ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

BIS(TRIMETHYLENEDIAMINE)COBALT(III) MEDIATED HYDROLYSIS OF TRIPOLYPHOSPHATE AND TRIMETAPHOSPHATE MECHANISTIC CONSIDERATIONS

CHARLES OKADIA
JULY 1991

Bis(trimethylenediamine)cobalt(III) Mediated Hydrolysis of tripolyphosphate and trimetaphosphate: Mechanistic Considerations

A Thesis Presented to the School of Graduate
Studies Addis Ababa University

In partial fulfillment of the degree of
Masters of Science in Chemistry

by

Charles Okadia

July 1991

Dedicated to my late father Mzee Otieno Owuor for his positive contributions to my education

Acknowledgments

I would like to thank my research adviser Dr. Fikru Tafesse for not only chosing the topic of this research but also for his friendly accessibility and fruitful discussions and invaluable assistance during the course of this work.

I would also like to express my deep gratitudes to the NAPRECA Executive Secretary and the Steering Committee for making it possible for me to join the programme. Thanks to the German Academic Exchange Programme (DAAD) for providing the research funds for this project through Natural Products Research for Eastern and Central Africa (NAPRECA) and the NAPRECA for arranging these scholarships through DAAD and lastly to the Ethiopian Flora Project for providing me with the computer facilities.

My deep appreciations also go to Dr. J.A. Ogur of University of Nairobi for his immense moral and material support during the course of this work; Nelly B. Atieno for being such an invaluable companion during the course of the work; W/z Mehret, Ato Sahlemichael Deme and to Dr. Oyugi Aseto of the Organization of African Unity (OAU) for their dear assistance during this work and to my laboratory mate Ato Kelemu Lemma for his cooperation, and all those who may have assisted in one way or another.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

ABSTRACT

The hydrolysis of tripolyphosphate (pppi) and trimetaphosphate (mpppi) at 10^{-3} M concentration by $[tn_2Co(OH)(OH_2)]^{2+}$, (tn = trimethylenediamine), using Eu^{2+}/H^+ quenching was investigated. The europium quenching was adopted since other methods interfere with phosphate analysis. The reactions were monitored by measurement of orthophosphate (pi), pyrophosphate (ppi), tripolyphosphate (pppi) and trimetaphosphate (mpppi) in the quenched aliquots of the reaction mixtures of the reagents. A method was developed to separate and determine the different phosphate species in the orthophosphate form using acetone-phosphomolybdate complex as the analytical reagent. The reactive species with regard to the hydrolysis were found to be a 3:1 $tn_2Co(III)$pppi and $tn_2Co(III)$mpppi complexes. The results showed larger amounts of hydrolytic products for the directly added systems than those of stepwise additions. The production of phosphites in the reaction solution that contain $[tn_2Co(OH)(OH_2)]^{2+}$ and trimetaphosphate was observed. Spectrophotometric determination of the phosphite was done by first oxidising it with iodine to orthophosphate and using acetone-phosphomolybdate complex as the analytical reagent. Factors affecting the formation and degradation of the reactive and unreactive species have been discussed and plausible mechanisns formulated.

1. INTRODUCTION

1.1. General

The role of phosphorus in biological systems, industry and agriculture cannot be underestimated. Phosphorus is usually present in the form of phosphate esters or condensed polyphosphates and metaphosphates. The chief form of metabolic free energy storage, ATP, is a phosphate ester whose hydrolysis during anabolic processes and synthesis during the catabolic processes leads to provision of energy for cellular functions [1,2]. In the soap industry tripolyphosphates are used as sequestrants while in agriculture, phosphates are used as fertilizers and pesticides [3,4].

The different biochemical reactions involving phosphates are enzyme dependent. The enzymes which are the catalysts for most of these reactions include phosphoryl transfer enzymes and nucleotydyl transfer enzymes. These enzymes usually contain divalent metal ions at their active sites [5,6]. The number of metal ions in an enzyme can vary. Usually two or more metal ions are required whereby one metal ion may be playing a structural role while the remaining one(s) play a catalytic role [5]. It has been established that enzymes that catalyse the hydrolysis of phosphate esters, pyrophosphates and ATP require metal ions such as Mg^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+} and Co^{2+} for their activity [8].

1.2. Mechanism of Phosphate Hydrolysis

There are two mechanisms by which hydrolytic cleavage of the phosphate moities of the phosphate esters can take place: acid catalysed and base catalysed mechanisms [9,10]. The acid catalysis consists of the protonation of the tripolyphosphate oxygen atoms in ATP to form an uncharged molecule. Water then acts as a nucleophile and attacks one of the phosphorus atoms with a subsequent elimination of a phospho group as phosphoric acid (H_3PO_4). Base catalysis however takes place in neutral or basic media in which the acidic protons have been removed producing a high negative charge. This negative charge can be neutralized or removed by using metal ions while at the same time creating a high electrophilicity around the phosphorus centre.

1.3. Role of Metal Ions

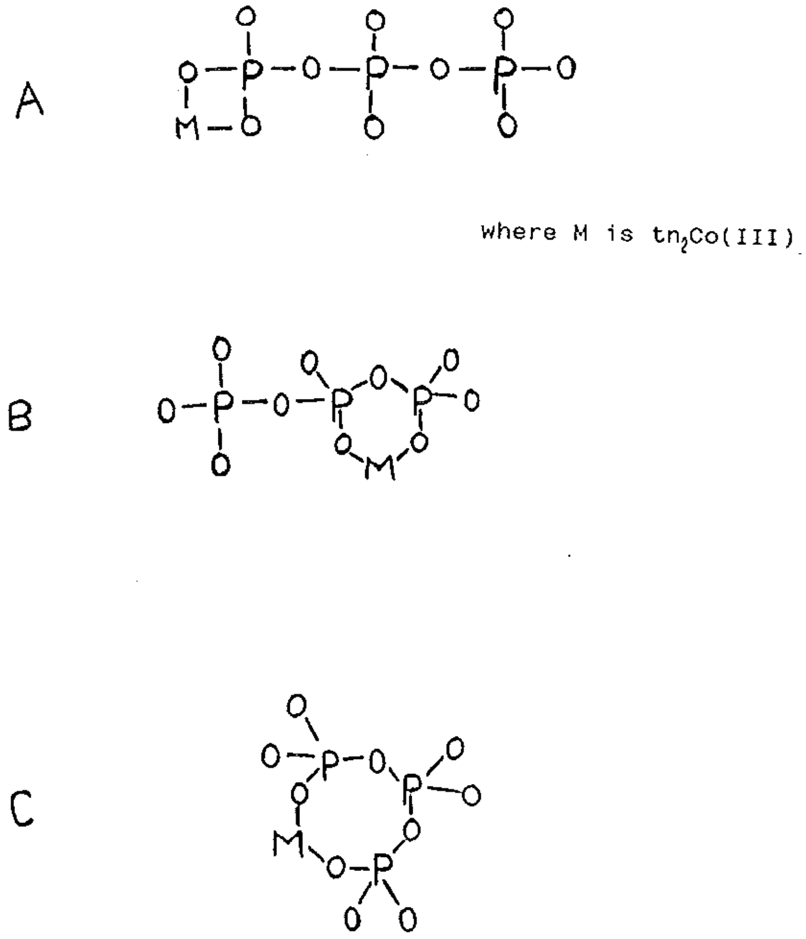
Divalent metal ions are required in most enzyme catalysed nucleophilic displacement reactions at phosphorus (V) centre hence are an ideal choice in model studies of catalytic hydrolysis of the condensed phosphates [6,11]. Metal ions have been investigated as catalysts for the hydrolysis of phosphate esters [12-14] with a view to revealing the mechanistic aspects and reasons for the rate enhancement during such reactions. The rapid enzymic hydrolysis of the P-O-P bonds in biological systems is some 10^{10} times faster than the slow hydrolysis of these bonds in aqueous laboratory conditions. For example the hydrolysis of the pyrophosphate by Yeast Inorganic Pyrophosphatase (YIP), occurs about 10^{11} fold faster than the hydrolysis in the absence of the enzyme [16].

To elucidate the role of the divalent metal ions, many investigations have been made on metal ions and some of their complexes that could serve as models for enzymes. Magnesium ion, despite its biological activity does not produce a marked increase in the rate of hydrolysis compared to the other metal ions in the absence of the enzymes [17]. Calcium on the other hand has shown some catalytic activity. However greater rate enhancements have been achieved by some transition metal ions [17-21]. It has been shown that the rate of hydrolysis is dependent on the concentration of the metal ion. A ratio of two metal ions per molecule of phosphate ester was found to be the most effective [17]. Recently it was reported that a metal to pyrophosphate ratio of 3:1 leads to larger rate enhancement [15]. The major drawback with the class of divalent metal ions is that they are very labile towards solvolytic ligand displacement. Coupled with this, the identification of the species involved in the hydrolysis is interfered with by the presence of monomeric , dimeric and hydrated complexes [22].

Metal ions can potentially play many roles in the hydrolysis of the phosphate esters [7,9]. Some of these are listed below.

(1) Metals coordinate to the phosphates thereby neutralizing the negative charge around the phosphorus atom hence allowing the approach of an incoming nucleophile,

(2) Metals can also help remove the electron density from the phosphorus centre making it prone to nucleophillic attack. This is possible if the metal coordinates to the phosphate groups of a tripolyphosphate (Figures 1.1 A, 1.1 B and 1.1 C) or trimetaphosphate(Figures 1.2 A, 1.2 B and 1.2 C)



where M is $tn_2Co(III)$

Figure 1.1. Possible modes of metal coordination to tripolyphosphate (charge and degree of protonation omitted)

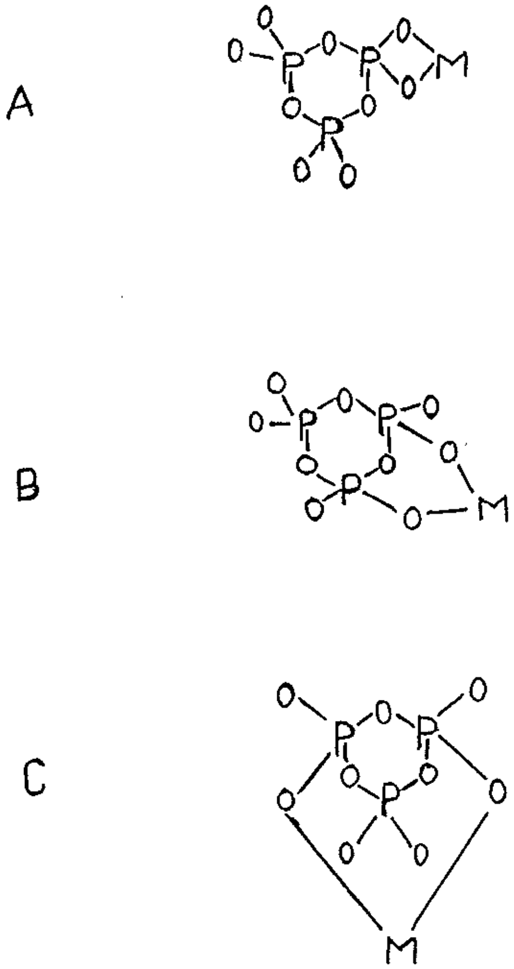


Figure 1.2. Possible modes of metal coordination to trimetaphosphate (charge and degree of protonation omitted)

(3) Template formation :

Coordination of the phosphate groups to a metal ion changes the geometry of the phosphate moiety allowing for an intramolecular hydrolysis. The catalytic role of the metal ions in providing a template reaction corresponds to the formation of a bi- or tridentate ligand in the transition state [23].

(4) Generation of a coordinated nucleophile :

A better coordinated nucleophile than water , for example hydroxide can be generated at a physiological pH. This is achieved by lowering the pH below the pK_a of the conjugate acid of the nucleophile. Reasonable concentrations of the coordinated nucleophile is achieved in this manner without the risk of free metal ion precipitation as hydroxide as would be the case.

(5) Ring strain :

In many biological systems, metals may be bound in coordination geometries distorted for the sake of catalytic efficiency. For example binding a metal ion to a single phosphoryl group in a bidentate form reduces the O-P-O bond angle. This lowers the activation energy necessary to attain transition state while at the same time allowing for an attack by a coordinated nucleophile.

1.4. Model Systems

Studies aimed at giving a better insight into the enzymatic reactions in biological processes have been done at great lengths [6,11,15,17,22,24.25]. Most of these have been geared towards getting more information about the active site in the enzymes. Although enzymes are complex macromolecules, model compounds of lower molecular weights have been synthesized and used to mimic their reactions. Substrate models have varied from pyrophosphates, tripolyphosphate, trimetaphosphate, AMP, ADP and ATP while the catalysts have recently been cobalt(III) amine complexes [6, 11, 24, 25, 26].
In most model systems, the essential biologically active metal ions such as Mg^{2+}, Ca^{2+}, K^{+} and Na^{+} have been replaced by transition metal ions. The transition metals have the advantage of being fixed in their positions around the substrate molecule which in this case would be the phosphate oxygens [21]. The transition metals have been modified by introducing ligands such as amines onto the metal centre. This restricts the geometry and the number of coordination sites available for substrate models [22, 24, 28, 29].

