ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

# MECHANISTIC STUDIES OF THE HYDROLYSIS OF PYROPHOSPHATE PROMOTED BY TETRAAMINECOBALT(III) IONS

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# Mechanistic Studies of the Hydrolysis of Pyrophosphate Promoted by Tetraaminecobalt(III) Ions

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## Table of contents

Page

## List of Tables

# List of Figures

## List of schemes

## Abbreviations

| | |
|---|---|
| aq | Aquo |
| ATP | Adonesine 5-triphosphate |
| en | Ethylenediamine |
| $[N_4Co(aq)]^{3+}$ | Tetraaminecobalt (III) ion |
| pi | Inorganic orthophosphate |
| Ppi | Inorganic pyrophosphate |
| tn | Trimethylene diamine |
| YIP | Yeast inorganic pyrophosphatase |

# Abstract

The hydrolysis of pyrophosphate was investigated at different concentrations ($2X10^{-3}$ M, $4X10^{-3}$ M and $10^{-2}$ M) of $[N_4Co(OH)(OH_2)]^{2+}$ [$N_4$ = $en_2$ and $tn_2$ (en = ethylenediamine, tn = trimethylenediamine)] using $Eu^{2+}/H^+$ and $OH^-$ quenching techniques. The reaction was monitored by measurement of orthophosphate (pi) in quenched aliquots of the reaction mixtures using Malachite Green phosphomolybdate complex as the analytical reagent. The reactive species with regard to hydrolysis was found to be a 3:1 $N_4Co^{III}$ppi complex. The results show a relatively larger amounts of degradation products for the directly added systems than for those mixed by step wise addition. Factors affecting the formation and degradation of the reactive species are discussed and plausible mechanisms are forwarded.

# 1. INTRODUCTION

## 1.1. General

The importance of phosphorus in the fields of agriculture and biochemistry is quite immense. Phosphorus is an indispensable element as a nutrient for all living organisms. It is taken up and utilized as phosphate and its derivatives such as esters and anhydrides. Phosphate esters and condensed phosphates including ATP are the major source of energy and play a vital role in energy transfer reactions including general synthesis and muscular movement of living systems. Phosphate is also required by nucleic acids.

The various biochemical reactions that take place in cells of all living systems are mediated by enzymes. An enzyme is a protein that is synthesized in a living cell to catalyse biochemical reactions. Similarly the biochemical reactions of phosphates are also controlled by phosphate utilizing enzymes. The enzyme catalysed transfers of phosphoryl, pyrophosphoryl and nucleotydyl groups are among the most important process in biological systems. These reactions are known to be catalysed by enzymes which contain divalent metal ions at their active sites as essential components [1-6]. Enzymes in general and phosphate utilizing enzymes in particular are specific for both the metal ions and sustrates [7].

It is established that the enzyme responsible for the hydrolysis of phosphate esters, pyrophosphates and ATP require divalent metal ions such as $Mg^{2+}$ , $Mn^{2+}$ , $Ca^{2+}$ and $Zn^{2+}$ for activity [8].

Since polyphosphates are essentail to life, understanding their hydrolysis and related phosphoryl transfer involving P-O-P bond cleavage is of relevance. As an example the enzyme catalysed hydrolysis of pyrophosphate is discussed in the following section.

## 1.2. Yeast Inorganic pyrophosphatase (YIP)

Yeast inorganic pyrophosphatase (YIP) is an enzyme consisting of two identical sub units [9] giving a molecular weight of the dimer as 64,000. This enzyme catalyzes the reversible hydrolysis of pyrophosphate to orthophosphate [9-11] and it is specific for the hydrolysis of pyrophosphate in the presence of $Mg^{2+}$ ions [10]. When $Mg^{2+}$ ions are replaced by other activator metal ions, the enzyme becomes less specific and also hydrolyses a number of pyrophosphate esters. The presence of inorganic ortho, meta and polyphosphates and organic phosphates appear to inhibit pyrophosphate hydrolysis only to the extent that they compete for $Mg^{2+}$ [10]

The YIP dimer binds divalent metal ions including $Mg^{2+}$ , $Mn^{2+}$, $Co^{2+}$ , $Zn^{2+}$ , $Ni^{2+}$ , $Fe^{2+}$ and $Cd^{2+}$ all of which activate the enzyme where as $Ba^{2+}$ and $Ca^{2+}$ inhibit it. It has been reported [6, 13] that free metal ions bind to the enzyme before substrate or inhibitors can be bound to it.

Cooperman et al [14] reported that the enzyme has two binding sites per sub unit, one of high affinity and the other with low affinity. Previous studies [11] have suggested that two divalent metal ions per enzyme subunit are required for maximal activity. Whether or not both metal ions participate directly in catalysis is not known.

However from NMR studies [15,16], it has been established that one of the $Mg^{2+}$ ions is bound tightly to one of the phosphate binding sites, the second ion is relatively far from both phosphate sites, thus may play simply a structural role. There is still some discussion as to whether the substrate is a chelate [12] or a monodentate species [14]. Cooperman et al are of the opinion that it is more likely that the natural substrate for the enzyme is the monodentate pyrophosphate [14]. There is ample evidence in favour of the chelate as the most abundant species [17]. Despite the large number of reported studies dealing with this topic, the mechanism of pyrophosphatase catalysis remain unsettled.

## 1.3. Role of Divalent Metal Ions

Enzyme catalysed transfers of phosphoryl, pyrophosphoryl and nucleotydyl groups are important in biological systems. This has provided stimulus for search designed toward revealing the mechanistic aspects and source of rate enhancement in the hydrolysis of phosphate esters.

The rapid enzymic hydrolysis of phospho-anhydride bonds contrasts with the extremely slow hydrolysis of these bonds under aqueous laboratory conditions, rate difference of the order of $10^{10}$ are observed. For example, the hydrolysis of pyrophosphate by yeast inorganic pyrophosphatase (YIP) occurs about $10^{11}$ fold faster than hydrolysis in the absence of the enzyme [10].

To characterise the role of divalent metal ions, many investigators have studied metal ion complexes that can serve as models for the activities of enzymes. Early workers [18-21] have shown that divalent metal complexes contribute impressive rate enhancements for hydrolysis of phosphate esters in neutral media. However the precise catalytic role of the metal ions and the source of rate enhancement have not been established due to the formation of various stoichiometry for the metal phosphate complexes involved [2], consequently, the divalent metal ions complexes, as a class, are extremely labile toward ligand displacement and the simultaneous presence of monomeric, dimeric and hydrated complexes in aqueous solution seriously complicates reliable identification of the species actually involvd in hydrolysis. Furthermore, in model system involving hydrolysis of polyphosphates, it has not been possible to obtain other than very modest catalytic effects by addition of labile aqua cations such as $Mg^{2+}$, $Co^{2+}$, $Zn^{2+}$, $Cu^{2+}$, $Mn^{2+}$ [2-3] compared to enzyme catalyzed reactions.

The implication of the above is that in such systems, there is at most the smallest fractions of polyphosphates in suitable reactive sites.

Various theories have been advanced regarding the role of metal ions in promoting hydrolysis of phosphate esters [1,22]. Among which are:

(i) Charge Nueutralization and Bond Polarization (Electron Density Polarization)

Coordination of a metal to a phosphate group changes the nature of the charge distribution at the phosphorus centre. The positive charge on the metal ion reduces the negative charge of the phosphates. Both effects allow the coordinated nucleophile to attack the phosphorus centre.

(ii) Ring Strain Induction

Binding a metal ion to a single phosphoryl group in a bidentate fashion reduces the O-P-O bond angle. As a result the activation energy necessary to attain a transition state is minimised. At the same time it may allow in line attack by a coordinated nucleophile.

(iii) Template Formation

Coordination to a metal ion fixes the reactive groups in an optimum stereochemical orientation for intramolecular hydrolysis.

The catalytic role of metal ions in providing a template for reaction corresponds to the formation of a bi-or tridentate ligand in the transition state [23].

## (iv) Generation of a Coordinated Nucleophile

The metal ion can generate appreciable concentrations of a Coordinated nucleophile at a PH lower than the $P^{k}a$ of the conjugate acid of the nucleophile. For example, a coordinated hydroxide, which is a better nucleophile than water, can be made available at a physiological $P^{H}$ in such a manner.

Other factors which bring about maximum rate enhancement of hydrolysis of phosphates by metal complexes include: the type of metal, metal ion to substrate ratio, the presence of two aqua groups coordinated to the metal which are cis-to each other, presence of maximum charge on the metal and the position of metal-substrate binding [25,26]. It is impossible to separate each of the above factors to the over all rate enhancement in a given model system, since the factors are interreleted.

Since polyphosphates are essential in biological systems, understanding their mechanisms of hydrolysis and related phosphoryl transfer involving cleavage within P-O-P bond is of relevance. Hence studies of the mechanistic aspects of hydrolysis of pyrophosphate in a well characterised kinetically inert complexes are useful undertakings to gain further insight into the roles of metal ions in hydrolytic cleavages. The selection of suitable model for such studies is discussed in the following section.

## 1.4 Choice of Model Systems

The aim of this project is to study the mechanistic aspects of the hydrolysis of pyrophosphate promoted by two tetraaminecobalt (III) complexes. This section emphasizes mainly on the design of the systems. The difficulties associated with using divalent metal complexes as model systems has been elaborated in the previous pages. In the present study, substitution inert metal ions such as cobalt(III) are utilized to overcome those drawbacks.

