METAL ION MEDIATED HYDROLYSIS OF 4-NITROPHENYLPHOSPHATE IN MICROEMULSION MEDIA: CATALYTIC VERSUS STOICHIOMETRIC EFFECTS

by

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DECLARATION

I, Kennedy Uchenna EGUZOZIE sincerely and solemnly declare that the work:

METAL ION MEDIATED HYDROLYSIS OF 4-NITROPHENYLPHOSPHATE IN MICROEMULSION MEDIA: CATALYTIC VERSUS STOICHIOMETRIC EFFFECTS
is my work and that all sources that have been quoted have been indicated and acknowledged by means of complete references

DEDICATION

This research dissertation is dedicated to:

The will of the Almighty God –

> *Lord, take my silver and my gold*
> *not a mite would I withhold*
> *Take my intellect O, Lord*
> *and use it as thou shall choose;*

my loving parents Mr. Denis Eguzozie and Mrs. Evelyn Eguzozie;

my brothers and sisters (Ngozi, Ada, Oby, Gladys, Chijioke, Harriet, Chinedu, Benson, Adaku, Emeka and Kelechi);

my loving wife (my angel from heaven) Dr Ogochukwu Kennedy Eguzozie -

'Honie, in you I have found love, affection, motivation and inspiration

What we share is worth more than all the gold on planet earth

I love you my darling';

my beautiful daughter (my bundle of joy) Mmesoma Kennedy Eguzozie –

'Baby, you are so lovely, I can see in you my dream come true.

Daddy loves you very much and thanks for all the joy that you bring me.'

ACKNOWLEDGEMENTS

ABSTRACT

The hydrolysis of 4-Nitrophenylphosphate (NPP) as model substrate in the presence of several cobalt (III) amine $[N_4Co(OH)(H_2O)]^{2+}$ and copper bipyridyl $[Cu(bpy)(H_2O)_2]^{2+}$ complexes in oil in water microemulsion media was investigated. The reaction was monitored by measuring the absorbance of the nitrophenolate ion produced in the reaction aliquots with time under the experimental conditions. The order of effectiveness of the microemulsion systems towards the hydrolysis of NPP in the presence of these metal ions were found to be cationic > anionic > aqueous at neutral pH. The results of the present investigation exhibits stoichiometric turnovers for the 1:1, 2:1 and 3:1 cobalt to NPP ratio and catalytic turnovers for the $[Cu(bpy)^{2+}$ to NPP ratio of 1:20. Catalysis in the microemulsion mediated reaction solutions was evident even in low concentrations of the metal ions in 1:2000 metal to NPP ratio. An explanation for the enhanced catalytic activity of the $[Cu(bpy)(OH)(H_2O)]^{2+}$ complex for the hydrolysis of NPP is afforded and the application of the above model systems for possible environmental decontamination of toxic organophosphates is anticipated.

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LIST OF ABREVIATIONS

AchE	Acetylcholinestase
ADP	Adenosine 5' Diphosphate
ATP	Adenosine 5' Triphosphate
BsPAP	Bovine spleen purple Acid Phosphatase
CTABr	Cetyltrimethylammonium bromide
CTACl	Cetyltrimethylammonium chloride
DNA	Deoxyribonucleic Acid
DNCB	2, 4- dinitroclorobenzene
DNFB	2, 4 dinitorfluorobenzene
en	Ethylenediamine
HSAB	Hard-soft-Acid-Base
IBX	Sodium 2-nitro-4-iodoxybenzoate
KbPAP	Kidney bean Purple Acid Phosphatase
ME	Microemulsion
NDP	Nucleoside 5`- diphosphate
NP	4 – Nitrophenol
NPP	4- Nitrophenyl Phosphate
NTP	Nucleoside 5`-triphosphate
O/W	Oil-in Water microemolsion system
o-IBA	o-iodoxybenzoate
PAP	Purple Acid Phosphatase
PNPA	p-nitrophenyl acetate
PNPDPP	p-nitrophenyl diphenyl phosphate
SDS	Sodium dodecylsulphate
TEPP	Tertraethylene Pyrophosphate
tn	Trimethylenediamine
tren	Tetraethylenediamine
trpn	Tetrapropylenediamine
W/O	Water- in Oil microemolsion system

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CHAPTER ONE

INTRODUCTION

1. INTRODUCTION AND GENERAL CONSIDERATIONS

1.1 PRELIMINARY REMARKS

Organophosphates can be classified as esters, amides and thiol derivatives of phosphoric and phosphonic acids as depicted below where R_1 and R_2 are usually

$$R_1\diagdown \diagup O \text{ or } S$$
$$P$$
$$R_2 \diagup \diagdown X$$
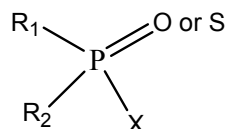
simple alkyl or aryl groups both of which may be bonded directly to phosphorous as in phosphonates or linked via - O – or – S – as in phosphates.

The leaving group X can be any one of a wide variety of substituted and branched aliphatic, aromatic or heterocyclic groups linked to phosphorous via a bond of some lability (usually – O – or – S -) [1].

They are perceived to be environmentally unfriendly compounds because they persist in the biosphere for a long period of time without degradation at ambient conditions of $25^{\circ}C$ and pH 7.0 [2] (i.e. they are resistant to both biological and chemical degradation even at high pH) as shown below in the rate of hydrolysis of some selected group of organophosphate esters.

Organophosphate esters	Rate of hydrolysis ($k_{OH}M^{-1}sec^{-1}$)
Carboxylic esters	1.5×10^{-1}
Amide	5.0×10^{-6}
Phosphate esters	6.8×10^{-12}

1.1.1 Organophosphates in daily use

Organophosphates which possess toxic properties have found wide applications as insecticides and pesticides [3]. Aryl organophosphates must be activated before becoming toxic, while alkyl organophosphates do not require activation for toxicity. Some common organophosphate insecticides include Malathion, Parathion, Methyl Parathion, Diazinon, Chlorothion and Dichlorovos.

More harmful chemicals in the same group include chemical warfare nerve agents. They are mostly nerve poisons (neurotoxins) and may cause cumulative damage to the nervous system and liver and as well as be a cancer risk. Nerve agents contain a P = O moiety while in insecticides, this is often replaced by a P = S group [4,5]. When a nerve agent is absorbed into the human body, it will react irreversibly with the enzymes responsible for the inactivation of neurotransmitters involved in the transmission of nerve impulses, thus leading to their being accumulated in the body. This will lead to over discharge of nerve signals along the nervous system to create a condition known as cholinergic overdrive which will result in instantaneous death. Nerve agents are perhaps the most feared of potential agents of chemical attack. While organophosphate compounds were synthesized in 1854; the first nerve agents were not developed until 1936 [6]. The Germans developed first Tabun (O-ethyldimethylamidophosphorylcyanide) which is easy to produce, while its precursor chemicals are also readily available. Later Sarin (isopropylmethylphosphono-flouridate) which is a colorless, odorless and tasteless liquid in its pure form was synthesized. Somann (pincolylmethylphosphono-fluoridate) which has slight camphor like odor was also developed [7].

Other nerve agents like VX (O-ethyl-S-diisopropylamnoethylmethylphosphothiolate) which is a colorless, odorless and oily liquid that can easily be absorbed through the human skin and is also lethal through inhalation has been produced in the United States [8]. The Russian military is believed to have produced a similar, if not identical agent including the one known as VR55 [9]. Nerve agents originally developed in Germany have been designated "G Series" agents. These include GA (Tabun), GB (Sarin), GD (Soman) and GF which has no common name. These agents are most hazardous when delivered through inhalation as vapour or aerosol. They may be absorbed through the eyes or skin, and are very toxic when ingested. They are less of a percutaneous threat than another group of agents known as the "V Series" agents. These include VE, VG, VM and the best known VX. A newer group of the series known as "GV" Series agents combines both properties of the other two classes posing a threat both through inhalation and through percutaneous absorption. In general V Series agents have greater persistency and potency than the G Series agents as shown in the tables 1.1 and 1.2 below.

Table 1.1: Physical Properties of Nerve Agents

	Vapor pressure	Volatility	Appearance	Odor	Solubility	Persistence
Tabun (GA)CAS #7781-6	0.037mmHg at 20°C	576-610mg/m^3 at 25°C	Clear to brown liquid	Fruity	9.8g/100g at 25°C	T ½ = 24-36hrs
Sarin (GB)CAS# 10744-8	2.1mmHg at 20°C	1640-22000 mg/m^3 at 25°C	Clear liquid	Odorless	Miscible	2-24hrs at 5- 25°C
Soman (GD)CAS #9664-0	0.4mm Hg at 25°C	3060-3900mg / m^3 at 25°C	Clear liquid	Fruity, oil of camphor	2.1g / 100g at 20°C	Relatively persistent
GFCAS# 32999-7	0.07 mm Hg at 20°C	59 ppm	Clear liquid	Odorless	3.7 g / 100 at 25°C	Unknown
VX CAS# 2082080-8	0.0007mmHg at 20°C	3-30,(10.5) mg/m^3 at 25°C	Thick clear to straw coloured liquid	Odorless	Miscible at<9.4°C "Slight" at 25°C	2 - 6days

The volatility of these agents is low (the most volatile Sarin, has a vapor pressure similar to that of water. Thus, the term "nerve gases" is a misnomer as military nerve agents are predominantly liquid at room temperature. These agents pose an inhalation threat primarily when dispersed as an aerosol. These were demonstrated in a Sarin attack on the Tokyo subway. A punctuated plastic container with a nerve agent was placed under a seat, and subsequently allowed to evaporate. Because the method of dispersal relied on evaporation, there were no severe fatalities [10].

Table 1.2 below depicts the estimated measures of toxicity of some common chemical warfare nerve agents.

Table 1.2: Estimated measures of Toxicity of some common Nerve Agents.

	LD$_{50}$ (percutaneous)	LC $_{50}$	LCt$_{50}$	IDLH
Tabun (GA)	1gm /person	2ppm	100 - 400mg x min	0.03ppm
Sarin (GB)	1.7gm /person	1.2 ppm	50 - 100mg x min	0.03ppm
Soman (GD)	0.35gm /person	0.9ppm	25 - 70mg x min	0.008ppm
GF CAS	0.03gm /person	Unknown	Unknown	Unknown
VX CAS	0.01gm /person	0.3 ppm	5 - 50mg x min	0.002ppm

Toxicity is usually measured in terms of a factor known as LD$_{50}$ which is the average minimum dosage, in milligrams per kilogram of body weight, required to kill 50% of a group of a particular species. Toxicity has also been registered in terms of LD$_{50}$ via skin (percutaneous) absorption per individual species and also in terms of a factor known as LCt$_{50}$ which is the lethal concentration in milligram-

minutes per cubic metre required to kill 50% of a species either by inhalation or percutaneous absorption.

LD_{50} : Nerve agents are among the most lethal agents available that have been developed for military use. The percutaneous LD_{50} or dose required to kill 50% of those exposed are in the milligram range for many agents. A drop of VX on the kin is potentially lethal.

LC_{t50} : The concentration – time product is a measure of exposure to vapour or aerosol over time. The LC_{t50} is the concentration- time product that is lethal to 50% of those exposed and reflects toxicity by the inhalational route.

IDLH : The IDLH is the concentration of toxin in air that is "immediately dangerous to life and health". For VX vapor in air, 2 parts per billion or 2ppb is likely to result in toxicity.

Studies[11] using a combination of pesticides and insecticides in mice suggested an alteration in the immune, endocrine and nervous systems functioning and these were shown to occur at concentrations that are prevalent in ground water. The research suggested that the most at risk are the fetus and very young children whose systems are still at the development stages. The report also suggested that persistent and consistent exposure to organophosphates pesticides and insecticides induces depression in humans and this was attributed to the decrease in serotonin levels in the body.

The prevalence of these toxic organophosphates in our environment has been attributed to human activities even as they pose serious threats as environmental pollutants. These activities include:

(1)   Improper management of stockpiles of expired pesticides

(2)   Improper domestic and agricultural applications of pesticides and

(3)   Their use as chemical warfare nerve agents in terrorist attacks
       or in combat situation in a war.

Hence the development, production, stockpiling and use of nerve and chemical warfare agents are prohibited by the Organization for the prohibition of chemical weapons (OPCW) and other environmental agencies.

Recently, much research consideration has been directed towards reducing the environmental problems caused by the presence of organophosphate esters that are toxic and are resistant to both biological and chemical degradation and can accumulate in the biosphere and organisms. The decontamination of nerve gases is required in battlefields, laboratories, storage and destruction sites. In the past, dilution was used as a solution to these pollution problems, but due to the fact that pollution levels in our rural and urban environment have increased so much in amount and toxicity, this approach is no longer acceptable. The most versatile

decontamination method commonly used is washing and spraying with water that contains soap or detergents. The disadvantage of this method is that, although most of the contaminants are diluted and removed, not all of them are neutralized or destroyed. To neutralize and destroy these contaminants, the addition of bleach (i.e. hypochlorite or other active chlorine containing compounds in alkaline aqueous solution (pH ~ 11) is utilized [12].

Several other techniques have been developed and reported to address the pollution threats caused by the presence of these toxic organophosphate esters in the environment. Such techniques like:

(1) Gamma irradiation

(2) Wet air oxidation

(3) Nuclear incineration

(4) Molten salt oxidation

(5) Plasma arc processes and

(6) Hypochlorite formulations have been employed over the years.

However, these known methods use compositions and formulations which have certain undesirable properties like corrosiveness, flammability and even toxicity. Hypochlorite formulations are very corrosive and toxic. Additionally, the application of hypochlorite decontaminants often requires substantial scrubbing for the removal and destruction of the organophosphate contaminant, a procedure which limits its use.

It has been reported that nerve gases can be destroyed by bleach through oxidation to less toxic inorganic phosphates and alkali through hydrolysis of the P-O or P-S bond [13,14]. One disadvantage of using bleach according to the report is that a large excess is required. Also the active chlorine content of bleach solutions decreases with time. Moreover, bleach is indiscriminately corrosive to any surface or compound it comes into contact with. Base hydrolysis also has some limitations such as the requirement for large quantities to maintain a high pH level. The report also suggested that a chemical nerve agent like VX (O-ethyl-S-[2-(diisopropylamino) ethyl] methylphosphonothiolate) has low solubility in alkaline solution and therefore its reaction with the base would be very slow. Catalytic hydrolysis involving metal ions (eg, Ag+, Hg^{2+}) [15-17] and enzymes (eg organophosphorous acid anhydrases) [13, 18-22] have been proposed but they have limitations too. For example Ag^+ is too expensive and Hg^{2+} is too toxic to be used as a decontamination agent. The enzyme organophosphorous hydrolase (OPH) has been reported [23] to be highly efficient for the hydrolysis of organophosphorous pesticides but much lower efficiency for the hydrolysis of VX ($k_{cat} = 0.3$ s^{-1}). Nanocrystalline metal oxides as catalyst for nerve agent destruction have also been investigated by various researchers [24-26] and more recently, studies conducted at the US Army Edgewood Chemical and Biological Centre at Aberdeen Proving Ground, Maryland [27] have shown aqueous solutions of

aluminum sulphate and sodium aluminate buffered at pH 4 to be very promising for destroying large quantities of VX, GB and GD. None of the above techniques have been accepted as an adequate solution to decontamination problems posed by this group of toxic organophosphate compounds [28]. Organophosphate pesticides are the most widely used type of pesticide and have replaced the environmentally persistent organochlorine reagents. Despite their potential environmental problems, the use of organophosphate pesticides has been increasing and is predicted to increase in the future because of the lack of suitable substitutes. Long-lived pesticides pose a threat when they spread beyond their intended application. The search for alternative agents for the decontamination of organophosphate nerve agents and pesticides still continues.