In the present study, bis(trimethylenediamine)-Cobalt(III) complex is used. Cobalt(III) amine complexes are known to be low spin. They are substitutionally inert, meaning that the nitrogen ligands do not readily dissociate hence retain their integrity for long periods of time in aqueous solution [25]. Cobalt(III) complexes of diamines and triamines are supposed to have fixed geometrical orientation. The choice of bis(trimethylenediamine)cobalt(III) complex was prompted by the fact that this complex is 100 % cis in the solution [30]. This would ensure maximum hydrolysis as it is envisaged that a complex of bis(trimethylenediamine)cobalt(III) and phosphate ester with cis geometry arrangement of a potential intramolecular nucleophile (i.e. OH) has a better chance of hydrolysis as opposed to the corresponding trans isomer. Despite the fact that enzymes do not utilize substitution inert metal ions since the reaction would be stoichiometric rather than catalytic, bis(trimethylenediamine) cobalt(III) ions could not be different from labile metal ions in terms of their ability to activate substrate and organise coordinated nucleophile [49].

In the present work an attempt will be made to develop analytical techniques to monitor all of the hydrolytic products formed in the reaction solution. This attempt is unique as earlier works were only geared towards determining the amounts of phosphate ions produced in

the solution without considering other condensed phosphate ions which are presumably formed in the course of the reaction. The results of this study will provide a better understanding of the roles played by metal ions in phosphoryl transfer reactions.
Europium (II) quenching technique [24, 25] will be employed to stop the reaction and release all bound phosphate species in the solution.

1.5. Analytical Technique for Phosphate Determination

The hydrolysis of phosphorylated compounds have been classically monitored by determining the inorganic phosphate released by the hydrolytic reaction. A plethora of methods for the determination of inorganic phosphate is available in the literature. These include titrimetric, ^{31}P NMR, gravimetric and spectrophotometric methods [6,31-38]. The most widely used procedures involve modification of the methodology based on a phospho- molybdate complex [33-38]. However most of these methods have major drawbacks in that they require filtration, extraction and are not useful in detecting all the phosphate species present in the solution at a given time. In others such as the malachite green method [33], the dye sticks on the wall of the spectrophotometric cell. This requires tedious cleaning of the cell after every sample reading.

In the present work, a modification of a more efficient and sensitive spectrophotometric method reported by Hirata and Appleman [38] has been adopted. The method utilizes the formation of a phosphomolybdate-acetone complex. The complex gives a yellow colour with a reasonable absorption at 328 nm. The method allows for efficient microdetermination of the phosphate samples. It also ensures efficient and selective determination of the orthophosphates without interference from other phosphate species such as pyrophosphate, tripoly phosphate or trimetaphosphate.

1.6. This Research

The major aim of this work is to make a comparative study of the hydrolysis of tripolyphosphate and trimetaphosphate by bis(trimethylenediamine)cobalt(III) complex at pH 6.5. The choice of $[tn_2Co(OH_2)_2](ClO_4)_3$ was not arbitrary. The complex exists absolutely in its cis- form in the solution. The trimethylene diamine moieties ensures that four of the six coordination sites around the cobalt centre are blocked. This leaves only two vacant sites to be occupied by aqua ligands.

At pH 6.5, the complex exists largely in the hydroxo-aqua form due to the deprotonation of one of the water molecules [25].

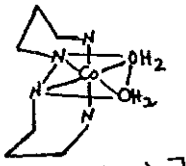
It is hoped that in carrying out the hydrolytic studies plausible mechanisms will be designed for the cobalt(III) mediated cleavages of tripolyphosphate and trimetaphosphate. To attain the objectives the following investigations will be carried out:

1. the amounts of orthophosphate (pi), pyrophosphate (ppi), tripolyphosphate (pppi) and metaphosphate (mpppi) present in the solution during the course of the reaction at pH 6.5 will be monitored spectrophotometrically;

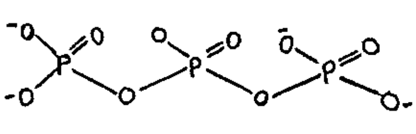
2. the effect of increased metal to condensed phosphate ratio on the rate of hydrolysis will also be studied; and

3. The study of the effect of order of addition of the $[tn_2Co(OH_2)_2]^{3+}$ complex on the rate of tripolyphosphate and trimetaphosphate hydrolysis will also be undertaken.

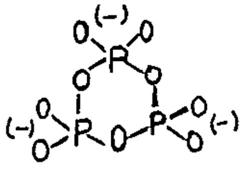
In carrying out these studies, cis-diaqua-bis(trimethylene diamine) cobalt(III) perchlorate $\{[tn_2Co(OH_2)_2](ClO_4)_3\}$, sodium tripolyphosphate ($Na_5P_3O_{10}$) and sodium trimetaphosphate $\{(NaPO_3)_3\}$ were synthesized. The synthesis procedures are outlined in section 2.4 and 2.7. The structures of $[tn_2Co(OH_2)_2]^{3+}$, pppi and mpppi are depicted in Figure 1.3.



cis-[tn$_2$Co(OH$_2$)$_2$]$^{3+}$



pppi



mpppi

Figure 1.3.Structures of diaquabis(trimethylenediamine)cobalt(III), ([tn$_2$Co(OH$_2$)$_2$]$^{3+}$), tripolyphophate and trimetaphosphate

2. EXPERIMENTAL

2.1. Reagents

Absolute ethanol (Riedel de Haen), ammonium molybdate tetrahydrate (Analar, BDH), barium chloride (BDH), calcium chloride (Riedel de Haen), cobalt(II) chloride hexahydrate (Riedel de Haen), cobalt (II) nitrate hexahydrate (BDH), copper (II) sulphate pentahydrate (Merck), diethyl ether (BDH), disodium hydrogen ortho phosphate (BDH), ethylene diamine (East Man Kodak), europium(III) oxide (Fluka), hydrochloric acid (Riedel de Haen), hydrogen peroxide (Riedel de Haen), iodine (BDH), magnesium chloride (Riedel de Haen), mercuric chloride (BDH), perchloric acid (Merck), potassium dihydrogen orthophosphate (BDH), potassium iodide (Riedel de Haen), potassium permanganate (Merk), nitric acid (Inter Chem), silver nitrate (Riedel de Haen), sodium dihydrogen orthophosphate dihydrate (BDH), sodium hydroxide (BDH), sodium thiosulphate (BDH), sulphuric acid (BDH), trimethylenediamine (Eastman Kodak), zinc granules (BDH), were used as supplied without further purification.

2.2. Instruments

The pH measurements were done using an Orion research microprocessor ion analyser model 901 fitted with a combined electrode (Fisher) (4 M KCl- Sat.Ag/AgCl).

Automatic pipettes with plastic tips were used to transfer measured quantities of reaction mixtures. The pH adjustments were done using glass stick doting of either NaOH or $HClO_4$. The adjustment was done while the solution was being stirred using a magnetic stirrer. A Beckman model DU 65 UV-Visible recording spectrophotometer was used to obtain electronic spectra and for collection of rate data. 1 cm cell(total path length) was used for the spectra and measurements. Extinction coefficients are quoted in M^{-1} cm^{-1}.

2.3. Orthophosphate Determination

The determination of orthophosphate was done by modifying the method put forward by Hirata and Appleman [38]. Concentration of the orthophosphate in the aliquots were found from calibration curve studies of known orthophosphate solutions.

2.3.1. Preparation of phosphate standards

A stock solution of orthophosphate with a concentration of 50 μg P ml^{-1} was prepared by dissolving 0.219 g of anhydrous potassium dihydrogen orthophosphate (KH_2PO_4) dried at 110^0 C for 2 hours in one litre ofdistilled water. Different aliquots of this stock solution were taken and diluted to 100 mls to give concentrations ranging from 1 μg P ml^{-1} to 10 μg P ml^{-1}.

2.3.2. Preparation of molybdate reagent

A 1.545 g of ammonium molybdate tetrahydrate [$(NH_4)6Mo_7O_{24}.4H_2O$] was dissolved in 400 ml of distilled water with the aid of 0.8 ml of 70 % perchloric acid ($HClO_4$). The resulting solution was diluted to one litre with distilled water to give a 1.25 mM solution.

2.3.3 Procedure for the spectrophotometric determination of orthophosphate

Accurately measured 3.2. ml of the sample solution was mixed with 0.8 ml of 43.75 % $HClO_4$ acid and 2.0 ml of the molybdate reagent. After thoroughly shaking for one minute, it was allowed to stand for one more minute. 4 ml of ice cold acetone was added to it using an automatic burette. The addition was done by inserting the tip of the burette below the surface of the solution. The resulting mixture was stoppered, shaken for one minute and allowed to stand for one more minute to complete its colour development. Readings were then taken at 328 nm against the blank.

From an average of triplicate readings, calibration graph was drawn by plotting concentrations versus time. The system obeyed Beers law between the ranges 1 ug P ml^{-1} and 6 μg P ml^{-1}. Molar extinction coefficient was found to be 1.696×10^4 M^{-1} Cm^{-1}

2.4 Preparation of the Condensed Phosphates [39]

2.4.1 Sodium tripolyphosphate($Na_5P_3O_{10}$)

A 10 g finely ground mixture of sodium dihydrogen orthophosphate dihydrate ($NaH_2PO_4.2H_2O$) and sodium hydrogen orthophosphate (Na_2HPO_4) in the mole ratio of 1:2 was placed in a platinum crucible and heated for two hours at 580^0 C in an electric furnace. The product formed was allowed to cool in the furnace and ground into fine powder using pestle and mortar. A yield of 7.59 g (90.78 %) was obtained.

2.4.2 Sodium trimetaphosphate ($Na_3P_3O_9$)

Some 20 grams of finely ground Sodium dihydrogen orthophosphate dihydrate ($NaH_2PO_4.2H_2O$) was placed in a platinum crucible and then heated at 550^0C for 5 hours in an electric furnace. The crucible and its contents were allowed to cool in the furnace. The product was transferred to a mortar and ground into fine powder. This gave a yield of 12.93 g (98.98 %).

2.5 Characterization of the Condensed Phosphates

A 1 % solutions of the prepared condensed phosphates were reacted with portions of 0.1N solutions of $CaCl_2$, $BaCl_2$, $ZnCl_2$, $MgCl_2$, $AgNO_3$ and 2% solution of $Coen_3Cl_3$. The results of the observations conformed with those cited in the literature [3,40]. The presence of orthophosphate as an impurity was detected in both tripolyphosphate and trimetaphosphate by using standard tests [35,38] to the extent of 2.35 % and 1.94 % respectively.

The trimetaphosphate was found to be free of other condensed phosphates while in the tripolyphosphate the presence of pyrophosphate was confirmed [40]. The amount of pyrophos phate was found to be 5.9 % in the tripolyphosphate preparations by precipitating out quantitatively the pyrophosphate with $Coen_3Cl_3$ solution at pH 6.5 [41].

2.6. Separation and Determination of Various Phosphate Species

A scheme for separation of the individual species in a solution of a mixture of orthophosphate (pi), pyrophosphate (ppi), tripolyphosphate (pppi) and trimetaphosphate (mpppi) was developed as given in scheme 2.1. The scheme was designed taking the following into consideration:

(i) lack of simple direct method for pppi or mpppi determination to date;

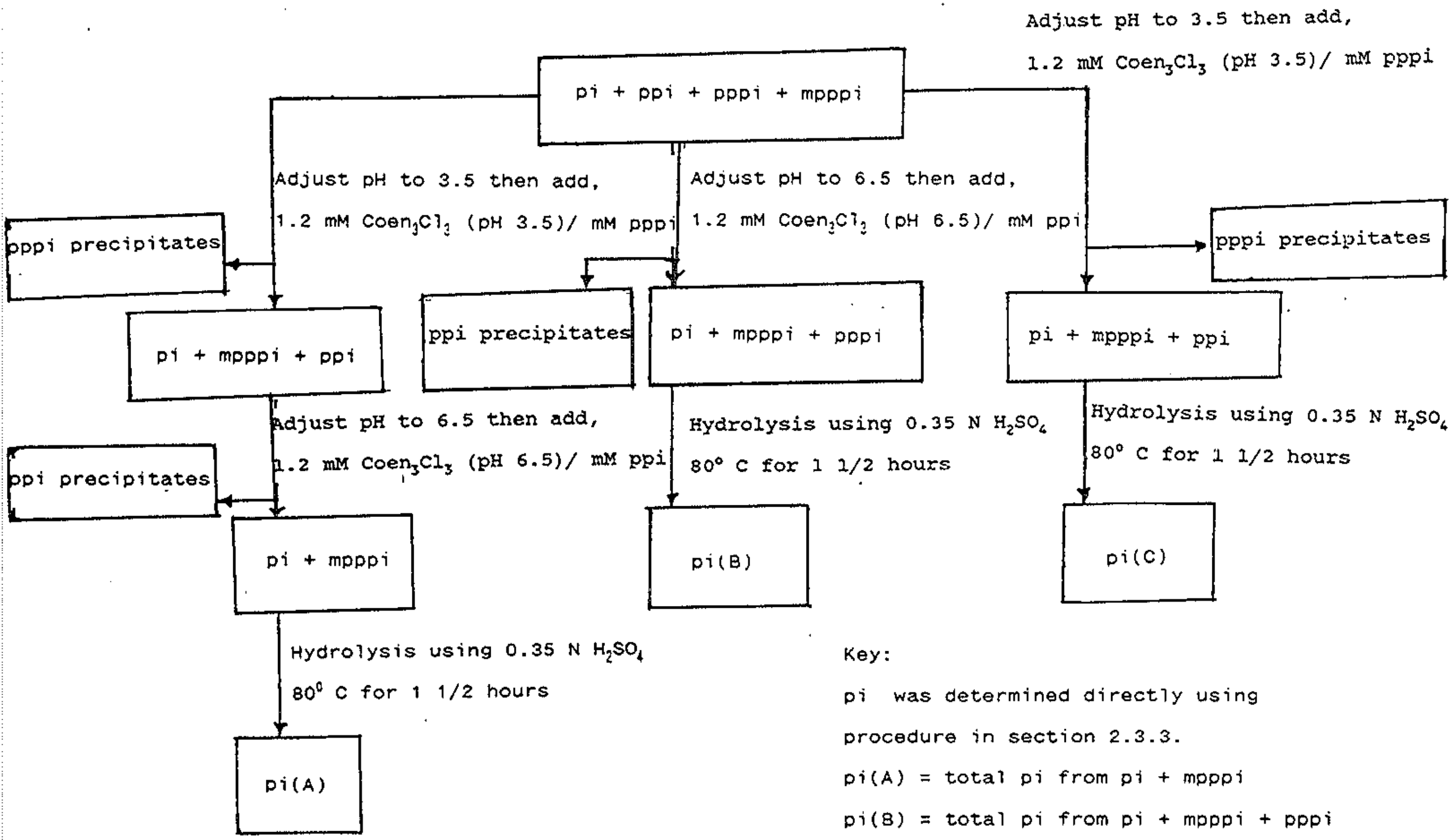
(ii) ppi isquantitatively precipitated by $Coen_3Cl_3$ at pH 6.5 [41];