The ability to coordinated metal ions, particularly cobalt(III) to catalyse the hydrolysis of both inorganic and organic phosphates has been demonstrated by several groups of workers [4,22,27].

Recently attention has been focused on the effects of tetraamine cobalt(III) complexes on hydrolysis of anionic phosphates such as pyrophosphate, ATP and triphosphate [22,27-29]. Tetraaminecobalt (III) complexes provide advantages over the labile hydrated divalent metal ion in that the cobalt(III) complexes are simple to synthesize and kinetically robust thereby allowing to characterise all the species present in solution. The kinetics and mechanisms of formation of these complexes are well understood [30,31]. In addition, cobalt(III) complexes provide advantage over the labile ones since the Co-N bonds are inert and retain their integrity for long periods of timein aqueous solution [32]. The choice of amines as blocking ligands is advantageous since they labilize the aqua ligands. By the use of such reagents it has been possible to enhance the rate of hydrolysis of polyphosphates such as pyrophosphate and triphosphate by up to $10^5$ fold [28,32,33].

In this work cobalt(III) complexes of ethylenediamine ($H_2NCH_2CH_2NH_2$) and trimethylenediamine ($H_2NCH_2CH_2CH_2NH_2$) were prepared and used to study the hydrolysis of pyrophosphate. The rate of hydrolysis were correlated with product yields and kinetic parpmeters, so that the pyrophosphate hydrolysis promoted by tetraaminecobalt(III) complexes provide models

Which can be useful in elucidating the importance of metal centres in pyrophosphatases and related enzymes. Despite the fact that enzymes do not utilize substitution inert metal ions since the reaction would be stoichiometric rather than catalytic, tetramminecobalt(III) ions could not be different from labile metal ions in terms of their ability to avtivate substrate and in organizing coordinated nucleophiles [29].

In order to get maximum probability of reaction one should choose the proper isomers. Thus, a complex with cis-geometric arrangements of a potential intramolecular nuleophile and coordinated pyrophosphate substrate, as opposed to the corresponding trans-isomer is expected to have a maximum hydrolysis. Other factors for maximum rate ehhancement are the effect of ring size and ring strain. Such factors can not be adequately revealed if labile metal ions are used.

The present study provides a better understanding of the roles of metal ions in phosphoryl transfer reactions. A relatively new analytical technique for quenching of reaction aliquots, based on fast electron exchange is employed.

## 1.5 Analytical Techniques for Phosphate Determination

Determination of phosphates is of importance, particularly in the fields of biochemistry, agriculture and environmental science. Hence a reliable method is needed for the determination of phosphate in solution which contain various types of substances. The method should be selective and sensitive.

There are a number of techniques for the determination of phosphate that have been used for the analysis of phosphates such as titrimetric, gravimetric, $^{31}$PNMR spectroscopy and spectrophotometric methods.

Most of the procedures [34] for the spectrophotometric determination of inorganic phosphate are based on the formation of heteropoly acids such as phosphomolybdate and phosphovanadomolybdate in acidic medium. The heteropoly acid of phosphate formed is used as a light absorbing species either as it is, after reduction to a heteropoly blue (molybdenum blue) species or after extraction in to an organic solvent as protonated species as an ion pair with bulky cation. The spectrophotometric determination of inorganic phosphate has been traditionally accomplished by reduction of the phosphomolybdate heteropoly complex to give the formation of the coloured complex molybdenum blue.

Recently a spectrophotometric method based on the formation of an ion pair between phosophomolybdate and Malachite Green which was subsequently modified, has been applied to the determination of phosphate in various sample [34-38]. However most of the published procedures have a common draw back in that they require filtration and extraction steps due to low solubility of Malachite Green.

In this work, a modified spectrophotometric technique, reported bu Bayakov et al [38], based on the formation of Malachite Green phosphomolybdate ion pair complex is utilized to measure the amount of phosphate produced as a result of the hydrolysis of pyrophosphate by tetraaminecobalt(III) complex.

$$HMG^{2+} + H_3PO_4Mo_{12}O_{36} \longrightarrow (MG^{+})(H_2PO_4MO_{12}O_{36}^{-})$$

The modified method is highly selective and very sensitive for phosphate determination [34,38]. The reaction of Malachite Green with phosphomolybdate results in the appearance of an intense absorbance at 620-650 nm. This method requirs only one pipetting step and eliminates the problem of dye solubility, extraction, filtration and allows many analysis in a short time.

Previous comparative studies have shown that Machite Green is the dye of choice for phosphate assay [34]. Various cationic dyes such as Methyl Green, Ethylviolet, Crystal Violet and Brilliant Green, which have similar structure with Malchite Green, have been investigated. It has been found that Ethyl Violet and Crystal Violet produced coloured precipitates easily; with Brilliant Green, the colour faded gradually; with Methyl Green no precipitate occurred but the colouration was not as intense as Malchite Green. With Malchite Green the precipitate was formed with difficulty and the colouration is the best of all. Hence Malchit Green is selected as the colour reagent. Therefore the advantage of Malchite Green over the other similar catioric dyes is that it gives a very intense colour with phosphommolybdate. In most of earlier similar studies [27,41] the production of phosphate by tetraaminecbalt(III) ions as a result of the hydrolysis of pyrophosphate and other condensed phosphates including ATP has been monitored by 31PNMR spectroscopy.

## 1.6. Aims of the work

The main objective of this study was to investigate the hydrolytic cleavage of pyrophosphate by cis-$[Co(en)_2aq]^{3+}$ and cis-$[Co(tn)_2aq]^{3+}$ in the middle pH region.

This investigation includes:

(i) the study of the rate of complex formation (anation) of the tetraaminecobalt(III) with pyrophosphate.

(ii) measurements of the amount of phosphate produced as a result of pyrophosphate hydrlysis by the two tetraaminecobalt(III) complexes.

(III) the study of the effect of concentration and increased metal to pyrophosphate ratio on the rate of hydrolysis.

(iv) the study of the effect of order of addition of the tetraaminecobalt(III) complex to pyrophosphate on the hydrolytic cleavage.

(v) the study of the effect of pH on the hydrolysis.

(vi) Conducting a comparative study on the two tetraamine cobalt(III) complexes towards their effectiveness in promoting hydrolysis.

(vii) elucidating a plausible mechanism for the hydrolysis of pyrophosphate promoted by the tetraaminecobalt(III) complexes.

In order to achieve the above objectives, cis-diaquabis-(ethylenediaminecobalt(III) perchlorate and cis-diaquabis-(treimethylenecobaltperchlorate(III) complexes were synthesised

. The structure of the ligands are depicted in Figure 1.1. The synthetic route followed and other experimental details are given in the experimental section.

**ethylenediamine** **trimthylenediamine**

Fig. 1.1. Ligand Structures.

# 2. EXPERIMENTAL

## 2.1. Reagents

Cobalt(II) nitrate hexahydrate (BDH), sodium perchlorate (BDH), Perchloric acid (Merck), ethylenediamine (Eastman Kodak), trimethylenediamine (Eastman kodak), hydrogen peroxide (Riedel-de Haen), sodiumdihydrogen orthophosphate dihydrate (BDH), disodiumdihydrogenpyrophosphate (BDH), Ammoniummolybdatetetrahydrate (Analar, BDH), Malachite Green (Riedel-de Haen), europium(III) oxide (Fluka and Aldrich), mercuric chloride (BDH), zinc granules (BDH) and sodium hydroxide (BDH) were used as such without further purifications.

## 2.2 Instruments

pH measurements were made using orion research microprocessor ion analyzer model 901 fitted to a combined electrode (Fisher) 4M KCl-Sat. Ag/AgCl.

Automatic pipets with disposable plastic tips, obtained from Rainin Company, were used to transfer measured quantities of reaction mixtures. "Glass stick dotting" was used to adjust the $P^H$ of reaction by adding drops of NaOH or $HClO_4$ from, a glass rod.

A Beckman model Du-65 uv-visible recording spectrophotometer was used to obtain electronic spectra and for collections of rate data. Quartz mixing cells (0.9 cm total path length) were used for the study of complex formation of ppi with tetraaminecobalt(III) complex, and 1 cm cells (total path length) were employed for routine spectra and absorbance measurements. Extinction coefficients are quoted in $M^{-1}$ $cm^{-1}$.

## 2.3 Synthesis and Characterization of the Tetraaminecobalt(III) Complexes

### 2.3.1. Synthesis of tetraaminecobalt(III) complexes

The diaqua complexes, $[N_4Co(H_2O)_2]^{3+}$ where $N_4$ = $(tn)_2$ or $(en)_2$, were prepared from the carbonato complexes, $[N_4CoCO_3]^+$, which in turn were prepared from sodiumtris (carbonato) cobaltate(III) trihydrate

$(Na_3[Co(CO_3)_3]\ 3H_2O)$.

$Na_3[Co(CO_3)_3]\ 3H_2O \dashrightarrow [N_4CoCO_3]ClO_4$ (1)

$[N_4CoCO_3]ClO_4 \dashrightarrow [N_4Co\ (H_2O)_2]\ (ClO_4)_3$ (2)

2.3.1.1. Preparation of $Na_3$ $[Co(CO_3)_3]\cdot 3H_2O$: Sodiumtris (carbonato) cobaltate(III), was prepared by oxidation of cobalt(II) nitrate hexahydrate according to the procedure given in the literature [39,40]. The compound can be stored in the dark at a low temperature for a brief period of time to prevent decomposition.