1.2 : Metal ion Catalysis

Metal ion catalysis for the hydrolysis of toxic organophosphates have been the subjects of previous reports [29,30] and most of these investigations were concentrated on enzyme – based detoxification techniques and the reaction media. Many of the toxic nerve agents and pesticides possess a P-O-C linkage in their structure. Cleaving the P-O-C bond could be a means of deactivating these toxic agents. This is convenient due to the nucleophilicity of the phosphorous and polar nature of the P-O and C-O bonds. Metal ion containing enzymes are capable of cleaving or hydrolyzing phosphate ester bonds without causing more harm to the environment and organized assemblies such as micelles, microemulsions, double emulsions and even colloids [31,32] have also been utilized as potent hydrolyzing agents in the last couple of years. It has been reported that binuclear boron halide chelate compounds can dealkylate a wide range of phosphates at ambient temperature [33-35]. In effect, the destruction of this group of compounds using metal ions now constitutes a challenging problem in organic reactivity which involves nucleotidyl and phosphoryl transfer reactions.

1.2.1 NUCLEOTIDYL AND PHOSPHORYL TRANFER REACTIONS

In biological systems, enzyme promoted reactions involving both phosphoryl and nucleotydyl transfer are of enormous importance especially in the storage and utilization of energy as in the formation and hydrolysis of ATP [36]. PO_3^- transfer reactions involve the rupture of normally very robust P(M)-O bonds as evidenced by the inertia of polyphosphates (ATP) and phosphate esters to uncatalyzed hydrolysis. Thus the ability of enzymes, literally to direct such processes (like the hydrolysis of ATP) to take place when the system needs energy and not allow the hydrolysis of structural phosphate esters to occur in the same system, creates an intriguing mechanistic problem. The fact that metal ions, usually Mg^{2+} participate in the enzymic processes has led to considerable research into the hydrolysis of polyphosphates and phosphate esters as catalyzed by or promoted by metal ions [37]. Our research group has had a modest interest in such reactions since researchers like Scout [38] and Sargeson [39,40] and their co-workers reported that oxidizing VO^{2+} complexes of PPPi and ATP produce a 1000 fold increase in the hydrolysis rates induced by VO^{2+}. The report also concluded that there was also a rate enhancement in the hydrolysis effected by complexes formed of

$N_4Co(OH)(OH_2)^{2+}$ complexes with polyphosphates using ^{31}P NMR which gives structural information on precursor complexes, intermediates and products. Through such reactions, it is possible to compare the behavior of labile, semi labile and inert metal ions, and the structural and kinetic factors promoting and inhibiting phosphoryl transfer processes.

1.2.2 THE ROLE OF DIVALENT METAL IONS IN PHOSPHORYL AND NUCLEOTIDAL TRANSFER REACTIONS

Metal ions involvement in the hydrolysis of phosphate esters is of particular interest because of the essential roles the metal centers play in biological phosphoryl transfer reactions. The requirements of divalent metal ions in both hydrolysis and condensation reactions in biological systems is well documented in the literature [41,42].Many of the enzymes involved in hydrolysis of phosphate esters are metalloenzymes that require metal ions for their reactivity. A number of these enzymes and enzymic models have been studied [43,44] in considerable details and various proposals which have been advanced regarding the role of metal ions in catalyzing or promoting the hydrolysis of phosphate esters are as follows:

(i) **Charge neutralization or shielding** ─ like protons metal ions are Lewis acids or electrophiles. They can serve as a general acid catalyst for reactions catalyzed by protons. Secondly phosphate derivatives usually carry many negative charges so that the approach of a negatively charged nucleophile such as a hydroxide ion is not favored on electrostatic grounds and so the neutralization or shielding of these negative charges is very necessary for a perfect nucleophilic attack at the phosphorous metal centre.

(ii) **Strain induction** ─ the bidentate coordination of a phosphate monoester to a metal ion could result in the formation of a strained chelate ring thus facilitating a neucleophilic attack at the phosphorus centre.

Nucleophilic attack
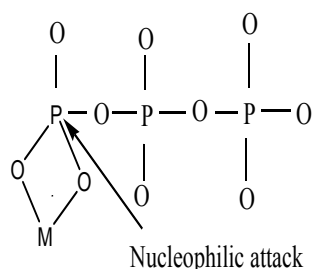
(iii) **Template formation for orientating substrate in optimum stereochemical position** ─ Metal ions enhance the optimal stereo-chemical orientation for intramolecular hydrolytic attack by the Nucleophile.

(iv) Generation of coordinated nucleophile at biological pH ─ Hydroxide is a better nucleophile than water but its concentration would be low at pH 6.5. This problem is overcome by the simultaneous coordination of water and phosphate esters to a metal atom. i.e. an effective nucleophile is provided at biological pH [45,46].

$$
\underset{\text{Acidic}}{M\!\!\begin{array}{l} OH_2 \\ OH_2 \end{array}} \longleftrightarrow \underset{\text{Neutral}}{M\!\!\begin{array}{l} OH_2 \\ OH \end{array}} \longleftrightarrow \underset{\text{Basic}}{M\!\!\begin{array}{l} OH \\ OH \end{array}}
$$
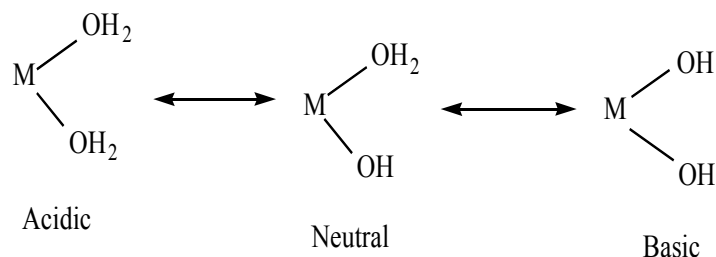
Bimetallic and polymetallic centers have been used as catalytic sites in many phosphoryl and nucleotydyl transfer reactions studies [47] of enzymes with Zn(II) ─ Zn(II) active sites that include phosphoproteins phosphatases, alkaline phosphatases and phosphotriesterase. The common occurrence of bimetallic/polymetallic active centre is rationalized by the two metal ion mechanism where presumably both the general Lewis acid properties and charge effects are considered. Two metal ion mechanism is operative with the main group metal ions as corroborated by the fact that both Zn^{2+} and Mg^{2+} are common in these Hydrolytic enzymes (alkaline phosphatases, yeast inorganic pyrophosphatases, kinases and purple acid phosphatases). The structural studies of both kidney bean purple acid phosphatase (PAPS) and calcineurins revealed the existence of Fe-Zn active sites. A short review of biochemical and structural information which had been established on enzymic phosphoryl transfer reactions in alkaline phosphatases, yeast inorganic pyrophosphatase and purple acid phosphatase is given below.

1.3 ALKALINE PHOSPHATASE

Alkaline phosphatase (AP) catalyzes the hydrolysis of phosphate monoesters with a rate maximum near pH 7-8. The AP which has been isolated from E.coli is a dimer of molecular weight 94000 and it contains at least two to four Zn^{2+} ions and Mg^{2+} ions per dimer. The metal ions appear to play two roles in the enzymes. Two Zn^{2+} ions are required for activity; these are called catalytic and are bound very strongly to the enzyme. The two further Zn^{2+} ions and the two Mg^{2+} ions, termed structural, tend to enhance the activity of the enzyme in addition to stabilizing its tertiary and quaternary structure. Several proposals for the mechanism of hydrolysis by AP 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 phosphorous centre, and/or activation of the nucleophile. The nucleophile in question may be the hydroxyl of serine 102 or

water. The nucleophile through coordination 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 neutralization of the bound phosphate. Although much is known about the structure of the active sites, the role of the metal ions in the mechanism is still unclear and the degree of involvement of the other functional groups surrounding the enzyme is yet to be established.

1.4 YEAST INORGANIC PYROPHOSPHATASE

The enzyme Yeast Inorganic Pyrophosphate (YIP) consists of two identical sub-units with a molecular weight of 6400 for the dimer. YIP catalyses the reversible hydrolysis of pyrophosphate. In the presence of Mg^{2+}, YIP is specific for pyrophosphate. However, when other metal ions are involved, the enzyme becomes less specific and it will hydrolyze a number of pyrophosphate esters [48]. 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+}, Fe^{2+} and Cd^{2+} all of which activate and Ba^{2+} and Ca^{2+} which inhibit it [49]. Several observations [50-52] conclusively show that one metal ion is required to form the activated substrate and at least one other divalent ion is required for activity. Several proposals [53,54] have been forwarded to explain the mechanism of YIP. The basic features include:

(1)   Metal binding to the pyrophosphate to produce a substrate

(2)   Production of a metal bound hydroxo ligand as a nucleophile by an adjacent basic amino residue and

(3)   Stabilization of the substrate by interaction with cationic amino acid residue.

1.5 PURPLE ACID PHOSPHATASES (PAP)

Purple Acid Phosphatases constitute diiron carboxylate proteins which hydrolyze orthophosphate monoesters under acidic conditions. Purple Acid Phosphatases are dinuclear iron containing non-heme proteins that catalyze the hydrolysis of the activated phosphoric esters. The enzyme exists in two forms, the reduced and the oxidized form –

   Reduced form (active) [Fe(III)-OH-Fe(II)]
   Oxidized form (inactive) [Fe(III)-OH-Fe(III)

Fe(II) of the reduced PAP is labile and can be substituted by the other divalent metal ions ; eg Zn^{2+} while it still retains its catalytic activity [55, 56].

1.6 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 [57]. Thus the overall catalyzed reaction may be written as:

$$Acceptor \ + \ NTP \ \leftrightarrow Phosphorylated \ acceptor \ + \ NDP$$

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 phosphorous atom becoming chiral. From the large number of kinases investigated [58], it appears that the enzymes will utilize only one conformation of the complex. This is typical of enzymes which are stereospecific even when the reactants and products are chiral.

For example, in the hydrolysis of guanosine (2',3') cyclic phosphate by dinuclear metal complex of Zn^{2+}, the formation of 2'-GMP and 3'-GMP as products was observed [59]. The reaction follows the same route as that of simple phosphate esters. When the substrate and catalyst are added together, there was rapid formation of the ternary complex (Michaelis- Menten) followed by the charge neutralization and phosphate activation through the binding of the phosphate oxygen to one of the Zn^{2+} as shown below.

B = Guanine
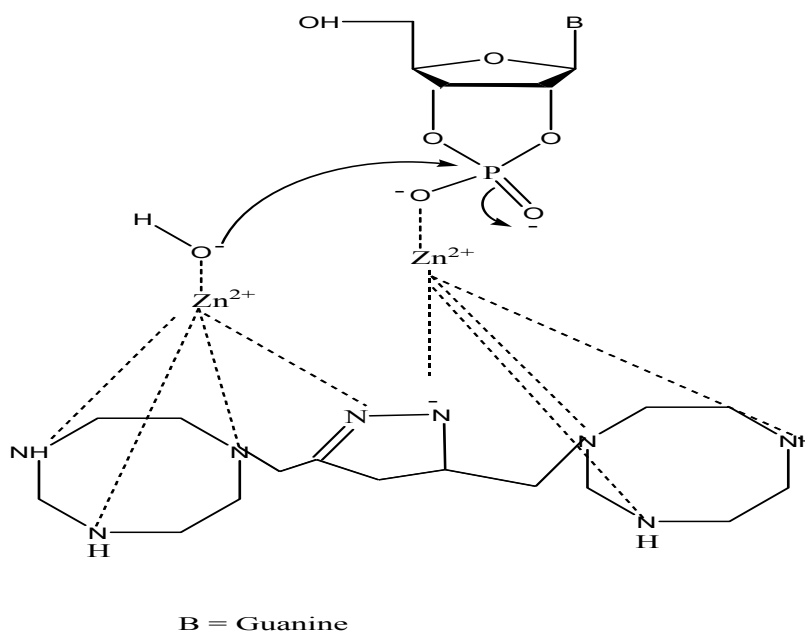
The substrate depicted above is activated by one metal ion through Lewis acid activation while the dianionic phosphate intermediate formed is stabilized by both metal ions. The 2nd Zn^{2+} presumably activates the nucleophile, followed by the subsequent activation of the leaving group. The presence of the hydroxide ion is always necessary as it is the most active nucleophile in most hydrolytic reactions. As a result of intramolecular attack by the hydroxide, the diester is cleaved to the monoesters 2'- GMP and 3'- GMP [60]

1.7 AIM OF STUDY

Tropical Africa which is predominantly rural, are plagued by acute shortage of food and wide spread of insect borne diseases such as malaria, yellow fever, dengue fever caused by the prevalence of such pest like mosquitoes, cockroaches bedbugs etc. The pressure to produce increased foods has led to the widespread use of sub standard and sometimes expired organophosphorous compounds in fertilizers, herbicides etc and the need for the control of these insect pests has also led to the use of these expired and/or substandard organophosphate compounds imported from the western countries either as insect repellants or as insecticides due to ignorance or the lack of proper technological detecting capability of these expired chemicals. Most of these organophosphate ester derivatives are persistent chemicals which contribute to the long term pollution problems in our biosphere thus constituting a health and environmental challenge. These health and environmental issues require solutions that are appropriate to the environ-mental, economic and social conditions of these African countries.

Also, tropical and sub-tropical climates which are characteristics of the tropics create conditions for organophosphate derivatives that favor the production of pollutants more severe than is obtained in the industrialized countries where most of the technologies for decontamination are being developed. Our research undertaking takes the above scenario into consideration and attempts to contribute positively to the safe disposal and management of toxic organophosphates in our environment. The aim of the present study therefore is to

(1) Understand the hydrolysis of phosphate esters by using 4-nitro phenlyphosphate as a substrate model for organophosphates.

(2) To mimic the effects of metal ions (Co(III) and Cu^{2+} complexes as an enzyme model.

(3) To study the effects of different microemulsion systems as an effective decontamination media for hazardous organophosphates.

(4) To formulate decontamination solutions which are environmentally friendly fast, efficient, cheap and effective under ambient conditions, with the ultimate aim being to contribute to the decontamination of hazardous organophosphate derivatives from our environment. In this study, microemulsion formulations have been employed for the hydrolysis of

organophosphate esters in conjunction with other hydrolyzing agents such as metal ions. The synergism between the hydrolyzing species and the reaction media is found to be important for hydrolytic rate enhancement.

1.8   CHOICE OF MODEL SYSTEMS

1.8.1 Co (III) MODEL SYSTEM

Although other metals such as magnesium, iron etc are metal cofactors, Co(III) was used as model system because of the following reasons [61].

(1)   Model systems based on cobalt (III) complexes are especially advantageous in that metal coordination sites open for phosphate substantitution can be limited, permitting studies of a type not readily available using labile metal centers.

(2)   Co(III) complexes are exchange inert and so its complexes are thermodynamically stable thus making the mechanisms and kinetics of Co(III) complexes to be well understood.

(3)   The (- Co-N- bonds) in Co(III) amine and polyamine complexes retain their integrity in aqueous and microemulsion media for a long time.

(4)   Co(III) complexes are easy to synthesize and are kinetically robust thereby permitting the characterization of all the species present in the solution.

(5)   Co(III) is a low spin d^6 ion; it is diamagnetic and therefore enables ^{31}P studies as a probe for monitoring the reactions.

(6)   Co(III) is semi-labile and hence does not readily undergo ligand exchange reactions.

