THE DEVELOPMENT OF HETEROGENEOUS CATALYSTS FOR THE HYDROLYSIS OF PHOSPHATE TRIESTERS

A Thesis
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MASTER OF PHILOSOPHY
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University of St Andrews

by

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United College of St. Salvator
and St. Leonards College

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ABBREVIATIONS

The following abbreviations are used for convenient nomenclature of the various ligands.

[12]aneN$_3$	1, 5, 9-triazacyclododecane
cyclen	1, 4, 7, 10-tetra-azacyclododecane
dpa	2, 2'-dipyridylamine
tmen	N, N, N', N'-tetramethylethylenediamine
trimen	N, N, N'-trimethylethylenediamine
tren	2, 2', 2"-triaminoethylamine

DECLARATION

I, Susan Elizabeth Blatchford hereby certify that this thesis has been composed by myself, that it is a record of my own work, and that it has not been accepted in partial or complete fulfilment of any other degree or professional qualification.

I was admitted to the Faculty of Science of the University of St Andrews under Ordinance No. 12 in October, 1991.

Signed: .....

July 1994.

ACKNOWLEDGEMENTS

I would like to thank Professor R. W. Hay for his supervision and patience. Professor Cole-Hamilton, Dr. G. Wooley and Dr. D. Smith for their advice. Ms. S. Smith, Dr. C. Glidewell, Mrs. M. Smith and Dr. F. Riddell for their help with analysis. Dr. A. Aitken for his guidance on safety. Bobby Cathcart and John Smith for their cheek! And, of course, all of the technical staff for their huge efforts and friendliness.

Lab. life would have been much duller without Sian, Thomas, Caroline and Andrew. Thanks for your company!

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I would also like to thank both Ian and Gill McEnaney, and John and Denise Howard for their support this year.

Last, but in no ways least, I do not know how to thank my Mum and Dad, brother, grandma and of course 'Tiger' for all their understanding and much needed backing, both emotional and financial especially throughout these last two years.

To mum. dad

and Paul.

With love.

DULCE ET DECORUM EST

Wilfred Owen

(It is sweet and meet to die for one's country. Sweet and decorous.
A Latin tag from Horace, Odes III ii .13)

Bent double like old beggars under sacks,
Knock-kneed, coughing like hags, we cursed through sludge,
Till on the haunting flares we turned our backs
And towards our distant rest began to trudge.
Men marched asleep. Many had lost their boots
But limped on, blood shod. All went lame; all blind;
Drunk with fatigue; deaf even to the hoots
Of tired, outstripped Five-Nines that dropped behind.

Gas! Gas! Quick, boys! - An ecstasy of fumbling,
Fitting the clumsy helmets just in time;
But someone still was yelling out and stumbling,
And flound'ring like a man in fire or lime...
Dim, through the misty panes and thick green light,
As under a green sea, I saw him drowning.

In all my dreams, before my helpless sight,
He plunges at me, guttering, choking, drowning.

If in some smothering dreams you too could pace
Behind the wagon that we flung him in,
And watch the white eyes writhing in his face,
His hanging face, like a devil's sick of sin;
If you could hear at every jolt, the blood
Come gargling from the froth-corrupted lungs,
Obscene as cancer, bitter as the cud
Of vile, incurable sores on innocent tongues, -
My friend, you would not tell with such high zest
To children ardent for some desperate glory,
The old lie: Dulce et decorum est
Pro patria mori.

ABSTRACT

The main emphasis of this work has been the development of solid support catalysts for the divalent metal ion based catalysed hydrolysis of phosphate triesters. It has previously been shown that copper (II) complexes are effective catalysts for the hydrolysis of substrates such as 2,4-dinitrophenyldiethylphosphate (DNPDEP) which is an excellent simulant for the toxic nerve agents.

Catalysts have been developed using both silica gel and polystyrene as the solid support. The activity of copper (II) Chelex 100 resin which contains iminodiacetate functional groups has also been investigated. Preliminary studies of the catalytic activity of the solid supported copper (II) complexes were carried out using DNPDEP.

Homogeneous catalysis with copper (II) tetramethylenediamine and copper (II) dipyridylamine complexes was studied with the substrates 4-nitrophenyl picolinate and ethyl glycinate. The picolinate ester was of value when spectrophotometric monitoring was required. These esters are non toxic and thus present many advantages for the initial surveys of catalytic activity. The temperature dependence of the water and base hydrolysis of 4-nitrophenylpicolinate has been investigated in detail and the activation parameters determined. For base hydrolysis $\Delta H^* =$ $14.6 kJmol^{-1}$ and $\Delta S^*_{298} = -154 JK^{-1}mol^{-1}$.

Several macrocyclic ligands have been synthesised and their solution chemistry investigated as they may be readily incorporated on solid supports. In addition suitable modification of the ligands could give interesting metallomicelles for catalytic work.

PREFACE

Chemical substances used in war for their direct physiological or chemical effects are called chemical agents.

Attempts at overcoming the enemy by the generation of poison gases have been made from the dawn of antiquity. In their war against the Athenians (431 - 404 B.C.), the Spartans besieged the cities of Platea and Belium, weakening the opposition by burning wood with pitch and sulphur under the walls of their cities, choking the defenders and rendering the attack less difficult. The use of poison gases continued throughout the Middle Ages, hand grenade bottles were projected through the air releasing noxious fumes, Prester John in the Eleventh Century, is said to have stuffed copper figures with explosives and combustibles, which when emitted from the mouths and nostrils of the effigies caused 'great havoc'!

