CHAPTER 1

1. INTRODUCTION

1.1 PRELIMINARY REMARKS

Inorganic phosphorus compounds are generally insoluble and move into the ground water mainly attached to soil particles or soluble organic moieties. Waste water in urban areas contain substantial amounts of inorganic phosphorus compounds resulting from detergents used for various purposes. Inorganic phosphorus compounds such as dibasic calcium phosphate, tribasic sodium phosphate and dibasic potassium phosphate are not considered to be toxic to humans or animals.[1] However, inorganic phosphates accelerate eutrophication of surface waters causing an increased growth of undesirable algae and aquatic weeds which result in shortages of dissolved oxygen in the water systems. This in turn results in massive bloom of cyanobacteria. These blooms cause a wide range of problems including fish kills, foul smells, unpalatability of drinking water and the formation of trihalomethane during water chlorination in treatment plants. Consumption of cyanobacterial blooms in water can kill livestock and pose a serious health hazard to humans due to the release of neuro and hepa toxins by the cyanobacterial blooms.[2]

A better understanding of the mechanism of hydrolysis of phosphate esters is required to address the environmental problems. For example, such knowledge is needed to evaluate the probability that during a chemical spill the organophosphate will reach water sources and contaminate the ground water.

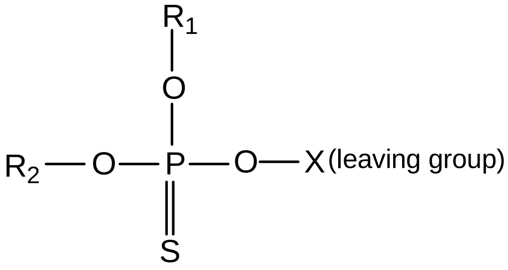
Of equal importance is “Pollution prevention” strategies in the form of safe decontamination procedures which will result as spin-off of the knowledge. This chapter discusses the summary of the chemistry of phosphorus and phosphates, roles of metal ions in phosphate ester hydrolysis, the model systems and the lessons learned, the chemistry of microemulsion systems and the aim of our investigation in the given order.

1.2 PHOSPHORUS AND PHOSPHATES

Phosphorus is one of the essential elements that support life by being a constituent of various compounds, such as DNA and proteins. It is one of the mass components in bones and teeth. Phosphorus is not found free in the environment but in phosphorus mineral deposits. Phosphorus exists in three varieties, namely: white, red, and black phosphorus. White phosphorus consists of discrete tetrahedral P_4 molecules.[3] The bond angles in the P_4 tetrahedron structures are strained, and this makes it very reactive. It is usually stored in water due to its high reactivity on exposure to air and this helps in prevention of oxidation by formation of a hydroxide coating on its surface. Red and black phosphorus are the derivatives of white phosphorus and are less toxic and less reactive compared to it.

When phosphorus is bonded to oxygen, the resulting compounds are often referred to as phosphates. The term also extends to those compounds that are formed by the replacement of one or more oxygens by a metal. Phosphates are mainly introduced into the environment as a result of mining activities[4] (phosphate mines), agriculture (phosphate containing fertilizer), from industrial products such as in food industry and from additives (such as Na_2HPO_4) in detergent industry for water softening and pH stabilization.

Phosphate derivatives are extremely important in biology as they form part of the backbone of DNA. Phosphate anhydrides such as ATP provide energy in the cell for the essential cellular functions such as phosphorylation and metabolism. The phosphate derivatives can be esters, amides and thiol derivatives of phosphonic acids. Their mode of reactivity and importance depends on the type of functional groups they possess. In the chemical structure below, R_1 and R_2 are usually simple alkyl or acyl groups, both of which may be bonded directly to phosphorus (in phosphonates) or linked via -O-, or -S- in phosphates.[5] In phosphoramidates, carbon is linked to phosphorus through NH- group. The leaving group, X, can be any one of a wide variety of substituted and branched aliphatic, aromatic or heterocyclic groups linked to phosphorus via a bond of some lability (usually -O-, or -S-). The double bonded atom is -S- (in phosphothionates) and -O- (in phosphates).



The phosphate esters of organic compounds are normally referred to as organophosphates and they differ in their toxicity according to the functional group they carry. Organophosphates can be classified into two groups; aryl organophosphates which must be activated by the liver microsomal enzymes before becoming toxic, and alkyl organophosphates which do not require activation for toxicity.[5] The toxicity of organophosphate compounds as anticholinesterase agents is attributed to the inhibition of acetylcholinesterase enzymes. Acetylcholinesterase enzymes play an important role in the transmission of chemical nerve impulses[6] in a synaptic cleft. Organophosphates are widely used as chemical warfare nerve agents and pesticides due to their toxicities. Some organophosphate compounds are utilized for the treatment of some health conditions such as glaucoma. The first organophosphate to be used for such treatment was physostigmine in the 1850s.[7] It is therefore necessary to give a brief description of some of the organophosphate compounds used as toxins.

1.2.1 ORGANOPHOSPHATE ESTERS AS NERVE AGENTS AND PESTICIDES

Most of the organophosphate nerve agents for military use were an outgrowth of the search for effective insecticides. They were named as nerve agents due to their effects on the nervous system of the victim. They are mostly absorbed through the skin or lungs during contact.

Organophosphate nerve agents consist of two types depending on the degree of their toxicity: G-type agents [GA (tabun), GB (Sarin), GD (Soman)] (which are all non persistent and get their way into the body by inhalation) and V-type agents [VX (o-ethyl-S-diisopropylaminomethylmethylphosphothiolate)] (which are persistent, and highly toxic). They find their way through by inhalation, skin contact, or mucosal absorption).[8] Included amongst such organophasphate nerve agents are;

- Tabun (o-ethyldimethylamidophosphorylcyanide) is easier to produce, as its precursor chemicals are readily obtainable.
- Sarin (isopropylmethylphosphono-fluoridate) is a clear, colourless, odourless and tasteless liquid in its pure form. Sarin was originally developed in Germany in 1938.
- Soman (pinacolylmethylphosphonofluoridate) has a slight camphor-like odour and was synthesized in Germany in 1944. It is the fastest killing nerve gas; it is lethal through both inhalation and skin contact.
- VX (o-ethyl-S-diisopropylaminomethylmethylphosphothiolate) is a clear, amber-coloured, odourless, oily liquid that was synthesized by a British scientist in the 1950s. It is easily absorbed through the human skin and is also lethal through both inhalation and skin contact.[9,10]

The major types of pesticides include insecticides, herbicides, biocides (algaecides, slimicides etc.). Some of the common organophosphate insecticides include malathion, parathion, methylparathion, diazinon, chlorothion and dichlorovos.[6,11] Parathion is an excellent and widely used insecticide because it is extremely toxic and cheap to produce.

Both nerve agents and pesticides are organophosphates, which act on the central nervous system of the victim by affecting the acetylcholinesterase enzymes. However, organophosphate nerve agents are much more poisonous than organophosphate pesticides[7] and this is because of the fact that, they are easily absorbed and transmitted throughout the body by the nervous system.

Some organophosphate esters are known to cause damage to the liver tissues thereby triggering cancer. The combinations of commonly used agricultural chemicals in concentrations that mirror the levels found in ground water, can significantly influence the immune and endocrine systems as well as neurological health.[12] A study, which was conducted using combinations of pesticides and nitrates in mice suggested the alteration in the immune, endocrine and nervous system functioning, and these have been shown to occur at concentrations that are prevalent in ground water.[11] Most at risk are the developing foetus and children, where their systems are still at the developmental stages. It has also been reported[12] that the exposure by agricultural use of organophosphates induces depression in human beings, which is mainly attributed to the decrease in serotonin levels in the body as a result of excess undesirable organophosphates. Clearly, this evidence indicates the problem that is faced by many communities who are made to believe that these chemicals are totally harmless.

1.2.2 ORGANOPHOSPHATE ESTERS AS POLLUTANTS

Organophosphates are generally insoluble in aqueous media and cannot be efficiently hydrolyzed even at high pH. Most toxic organophosphates in the environment are the results of human activities and they pose a serious threat as environmental pollutants. Pollution due to organophosphates is attributed mainly to improper management of stock piles of expired pesticides, and chemical warfare nerve agents. When using any chemical for whatever purpose one has to ask this important question ‘how long the applied or dumped chemical will remain in the environment?’ Some of the widely used compounds are generally regarded as ‘safe’ due to ‘fast’ degradation. However, the environmental rates for their degradation vary as a function of microbial composition of the soil, the type of the contaminant, the pH and the availability of sunlight. Tests conducted for the degradation of these compounds under laboratory conditions (25 ^{o}C, pH 7) reveal that biodegradation is about one order of magnitude faster than chemical hydrolysis, which in turn is ten times faster than photolysis.[13] In ground water (pH 6, 5) hydrolysis reaction of organophosphates was found to have a half-life of one year[13] and this was found to be ten days under laboratory conditions. This evidence proves that organophosphates persist in the environment for longer periods of time. Most of these chemicals are classified as persistent because they are stable under ambient environmental conditions.

The developing nations are the most affected since they are unaware of the dangers of these chemicals and also the lack of the expertise in addressing the problem. The other major problem is the lack of financial resources and technical facilities that are required to get rid of the chemicals.[14] Several techniques have been developed to address the pollution threat posed by organophosphates. Included amongst such techniques are *gamma* irradiation, incineration, wet air oxidation, plasma arc process, molten salt oxidation and enzyme-based detoxification.[5] All of these technologies are still under investigation. Much research is now concentrated on the enzyme-based detoxification and on the reaction media. It has been shown in several studies[15-17] that the metal ion containing enzymes are capable of hydrolyzing the phosphate ester bond. Also, organised assemblies such as microemulsions have been utilised as potent hydrolysing agents during the last few years.[18-20] Organised assemblies have been found to enhance the rate of hydrolytic reaction.

1.3 HYDROLYSIS OF ORGANOPHOSPHATE ESTERS: LITERATURE REVIEW

In biological systems enzyme promoted reactions involving both phosphoryl transfer and nucleotidyl transfer are of numerous importance.[21] In many of these reactions the enzymes are activated by metal ions, yet the detailed roles of the metal ions are generally not clearly understood. A short review of biochemical and structural information which has been established on enzymic phosphoryl transfer is provided below:

1.3.1 ALKALINE PHOSPHATASES

Alkaline phosphatases catalyse the hydrolysis of phosphate monoesters with a rate maximum near pH 7 to 8. The phosphatases which have been isolated from mammalian and bacterial sources have been found to contain catalyticaly essential Zn^{+2} and Mg^{+2} ions.[22] Alkaline phosphatase isolated from *E. coli* is a dimer of molecular weight 94,000 and it contains at least two and up to four Zn^{+2} ions and two Mg^{+2} ions per dimer. The metal ions appear to play two roles in the enzymes. Two Zn^{+2} are required for activity; these are called catalytic and are bound very strongly to the enzyme. The two additional Zn^{+2} ions and the two Mg^{+2} ions, termed structural, enhance the activity of the enzyme in addition to stabilizing its tertiary and quaternary structure.[23]

Several proposals for the mechanism of hydrolysis by alkaline phophatase have been advanced. The usual role of the metal ion is considered to involve activation of the substrate by coordination, thereby increasing the electrophilicity at the phosphorus center, and or activation of the nucleophile. The nucleophile in question may be either the hydroxyl of serine 102 or water. The nucleophile, by coordinating to the metal ion, becomes more acidic and can be deprotonated more easily thereby increasing its nucleophilicity. The catalytically essential amino acid arginine 166 is located close to the phosphate binding site and is probably involved in charge nuetralization of the bound phosphate. Although much is known about the structure of the active site, the role of the metal ions in the mechanism is still unclear and the degree of involvement of other functional groups surrounding the enzyme has yet to be established.