2.3.1.2. Preparation of $[en_2CoCO_3]ClO_4$ and $[tn_2CoCO_3]ClO_4$: Bis (ethylenediamine) carbonatocobalt(III) perchlorate and bis (trimethylenediamine) carbonatocobalt(III) perchlorate were synthesised by modifying the published prodedures [27,41].

Preparation of $[en_2CoCO_3]ClO_4$ 17.5 ml (26.8 g) of 60% $HClO_4$(w/v) was mixed to a diluted solution of 5.3 ml (4.8 g) of 100% (v/v) ethylenediamine and the pH of the mixture was brought to 5. This mixture was added drop to a warmed slurry of 14.4 g (0.04 mole) of $[Na_3Co(CO_3)]$. $3H_2O$ with a constant stirring using a magnetic stirrer hot plate and the temperature of the r reaction was raised to 70-75$^0$C.

The reaction mixture was stirred at this temperature for about 30 minutes until a dark red solution was obtained. On cooling red crystals were separated and purified from the mixture by decantation and washing with portions of distilled water repeatedly. Finally the crystals were washed with ethanol and then dryed with ether.

Preparation of $[tn_2\ CoCO_3]ClO_4$. 17.5 ml (26.8 g) of 60 % (w/v) $HClO_4$ was added slowly drop by drop to 6.7ml (5.92 g) 100%(v/v) trimethylenediamine to bring the pH to neutral. This mixture was added drop by drop to a warmd slurry of 14.4 g (0.04 mole) of $Na_3[Co(CO_3)_3].3H_2O$ with constant stirring using a magnetic stirrer hot plate. The temperature of the reaction mixture was raised to 75-80$^0$C and heated with constant stirring until a red solution was produced. This mixture was filtered while hot. The residue was washed with small portions of distilled water. The filtrate and washing were combined together and refiltered until no residue was seen on the filter paper. Finally the solution was evaporated until a scum was formed. Cooling to room temperature and adding ethanol gave bright red square plate crystals.

The crystals were filtered and washed with ethanol and then dryed with ether.

### 2.3.1.3. Preparation of $[en_2Co(H_2O)_2]$ $(ClO_4)$ and $[tn_2Co(H_2O)_2]$-$(ClO_4)_3$:

The conversion of both carbonato complexes to the corresponding diaqua species was achieved by adding 2.5 mmoles of $HClO_4$ per mmole of finely divided carbonato complexes and stirring under an aspirator vacuum for about 20 minutes in the dark at a temperature of $50^0C$.

## 2.3.2 Characterization of the tetraaminecobalt(III) complexes

Characterization of the diaqua and the carbonato complexes was achieved by un-visible spectroscopy. The molar extinction coefficients and absorbance maxima and minima of the complexes were found to match with those available in the literature [32,42,43]. The values are depicted in Table 2.1 .

Table 2.1 Absortance Maxima and Minima of the Compounds synthesized

| Complex | Litetature value | | | | Experimental value | | |
|---|---|---|---|---|---|---|---|
| | $\lambda_{max}$ (ε) nm $m^{-1}cm^{-1}$ | $\lambda_{min}$ (ε) nm $m^{-1}cm^{-1}$ | $\lambda_{max}$ (ε) nm $m^{-1}cm^{-1}$ | $\lambda_{min}$ (ε) nm $m^{-1}cm^{-1}$ | $\lambda_{max}$ (ε) nm $m^{-1}cm^{-1}$ | $\lambda_{min}$ (ε) nm $m^{-1}cm^{-1}$ | $\lambda_{max}$ (ε) nm $m^{-1}cm^{-1}$ |
| $Coen_2CO_3ClO_4$ (42) | 510(129) | – | 360(115) | – | 510(130) | 429(21) | 360(118) |
| $Coen_2(H_2O)_2ClO_4)_3$ (43) | 495(79) | – | 360(64) | – | 493.5(79) | 415.2(14) | 361(68) |
| $Cotn_2CO_3ClO_4$ (32) | 520(106.8) | 430(12) | 359(126.3) | 327(50.8) | 520.2(106) | 430.6(128) | 360.6(1[illegible]1) |
| $Cotn_2(H_2O)_2(ClO_4)_3$ (32) | 506(53.5) | – | 362(65.4) | | 506.2(54.8) | | 361(65.8) |

It is to be noted that in the middle $p^H$ region the major species that previal in the solution is the hydroxoaqua ion.

found that the colour reagent interacts selectively with phosphate and no interference was seen with $Co^{2+}$, $Eu^{2+}$, $Eu^{3+}$ and the condensed phosphates. PH dependence study was also conducted on the method and standard method was adopted.

## 2.5 Analytical Techniques for the Study of the Hydrolytic Reactions

The analytical techniques for the stydy of the hydrolytic cleavage of pyrophosphate was investigated at different pyrophosphate concentrations with $[tn_2Co(aq)]^{3+}$ and $[en_2Co(aq)]^{3+}$ at PH 5, 6.5 and 8 for different metal to pyrrophosphate ratio and different order of addition. The study was based on analysing for inorganic phosphate, pi, produced in the quenched reaction solution aliquots by the method outlined above. 1 ml aliquots withdrawn from the reaction mixtures at different intervals of 5, 10, 20, 30, 40, 50, 60, 90, and 120 minutes were quenched by two separate methods: (I) with europium(II) and $H^+$ to reduce cobalt(III) to cobalt(II) and release all bound phosphate and pyrophosphate species and (2) with OH (2 M) after quenching, where all phosphate are released after 20 hrs [32].

Solutions prepared according to the above procedures were maintained at constant PH by addition of NaOH or $HClO_4$ using glass stick dotting. The hydrolysis of ppi was monitored by observing the production of pi with time. Aliquots were withdraw at intervals of time and quenched to stop the reaction and release all bound phosphate before assaying.

The following were investigated for both systems by Eu(II) and OH quenching techniques.

| | Cobalt to ppi ratio | Reaction equation[a] |
|---|---|---|
| 1 | 1:1 | $N_4Co(aq)$ + ppi[b] |
| 2 | 2:1 | $2N_4Co(aq)$ + ppi[b]<br>$N_4Co(aq)$ + $N_4Coppi$[c] |
| 3 | 3:1 | $3N_4Co(aq)$ + ppi[b]<br>$2N_4Co(aq)$ + $N_4Coppi$[c]<br>$N_4Co(aq)$ + $(N_4Co)_2$ ppi[d] |

$N_4Co(aq) = [en_2Co(aq)]^{3+}$ or $[tn_2Co(aq)]^{3+}$

a. Charge and degree of protonation omitted.

b. Direct addition.

c. Preformed 1:1 complex plus reactant.

d. Preformed 2:1 complex plus reactant.

## 2.7 Quenching Techniques

### 2.7.1 Europium(II) Quenching Method

A solution which is 0.1 M in Eu(III) was prepared by dissolving 0.8798 g of $Eu_2O_3$ in enough 2 M HCl to make 50 ml of the reagent. Eu(III) was reduced to Eu(II) by adding about 10 ml of the reagent to amalgamated zinc in an atmosphere of dry nitrogen and leaving for a period of 30 minutes. Amalgamated zinc was produced by soaking zinc granules in solution of mercuric chloride ($HgCl_2$) in HCl. 0.1 ml portions of the sample aliquots were quenched with 0.1 ml of Eu(II) which reduces Co(III) to Co(II) and releases all bound phosphate and pyrophosphate species instantly. All excess Eu(II) must be oxidized completely because $Eu^{2+}$ forms blue complex with ammoniummolybdate and interferes with the phosphate analysis. The reduction of Co(III) can be seen by the complete decolourizaion of the solution. The remaining Eu(II) in the solution is then air oxidized. This is achieved within a minute [44] and the analysis of phosphate was carried out immediately.

The effectiveness of the Eu(II) quenching was investigated with different cobalt complexes. It was found that [Coen$_3$]$^{3+}$, [tn$_2$Co(H$_2$O)$_2$]$^{3+}$, [en$_2$Co(H$_2$O)$_2$]$^{3+}$, aquated cis-and trans-[en$_2$CoCl$_2$]$^+$ were not decolorized as a result of addition of Eu(II). However [en$_2$CoCO$_3$]$^+$, [en$_2$Copi] , [en$_2$Coppi], [tn$_2$Copi], [tn$_2$Coppi] and fresh solutions of cis- and trans[en$_2$CoCl$_2$]$^+$ were found to be decolourized instantly upon addition of the reagent. The reasons for the above observations are unknown.

### 2.7.2 Hydroxide Quenching Techinique

One ml portions of the sample aliquots were withdrawn and quenched by addition of 1 ml 4 M NaOH (2 M after quenching). The quenched solutions were left for about 20 hours to release all bound phosphate and pyr ophosphate [32,45,46] before bringing the p$^H$ to 6.5 with HClO$_4$ and subjecting it to phosphate analysis.

It has been reported that compared to europium quenching the hydroside (OH) quenching method gives additional phosphate [32,45,47], which can arise through a conventional $SN^1CB$ mechanism or by the direct attack of hydroxide ion on electrophilic phosphorous centre ($SN^2$ mechanism). In our investigation the hydroxide quenched reaction solution aliquots gave a negligible percentage hydrolytic product compared to those quenched with Europium(II). This is presumably attributed to reanation of the unbound phosphate species with the metal ions to form complexes. This is possible as the analytical technique for the determination of phosphate requires an incubation of the sample with the reagent for 5 minutes. It is to be noted that complex formation of phosphate and pyrophosphate is fast in the middle $P^H$ region [27]. This problem did not affect previous investigations [32,45,47] since their method of phosphate analysis did not require the above protocol. Hence in this work europium(II) quenching was utilized.