1.8.2 4-NITROPHENYLPHOSPHATE AS A PHOSPHATE ESTER   SUBSTRATE MODEL

4-Nitrophenylphoshate was used as a substrate model because it allows one to measure the absorbance of NP in the reaction media at 400nm without any interference from NPP as depicted in the fig 1.1
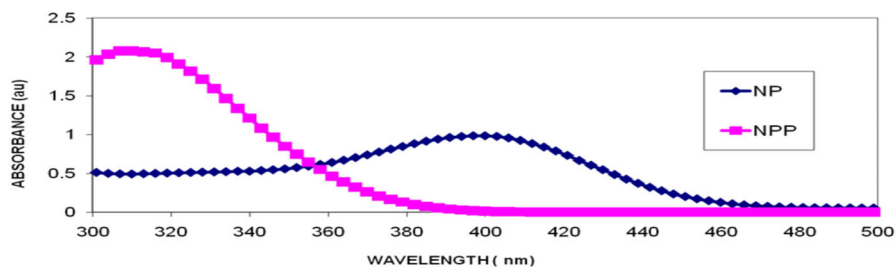
Figure 1.1: Absorption spectra of NPP and NP at pH 7.0

Calibration curve studies were done by preparing a series of 4-nitrophenol solutions ranging from a concentration of 10^{-3}M – 10^{-6}M and the pH adjusted to 6.5. One milliliter of this solution was mixed with 1mL of phosphate buffer and 3mL of water and its absorbance measured at 400nm. The absorbance values were plotted against concentration and the molar absorptivity determined as 4.925×10^3LMol^{-1}cm^{-1} at 400nm. Under slightly basic conditions, the 4-nitrophenol is deprotonated to form the 4-nitrophenolate ion which is bright yellow and has an absorbance maximum at 400nm, quite distinct from NPP which has an absorbance maximum at 310nm. The system conformed to Beer's law over the concentration range of 4-nitrophenol when measured at 400nm in a 1cm cell. The amount of nitrophenolate ion produced was determined from the calibration curve. The percentage hydrolysis was calculated assuming 100% 4-nitrophenolate production for complete hydrolysis.

1.8.3 Cu (II) MODEL SYSTEM

There are no records of any occupational diseases attributable to copper among people who have worked for many years with the metal or its salts. Indeed, it has sometimes been said that such people often appear healthier and generally suffer less from colds and other ailments. It is worth noting that copper is an indispensable constituent of all living tissues and is essential for the normal growth and well being of plants and animals. The minute quantities of copper needed for human health are usually obtained through the normal intake of food and water. Cu^{2+}_{aq} have a distorted square bipyramidal structure which arises from the Jahn-Teller effect. Four of the coordinated water molecules are situated in an equatorial square planar position about the central Cu^{2+} while the remaining two H_2O are located at a greater distance along the central axis perpendicular to the square planar. In the formation of its ternary complexes, Cu^{2+} shows a strong preference for square planar structures. The square bipyramidal $[Cu(H_2O)_6]^{2+}$ ion is somewhat more strongly distorted towards a square planar coordination sphere by the coordination of 2'2-bipyridyl, thus creating the "right" geometry for the coordination of the second ligand like 4-Nitrophenylphosphate whose donors are negatively charged oxygen atoms. By definition, ternary complexes consist of a metal ion and two different ligands, other than the solvent [62]. An example of a ternary complex is $[Cu(bpy)(OH)(H_2O)]^+$. Such ternary complexes are very important in analytical chemistry and metal catalyzed reactions [63,64], they

appear in biological fluids [65,66], and manifest themselves as enzyme- metal-ion substrate complexes [67,68].

Possible explanation for the great stability of ternary Cu^{2+} complexes containing aromatic N ligands, like 2,2'-bipyridyl could be attributed to the Pearson "hard and soft rule". Hard acids prefer to associate with hard bases and soft acids prefer to associate with soft bases. According to Pearson's, Cu^{2+} is neither hard nor soft but is borderline; the same is true for the pyridyl group. Going across the 3rd transition metal series from Ca^{2+} to Zn^{2+}, the ions becomes softer as a consequence of the increasing number of d electrons. Since π bonds are formed and back- donation occurs from the coordination of 2,2'-bipyridyl to Cu^{2+}, the d electron content on Cu^{2+} decreases; i.e. it becomes harder. Hence, through the combination of the borderline 2,2'-bipyridyl with the borderline Cu^{2+}, a harder species is created which is more favoured to combine with O than with the N ligands (the hardness increasing in the series $F^- > OH^- > NH_2^- > CH_3^-$) thereby obeying Pearson's "hard" and "soft" rule of hard acids prefer to associate with hard bases and soft acids prefer to associate with soft bases [69].

Copper is not toxic, and there has been no reported cases in the literature where copper is known to have constituted any environmental hazard and so would require minimal logistical support. Geometric consideration like the Jahn Teller distortion in copper coupled with other consideration like its availability and affordability which will be discussed later are other reasons for considering copper as an enzyme model.

1.8.4 MICROEMULSION AS HYDROLYZING MEDIA

Microemulsions and micellar systems showed better promise, as have been reported in previous investigations [70]. The microemulsion based system is simple, inexpensive, mild and relatively rapid. These features arise from the fact that microemulsions represent a community of molecules that function only by virtue of cooperative action. Each of the components (water, hydrocarbon, surfac-tant, cosurfactant, organophosphate agent and metal ion) has its own residence site and its own role in the reaction. This kind of self assembly inherent to microemulsions, results in the formation of an organized system that helps in assisting hydrolysis of organophosphates. A brief description of microemulsions and some other related organized assemblies is given below.

Microemulsions are transparent vesicles containing large amounts of both water and hydrocarbon. They are colloidally dispersed systems and hence show an essential distinction from molecular solutions of hydrocarbon and water. In microemulsion, the hydrocarbon/water colloidal solution is stabilized by a combi-nation of a surfactant and a medium chain length alcohol such as butane-1-ol. Microemulsions have found a great variety of applications and they are not only scientifically but technologically well established. According to Friberg, micro-emulsions are at present the focus of extensive research efforts [70], however their definition is open to debate.

Microemulsions are generally ternary fluid made up of an aqueous phase, an oil phase, a surfactant and cosurfactant [71]. The resultant system is composed of micro droplets or swollen micelles, consisting of an oily or aqueous centre surrounded by a mixed film of surfactant and cosurfactant. Microemulsions are defined as transparent, optically isotropic and thermodynamically stable homogeneous solution of oil and water, stabilized by the addition of a surfactant and a cosurfactant [72] They are droplets type of dispersions, either of oil-in-water(o/w) or water-in-oil(w/o). They can also be of oil-in-water-in-oil(o/w/o) or water-in-oil-water(w/o/w) and these are referred to as double microemulsions. Since they are mixtures of polar and non-polar compounds, they are stabilized by addition of a surfactant/cosurfactant mixture which is sometimes called emulsifiers. The surfactant usually forms a monolayer at the oil/water interface, and is positioned in such a way that the polar head groups are in contact with the water whilst the non-polar tail reside in the oil phase, thereby solubilizing the mixture of the two immiscible liquids by reducing the interfacial tension between the two phases.

The ternary fluids referred to as Microemulsions were first discovered by Schulman and Hoar around 1940's. They discovered that by titration of opaque emulsion with a medium chain alcohol such as butanol, a transparent system is produced. The additional component, the alcohol is called a co-surfactant. Further study by Sherman and Prince [73] confirmed that such transparent emulsions had small dispersed phase particles that are smaller than 0.1um. Microemulsions are currently the subject of investigations because of their wide range of potential and actual utilization in enhanced oil recovery [74]. This utilization rest on the unique capability of microemulsions to unite in a single phase of low viscosity and their very low interfacial tension with excess oil or water [75].

The microscopic structure of microemulsions depends on the volume fraction of the dispersed phase, temperature and the chemical properties of its components.

(a) Oil-in-water (o/w): Here oil is dispersed in water such that the hydrocarbon tails of the surfactants form the core and the polar head group is in contact with the aqueous medium.

(b) Water-in-oil (w/o): At low water concentration, the situation is reversed and small water droplets are dispersed in oil. Here the polar groups form the core and the hydrocarbon tails now is in contact with the oil [76,77].

(c) Double microemulsions : Double microemulsions are colloidal systems that is formed by the dispersion of one phase in another which is normally immiscible. They can either be oil-in-water-oil (o/w/o) (with the dispersed aqueous globules containing smaller dispersed oil droplets) or water-in-oil-water (w/o/w) (with the dispersed oil globules, themselves containing smaller aqueous droplets)

To formulate a double microemulsion, it is necessary to choose at least two surfactants with one low in HLB (high lipophilic balance) number and the other with a high HLB [78]. Preparation of a (o/w/o) microemulsion involves dissolving

a low HLB (<10) surfactant in oil, followed by the addition of water. This leads to the formation of water in oil (w/o) emulsion, which is then further emulsified in aqueous solution of a surfactant with a high HLB number (>10) to produce a (o/w/o) microemulsion. The opposite applies for (w/o/w) microemulsion system.

Double emulsions are very important in the pharmaceutical, food and cosmetic industries as they are able to encapsulate both hydrophilic and hydrophobic molecules [78]. Their importance lies in the fact that they can release the encapsulated molecules slowly from their internal droplets when administered in vitro due to their double compartment structure. 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 or cosurfactant used during the formulation. The important properties of microemulsion are depicted in the table 1.3

Table 1.3: Properties of emulsions and microemulsions

Property	Emulsions	Microemulsions
Disparity	Particles > 0.1 micrometer, visible under microscope	Particle is usually 0.01-0.1 micrometers, invisible under microscope.
Light transmittance	Non transparent	Transparent
Stability	Non stable, can be stratified by centrifuge.	Thermodynamically stable, can't be stratified by centrifuge.
Amount of surfactant	Small addition of surfactant, cosurfactant not necessary	Large amounts of surfactants, and cosurfactants is a must
Miscibility	o/w type is immiscible with oil, w/o type immiscible with water	Miscible with oil and water within some limits

In recent years, micellar solutions and microemulsions have been investigated as media for various chemical reactions [79,80]. Among these investigations, a particular challenging field was in the use of microemulsions in biocatalysis. Studies [80] have shown that the influence of structured fluids like micro-emulsions on chemical reactions can be described by the following properties

(i)   Solubilization of a broad spectrum of substances in one phase system to overcome reagent incompatibility problems.

(ii) Enhancement of specific rate of reaction due to the partitioning and concentration of reactants and products.

(iii) The structured fluid can influence reaction regioselectivity due to orientation of reactants at the interfacial region.

Microemulsions are also employed extensively in the synthesis of nanometer-size particles [81,82]. (e.g.) spherical copper nanoparticles were synthesized in SDS/isopentanol/cyclohexane/water microemulsion formulation with sodium borohydride as the reducing agent.

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 have to be taken into account for a comprehensive understanding of chemical reactions carried out in these media. They are dynamic systems whereby continuous movement and collision with each other is occurring. In each collision, material interchange can take place. It is well established that not all droplet collisions are effective for material exchange; therefore there are other factors such as temperature, surfactant mixture, HLB number and cosurfactant that play a significant role in the rate enhancement. It is therefore necessary to look at other factors that can affect the performance of microemulsions in chemical reactions.

(a)   Surfactants: It is necessary to choose the right surfactant in developing microemulsions as they have pronounced effects on their performance and stability. The charge of a surfactant can also affect the rate of reaction between a lipophilic organic molecule and a charged polar nucleophile. The general rule is that cationic surfactants accelerate the reaction involving anionic nucleophiles and vice versa. However, it has been found that for cationic surfactants, the choice of counter ion is decisive. The choice of counter ion is less important for anionic surfactant.

(b)   High Lipophilic Balance: A good surfactant should have a low solubility in both the dispersed and continuous phase. This maintains the stability of the microemulsion by allowing the surfactant to reside on the interface and not solubilizing in one of the phases. Surfactants having the HLB number of 9 to 13 are good for the formulation of microemulsions. This is because their solubility is intermediate between highly oil and water soluble surfactants.

(c) Cosurfactants: Maximum solubilization of dispersed and continuous phase in each other is achieved by the addition of a cosurfactant. The presence of a cosurfactant reduces the interfacial tension close to zero, thereby effecting the maximum solubilization between the two phases. Cosurfactants can be a short to medium chain alcohol. Cosurfactants can be mixed so as to improve the microemulsion quality which also has a positive effect on the solubilization of the two immiscible phases.

(d)   Temperature: It has been suggested that the HLB number of non-ionic surfactant should be a function of temperature, as non-ionic surfactants become increasingly hydrophobic with temperature. Phase inversion temperature is an

important parameter to microemulsion with anionic surfactant because as temperature increases, the microemulsion will go through a change from oil-in-water to water-in-oil. Surfactant Mixture: Surfactants reduce the interfacial tension between two droplets. A single surfactant might not mean high solubilization, but a mixture of suitable surfactants improves the solubility of the two phases in each other. The surfactant mixture can be of the same charge or can be of different charge. The use of surfactant mixtures with high oil and water solubility improves the performance of the existing microemulsions.

Microemulsions have also been employed in chemical reactions for various purposes such as overcoming reagent incompatibilities and release of trapped drug as employed in pharmaceutical industries. Reagent incompatibility is the problem that is often encountered in organic reactions since organic compounds have different solubility's in different solvents. It is therefore necessary in these situations to bring the reactants together and thus make them to react with polar apriotic solvents, phase transfer catalyst or mechanical mixing. They are often employed in this instance as they represent the intermediate route between organic and inorganic compounds. In microemulsions, high concentrations of both the water soluble and the water insoluble compounds are presumed to dissolve simultaneously. The oxidation of mustard has been reported in oil-in-water microemulsion where the compound was oxidized either in or on the oil droplets with the help of microemulsion. It was observed that the surfactant (butan-1-ol) also assisted in the reaction.

Microemulsions can accelerate the rate of a chemical reaction by other means than just supplying a large interfacial surface area. If the surfactant head carries a charge as in the case of anionic and cationic surfactants, reagents of opposite charges will be confined to the interior of the droplets of water-in-oil micro-emulsions. Such compartmentalization and concentration of the reagents leads to a rate enhancement in such reactions. 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 boundary where the reaction normally occurs. This type of rate enhancement has been referred to as microemulsion/micellar catalysis in comparison to micellar catalysis for these systems.

1.9   SOME OTHER RELATED SYSTEMS TO MICROEMULSIONS

EMULSIONS

Emulsions refer to the dispersion of two systems/phases into each other, which are normally immiscible [83]. Their composition is the same to that of microemulsion in the sense that they are both composed of immiscible liquids. Emulsions normally do not contain a surfactant and they are milky in colour. They are usually unstable and are therefore stabilized by the addition of a surfactant.

Water-in-oil emulsions are miscible with oil and oil-in-water emulsions are miscible in water. This is one of the simple ways in which one can be able to test the emulsion whether it is o/w or w/o. Emulsions tend to have a cloudy or milky appearance and these is because they are able to scatter light when it passes through them thus making them to have a limited period of stability.

SURFACTANTS

Surfactants or surface active agents are usually organic compounds. They are sometimes referred to as wetting agents because they have the ability to lower the surface tensions of liquids. Surfactant molecules are made up of two parts with different affinities for the solvent. It is made up of the hydrophobic portion which is usually a hydrocarbon chain which is attached to the hydrophilic functional group. They have the ability to solubilize one phase into the other by intervening in between the two immiscible phases. Surfactants are categorized according to the charge which they carry on their hydrophilic head after dissociation in aqueous solution.

(i)   Anionic surfactant : Anionic surfactants form a negative charge on their hydrophilic head upon dissociation in aqueous medium and a positive

(ii)   counter ion eg Sodium Dodecylsulphate (SDS). They are good foaming agents and so have found wide applications in the soap and detergent industries. They are also the most widely used surfactant on accounts of cost, performance and reduced toxicity.

(iii)   Cationic surfactants : Cationic surfactants form a positive charge on the hydrophilic head group upon dissociation in aqueous medium and a negative counter ion. eg quaternary amines, fatty amine salts, and cetyl-trimethylammonium chloride (CTACl). There is some limit in the use of cationic surfactants due to their high toxicity.

(iv)   Nonionic surfactant: Nonionic surfactants have no charge on their hydrophilic head. The hydrophilic group is made of some other very water soluble moiety (a short water soluble polymer chain) rather than charged specie [84,85]. e.g. alkyl polyglycosides, whose hydrophilic head is a sugar moiety. These sugars are just polysaccharides, but they can be made from disaccharides, trisaccharides and various other sugars.

(v)   Amphoteric surfactants: Amphoteric surfactants contain both negative and positive charges on their hydrophilic head groups. They have both acid and alkaline properties, which make them to be stable over a wide pH range. The key functional groups in the chemical structures of the amphoteric surfactants are the more or less quaternized nitrogen and the carboxylic groups [86].