1.3.2 YEAST INORGANIC PYROPHOSPHATASE

The enzyme yeast inorganic pyrophosphatase consist of two identical subunits with a molecular weight of 64,000 for the dimer. Yeast inorganic pyrophosphatase catalyzes the reversible hydrolysis of pyrophosphate. In the presence of Mg^{+2}, yeast inorganic pyrophosphatase is specific for pyrophosphate; however, when other metal ions are employed, the enzyme becomes less specific and it will then hydrolyze a number of pyrophosphate esters.[24] In the absence of phosphate the native dimer binds divalent metal ions, while three divalent metal ions are bound with high affinity in the presence of phosphate. These metal ions include Mg^{+2}, Mn^{+2} , Co^{+2} , Zn^{+2} , Ni^{+2} , Fe^{+2} and Cd^{+2}, all of which activate the enzyme, and Ba^{+2} and Ca^{+2} which inhibit it.[25] Several observations[26,27-29] conclusively show that one metal ion is required to form the substrate and at least one other divalent metal ion is required for activity. Several proposals have been fowarded to explain the mechanism of yeast inorganic pyrophosphatase. The basic features for the mechanism of yeast inorganic pyrophosphatase reactions[25,30] include: metal ion binding to the pyrophosphate to produce a substrate; production of a metal bound hydroxo ligand as a nucleophile by an adjacent basic amino acid residue and stabilization of the substrate by interaction with cationic amino acid residue (in this case arginine).

1.3.3 KINASES

The class of enzymes which catalyze the reversible transfer of the terminal PO_3^- groups from nucleoside 5`-triphosphates to water are named nucleoside 5`-triphosphatases. When the acceptor molecule is other than water they are termed kinases. Kinases generally require at least one divalent metal ion (frequently Mg^{+2}) for activity.[31] Thus the overall catalyzed reaction may be written as:

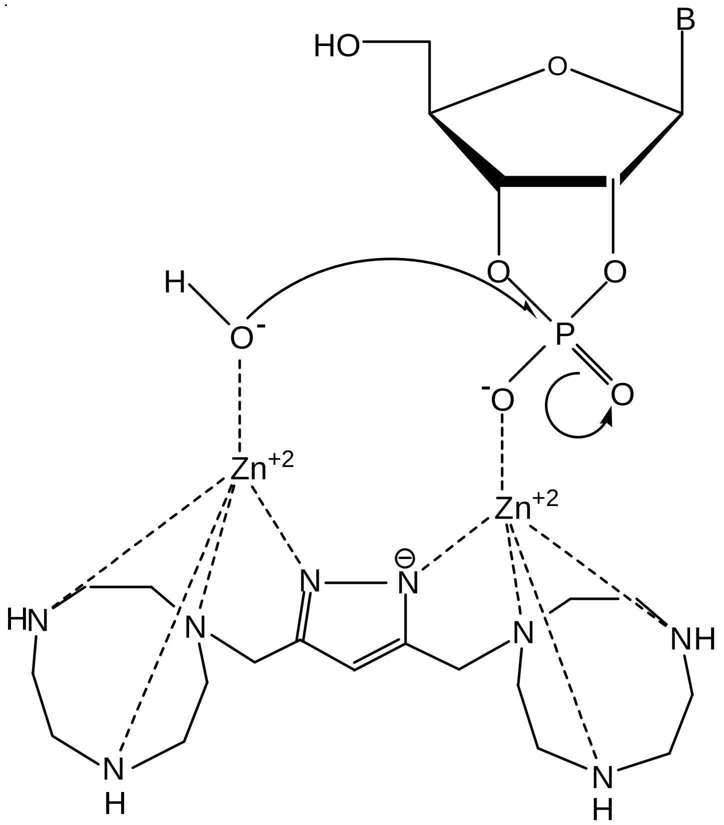
$$\textbf{Acceptor + NTP} \leftrightarrow \textbf{Phosphorylated acceptor + NDP}$$

In most of these reactions, the metal ion seems to participate in the reaction as the nucleoside 5`-triphosphate complex by forming the β,γ-bidentate chelate. In general, kinases require the NTP to be in the form of a M^{+2} (usually Mg^{+2}) complex before accepting it as a substrate. This results in the β phosphorus atom becoming chiral. From the large number of kinases investigated[32-34], it appears that the enzymes will utilize only one conformation of the complex. This is typical of the enzymes, which are stereospecific even when the reactants and products are chiral. With continued interest in the hydrolysis of phosphate esters by metal ion containing enzymes in biological systems a lot of work has been done to date.

Some ideas on what is exactly happening at the active site of an enzyme has been obtained by the use of enzyme mimics and this knowledge of metal ion hydrolysis on phosphate esters has been tried on real biological phosphate esters by several researchers.[35-40] Almost all the reported mechanisms for the hydrolytic reaction of phosphates by metal ions have reported a common pathway indicating the necessity of the metal ion in hydrolytic reaction.

In the hydrolysis of biologically relevant compounds such as guanosine(2`-3`) cyclic phosphate ((2`-3`)cGMP) [35] by dinuclear metal complexes of Zn^{+2}, the formation of 2`-GMP and 3`GMP as products was observed. The reaction follows the same route as that of simple phosphate esters. When the substrate and the catalyst are added together there is rapid formation of the ternary complex (Michaelis-Menten) followed by the charge neutralisation and phosphate activation through the binding of the phosphate oxygen to one of the Zn^{+2}.[35,37]

The substrate shown in the following diagram is probably activated by one metal ion through Lewis acid activation while the dianionic phosphate intermediate is stabilized by both metal ions. The second Zn^{+2} activates the nucleophile, which is followed by the activation of the leaving group. The presence of the hydroxide ion is always necessary as it is the most active nucleophile in most hydrolytic reactions. As a result of intramoecular attack by hydroxide the diester is cleaved to the mono esters 2`-GMP and 3`GMP.



B = Guanine

***SCHEME* 1.1: BINUCLEAR HYDROLYSIS OF GUANOSINE(2`-3`) CYCLIC PHOSPHATE ((2`-3`)cGMP)** [35]

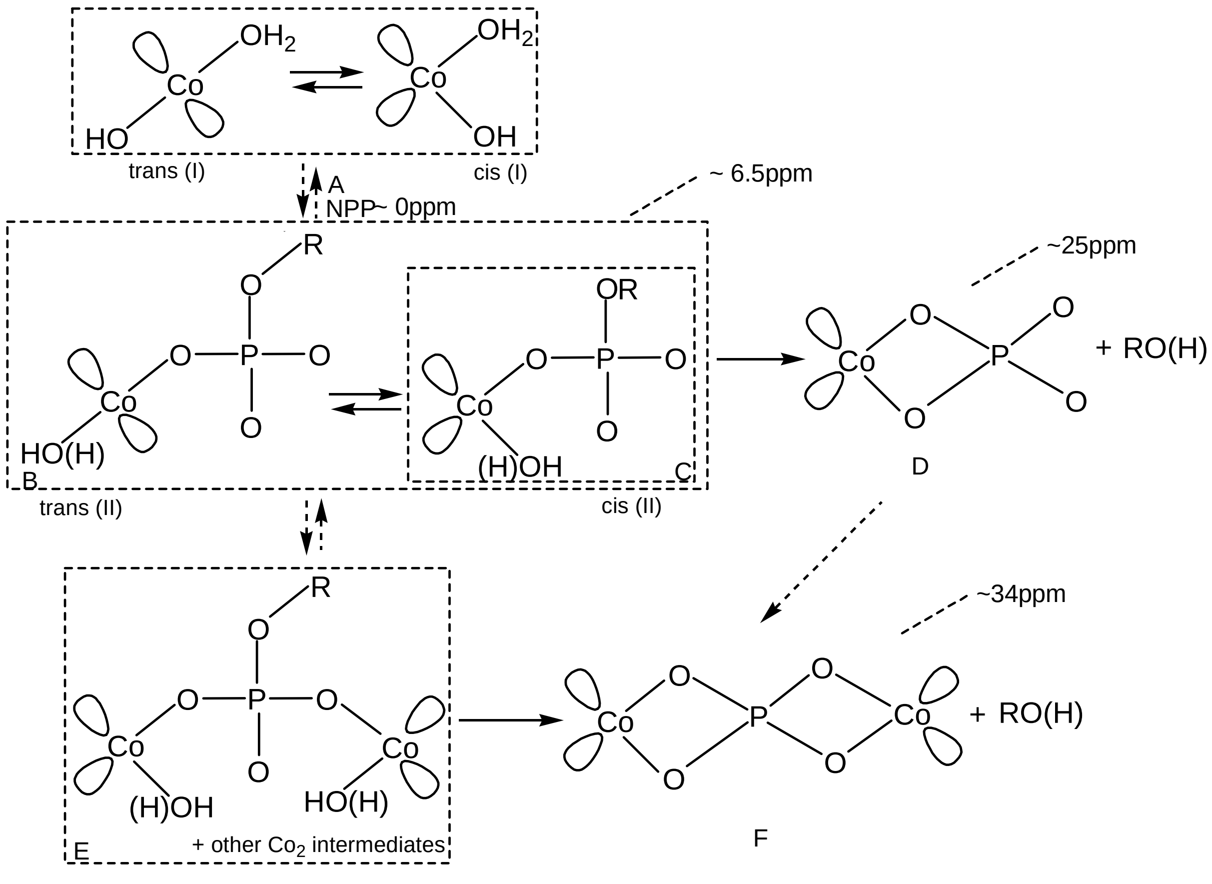
1.4 MODEL SYSTEMS IN HYDROLYSIS OF ORGANOPHOSPHATES

Model systems have continuously provided us with the basis for increasing our knowledge of the hydrolysis of phosphate esters. Different researchers[39-41] at different times have emphasized the importance of metal ion requirements for efficient hydolysis. Initial research was based on divalent metal ions. A major problem with those systems was that the divalent metal ions are substitutionally labile and form a variety of complexes which are in rapid equilibrium. As a result it was difficult to define any particular complex or the binding mode as being responsible for the observed rate enhancement. It was this reason that led to the use of model systems for the study of these hydrolytic reactions. Substitution reactions in biological systems use primarily the first row transition metal ions as co-factors. Due to the lability of the divalent metal ions and the complexity of enzymic molecules it was not easy to follow the hydrolytic path. The identification and monitoring of the intermediates formed in the reaction was also difficult. Therefore later investigations were conducted using semi-labile metal ion complexes with simple coordinated ligands such as those of cobalt, and lanthanides and other semilabile metals ions. The model systems mimic the real bioenzymes that act under physiological conditions. This has shed some light in the understanding of the kinetics and mechanisms of phosphate ester hydrolysis. Also the direct study of real organophosphate ester pesticides and chemical warfare agents could not be undertaken due to their toxicity and to the fact that one has to obtain authorisation to undertake such investigation from OPCW. Much investigation has also been undertaken on simple organophosphate ester simulants such as 4-NPP[5,6,42] and BNPP[41].

In model systems, the most effective and well studied metal centres in promotion of phosphate and polyphosphate ester hydrolysis have been found to be those of Co(III) (in such complexes as $[Co(trpn)(HO)(H_2O)]^{+2}$and $[Co(tn)_2(HO)(H_2O)]^{+2}$).[41] Co(III) polyamine complexes have been proven to have up to 10^8 rate enhancement in the hydrolysis of activated phosphate esters and have been studied as phosphatase and ATPase models. What is more significant about Co(III) complexes compared to other metal ion complexes, is that it offers some important advantages over other metal complexes.

- Co(III) complexes are easy to prepare and are kinetically robust, thereby permitting the characterisation of all the species present in the solution,
- Kinetics and mechanisms of Co(III) complex substitutions are well understood.
- For NMR studies, cobalt is a low spin d^6, therefore diamagnetic,
- cobalt is substitution inert,
- Various forms of cobalt complexes can be separated by chromatography and
- -Co-N bonds retain their integrity in aqueous media for a long time.[41]

In a recent study[42], 4-nitrophenyl phosphate hydrolysis by Co(III) was monitored using a combination of uv-visible and ^{31}P NMR techniques. UV-visible spectrophometer was used to follow the rate of the reaction while ^{31}P NMR was used for the identification of the intermediates formed in the reaction. This study provided an additional evidence on the mechanism of hydrolysis of phosphate esters. The overall mechanistic scheme and a brief observations of such reaction follows:



(Charges mostly omitted: R- =4-nitrophenyl-)

***SCHEME* 1.2: REACTION PATHWAY FOR THE HYDROLYSIS OF 4-NPP BY Co(III)**[42]

It is generally known that the $Co(tn)_2(aq)$ complexes occur exclusively as trans species in the solid form but in aqueous form it occurs in equilibrium as cis and trans with cis being the predominant species. The preference of the cis species in the aqueous state is due to the ease at which hydrogen bonding on the adjacent hydrogen atoms of the hydroxoaqua coordinated nucleophiles can take place. The reaction occurs by both forms but cis is mainly preferred as the effective nucleophile is adjacent to the substrate. The trans isomer forms a minor product and reacts much slower than the cis isomer.