(iii) pppi is quantitatively precipitated by $Coen_3Cl_3$ at pH 3.5 [41];

(iv) pi can be determined directly by the method of Hirata and Appleman [38] without interference from the condensed phosphates;

(v) condensed phosphates can be completely degraded into orthophosphates by heating them in 0.35 N H_2SO_4 at 80^0 C for a period of 1 1/2 hours; and

(vi) $Coen_3Cl_3$ does not react with trimetaphosphate or orthophosphate under the experimental conditions (i.e. pH 3.5 and pH 6.5).



Key:

pi was determined directly using procedure in section 2.3.3.

pi(A) = total pi from pi + mpppi

pi(B) = total pi from pi + mpppi + pppi

pi(C) = total pi from pi + mpppi + ppi

Scheme 2.1. Assay of phosphate species

The scheme was tested by preparing a solution containing known mixtures of pi, ppi, pppi and mpppi. The average result from triplicate analysis is depicted in Table 2.1.

Table 2.1. Testing of the phosphate determination Scheme

Phosphate species	Calculated* amount (μg P/ml)	Actual** amount (μg P/ml)	Experimental amount (μg P/ml)
Pi	0.61	0.67	0.66
PPi	0.84	0.82	0.83
PPPi	1.31	1.20	1.05
mPPPi	1.20	1.18	1.12

* Calculated amounts were made assuming 100 % purity of all the phosphate species used and taking into consideration that one mole of pppi or mpppi is equivalent to three moles of pi while one mole of ppi is equivalent to two moles of pi.

** Actual amounts are those values obtained after corrections due to :

(1) presence of 2.35 % pi and 5.9 % ppi in the prepared pppi;

(2) presence of 1.94 % pi in prepared mpppi and

(3) presence of 2.0 % pi in the supplied ppi.

The corrections were necessitated by the difference observed between the calculated amounts and the experimental amounts.

2.7. Preparation of Cobalt(III) Complexes.

2.7.1. $[tn_2CoCO_3]ClO_4$

bis(trimethylenediamine)carbonatocobaltate(III) perchlorate, ($[tn_2CoCO_3]ClO_4$), was synthesized by modifying the literature procedures [15,25,42,43].

To a warm slurry of 7.2 g (2.76 mM) of sodium tris-carbonato cobaltate trihydrate ($[Na_3Co(CO_3)_3].3H_2O$) dissolved in a minimum amount of water and kept on a magnetic stirrer hot plate at 60^0 C, acidified trimethylenediamine was then added dropwise with continuous stirring. The acidified trimethylenediamine solution (pH 4-5) was prepared separately by adding 7.5 ml of 70 % $HClO_4$ dropwise to 3.35 ml (0.04 M) of the ligand. The temperature of the solution was then raised to 75-80^0 C and heating continued till a red solution was produced. The solution was filtered while hot. The residue was washed and the filtrate combined with the washings. Refiltration was done till no residue remained on the filter paper. The solution was then evaporated at 60^0 C on a hot plate till a scum formed on the surface.

This was cooled to room temperature and absolute ethanol added to it. This gave red square crystals which were washed with ethanol followed by using diethyl ether and then dried. The yield was 2.77 g (38 %) which was conforming to the literature value.

2.7.2. $[tn_2Co(OH_2)_2](ClO_4)_3$

The diaqua bis(trimethylenediamine)cobalt(III) perchlorate, ($[tn_2Co(OH_2)_2](ClO_4)_3$ was prepared according to the following reactions:

$Na_3[Co(CO_3)_3].3H_2O + tn_2.HClO_4$ ----$[tn_2CoCO_3]ClO_4$...(i)

$[tn_2CoCO_3]ClO_4 + HClO_4$-----$[tn_2Co(OH_2)_2](ClO_4)_3$...(ii)

The conversion of the carbonato to the diaqua was achieved by addition of 2.5 mM 70 % $HClO_4$ per millimole of finely divided carbonato complex and stirring under an aspirator vacuum at 50^0 C for 20 minutes [15]. The resulting solution was used without crystallization. The presence of the target complex was confirmed by UV spectra (see Table 2.2)

The diaqua-bis(trimethylenediamine)cobalt(III) ion can exist as diaqua, hydroxoaqua or dihydroxo species depending on the pH. In the lower pH regions of pH 1.0 to pH 4.0, the diaqua species predominates. In the middle pH regions, the hydroxoaqua is predominant. The hydroxoaqua species does not exist in the crystalline state as monomeric units. cis-Hydroxoaqua ions exist as dimers bridged by two $H_3O_2^-$ ligands while the trans-hydroxoaqua ions are polynuclear chains of metal atoms bridged by single $H_3O_2^-$ bridges [44].

Bridging by $H_3O_2^-$ species is accomplished by formation of a strong and short symmetrical hydrogen bonding [44,45] between the hydroxo ligand of one metal ion and the aqua ligand of the other metal ion. In concentrated solutions of the hydroxoaqua metal ions, olation reactions are presumed to take place leading to formation of polynuclear species. Olation reactions may also occur in aged solutions of hydroxoaqua metal complexes [25]. To minimise these problems, freshly prepared hydroxoaqua complexes of low concentrations were used for all the experiments.

2.7.3. tris(1,2-ethylenediamine)cobalt(III) chloride, ($Coen_3Cl_3$)

To 5.2 ml of 100 % ethylenediamine, 3.4 ml of 6 N HCl was added. The resulting mixture was poured into a solution of 4.8 g (0.02 M) of cobalt(II) chloride hexahydrate ($CoCl_2.6H_2O$) in 15 ml of distilled water. A stream of air was then bubbled through the solution for three hours. After the solution was evaporated in vaccuo to a third of its original volume, 3 ml of conc. HCl and 6 ml of absolute ethanol was added. The solution was then left to stand for one hour to effect crystallization. The crystals were filtered, washed with ethanol till the washings were colourless and dried with diethyl ether. The yield was 3.69 g (52.94 %).

2.7.4. Characterization of cobalt (III) amine complexes

Characterizations were done using UV-Visible spectroscopy. The molar extinction coefficients, the absorption maxima and minima were found to match well with the literature values [25] as depicted in Table 2.2.

Table 2.2. Absorption maxima and minima values for cobalt(III) complexes

Complex	Experimental value		Literatue value	
	λ_{max} (ε)	λ_{min} (ε)	λ_{max} (ε)	λ_{min} (ε)
$[tn_2CoCO_3]ClO_4$	520 (106.90)	-	520 (106.8)	430 (12)
	360 (134.47)	-	359 (126.3)	327 (50.8)
$[tn_2Co(OH_2)_2]$-$(ClO_4)_3$*	508 (53.2)	-	506 (53.3)	–
	357 (56.5)	-	362 (65.1)	–
$[Co(en)_3]Cl_3$	466.8 (76.3)	-	–	–
	339.8 (68)	-	–	–

* In the middle pH ranges the major species that prevails in the solution is the hydroxoaqua ion.

2.8. Hydrolytic Studies

Solutions containing bis(trimethylenediamine) cobalt(III) tripolyphosphate (tn_2Copppi) and bis(trimethylenediamine)cobalt(III) trimetaphosphate (tn_2Compppi) were prepared with metal to phosphate ratio of 1:1, 2:1 and 3:1 in such a way that the final concentration would be 1 X 10^{-3} M in the desired complex. 1 M $NaClO_4$ was used for ionic strength control in such a way that the solution would be 0.1 M in $NaClO_4$. During the course of the reaction, the solution was continuously stirred using a magnetic stirrer. An example of the procedure for the preparation of a solution of 1 X 10^{-3} M in tn_2Copppi (1:1 metal to pppi ratio) is given below.

Accurately measured 8.0 ml of pppi (5 X 10^{-3} M, pH 6.5) was mixed with 4 ml of $NaClO_4$ (1 M, pH 6.5) and 20 ml of distilled water. The pH of this solution was adjusted to 6.5. 8 ml of hydroxoaqua-bis(trimethylenediamine)-cobalt(III) complex (5 X 10^{-3} M, pH 6.5) was added dropwise to the above solution with continuous stirring. The final volume was 40 ml. For the higher metal to pppi ratios (i.e. 2:1 and 3:1), the amount of water was reduced accordingly to maintain the final volume at 40 ml.

The hydrolysis of either pppi or mpppi was monitored by correlating the amount of pi, ppi, pppi and mpppi present in the solution with respect to time.

2 ml aliquots were withdrawn at different intervals (2, 5, 10, 20, 30 and 60 minutes) and quenched with Eu(II) to stop the reaction and release all the bound phosphate species before assaying.

The europium quenching technique is corroborated in section 2.8.6.

The following were investigated using the above procedure:

Cobalt/Cpi[a] ratio	Reaction equation [b]
1:1	$tn_2Co(aq) + Cpi$ [c]
2:1	$2\ tn_2CoCpi + Cpi$ [c]
1:1 +1	$tn_2CoCpi + tn_2Co(aq)$ [d]
3:1	$3\ tn_2Co(aq) + Cpi$ [c]
1:1+ 2	$tn_2CoCPi + 2\ tn_2Co(aq)$ [d]
1:1+1+1	$tn_2CoCpi + tn_2Co(aq) + tn_2Co(aq)$[e]

where:

a: Cpi designates pppi or mpppi,

b: charge and degree of protonation omitted,

c: direct addition,

d: preformed 1:1 metal to condensed phosphate complex plus 2 molar ratio of the metal complex,

e: preformed 1:1 metal to condensed phosphate complex plus addition of two molar ratio of the complex

2.8.3. Trimetaphosphate (mpppi) assay

The pH of quenched dilute aliquots of the above solution was adjusted to 3.5 with $HClO_4$. To some 75 ml of the aliquot, 2.5 mls of 1.2 mM tris(ethylenediamine)-cobalt(III) chloride, ($Coen_3Cl_3$), at pH 3.5 was added to precipitate out pppi. This was filtered and the pH of the resulting filtrate adjusted to 6.5. Excess $Coen_3Cl_3$ was added to the filtrate to precipitate out the ppi present. Again filtration was carried out and the final filtrate hydrolyzed by adding 15 ml of 0.35 N H_2SO_4 and heating in a water bath at 80^0 C for 1 1/2 hours. The final volume of the solution was made up to 100 ml with distilled water and its pH adjusted to 6.5. The total pi in the solution due to mpppi and pi designated as pi(A), was then assayed using the procedure outlined in section 2.3.3.

mpppi (in terms of pi) = pi(A)-pi.

Therefore the concentration of mpppi alone is [pi(A)-pi]/3

Note: The concentration of free pi was determined dsreotlyned8aBove in section 2

2.8.4. Tripolyphosphate (pppi) assay

A 75 ml of the diluted aliquot sample (pH 6.5) was taken and 1.5 ml of 1.2 mM $coen_3Cl_3$ solution (pH 6.5) added to it to precipitate out ppi. The solution was then filtered and 15 ml of 0.35 N H_2SO_4 added to it. The resulting solution was hydrolyzed at 80^0 C in a water bath for 1 1/2 hours. The final volume of this solution was raised to 100 ml and its pH adjusted to 6.5. The total free orthophosphate due to pi, mppi and pppi designated as pi(B) was determined according to section 2.3.3. Hence pppi (in terms of pi) = pi(B)-pi(A). Therefore pppi alone = [pi(B)-pi(A)]/3, where pi(A) is total pi due to pi and mpppi.