Surfactants are also identified by their high lipophilic balance (HBL). High lipophilic balance or (HLB) number is a parameter that has been assigned to each surfactant molecule. These numbers indicate the solubility of:

a surfactant in a microemulsion. A high HLB number represents a more water a soluble (hydrophilic) surfactant while a lower number represents a more oil a soluble (hydrophobic) molecule [87].

The formation of an emulsion involves the increase in the interfacial areas between the two phases and always accompanied by an increase in the free energy of the system [88]. Hence the ease at which emulsions are formed can therefore 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. The addition of an emulsifying agent which is normally absorbed at the interface lowers the interfacial tension and therefore is usually necessary if a stable emulsion is to be formed. Unlike microemulsions, emulsions do not remain stable indefinitely.

MICELLES

Micelles are very dynamic structures which are formed by loosely bound surface-tant aggregates i.e. they are surfactants at high concentrations. They have lifetimes as short as milliseconds in contrast to emulsion droplets which have long lifetimes. There is equilibrium and a constant exchange between surfactant monomers and surfactant in the micellar aggregation. They are essential for most of the processes that use surfactants as they allow the creation of a hydrophobic domain in aqueous environment [89,90]

COLLOIDS

Colloids are mixtures in which one substance is divided into minute particles (colloidal particles) and dispersed throughout a second substance. Colloidal particles are larger than molecules specifically between 10^{-7} and 10^{-5}cm. 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.

DOUBLE EMULSIONS

Double emulsions are colloidal systems that are formed by the dispersion of one phase into another which are normally immiscible. They are the same as other colloidal systems such as emulsions and microemulsions in the sense that they also consist of two phases. The only difference is in their formulation. They can either be oil-in-water-in-oil (o/w/o) with the dispersed aqueous globules containing smaller dispersed oil droplets or water-in-oil-in-water (w/o/w) with the dispersed oil globules containing smaller aqueous droplets. To formulate a double emulsion, it is necessary to choose, at least two surfactants with a low in HLB number and the other with a high HLB number [91]. The two surfactant used are of different

solubility. Preparation of o/w/o emulsions 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. The 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 emulsion. The opposite applies for o/w/o emulsion systems. 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 [92].

LIQUID CRYSTALS

Under certain conditions, a combination of oil, water and surfactant may result in a phase where there are orderly lanes of oil and water separated by monomolecular layers of surfactants. These results in the formation of liquid crystals [75].Surfactant molecules (eg phospholipids, soaps, detergents and block copolymers) self assemble in selective solvents (eg water) to form bilayer membranes and micelles of several structures which can organize in a variety of patterns.   Such   self assembled structures may be swollen by adding an organic solvent to the system, which remains stable as a clear single phase. Such two-solvent composites are called microemulsions. Repulsive forces between the surfactant head groups organize the bilayers on longer length scales. This process may form lamellar stacks, sponge phases, or microemulsions depending on whether there are one or two solvents. Even more relevant to biomolecular systems is the formation of closed-film structures called vesicles, which are already being employed as simple containers to transport drugs within the blood system.

CO-SURFACTANTS

Cosurfactants are generally low molecular weight alcohols [87]. They are critical in reducing the interfacial tension between the droplets and the continuous phase to near zero. They increase the fluidity of the interface and destroy the liquid crystalline or gel structures that prevent the formation of microemulsions. Single surfactants do not produce best microemulsions, hence there is a need for a cosurfactant.

CHAPTER TWO

EXPERIMENTALS

2. INSTRUMENTS AND REAGENTS

All reagents used were either analytical reagent grade or the purest available comercially and were used without further purification. Measurement of pH was made with the Metrohm 632-pH meter equipped with a combination electrode. The pH electrode was conditioned prior to use in a pH range 4 to 9. A thermospectronic Genesys 2 UV/vis Spectrophotometer was used to obtain spectra and a Genesys 10 UV/vis Spectrophotometer to collect rate data. The pH of the reaction mixture was maintained by adding drops of 0.1M, 0.01M, and 0.001M of NaOH or $HClO_4$ using different marked dotting glass rods in a simple technique referred to as glass stick dotting. The diaqua complexes $[CoN_4 (H_2O)_2]^{3+}$ where $N_4 = en_2$, tn_2 were prepared in solution from the carbonato complexes $[CoCO_3N_4]$ as will be described later. The temperature of the reaction was controlled using a thermostated water bath, which allows the water to circulate via a jacketed reaction vessel as shown in the experimental set up below.

Figure 2.1: Experimental setup

Microemulsions used were prepared using the available literature methods [93] by mixing the chosen surfactant, hexane, butan-1-ol, and titrating the slurry with the required amount of water, agitating mildly to give a clear solution. The following compositions by mass were used:

ME1 H_2O (90%), hexane (2%), SDS or CTAC (20%), butane-1-ol (6%).

ME2 H_2O (82%), hexane (3%), SDS or CTAC (5%), butane-1-ol (10%).

ME3 H_2O (60%), hexane (4%), SDS or CTAC (18%), butane-1-ol (18%).

ME4 H_2O (43.2%), hexane (10.4%), SDS or CTAC (14.8%), butane-1-ol (31.6%).

The ME2 formulation was chosen for this study since previous investigations showed maximum hydrolysis of organophosphates in metal ion catalyzed hydrolysis reactions in ME2 reaction solutions [93]. The substrate model 4-Nitrophenylphosphate used in the reactions were prepared and used in the same day as its chemical composition deteriorates upon standing.

An investigation of all the reagents used in the experiment showed little or no interference in the absorbance region of interest. The absorbance detected was utilized to determine the net absorbance of the reaction aliquots. 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 contained ($1x10^{-3}$M) 4-Nitrophenylphosphate after mixing.

2.1 SYNTHESIS OF SODIUM TRIS-CARBONATOCOBALTATE (III) TRIHYDRATE BY THE BAUER AND DRINKARD METHOD

In an attempt to avoid the difficulties associated with other general methods of cobalt (III) complex compounds, Bauer and Drinkard investigated the utility of a new interme-diate complex of cobalt (III) [94]. The compound which they isolated was represented as sodium tris-carbonatocobaltate (III) trihydrate, $Na_3[Co(CO_3)_3].3H_2O$. This intermediate has the following advantage as a precursor for the preparation of a representative group of cobalt (III) coordination compounds.

(1) The acid form of the ligands may be used instead of the basic form that aid oxidation and absorption of carbondioxide from the surrounding thus increa-sing volatility problems.

(2) The intermediate is very reactive under mild conditions

(3) Conditions for the preparation of this intermediate are not critical or time consuming, and the starting materials are readily available.

(4) The intermediate is very stable on storage, if kept dry and away from UV rays.

(5) Since the acid form of the ligand could be used, the evolution of carbondioxide gas presumably can drive the reaction to completion even under mild conditions.

The intermediate was then synthesized using the available literature method by Bauer and Drinkard.

A 50 mL solution of 29.1g of $Co(NO_3)_2.6H_2O$ (0.10mole) and 10 mL of 30% hydrogen peroxide (excess) was added dropwise with stirring to a cold slurry of 42.0g of sodium bicarbonate (0.50mole) in 50 mL of H_2O. The mixture was allowed to stand at 0^oC for 1hr with continuous stirring. The olive product was filtered, washed on the filter with three 10 mL portions of ice cold water, and then thoroughly washed with absolute alcohol and dry ether.

Yield was 38.33g, % yield = 92.32% of theoretical.

The elemental analysis and other characterization of this intermediate had been performed by Bauer and Drinkard and this is consistent with our investigation. Hence no further characterization was performed. The flow chart depicted below is a schematic representation of the Bauer and Drinkard synthetic pathway.
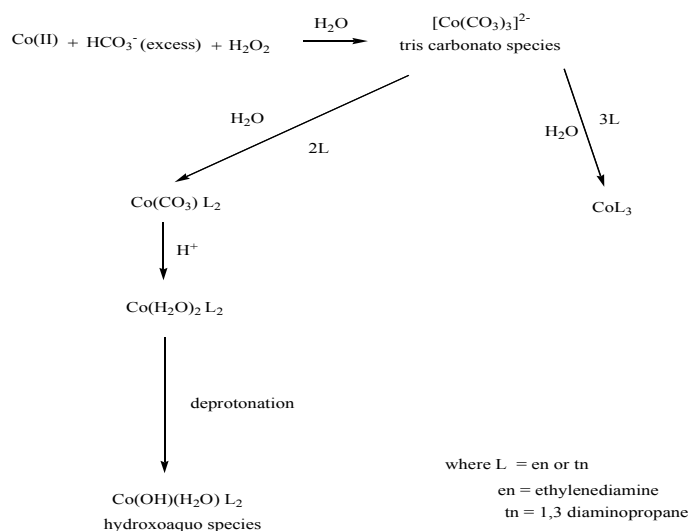
Schematic representation of the Bauer and Drinkard synthetic pathway.

It is important to mention that the number of base equivalents per mole of intermediate is six. Thus, the number of coordination positions per mole is equal to the number of base equivalents per mole. Ligands may be added in their acid form to liberate CO_2 and produce the desired complex. Volatility of CO_2 will drive the reaction to completion. Hence this intermediate was used as a precursor for the preparation of a representative group of cobalt(III) coordination compounds in

which the mole ratio of the intermediate to ligand $(Na_3[Co(CO_3)_3].3H_2O$: Ligand) was 1:2 in the en_2 and tn_2 series and 1:3 in the en_3 and tn_3 series where Ligand = bidentate ligand of ethylenediamine and 1,3diaminopropane. From the above molar relationship, the following cobalt (III) complexes were prepared.

2.2 CONVERSION OF THE CARBONATOCOBALTATE(III) SPECIES TO THE CARBONATOCOBALT (III) SPECIES

(1) Carbonato Tris-ethylenediaminecobalt (III)perchlorate
$[Co(en)_3CO_3)](ClO_4)_3$

A slurry of 3.6g of $Na_3[CoCO_3)_3].3H_2O$ (0.01mole) was mixed with 0.03mole of ethylenediamine and 0.06mole of perchloric acid and warmed in a steam bath for 2 hours. The reagent mixture was then filtered hot and the filtrate was saturated with absolute alcohol to precipitate an orange product which was further recrystallized by placing it in the refrigerator for about 12 hours in an ethanol-water solution.

NOTE: A pronounced improvement in both the quantity and quality of the yield was observed when the reagent mixture was filtered hot and the filtrate was reduced to about 1/3 of its original volume using reduced evaporation technique after which further precipitation and recystallization as mentioned earlier was carried out

Yield = 3.09g,   % yield = 89.1%

(2) Aqua carbonato-bis-ethylenediaminecobalt(III) perchlorate
$[Co(en)_2(CO_3)(H_2O)](ClO_4)$

A slurry of 3.6g of $Na_3[CoCO_3)_3].3H_2O$ (0.01mole) was mixed with 0.02mole of ethylenediamine and 0.04mole of perchloric acid and warmed in a steam bath for 2 hours. The reagent mixture was then filtered hot and the filtrate was saturated with absolute alcohol to precipitate an orange product which was further recrystallized by placing it in the refrigerator for about 12 hours in an ethanol-water solution.

Yield   = 3.12g, % yield = 91%

(3) Carbonato tris-trimethylenediaminecobalt(III) perchlorate
$[Co(tn)_3(CO_3)(ClO_4)_3$

A slurry of 3.6g of $Na_3[CoCO_3)_3].3H_2O$ (0.01mole) was mixed with 0.03mole of 1,3diaminopropane and 0.06mole of perchloric acid and warmed in a steam bath for 2 hours. The reagent mixture was then filtered hot and the filtrate was saturated with absolute alcohol to precipitate an orange product which was further recrystallized by placing it in the refrigerator for about 12 hours in an ethanol-water solution.

Yield = 3.04g,   % yield = 83%

(4) Aquacarbonatobis-trimethylenediaminecobalt(III)perchlorate
[Co(tn)₂(CO₃)(H₂O)](ClO₄)

A slurry of 3.6g of $Na_3[CoCO_3)_3].3H_2O$ (0.01mole) was mixed with 0.02mole of 1,3diaminopropane and 0.04mole of perchloric acid and warmed in a steam bath for 2 hours. The reagent mixture was then filtered hot and the filtrate was saturated with absolute alcohol to precipitate an orange product which was further recrystallized by placing it in the refrigerator for about 12 hours in an ethanol-water solution.

Yield: 3.19g; 85% of theoretical

2.3 CONVERSION OF CARBONATO TO THE DIAQUO SPECIES

The conversion of the carbonato species to the aqua species was achieved by adding 2.5mmol 6M perchloric acid to 1mmol of finely divided carbonato species. The solution was then stirred in an aspirator vacuum for 30mins at 50^0C and the pH adjusted to 7.0.

2.4 PREPARATION OF THE BUFFER SYSTEM

In an unbuffered solution, the acid-base reaction of 4-Nitrophenol changes in concentration resulting in a shift in the equilibrium position and a consequent deviation from Beers law as shown below

4-Nitrophenol

4-Nitrophenolate ion
or
p- Nitrophenolate ion
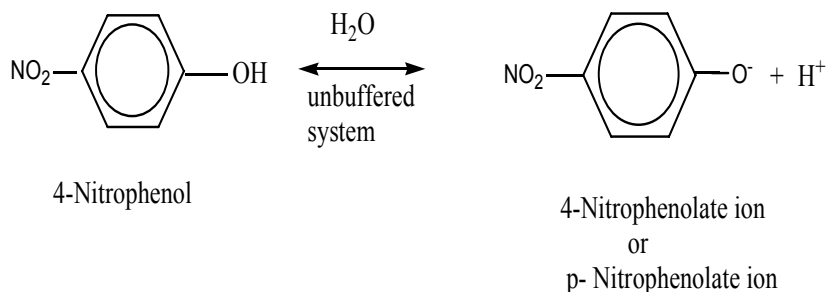
In a buffered system, the pH of the system would be fixed and the ratio of the concentration of the p-nitrophenolate ion to the p-nitrophenol would be constant thereby obeying Beers law. Hence the buffer was used in order to monitor the nitrophenol or nitrophenolate ion production in the system. The buffer system was then prepared by 8.62g sodium phosphate dibasic and 5.42g of sodium phosphate monobasic salts and making up to 1000 mL with carbondioxide free distilled water.

2.5 Preparation of Cu(II) enzyme model

In phosphate ester complexation, the effectiveness of Cu(II) as a catalyst in the series of aquo complexes cannot be explained simply by degree of complex

formation alone. The reason for the effective hydrolysis of phosphate esters by Cu^{2+} probably results from the low pK_a of water bound to Cu^{2+}, of which none of the metal aquo complexes are deprotonated to a significant extent at neutral pH. As observed for $[Cu(bpy)^{2+}]$ vs $[Cu(bpy)(OH)(OH_2)]^+$, the hydrolysis by a metal hydroxide complex has been reported [95] to occur faster than hydrolysis by a metal aquo complex. Also reasonable formulation for a Cu(II) hydroxide – phosphor ester intermediate is that it has hydroxy and phosphate ester ligands bound cis to $[Cu(bpy)^{2+}]$. The copper (II) complex plays a bifunctional role of effecting hydrolysis by the electrophilic activation of the coordinated phosphate ester and by providing a cis proximity for the hydroxide nucleophile. Lastly, $Cu(bpy)^{2+}$ unlike other metal complexes, exchanges aquo ligands rapidly on the time scale of hydrolysis, thus enhancing the possibility of both inter and intra molecular hydrolyses.