Mixing the phosphate and the metal ion in a 1:1 molar ratio leads to the immediate formation of a monocoordinated phosphate complex of cis(II) and trans(II) geometry as depicted in the scheme above. The formation of a monocoordinated complex of cobalt is immediately followed by nucleophilic attack on the phosphorus center by the coordinated hydroxide on the cis position. This leads to the activation of the phosphorus-oxygen bond thereby releasing 4-nitrophenol as a leaving group and the formation of a four-membered ring system of cobalt and phosphate. Alternatively coordinated hydroxide can also pick up a proton from the solution to form water thereby leaving the coordination sphere of the cobalt. An attack by the phosphate oxygen on the cobalt center leads to the formation of the four-membered ring system of cobalt without the production of 4-nitrophenol. The coordination of a second metal to form a bicoordinated complex leads to immediate breaking of the phosphorus-oxygen bond similar to what has been described above. Hydrolysis of the monocoordinated trans species is presumed to proceed by this process. The end result is the formation of 4-nitrophenol and two four-membered ring systems of the phosphate and the cobalt.

The reaction was also monitored by ^{59}Co NMR to identify the cobalt chelates formed in the solution. The intermediates were also monitored using ^{31}P NMR spectroscopy. It is therefore necessary to look at some of the nuclear magnetic resonance properties and requirements for the effective identification of the species. The identification of compounds by solution ^{31}P NMR is based on their chemical shift relative to an external H_3PO_4 standard. The chemical shift is defined by:

$$(V_S - V_R)/ V_R \ x\ 10^6,$$

Where V_S and V_R are the frequencies of the sample and the reference standard, relative to that of the applied magnetic field.[43] Chemical shift values are dimensionless and expressed in parts per million (ppm) with the standard set to 0ppm: Chemical shifts depend primarily on the degree of molecular shielding around the P nuclei, but are modified by the surrounding chemical environment. For example, variations in ionic strength, pH, probe temperature, and the presence of paramagnetic ions can all induce subtle changes in chemical shift that complicate signal assignments.[44] With a spin quantum number of ½, the phosphorus nucleus ^{31}P (the only natural isotope of this element) will give a single spectral line, whose chemical shift is highly indicative of the particular phosphorus functional group, and is generally predictable from studying the effects of structural change. Having said that, it is well recognised that ^{31}P NMR shifts can be influenced by structural changes, and anyone reporting ^{31}P chemical shifts should rationalize the data with the structure at phosphorus to avoid assignment errors. Chemists and Analysts should be observant about any unusual effects that might be operating on the shielding phenomenon. Some of the nuclear magnetic resonance properties for iron[45], phosphorus and other nuclides are depicted in table1.

Isotope	NMR Frequency	% Abundance	I(spin)	Sensitivity
^{1}H	42.6	99.98	½	1
^{31}P	17.2	100	½	0.4
^{13}C	10.7	1.1	½	0.25
^{59}Co	10.0	100	7/2	0.28
^{57}Fe	3.2	2.2	½	$3.37x10^{-5}$

Table 1: NUCLEI PROPERTIES FOR SELECTED NUCLIDES

Phosphates are generally not active in the uv-visible region unless coordinated to some special compounds, therefore measuring the amount of 4-nitrophenol produced from 4-nitrophenyl phosphate provides a direct measure of the hydrolytic rate of the organophosphate compound.

1.4.1 OTHER MODEL SYSTEMS

Although cobalt is the most well investigated metal ion as model for the hydrolysis of phosphate ester, a number of other metal ions have also been utilised. A few of them will be mentioned below as examples. Several studies[39,46] on the use of lanthanide complexes for the hydrolysis of phosphate esters has been reported. There are reports on the effect of lanthanide ions in the hydrolysis of phosphate esters either as individual, synergism between them or synergism with other metal ions. The reported mechanisms are in line with other reports for the hydrolysis of phosphate esters. In the study of lanthanide hydroxide gel for the hydrolysis of different phosphate monoesters[46], it was found that the mechanism goes through the formation of a four-membered cyclic species. The bond breaking is reported to occur between the phosphorus and oxygen not between the carbon and oxygen and also the best hydrolytic rate is at the pH range of 4 to 8. There are reports that lanthanide(III) complexes of tetraazamacrocycles containing the hydroxyalkyl groups[47] have a rate enhancement of 10^7 for bis(4nitrophenyl) phosphate. Also the cooperative hydrolysis of lanthanides, non lanthanum[48] ions and hydrogen peroxide[49,50] for the hydrolysis of phosphate diesters has been studied as a model for ribosome chemistry catalysis. In short lanthanides are amongst the late investigated metal ions for the hydrolysis of phosphate esters. In all the hydrolytic studies that involve binuclear metal complexes synergism has been reported to be prevalent. This synergism has been shown to be affected by a number of factors such as the type of ligand and the identity of the metal ion involved. Double Lewis acid activation is the popular reaction path way for the mechanism of hydrolysis reaction. Binuclear lanthanide macrocyclic complexes have also been studied for the hydrolysis of phosphates[51] and have been found to be amongst the effective complexes for hydrolysis reaction. Binuclear Cu(II) complexes of two cis-inositol derivatives on the hydrolysis of *bis*(4-nitrophenyl)[52] phosphate have been found to be selective for phosphate monoesters and diesters and have been shown to have a rate enhancement of $1x10^5$ to $4x10^5$ fold.[50] Model metal ion complexes are also selective in the type of organophosphate esters they hydrolyse just like enzymes.

1.5 ROLES OF METAL IONS IN ORGANOPHOSPHATE ESTER HYDROLYSIS

Many of the enzymes involved in the hydrolysis of phosphate esters are metalloenzymes that require metal ion for their activity. A number of these enzymes and enzymic models have been studied in considerable detail and have been shown to use a variety of structures, functional groups and chemical mechanism to accelerate the rate of hydrolysis reaction.[53-56] Metalloenzymes may contain one metal ion such as in carboxypeptidase and thermolysin, two metal ions catalysing the hydrolysis of phosphate monoesters, diesters, including DNA and RNA or polymetallic as has been studied in many phosphoryl-transfer enzymes such as that of kidney bean purple acid phosphotases(kbPAPs) and bovine spleen purple acid phosphotases (bsPAPs).[53,54,57,58] Hydrolases that contain dinuclear active sites utilise a wide range of first row transition metals whose structural properties regulate their Lewis acidities, which in turn controls the level of hydrolytic activity.[59] The central metal ion plays an important role in the enhancement of the hydrolytic reaction. Such information has been obtained in several studies[60-62] including those of Cobalt (III) complexes, which have been found to be efficient catalysts in promoting hydrolysis of phosphate diester with good or poor leaving groups. The mechanism which the hydrolysis reactions undergo is believed to be SN_2 type[59], and usually assumes the inversion of configuration with respect to the phosphorus center. There are three direct modes of activation that a metal ion can provide for accelerating the rate of phosphate ester hydrolysis. These include amongst others, the general Lewis acid activation (coordination of phosphoryl oxygen(s) to the metal), nucleophilic activation (coordination of the nucleophile such as hydroxide/ water to the phosphorus center) and the activation of the leaving group.[63] Since phosphatases often have two or more coordinatively unsaturated metal centers in the active site, one of the metals is presumed to be responsible for structural stabilization purposes and holding of the substrate in place while the second is responsible for catalysis in view of the fact that it has coordinated nucleophile. The necessity of a coordinated hydroxide has been confirmed[64-67] by several studies. The studies[50] on two metal centered lanthanide complexes that are bridged by peroxide has confirmed this notion. The bridging peroxides also act as nucleophiles as they are able to attack the phosphorus center, thereby enhancing the rate of hydrolysis. Included are several parameters such as the type of ligand, the reaction pH, the type of metal ion and its valence state. The spatial arrangement and the distance between the metal ions also play a crucial role in the effectiveness of these metal ions towards the hydrolysis of phosphate esters.

A number of mechanisms by which metal ions and metal complexes could facilitate the reactions of phosphate esters are;[63]

- Electrophilic activation of the phosphorus center
- Charge neutralisation effects. Phosphate derivatives usually carry many negative charges so that the approach of a negatively charged nucleophile such as hydroxide ion is not favoured on electrostatic grounds.
- Provision of an effective nucleophile at biological pH. For example coordinated water or hydroxide to a metal center
- Organisation of the reactants so that an intramolecular reaction can occur
- Orientation of the substrate for the enzyme action
- Activation of the substrate in a strained chelate.

In the present study an attempt will be made to get a better understanding of the roles played by metal ions in different reaction media for the hydrolytic reaction of phosphate esters. The following section discusses the chemistry of microemulsions as our investigation is centered on comparative studies of hydrolysis of phosphate esters in aqueous and different microemulsion systems.

1.6 MICROEMULSIONS

Microemulsions are transparent, optically isotropic and thermodynamically stable homogeneous solutions of oil and water, stabilized by addition of a surfactant and usually a cosurfactant.[68,69] They are frequently droplet type of dispersions, either of oil-in-water(o/w) or water-in-oil(w/o). Whether one forms an oil-in-water or water-in-oil type of microemulsion depends on the proportions of the two phases. They can also be of oil-in-water-in-oil(o/w/o) or water-in-oil-in-water(w/o/w) and these are referred to as double miroemulsions. Since they are mixtures of polar and non-polar compounds they are stabilized by addition of surfactant/cosurfactant mixture, which is normally referred to as emulsifiers in food industry. In microemulsion systems, the surfactant forms a monolayer at the oil/water interface, and oriented in such a way that the polar head groups are in contact with the water whilst the non-polar tails reside in the oil phase, thereby solubilizing the mixture of the two immiscible liquids by reducing the interfacial tension between the two phases. Microemulsions are macroscopically homogeneous but microscopically exist as well defined regions/droplets of polar and non polar compounds, that is oil and water separated from each other by a layer of surfactant and the cosurfactant mixtures.[69,70] It is conclusive that microemulsions are formed exclusively by the dispersion of one or more phase(s) such as water, oil or air in another in which they are normally immiscible.[68] Microemulsions are thermodynamically stable, and they remain clear indefinitely.

Microemulsions consist of two parts; the main phase in which the other is dispersed often referred to as continuous phase and the dispersed phase referred to as discontinuous phase. Microemulsion droplets are usually 0.01-0.5 micrometers in diameter. They were first discovered empirically by Schulman, who found that the addition of a fourth component (often a short chain alcohol) to emulsion containing oil, water and a surfactant led to the formation of clear, apparently homogeneous phase. Microemulsions are formed spontaneously when the following components are mixed in the right proportions: water, oil, surfactant and the cosurfactant (generally a low molecular weight alcohol). The presence of a cosurfactant is critical in reducing the interfacial tension between the droplets of a dispersed phase and the continuous phase to near zero.[71,72] In the absence of a cosurfactant at the droplet interface, the mixture becomes milky due to diffraction of light and becomes unstable owing to the creation of much larger droplets referred to as emulsion.

Microemulsions can be charged (ionic) or uncharged (nonionic). Whether one wants to form an ionic or nonionic microemulsion depends on the choice of the surfactant/cosurfactant used during formulation and this also depends on the application intended for. A brief description of the chemistry of surfactants follows below.

1.6.1 SURFACTANTS

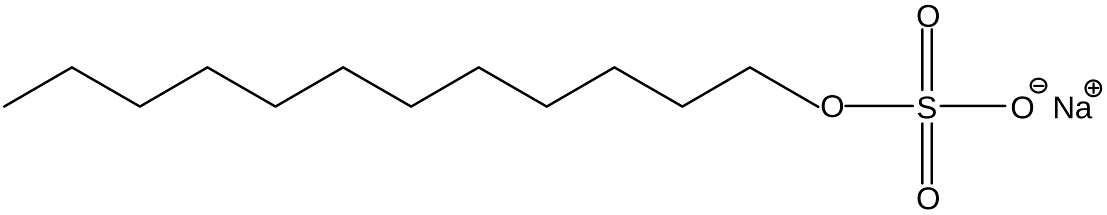
The name surfactant is derived from " surface active" agent as its effect is manifested on surface properties. Usually surfactants are organic compounds, and they are also referred to as wetting agents, because they have the ability to lower the surface tension of liquids. Surfactant molecules are made up of two parts with different affinities for the solvent. It is made up of hydrophobic portion, usually a hydrocarbon chain attached to a hydrophilic functional group. They have the ability to solubilize one phase into another by intervening in between the two immiscible phases. A little quantity of the surfactant molecule rests upon the water-air-interface and this result in decrease in water surface tension (this prevents the water/oil droplets from coalescence and keeps microemulsion stable).