2.8.5. Pyrophosphate (ppi) assay

The procedure was similar to that given in section 2.8.4 despite the fact that the pH was adjusted to 3.5 initially. At this experimental condition pppi precipitates. The remaining phosphpate species were hydrolyzed with H_2SO_4 to produce orthophosphates. The total free orthophosphate due to pi, mpppi and ppi designated as pi(C) was determined. The concentration of ppi in terms of pi was calculated.

ppi (in terms of pi) = pi(C)-pi(A).

Therefore, concentration of ppi = [pi(C)-pi(A)]/2, where pi(A) is total pi due to pi and mpppi.

2.8.6. Europium(II) quenching technique [15]

0.1 M solution of Eu(III) was prepared by weighing accurately 0.88 g of Eu_2O_3 and dissolving it in 50 ml of

2 M HCl. Eu(III) was reduced to Eu(II) by addition of 5 ml of the reagent to amalgamated zinc in an atmosphere of dry nitrogen and leaving for a period of 30 minutes. The Eu(II) was usually prepared just before use and all the amalgamated zinc granules removed as soon as the experiments were over. This was to avoid the formation of a white precipitate. The precipitate could be Zn(II) and Eu(II) salts. The white precipitate usually appeared when the amalgamated zinc was prepared by soaking zinc granules (previously rinsed with dilute HCl) in a 2 % solution of mercuric chloride ($HgCl_2$) in HCl. For typical reactions, 2 ml portions of the sample aliquots were quenched with 0.2 ml of Eu(II). This reduces all the Co(III) to Co(II) and release all the bound phosphate species instantly. The completion of the quenching was achieved by observing the complete declourization of the quenched aliquot sample. After quenching and diluting the aliquot samples to 100 ml, the volumetric flasks were usually left open for about five minutes. This was to ensure complete aerial oxidation [47] of any excess Eu(II) to Eu(III) since Eu(II) interferes with phosphate determination [15].

2.9. Preparation of phosphate buffer solution

Accurately measured 50 ml of 0.1 M sodium dihydrogen orthophosphate dihydrate ($NaH_2PO_4.2H_2O$) solution was thoroughly mixed with 36 ml of 0.1 M sodium hydroxide solution to give a buffer of pH 7.3.

2.9.1. Phosphite oxidation and analysis

A procedure for the phosphite oxidation was as follows: 25 ml of the phosphate buffer solution was put in a 100 ml volumetric flask. To this, 25 ml of the sample and 50 ml of 0.1 N iodine solution was added. The flask was then stoppered, shaken gently and left in the dark for one hour. The standard 0.1 N iodine solution was prepared using standard methods [48]. To 10 ml of the iodine oxidised samples, stoichiometric amounts of 0.1 N sodium thiosulphite pentahydrate ($Na_2S_2O_3.5H_2O$) solution was added to reduce excess iodine to the iodide. The solution was then diluted to 100 ml, its pH adjusted to 6.5, and the amount of phosphate determined spectrophotometricaly. A control oxidation experiment was carried out by taking 25 ml of buffer solution then mixing it with 25 ml of distilled water and 50 ml of standard 0.1 N iodine solution. The flask was shaken lightly and left in the dark for one hour. After this, 10 ml of this solution was taken and stoichiometric amount of the 0.1 N sodium thiosulphate solution added. The resulting solution was diluted to 100 ml, its pH adjusted to 6.5 then used as blank for spectrophotometric determinations.

3. RESULTS AND DISCUSSIONS

3.1 Hydrolytic Reactions

Some 1 x 10^{-3} M solutions of $tn_2Copppi$ and $tn_2Compppi$ were prepared at pH 6.5 with different cobalt to condensed phosphate ratio as outlined in section 2.8. After this, 2.0 ml aliquot samples were withdrawn at different times (2, 5, 10, 20, 30 and 60 minutes) and subjected to europium quenching as outlined in section 2.8.6. The quenched solutions were diluted to 100 mls and used for both orthophosphate and condensed phosphate assay. Blank determinations were carried out by using distilled water instead of the phosphate and then following the procedure given in section 2.3.3. The concentration of the orthophosphate or the condensed phosphates (in terms of orthophosphate) present in the solution were then determined according to the procedure given in section 2.3.3. Percentages of orthophosphate, pyrophosphate, tripolyphosphate and trimetaphosphate present in the solution were calculated as follows:

$\%\ pi = [pi]/[pi\ total] \times 100$

$\%\ ppi = [ppi]^*/[pi\ total] \times 100$

$\%\ pppi = [pppi]^*/[pi\ total] \times 100$

$\%\ mpppi = [mpppi]^*/[pi\ total] \times 100$

where:

* indicates that the concentration of ppi, pppi and mpppi are in terms of orthophosphate. The [pi total] was determined according to the procedure given in section 2.8.1.

3.1.1. Results

The results for the hydrolytic reactions of pppi and mpppi using $tn_2Co(III)$ (aq) {pH 6.5, Eu(II) quenching technique} are shown in the following tables and figures. All the values in the tables are expressed as percentages. All the results were subjected to student's t test [59] taking 95 % confidence limit as shown in Table 3.1.

The summary of pppi hydrolysis mediated by $[tn_2Co(OH)(OH_2)]^{2+}$ (5×10^{-3} M, ambient temperature, pH 6.5, 1 M $NaClO_4$) is shown below.

Table 3.1 % Hydrolytic products for different metal to pppi ratio

Time(min.)		2	5	10	20	30	60
1:1[a]	pi	10.78	14.5	27.38	36.51	39.83	43.57
	ppi	22.00	39.83	32.79	30.70	28.64	26.55
	pppi	66.40	43.57	36.93	32.37	30.70	28.21
	Totals	99.18	97.92	97.10	99.58	99.17	98.33
		+0.55	+0.61	+0.26	+1.36	+ 2.10	+2.10
2:1[b]	pi	17.61	22.22	33.13	46.96	60.38	62.89
	ppi	35.22	44.02	48.64	38.99	27.25	24.74
	pppi	45.70	32.70	17.18	13.00	10.90	10.91
	Totals	98.53	98.94	98.95	98.95	98.53	98.54
		+0.15	+1.01	+1.44	+0.44	+0.29	+0.22
1:1+1[c]	pi	17.30	22.78	29.96	39.66	50.20	54.42
	ppi	23.20	36.70	44.30	38.40	29.54	25.32
	pppi	59.07	39.24	24.47	19.83	17.72	16.46
	Totals	99.57	98.72	98.73	97.89	97.46	96.20
		+0.30	+0.16	+0.36	+0.19	+1.09	+0.24
3:1[d]	pi	4.22	20.68	36.70	64.13	78.06	87.76
	ppi	5.06	21.52	34.60	24.90	14.34	3.80
	pppi	86.07	50.63	21.52	3.80	1.26	0.84
	Totals	95.35	92.83	92.82	92.83	93.66	92.40
		+0.07	+0.93	+0.65	+0.89	+0.60	+0.50
1:1+2[e]	pi	20.67	27.42	38.82	56.54	70.04	77.63
	ppi	39.25	44.30	45.14	35.86	24.04	17.73
	pppi	38.82	26.16	14.34	5.90	3.80	3.38
	Totals	98.74	97.88	98.30	98.30	97.88	98.74
		+0.76	+0.67	+0.66	+0.50	+0.41	+0.97
1:1+1+1[f]	pi	24.79	28.15	36.55	51.68	63.02	71.84
	ppi	31.51	41.60	44.95	37.81	30.67	21.84
	pppi	42.85	29.00	16.39	8.82	5.47	5.47
	Totals	99.15	98.75	97.89	98.31	99.16	98.32
		+0.33	+0.52	+0.35	+0.74	+0.77	+1.20

a: Equimolar metal to mpppi ratio.
b: Direct addition 2:1 metal to mpppi ratio.
c: Preformed 1:1 plus one molar ratio of metal ion.
d: Direct addition 3:1 metal to mpppi ratio.
e: Preformed 1:1 plus two molar ratio of metal ion.
f: Preformed 1:1 plus addition of two molar ratio of metal ion.

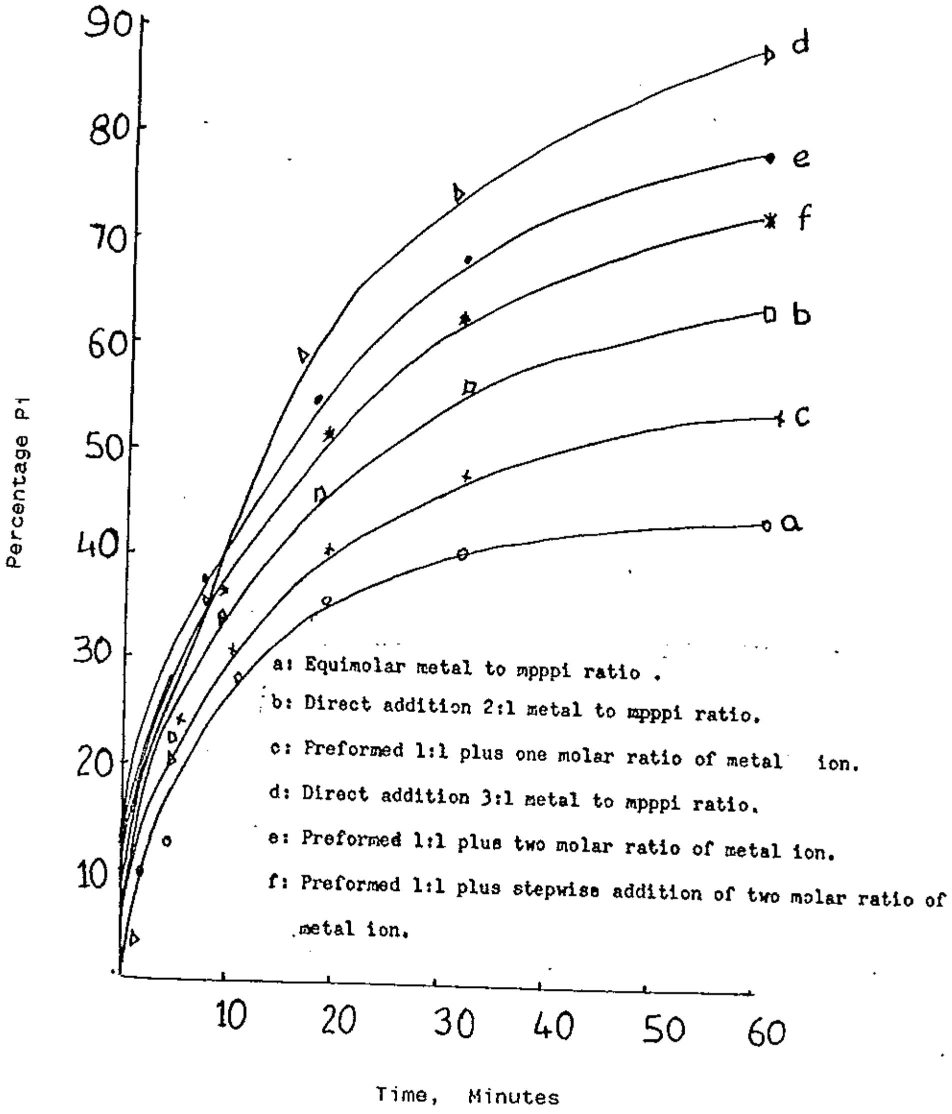


Figure 3.1. Production of pi during [tn$_2$Co(OH)(OH$_2$)]$^{2+}$ mediated hydrolysis of pppi

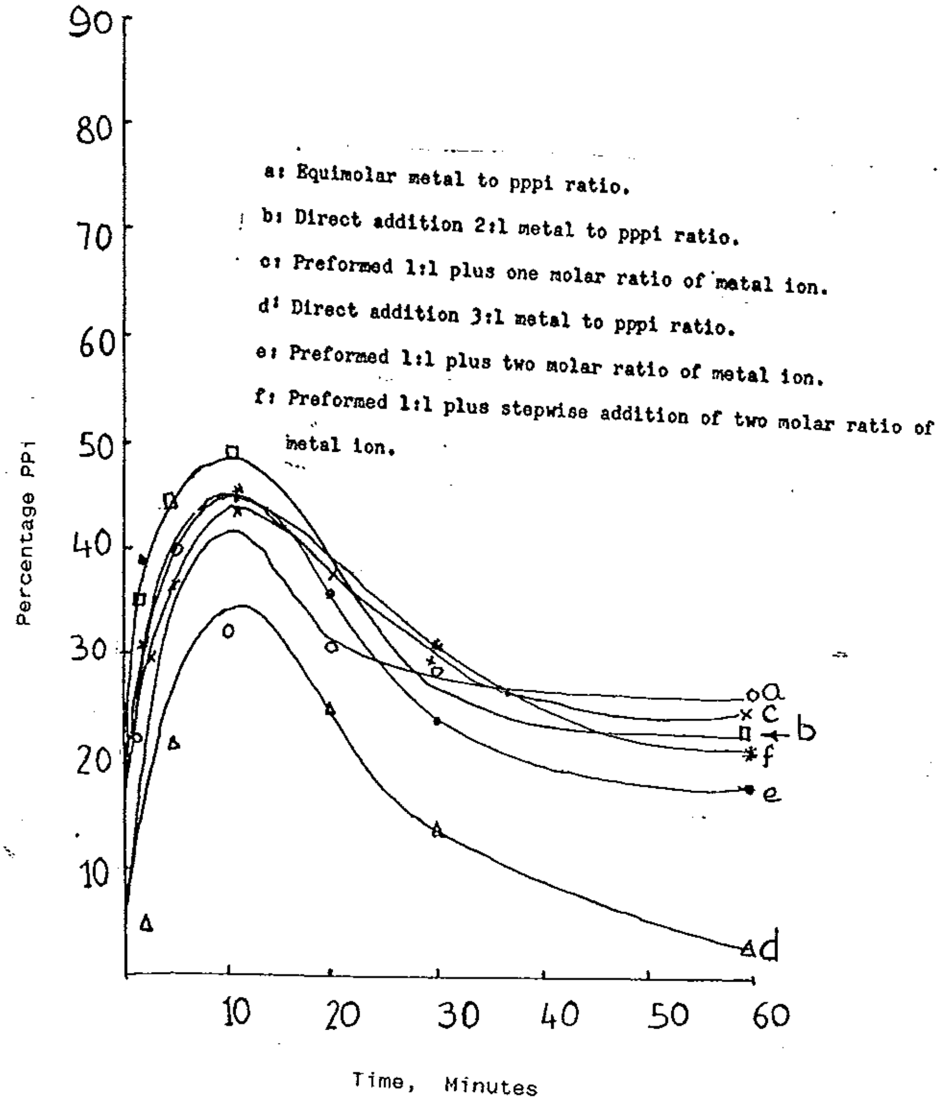


Figure 3.2. Production and depletion of ppi during $[tn_2Co(OH)(OH_2)]^{2+}$ mediated hydrolysis of pppi