## 2.8 Reactivities of Hydroxoaquametal Ions

Tetraamine cobalt(III) aqua ions, $[N_4Coaq]^{3+}$, exist as diaqua, hydroxoaqua and dihydroxo species depending on the $P^H$ of the solution. In the middle PH region, the major species is presumed to be the hydroxoaqua ions. The hydroxoaqua ions do nor exist as monomers, but as dimers and polymers in the solid state. cis-hydroxoaqua ions are dimers bridged by two $H_3O_2^-$ ligands and trans-hydroxoaqua ions exist as polynuclear chains of metal atoms bridged by single $H_3O_2^-$ ions. Bridging by $H_3C_2^-$ species may be accomplished by formation of a strong and short symmetrical hydrogen bonding [48,49] between the hydrcxo ligand of one metal ion and the aqua ligand of the other metal ion. It is generally assumed that the mechanism of the solid state is formulated as an elimination of water molecules from $H_3O_2^-$ bridges [31,48]. The formation of polynuclear ions also takes place in aged and highly concentrated solutions [50,51]. To minimize these complications freshly prepared solutions were used in all of our investigations.

# 3. RESULTS AND DISCUSSION

## 3.1 Complex Formation by Tetraaminecobalt(III) Ions with Pyrophosphate

The study of complex formation $N_4Co(III)$ (aq) ($N_4$ = $tn_2$ or $en_2$) with pyrophosphate in the middle $p^H$ region was undertaken by mixing a fixed concentration of the tetraaminecobalt(III) ions (4 X $10^{-3}$ M) with varying amount of ppi. Equal volumes of solutions of the tetraaminecobalt(III) ions and pyrophosphate were taken in a mixing cell with 1:1, 1:5, 1:10, 1:15 and 1:20 tetraamine complexes to pyrophosphate ratios at $p^H$ 6.5 and 0.1 M $NaClO_4$ (for ionic strength control). Absorption spectra of the $N_4$Coppi complexes in the middle $p^H$ region are depicted in Figures 3.1 and 3.2.

The first order rate constants $k_{obs}$ were calculated by using

$$\left[\frac{A_\infty - A_t}{A_\infty - A_0}\right] = -kt$$

plot of -ln ($A_\infty$ -$A_t$) versus t, gave a straight line, where $A_0$, $A_t$ and $A_\infty$ are the absorbances initially, after time t and after completion of the reaction. The first order rate constants

($K_{obs}$) obtained from the slope of $-\ln (A_\infty - A_t)$ versus t for $[tn_2\text{-}Co(OH)(OH_2)]^{2+}$ systems are $1.5 \times 10^{-2}\ s^{-1}$, $2 \times 10^{-2}\ s^{-1}$, $2.67 \times 10^{-2}\ s^{-1}$, $3 \times 10^{-2}\ s^{-1}$, and $3.08 \times 10^{-2}\ s^{-1}$ for the ratios of 1:1, 1:5, 1:10, 1:15 and 1:20 (tetraamine cpmplex to ppi) respectively.

The first order rate constonts calculated for the $[en_2Co(OH)(OH_2)]^{2+}$ systems were $3.9 \times 10^{-3}\ s^{-1}$ $5 \times 10^{-3}\ s^{-1}$ and $1.1 \times 10^{-2}\ s^{-1}$ for 1:1, 1:5 and 1:10 (tetraamine complex to ppi ratios) respectively. The half lives were calculated to be in the ranges of 40.6 seconds to 22.5 seconds for the $[tn_2Co(OH)OH_2]^{2+}$ containing systems, and the rate increases with the increase of the ratio of ppi to tetraamine cobalt(III) ions. A limiting rate is attained at higher ppi to tetrramine complex ratio due to complete ion pair formation [30]. In order to explain such a variation of rate, the following reaction scheme is proposed [52]

$$\text{cis-}[Cotn_2(OH)(OH_2)]^{2+} + \text{ppi} \xrightleftharpoons[\text{fast}]{K_F} \underset{\text{ion pair}}{\text{cis - }[Cotn_2(OH)(OH_2)]^{2+}\cdot\text{ppi}}$$

$$\text{cis-}[Cotn_2(OH)(OH_2)]^{2+}\cdot\ \text{ppi} \xrightarrow{K_a} [Cotn_2ppi] + OH^- + H_2O$$

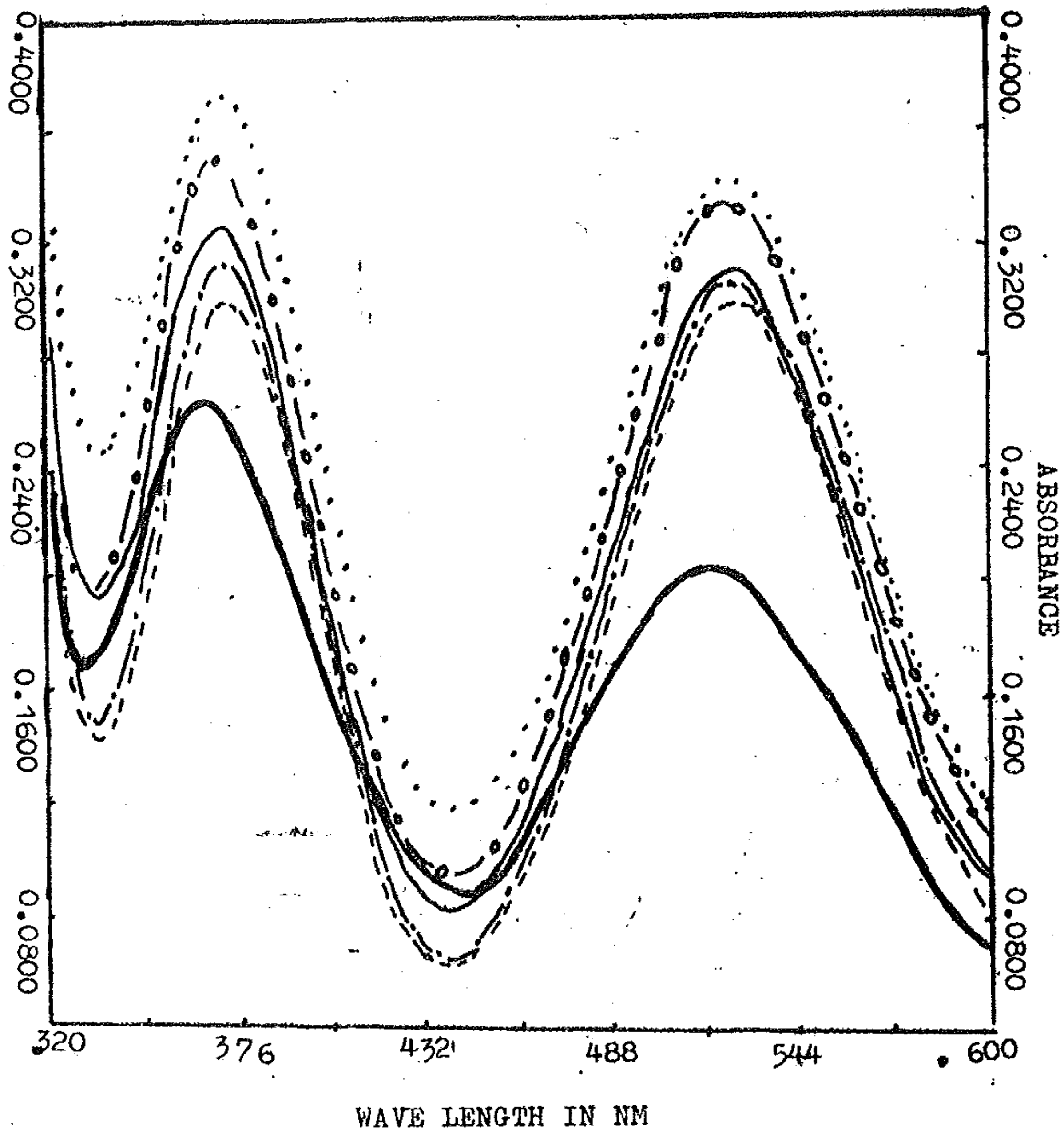


Fig. 3.1 Absorption spectra of $tn_2$Coppi in aqueous media with different $[tn_2Co(OH)(OH_2)]^{2+}$ to ppi ratios

(▬) = $[tn_2Co(OH)(OH)_2]^2$, (----)= 1:1, (-.-.-.) = 1:5 ,

(___) = 1:10, (—o—o—)= 1:15, (.....) = 1:20

The rate law from the above scheme can be derived [52].

$$\frac{d[\mathrm{Cotn_2ppi}]}{dt} = \frac{K_aK_E\ [\mathrm{Cotn_2(OH)(OH_2)}]^{2+}\ \mathrm{total}\ [\mathrm{ppi}]}{1 + K_E\ [\mathrm{ppi}]}$$

$$= K_{obs}[\mathrm{Cotn_2(OH)(OH_2)}^{2+}\ \mathrm{total} \qquad (1)$$

Where $k_a$ is anation rate constant, $K_E$ is ion pair equilibrim constant and $[\mathrm{Cotn_2(OH)(OH_2)}]^{2+}$ total is concentration of total unreacted complex. Hence

$$k_{obs} = \frac{K_aK_E\ [\mathrm{ppi}]}{1+K_E\ [\mathrm{ppi}]} \qquad (2)$$

$$\frac{1}{k_{obs}} = \frac{1}{k_a} + \frac{1}{k_aK_E\ [\mathrm{ppi}]} \qquad (3)$$

From equation 3, a plot of $\frac{1}{K_{obs}}$ versus $\frac{1}{[\mathrm{ppi}]}$

is linear with slop = $\frac{1}{k_aK_E}$ and intercept $\frac{1}{k_a}$

The $k_a$ and $K_E$ values thus can be calculated from the slop and intercept.