Variations are possible in the head and the tail groups of surfactants. The variation in the molecular structure of the surfactant allows variation in their interfacial properties.

Surfactants are mostly categorized according to the charge they carry on their hydrophilic molecules after dissociation in aqueous solution.[71,72] Hence names such as:

- Anionic surfactants: Anionic surfactants form a negative charge on their hydrophilic head upon dissociation in aqueous medium and a positive counter ion. They are good foaming agents and therefore have found applications in detergent industry. They are the most widely used surfactants on accounts of cost, performance and reduced toxicity. Examples include alkyl sulphates. [73]

Shown below is an example of a surfactant structure, a simple molecule of anionic surfactant (SDS):



SODIUM DODECYLSULPHATE

- Cationic surfactants: Cationic surfactants form a positive charge on the hydrophilic head group upon dissociation in aqueous medium and a negative counter ion. Examples are quaternary amines[74], Fatty amine salts and cetyltrimethylammonium chloride. These ammonium salts of cationic surfactants can be sensitive to high pH levels where they might be deprotonated. Quaternary ammonium salts are more stable. Cationic surfactants include two main groups, i.e. betaines[75] and the real amphoteric surfactants based on fatty alkyl imidazolines. There is some limit in use of cationic surfactants due to their toxicity.

- Nonionic surfactants: Nonionic surfactants have no charge on the hydrophilic head. The hydrophilic group is made of some other very water-soluble moiety, (e.g. a short, water-soluble polymer chain) rather than a charged species. Traditionally, nonionic surfactants have used a poly(ethylene oxide) polymer as the hydrophilic group. Poly(ethylene oxide) polymers used in nonionic surfactants are typically 10-100units long.[76,77] They are usually mild and good wetting agents and solubilisers. The characteristics of surfactants are largely dependent on their head groups. Examples include alkyl polyglycosides, whose hydrophilic head is a sugar moiety. These sugars are just polysaccharides, but can be made from disaccharides, trisaccharides and various other sugars.
- Amphoteric surfactants: Amphoteric surfactants contain both negative and positive charges on their hydrophilic head groups. The amphoteric surfactants have both acid and alkaline properties, which makes them to be stable over a wide pH ranges. The key functional groups in the chemical structures of the amphoteric surfactants are the more or less quaternized nitrogen and the carboxylic groups.[74]

In addition to their distinction in terms of charge, surfactants are often named in association with their technological applications; hence names such as detergents, wetting agents, emulsifiers and dispersants arise. Surfactants are also identified by their HLB (high lipophilic balance) numbers.

- High lipophilic balance: high lipophilic balance (HLB) number is a parameter that has been assigned to each surfactant molecule. It has been proven to be a useful tool when one is to design a microemulsion system. HLB number indicates the solubility of a surfactant in a microemulsion. High lipophilic balance (HLB) number has a range of 0-20 for nonionic surfactant.[74] High number represents a more water soluble (hydrophilic) surfactant while a lower number represents a more oil soluble (hydrophobic) molecule.

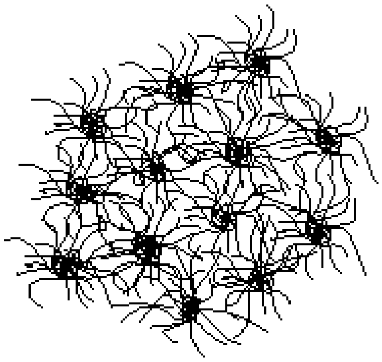
1.6.2 SURFACTANT MIXTURES

The use of surfactant mixtures often give rise to enhanced performance over their individual components, and so surfactant blends are employed in a wide variety of practical applications.[76] The use of mixtures of nonionic surfactant is essential because the benefit of a cosurfactant such as short chain alcohol is not always available. These alcohols are not considered as edible and may be irritating to the skin, therefore their application is limited. In order for one to understand the molecular origins of surfactant ‘synergism’ it is necessary to measure directly the interfacial composition at different types of interface. Particularly air/water, oil/water and liquid/solid. Neutron reflectance and X-ray scattering are used to study the surfactant mixtures at an air/water interface. Mixtures of oppositely charged surfactant (cationic and anionic mixture) exhibit novel solution and interfacial properties.[78,79,80] The interplay between electrostatic effects and surfactant molecular geometry allows a rich diversity of phase behaviour. A phase-behaviour and microstructures of several mixtures of cationic and anionic surfactants have been explored.[78]

Surfactant mixtures are often used in commercial products because they improve product performance. Also using mixture of surfactant can provide different types of performance in a single product. The physical properties of surfactant such as critical micelle concentration and wettability of surfaces can be notably changed after mixing. Micelles are formed by the addition of surfactant monomers beyond the point of saturation and this affects the performance of microemulsions either positively or negatively.

1.6.3 MICELLES

Micelles are aggregates of surfactant monomers that are formed in microemulsions when they are saturated with a surfactant. Aggregate is a fairly general term which describes a particle made up of one or several types of these subunits. They are also formed generally by amphiphilic molecules in aqeuous environment. The points at which micelles are formed in an emulsion are often referred to as critical micelle concentration. Beyond the critical micelle concentration any surfactant monomer added to the aqueous solution do not add to the solution but contributes further to the formation of micelles.[74] The following structure shows an example of micelle, where the dark portions represent coaggregation of the polar head groups while the tails point out. Micelles are of various shapes and sizes, and amongst such shapes are cylindrical, and lamellar.



AGGREGATES OF SURFACTANT MONOMERS

The monomers in the micelle are arranged in such a way that their polar heads point outside the centre while their non polar tails point inside.

1.6.4. EMULSIONS

Emulsion refers to the dispersion of two systems/phases into each other, which are normally immiscible (such as oil and water).[81] Their composition is similar to that of microemulsion in the sense that they are both composed of two immiscible liquids. Note that an emulsion does not contain a cosurfactant. Such dispersions are usually milky, hence the term emulsion was adopted. Like microemulsion, whether one forms an oil-in-water or water-in oil depends on the proportion of the two phases. Emulsions are usually unstable and they are stabilised by the addition of surfactants, which are normally referred to as emulsifiers. They have a limited period of stability. Both types of emulsion (Water-in-oil and oil-in-water) are miscible with oil and water, respectively. This is one of the simple methods for testing whether the emulsion is w/o or o/w.

To form an emulsion one needs mechanical energy in the form of vigorous mixing. The success of an emulsifying process depends in large measure on the interfacial tension between the two liquids and this is achieved by addition of an appropriate emulsifying agent. Examples of emulsion include butter, margarine, mayonnaise etc.

The formation of emulsion, which involves an increase in interfacial areas between the two phases is accompanied by an increase in free energy. The ease of formation of emulsions can be measured by the amount of work needed for its formation. The lower the interfacial tension the less work is needed and the more readily the emulsion is formed.

1.6.5 DOUBLE EMULSIONS

Double emulsions are colloidal systems that are formed by the dispersion of one phase in another which are normally immiscible. They are the same as other colloidal systems such as emulsion and microemulsion in the sense that they also consist of two phases. The only difference is in the formulation. They can either be oil-in-water-in-oil (o/w/o)[81] (with dispersed aqueous globules containing smaller dispersed oil droplets) or water-in-oil-in water (w/o/w) (with dispersed oil globules, themselves containing smaller aqueous droplets).

To formulate a double emulsion it is necessary to choose, at least two surfactants, one low in HLB number and the second with a high HLB number.[81] The two surfactants used are of different solubility. Preparation of o/w/o emulsion involves a low-HLB (<10) surfactant, which is first dissolved in oil, followed by the addition of water. This leads to the formation of water-in-oil (w/o) emulsion. Then water-in-oil (w/o) system is further emulsified in aqueous solution of a surfactant with high HLB number (>10) to produce a w/o/w. The opposite applies for o/w/o emulsion system. Double emulsions are important in the pharmaceutical, food and cosmetic industries, as they are able to encapsulate both hydrophilic and hydrophobic molecules. Their importance lies in the fact that they release the encapsulated molecules slowly from their internal droplet when administered in vitro due to their double compartment structure.[77]

1.6.6 COLLOIDS

Colloids are formed by dispersion of one component to another. Their particle size ranges from several nanometers(nm) to several millimeters(mm). Colloids bridge the gap between solutions and suspensions.[80] In colloidal systems the particles are dispersed without appreciable bonding to solvent molecules, and they do not settle out on standing. As compared to solutions, colloids may exist in any of the three states of matter. Amongst other properties of colloids include;

- Optical effect
- Motion effect
- Electrical charge effect, and
- Adsorption effect. [80,82]

They consist of a dispersed phase distributed uniformly in a finely divided state in a dispersion medium. Examples of colloidal systems include the following; mists, smoke, milk and mud.

1.6.7 USE OF MICROEMULSIONS AND SURFACTANTS

Surfactants find numerous applications in the chemical process industries such as foods, pharmaceuticals, household products, agricultural chemicals and mineral processing industries. The natural occurring surfactants in plants, animals and humans have important biological roles on physiological functions. With the knowledge that a cell contains about ninety six percent water, there is a view that it also has some dissolved oil amongst other components, which would then make the cell cytoplasm an oil-in-water (o/w) type microemulsion. The biological processes function perfectly in these systems. The widespread applications of surfactants and microemulsions originate from the duality of their molecular characteristics and compositions. They are composed of polar and non polar molecules.

Cationic surfactants are mostly used as conditioners and softeners. Their application in food industries is limited due to their inherent toxicities. Nonionic surfactants are predominantly used in foods and drinks and skin-care products. They are also used as solubility enhancers in pharmaceutical[83] formulations. Aqueous formulations of drugs often require the use of solubility enhancers that increase the concentration of the active substances to therapeutically acceptable levels.[83,84] Anionic surfactants are good foaming agents, therefore they are mainly used in soap industries as shampoos, dishwashing detergents and in washing powders. Commercially, they are mostly used in conjunction with nonionic surfactants so as to provide further stability.

Micelles have a variety of applications (which often depend on the molecules from which they are formed), such as physical models for more complicated biological systems, prototype drug delivery systems or encapsulants for other hydrophobic molecules, or constituents of larger aggregates.

Microemulsions are normally referred to as microreactors on the view that reactions occur inside the microdroplet with orientation of reactants in such a way that they meet each other for the reaction to take place, hence are employed in a number of chemical processes[85]. The main highlights of microemulsions are;

- The droplets in microemulsion are continuously diffusing through the continuous phase, leading to collision between droplets,
- The collisions are elastic; they are often called sticky collisions because they result in the coalescence of droplets and exchange of material between droplets before they break apart,
- For a reaction in water-in-oil microemulsion involving reactant species totally confined within the dispersed water droplets, a necessary step prior to their chemical reaction is the transfer of reactants into the same droplet.

- When the chemical reaction is faster(close to being diffusion-controlled), the overall reaction rate is likely to be controlled by the rate of interdroplet transfer of the reacting species or solubilization exchange. Therefore the rate of the collision process of droplets in the microemulsion and the mechanism of transfer of reactant between two droplets (coalescence of droplets) are of significance to the reaction kinetics,

- Reactions on the nanosecond time scale droplets are essentially isolated, and no exchange occurs between droplets.

An attempt has been made [6,86-88] to employ microemulsion system as a decontaminating solutions for organophosphate ester hydrolysis. Synergistic effects of metal ions in microemulsion have been found to give a promising result in decontamination endeavours.