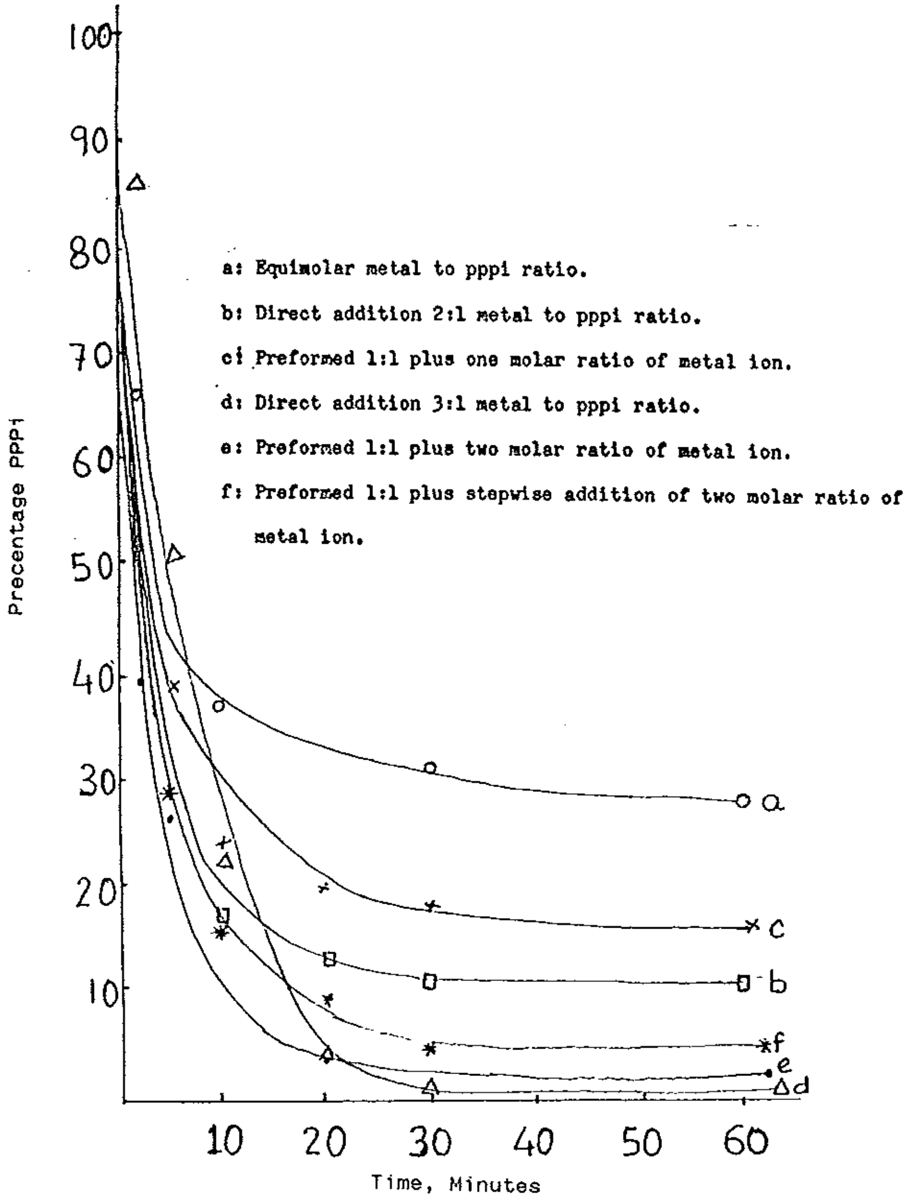


Figure 3.3. Depletion of pppi during $[tn_2Co(OH)(OH_2)]^{2+}$ mediated hydrolysis of pppi

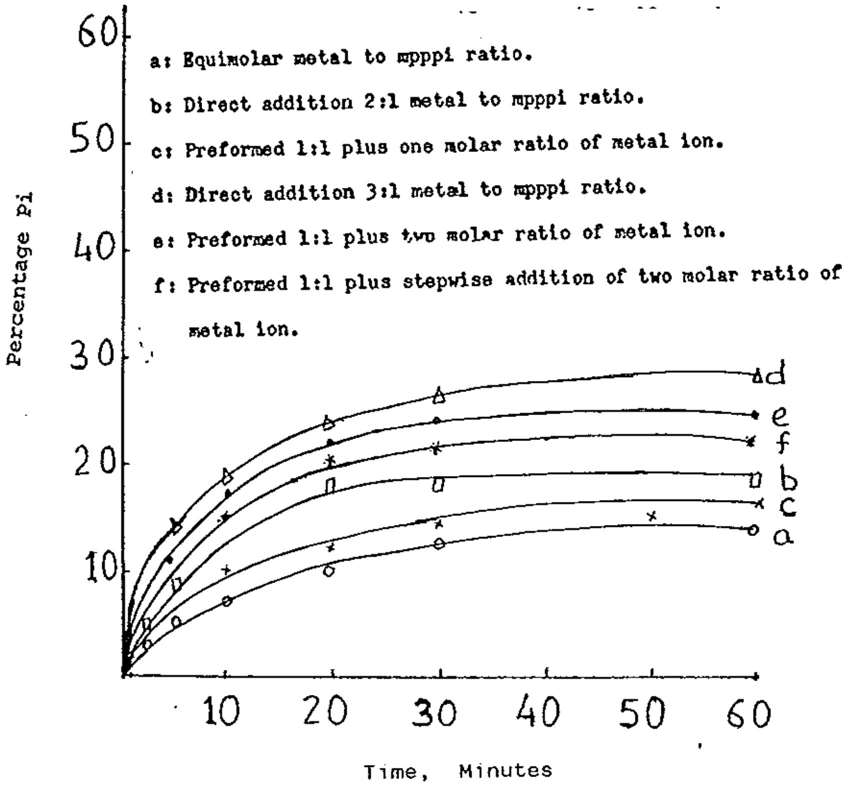


Figure 3.4. Production of pi during $[tn_2Co(OH)(OH_2)]^{2+}$ mediated hydrolysis of mpppi

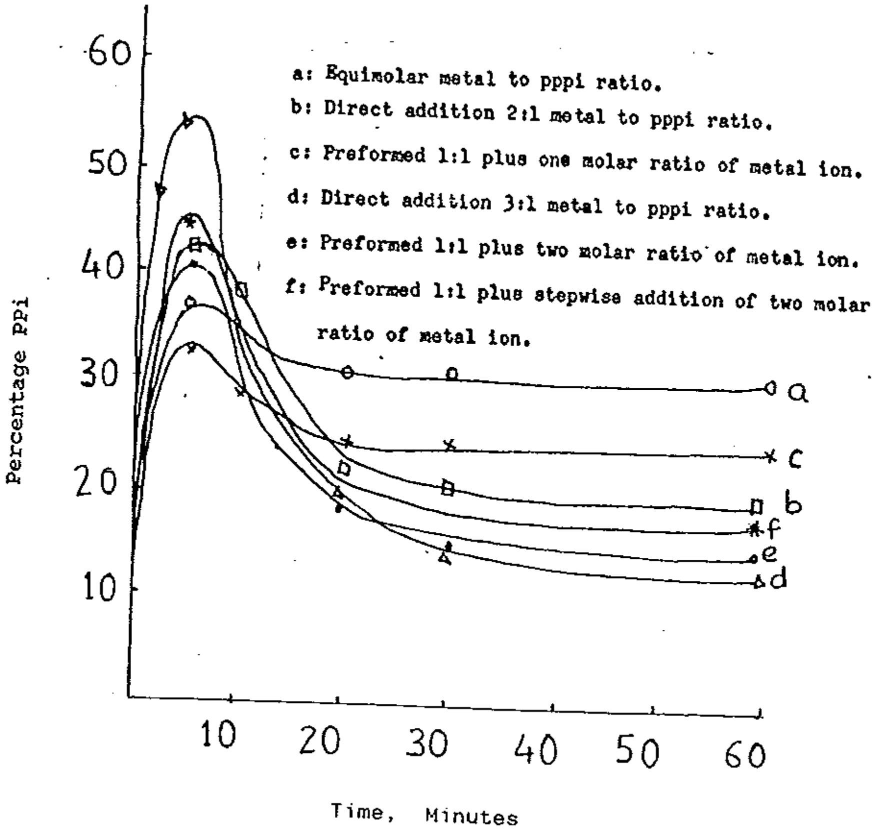


Figure 3.5. Production and depletion of ppi during $[\mathrm{tn_2Co(OH)(OH_2)}]^{2+}$ mediated hydrolysis of mpppi

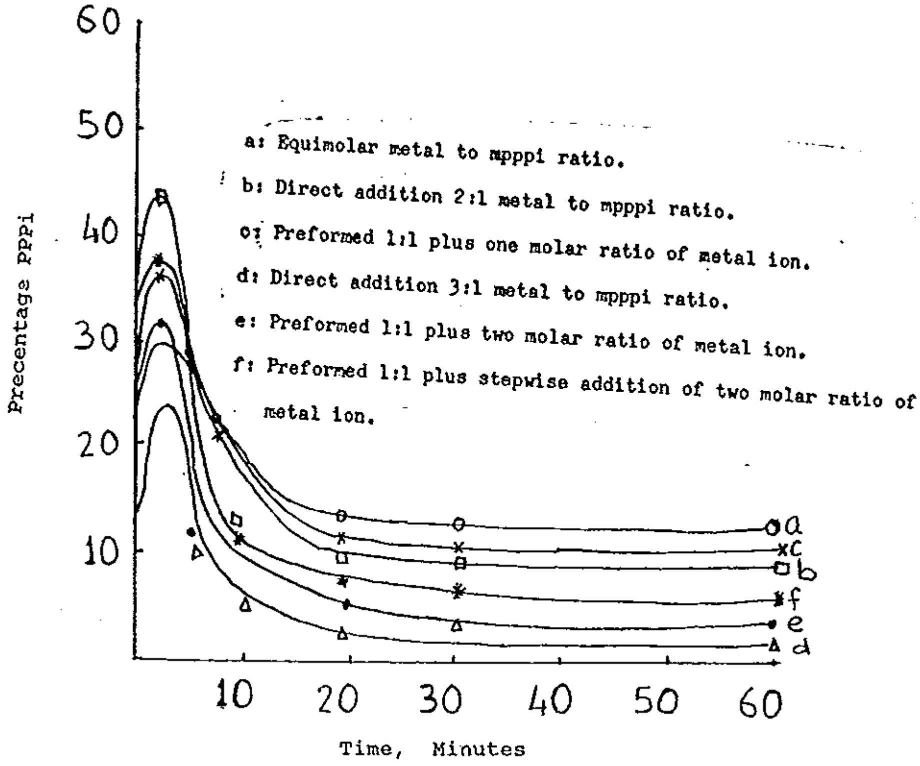


Figure 3.6. Production and depletion of pppi during $[tn_2Co(OH)(OH_2)]^{2+}$ mediated hydrolysis of mpppi