There have always been campaigns against the use of chemicals in war. The Hague Conference in 1899, persuaded several of the more prominent nations in Europe and Asia to pledge themselves against the use of projectiles whose only object was to emit suffocating or poisonous gases. Germany subsequently signed the Convention in 1900, the United States of America, however, never signed, their reasoning was, reported by Admiral Mahon (the US delegate) : [1]"The reproach of cruelty and perfidy addressed against these supposed shells was equally uttered previously against firearms and torpedoes although both are now employed without scruple. It is illogical and not demonstrably humane to be tender about asphyxiating men with gas when all are prepared to the blow the bottom of an iron clad at midnight, throwing four or five hundred men into the sea to be choked by the water with scarcely the remotest chance to escape."

During the American Civil War gas attacks were used to disable the enemy. The advantages of this form of attack were considered to be a reduction in the sanguinary character of the battlefield hence rendering the conflicts results more decisive and the deaths more humane.

It was just after 1700 hours on Thursday 22 April 1915 that the Germans released 150 tonnes of Cl_2 gas along 7000 metres of their front line, which was at some points only 50 metres away from the Allies. The yellow green cloud sprang on an unprepared enemy. Lifted by the temperature of the ground its effectiveness was decreased but the psychological shock was

optimised. The French front line watched the cloud spring out of the ground and roll towards them filling every crevice, dug out and trench as it came. The soldiers in the front and support lines, with no masks and no protection, broke line and fled. The cloud following them, enveloped them as they gasped and choked, panicking in the lack of air. Thus the trench stalemate was broken in Flanders.

It can only be expected, that the initial feelings towards gas warfare were a mixture of amazement, panic and blinding terror.

Rev. O.S. Watkins in the Methodist Recorder (London) described the arrival of poison gas : (1)"Going into the open air for a few moments relief from the stifling atmosphere of the wards our attention was attracted by very heavy firing to the North where the line was held by the French. Evidently a hot fight and eagerly we scanned the country with our field glasses hoping to glean some knowledge of the progress of the battle. Then we saw that which almost caused our hearts to stop beating - figures running wildly and in confusion over the fields. 'the French have broken,' we exclaimed. We hardly believed our own words... The story they told us, we could not believe. We put it down to their terror stricken imaginings - a greenish dense cloud had swept down upon them turning yellow as it travelled over the country, blasting everything it touched, shrivelling up the vegetation. No human courage could face such a peril."
"Then, there staggered into our midst, French soldiers blinded, coughing, chests heaving, faces an ugly purple colour - lips, speechless with agony, and behind them in the gas choked trenches we learned that they had left hundreds of dead and dying comrades. The impossible was only too true."

If the Germans had had more confidence in their new weapons and been more aware of their effects, and if Haber, known for nitrogen fixation, and the acknowledged Father of chemical warfare, had implemented his 'brainchild' more successfully, the outcome of World War I could well have been very different.

Thus large scale chemical warfare became a reality, and the particular horror associated with it contributed to its effectiveness.

The next major attempt to form an agreement on the use of chemical arms, was the Geneva Protocol of 1925. In effect both the Hague Conference and Geneva Protocol acted as a 'no first use' ban - all signatories were forbidden from use against other signatories, but in the case of attack by non signatories and belligerents, defence with chemical warfare was allowed.

From this time, it seems that the strategic value of battle deaths has been greatly diminished. It is seen to be much more valuable to laden an enemy with men put out of action by non-fatal battle wounds, casualties, who hence become military liabilities. Gas was seen to be four times as effective in securing such injuries as causing battle deaths[2].

Gas affects the whole area it crosses equally. It seeps into a copse of trees, a room, an empty building. It contaminates and poisons grain and food supplies, it diminishes industrial and commercial activities and causes wide spread discontent, anxiety, disablement and fear amongst civilians.
In contrast, conventional munitions cause injuries only in the immediate area of attack. The effects are instantaneous, two soldiers standing side by side may suffer completely opposite fates. Trenches and dugouts which may provide protection from artillery fire are no defence from poisonous gases which can remain long after the initial attack in gas pockets.
"Gas follows no narrow trajectory, it permeates the air and overcomes all incidental obstacles of terrain to stalk its quarry relentlessly."[3]

Chlorine attacks were followed by phosgene ($COCl_2$) attacks, which in turn were followed by the German attack of mustard gas in the Ypres section of Flanders in July 1917.

We were slow to develop our ideas. It seems we went through an examination of practically every compound in the whole catalogue of chemicals that offered any promise of military utility and actually trialled scores of potential chemical agents. Reports suggest we adopted a haphazard, 'suck it and see', type approach towards chemical weapon development, our Scientists appeared to be working aimlessly and writing copiously.

Research on both sides of the Channel continued between the wars. The development of the first nerve agent TABUN in December 1936 by the German, Dr. G. Schrader, during his

investigations into insecticides, under the instructions of Hitler, as the German Chancellor, opened the door to the most horrific of all weapons. The production of Sarin, Soman and eventually the 'V' agents, which were produced by the British in the 1950s, followed.
VX has the volatility of heavy diesel oil, and is the worst of the class, together known as nerve agents. All are colourless, odourless and tasteless liquids or gases, their effects infinitely worse than their predecessors.
Fearfully the opponents anticipated their use in World War II, fortunately they were never employed. In 1945, however, when Hitler felt that Germany had failed him, he gave orders to use nerve agents in a final holocaust - only the unexpected speed with which the Reich fell prevented this.

Churchill, aware of the possibility that chemical warheads on V-2 rockets could be used to bomb Britain, said, "I should be prepared to do anything that would hit the enemy in a murderous place. I may certainly have to ask you to support me in using poison gas. We could drench the cities in Germany in such a way that most of the population would be requiring constant medical attention."(2)

The fears surrounding the suffocating powers of chlorine gas and phosgene, and the skin degeneration and blistering caused by sulphur mustard, were superseded by reports on the physiological effects that nerve agents elicit .