The $k_a$ and $K_E$ values were found to be $3.6 \times 10^{-2}$ $s^{-1}$ and 78.6 $M^{-1}$ respectively.

## 3.2 Hydrolytic Reactions

The experiments were carried out at ambient temperature and 0.1 M ionic strength ($NaClO_4$). Solutions of $N_4$Coppi, ($N_4$ = $tn_2$or $en_2$) were prepared with concentration of $10^{-3}$ M, 2 X $10^{-3}$ M, 4 X $10^{-3}$ M and $10^{-2}$ M at the desired $p^H$ as given in section 2.6. The effect of order of addition on the hydrolytic cleavage was also studied. One ml sample aliquots were withdrawn and quenched with 0.1 ml europium(II) to stop the reaction and release all bound phosphate species. Then at various times, further aliquots were withdrawn and similarly quenched with europium(II). The quenched solutions were subjected to phosphate analysis by the Malachite Green phosphomolybdate procedure [38]. Similar experiments were performed using hydroxide quenching technique. A 1 ml sample was withdrawn and quenched with 1 ml of 4 M NaOH. Further aliquots were also withdrawn at various times and quenched. The quenched solutions were kept in the dark for a period of 20 hours, and then subjected to phosphate analysis after neutralization of the excess hydroxide in the test solution which was introduced as a result of the quenching procedure. The test solutions were incubated with the analytical reagent for a period of 5 minutes after which the absorbances were measured at 650 nm (1 cm cell against a reagent blank). Blank determination was carried out by taking one ml of the pyrophosphate solution and following the procedure outlined above. The concentrations of the phosphate produced were calculated from extiinction coefficient value which was obtained from calibration curve using standard phosphate solutions. Percentage inorganic phosphate produced in the reaction was calculated as follows.

$$\% \text{ pi} = \frac{[\text{pi}]}{2 \times [\text{ppi}]_i} \times 100$$

where % pi = percentage phosphate,

[pi] = concentration of inorganic phosphate determined

[ppi]i = concentration of intial pyrophosphate.

In the above formula it is assumed that one molecule of ppi produces two molecules of pi species.

### 3.2.1 Results

The results for a typical study for the reaction of ppi with $tn_2Co(III)(aq)$ by europium quenching and with different order of addition in the middle $^{pH}$ region are given in Table 3.1 and Figure 3.3

Table 3.1 Hydrolysis of ppi promoted by $[tn_2Co(OH)(OH_2)]^{2+}$ ($4X10^{-3}$ M, ambient temperature, pH 6.5, 0.1 M $NaClO_4$)

a, equimolar metal to ppi ratio,

b, direct addition (2:1 metal to ppi ratio),

c, preformed 1:1 plus 1 molar ratio of the metal,

d, direct addition (3:1 metal to ppi ratio),

e, preformed 1:1 plus two molar ratio of the metal,

f, preformed 1:1 plus step wish addition of the metal.

| | % pi produced | | | | | |
|---|---|---|---|---|---|---|
| | Metal to ppi ratio | | | | | |
| Min | $1:1^a$ | 2:1 | | 3:1 | | |
| | | $2:1^b$ | $1:1+1^c$ | $3:1^d$ | $1:1+2^e$ | $1:1+1+1^f$ |
| 5 | 0.25 | 0.60 | 0.71 | 0.47 | 0.59 | 0.49 |
| 10 | 0.31 | 0.86 | 0.90 | 0.78 | 0.84 | 0.69 |
| 20 | 0.50 | 1.06 | 1.04 | 1.30 | 0.95 | 0.94 |
| 30 | 0.64 | 1.26 | 1.12 | 1.56 | 1.45 | 1.18 |
| 40 | 0.69 | 1.47 | 1.20 | 1.86 | 1.78 | 1.38 |
| 50 | 0.83 | 1.54 | 1.26 | 2.25 | 2.10 | 1.61 |
| 60 | 0.86 | 1.58 | 1.31 | 2.64 | 2.45 | 1.82 |

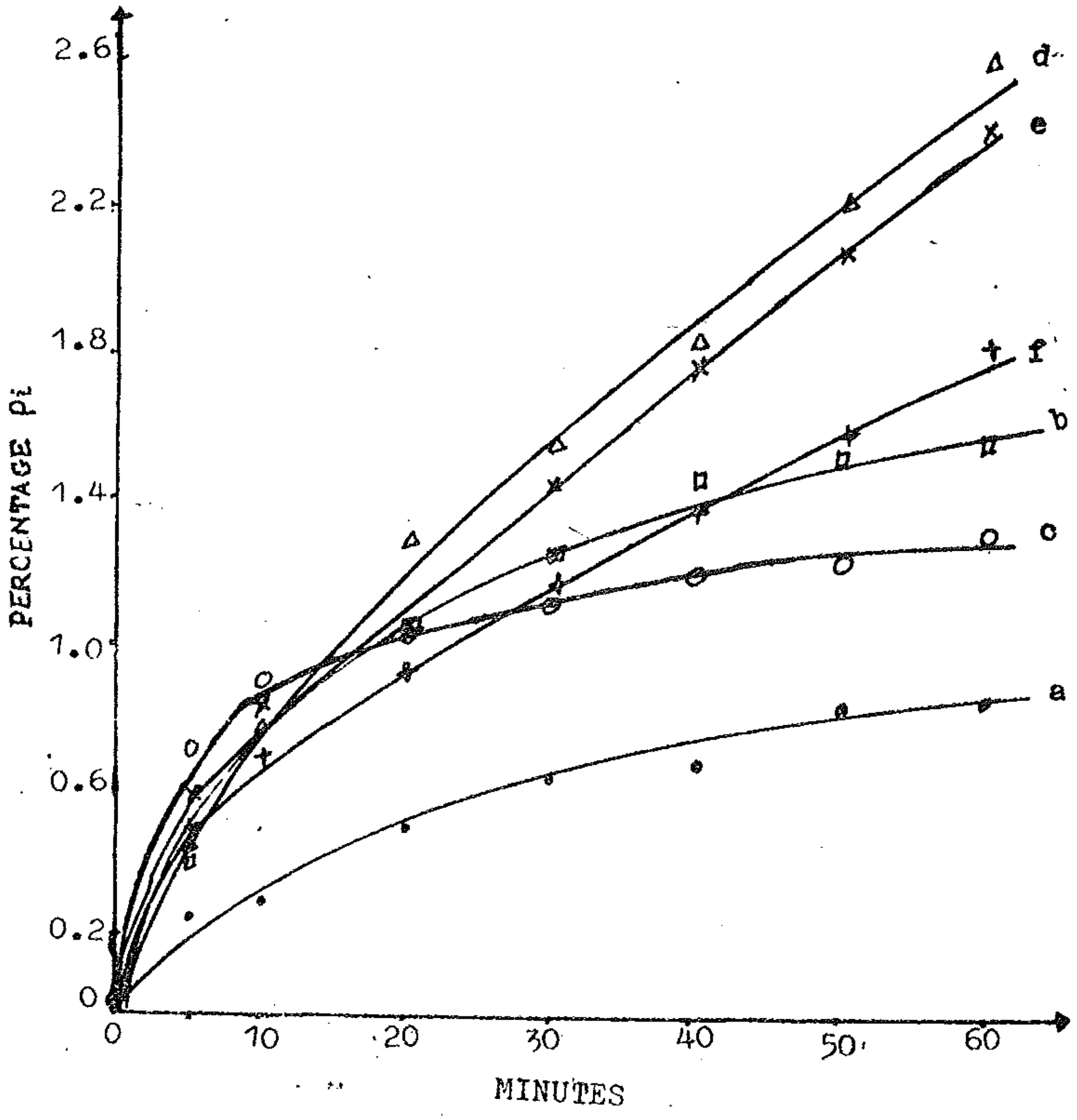


Fig.3.3. Hydrolysis of ppi promoted by $[tn_2Co(OH)(OH_2)]^{2+}$ for different metal to ppi ratios and different order of addition.

($4 \times 10^{-3}$ M, ambient temperature, pH 6.5 0.1 M $NaClO_4$)

a; equimolar metal to ppi ratio,

b, direct addition (2:1 metal to ppi ratio),

c; preformed 1:1 plus 1 molar ratio of the metal,

d, direct addition (3:1 metal to ppi ratio),

e, preformed 1:1 plus two molar ratio of the metal,

f, preformed 1:1 plus stepwise addition.

Experiments were also conducted on the 3:1 (cobalt to pyrophosphate ratio) systems at $10^{-2}$ molar concentration in the middle $P^H$ region to investigate the effect of concentration and order of addition on the hydrolytic cleavage. The result of the $tn_2Co(III)$ aq systems are shown in Table 3.3 and Figure 3.5. The $en_2(Co(III)$ aq containing systems did not behave well for the 2:1 and 3:1 cobalt to pytophosphate ratio in the middle $P^H$ region as the test solution resulted in precipitation.