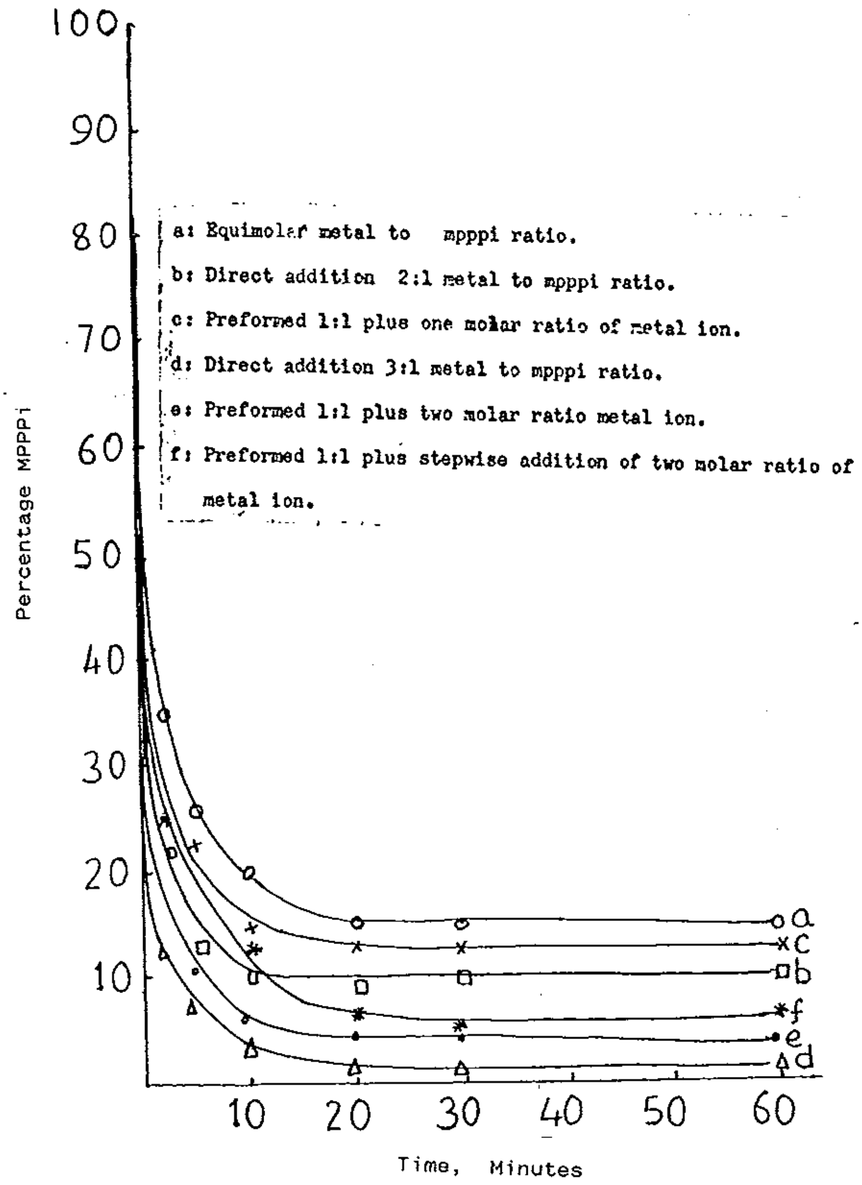


Figure 3.7. Depletion of mpppi during $[tn_2Co(OH)(OH_2)]^{2+}$ mediated hydrolysis of mpppi

3.1.1.1 Qualitative and quantitative analysis of trimetaphosphate hydrolytic products.

It was noted that during the course of the $[tn_2Co(OH)(OH_2)]^{2+}$ mediated hydrolysis of mpppi, the total sum of the hydrolytic products do not add up to 100 % and it is evident that there was a steady decrease in the total amount of the total phosphate concentration with increased time (see Table 3.2). As a result qualitative tests were carried out to find out if other oxy-acid salts of phosphorus prevail in the solution. The qualitative tests [40] indicated the presence of phosphites in the solution.

Quantitative analysis was then carried out by first oxidising the orthophosphite (PO_3^{3-}) to the corresponding orthophosphate (PO_4^{3-}) using iodine [48] according to the equation:

$$PO_3^{3-}\ (aq) + I_2\ (aq) + H_2O\ (l) \text{----} PO_4^{3-}(aq) + 2I^-\ (aq) + 2H^+\ (aq)$$

The PO_4^{3-} so formed was then determined spectrophotometrically using the protocol given in section 2.3.3.

A representative summary of the total sum of the hydrolytic products of mpppi by $[tn_2Co(OH)(OH_2)]^{2+}$ before and after oxidation by iodine is shown in Table 3.3.

Table 3.3. Percentage variation of total Pi with respect to metal to mPPPi ratio

Sample*	Total Pi before oxidation	Total Pi after oxidation	Pi due to oxidation of PO_3^{3-}
1:1	71.5 % (65.1 μg P/ml)	99.83 % (90.9 μg P/ml)	28.33 % (25.8 μg P/ml)
2:1	56.78 % (51.7 μg P/ml)	99.83 % (90.9 μg P/ml)	43.05 % (39.2 μg P/ml)
3:1	43.05 % (39.6 μg P/ml)	99.83 % (90.9 μg P/ml)	56.05 % (51.3 μg P/ml)

* All the samples for the different metal to mpppi ratios were taken after 60 minutes of reaction time. The values in brackets show concentrations in ug P ml^{-1}. The percentages were calculated relative to the total value of 91.05 ug P ml^{-1} (see section 2.8.1). In Table 3.4 the production of orthophosphite (PO_3^{3-}) with respect to time for different metal to mpppi ratio is depicted. All the values in the table were calculated as percentages using the following equation:

% PO_3^{3-} = $[PO_3^{3-}]/[pi\ total]$ X 100 where $[PO_3^{3-}]$ is the concentration of the PO_3^{3-} in terms of its oxidised form, PO_4^{3-} and [pi total] corresponds to the sum of the phosphate species in the solution in terms of orthophosphates (i.e. 91.05 ug P ml^{-1})

Table 3.4. Production of orthophosphite (PO_3^{3-}) with respect to time for different metal to mpppi ratio

Time (min.)	Percentage phosphite for different metal to mpppi ratio					
	1:1[a]	2:1		3:1		
		2:1[b]	1:1+1[c]	3:1[d]	1:1+2[e]	1:1+1+1[f]
2	1.68	0.90	2.10	5.45	0.85	1.67
5	2.61	5.85	4.03	11.83	25.52	7.12
10	20.92	28.04	26.78	43.10	28.87	33.47
20	28.87	41.85	37.65	52.31	50.63	45.60
30	28.87	42.68	38.48	53.55	52.30	48.27
60	28.33	43.05	39.33	56.33	52.72	48.27

a : Equimolar metal to mpppi ratio.

b : Direct addition 2:1 metal to mpppi

c : Preformed 1:1 plus one molar ratio of metal ion.

d : Direct addition 3:1 metal to mpppi ratio.

e : Preformed 1:1 plus two molar ratio of metal ion.

f : Preformed 1:1 plus addition of two molar ratio of metal ion.

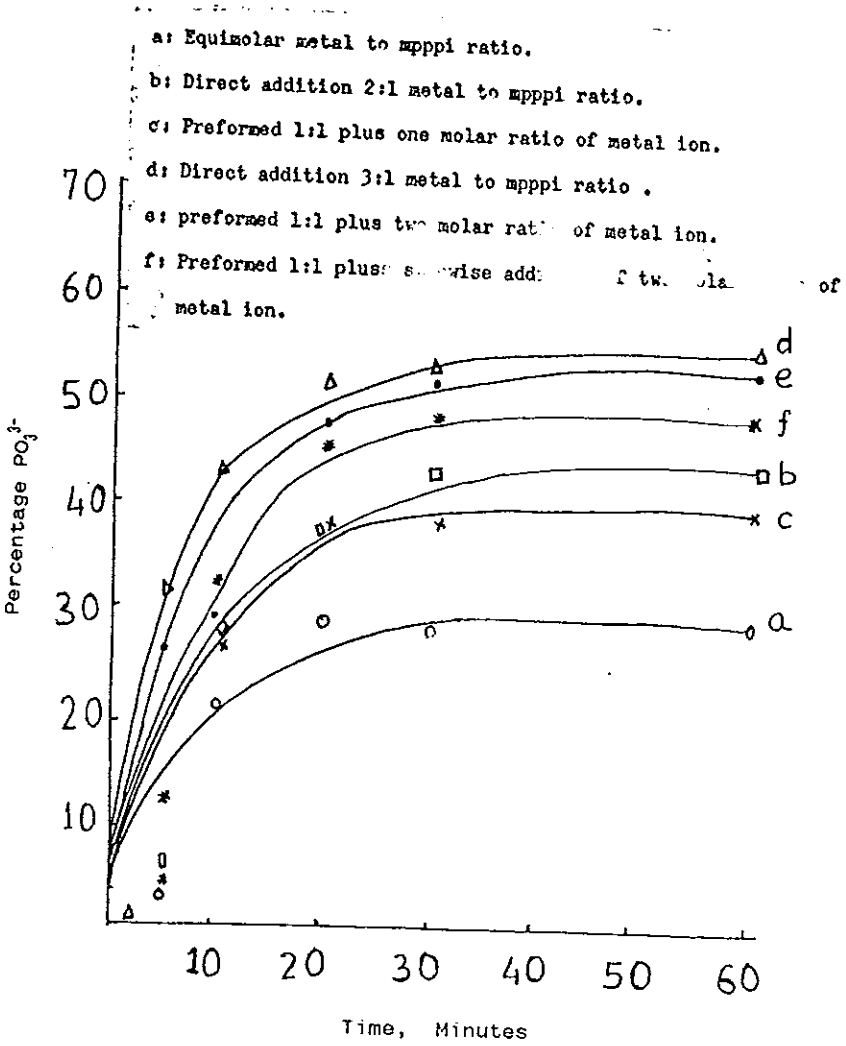


Figure 3.8. Production of PO_3^{3-} during $[tn_2Co(OH)(OH_2)]^{2+}$ mediated hydrolysis of mpppi

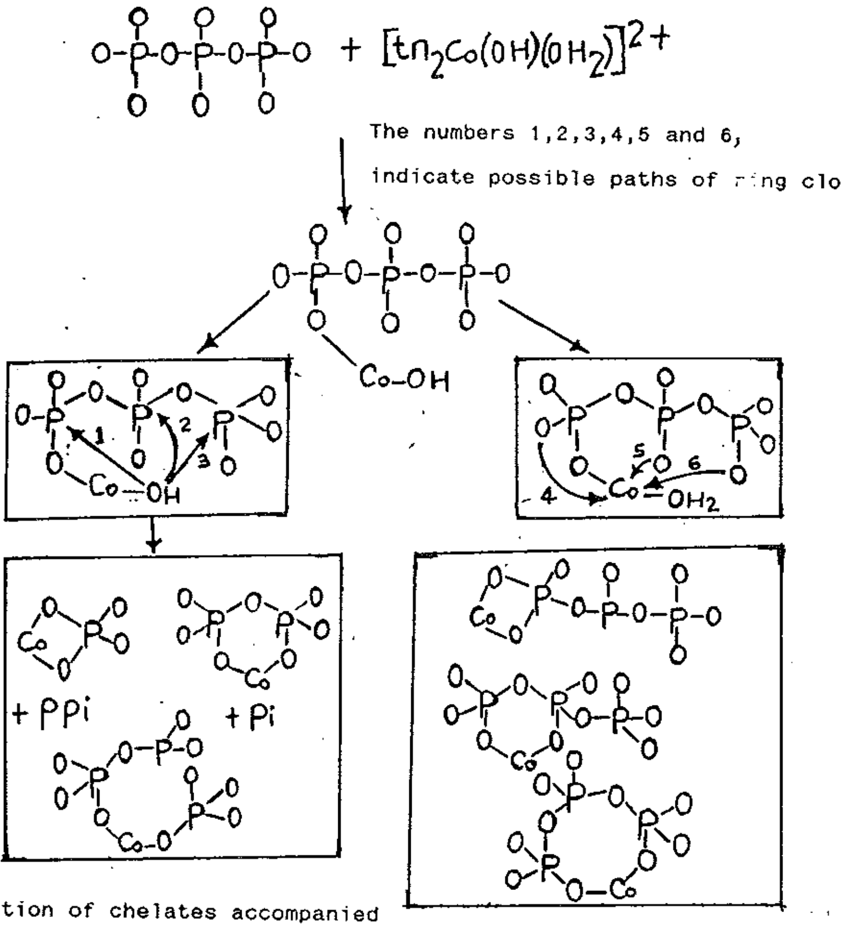
3.2 Discussion

From the results presented, salient features have been noted. The following explanations will be dedicated to elucidating them. The reactive species, hyroxoaqua-bis(trimethylenediamine)cobalt(III) ion, ([tn2Co(OH)(OH2)]2+), predominates at pH 6.5 [15,25,57]. It has a strong nucleophile (hydroxide) and a labile water molecule coordinated to the cobalt centre. The hydroxoaqua complex is generated from the diaqua complex which exists at mildly acidic pH ranges. It has been observed that a coordinated hydroxide is a very effective nucleophile in the intramolecular hydrolysis of the nitriles, olefins, amino acid esters [49] and phosphate esters [6,15,25,50]. The reactivity of the hydroxo aqua complex has been attributed to the electrons freed from the diaqua complex by loss of a proton. This labilizes the coordinated water molecules by increasing the electron density on the cobalt centre [51]. The possibility of different conformation states enjoyed by the six membered chelate rings of the trimethylenediamine moieties around the cobalt centre, has been presumed to enhance the reactivity of the coordinated hydroxide.