The symptoms of nerve poisoning are lacrimation, salivation, violent contractions of gastrointestinal tract and bladder and bronchoconstriction (accumulation of ACh at parasympathetic nerve endings); muscular twitching, paralysis (caused by accumulation of ACh at myoneural junctions of striated muscles), vaso constriction (caused by excess ACh at sympathetic ganglia and adrenal glands), hyperexcitability and central paralysis effects.

The longer the contact time, and the higher the concentration of nerve agents and the worse the effects. Exposure to nerve agents can result in death by asphyxia or, severe brain damage at sub lethal doses, caused by repeated convulsions.

It is essential that we defend our Armed Forces against the possibility of chemical attack.

Personnel protection has progressed from simple gas masks - cotton taped onto the face, steeped in sodium thiosulphate (1915), to the British N.B.C. suits, offering complete protection to our armed forces from Nuclear, Biological and Chemical attacks, in war time.

In the early 1960s Britain abandoned its offensive chemical warfare programme. However defence programmes are still intense, and troops still undergo regular training for defence against chemical warfare.

After the war between, 1945 and 1950, the U.S.S.R. continued to build up stocks. It would appear that Britain is bravely pioneering unilateral disarmament . The U.S. have kept their stockpiles, but stopped production, concentrating their efforts on nuclear warfare.
NATO's defensive stockpile, potentially, 400,000 tonnes munitions, is enough to kill the world's population 8,000 times over. In the 1960s USA reinitiated offensive research - the Binary Munitions Program - in these weapons chemicals are kept separately, and only combined in the missile head or at the point of explosion, resulting, in the production of substances which can cause severe harm.

The last build up of stocks in USA and USSR occurred in 1960s.

If a disarmament treaty is ever to be signed, by the superpowers, continuous monitoring of use of potential raw materials and in-house, on-site checks of chemical plants will be essential.

Prior to the use of chemical and nuclear warfare, wars were carried out on the principle that 'might made right'. There are two schools of thought on the philosophy of battle.
School 1 suggests that the ends of the war justify the means and that there is no limit to the degree of force which may be employed to attain victory.
School 2 suggests that no greater degree of force should be employed in war than is necessary to achieve victory in battle and that the ruthless destruction of life and property is not warranted in the conduct of warfare.

I suggest that both chemical and nuclear weapons fall into School 1, and conventional weapons into School 2.

We can't turn back the clock, nor can we forget what we already know, but we must try to prevent any more battles in which the degree of damage caused in attaining victory is unjustified.Perhaps this could be achieved by multi-lateral disarmament, or the build up of stocks as deterrents, or the development of better protection and decontaminants. Of these options the latter, I feel, will sadly be the easiest to achieve.

REFERENCES

1. Fries and West, Chemical Warfare, Chapt. 1, McGraw-Hill Book Co., New York (1921).
2. M. Sheeham and M. Robertson, Arms Race, Oxford (1983).
3. A.M. Prentiss and G.J.B. Fisher, Chemicals in War, Introduction, McGraw-Hill Book Co., New York (1921).
4. L.F. Haber, The Poisonous Cloud, Clarendon Press, Oxford (1986).
5. M.B. Jacobs, War Gases, Their Identification and Decontamination, InterScience Publishers INC., New York (1942).