1.7 AIM OF THE STUDY

African countries, who are predominantly rural, are often plagued by acute shortage of foods and widely spread diseases resulting from malnutrition. The pressure for increased food production has led to the widespread use of organophosphorus compounds such as fertilizers, herbicides and insecticides which have overloaded the ecosystem. Most organophosphate compounds are persistent chemicals which contribute to the long term pollution problems in the biosphere. Pollution is defined as the excess of substances in the environment beyond acceptable levels. These health and environmental issues require solutions that are appropriate to the environmental, economic and social conditions of these countries. Tropical and sub-tropical climates and the surroundings create conditions for more severe organophosphate pollution than found in industrialized countries in the north where most of the technology for remedition is being developed. Our research undertaking takes the above scenario into consideration and attempts to contribute positively to the safe disposal; and management of organophosphates in our environment. The main aims are:

- To understand the hydrolysis of phosphate esters by using 4-nitrophenyl phosphate as a model for organophosphates.
- To mimic the effect of metal ions in kinases and phosphatases and get an insight into their reaction mechanisms.
- To study the effect of different microemulsion systems as effective decontamination agents
- To investigate the synergistic effects of iron in ferrous and ferric forms with microemulsion to bring about effective hydrolysis in phosphate esters
- To formulate decontamination solutions which are fast, cheap and effective under ambient conditions. The ultimate aim being to contribute to decontamination of the environment from the undesirable products of organophosphates.

1.7.1 THE CHOICE OF IRON AS MODEL SYSTEM

Iron was chosen as our model because of its ubiquitous nature and its involvement in similar enzymic systems and model studies.[89,90] Iron is one of the essential micronutrients that takes part in many biological processes. An average human being contains about 3-4g of iron depending on the body volume. This iron is mainly bound to oxygen transporting haemoglobin in the red blood corpuscles; in protein myoglobin.[50] Iron atom binds reversibly to an oxygen molecule and remains in the ferrous (Fe(II)) state in oxygenated form. Iron is also a component of the *cytochrome c* oxidase, which is a large multi-component membrane-bound protein located in the inner membrane of the mitochondrion and of many other bacteria.[91] This cytochrome structure comprises two iron atoms containing heme moieties, two copper centers and additional metal binding sites for Zn and Mg. Catalase and the peroxidase enzymes also contain iron as a cofactor. The enzymes contain active centers, which closely resembles those found in haemoglobin and myoglobin. In these enzymes, the iron is a high-spin Fe(III). Iron plays an important role in photosynthesis(I) and photosynthesis(II).[91] Various iron containing enzymes are also known.

Prussian blue is a mixed-valence cyanide-bridged cubic network containing a low spin Fe(II) surrounded by carbon and a high spin Fe(III) linked to the nitrogen atoms of the cyanide bridge.[60,92] Prussian blue and Turnbull's blues are all identical, they are just ferric ferrocyanides. Prussian blue consists of two forms, soluble and insoluble form. In reality all Prussian blue complexes are insoluble. The Prussian blue referred to as soluble is itself insoluble, but is able to form fine suspensions in water which makes it appear like a true solution. There exists a whole range of such iron blues having compositions depending on the method of preparation at molecular level. They all have in common a characteristic cubic structure, but this lattice can accommodate variable amounts of water and metal ions within it. The formulae range from $K[Fe(CN)_6].5H_2O$ (the so called 'soluble' Prussian blue) to $Fe_4[Fe(CN)_6]_3.15H_2O$ (the so called 'insoluble' Prussian blue).

The solid structure of the insoluble Prussian blue corresponds to a structure having a hexacoordinate low spin Fe(II) bonded through the nitrogen atoms of the cyanide. To achieve this stoichiometry one fourth of the Fe(II) sites are occupied by water molecules. The structure consists of bridging cyanide groups [Fe(II)-CN-Fe(III)] which resembles the reduced (active) forms of the purple acid phosphatases. The enhanced hydrolysis achieved by Prussian blue seems logical as the complex satisfies the requirement of two metal centers: The structural metal center and the active metal center like most enzymes.

In biological systems, Iron is an essential component of the purple acid phosphatases (PAPs), which are dinuclear iron enzymes that catalyze in vivo hydrolysis of phosphate esters under weakly acidic (or neutral) conditions.[41] The presence of binuclear centers have also been reported[53] in aminopeptidase enzyme extracted from *Escherichia coli*, which is one of the proline specific enzyme that catalyzes the hydrolysis of peptide bonds. The enzyme contains two divalent Mn^{+2} ions, which play a vital role in catalytic activity. In this enzyme the Mn^{+2} ions can also be replaced by other divalent metal ions such as Co^{+2}, Zn^{+2}, and Ca^{+2} without much loss in its activity. This also depends on the substrate to be catalyzed as enzymes are specific by nature. Amino peptidase was also found to have some degree of activity for the hydrolysis of a wide range of organophosphorus compounds.

The mamalian purple acid phosphotase (PAP) contain two iron ions while the plant PAP from kidney bean (kbPAP) have one iron and one zinc. The two PAPs have similar properties. The mamalian PAP enzyme exists in the reduced form (active); Fe(III)-OH-Fe(II), and oxidized form (Inactive); Fe(III)-OH-Fe(III). Iron(II) of the reduced PAP is labile and can be substituted by other divalent metal ions, in particular Zn(II), retaining catalytic activity. The mechanism for the operation of this enzyme involve the the binding of phosphate ester on the labile Fe(II) and the attack by the hydroxo ligand of the Fe(II). In conclusion iron is involved in a number of biological and environmental processes as it is also found in many soils.

CHAPTER 2

2. EXPERIMENTAL SECTION

2.1 INSTRUMENTS AND REAGENTS

All reagents used were either analytical reagent grade or the purest available commercially and were used without further purification. pH was measured with a Metrohm 632-pH meter equiped with a Metrohm combination electrode. The pH electrode was conditioned prior to use in a pH range 4 to 9. A thermo spectronic Genesys 2 uv/vis spectrometer was used to obtain spectra for interference comparison of the reacting species and a Genesys 10 uv/vis spectrometer to collect rate data.

The pH of the reaction mixture was adjusted by adding drops of NaOH or $HClO_4$ using a glass rod, a simple method referred to as glass stick dotting, i.e. dipping the tip of a glass rod which has been immersed in perchloric acid or in sodium hydroxide according to pH changes desired. The temperature of the reaction was controlled using a thermostated water bath, which allows the water to circulate via a Jackected reaction vessel.

Microemulsions were prepared by mixing hexane, the surfactants (SDS or CTAC), 1-butanol and water. The following compositions by mass were used for the cationic and anionic o/w microemulsions respectively, SDS or CTAC 5% / Hexane 3% / 1-butanol 10% /water 82%. Microemulsions used were prepared using the available literature[72] methods of mixing the chosen surfactant, hexane and 1-butanol and titrating with a small amount of water. A white paste, which was formed initially, was further titrated with vigorous stirring causing the white paste to become clear.

All the metal ion solutions used in the reactions were prepared and used in the same day as some of the iron solutions, particularly Fe(II) which is oxidized upon standing. For those compounds that were water insoluble, the amount used was calculated from the moles that were to be used in a certain volume of the solution. An investigation of all the reagents used in the experiment showed little or no intereference in the absorbance region of interest. The absorbance detected were utilized to determine the net absorbance of the reaction aliqouts. The absorbance of 4-nitrophenol($1x10^{-3}M$ after mixing) at 400nm was taken as the value for 100% dissociation of 4-nitrophenyl phosphate under the same set of experimental conditions. The reagents were prepared in such a way that the final solution contain $1x10^{-3}M$ 4-nitrophenyl phosphate after mixing.

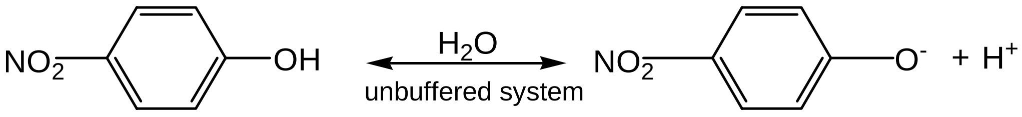
The experimental set up of the apparatus used in the investigation is depicted in the following diagram.

Figure 2.1: EXPERIMENTAL SETUP

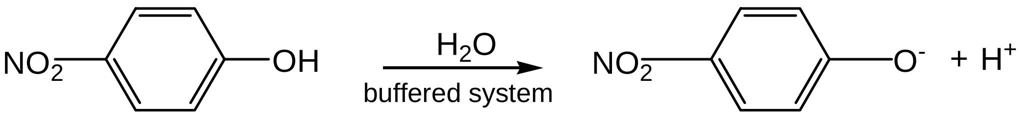
2.2 PROTOCOL OF THE STUDY

The protocol for the study involved mixing of 8 ml (5 x 10 $^{-3}$M) solution of 4-nitrophenyl Phosphate(4-NPP) in a thermostated reaction vessel with 4ml of $NaClO_4$ (1M) and 20 ml of the solvent. The solvent was either water or one of the microemulsions. The pH of the solution was adjusted to 6.5. An 8 ml volume of temperature equilibrated metal ion solution (5 x 10 $^{-3}$ M), was then added, making the total volume to 40 ml. Reaction aliquots (2 ml) were withdrawn from the reaction vessel at 1, 3, 5, 10, 15, 30, and 40 minutes, respectively, and mixed with 2 ml of phosphate buffer (pH 7.4). The buffer system was prepared by mixing 8.62 grams of sodium phosphate dibasic and 5.42 grams of sodium phosphate monobasic salts and making up to 1000 ml with carbon dioxide free, distilled water.

The absorbance of the reaction solution was then measured at 400 nm after mixing. The percentage hydrolysis was calculated from the net absorbance values of the aliquots over the absorbance value of the same concentration of 4-nitrophenol assuming 100% hydrolysis. In an unbuffered solution the acid–base reaction of 4-nitrophenol changes in concentration resulting in a shift in the equilibrium position as shown below.



In a buffered solution, the pH would be fixed and the ratio of the concentration of *p*-nitrophenolate to the 4-nitrophenol would be constant thereby obeying Beer’s law.



All absorbances were taken against a water blank. Interference from other compounds in the analyte was not detected. Under slightly basic condition the 4-nitrophenol is deprotonated to form the 4-nitrophenolate ion which is bright yellow and has an absorbance maximum at 400 nm, quite distinct from 4-nitrophenol which has an absorbance maximum at 310 nm. The molar extinction coefficients were determined from the calibration curve studies with the standard 4-nitrophenol solutions as 3.67×10^{3} M^{-1}.

The system conformed to Beer's law over the concentration range 0–20 mg l^{-1} of 4-nitrophenol when measured at 400 nm in a 1 cm cell.[5,6]

The hydrolysis on 4-NPP was calculated from the net absorbance measurements of the aliquots at different times. An example is given below to illustrate the protocol.

Net absorbance = Absorbance of reaction aliquot - Absorbance of metal ion

%Hydrolysis = (Net absorbance/4-NP absorbance) x 100

From table 1, the absorbance (A) for Fe(II) at 40 minutes in cationic microemulsion medium was calculated as follows;

1st experiment	+	2nd experiment	+	3rd experiment
0.507		0.507		0.509

Average absorbance = 0.508

Absorbance of blank $FeSO_4.7H_2O$ was found to be 0.165A. Therefore, net absorbance(A) is;

= 0.508 - 0.165

= 0.343

Absorbance of blank 4-nitrophenol is equal to 3.696A,

% Hydrolysis = (0.343A/3.696A) x 100

= 9.28%

Alternatively, one may use the molar extinction coefficient to determine the percentage hydrolysis. The molar extinction coefficient

$\varepsilon = 3.67\text{x } 10^3$

$A = 0.343$

Using Beer's Law : $A = \varepsilon c l$, where, l is the curvette path length, c is the concentration and ε the extinction coefficient.

$C = A/\varepsilon$

$= 0.343/(3.67\text{x}10^3)$

$= 0.000092828$

% Hydrolysis $= (0.000092828/1\text{x}10^{-3}) \text{ x } 100$

$= 9.28\%$

CHAPTER 3

3. RESULTS AND DISCUSSION

3.1 RESULTS

The results of the investigation are provided in the following tables and are the average values for triplicate analysis of each system at a given temperature.

Table 3.1: HYDROLYSIS OF 4-NPP[a] BY IRON IN AQUEOUS AND MICROEMULSION MEDIA AT VARIOUS TEMPERATURES.