This may be so especially towards intramolecular attack on the phosphorus centre by creating an optimum stereochemical orientation. During the hydrolysis of the condensed phosphates, anation reaction takes place and the labile water molecule is presumably substituted by a condensed phosphate anion. This is supposed to lead to formation of a monodentate species such as $[tn_2Co(OH)(P_3O_{10})]^+$ and $[tn_2Co(OH)(P_3O_9)]^+$ in the 1:1 metal to tripolyphosphate and trimetaphosphate solutions respectively. In the hydrolysis of both pppi and mpppi, it was noted that the rate of hydrolysis increases as the metal to condensed phosphate ratio increases. The highest rates were observed with the direct addition 3:1 ratio. This is evidenced by the highest production of hydrolytic products and low concentrations of pppi and mpppi present in the solution during the course of the reaction. This observation was found to be in line with the notion that two or more metal ions per mole of substrate are essential for effective hydrolysis of condensed phosphates [42,52,53,54]. The 3:1 metal to condensed phosphate ratio causes high charge neutralization in the coordinated phosphates. Hence this could contribute to the high rate of hydrolysis observed. The other possible roles of the metal ions have been outlined in section 1.3.

The hydrolytic cleavages promoted by the 1:1 metal to pppi ratio is depicted in scheme 3.1. It is evident from the scheme that the initial stage of the reaction involves anation. The monodentate Copppi complex formed can then undergo further reaction giving probably four membered, six membered and eight membered ring systems. Some of these reactions would lead to the formation of the chelate rings accompanied by hydrolytic cleavage while others form less reactive chelate rings which do not undergo further cleavage.

However from earlier ^{31}P NMR studies, the six membered ring systems were found to be more prevalent than either the four membered or the eight membered systems [42]. It can be seen from Table 3.1 that appreciable amounts of ppi and pppi remain in solution at the end of the reaction time. This can be attributed to the presence of coordinated and uncoordinated ppi in the solution which either undergo hydrolysis at a very slow rate or do not degrade at all. PPi can also be formed by condensation reactions between Copi and pi [56]. The presence of pppi at the end of the reaction is also presumed to arise partially from condensation reactions between the coordinated ppi and pi.



The numbers 1,2,3,4,5 and 6, indicate possible paths of ring closure

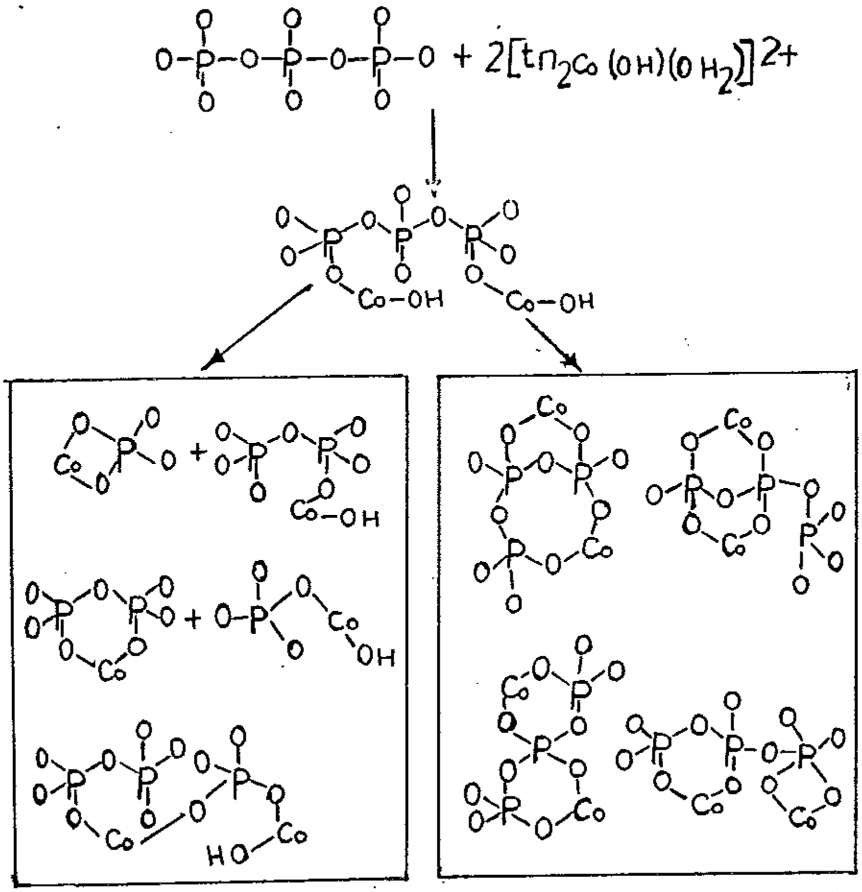
Formation of chelates accompanied by hydrolytic cleávage.

Formation of chelates without hydrolytic cleavage.

Scheme 3.1. Suggested features for the overall mechanism for [tn$_2$Co(OH)(OH$_2$)]$^{2+}$ mediated hydrolysis of tripolyphosphate, pH 6.5, ambient temperature, 0.1 M NaClO$_4$ and 1:1 metal to pppi ratio (charge and degree of protonation omitted)

In scheme 3.2 the hydolytic cleavage promoted by direct addition of 2:1 metal to pppi ratio is postulated as is evident from the scheme. Different chelates will be formed out of which some are more prone to hydrolytic cleavage than others. However the prevalence of less reactive complexes is not as high as in the 1:1 case. This is shown by the lower value of pppi remaining in solution at the end of the reaction time for the 2:1 case compared to that of the 1:1. The trend of pppi depletion is similar to that of the 1:1. The trend of the pi and ppi production is also similar to that of the 1:1 case. The suggested features for the 3:1 (direct addition) metal to pppi hydrolysis reaction is depicted in scheme 3.3.

Similar features to those of the 1:1 and 2:1 metal to pppi reaction solution also prevail in the 3:1 case. However the formation of reactive complexes that presumably undergo complete degradation seem to predominate under the experimental conditions.



Formation of chelates accompanied by hydrolytic cleavage

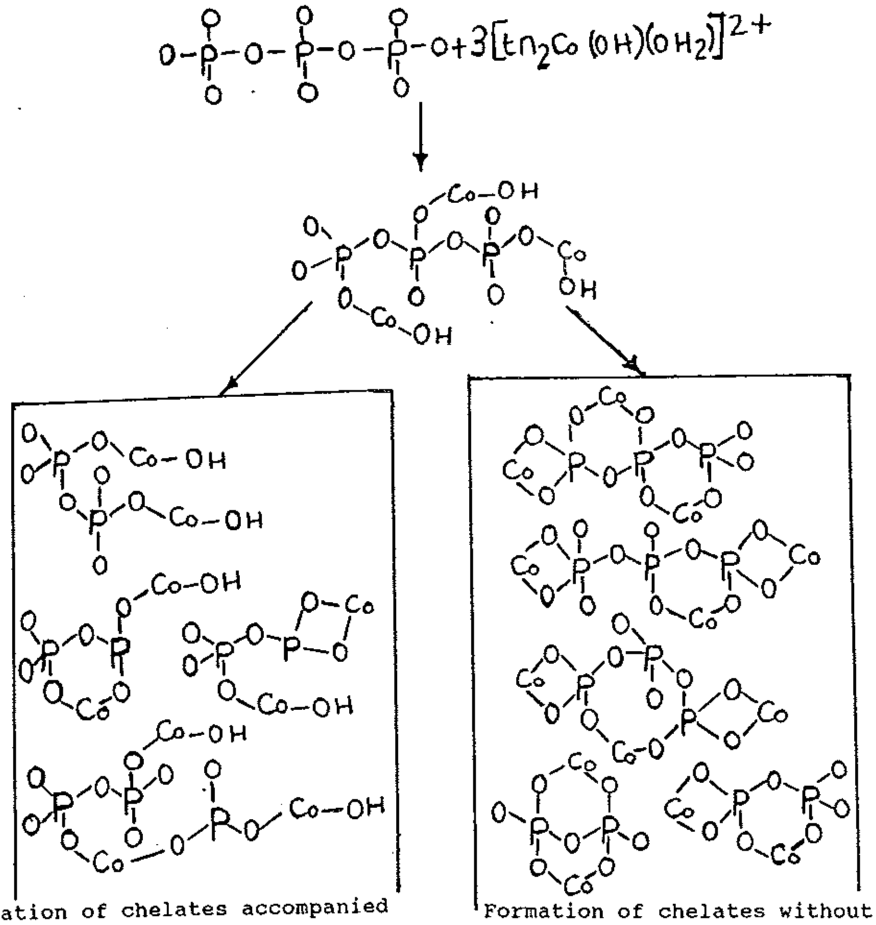
Formation of chelates without hydrolytic cleavage.

Scheme 3.2. Suggested features for the overall mechanism for $[tn_2Co(OH)(OH_2)]^{2+}$ mediated hydrolysis of tripolyphosphate, pH 6.5, ambient temperature, 0.1 M $NaClO_4$ and 2:1 metal to pppi ratio (charge and degree of protonation omitted)

The production of pi as a result of hydrolysis was found to be lower in the stepwise additions of the various metal to pppi ratio compared to those of direct addition. This could be due to the formation of predominant reactive chelates which eventually undergo complete degradation in the case of the direct addition systems. Addition of $[tn_2CO(OH)(OH_2)]^{2+}$ to pppi at 1:1 molar ratio results in rapid water substitution to form the monodentate complex which eventually forms ring systems. The rate of formation is presumed to be relatively fast. Addition of another molar ratio of $[tn_2CO(OH)(OH_2)]^{2+}$ to the preformed 1:1 complex at pH 6.5 results in the formation of several chelates out of which the predominant species are presumed to be stable towards hydrolysis.

For the direct addition systems, more than one metal ion reacts with every molecule of pppi. The random attack of the metal ion on the pppi would effect the production of reactive chelates. For the stepwise additions, the first bond produced with the preformed complex would dictate the position to which the second metal ion would coordinate thereby effecting the formation of less reactive chelates.

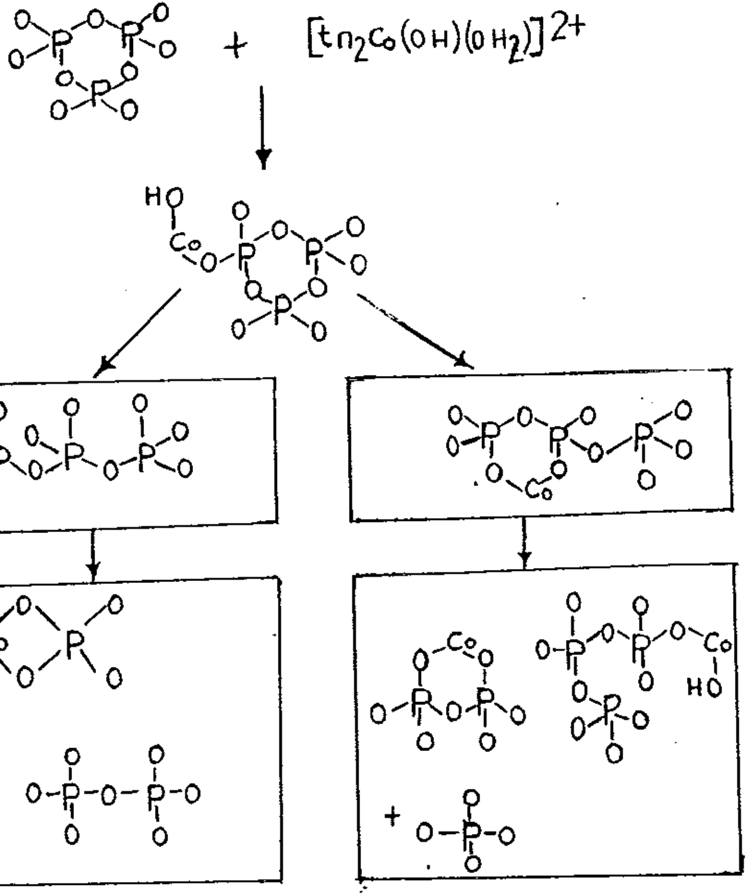
The hydrolysis of mpppi was also carried out using different metal to mpppi ratios. From the mpppi hydrolysis data (see table 3.2), it is apparent that two distinct features are revealed in the hydrolytic reaction. One feature conforms to the general theory [3,55,56] that mpppi hydrolysis proceed via ring opening (scheme 3.4) to pppi which then degrades to the pyrophosphate and eventually to orthophosphate. The other feature is suggested by scheme 3.5, according to which the production of phosphite is rationalized. The type of mechanism suggested in scheme 3.5 is analogous to the reactions involved in the epoxidation of the alkenes [58].



Formation of chelates accompanied by hydrolytic cleavage. Other possible chelates are same as those shown in schemes 3.1 and 3.2

Formation of chelates without hydrolytic cleavage. Other possible chelates are same as those shown in schemes 3.1 and 3.2

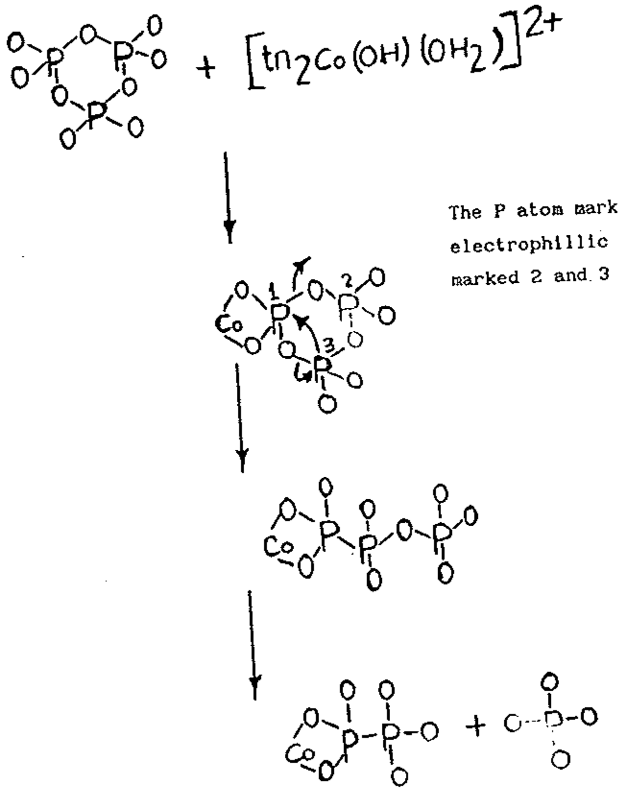
Scheme 3.3. Suggested features for the overall mechanism for $[tn_2Co(OH)(OH_2)]^{2+}$ mediated hydrolysis of tripolyphosphate, pH 6.5, ambient temperature, 0.1 M $NaClO_4$ and 3:1 metal to pppi ratio (charge and degree of protonation omitted)



Formation of chelates accompanied by hydrolytic cleavage.