The following copper complexes are present in an aqueous solution of Cu^{2+} and 2,2'-bipyridine in a 1:1 ratio $K_o = k_1 [Cu(bpy)^{2+}] + k_2 [Cu(bpy)(OH)]^+ + k_3 [Cu(bpy)_2^{2+}] + k_4 [Cu(bpy)(OH)_2^{2+}] + K_5 [Cu^{2+}]$. The equilibrium concentrations of these copper complexes at various 2,2' bipyridine to copper ratios are listed in table 2.1 below. At a 1:1 ratio of Cu^{2+} to 2,2'-bipyridine (5×10^{-5}M, pH 7.85),the hydrolysis of a phosphate diester by Cu^{2+} ($k_5 = 5.0 \times 10^{-3}$M^{-1}s^{-1}, or by $Cu(bpy)^{2+}$ ($k_1 = 1.9 \times 10^{-3}$M^{-1}s^{-1}).

Table 2.1: Effects of concentration (M) of 2,2'-Bipyridine on the Concentrations (M) of copper (II) Complexes in Water at 75°C [95].

[bpy]	[Cu^{2+}]	[Cu(bpy)$^{2+}$]	[Cu(bpy)$_2^{2+}$]	[Cu(bpy)(OH)$^+$]	[Cu(bpy)(OH)$_2^{2+}$]
2.5 X 10^{-5}	2.5 X 10^{-5}	1.5 X 10^{-6}	1.1 X 10^{-9}	9.1 X 10^{-6}	7.2 X 10^{-6}
5.0 X 10^{-5}	6.7 X 10^{-7}	2.2 X 10^{-6}	9.3 X 10^{-8}	1.4 X 10^{-5}	1.6 X 10^{-5}
1.0 X 10^{-4}	7.5 X 10^{-7}	2.1 X 10^{-6}	7.1 X 10^{-6}	1.3 X 10^{-5}	1.4 X 10^{-5}
2.0 X 10^{-4}	2.0 X 10^{-9}	1.7 X 10^{-6}	1.8 X 10^{-5}	1.1 X 10^{-5}	9.8 X 10^{-6}

bpy = 2,2'-bipyridine; pH 7.85; $[Cu^{2+}] = 5.0 \times 10^{-5}$M.

Hence in a hydrolysis of a phosphate ester effected by $[Cu(bpy)^{2+}]$ and $[Cu(bpy)(OH)^+]$

$$K_o = k_1[Cu(bpy)^{2+}] + k_2[Cu(bpy)(OH)^+]$$

Therefore,

$$k_o = \left(\frac{k_1[H^+] + k_2 k_a}{[H^+] + k_a} \right) [Cu(bpy)^{2+}]_T$$

where $[Cu(bpy)^{2+}]_T$ is the total concentration of copper complex, k_a is the acid dissociation constant for the coordinated water molecule, k_2 is the second-order rate constant for $[Cu(bpy)(OH)^+]$ mediated hydrolysis of the phosphate ester.

The relationship between equilibrium constant and standard free energy change of the reaction is given by the equation:

$$\Delta G^o = -RT \ln K_{eq} \ldots\ldots\ldots\ldots(1)$$

where ΔG^o = standard free energy change
K_{eq} = equilibrium constant
T = temperature.

Also the standard free energy change ΔG^o upon reaction is related to both the standard enthalpy change ΔH^o and the standard entropy change ΔS^o by the equation at constant temperature

$$\Delta G^o = \Delta H^o - T\Delta S^o \ldots\ldots\ldots\ldots (2)$$

Although the standard free energy change ΔG^o is dependent on temperature (eq 1 & 2), the standard enthalpy change upon reaction are not temperature dependent (or at least not very much so). Hence the enthalpy and entropy changes are regarded as constants with respect to temperature.

The relative change in equilibrium constants as a function of temperature is expressed by the Van't Hoff equation

$$\ln (k_2/k_1) = -\Delta H^o / R (1/T_2 - 1/T_1) \ldots\ldots(3)$$

Hence the relative changes in the values of the equilibrium constants for the reactions at 75°C (348 K) and 25°C (298 K) is minimal under our experimental conditions. It is with this valid assumption that we were able to estimate the equilibrium concentrations of the different copper complexes in the various solutions.

The equilibrium concentration of the active species was multiplied by a factor of 2000 to conform to experimental protocol at 25 ^{o}C or 298 K. Hence the following estimated equilibrium concentrations of all the species were now presumed to exist in the reaction solution,

$[Cu (bpy) (H_2O)_2]^{2+}$ ……………… 4.4×10^{-3}M
$[Cu (bpy) (OH) (H_2O)]^{+}$……………$2.8 \times 10^{-2}$M
$[Cu (bpy)_2]^{2+}$………………………1.86×10^{-6}M
$[Cu (bpy) (OH)_2]^{2+}$…………………3.2×10^{-2}M
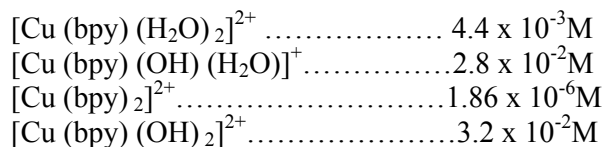
2.6 Preparation of stock solution of 2.8×10^{-2}M $[Cu(bpy)(OH)(H_2O)]^{+}$

A stock solution of the $[Cu (bpy) (OH) (H_2O)]^{+}$ was prepared by mixing 0.0669g (2.68×10^{-4}moles) of $CuSO_4.5H_2O$ with 3.1238g (0.02moles) of 2, 2'-bipyridine in a 250 mL beaker with 170 mL of the solvent. The solvent used in this case were

water or either of the microemulsions (anionic or cationic). The mixture was stirred continuously for about 15 mins with the temperature maintained at 75°C. The mixture was now made up to 200 mL with the appropriate solvent still at 75 °C and the pH adjusted to 7. Several dilutions of 2.8 x 10^{-3}M, 2.8 x 10^{-4}M, 2.8 x 10^{-5}M and 2.8 x 10^{-6}M were then made and these were used as the working standard stock solution.

2.7   PROTOCOL OF THE STUDY

The protocol of the study involves mixing 2mls of the 5×10^{-3}M NPP solution in a thermostated reaction vessel equilibrated at 25°C with 6 mL of the solvent (water or microemulsion) and the mixture was stirred continuously for about 15 mins. The pH of the solution was then adjusted to 7.0**.** A 2 mL volume of temperature equilibrated metal ion solution (5×10^{-3} M) pH 7.0 hydroxoaqua form predominant $[Cu(bpy)(OH)(H_2O)]^+$ for the catalytic experiments or $[N_4Co(OH)(H_2O)]^+$ for the stoichiometric experiments) was added into the mixture making the total volume to be 10 mL, resulting in a 1×10^{-3} M for the mixture. Reaction aliquots (1 mL) were drawn from the reaction vessel at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 5.0, 10.0, 15.0, 25.0 and 30.0 min respectively and mixed with 1 mL of phosphate buffer (pH 7.4) and 3 mL of water bringing the total volume to 5 mL. The absorbance of the reaction solution at each of the times was measured at 400m. The amount of nitrophenolate ion produced was determined from a calibration curve and the % hydrolysis was calculated assuming 100% nitrophenolate production for complete hydrolysis. Calibration curves were prepared from a series of 4-nitrophenol solutions ranging from a concentration of 1.0 x 10^{-3}M to 1.0 x 10^{-6}M and the pH adjusted to 6.5. One mL of this solution was mixed with 1mL of phosphate buffer and 3mL of water and its absorbance measured at 400nm. The absorbance values were plotted against concentration and the molar absorptivity determined as 4.925 x 10^3LMolcm^{-1} at 400nm. Triplicate analysis was done for each system. The average values were used for analysis of the data, all absorbances were taken against 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 400nm, quite distinct from NPP which has an absorbance maximum at 310nm as depicted in fig 1.1 in 1.8.2 pg 12

CHAPTER THREE

RESULTS AND DISCUSSION

3   RESULTS AND DISCUSSION

The results of the investigation are provided in the tables below and are the average values of the triplicate studies of the hydrolytic effects of the cobalt amine $[CoN_4(OH)(H_2O)]^{2+}$ and $[Cu(bpy)(OH)(H_2O)]^+$ complexes in the aqueous and microemulsion media.

$$[N_4] = (en) = \text{ethylenediamine}, (tn) = 1, 3 \text{ diaminopropane}$$

Table 3.1: Percentage hydrolysis of 4-nitrophenylphosphate by CoL_3 and $[CoL_2 (OH)(H_2O)]^{2+}$ complexes at different NPP to metal ratio in the different reaction media [10^{-3}M, 25^oC, pH 7.0]

SYSTEM	Metal : NPP Ratio	MEDIUM	% 4-NITROPHENOL PRODUCED WITH TIME						
			1 min	3 min	5 min	10 min	15 min	25 min	30 min
Co(en)₃ + NPP	1:1	WATER	0.508	0.629	0.670	0.668	0.668	0.665	0.664
2Co(en)₃ + NPP	2:1	WATER	0.809	0.930	0.970	0.968	0.965	0.963	0.963
3Co(en)₃ + NPP	3:1	WATER	1.009	1.13	1.17	1.15	1.15	1.14	1.13
Co(en)₃ + NPP	1:1	AME2	0.804	0.925	0.967	0.969	0.968	0.968	0.964
2Co(en)₃ + NPP	2:1	AME2	1.109	1.245	1.264	1.265	1.263	1.260	1.260
3Co(en)₃ + NPP	3:1	AME2	1.714	1.699	1.699	1.698	1.695	1.695	1.694
Co(en)₃ + NPP	1:1	CME2	1.304	1.425	1.468	1.469	1.468	1.467	1.463

SYSTEM	Metal : NPP Ratio	MEDIUM	% 4-NITROPHENOL PRODUCED WITH TIME						
			1 min	3 min	5 min	10 min	15 min	25 min	30 min
2Co(en)$_3$ + NPP	2:1	CME2	1.608	1.864	1.866	1.865	1.865	1.864	1.864
3Co(en)$_3$ + NPP	3:1	CME2	2.214	2.422	2.482	2.481	2.480	2.480	2.480
Co(en)$_2$ + NPP	1:1	WATER	7.09	7.09	7.11	7.10	7.10	7.09	7.09
2Co(en)$_2$ + NPP	2:1	WATER	11.11	11.11	11.13	11.11	11.11	11.10	11.09
3Co(en)$_2$ + NPP	3:1	WATER	12.60	12.63	12.66	12.64	12.64	12.63	12.62
Co(en)$_2$ + NPP	1:1	AME2	7.34	7.40	7.41	7.43	7.41	7.40	7.40
2Co(en)$_2$ + NPP	2:1	AME2	11.60	11.63	11.65	11.63	11.62	11.62	11.60
3Co(en)$_2$ + NPP	3:1	AME2	14.40	14.42	14.44	14.44	14.42	14.42	14.41
Co(en)$_2$ + NPP	1:1	CME2	13.82	13.93	13.95	13.93	13.93	13.91	13.90
2Co(en)$_2$ + NPP	2:1	CME2	17.87	17.96	17.98	17.97	17.96	17.94	17.92
3Co(en)$_2$ + NPP	3:1	CME2	19.75	19.74	19.88	19.85	19.84	19.82	19.82
Co(tn)$_3$ + NPP	1:1	WATER	1.02	1.08	1.12	1.09	1.09	1.06	1.06
2Co(tn)$_3$ + NPP	2:1	WATER	3.12	3.19	3.27	3.21	3.19	3.19	3.19
3Co(tn)$_3$ + NPP	3:1	WATER	4.56	4.68	4.70	4.65	4.65	4.63	4.63
Co(tn)$_3$ + NPP	1:1	AME2	1.99	2.05	2.08	2.03	2.03	2.02	2.02
2Co(tn)$_3$ + NPP	2:1	AME2	4.12	4.19	4.27	4.22	4.21	4.20	4.20
3Co(tn)$_3$ + NPP	3:1	AME2	4.56	4.68	4.70	4.69	4.67	4.65	4.63

SYSTEM	Metal : NPP Ratio	MEDIUM	% 4-NITROPHENOL PRODUCED WITH TIME						
			1 min	3 min	5 min	10 min	15 min	25 min	30 min
Co(tn)₃ + NPP	1:1	CME2	2.14	2.44	2.84	2.80	2.79	2.78	2.78
2Co(tn)₃ + NPP	2:1	CME2	4.65	4.81	4.83	4.83	4.82	4.80	4.80
3Co(tn)₃ + NPP	3:1	CME	5.69	5.89	5.91	5.90	5.90	5.89	5.89
Co(tn)₂ + NPP	1:1	WATER	16.91	16.99	17.02	16.99	16.98	16.96	16.95
2Co(tn)₂ + NPP	2:1	WATER	20.18	20.28	20.30	20.28	20.26	20.26	20.26
3Co(tn)₂ + NPP	3:1	WATER	21.24	21.30	21.34	21.28	21.27	21.27	21.26
Co(tn)₂ + NPP	1:1	AME2	19.32	19.46	19.48	19.46	19.46	19.46	19.45
2Co(tn)₂ + NPP	2:1	AME2	22.59	22.70	22.75	22.73	22.73	22.72	22.71
3Co(tn)₂ + NPP	3:1	AME2	23.70	23.74	23.75	23.72	23.72	23.71	23.70
Co(tn)₂ + NPP	1:1	CME2	30.58	30.82	30.84	30.82	30.82	30.80	30.80
2Co(tn)₂ + NPP	2:1	CME2	46.52	46.60	46.64	46.62	46.60	46.60	46.59
3Co(tn)₂ + NPP	3:1	CME2	50.80	51.03	51.07	51.04	51.03	51.02	51.02

Table 3.2: Percentage hydrolysis of 4-nitrophenylphosphate for the reaction of various concentrations of $[Cu\,(bpy)\,(OH)(H_2O)]^+$ complexes with (5.0×10^{-3}M) NPP in the different reaction media [10^{-3}M, 25^oC, pH 7.0]

[ ]	Cu : NPP Ratio	MEDIUM	% NITROPHENOL PRODUCED WITH TIME IN MINUTES						
			0	1	2	5	10	15	30
2.8×10^{-2}	5:1		0	10.58	20.18	33.30	48.7	55.10	60.01
2.8×10^{-3}	1:2		0	18.50	28.90	42.40	56.06	61.01	64.5
2.8×10^{-4}	1:20	WATER	0	35.30	46.50	56.30	62.60	65.20	69.01
2.8×10^{-5}	1:200		0	8.52	12.50	15.50	19.40	23.00	32.30
2.8×10^{-6}	1:2000		0	5.43	7.40	9.70	12.21	14.23	17.02
2.8×10^{-2}	5:1		0	15.92	36.00	47.81	54.20	58.50	61.02
2.8×10^{-3}	1:2		0	34.40	56.33	64.30	70.42	73.21	77.10
2.8×10^{-4}	1:20	AME2	0	55.01	71.20	78.04	83.72	86.21	89.60
2.8×10^{-5}	1:200		0	44.73	62.01	71.20	76.91	79.30	83.02
2.8×10^{-6}	1:2000		0	23.70	48.20	57.32	64.00	67.01	70.02
2.8×10^{-2}	5;1		0	39.25	56.90	66.91	69.66	72.20	73.19
2.8×10^{-3}	1:2		0	52.14	67.30	76.41	80.02	81.30	81.39
2.8×10^{-4}	1:20	CME2	0	53.14	75.01	82.80	86.50	88.80	90.91
2.8×10^{-5}	1:200		0	22.33	40.40	46.41	48.35	50.22	50.60
2.8×10^{-6}	1:2000		0	18.02	33.05	37.50	40.33	42.65	42.96

All the six coordination sites of cobalt are presumed to be fully occupied by the bidentate ligands of either en or tn in the CoL_3 moieties. Hence no hydrolytic reaction is expected in this system. In the $Co(H_2O)L_2$ moieties, it is assumed that four of the six coordination sites of cobalt have been occupied by two of the bidentate ligands, thus allowing the remaining two sites to still be occupied by water. Hence the

(a)   Diaqua species

(b)   The hydroxoaquo species and

(c)   The dihydroxo species exist in the solution depending on the pH of the solution.

