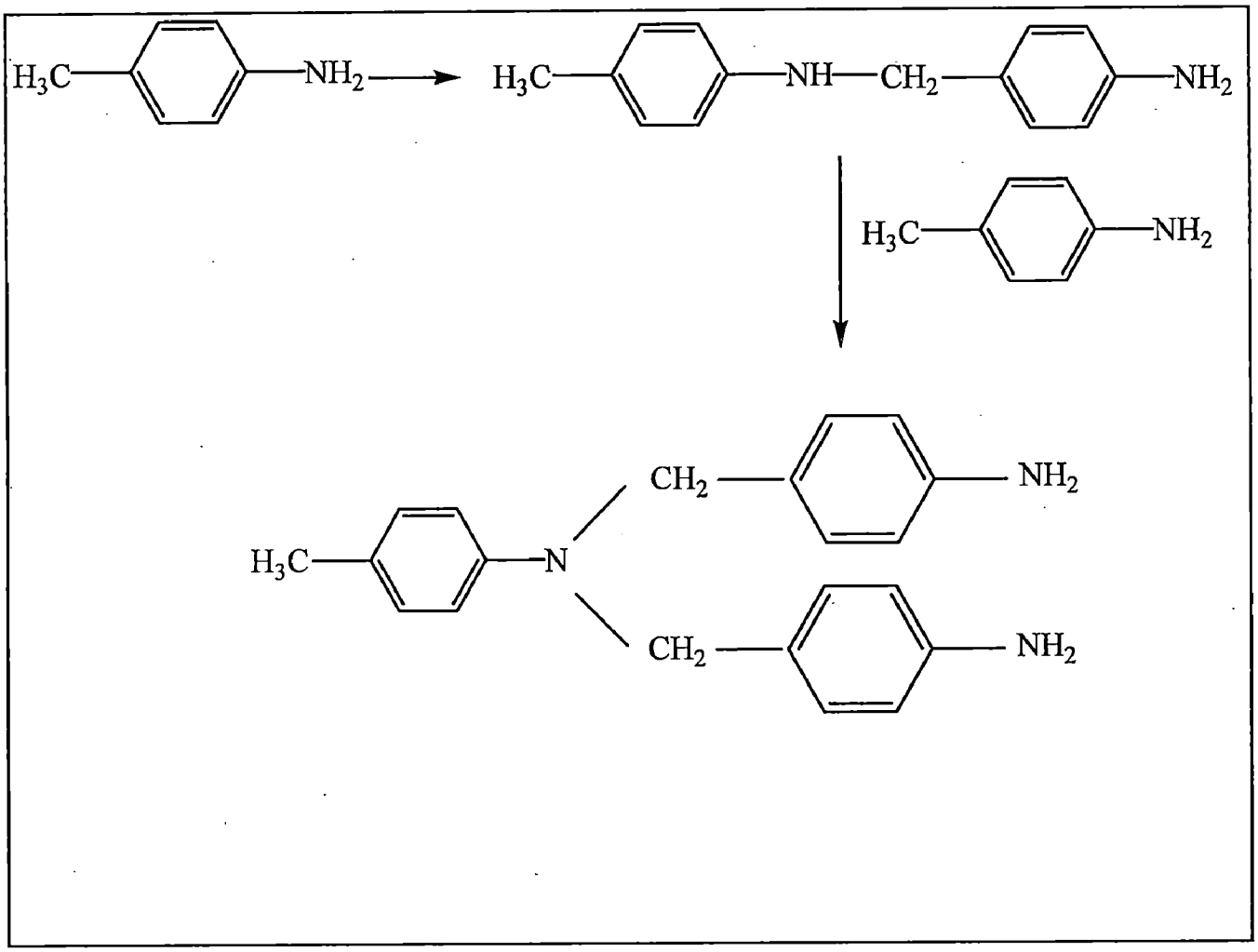
Proposed mechanism for the formation of dimer of p-anisidine

Figure 6.5.9



Proposed mechanism for the formation of dimer of p-chloroaniline

Figure 6.5.10



Proposed mechanism for the formation of dimer of p-toluidine

Figure 6.5.11

References:

1. Bernal, J.D., "The Physical Basis of Life". Routledge and Kegan Paul, London, 1951, p. 34.

2. Greenland, D.J., Laby, R. and Quirk, J.P. "Adsorption of glycine and its di-, tri- and tetra-peptides by montmorillonite". Trans. Taraday Soc., **58**, 829 (1962).

3. Greenland, D.J., Laby, R. and Quirk, J.P. "Adsorption of amino-acids and peptides by montmorillonite and illite. Part 2.—Physical adsorption". Trans. Taraday Soc., **61**, 2024 (1965).

4. Graf, G. and Laganly, G. "Interaction of clay minerals with adenosine-5-phosphates". Clays Clay Miner., **28**, 12 (1980).

5. Ferris, J.P., Hagan, W.J. "The adsorption and reaction of adenine nucleotides on montmorillonite", Jr. Orig. Life, **17**, 69 (1986).

6. Ferris, J.P. and Kamaluddin "Oligomerization reactions of deoxyribonucleotides on montmorillonite clay: The effect of mononucleotide structure on phosphodiester bond formation". Orig. Life Evol. Biosphere, **19**, 609 (1979).