CHAPTER ONE

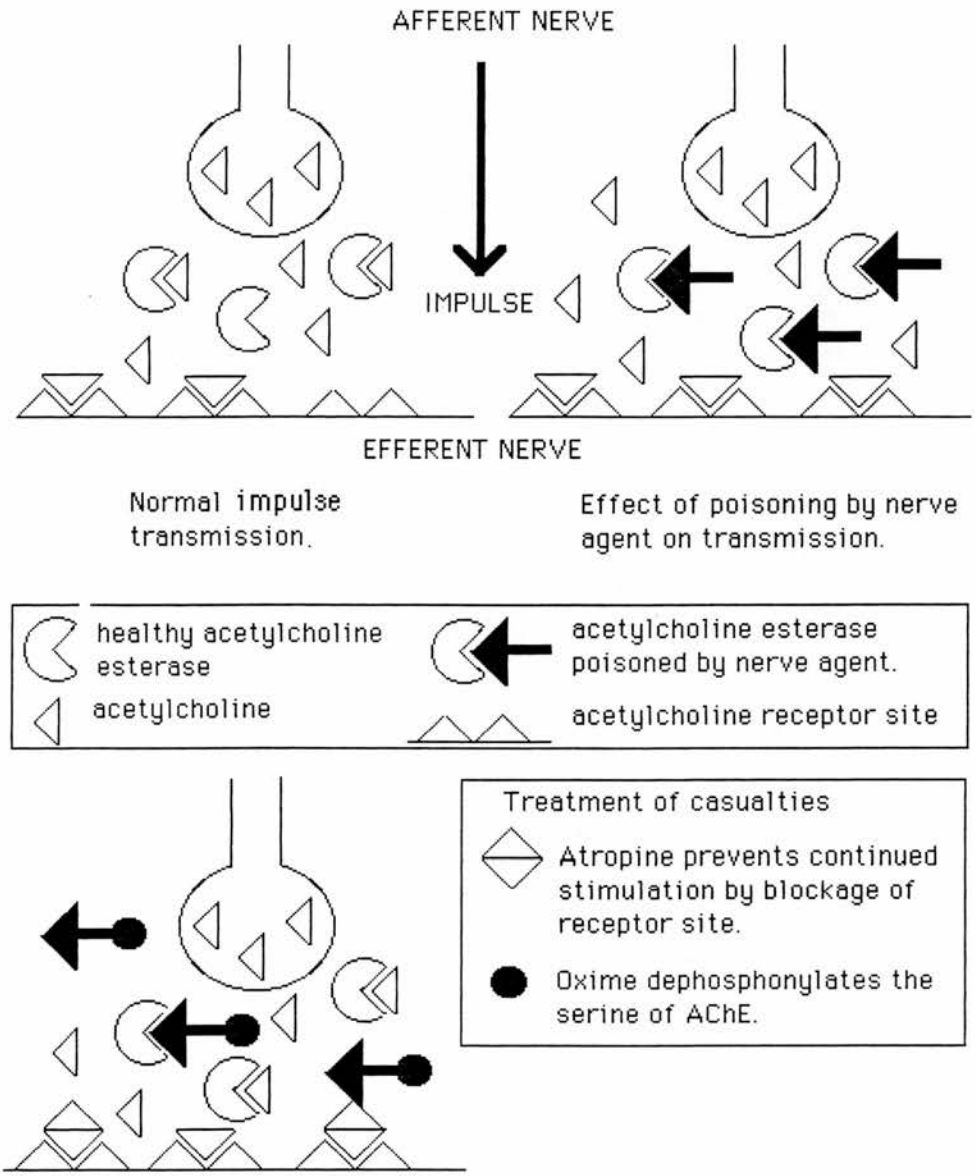
1.1 INTRODUCTION

The risk of chemical contamination is not just a concern on the battlefield during war time but it is also present in the home, in industry, on the farm and in research laboratories.
The research carried out into decontamination of organic phosphorus esters is, of course, primarily in relation to the treatment of soldiers and their equipment following an attack by chemical agents during hostilities.

The key elements of protective strategy against chemical attack can be broken down into four areas.
1.Early detection.
2.Protective measures, adjusting suits and respirators for personnel.
3.Decontamination of surfaces onto which agent has fallen.
4.Medical counter measures for those effected directly by the attack.

Nerve agents may enter the body through the respiratory tract and may also be absorbed through the skin. They are so called because their primary targets are sites in the body where large concentrations of acetylcholine esterase exist i.e. at nerve endings.
Scheme 1.1 represents the physiological processes involved in nerve agent poisoning. Basically, when a nerve impulse is transmitted from the afferent nerve to the efferent nerve it is so by a series of changes in electrical impulses and changes in the concentrations of the chemicals present. Acetylcholine (ACh) is released from the afferent nerve, and diffuses directly to receptor sites on the efferent nerve, where a sufficiently high concentration of ACh triggers a nerve impulse in the efferent nerve. The ACh is then released back into the synapse, and to prevent continued stimulation it is hydrolysed immediately to an inactive form by the enzyme acetylcholine esterase (AChE).
Nerve agents inhibit the function of AChE - they promote the phosphorylation of serine, thus a build up of the active ACh occurs and hence continuation of nerve stimulation at the efferent nerve occurs.
There are few forms of treatment for nerve agent poisoning: Atrophine, an anti-cholinergic drug blocks the receptor sites of the efferent nerve, preventing continued stimulation. P2S (N-methyl pyridinium-2-aldoxime methane sulphonate) acts by

Scheme 1.1 The physiological effects of nerve agents.[1]



removing the phosphoryl group from the AChE thus regenerating it.

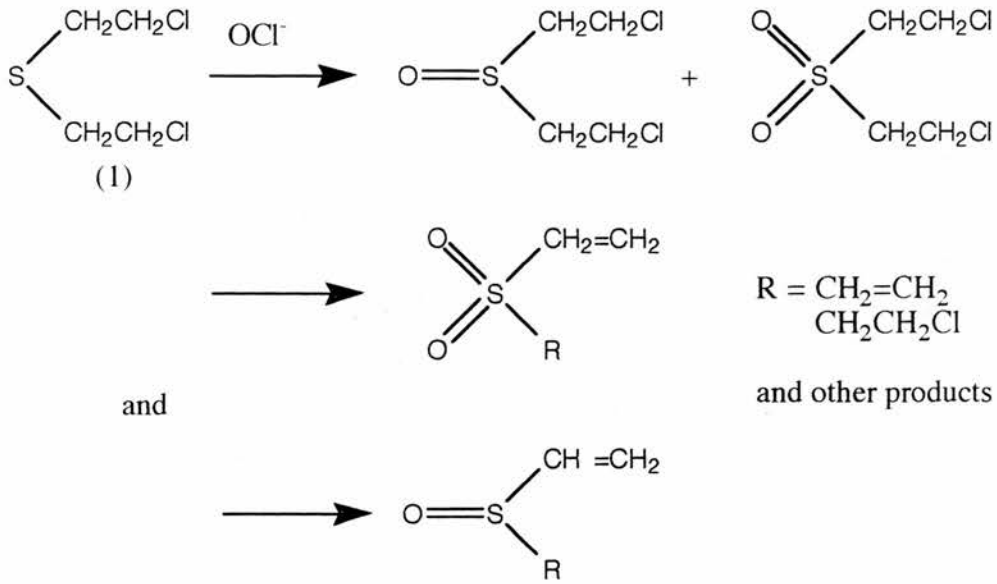
The lethal dose of these agents is so low (LD_{50} of Sarin ranges from 230μg/Kg in mice to about 20μg/Kg in primates) that if inhaled they will usually prove fatal. Their lack of smell and colour generally renders them impossible to detect prior to the onset of physical symptoms, by which time most remedies are ineffective, and death, or severe permanent effects will follow. Protection by specially designed clothing, followed by rapid decontamination of the immediate area, is preferential to the medical treatments which would be necessary following the development of symptoms.