Table 3.3. Hydrolysis of ppi promoted by $[tn_2Co(OH)(OH_2)]^{2+}$ for 3:1 metal to ppi ratios with different order of addition. ($10^{-2}$ M, $P^H$ 6.5, ambient temperature and 0.1 M $NaClO_4$)

a, direct additon (3:1 metal to ppi ratio),

b, preformed 1:1 plus two molar ratio of the metal,

c, preformed 1:1 plus stepwise addition.

| Min | % pi produced | | |
|---|---|---|---|
| | Metal to ppi ratio | | |
| | 3:1[a] | 1:1+2[b] | 1:1+1+1[c] |
| 5 | 1.00 | 1.20 | 1.50 |
| 10 | 1.39 | 1.78 | 2.00 |
| 20 | 2.39 | 2.91 | 3.93 |
| 30 | 4.06 | 3.93 | 4.67 |
| 40 | 4.13 | 5.02 | 5.35 |
| 50 | 4.84 | 6.63 | 6.54 |
| 60 | 5.97 | 6.88 | 5.73 |
| 90 | 7.53 | 7.91 | 7.56 |
| 120 | 9.45 | 9.05 | 8.50 |

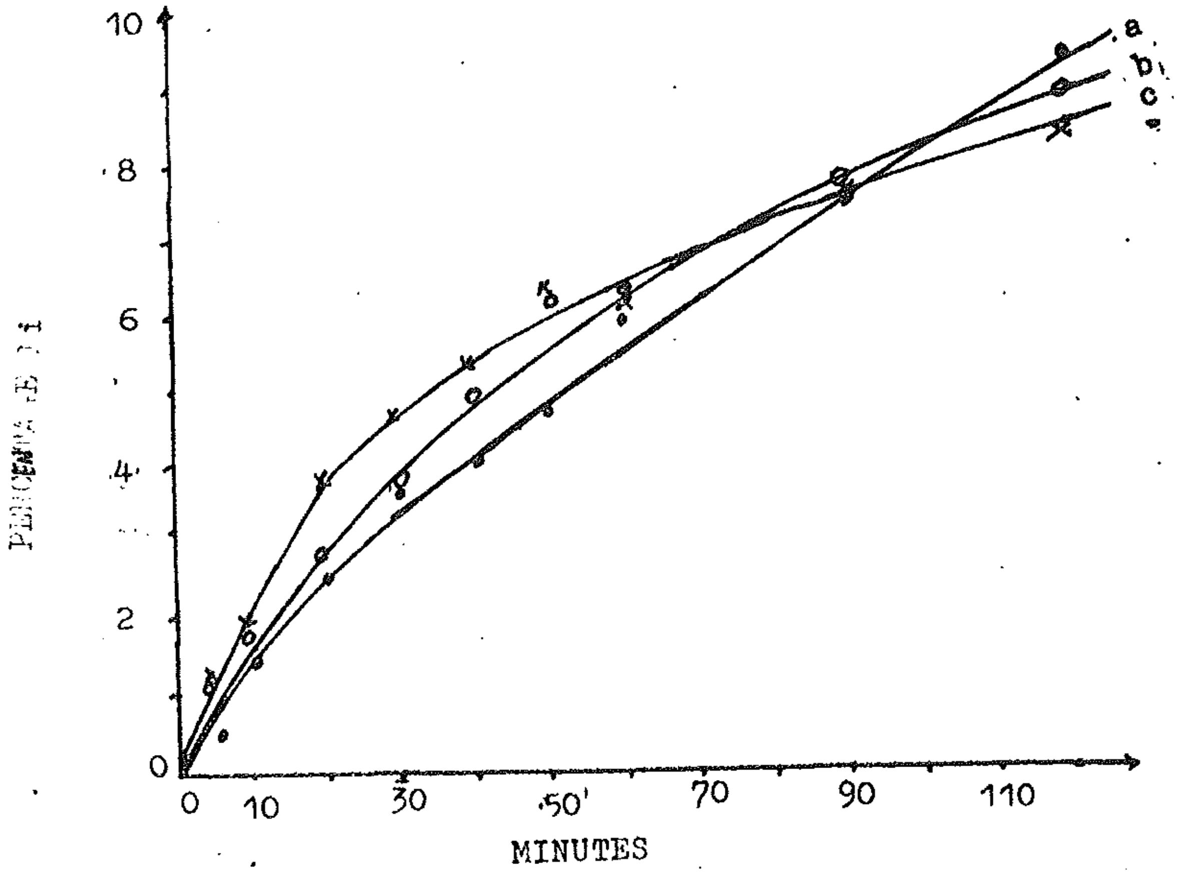


Fig.3.5 Hydrolysis of ppi promoted by $[tn_2Co(OH)(OH_2)]^{2+}$ for 3:1 metal to ppi ratios with different order of addition ($10^{-2}$ M, pH 6.5, ambient temperature and 0.1 M $NaClO_4$)

a, direct addition (3:1 metal to ppi ratio),

b, preformed 1:1 plus two molar ratio of the metal,

c, preformed 1:1 plus stepwise addition.

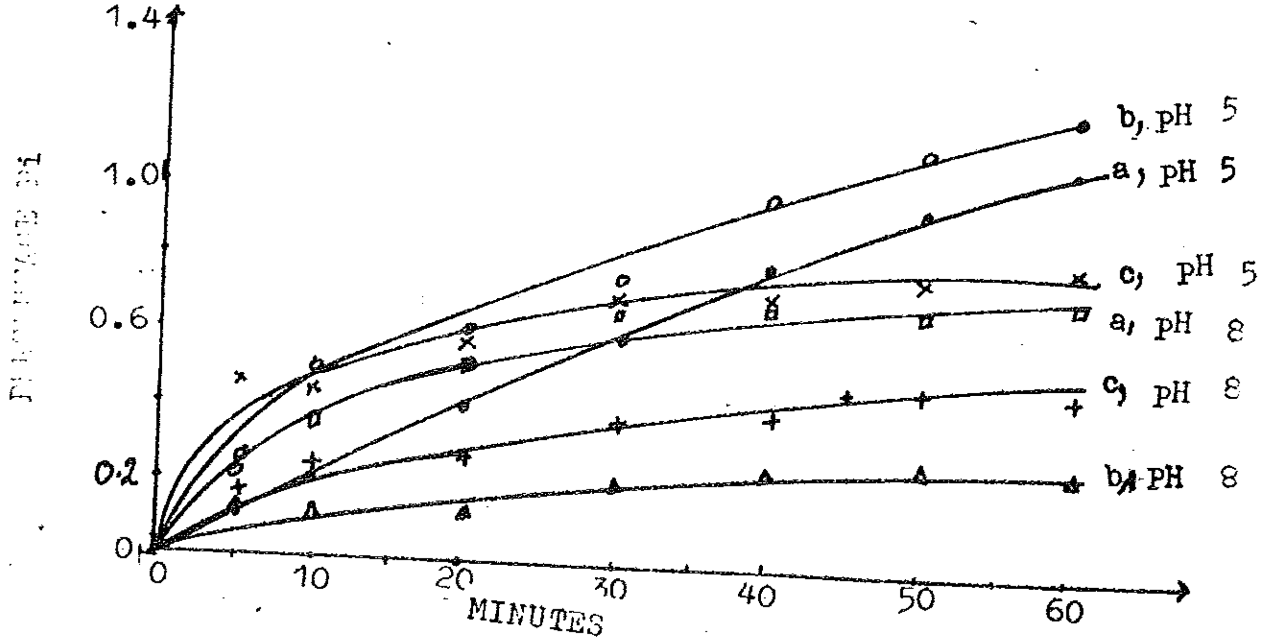


Fig.3.6. Hydrolysis ppi promoted by $tn_2Co(III)(aq)$ for 3:1 metal to ppi ratio with different order of addition (4 X $10^{-3}$ M, pH 5 and 8, ambient temperature and 0.1 M $NaClO_4$)

a, direct addition (3:1 metal to ppi ratio),

b, preformed 1:1 plus two molar ratio of the metal,

c, preformed 1:1 plus stepwise addition.

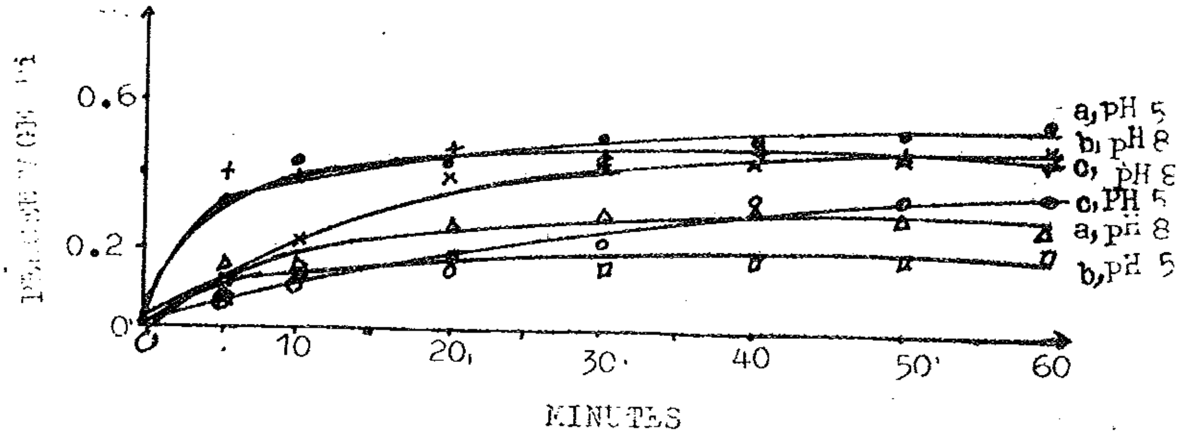


Fig.3.7. Hydrolysis of ppi promoted by $en_2Co(III)(aq)$ for 3:1 metal to ppi ratio with different order of addition ($4 \times 10^{-3}$ M, pH 5 and 8, ambient temperature and 0.1 M $NaClO_4$).

a, direct addition (3:1) metal to ppi ratio,

b, preformed 1:1 plus two molar ratio of the metal,

c, preformed 1:1 plus stepwise addition.