Sytem[b]	% 4-Nitrophenol produced[c]									
	Temp. in ^{O}C	Media	1min	3min	5min	10min	15min	20min	30min	40min
4-NPP + Fe^{2+}	20	WATER	2.4	2.7	2.8	2.9	3.0	3.2	3.5	3.9
		AME2	3.2	3.4	3.5	3.7	3.8	4.2	4.8	5.1
		CME2	5.8	6.1	6.3	6.6	6.8	7.2	7.4	7.6
	30	WATER	3.5	3.6	3.7	3.7	4.0	4.2	4.5	4.8
		AME2	4.2	4.4	4.5	4.6	4.9	5.3	5.7	6.4
		CME2	7.9	8.5	8.8	8.9	9.1	9.2	9.2	9.3
	40	WATER	4.9	5.0	5.2	5.4	5.5	5.9	6.1	6.3
		AME2	5.8	6.2	6.1	6.3	6.9	7.1	7.3	7.5
		CME2	9.4	9.7	9.9	10.4	10.8	11.6	11.9	12.1
	50	WATER	7.0	7.9	8.1	8.3	8.6	9.0	9.2	10.1
		AME2	6.9	7.3	7.5	7.7	8.1	8.4	8.6	8.7
		CME2	13.4	13.9	14.5	14.9	15.7	15.9	16.0	16.2
4-NPP + 2 Fe^{2+}	20	WATER	2.8	3.2	3.3	3.5	3.6	3.8	4.2	4.6
		AME2	2.2	2.4	2.4	2.6	2.7	2.9	3.2	3.4
		CME2	7.7	8.8	9.0	9.4	9.6	9.8	10.1	10.2
	30	WATER	5.4	5.6	5.7	5.8	5.9	6.0	6.2	6.4
		AME2	4.2	4.3	4.5	4.5	4.6	4.7	4.7	4.7
		CME2	11.4	11.5	11.6	11.6	11.8	11.8	11.9	12.1
	40	WATER	8.6	8.9	9.0	9.1	9.3	9.4	9.6	9.6
		AME2	6.5	6.6	6.6	6.7	6.8	6.6	6.8	6.8
		CME2	16.2	16.4	16.5	16.7	16.8	17.0	17.1	17.2
	50	WATER	10.5	10.7	10.9	11.1	11.4	11.5	11.6	11.6
		AME2	7.8	8.1	8.4	8.5	8.6	8.7	8. 8	8.9
		CME2	18.1	18.6	18.9	19.2	19.5	19.6	19.7	19.7

System	% 4-Nitrophenol produced									
	Temp. In ^{O}C	Media	1min	3min	5min	10min	15min	20min	30min	40min
4-NPP + Fe^{3+}	20	WATER	5.7	5.8	5.8	5.9	6.0	6.1	6.1	6.2
		AME2	6.2	6.6	6.7	6.7	6.9	6.9	7.2	7.2
		CME2	9.7	9.9	10.2	10.4	10.8	10.7	10.9	10.9
	30	WATER	11.2	11.4	11.5	11.7	11.7	11.9	12.0	12.0
		AME2	12.8	13.1	13.4	13.6	13.8	13.7	13.9	13.9
		CME2	14.5	15.0	15.4	15.9	16.0	16.0	16.2	16.2
	40	WATER	11.8	12.0	12.2	12.4	12.4	12.5	12.7	12.8
		AME2	14.2	14.5	14.7	15.0	15.2	15.3	15.5	15.5
		CME2	15.7	16.1	16.4	16.6	16.8	17.0	17.0	17.1
	50	WATER	13.6	13.9	14.0	14.0	14.1	14.2	14.2	14.2
		AME2	16.1	16.7	16.7	17.0	17.0	17.1	17.1	17.2
		CME2	17.5	17.8	17.8	18.1	18.3	18.5	18.5	18.6
4-NPP + 2 Fe^{3+}	20	WATER	7.7	7.9	7.9	8.1	8.2	8.2	8.4	8.4
		AME2	9.8	10.1	10.3	10.3	10.5	10.6	10.7	10.7
		CME2	11.1	11.7	11.8	11.9	12.3	12.5	12.9	13.1
	30	WATER	14.4	14.7	15.1	15.3	15.4	15.7	15.8	15.8
		AME2	16.3	16.8	17.0	17.2	17.4	17.5	17.5	17.5
		CME2	18.3	18.5	18.6	18.6	18.6	18.7	18.7	18.8
	40	WATER	15.2	15.7	15.9	16.0	16.1	16.1	16.3	16.4
		AME2	17.6	17.8	18.2	18.3	18.5	18.5	18.8	18.7
		CME2	20.9	21.2	21.3	21.5	21.5	21.7	21.7	21.9
	50	WATER	16.4	16.8	17.1	17.2	17.2	17.3	17.4	17.6
		AME2	18.2	18.3	18.3	18.4	18.5	18.8	19.0	19.2
		CME2	24.1	24.4	24.5	24.7	24.7	24.9	24.9	25.1

System	% 4-Nitrophenol produced									
	Temp. In ^{O}C	Media	1min	3min	5min	10min	15min	20min	30min	40min
4-NPP + Turnbull's blue (sol.)	20	WATER	8.0	8.2	8.3	8.4	8.6	8.7	8.8	8.9
		AME2	4.6	4.7	4.8	4.8	5.0	5.0	5.1	5.1
		CME2	13.2	13.4	13.4	13.5	13.6	13.6	13.6	13.6
	30	WATER	13.0	13.2	13.3	13.4	13.7	13.9	13.9	14.1
		AME2	6.8	6.9	7.0	7.2	7.3	7.3	7.3	7.3
		CME2	19.2	19.4	19.4	19.6	19.7	19.9	19.9	19.9
	40	WATER	15.4	15.9	16.1	16.2	16.4	16.6	16.6	16.6
		AME2	8.0	8.2	8.3	8.3	8.5	8.6	8.6	8.6
		CME2	21.5	21.7	21.7	21.8	22.1	22.1	22.1	22.2
	50	WATER	17.2	17.7	17.8	18.0	18.6	18.6	18.6	18.8
		AME2	9.4	9.5	9.5	9.7	9.8	9.8	9.8	9.9
		CME2	26.0	26.3	26.4	26.5	26.7	26.7	26.7	26.8
4-NPP + Prussian blue (insol.)	20	WATER	10.3	10.8	11.0	11.2	11.5	11.7	11.8	12.0
		AME2	13.9	14.3	14.4	14.4	14.8	14.9	15.1	15.3
		CME2	18.0	18.3	18.3	18.5	18.5	18.7	18.7	18.7
	30	WATER	22.6	23.1	23.2	23.5	23.6	23.9	24.0	24.3
		AME2	25.6	25.8	26.0	26.1	26.4	26.5	26.7	26.9
		CME2	28.5	28.7	28.8	29.0	29.1	29.1	29.2	29.2
	40	WATER	26.3	26.7	26.7	26.8	27.0	27.2	27.3	27.5
		AME2	30.0	30.3	30.3	30.5	30.7	30.8	31.0	31.6
		CME2	36.1	36.3	36.4	36.5	36.6	36.7	36.8	36.8
	50	WATER	27.3	27.8	28.2	28.2	28.4	28.5	28.6	28.7
		AME2	29.8	30.0	30.2	30.4	30.5	30.9	31.2	31.4
		CME2	39.2	39.4	39.5	39.6	39.7	39.8	41.0	41.1

a. The degradation of 4-nitrophenyl phosphate in the absence of metal ions using the same protocol was negligible. The experimental conditions for aqueous and anionic microemulsion systems were:

$C = 10^{-3}$ molar, I = 1M $NaClO_4$, Initial pH 6.5

b. AME = Anionic microemulsion (82% water, 3% hexane, 5%SDS, 10% 1-butanol

CME = Cationic microemulsion(82% water, 3%hexane, 5% cetyltrimethylammonium chloride, 10% 1-butanol)

The experiments with Fe(II) were conducted using Ferrous ammonium sulfate (Mohr's salt) or ferrous sulfate as the source of ferrous ions. The results of those investigations agreed well within experimental errors. Similarly Ferric ammonium sulfate or Ferric chloride were utilised as a source for ferric ions. The results of both analysis were comparable.

c. Triplicate analysis was done for each system at a given temperature. The values given in the table are average values of those analyses. The standard deviation for the triplicate analysis ranged from 0.1 to 0.2 in each case.

Production of 4-nitrophenolate ion appears to be biphasic. There is an initial burst in one minute which levels off dramatically between the second minute and the fourtieth minute. If one just looks at the one minute data, it is clear that there is roughly a 2-fold increase in 4-nitrophenolate production with cationic micremulsion compared to what is seen in water. The investigation was limited to 40 minutes reaction time as the amount of additional hydrolysis product decreased markedly after 30 minutes of reaction time.

A plot for the average hydrolysis of 4-nitrophenyl phosphate in various reaction media for some of the systems is given in figure 3.1.

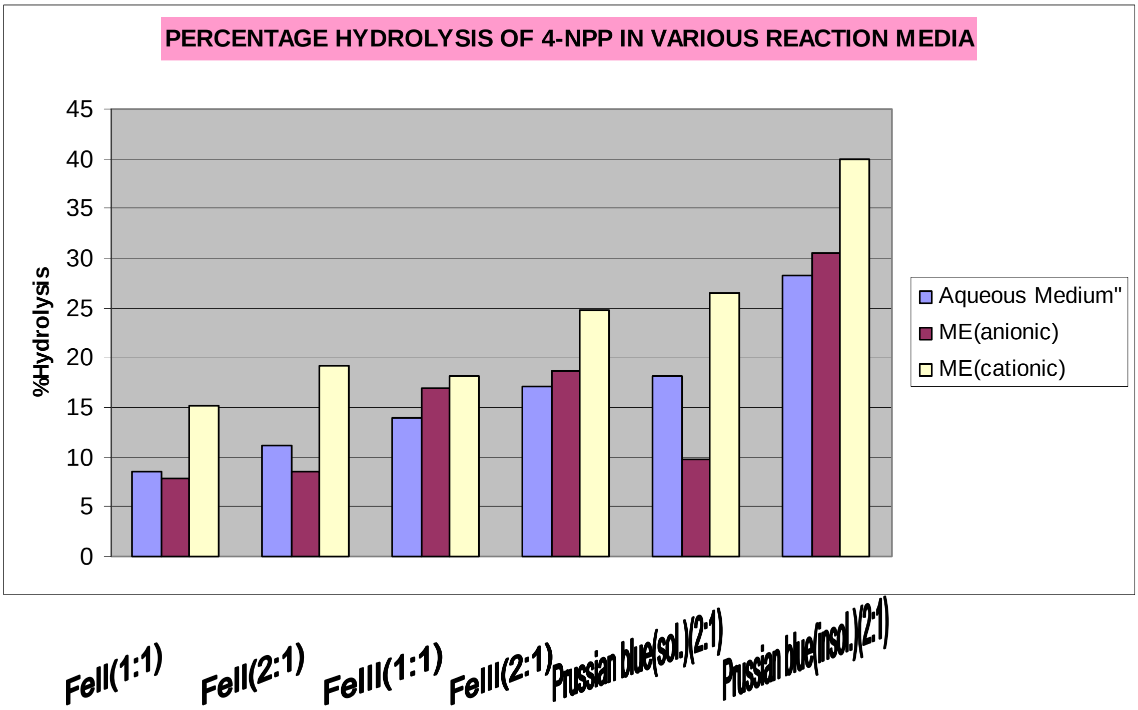
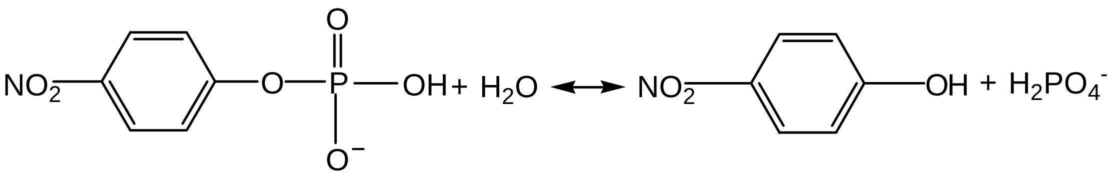