The rapid and efficient decontamination of the environment following assault enables the defending troops to resume full duties swiftly, by removal of protective clothing, and quells further danger from poisoning by residual agents.
With the continuous development of more complex and dangerous chemicals the challenge to discover adequate means for their neutralisation becomes more critical. The combined knowledge of both engineers and chemists are increasingly relied upon to provide efficient protective measures for our defending forces.

Before the development of mustard gas there were no requirements for decontaminants on the battle field. Chlorine and phosgene, both gases, diffused from the scene of the attack as quickly as weather conditions would allow. Mustard gas, however, due to its persistency, demanded active cleansing of the site affected. Physical methods of cleansing a contaminated environment, such as high powered jets of steam, water, sea water and detergents, simulating accelerated natural weather decay, are effective, however not feasible, or rapid enough, in every situation.

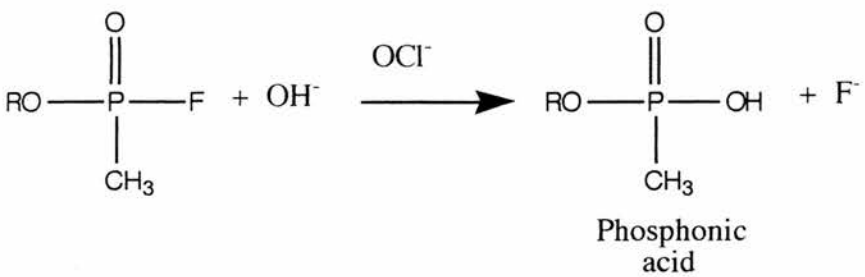
The first chemical decontaminates were bleaching powders and to a lesser extent potassium permanganate. The sulphur mustard[2] undergoes a series of oxidation and elimination reactions in strongly basic solution to give non-toxic products, (Scheme 1.2).
During World War II super chlorinated bleaches were the main decontaminants, to be superseded by the more stable, less corrosive and less alkaline N-chloro compounds (i.e. Fichlor (sodium dichloride isocyanurate in a borate buffer (pH 10.2)), chloroamines).
The first decontaminants for the nerve agents (G agents) were solutions of alkali salts; these

rapidly detoxify, with hydrochloric acid liberation.



[Scheme 1.2]

Throughout the following decades the main chemical principle for detoxification of the nerve agents was related to the use of the strong alkali and acid solutions[2] developed in World War II. The main disadvantages of these decontaminants are that they are too aggressive for use on delicate materials and equipment, and especially caustic for personal use, Scheme 1.3.



R = $(CH_3)_2CH$ Sarin
$(CH_3)_3CCH(CH_3)$ Soman

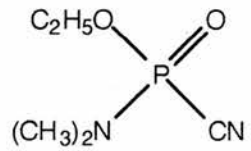
[Scheme 1.3]

The surfaces of equipment entering battle zones are required to be of a very high standard, cracks and screws on surfaces pose problems for even the most searching, cleansing treatment. Natural weathering and other man made physical methods are hindered by the high surface tension of the agents, so residues can sometimes remain until the surrounding temperature is sufficient to evaporate the agent, hence further contamination of unsuspecting personnel can occur some time after the initial attack. Therefore the immaculate design of protective suits,

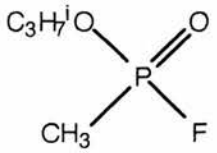
transport and equipment is imperative.

Ultimately it is hoped that self decontaminating materials will be developed which will coat equiptment and hence reduce the amount of decontaminants that are carried into battle areas. Thus lessening the load and logistical burdens of the servicemen.

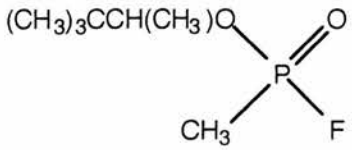
Typical phosphate ester based nerve agents are :



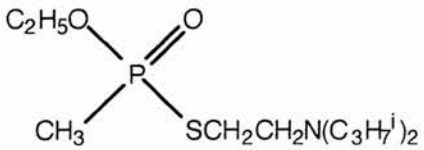
GA Tabun



GB Sarin



GB Soman

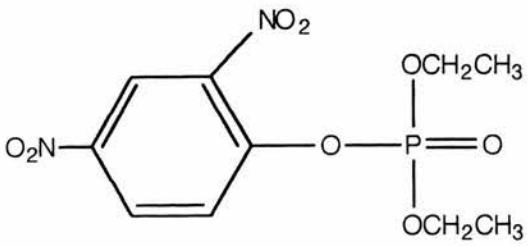


VX

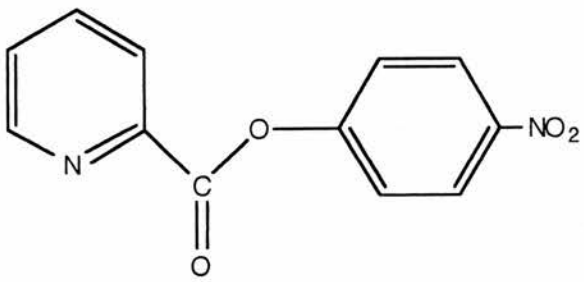
The approach adopted in the present work is to continue the research in the development of metal catalysts for the hydrolysis, detoxification, of phosphate esters. With an emphasis on the loading of such catalysts onto a variety of polymeric supports.

Simulants

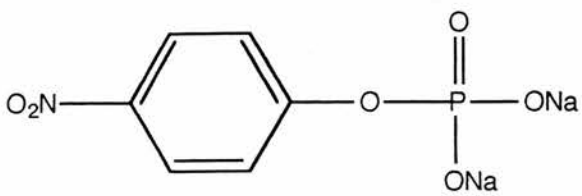
Owing to the problems faced when working with nerve agents several simulants were used in our research. The simulants used were 4-nitro phenylpicolinate, ethyl glycinate and 2,4-dinitrophenyldiethyl phosphate (DNPDEP). The hydrolysis of these carboxylic and phosphate esters were studied in the presence and absence of copper catalysts.