The identification of the active absorbing intermediate species in the $CoL_2(H_2O)_2$ moieties in the solution was necessary in order to monitor the hydrolytic path of the reactions and to identify which of the binding modes was responsible for the observed rate enhancements. Spectrophotometric studies of the Co $(H_2O)L_2$ complexes was then carried out to identify the active absorbing species in the reaction solution. The UV/vis spectra of the metal complexes at pH 4, 5, 6, 7, and 8 are depicted in the figure 3.1 below.
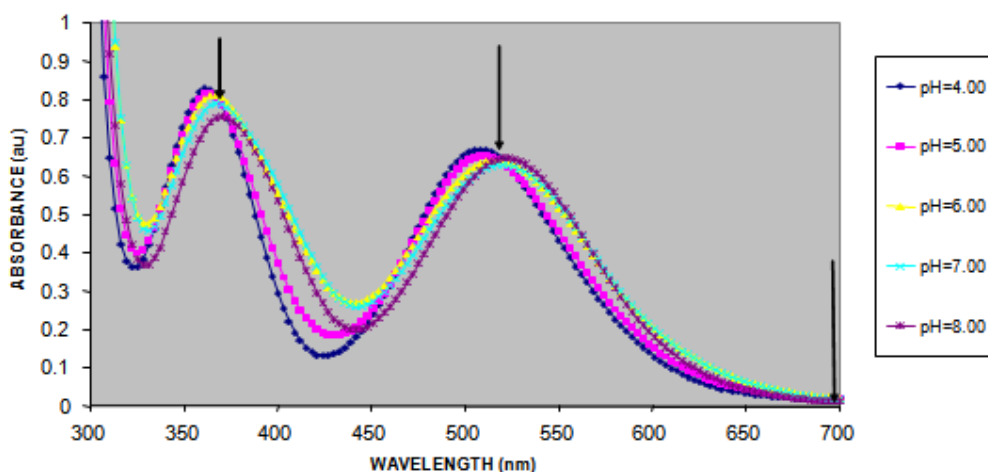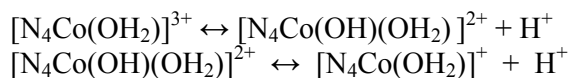
Fig 3.1: UV-vis absorption spectra of $[Cotn_2(OH)(H_2O)]^{2+}$ at 10^{-2}M Concentration at various pH values

The UV-vis absorption spectra in the figure 3.2 above demonstrate the identification of the presumed absorbing species in the enzyme model at pH 7.0 under our experimental conditions and the following assumptions are possible.

(i)   The predominance of the hydroxoaquo species in the pH range of 4 – 8 was noted by the presence of isobestic points in the UV/vis spectral studies of the enzyme model Co (III) complexes.

(ii)   No isobestic point or points were observed at pHs below 4 and above 8

(iii)   The existence or presence of an isobestic point or points is often considered proof of the existence of two absorbing species in the analyzed solution [96,97]. Hence the presence of both the hydroxoaqua and the diaqua species is predicted to prevail between the pH ranges of 4 – 6 and the hydroxoaqua and dihydroxo species between the pH ranges of 6 – 8

(iv)   It is presumed that at pH around 6.5 to 7.0, the hydroxoaqua species is predominant. The values for pk_1 and pk_2 as determined by potentiometric titration under conditions where cis-trans isomerization had proceeded to equilibrium are 9.0, 12.1, 4.98, and 7.2 for en_2 and tn_2 complexes respectively. Diaquabis(ethylenediamine)cobalt(III) in solution will be a mixture of cis and trans isomers as reported by previous investigators [98]. However, diaquabis(trimethylenediamine)cobalt(III) will prevail as cis isomers in solution due to fast cis-trans isomerization ($t_{1/2}$ = 1sec at 25°C) under the experimental conditions Literature values of pk_1, 4.98 and pk_2, 7.22 for $[Cotn_2(H_2O)_2]^{3+}$ support the above assumption [99]. i.e. the pH

corresponding to the rate maxima are all between the pk_1 and pk_2 of the acid-base equilibrium for the cobalt complex, indicating that the aquahydroxo form is the active species that promotes the hydrolysis. The enhanced reactivity for the hydroxoaqua ions in comparison to the dihydroxo or diaquo species was noted for the system and this is in line with literature reports [100]. The reactivity of the hydroxoaqua complex has been attributed to the presumption that the electron freed from the diaqua complex by loss of a proton to form the hydroxoaquo moiety will labilize the coordinated water [101] as demonstrated in the acid-base equilibrium shown below. The diaqua, aquahydroxo and dihydroxo tetraamine cobalt (III) complexes are related by the acid-base equillibria shown below.

$$[N_4Co(OH_2)]^{3+} \leftrightarrow [N_4Co(OH)(OH_2)]^{2+} + H^+$$
$$[N_4Co(OH)(OH_2)]^{2+} \leftrightarrow [N_4Co(OH_2)]^{+} + H^+$$

This leads to the weakening of the Co – H_2O bond and in effect forcing the coordinated water to leave thus favouring the coordination of the O donor ligand like NPP. Hence at pH around 7.0, the $[N_4Co\ (OH)\ (H_2O)]^{2+}$ intermediate complex and NPP react to form a cis hydroxo monodentate – NPP species as shown in the reaction mechanism below. It has been proposed in previous investigations [102-104] that the mechanism of the hydrolysis involves the coordination of the phosphate ester to the cobalt complex followed by intramolecular metal hydroxide attack on the coordinated phosphate to produce 4-nitrophenol and phosphate derivative.
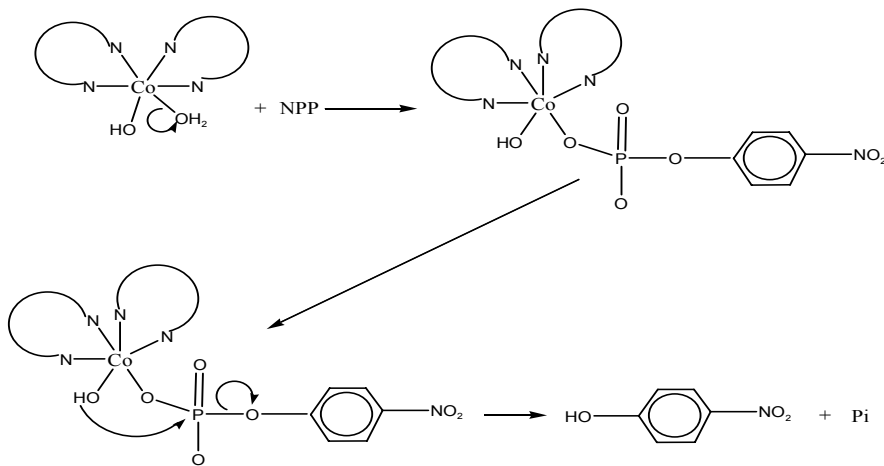
Plausible mechanism of the reaction between cis $[N_4Co\ (OH)\ (H_2O)]^{2+}$ and NPP to form the cis – hydroxomonodentate – NPP species which undergoes hydrolysis to form phenol and the phosphate derivative

The observed rise in the pH of the reaction reflects the second step which involves the intramolecular attack by the coordinated hydroxide on the phosphorous centre thereby promoting the hydrolysis of the NPP.

3.1 Effects of the Coen$_3$ and [Coen$_2$(OH)(H$_2$O)]$^{2+}$ complexes on the Hydrolysis of NPP

The reaction profiles depict the plot of percent hydrolysis versus the reaction time of between 0 – 30 minutes in the [Co(en$_3$)] and [Co(en$_2$)(OH)(H$_2$O)$^{2+}$] complexes in the aqueous, anionic and cationic microemulsion reaction media respectively.
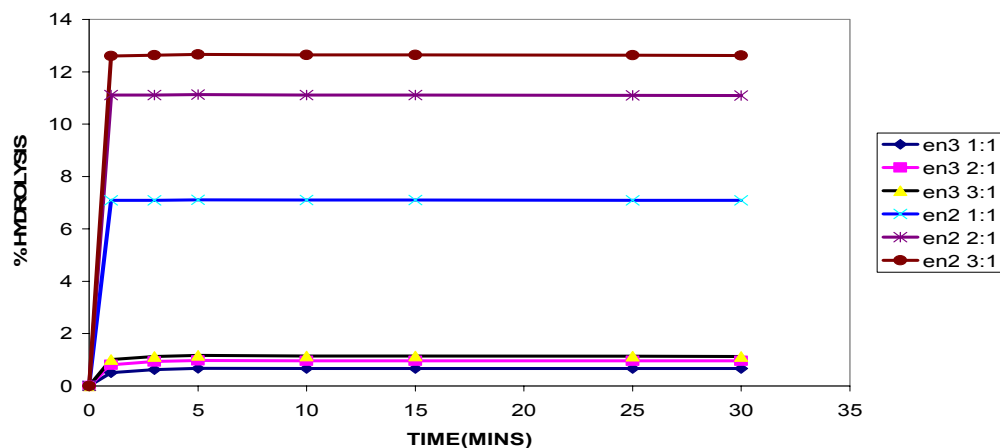
Fig 3.2: Reaction profile for the hydrolysis of NPP (10^{-3}M after addition) promoted by [Co(en$_2$)(OH)(H$_2$O)]$^{2+}$and [Co(en$_3$)] complexes at 1:1, 2:1 and 3:1 cobalt to NPP ratio at pH 7.0 and 25°C in aqueous media.
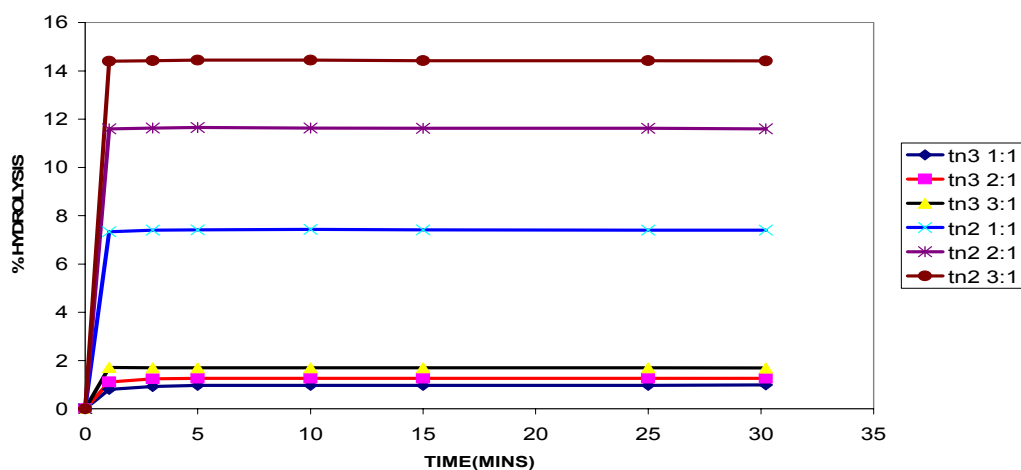
Fig 3.3: Reaction profile for the hydrolysis of NPP (10^{-3}M after addition) promoted by [Co(en$_2$)(OH)(H$_2$O)]$^{2+}$and[Coen$_3$] complexes at 1:1,2:1 and3:1 cobalt to NPP ratio at pH 7.0 and 25°C in anionic microemulsion media.

Fig 3.4: Reaction profile for the hydrolysis of NPP (10^{-3}M after addition) promoted by $[Co(en_2)(OH)(H_2O)]^{2+}$ and $Co(en_3)$ complexes at 1:1,2:1 and 3:1 cobalt to NPP ratio at pH 7.0 and 25^oC in cationic microemulsion media.

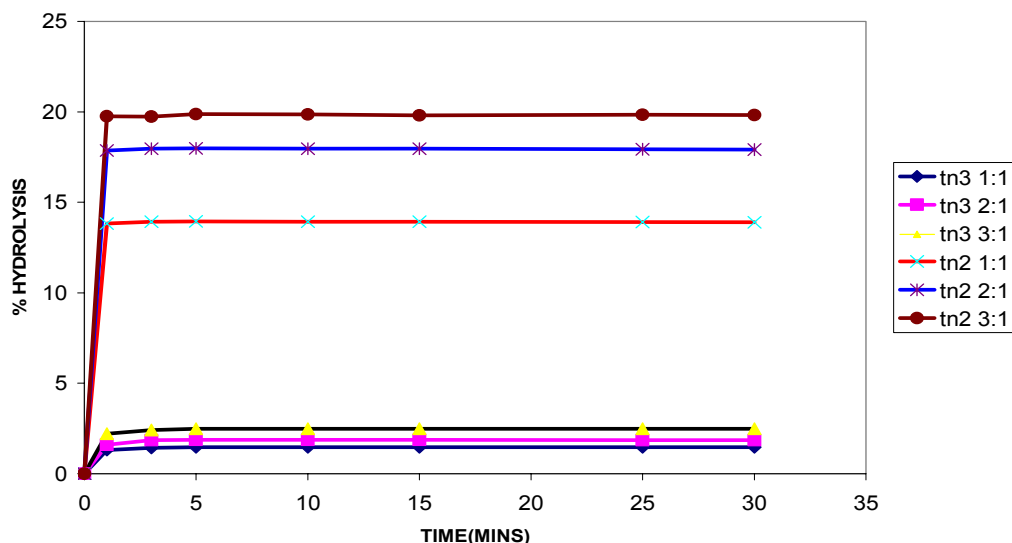(1) The production of the nitrophenolate ion appears to be biphasic as depicted in all the figures. There was an initial burst within 0.5 minute which levels off dramatically after that. It was not possible to investigate the reaction profile between the time of addition (0 time and 0.5 minutes) under the experimental protocol.

(2) The effects of the concentration of metal ion to phosphate ratio were also observed in all the reaction profiles depicted. An enhancement of hydrolysis was noted in the reaction solutions that contained 2:1 metal to phosphate ratio. This can be explained by the fact that the second metal ion available in the solution coordinates to one of the available oxygen's on the NPP thereby creating a possibility of intramolecular hydrolytic attack on the phosphorous centre by the coordinated water or hydroxide of the metal ion. No significant additional hydroysis was observed for reaction solutions that contained more than 3:1 metal to NPP ratio.

Since the rate increase observed in the reaction was attributed to stoichiometric reasons and not due to any catalytic reasons, it was anti-cipated that with an increase in the metal to phosphate ratio, the rate of hydrolysis would increase. However, above 3:1 metal to phosphate ratio, the concentration of the hydroxoaqua species in the system becomes large presumably triggering an olation reaction resulting in the production of unreactive dimeric species in the solution which in effect slows down the rate of the reaction accordingly as has been reported in similar studies [105,106]. In addition, a simple phosphate like NPP is liable to undergo condensation reactions at high metal concentrations as has been reported also [107]. Generally, the absence of reactive hydroxoaqua moieties ex-plains the rate saturation in systems containing large metal to phosphate ratios.

(3) An increase in hydrolysis was noted in microemulsion media compared to that in aqueous media. The anionic micelle data is more or less similar to that of the aqueous media. Possible explanations to the above include enhanced substrate solubilization in the microemulsion systems thereby aiding hydrolysis by bringing reacting species closer to each other. Microemulsions are thermodynamically stable transparent dispersions of two immiscible liquids, stabilized by an artificial film of surfactants. Each of the microemulsion components has its own function that makes the system very useful.

Water acts as the solvent and hexane suspended as microdroplets serve as a residence for the water insoluble reactants. The surfactant (SDS or CTAC) absorbs to the hexane/water interface and stabilizes the droplets. The presence of the co-surfactant is critical in reducing the interfacial tension between the droplets and the continuous phase to near zero.

Cosurfactants also have the ability to solubilize and disperse the maximum amount of the substrate thereby enhancing the possibility of hydrolysis by the metal centers.

Microemulsions consist of too many tiny droplets which are continuously diffusing through the continuous phase, thus leading to collision between the droplets. These collisions are presumably elastic because they result in the exchange of materials between the droplets which results in an increase in the surface area at the interfacial boundary thus enhancing the maximum solubilization and partitioning of the reactants, thereby increasing the possibility of hydrolysis by the metal ion. Their hydrophilic (water soluble) and a hydrophobic (oil soluble) interface tends to enhance the proper mixing of the polar and non polar reactants at the interfacial boundary thus increasing the rate of the reaction.