Figure 3.1: PERCENTAGE HYDROLYSIS OF 4-NPP IN VARIOUS REACTION MEDIA

3.2 DISCUSSION

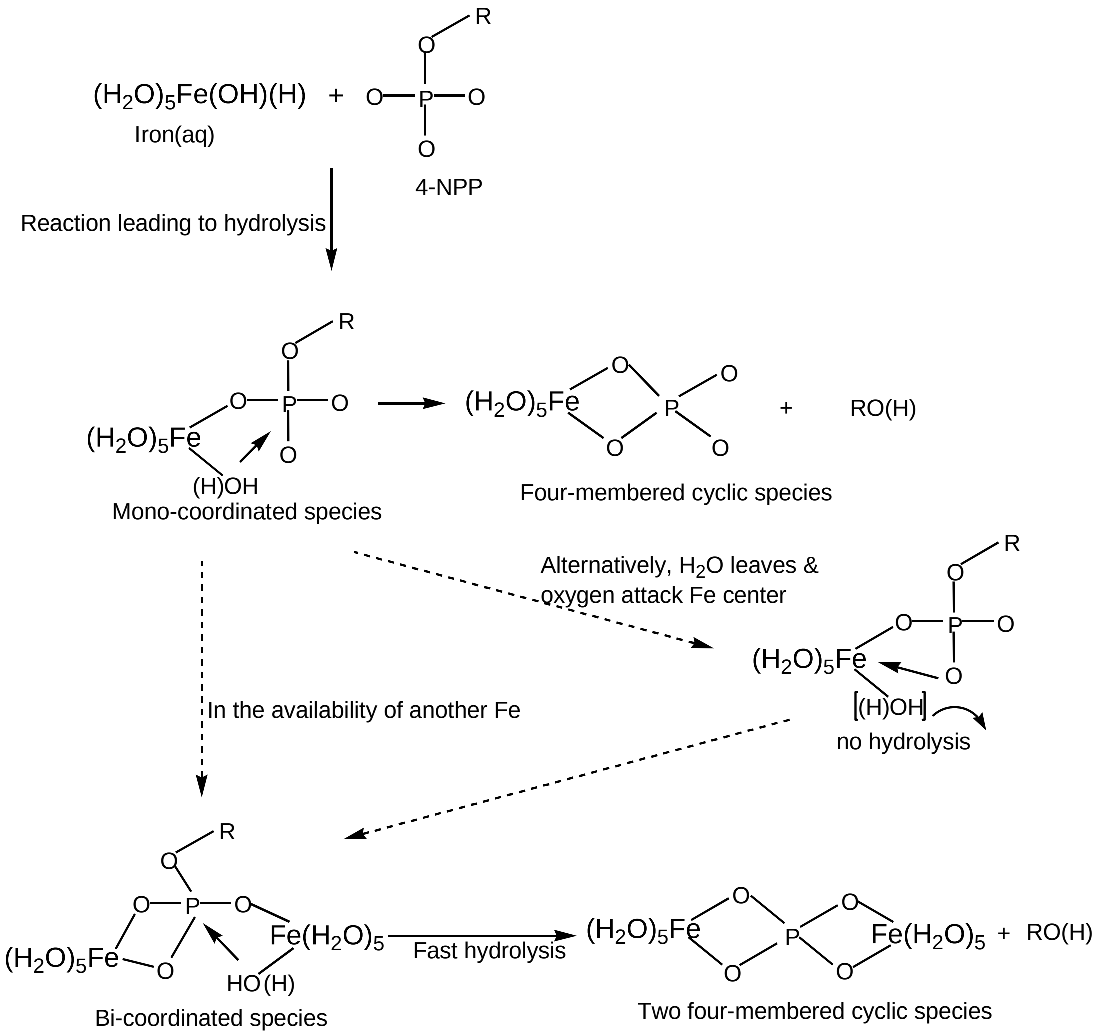
The overall hydrolysis of 4-nitrophenyl phosphate around neutral pH can be described by the following reaction equation.



The rate of the reaction in the pH range from 4 to 9 is both essentially constant and very slow.[93] Hydrolytic reaction is effectively enhanced by the presence of metal ions.

Some observation, which could be drawn for the different systems examined in the present investigations are:

1. Mixing the solution of a metal ion and 4-nitrophenyl phosphate at 1:1 molar ratio results in the formation of a monocoordinated complex by metal coordination to one of the anionic oxygens of 4-nitrophenyl phosphate. The mechanism follows a Lewis acid activation, which is operative in hydrolysis of phosphates. Once the metal coordinates, the electron deficient oxygen pulls an appreciable amount of electrons from the phosphorus center. This make phophorus vulnerable to the intramolecutar attack by the coordinated water or hydroxide of the metal. Nucleophilic attack of the phosphorus center by the coordinated hydroxide weakens the phosphorus-oxygen bond of the ester linkage resulting in cleavage/hydrolysis. This is a step by step process in the sense that a phosphate ester bond cannot break before the coordination step. The formation of the cyclic intermediate species of the metal ion and the substrate is important as it creates a ring strain on the chelate, thereby aiding the hydrolysis reaction. The four-membered ring chelate systems are generally unstable except for those of Co(III) complexes.[60] This will eventually end up in hydrolysis of the phosphate moiety.[94,95] The ring opened monocoordinated complexes are also present in the solution at the pH of the present study. This has been revealed by the previous studies.[6] Several other coordinated complexes are also envisaged to exist in the solution as depicted in the scheme below.



(Charges mostly omitted; $Fe^{+2/+3}$; R- = 4-nitrophenyl-)

***SCHEME* 3.1: PLAUSIBLE REACTION SCHEME FOR THE HYDROLYSIS OF 4-NPP BY IRON**

2. An enhanced rate of hydrolysis was noted in reaction solutions that contain a 2:1 ratio of metal ion to 4-NPP. This can be explained by the fact that, the first metal coordinates to one of the anionic oxygens of the 4-nitrophenyl phosphate without hydrolysis taking place. Instead of intramolecular attack, the coordinated water on the metal center leaves. Generally it is known that water is a good leaving group. This creates a room

for coordination of the phosphate oxygen to the metal center. The second metal ion available in the solution coordinates to one of the available oxygen anions on the 4-NPP thereby creating a possibility for intramolecular attack on the phosphorous center by cis coordinated water or hydroxide of the metal ion. Coordinated water or hydroxide are not in a conducive arrangement to effect intramolecular attack on the phosphorus center. As a result two ring systems containing four-members are likely to be formed.[34] Some of the above intermediates have been identified[42] by the ^{31}P NMR previous studied on related systems. No significant additional hydrolysis was observed for reaction solutions that contained more than 3:1 metal to 4-NPP ratio.

3. An enhanced hydrolytic rate was noted with increase in temperature, which is in line with earlier reports.[6] This is not suprising as an increase in temperature generally leads to an increase in the rate of most reactions.

4. An increase in the rate of hydrolysis was also noted in microemulsion media compared to aqueous media. Microemulsions are thermodynamically stable transparent dispersions of two immiscible liquids, stabilized by an interfacial film of surfactants.[66] Since they are a mixture of polar and non polar compounds they have the ability to solubilize and disperse the maximum amount of the reactants thereby enhancing the possibility of hydrolysis by the metal centers. Microemulsions can accelerate or dececlerate the rate of chemical reactions in various ways. This depends, amongst other factors on their composition in the terms of surfactants and phase ratio (o/w or w/o) used.

   In a two phase system reaction occurs at the interface. Microemulsions, therefore have the ability to increase the surface area at which the reaction is taking place thereby increasing the rate of the reaction. The microemulsion droplets act as microreactors at molecular levels. In microemulsion if both reactants are soluble in a same phase the reaction takes place in that phase but if they are soluble in different phases then the reaction takes place in a droplet interphase. This is achieved when the surfactant used is able to attract both reactants into the interface.

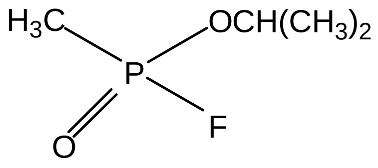
Cationic microemulsions consistently showed good rate accelerations in terms of their hydrolysing abilities towards 4-nitrophenyl phosphate. It is presumable that cationic microemulsion system results in coaggregates where the net cationic character is increased thereby increasing the positive character on the phosphate ester. The coordinated nucleophile (hydroxide or water) will then have better chance of attack on the phosphorous center compared to those in anionic microemulsion coaggregates. Surprising though, were the instances where the aqueous media showed higher rates of hydrolysis compared to anionic microemulsion media for Fe(II) and soluble prussian blue. This might possibly be explained by the solubility difference of the reactants in those media. Overall anionic microemulsion system showed enhanced rates of hydrolysis compared to those in aqueous media.

Microemulsions have been used as chemical microreactors because of their special interfacial properties allowing an intimate contact at nanoscale level of hydrophilic and hydrophobic molecules. The dynamic character of these nanosize-reactors is one of the most important features, which has to be taken into account for a comprehensive understanding of chemical reactions carried out in these media. Microemulsions are dynamic systems whereby continuous movement and collision with each other is occurring. In each collision, material interchange can takes place. It is well known that not all droplets collisions are effective for material exchange, therefore there are also other factors such as temperature, surfactant mixture, HLB number and cosurfactant that plays a significant role in the rate enhancement. These factors have been discussed in detail in the previous sections.

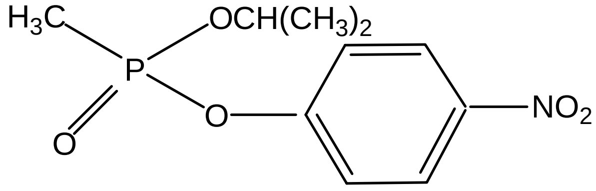
The synergistic effect of microemulsions for the oxidation of half mustard was reported[72] in oil-in-water microemulsion where the compound was reported to be oxidized either in or on the oil droplets. It was observed that the cosurfactant (which was 1-butanol) also assisted in the reaction. Hydrolysis of phosphate triesters, diesters and other phosphorus(V) compounds in microemulsion were reported.[96,97] The effect of microemulsions including those of droplet surfactants on the reactions of the phosphate esters is briefly outlined in the next two paragraphs.

Microemulsions can accelerate the reaction by other means other than supplying a large interfacial area. If the surfactant head group carries a charge as is the case with anionic and cationic surfactants, reagents of opposite charge will be confined to the interior of the droplets of water-in-oil microemulsions. Such a compartmentalisation and concentration of the reagents leads to a rate enhancement. The surfactant monolayer can also accelerate the reaction by attracting reagents of opposite charge situated in the water/oil domain, thus increasing its concentration in the interfacial zone, where the reaction occurs. This type of rate enhancement has been referred to as microemulsion/micelle catalysis in analogy with micellar catalysis for these systems.

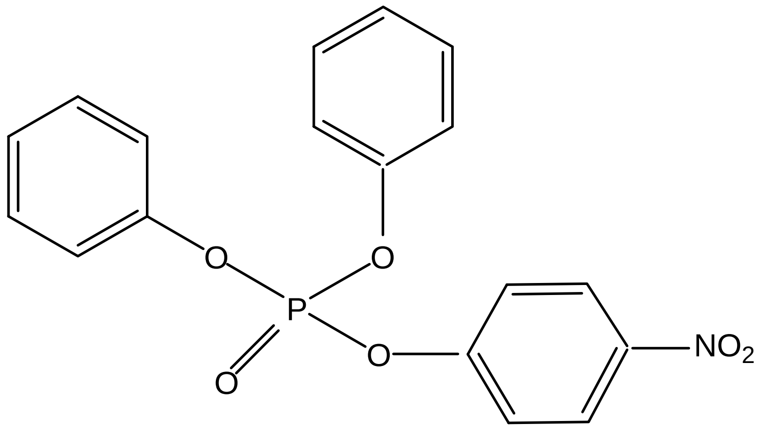
In our investigation microemulsion formulations have been employed for the hydrolysis of organophosphate esters in conjunction with other hydrolysing agents, such as metal ions. The synergism between the hydrolysing species and the reaction media is found to be important for hydrolytic rate enhancements. Several investigators attempted to study the hydrolysis of nerve agent and simulants as have been shown below with the hope of designing effective hydrolysis solution.



sarin (1)



4-nitrophenyl isopropyl methyl phosphate (2)