7. Ertem, G. and Ferris, J.P. "Template-Directed Synthesis Using the Heterogeneous Template Produced by Montmorillonite Catalysis: A Possible Bridge Between the Prebiotic and RNA Worlds". J. Am. Chem. Soc., **119**, 7197(1997).

8. Furukawa, T. and Brindley, G.W. "Adsorption and oxidation ofbenzidine and aniline by montmorillonite and hectorite". Clays Clay Miner., **21**, 279 (1973).

9. Julian C. Baker and Suzanne D. Golding "Occurrence and palaeohydrological significance of authigenic kaolinite in the aldebaran

sandstone, denison trough, queensland, Australia". Clays Clay Miner., **40**, 273 (1992).

10. Kowalska, Maria W., Ortego, J. and Dale, Jezierski Adam "Transformation of 2-žtrifluoromethyl/ aniline over ion-exchanged montmorillonite: formation of a dimer and cyclic trimer". Applied Clay Science, **18**, 233(2001).

11. Boyd, S.A. and Mortland, M.M. "Radical Formation and Copper (11)-Smectite". Environ. Sci. Technol., **20**, 1056 (1986).

12. Friedmann, N. and Miller, S. L. "Phenylalanine and Tyrosine Synthesis under Primitive Earth Conditions". Science **166**, 766 (1969).

13. Ali, S., Alam, T. and Kamaluddin "Interaction of Tryptophan and Phenylalanine with Metal Ferrocyanides and its Relevance in Chemical Evolution". Astrobiology, **4**, 420 (2004).

14. Ali, S. and Kamaluddin "Interaction of Aromatic Amino Acids with Metal Hexacyanochromate (III) Complexes: A Possible Role in Chemical Evolution". "Bull. Chem. Soc. Jpn., **79**(10), 1541(2006).

15. Ali, S., Ahmad, J. and Kamaluddin "Interaction of Ribose Nucleotides with Metal Ferrocyanides and its Relevance in Chemical Evolution," Colloids and Surfaces A: Physicochem. Eng. Aspects **236**,165(2004).

16. Ali, S. and Kamaluddin "The Interaction of Ribose Nucleotides with Metal Hexacyanochromates (III) and the Relevance to Chemical Evolution". Bull. Chem. Soc. Jpn, **77**, 1681(2004).

17. Ali, S. and Kamaluddin, "Interaction of Ribonucleotides with metal Hexacyanocobaltate (III): A possible Role in Chemical Evolution". Orig. Life. Evol. Biosph., **37**, 225 (2007).

18. Alam, T., Tarannum, H., Ravi, M.N.V., and Kamaluddin "Adsorption and Oxidation of Aromatic Amines by Metal Hexacyanoferrate (II)". Talanta, **51**, 1097 (2000).

19. Alam, T., Gairola, P., Tarannum, H., Kamaluddin and Ravi M. N. V. "Conversion of Anilines to their Oligomers by Copper Hexacyanoferrate (II)". Indian J. Chemical Technology, 7, 230(2000).

20. Kamaluddin "Studies on Metal Ferrccyanides as Prebiotic Catalyst". in Chela-Flores et al. (eds), First Steps in the Origin of Life in the Universe, Kluwer Academic Publishers, Netherlands.95(2001).

21. Alam, T., Tarannum, H., Ali, S., and Kamaluddin "Adsorption and Oxidation of Aniline and Anisidine by Chromium Ferrocyanide". J. Colloid & Interface Science, **245**, 251 (2002).

22. Viladkar, S., Alam, T. and Kamaluddin "Adsorption of Aromatic Amines on Metal Ferrocyanides". J. Inorg. Biochemistry, **53**, 69 (1994).

23. Alam, T. and Kamaluddin "Interaction of Aminopyridines with Metal Hexacyanoferrates (II)". Bull. Chem. Soc. Jpn. **72**, 1697 (1999).

24. Alam, T., and Kamaluddin, "Interaction of 2-amino, 3-amino and 4-aminopyridines with nickel and cobalt ferrocyanides". Colloids Surf. **162**, 89 (2000).

25. Alam, T., Tarannum, H., Kumar, N. and Kamaluddin "Interaction of 2-Amino-, 3-Amino-, and 4-Aminopyridines with Chromium and Manganese Ferrocyanides". J. Colloid Interface Sci. **224**, 133 (2000).

26. Kobayashi, K. and Ponnamperuma, C. "Trace elements in chemical evolution I". Orig. Life, **15**, 55 (1985).

27. Kobayashi, K. and Ponnamperuma, C. "Trace elements in chemical evolution II". Orig. Life, **16**, 67 (1985).

28. Brij Bhushan, Uma Shanker and Kamaluddin "Adsorption of Ribose Nucleotides on Manganese Oxides with Varied Mn/O Ratio: Implications for Chemical Evolution". Orig. Life. Evol. Biosph., **41**,469 (2011).

29. Giles, Charles H D'Silva, Anthony P. and Easton, Ian A. "General Treatment and Classification of the Solute Adsorption Isotherm Part II. Experimental Interpretation". J. Colloid & Interface Science, **47**, 766 (1974).