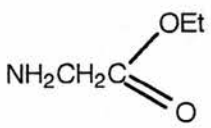
DNPDEP



4-Nitrophenyl picolinate



4-nitrophenyl phosphate



Ethyl glycinate

1.2 REFERENCES

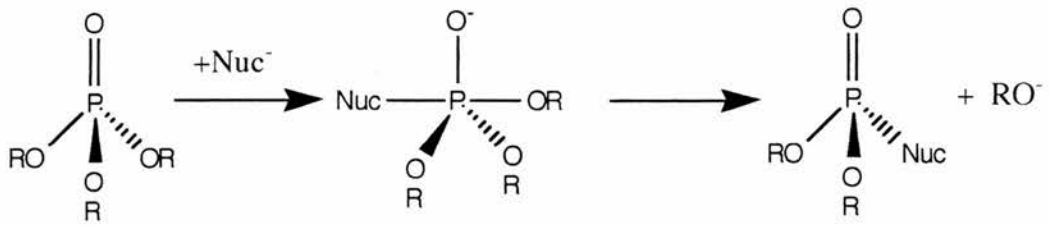
1. L. Leadbeater, Chem. Britain, 24, 683 (1988).
2. Y-C. Yang, J.A. Baker and J.R. Ward, Chem Rev, 92, 1729 (1992).

CHAPTER TWO

2.1 INTRODUCTION

The Hydrolysis of Phosphate Esters

In the absence of metal ions the nucleophilic substitution of phosphate triesters appears to occur exclusively by an associative type mechanism as depicted below.

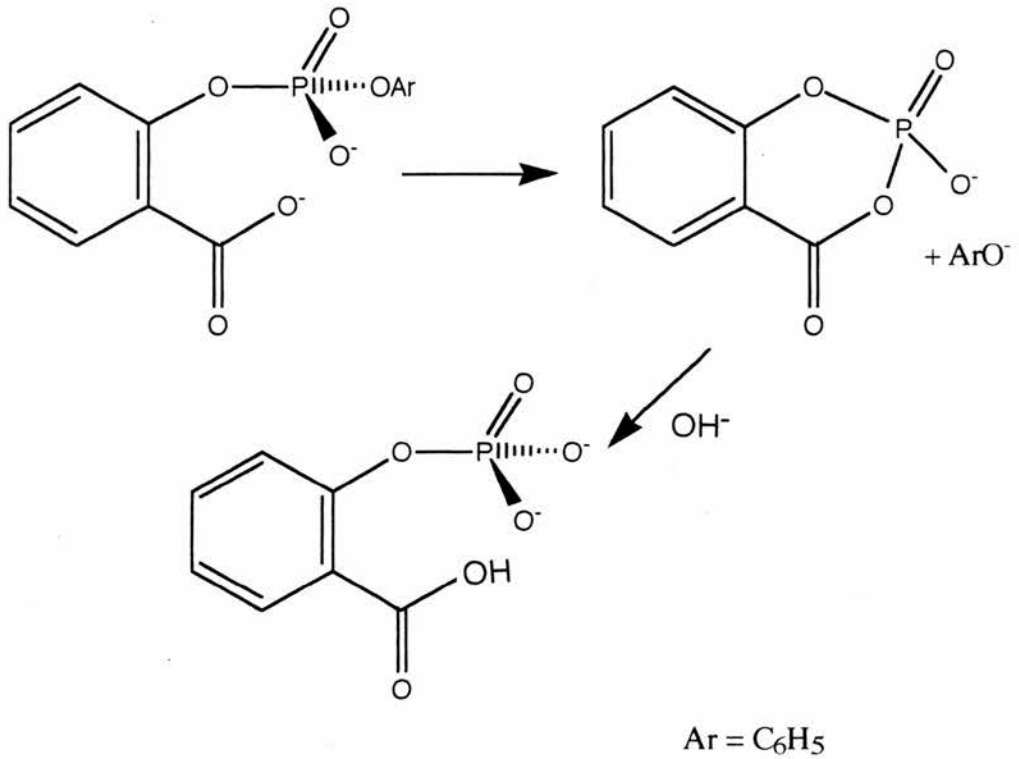


[Scheme 2.1]

An addition-elimination type of reaction occurs by addition of the nucleophile at phosphorus to generate a 5 coordinate intermediate or activated complex. The phosphorus is able to use its d-orbitals to expand its coordination number, and, it is argued, the attacking OH^- ion forms a 5 coordinate phosphorane intermediate.

In general it is suggested that the intermediate is trigonal bipyramidal in structure. By X-ray crystallographic techniques it can be shown that the apical bonds are longer and weaker than the equatorial bonds in the phosphorane intermediate. The apicophilicity of a substituent (its preference for the apical site), has been deduced for many substituents by NMR studies. The scale spans from F^- ions to alkyl groups in the order of decreasing apicophilicity. The phosphorane intermediates are stabilised by 4, 5 and 6 membered rings incorporating the phosphorus.