(4) If one just looks at the 0.5 to 1 minute data in all the figures, it is clear that there is roughly a 2-fold increase in nitrophenolate production with the cationic micelle (fig 3.4) compared to what is seen in the aqueous media (fig 3.2). This is presumably due to the fact that cationic microemulsion systems result in co-aggregates where the net cationic character is increased thereby increasing the positive character on the phosphate moiety. The coordinated nucleophile (water or hydroxide) will then have a greater possibility of attack on the phosphorous centre compared to those in anionic microemulsion co-aggregates. Also on electrostatic grounds, the positive counter ion which a cationic microemulsion produces in solution presumably will readily attract the negatively charged 4-Nitrophenylphosphate, but the negatively charged anionic microemulsion would repel the already negatively charged 4-Nitrophenylphosphate. The net result is that in the anionic microemulsion systems, one might not expect much enhancement of hydrolysis as

compared to the cationic microemulsions. Overall anionic microemulsion system showed enhanced rates of hydrolysis compared to those in aqueous media.

3.2 Effects of the Co(tn$_3$) and [Co(tn$_2$)(OH)(H$_2$O)] $^{2+}$ complexes on the Hydrolysis of NPP.

The results of the percent hydrolysis of NPP by the [Cotn$_3$] and [Cotn (OH)(H$_2$O)$^{2+}$] complexes were also plotted against the reaction time in all the media investigated as depicted in the reaction rate profiles below.
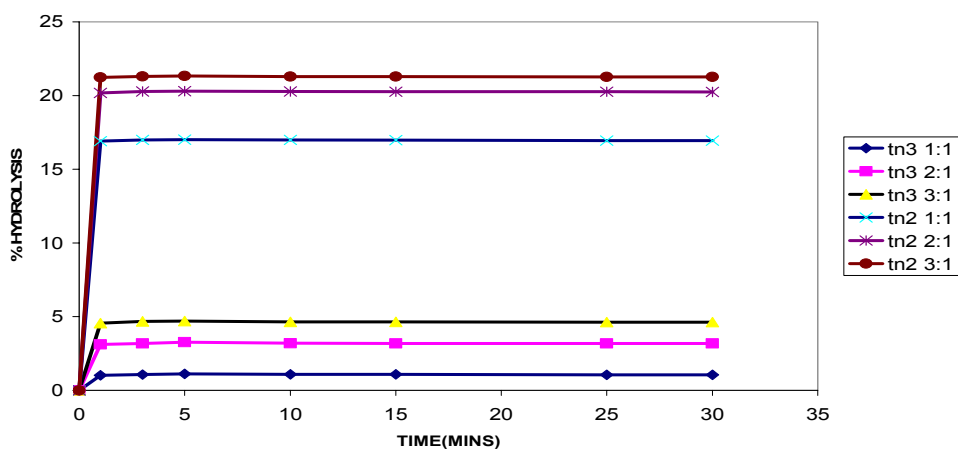
Fig 3.5: Reaction profile for the hydrolysis of NPP (10^{-3}M after addition) promoted by [Co(tn$_2$)(OH)(H$_2$O)] $^{2+}$and Cotn$_3$ complexes at 1:1, 2:1 and 3:1 cobalt to NPP ratio at pH 7.0 and 25°C in aqueous media.
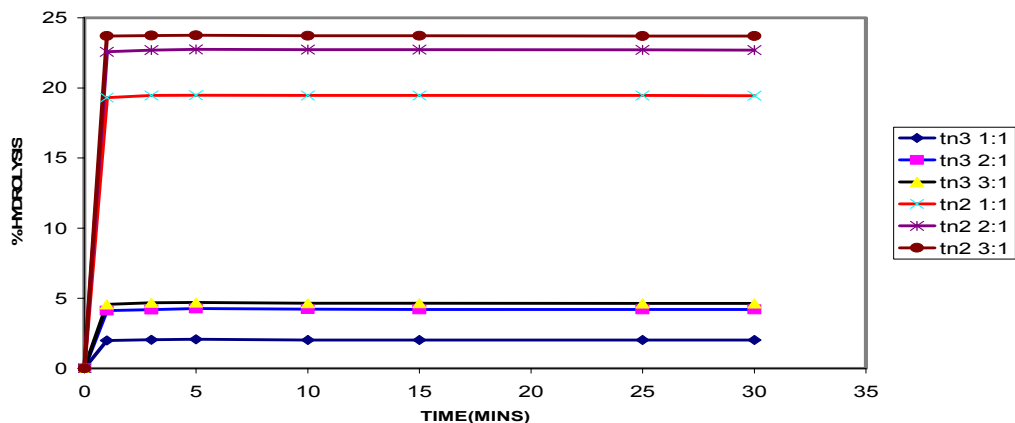
Fig 3.6: Reaction profile for the hydrolysis of NPP (10^{-3}M after addition) promoted by [Co(tn$_2$)(OH)(H$_2$O)]$^{2+}$and Cotn$_3$ complexes at 1:1, 2:1 and 3:1 cobalt to NPP ratio at pH 7.0 and 25°C in anionic microemulsion media.

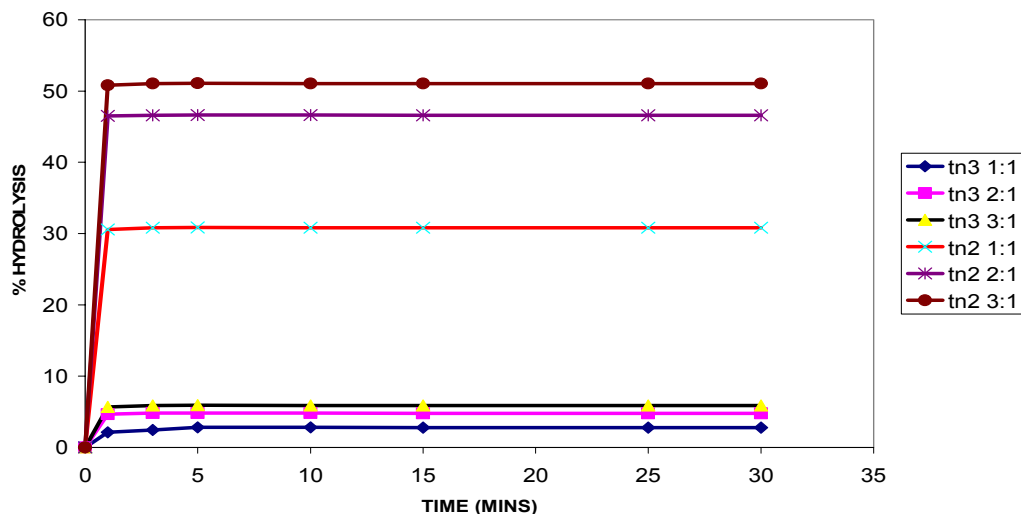**Fig 3.7:** Reaction profile for the hydrolysis of NPP (10^{-3}M after addition) promoted by $[Cotn_2(OH)(H_2O)]^{2+}$ and $Cotn_3$ complexes at 1:1, 2:1 and 3:1 cobalt to NPP ratio at pH 7.0 and 25°C in cationic microemulsion media.

(1) As observed in all the systems studied, the chelate ligands of tn_3 did not show very appreciable rate enhancements. This could be attributed to the fact that the chelate ligands of tn_3 form stable complexes with cobalt making it unavailable for coordination with the phosphate moiety for further reaction leading to hydrolysis. The small enhancement observed could be attributed to impurities of some tn_2 species in solution as the ligand sequentially coordinates to the metal to form the tn_3 moiety.

(2) $[Cotn_2(H_2O)(OH)]^{2+}$ continued to show an increase in hydrolysis compared to the $[Coen_2(H_2O)(OH)]^{2+}$ as observed in the rate profiles in figures 3.5, 3.6 and 3.7 respectively. This could be attributed to the fact that the greater the electron donating inductive effect of the coordinated ligands, the greater the electron density on the metal cobalt which in turn increases the positive character at the phosphorous centre thus making it very susceptible to a nucleophilic attack by either the coordinated water or hydroxide. The three methylene group in trimethylenediamine makes its electron donating inductive effect to be higher than that of ethylenediamine which has only two methylene groups.

The enhanced reactivity of the $[tn_2Co(OH_2)_2]^{3+}$ over $[en_2Co(OH_2)_2]^{3+}$ could also be attributed to the activation of the nitrophenylphosphate substrate in a strained chelate due to the fact that it is labile and can exhibit fast reactions such as aquation and isomerization depending on the nature of the ligands.. Similar influences of N_4 on substitution of water in $[Co(N_4)(OH)(H_2O)]^{2+}$ ions have also been reported in the literature [108].

The order of effectiveness of the N_4 ligands in Co (III) N_4 (aq) complexes at pH 7.0 is $tn_2 > en_2 > tn_3 > en_3$ towards hydrolysis of organophosphate simulants parrarels the water substitution rates in the complexes.

It can therefore be concluded that for Co (III) N_4 (aq) promoted hydrolysis, it seems likely that there would be closely related influences of the N_4 ligands on the rates of both Co (III) (N_4) substitution and Co (III) (N_4) promoted hydrolysis reactions as observed in the reaction profiles depicted above. This would suggest the greater the tendency for the Co-OH$_2$ bond to break in [Cotn$_2$ (H$_2$O) (OH)] $^{2+}$ complexes compared to those of [Coen$_2$ (H$_2$O) (OH)] $^{2+}$ thus effecting hydrolysis.

(3) An increase in hydrolysis was also noted in microemulsion media compared to that in aqueous media and the cationic microemulsion consistently showed good rate accelerations in terms of their hydrolyzing abilities towards p-nitrophenylphosphates. We presume that this rate enhancements could also be attributed to the solubilization properties of this media as has been described already.

The rate enhancements observed in the cobalt (III) amine complexes (enzyme model) on the hydrolysis of 4 – Nitrophenylphosphate (substrate model) could be attributed to stoichiometric reasons as the rate enhancements were observed only as the metal ion to NPP ratios were increased from 1:1, 2:1 and 3:1 molar ratios. The highest rate enhancement of about 52% was observed in the 3:1 metal to NPP ratio. This implies that more than one application or subsequent multiple applications would be required to attain 100% hydrolysis of the substrate. In effect, large volume of fluid that might be generated and the large amount of the metal complexes that would be used in such multiple application could result in some logistical challenges in which contaminated runoffs from this decontamination procedure could lead to undesirable additional environ-mental pollution problems. Also since the observed rate enhancements were attributed to stoichiometric reasons, this model system could not be deployed rapidly. The need for a model system that would

(1)   Be more efficient i.e. a system that would attain 100 % hydrolysis of the substrate at one application

(2)   May require less logistical support

(3)   Have minimal runoffs of fluid and metal complexes no lasting impact on the environment i.e. a system that is environmentally friendly and

(4)   A system that can be deployed rapidly was envisaged using the same protocol of study. Hence the choice for [Cu(bpy)(H$_2$O)]$^{2+}$ as an enzyme model.

The anticipated outcome of the use of [Cu(bpy)(OH)(H$_2$O)] $^{+}$ complexes as an enzyme model is expected to shed some light on the possibility of formulating a decontamination model system that will require less

logistical support, would have minimal runoffs of fluids and metal complexes, no lasting impact on the environment and can be deployed rapidly to address the pollution problems of toxic organophosphate contaminants in the environment.

3.3 Effects of [Cu (bpy)(OH)(H$_2$O)]$^+$ complexes on the hydrolysis of NPP

A series of experiments were conducted to investigate the effects of mixed media on the hydrolysis reactions. To test these effects, the Cu catalyst was prepared in aqueous solution and NPP substrate in one of the microemulsions. Similarly, the Cu catalyst was made in one of the microemulsions and NPP substrate was prepared in aqueous media. The results of the investigation showed the same reaction profile as the reactions in the microemulsion systems, but the ensuing hydrolysis was about 12% lower than those in the microemulsion based reaction systems. Analogously, the percentage hydrolysis was higher by about 10% compared to the aqueous systems when the Cu catalyst was made in the cationic microemulsion and NPP substrate was also prepared in a cationic microemulsion. The results of the percent hydrolysis of NPP by the [Cu(bpy)$^{2+}$] complexes were then plotted against the reaction time in all the media investigated to obtain the reaction profiles in the aqueous, anionic and cationic microemulsion media as depicted in figures 3.8, 3.9 and 3.10 respectively.
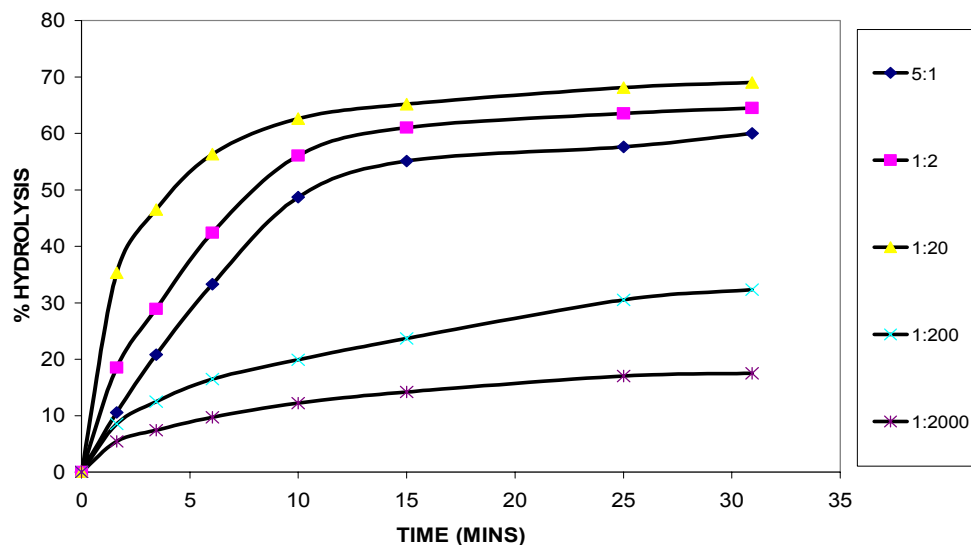
Fig 3.8: Reaction profile for the hydrolysis of NPP (10^{-3}M after addition) catalyzed by [Cu(bpy)(OH)(H$_2$O)]$^+$ complexes at different Cu:NPP ratio at pH 7 and 25°C in aqueous media.
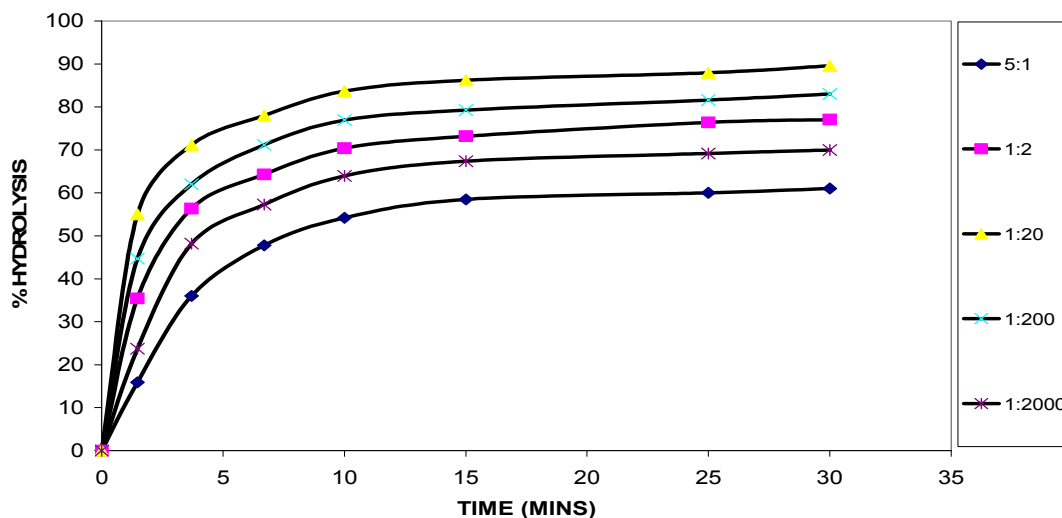
Fig 3.9: Reaction profile for the hydrolysis of NPP (10^{-3}M after addition) catalyzed by [Cu (bpy)(OH)(H$_2$O)]$^+$ complexes at different Cu:NPP ratio at pH 7 and 25°C in anionic microemulsion media.
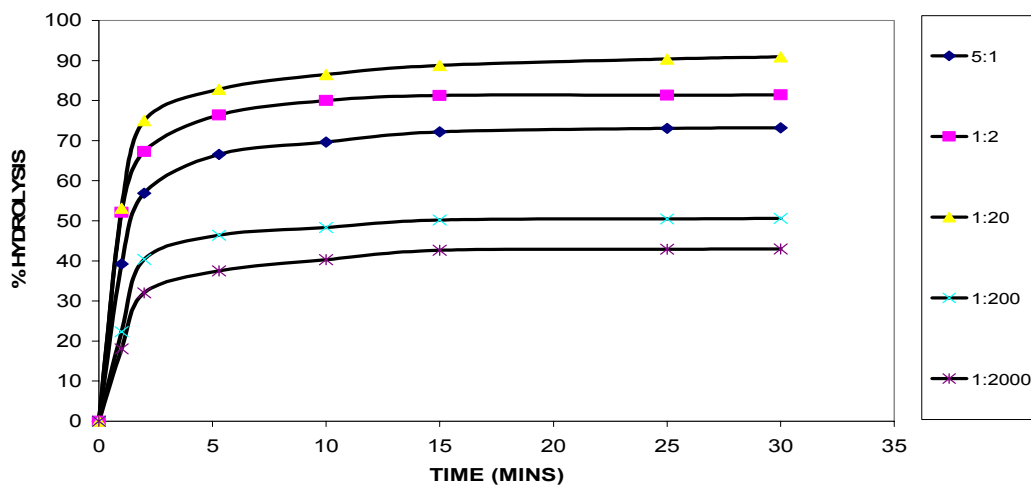
Fig 3.10: Reaction profile for the hydrolysis of NPP (10^{-3}M after addition) catalyzed by [Cu(bpy)(OH)(H$_2$O)]$^+$ complexes at different Cu:NPP ratio at pH 7 and 25°C in cationic microemulsion media.