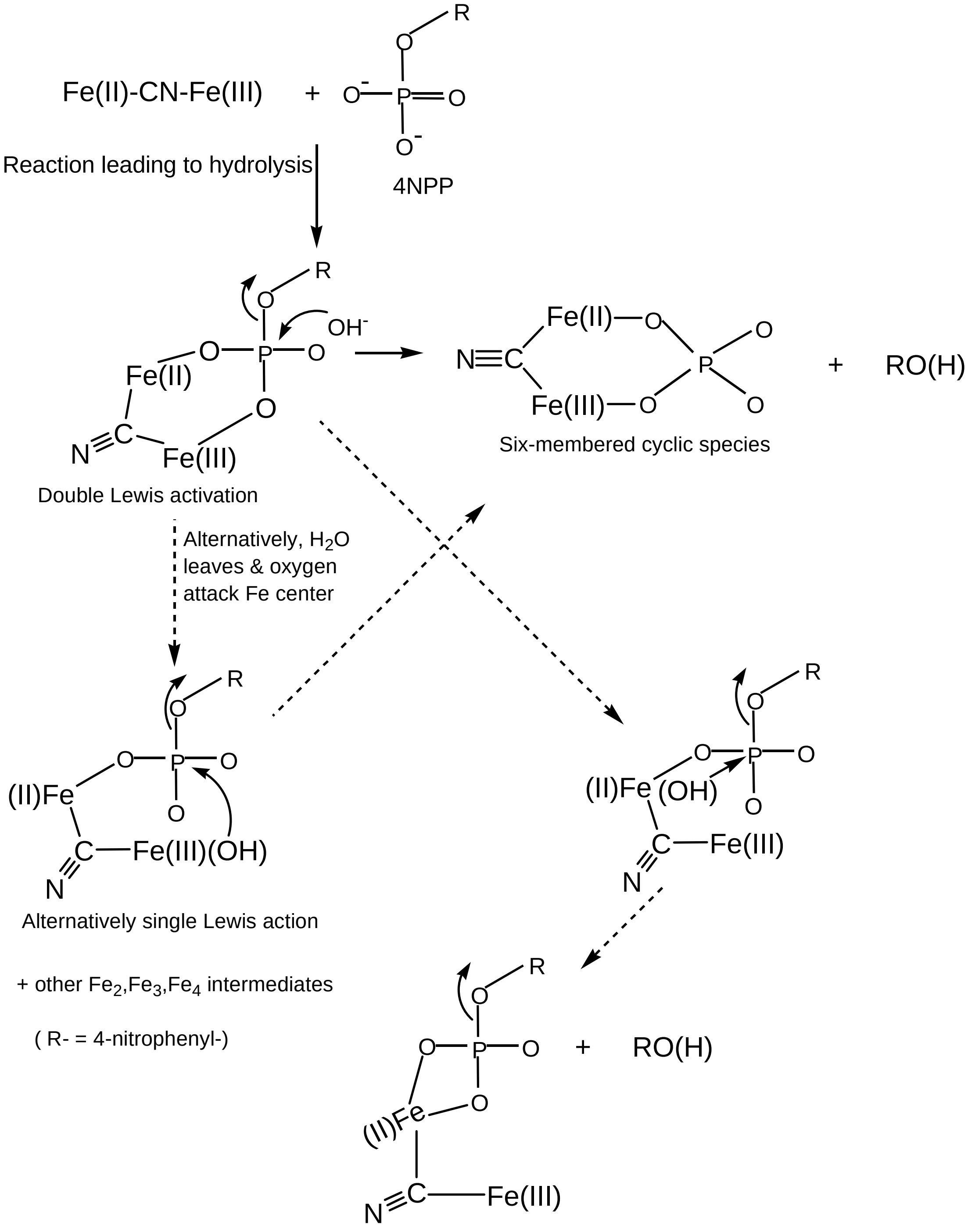
4-nitrophenyl diphenyl phosphate (3)

The hydrolysis of the above compounds (sarin, 4-nitropheny diphenyl phosphate and 4-nitrophenyl isopropylmethyl phosphate) in cationic micelles (Cetyltrimethyl Ammonium Bromide) with the assistance of hypochlorite as the nucleophile has been reported.[97] It was found that all the compounds were 100% hydrolysed in cationic micelles in the presence of hypochlorite, which was the effective hydrolyzing agent. The surfactant was observed to enhance the hydrolytic rate by 20 and 4 times for compounds 3 and 2 respectively. The more enhanced rate for compound 3 was attributed to its good association with the micellar (CTABr). The rates were found to be 1500 and 450 times for 3 and 2 respectively in the presence of hypochlorite suggesting that it is really indeed a good nucleophile. This is because the cationic micelles are capable of bringing the reactants closer together by binding to the substrate hydrophobically and at the same time coulombically[97] attracting the negatively charged nucleophile. Also hypochlorite catalyzed reactions in the presence of bromide might lead to the generation of hypobromide ion by halide exchange. The hypobromide is also an alpha nucleophile that might further contribute towards hydrolysis. In addition, both reactants are soluble in the same phase therefore are in a position to be confined and concentrated[96] in the same compartment. This will enhance the chances of the reactants meeting together for the reaction to take place. The enhancement of the hydrolytic rate in micelles is also brought about by the fact that micelles are capable of enhancing hydrolysis of the substrate even in the absence of a nucleophile. Micelles are closely related systems to microemulsions as they are all liquid dispersions that contain surfactants monomers, therefore their properties are not very different.

The system was also extended to related compounds of high interest such as sarin(1). It was observed that within ten minutes sarin was 100% hydrolyzed in the presence of CTABr and hypochlorite. The hydrolysis of these compounds using cationic micelles is in line with our observations as cationic microemulsion have shown more increased rate of phosphate ester hydrolysis.

5. An increased degree of hydrolysis was noted for the polymetallic complex of Prussian blue compared with the other metal ions studied. It is established in the literature that

the hydrolysis of phosphate ester is facilitated by enzymes containing one or more metal ions. The dinuclear or mononuclear metal site in a complex or enzyme is the active center. In a dinuclear metal site one of the metal ions stabilize the structure of the metal substrate complex while the second metal plays the reactive role. The plausible reaction mechanism operating in a binuclear metal complexes is discussed in the next pages.



SCHEME 3.2: PROPOSED SCHEME FOR THE HYDROLYSIS OF 4-NPP BY PRUSSIAN BLUE

The three possible pathways outlined in reaction scheme 3.2 are discussed below.

- The first path involves a single Lewis acid activation by the labile metal ion(Fe(II)) followed by an attack on the phosphorus center by a coordinated hydroxide acting as a nucleophile. The nucleophile can be one that is coordinated to the same metal Fe(II) or an Fe(III) hydroxide under the present pH conditions. The intramolecular attack by the hydroxide activates the leaving group of 4-NPP, therefore cleavage of the phosphate ester bond follows. The second metal ion in this mechanistic path might serve the structural requirement and for positioning of the catalytically involved iron for better coordination to the 4-NPP.
- The second possible mechanistic route goes through by a single Lewis activation/coordination by the labile metal ion(Fe(II)) and this is followed by the hydroxide attack that is coordinated to the second metal ion(Fe(III)). The presence of a large number of coordinated water molecules in the lattice of Prussian blue increases the chances of attack on the phosphorus center, which is depicted by an increased degree of hydrolysis. It is established in the literature[60] that hydrolases all have at least one bound water molecule for effective hydrolysis.

- Dinuclear metal complexes should be able to provide a double Lewis activation for the hydrolysis of phosphate esters by initially bridging the two metal centers with two phosphoryl oxygens. The nucleophile in this case is either a coordinated hydroxide in the labile metal center or a hydroxide picked up in the reaction solution. The other possible source for nucleophile might be the bridging cyanide group as it has been reported[50] in the literature for related systems. The hydrolysis of phosphates by a dinuclear lanthanide complex that is bridged by peroxides was reported to be assisted by a bridging peroxide. Peroxides act as nucleophiles in the reaction.[50] The hydrolysis of organophosphates is presumed to proceed via SN_2 mechanism with a possible inversion of the phosphorus configuration. For an SN_2 reaction mechanism, the effect of solvent polarity is usually much less, but the ability of the solvent to solvate the nucleophile is important. The double Lewis activation mechanisms in the model systems of phosphate diesters cleavage have been reported to have about $4x10^5$-fold rate acceleration, fairly independent of the leaving group.[98] The effect of the dinuclear mixed valent metal complex on the hydrolysis of 4-nitrophenyl phosphate has clearly been demonstrated by the comparison of the metal ions used in the investigation. Also a charge transfer between the iron(III)) and iron(II) is also presumed to help in aiding complex formation and hydrolysis.

Since turnbulls blue is sparingly soluble in water, the mechanism proposed for solid support materials is also presumed to be applicable in line with previous findings.[99,100] The hydrolysis of phosphate esters by insoluble zirconium hydroxide and by thermally activated MgO surface are worth noting. Zirconium hydroxide has been reported to form suspensions in the phosphate ester solution as it was also the case with the Prussian blue. The partial explanation for zirconium hydroxide having enhanced hydrolysis was attributed to its molecular structure and or the affinities if any for the substrate, similar to that of Prussian blue.

Also presumed to be responsible for such high rate is the presence of the coordinated bridging cyanides on irons. Cyanides are electrophilic groups and poor back donors when used as ligands. Poor back donation by these ligands results in reduction of the electron cloud arround the irons. The reduction in the electron cloud in the iron center causes the metal to seek an electron rich species, which is anionic oxygen of the phosphate. The coordination of oxygen to metal further pulls the electrons from the phosphorus center making it vulnerable to nucleophilic attack. The end result is the weakening of the phosphorus-oxygen bond of the ester.

The degree of hydrolysis for the 4-NPP by these metal ions/complexes is in the following order: Prussian blue (insoluble) > Prussian blue (soluble) > Fe(III) > Fe(II) in all of the three reaction media studied. A more enhanced rate of hydrolysis by iron(III) compared to iron(II) needs to be explained; Trivalent metal ions are generally good catalysts in chemisty. However, for ligand exchange reaction they are generally slow compared to divalent metal ions. They are able to bind to phosphates because of hard acid, hard base principle. Iron(III) is expected to form most stable bonds with hard ligands such as the charged oxygen atoms of the phosphate esters thereby aiding for activated substrate formation. In contrast iron(II) has a lower charge density, therefore prefers soft ligands. It is also known that ligands that prefer iron(II) retain an appreciable affinity for other biologically important bivalent metal ions such as copper(II) and zinc(II).[60]

Much consideration is given to reducing pollution caused by organic phosphate esters in the environment. The compounds are generally resistant to biological degradation and can potentially accumulate in the biosphere and biological organisms. Establishment of efficient functional models will enable us to understand facile degradation of toxic organic phosphate esters and thereby help us to formulate decontamination solutions. The reactions are rapid under mild conditions and require no specialized equipment for the procedure. Hence it might be adapted to situations where decontamination is anticipated.

3.3 CONCLUSIONS

Some conclusions that can be drawn from the study are given below in a bulleted format:

- The pH at which the reactions were carried conforms with previous reports that the hydrolysis of phosphates esters occurs in the pH range of 4 to 9. The reactions showed that 4-nitrophenyl phosphate is being hydrolysed in the presence of metal ion or complex at the pH of 6.5 and under the present reaction temperatures. The results showed that from the first minute, the reaction was already at its maximum and levels off. This suggests that as soon as the metal ion is added to 4-nitrophenyl phosphate solution, cleavage of the phosphorus-oxygen bond of the ester occurs.

- Prussian blue, both soluble and insoluble form gave a better enhanced rate of hydrolysis compared to Ferric and Ferrous ions in all the reaction media. The degree of hydrolysis in ascending order is as follows; Fe(II) < Fe(III) < Prussian blue (soluble) < Prussian blue (insoluble). More enhanced rates by Prussian blue lies more with its geometric structures compared to other metal ions. Its structure resembles those that are found in some biological enzymes with the synergism of its mixed valent state metal ions playing a major role in catalysis.

- The effectiveness of the reaction media in the hydrolysis reactions clearly show that the cationic microemulsions have more enhanced rate of hydrolysis compared to others.The degree of hydrolysis for these reaction media is as follows; aqeuous < anionic microemulsion < cationic microemulsion. Microemulsion systems performed better because of their ability to solubilise and disperse the maximum amounts of the reactants at molecular level for the hydrolysis reaction to take place.

- The reaction between the metal ions and the phosphate substrate is fast and rapid, and maximum amount of hydrolysis was achieved within one minute of mixing. The increase in hydrolysis was not so marked afterwards. The system reached saturation after about three minutes of the reaction time. Hence, it is anticipated that repeated spraying of the phosphate ester contaminant every three minutes with a solution of the metal ions in microemulsions will achieve a maximum degree of hydrolysis in field situations where decontamination is anticipated. The investigation also depicts that systems containing cationic microemulsions and the polymetallic complexes will be a method of choice for low temperature destruction of phosphate ester chemical agents in field situation. The method can also be adapted for high temperature destruction of chemical agents in bulk destruction scenarios at storage sites.

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