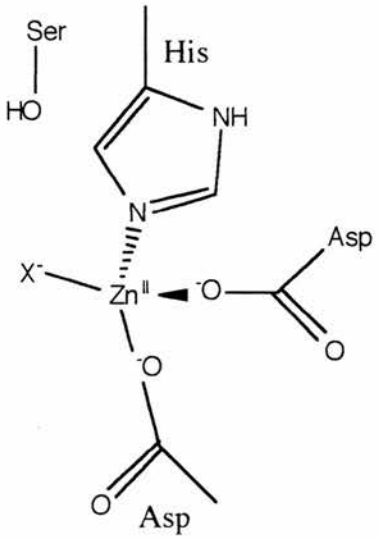
Pentacoordinate phosphoranes are not saturated and can quite easily give hexacoordinate species. There are many examples of such species e.g. PF_6^-, $P(OCH_3)_6^-$, and these like phosphoranes are also stabilised by electronegative substituents. In agreement with the associative mechanism suggested, it can be seen that 4 coordinate phosphorus has no problem in expanding to a 5 or 6 coordinate centre. For this reason a pendant nucleophilic functional group in a suitable position is able to participate in intramolecular nucleophilic substitution reactions thus greatly enhancing the rate of reaction. For example the intramolecular hydrolysis of phenyl salicyl phosphate is accelerated 10^7-10^8 fold over the analogous intermolecular pathway. Scheme 2.2[1].



[Scheme 2.2]

Enzymatic Hydrolysis of Phosphate Esters

Phosphoryl and nucleotidyl transfer enzymes are widespread and extremely important in biology. Their functions include the catalysis of nucleophilic reactions of phosphorus esters. Many such enzymes involve metal ions: a typical example is alkaline phosphatase, 2.1, which has a zinc (II) at the active site. The alkaline phosphatase from E. coli has a molecular weight of 94058 and has four zinc atoms per mole. The enzyme also contains approximately one atom of Mg(II). Recent X-ray studies of the enzyme containing bound PO_4^{3-} have established that two of the oxygen atoms of the phosphate are bound to two different zinc ions. Zinc appears to be involved in both the bonding and activation of the monoester substrate.

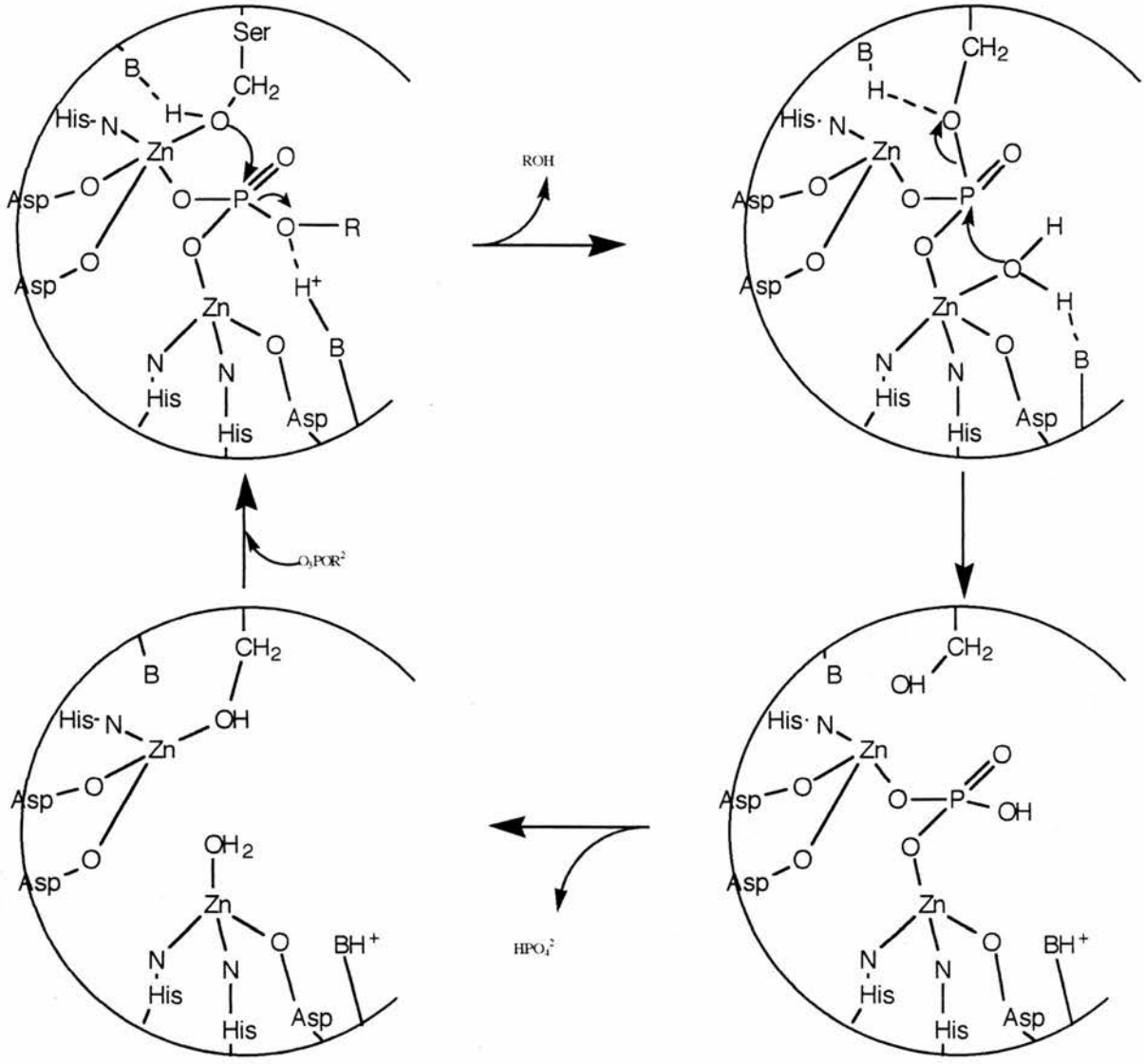


2.1 Active site of Alkaline phosphatase
(X^- = phosphate ester substrate)

A possible scheme by which phosphate monoesters are hydrolysed by E. coli alkaline phosphatase [1,2] is shown in scheme 2.3.