Formation of chelates without hydrolytic cleavage.

Scheme 3.4. Suggested features for the overall mechanism for $[tn_2Co(OH)(OH_2)]^{2+}$ mediated hydrolysis of trimetaphosphate, pH 6.5, ambient temperature, 0.1 M $NaClO_4$ and 1:1 metal to mpppi ratio (charge and degree of protonation omitted)



The P atom marked more
electrophilic th se
marked 2 and 3

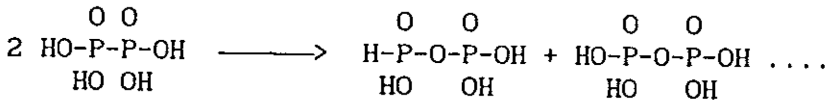
coordinated hypophophorus acid

Scheme 3.5. Suggested features for an alternative overall mechanism for $[tn_2Co(OH)(OH_2)]^{2+}$ mediated hydrolysis of trimetaphosphate, pH 6.5, ambient temperature, 0.1 M $NaClO_4$ and 1:1 ratio
(charge and degree of protonation omitted)

An increased degree of hydrolysis was observed for 3:1 metal to trimetaphosphate ratio. With the 3:1 metal to mpppi ratio, the highest hydrolytic cleavage was noted for the direct addition systems, followed by the system where two molar ratio of $[tn_2CO(OH)(OH_2)]^{2+}$ was added to the preformed tn_2Co mpppi. The lowest hydrolytic cleavage was seen for the systems with stepwise addition. This observation is in line with the findings with tripolyphosphate. The role of the metal ions in both cases is attributed among other factors to activation, substrate formation and structural consideration.

It is evident that the total sum of pi from the hydrolytic products of the trimetaphosphate solution decreases with increasing reaction time. However at initial stages of hydrolysis, the sum was found to be approximately 100 % (Table 3.2) for all of the metal to mpppi ratios. The loss in the total phosphate was found to be highest for the 3:1 and lowest for the 1:1 metal to mpppi ratios. Qualitative analysis showed the presence of ortho phosphite (PO_3^{3-}) in the solution. This indicated that apart from the expected mpppi, pppi, ppi and pi some PO_3^{3-} was also being produced. The production of PO_3^{3-} in the solution led to the postulation of scheme 3.5.

The phosphites detected are likely to have been produced from hypophosphorus acid. The degradation of hypophosphorus acid into orthophosphite proceeds according to the following equations [3]:

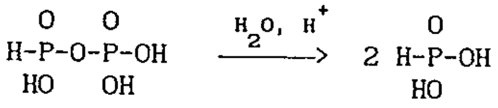


$$2\ \mathrm{HO{-}\underset{HO}{\overset{O}{P}}{-}\underset{OH}{\overset{O}{P}}{-}OH} \longrightarrow \mathrm{H{-}\underset{HO}{\overset{O}{P}}{-}O{-}\underset{OH}{\overset{O}{P}}{-}OH} + \mathrm{HO{-}\underset{HO}{\overset{O}{P}}{-}O{-}\underset{OH}{\overset{O}{P}}{-}OH} \quad \ldots.\ (1)$$

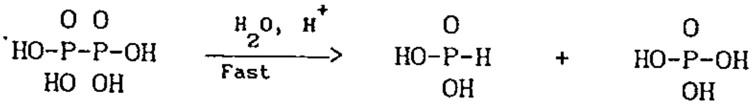
Hypophosphorus acid

Pyrophosphorus acid

Pyrophosphoric acid



$$\text{Then,}\ \mathrm{H{-}\underset{HO}{\overset{O}{P}}{-}O{-}\underset{OH}{\overset{O}{P}}{-}OH} \xrightarrow{H_2O,\ H^+} 2\ \mathrm{H{-}\underset{HO}{\overset{O}{P}}{-}OH}$$



$$\mathrm{HO{-}\underset{HO}{\overset{O}{P}}{-}\underset{OH}{\overset{O}{P}}{-}OH} \xrightarrow[\text{Fast}]{H_2O,\ H^+} \mathrm{HO{-}\underset{OH}{\overset{O}{P}}{-}H} + \mathrm{HO{-}\underset{OH}{\overset{O}{P}}{-}OH} \quad \ldots.\ (2)$$

Orthophosphorus acid

Orthophosphoric acid

The P-P bond can be formed intramolecularly given appropriate geometric and angular orientations in the metal-trimetaphosphate complex. Upon coordination of the metal to the trimetaphosphate, the phosphorus atom to which the metal is directly bonded via an oxygen bridge is made relatively more electrophillic than the remaining two which can now be considered as "nucleophiles". Either of the two nucleophillic phosphorus atoms can then attack the electrophillic one to form the P-P bond. The reaction is statistically favoured by the presence of two nucleophillic atoms per electrophile (phosphorus atom). The structure of the trimetaphosphate ring is presumed to ensure that the two phosphorus groups stay in close proximity for a reasonable time to effect the bond formation. The formation of the hypophosphorus acid would be in line with the idea that intramolecular reactions are time, angular and distant dependent [57]. The absence of hypophosphites or pyrophosphites in the solution was not surprising since between pH 1.5 and pH 2 they all degrade into orthophosphites [3]. The presence of the orthophosphites was further confirmed by its quantitative oxidation to the orthophosphate using iodine solution. It was noticed that the total pi after oxidation (90.90 ug P ml^{-1}) was approximately equal to the experimentally determinable total pi (91.05 ug P ml^{-1}). This indicated that the differ XXence in the total pi before oxidation and the experimentally determinable total pi was due to the phosphite.

The production of orthophosphite from the hypophosphorus acid is mainly a result of the fast process depicted in equation (2). However to some degree equation (1) also contributes to the degradation. This is seen by the tremendous initial increase in the amount of pyrophosphate detected in the solution during the course of hydrolysis.

CONCLUSION

The results of this work have shown that $[tn_2Co(OH)(OH_2)]^{2+}$ complexes catalyze the hydrolysis of tripolyphosphate and trimetaphosphate in the middle pH region with enhanced reactivity. The mode of hydrolysis is believed to be a combination of factors by the metal complexes including charge neutralization, electron density polarization, the provision of an appropriately placed nucleophile and close proximity of the reacting groups for a reasonable period of time which can be facilitated by the different conformation enjoyed by six membered chelates. This work has shown for the first time that phosphites are produced during the hydrolysis of trimetaphosphates to the extent of 50 % in 3:1 metal to mpppi ratio. The separation and quantification of different phosphate species in solution is also the first attempt in this endeavour.

REFERENCES

1 Bodner, G.M.; J. Chem. Educ. 7, 566 (1986).

2 Benet, T.P. and Frieden, E.; Modern Topics in Biochemistry, Macmillan company, New York, 1968.

3 Toy, A.D.F.; Comprehensive Inorganic Chemistry pp 468-528, Pegamon press, 1973.

4 Austin, G.T.; Shreeve's Chemical Process Industries, 5th Ed., chapter 26, McGraw Hill New York, 1984.

5 William, D.R.; The Metals of life, Camelot press, London 1971.

6 Hendry, P. and Sargeson, A.M.; Inorg. Chem. 29, 92 (1990).

7 Harrowfield, J.M.; Jones, D.R.; Lindoy, L.F. and Sargeson, A.M. ; J. Am. Chem. Soc. 102, 7733 (1980).

8 Morrison, J.F and Heyde, E.; Anu. Rev. Biochem. 41, 29 (1972).

9 Jencks, W.P.; Catalysis in Chemistry and Enzymology, McGraw Hill , New York, 1966.

10 Walsh, C.; Enzyme Reaction Mechanisms, W.H. Freeman and Company, San Francisco, 1979.

11 Morow, J.R. and Trogler, W.C.; Inorg. Chem. 23, 2330 (1989).

12 Gelman, S.H.; Peter, R.; Breslow, R.; J. Am. Chem. Soc. 108, 2388 (1986).

13 Menger, F.M.; Gan, L.H.; Johnson, E.; Durst, D.H.; J. Am. Chem. Soc. 109, 2800 (1987).

14 Morrow, J.R. and Trogler, W.C.; Inorg. Chem. 27, 3387 (1988).

15 Zewdu, A.; M.Sc. Thesis, Addis Ababa University, Addis Ababa, 1990.

16 Buttler, L.G.; The Enzymes, 3rd Ed. Boyer,P.D(Ed). Academic press, New York, vol. 4, pp 529-535, 1972.

17 Milburn, R.M.; Gautam-Basak, M.; Tribolet, R.; Sigel, H.; J. Am. Chem. Soc. 107, 3315 (1985).

18 Tetas, M.; Lowenstein, J.M.; Biochem. 2, 350 (1963).

19 Wagner-Jauregg, T.; Hackley, B.E.; Lies, T.A.; Owens, O. O.; Proper, R.; J. Am. Chem. Soc. 77, 922 (1955).

20 Gautafson, R.L.; Chaberek, S.; Martel, A.E. J. Am. Chem. Soc. 85, 598 (1963).

21 Epstein, J.; Mosher, W.A.; J. Phys. Chem. 72, 622 (1968).

22 Kenley, R.A.; Fleming, R.A.; Laine,R.M.; Tse,D.S. and Winter, J.S. Inorg.Chem. 23, 1870 (1984).

23 Bruce, T.C. and Benkovic, S.J.; Bioorganic Mechanism, I.W.A. Benjamin,New York, 1966.

24 Tafesse, F.; Massoud, S.S.; Milburn, R. M.; Inorg. Chem. 24, 2591 (1985).

25 Tafesse, F.; Doctorial Thesis, Boston University, Boston, 1986.

26 Chin, J. and Zon, X.; J. Am. Chem. Soc. **110**, 223 (1988).

27 Martin, R.M.; Mariam, Y.H.; Metal Ions in Biological Systems, vol. 8, p 57, 1979.

28 Haight Jr., G.P.; Hambley, T.W.; Hendry, P.; Lawrence, G.A.; Sargeson, A.M.; J. Chem. Soc. Chem. Commun. 488 (1985).

29 Norman, P.R. and Cornelius, R.D.; J. Am. Chem. Soc. **104**, 2356 (1982).

30 Fowless, A. D. and Stranks, D. R.; Inorg. Chem. **16**, 1276 (1977).

31 Bell, R.N.; Anal. Chem. **19**, 97 (1947).

32 Motomizu, S.; Wakimoto, T. and Toei, K.; Analyst, **108**, 361 (1983).

33 Baykov, A.A.; Evtushenko, O.A. and Avaeva, S.M; Anal. Biochem. **171**, 266 (1988).

34 Fiske, C.H. and Subarrow, Y.; J. Biol. Chem. **66**, 375 (1925).

35 Martin, J.B. and Doty, D.M.; Anal. Chem. **21**, 965 (1949).

36 Ueda, I. and Wada, T.; Anal. Biochem. **37**, 169 (1970).

37 Terasaki, W. and Booker, G.; Anal. Chem. **75**, 447 (1976).

38 Hirata, A. A. and Appleman, D.; Anal. Chem. **31**, 2097 (1959).

50 Kruse, W. and Taube, H.; J. Am. Chem. Soc. 83, 1280 (1961).

51 Miller, D.L. and Westheimer, F.H.; J. Am. Chem. Soc. 88, 1514 (1966).

52 Sigel, H.; Hofster, F.; Martin, R.B.; v <F7 Milburn,R.M.; Scheller-Kratinger and Scheller,K.H; J. Am. Chem. Soc. 106, 7935 (1984).

53 Sargeson, A.M.; J. Chem. Soc. Chem. Commun. 1568 (1984).

54 Osterheld, R.K.; Topics in Phosphorus Chemistry, Interscience, NewYork, 1972, vol. 7, p 103.

55 Thilo, E. Angew. Chem. Internat. Edit. 4, 1061 (1965).

56 Tafesse, F.; Transition Met. Chem., 16, 114 (1991).

57 Menger, F.M.; Acc. Chem. Res. 18, 128 (1985).

58 Kwart and Hoffman; J. Org. Chem. 31, 419 (1966).

59 Pecksoc, R.L; Shields, L.D.; Cairns, T. and McWilliam, G.I., Modern methods of Chemical Analysis, pp 490-491, 2nd Ed. John Willey and sons, New York, 1968.