## 3.3 Discussion

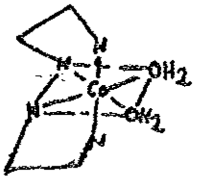
The major observations noted from the above studies are given below with possible explanations of the observed trend.

The Malachite Green phosphomolybdate method of analysis can not be used with hydroxide quenching. The method was found to be affected with a change in $P^H$. When the hydroxide quenched test solution was neutralized with perchloric acid prior to the addition of the analytical reagent, reanation of the Co(III) ions with the phosphate species is possible. Hence detection of the phosphate ions released as a result of hydrolytic cleavage was impossible. It is to be noted that the analytical technique for pi determination requires at least 5 minutes of incubation with the reagent in the middle PH region.

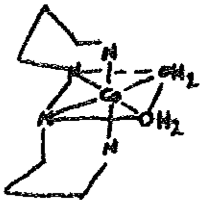
Complex formation of $N_4$Copi and $N_4$Coppi was achieved in less than five minutes. The half lives for the reaction of $tn_2$Co(III)aq with pi and ppi (1:1 metal to pi or ppi ratio) were found to be 110 seconds and 46 seconds respectively. Similarly the half life for the reaction of $en_2$Co(III)aq with ppi (1:1 metal to ppi ratio) was 2 minutes.

Increased hydrolysis of pyrophosphate was noted in the middle PH region (PH 6.5). There was no dignificant hydrolysis in both acidic ($P^H$ 5) and basic (PH 8) regions. This is attributed to the presence of hydroxoaqua ion as the major species for the tetraaminecobalt(III) complex in the middle $P^H$ region where as in acidic and basic media the predominant species are diaque and dihydroxo ions respectively. The hydroxoaqua ion is extraordinarily labile due to the fast trans to cis-isomerisation and fast exchange of coordinated water molecules compared tp the diaqua and the dihydroxo complexes [41]. This isomerisation proceeds by dissociative release of the aqua ligand due to trans activation by the hydroxo ligand [41]. Enhanced reactivity for the hydroxoaqua ions can be explained by assuming that the electrons freed from the diaqua complex by the loss of a proton labilize the coordinated water moledule by increasing the electron density on the central cobalt ions [53]. Complex formation of the monodentate tetraaminecobalt(III) pyrophosphate will be achived very fast for both diaqua and hydroxoaquo species. However the coordinated hydroxide can presumably attack the phosphorus centre to effect hydrolysis or from the bidentate chelate in the case of hydroxoaquo complexes, while the latter is only produced by the diaqua ions without significant hydrolysis. In the case of dihydroxotetraaminecobalt(III) ions, the strong Co-OH bond hampers the formation of the tetraamine cobalt(III) pyroophosphate complex, thereby showing no hydrolytic cleavage of the phosphoanhydride bonds.

The $tn_2Co(III)(aq)$ systems are much more effective in promoting hydrolytic cleavages of pyrophosphate than those containing the $en_2Co(III)(aq)$ ions. This is presumably attributed to the geometry of the tetraamine chelates of these complexes. The structures of the complexes are depicted in Figure 3.8.



cis-$[en_2Co(H_2O)_2]^{3+}$



cis-$[tn_2Co(H_2O)_2]^{3+}$

Fig. 3.8. Structure of $[N_4Co(H_2O)_2]^{3+}$ ions.

From the structure one can conclude that the six membered chelate of the trimethylene complex can take different conformations such as boat, chair and skewboat. The changes in conformation can affect the reactivities of the coordinated hydroxide towards intramolecular attack on the phosphorus centre. This is presumably due to the exclusion of the solvent molecules there by bringing the reactive groups (i.e. coordinated hydroxide and the electrophilic phosphorus center) in close proximity to each other. The five membered chelate of the ethylenediamine complex is more rigid and enjoys less degree of conformational varieties compared to trimethylene complexes. As a result the solvent molecules can reside between the reacting groups preventing the phosphorus center from being attacked by the coordinated hydroxide.

An increased degree of hydrolysis was observed for 3:1 metal to pyrophosphate ratio. This observation is in agreement with previous findings where the requirement of more than two metal ions per mole of the substrate was found to be essential for effective hydrolysis [27,54]. The role of metal ions is presumably attributed among other factors to activation, substrate formation, and structural considerations.

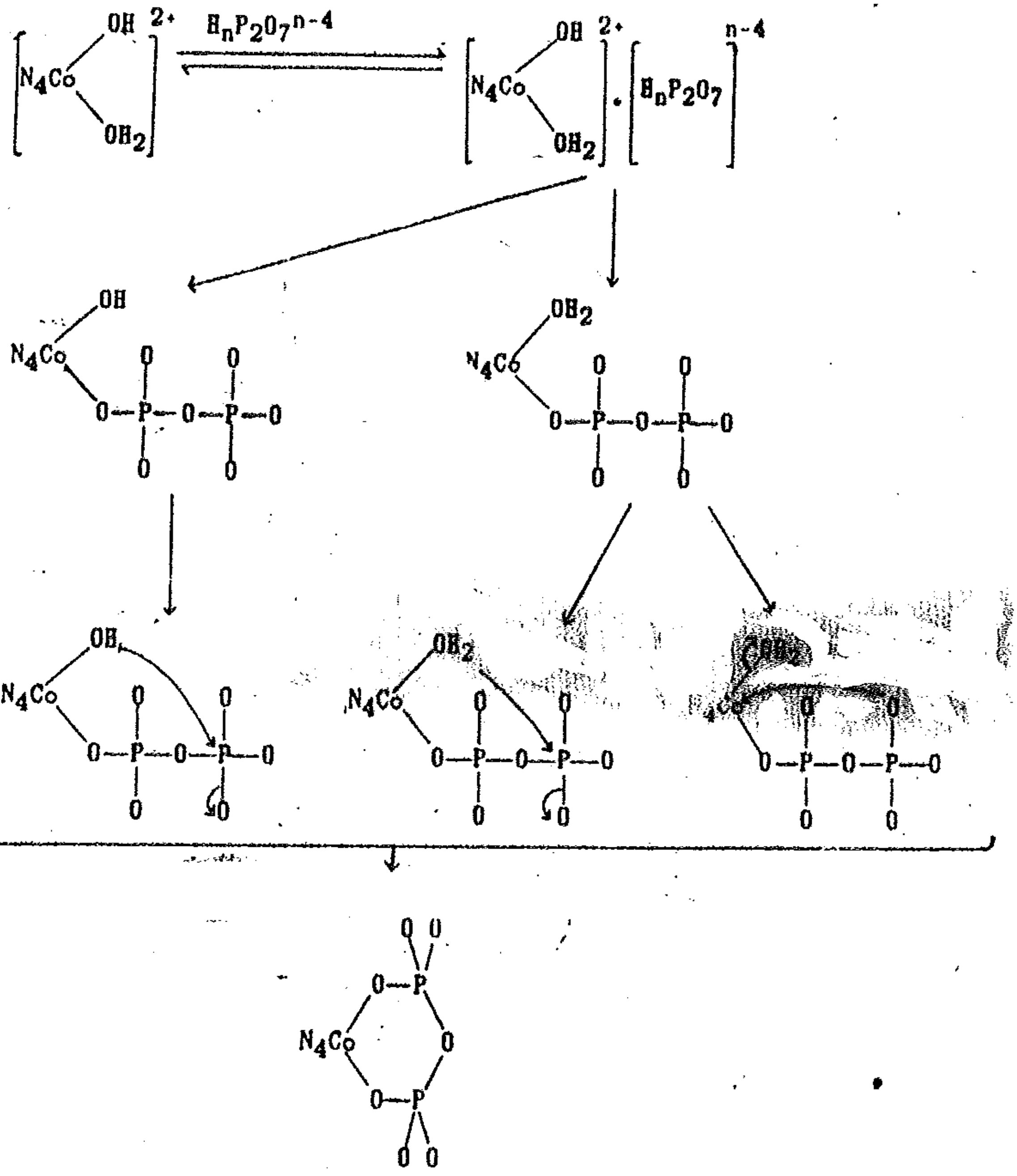
Within the 3:1 metal to pyrophoaphate ratio the highest phosphate release was noted for the direct addition systems, followed by the system where 2 molar ratio of $tn_2Co(III)(aq)$ was added to the preformed $tn_2Coppi$. The lowest hydrolytic cleavage was seen for the systems with stepwise addition. This is presumably due to the formation of different chelated pyrophosphate species where some are labile to hydrolytic cleavage while the others seem stable. Addition of $N_4Co(III)(aq)$, ($N_4$= $tn_2$ or $en_2$) to ppi at $p^H$ 6.5 where $[N_4Co(OH)(OH_2)]^{2+}$ predominates at a 1:1 molar ratio results in rapid water substitution to form the monodentate complex. Intramolecular cyclization of the monodentate complex produces a six membered chelate. As given in section 3.1 the rate of formation is relatively fast ($tn_2$ = 46 seconds) for 1:1 metal to pyrophosphate ratio for $[tn_2Co(OH)(OH)_2]^{2+}$ containg system. The small degree of hydrolysis is not due to the slow rate of anation reaction but it is attributed to the formation of a stable six membered chelate as shown in scheme 3.1.