As will be discussed later, the reactive species with respect to the hydrolysis of 4-nitrophenylphosphate is [Cu (bpy) (OH) (H$_2$O)] $^+$. This species is produced as a result of deprotonation of [Cu (bpy) (H$_2$O) $_2$]$^{2+}$ according to the equation.

[Cu (bpy) (H$_2$O) $_2$]$^{2+}$ + OH$^-$ $\rightarrow$ [Cu (bpy) (OH) (H$_2$O)] $^{2+}$ + H$_2$O
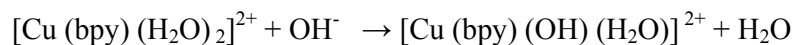
Percentage hydrolysis for the reaction of various concentrations of [Cu (bpy) (OH) (H$_2$O)] $^+$ to NPP ratio and pH 7.0 after 5 mins of reaction in the different media was plotted for clarity as depicted in the 3.11
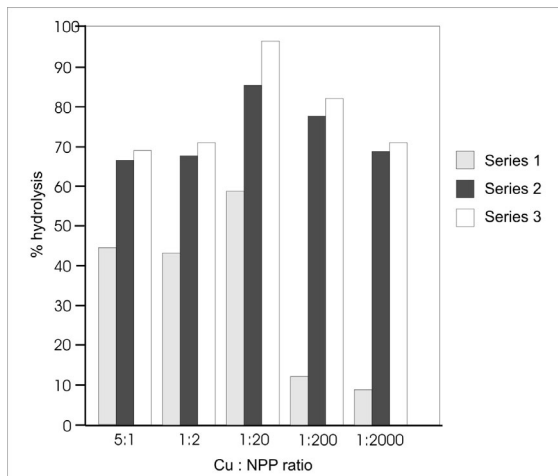
Figure 3.11: Plot of % hydrolysis versus Cu: NPP ratio for different reaction media after 5mins of reaction at 25°C and pH 7.0

Series 1 = aqueous, Series 2 = AME2, Series 3 = CME2

Initial concentration of [Cu(bpy)(OH)(H$_2$O)]$^+$ in moles per liter before mixing. Note that the final concentration of the species in the reaction solution is a fifth of the initial concentration taking into account the dilution factor that emanates from the protocol. The Cu: NPP ratio in the reaction solution is thus approximated as 5:1, 1:2, 1:20, 1:200, and 1:2000.

The degradation of NPP in the absence of copper using the same protocol was negligible.

(1) The curve is like a parabola in shape with the highest point observed at the 1:20 Cu:NPP ratio with a decrease in catalytic turnover at higher concentrations of 5:1 and 1:2 as well as a decrease again in catalytic turnover at lower concentrations of 1:200 and 1:2000 respectively. An increased hydrolysis was observed for systems that contained a Cu: NPP ratio of 1:20 in all the systems studied as depicted in figure 3.11. For the 1:20 Cu: NPP ratio, the concentration of the copper complex [Cu(bpy)(OH)(H$_2$O)]$^{2+}$ in the reaction solution after mixing was 5.6x10^{-5}M. It is well known that at concentrations greater than 5x10^{-5}M, dimerization to the catalytically inactive hydroxy-bridged dimer [Cu(bpy)(OH)$_2$]$^{2+}$ predominates [95], and presumably this resulted in the decrease in the concentration of the catalytically active species in the solution with a resultant decrease in catalytic activity as observed in these

systems. For systems that contain less than 5×10^{-5}M copper complex, the unavailability of the active catalytic species in sufficient amounts in the reaction explains the decrease in the percentage hydrolysis. The above phenomenon was markedly evident in the aqueous system and to a lesser degree in the microemulsion reaction solution.

(2) An appreciable catalytic turnover was also observed even at very low concentrations of 1:2000 Cu: NPP ratio. This phenomenon was markedly evident in the microemulsion media than in the aqueous media as depicted in the figures.

(3) The catalytic rate enhancement observed in the systems studied could be attributed to the interaction between the phenyl group of NPP and the π electrons of 2, 2'-bipyridine. The addition of $[Cu(bpy)(OH)(H_2O)]^{2+}$ to NPP results to an immediate interaction between the phenyl group of NPP and the π electrons of 2,2'-bipyridine resulting in the formation of a monodentate complex which can undergo intramolecular attack by the coordinated water or hydroxide of the metal complex on the phosphorous centre, thereby effecting hydrolysis. In previous studies [109], the formation of a four membered chelate ring system has been noted for related systems along with ring opened monodentate species. The copper promoted reactions exhibit catalytic properties and hydrolysis was complete within five minutes of reaction. The coordination of $[Cu(bpy)(OH)(H_2O)]^{2+}$ to NPP leads to an immediate interaction between the phenyl group of the NPP and 2,2'-bipyridyl leading to stacked moieties as depicted in the tentative structure below

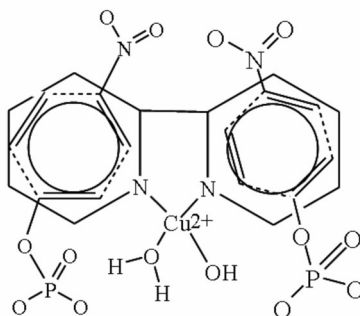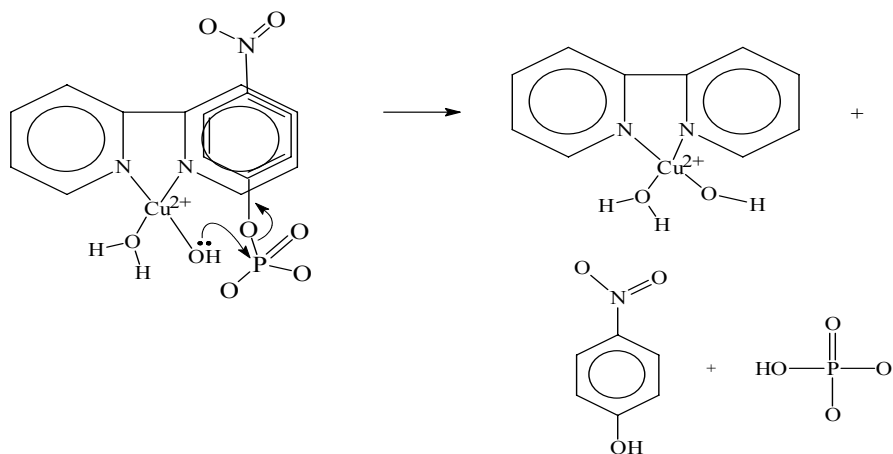***Figure 3.12:*** *Tentative structure for the ternary complexes formed between NPP and $[Cu(bpy)(OH)(H_2O)]^{2+}$*

The coordinated hydroxide or water in the stacked structure is positioned ideally to effect nucleophilic attack on the phosphorous centre thereby effecting hydrolysis as depicted in the proposed reaction pathway below.

Proposed reaction pathway for the nucleophillic attack of the phosphorous center by the copper dipyridyl complex

The active species of $[Cu(bpy)(OH)(H_2O)]^+$ is regenerated in solution and will therefore will be available for further catalytic reaction for the hydrolysis of NPP. This could account for the reason why catalysis in both the aqueous and the microemulsion mediated reaction was evident even in very low concentrations of the metal complex for example, 1:2000 metal to NPP ratio.

The importance of a ternary complex like $[Cu(bpy)(OH)(H_2O)]^+$ in metal ion catalyzed reactions are well known. eg the Cu^{2+} catalyzed decomposition reaction of H_2O_2 is strongly dependent on the kind of ligand bound to the metal ion [110,111]. In addition, the hydrolysis of diisopropylfluorophosphate has been reported to be catalyzed through several Cu^{2+} complexes. The rate of the metal ion catalyzed decarboxylation of acetonedicarboxylate and dimethyloxaloacetate was reportedly increased by coordination of the metal ion to an aromatic ligand like 2'2-bipyridyl compared to aqueous metal ions [112] while no enhancement is observed with aliphatic amines [112,113]. Also, the hydrolysis of diisopropyl fluorophosphates is catalyzed by several Cu^{2+} complexes among which the Cu^{2+} - 2, 2'-bipyridyl 1:1 complex is especially effective.

Such observation is consistent with our results suggesting that the difference in the reaction rate is closely connected to the stability of the ternary complexes formed during the reactions. This stability could be attributed to

(a)   formation of π bonds

(b)   hydrogen bonds between the ligands

(4) The 2, 2'-bipyridine is bound to Cu^{2+} through a σ donor bond and also through a π acceptor bond. Hence the resulting charge on the Cu^{2+} ion in the 1:1 complex is high due to back donation. The distorted square bi-pyramidal $Cu (H_2O)_6^{2+}$ ions will be strongly distorted towards a square planar coordination as a result of coordination to 2, 2'-bipyridine, thus creating the right geometry for the coordination of the second ligand which will especially favor negatively charged O atoms. It has been reported that the presence of an aromatic amine is crucial for the high stability of a ternary complex [113,114] and this was attributed to π back-bonding from the metal ion to the aromatic amine [115].

(5) Hydrogen bond formation between the two ligands bound to a metal ion is possible in ternary complexes, especially in biological systems where ligands like proteins and nucleic acids participate in complex formation. It is very imperative that the ligands forming the higher order complex are not only bridged via the central metal ion, but are also directly "linked" together. This inevitably contributes to the stability of the complex and ultimately to their catalytic enhancement towards the hydrolysis of 4-nitrophenylphosphate.

6) Lastly a number of mechanisms by which the copper bipyridyl complex, $[Cu(bpy)(OH)(H_2O)]^+$ could facilitate the hydrolysis of NPP as observed in our results can be summarized as follows in line with what has been forwarded for hydrolysis reactions of phosphate ester derivatives [116]

(i) Electrophillic activation of the phosphorous center in NPP which tends to increase the positive character at the phosphorous center thus making it susceptible to a nucleophillic attack by the coordinated nucleophile.

(ii) Charge neutralization effects. Since NPP carries negative charges, the approach of a negatively charged nucleophile such as OH^- on to the phosphorous centre is not favored on electrostatic grounds. Cu^{2+} ions in effect will increase the positive character at the phosphorous center thus making it susceptible to a nucleophilic attack by the coordinated nucleophile.

(iii) The prevalence of the coordinated hydroxide on the copper complex at neutral pH 7.0 is achieved by the simultaneous coordination of the nucleophile and NPP to the metal ion. The coordinated hydroxide can then function as an effective nucleophile towards the electrophillic phosphorous centre of NPP.

(iv) Activation of the NPP substrate in a strained chelate helps in organizing the reactants for effective intramolecular nucleophillic attack by the coordinated hydroxide.

CONCLUSION

(1) It is worth noting that copper is an indispensable constituent of all living tissues and is essential for the normal growth and well being of plants and animals. The minute quantities of copper needed for human health are usually obtained through the normal intake of food and water. Copper and its compounds are not like other metals such as lead or mercury. There are no records of any occupational diseases attributable to copper among people who have worked for many years with the metal or its salts. Indeed, it has sometimes been said that such people often appear healthier and generally suffer less from colds and other ailments. Hence contaminated run offs of the decontamination procedures with copper complexes do not pose additional environmental hazard; advisably the results of the investigation can be adapted to situations where decontamination of toxic organophosphates contaminants is anticipated.

(2) The average pH at which maximum hydrolysis of phosphate esters is in the pH region in which the hydroxoaquo ion predominates (pH region of 4 to 8). The results showed that 4-Nitrophenylphosphate was hydrolyzed in the presence of different metal ions or complexes at the physiological pH of 7.0 under ambient conditions. This observation is in accord with high reactivity's for hydroxoaquo complexes in comparison to the diaquo and dihydroxo species for reactions involving coordinated water molecules and is in conformation with reports from similar studies [117]. The results depict that from the first minute, the reaction is already at its maximum and levels off immediately after. This suggest that as soon as the metal ion was added to the 4-Nitrophenylphosphate solution, cleavage of the phosphorous-oxygen bond of the ester occurred. The effectiveness of the hydrolyzing media in the hydrolysis of 4-Nitrophenylphosphate clearly shows that the cationic microemulsions persistently show a rate enhancement compared to the anionic microemulsions and the aqueous media. The degrees of hydrolysis for these systems are as follows: cationic > anionic > aqueous media. The high hydrolytic cleavage observed in the microemulsion media rest on the composition and functioning of microemulsions as they have the ability to solubilize and disperse the maximum amounts of the reactants and substrates at the interfacial region for effective hydrolysis by increasing the surface area of the reaction.

(3) The findings of the study are important as it depicts ubiquitous transition metal complexes being effective as catalyst for the hydrolysis of aryl organophosphates in microemulsion systems. i.e., the synergistic effects of ubiquitous transition metal ions complexes in micro emulsions for the decontamination of toxic organophosphates have been established. Hence our experiments can be adapted to situations where decontamination of aryl organophosphate contaminants is anticipated.

(4) The study has established efficient functional models which have made understanding the facile degradation of toxic organophosphate esters (pesticides and chemical warfare nerve agents) in the biosphere easy. Hence the findings could help in the formulation of a decontamination model that would require less logistical support, would have minimal runoffs of fluids and in effect no lasting impact on the environment, and can be deployed rapidly and therefore is recommended as a decontamination model solution.

(5) The effect of copper complexes in microemulsions for the hydrolysis of unactivated phosphate esters remains to be investigated.

(6) The reactions described in the investigation are rapid under mild conditions and require no specialized equipment. Hence it can be adapted to situations where decontamination is needed especially in rural sub-Saharan Africa.

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