Addition of 1 molar ratio of $[tn_2Co(OH)(OH_2)]^{2+}$ to the preformed 1:1 complex at $p^H$ 6.5 results in the formation of a double six membered chelate in a manner analogous to that described above. The existence of one six membered ring and another four membered ring or the formation of two four membered rings as a result of ring opening of the preformed six membered chelate can be presumed as possible.

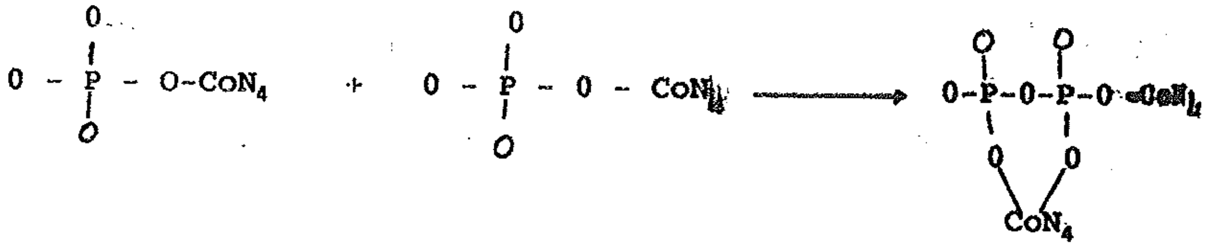
Previous workers [27,54] have shown the existence of the above species by $^{31}$PNMR studies in solutions containing pyrophosphate and $N_4Co(III)(aq)$ in the middle $P^H$ region. Addition of another molar ratio of $[tn_2Co(OH)(OH_2)]^{2+}$ to the above can presumably result in the formation of tetraaminecobalt(III) pyrophosphate species with coordinated hydroxide on one of the cobalt.
All the three metal ions would assist the hydrolysis by helping to neutralize the negative charge on the phosphate residue and by polarizing the phosphorus atom to allow efficient attack by the coordinated hydroxide.

Presumably some of the 3:1 species lead to hydrolysis while the other produce stable complexes as shown in scheme 3.2. The direct addition method probably forms largely species I and II which are active towards hydrolytic cleavage. The stepwise method of addition presumably produces largely species III which is more stable towards hydrolysis compared to species I and II.

Scheme 3.1. Proposed mechanism for the formation of $N_4$Coppi by intramolecular cyclization of the monodentate complex (charges and degree of protonation omitted).



An increased rate of hydrolysis during the initial stages of the reaction for all of the systems show a levelling off of the rate culminating at saturation. A possible explanation for the rate saturation phenomenon was suspected to be transphosphorylation as depicted in the following equation.



The investigation in this aspect did not give positive result. Hence rate saturation is presumably attributed to the formation of stable chelates.

# 4.CONCLUSION

The results of this work have shown that efficient hydrolysis requires the formation of a complex containing more than a single metal centre to greatly enhance the rate of pyrophosphate hydrolysis. Chelate formation and subsequent hydrolysis of pyrophosphate by the tetraaminecobalt(III) complexes depend on the order of addition of the metal ions to pyrophosphate. The rate enhancement for $[Cotn_2(OH)(OH_2)]^{2+}$ containing systems at PH 6.5 compared to uncatalysed reaction is as large as $10^5$. The mode of hydrolysis is believed to be a combination of effects including charge neutralization electron density polarization and the provision of an appropriately placed nucleophile, i.e. a metal bound hydroxide.

This work will provide further insight into the role of metal ions in hydrolytic reactions.

14. K.M. Welsh. I.M. Armitoe, and B.S. Cooperman, Biochemistry, 22, 1046,1983.

15. D.J. Hann and B.S. Cooperman, Biochemistry, 17, 4033, 1981.

16. J.W. Spiro and L.G. Butler, J. Biol. Chem., 251, 2611, 1976.

17. W.B. Knight, S.S. Ting, S. Chuang, D. Dubaway-Mariano, T. Haromy and M. Sundralingam, Arch. Biochem. Biophys., 227, 320, 1983.

18. T. Wagner-Jauregg, B.E. Hackley, T.A. Lies, O.O. Owens, and R. Proper, J. Am. Chem. Soc., 77, 922, 1955.

19. R.C. Courtney, R.L Gustafson, S.J. Westerbacks, H. Hyytiainen, S.C. Chaberek and A.E. Martell, J. Am. Chem. Soc., 79, 3030,1957.

20. R.L. Gustafson, and A.E. Martell, J. Am. Chem. Soc., 84, 2309,1962

21. J. Epstein and D.H. Rosenbatt, J. Am. Chem. Soc., 80, 3596, 1958.

22. B. Anderson, R.M. Milburn, J.M. Harrowfield, G.B. Robertson and A.M., Sargeson, J. Am. Chem. Soc., 99, 2652, 1977.

23. T.C. Bruce and S.J. Benkovick, Bioorganic Mechanism, I.W.A. Benjamin, 1966, p.125

24. R.A. Kenley, R.H. Flemingo, R.M. Laine, D.S. Tse, and J.S. Winterle, Inorg. Chem, 23, 1870, 1984.

25. H. Sigel, F. Hofstetter, R.B. Martin, R.M. Milburn, K.V. Scheller, and K.H. Scheller, J. Am. Chem.Soc., 106, 7935, 1984 and reference therein.

26. R.C. Courtney, R.L. Gustafson, S.J. Westerback, H.Hyytiainen, S.C. Chaberek, and A.E. Martell, J. Am. Chem. Soc., 79, 3030, 1957.

27. P.W.A. Hubner, and R.M. Milburn, Inorg. Chem., 19, 1267, 1980.

28. P.R. Norman and R.D, Cornelius, J. Am. Chem. Soc. , 104, 2356, 1982.

29. D.R. Jones, L.F. Lindoy, A.M. Sargeson, J. AM. Chem. Soc. , 105, 7327, 1983.

30. F. Basolo and R.G. Pearson, Mechanisms of Inorganic Reactions, 2nd ed., Wiley, New York 1967.

31. F.A. Cotton and G. Wilkinson, Advanced Inorganic Chemistry, 4th ed. Wiley, New York 1980.

32. Fikru Taffesse, Doctotial thesis, Boston University, Boston, 1986.

33. G.P. Haight, Jr. T.W. Hambley, P. Hendry, G.A. Lawrance, and A,M. Sargeson, J. Chem. Soc. Chem. Commun. 488, 1985.

34. S. Motomized, T. Wakimato and Kyojitoie, Analyst, 108, 361, 1983.

35. C.L. Penney, Anal. Biochem., 75, 201, 1976.

36. B. Anner and M. Mosmayer, Anal. Biochem., 65 305, 1975.

37. D.J. Stewart, Anal. Biochem., 62, 349, 1974.

38. A.A. Baykov, O.A. Evtushenko and S.M. Avaeva, anal Biochem. 171, 266, 1988.

39. H.F. Bauer and W.C. Drinkard, J. Am. Chem. Soc., 82, 5031, 1960.

40. H.F. Bauer and W.C. Drinkard, Inorganic Synthesis, McGraw-hill, New York, volume VIII, p. 202, 1957

41. I.R. Jonasson, S.F. Lincoln and D.R. Stranks, Aust. J. Chem., 23, 2267, 1970.

42. A.M. Sargeson and G.H. Searle, Inorg. Chem., 6 , 794, 1967.

43. R.D. Gillard and G. Wilkinson, J. Chem. Soc., 3193, 1963.

44. H.P. Malan and H. Munzel, Radiochim. Acta,, 5 , 20, 1966.

45. Fikru Taffese and R.M. Milburn, Inorg. Chem. Acta., 135, 119, 1987.

46. R.M. Milburn, M. Gautam-Basak, R.R. Ribolet and H. Sigel, J. Am. Chem. Soc., 107,3315, 1985.

47. Fikru Taffese, S.S. Massoud, R.M. Milburn, Inorg. Chem., 24, 2591, 1985.

48. A. Bino, and D. Gibson, J. Am. Chem. Soc., 104, 4383, 1983.

49. C.L. Rohlting, L.C. Allen, and M.C. Cook, J. Chem. Phys., 78, 2498, 1983.

50. M. Ardon, and R.A. Plane, J. Am. Chem. Soc., 81 , 3197, 1959.

51. H. Stunzy, and W. Maty, Inorg. Chem., 22 , 2145, 1983.

52. D. Chatterjee and G. Sankar De. Transition Met. Chem., 12, 193, 1987.

53. W. Kruse and H. Taube, J. Am. Chem. Soc., 83, 1280, 1961.

54. I.I Creaser, G.P. Haight, R. Peachy, W.T. Robinson, and / M. Sargeson, J. Chem. Soc. Chem. Commun., 1568, 1984.