The mechanism can be summarised under the following headings:

1. The phosphate ester binds directly to both the zinc ions activating the phosphorus centre to attack by the deprotonated serine nucleophile. The deprotonation of the serine hydroxyl is enhanced by binding to the metal ion and intramolecular removal of the proton assisted by an enzyme base. The alkoxide leaving group is thus encouraged to leave and is facilitated by protonation by an enzymic acid. Inversion at the phosphate centre occurs.
2. A water molecule coordinated to the second zinc ion, deprotonated by an enzyme base, functions as an intramolecular nucleophile resulting in elimination of the serine alkoxide ion protonated by an enzymic acid. Inversion at the phosphorus centre occurs.
3. Cleavage of the phosphate ester occurs with overall retention of configuration as two stereochemical inversions occur.

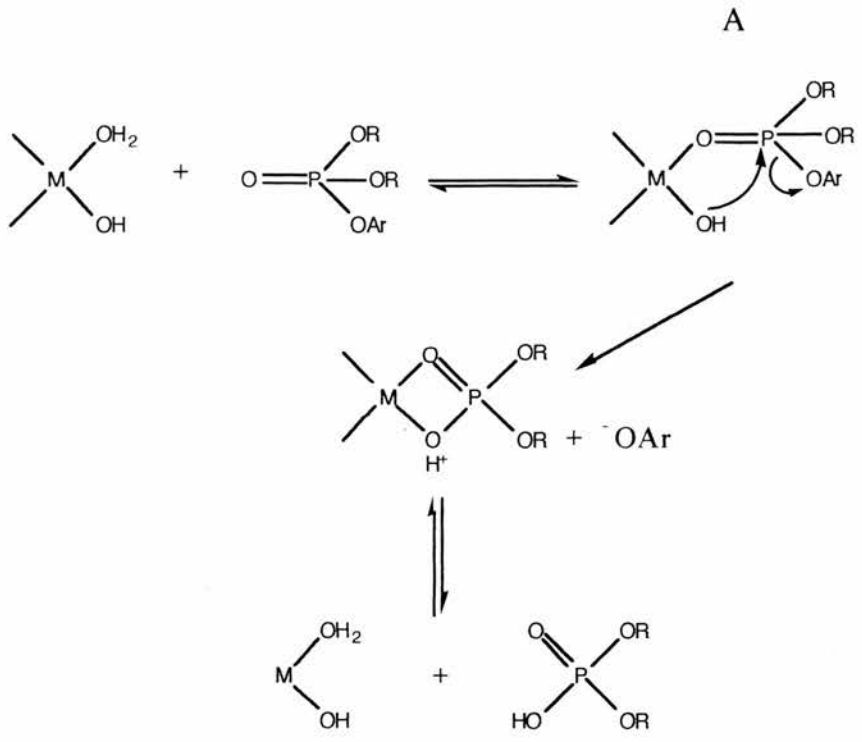


Scheme 2.3

Catalysis by Metal Complexes.

Metal ions and metal complexes catalyse the hydrolysis of a variety of mono-, di- and triesters of phosphoric acid and extensive reviews[3,4,5,6] on the topic are available. Catalytic effects have been studied using both kinetically inert cobalt (III) complexes and labile copper (II) complexes. The reactions involving cobalt (III) complexes tend to be stoichiometric in character as the products of the hydrolysis remain bound to the metal centre. However such reactions are frequently of value as it is easier to define the mechanism of the hydrolysis.

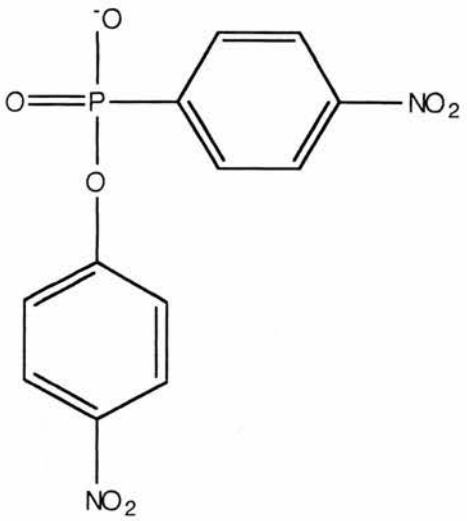
Previous work has shown that cis-hydroxoaqua complexes are excellent catalysts for the hydrolysis of triesters of phosphoric acid. Catalysis appears to involve the reactions shown in Scheme 2.4.



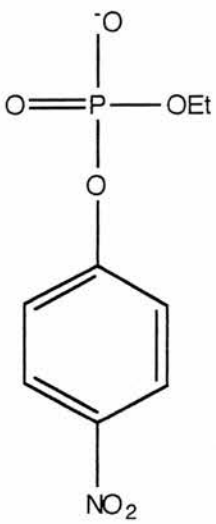
[Scheme 2.4]

In the complex (A) binding of the phosphoryl oxygen atom to the metal ion leads to electron withdrawal from phosphorus centre so that ready intramolecular attack by metal bound hydroxide ion occurs. The kinetic pK=7 for the hydrolysis of DNPDEP by this complex ion is close to the measured pKa (7.1) for $[Cu(bipy)(OH_2)_2]^{2+}$. The phosphorane intermediate (not shown), then breaks down by loss of the OAr^- leaving group. The bound phosphate diester is in equilibrium with the hydroxoaqua complex so that turnover can occur.

Some typical examples of this type of reaction are now considered. Copper (II) complexes of bipyridine, $[Cu(bipy)(OH)(OH_2)]^+$ catalyse the hydrolysis of the two phosphate diesters bis(4-nitrophenyl)phosphate (2.2)=BNPP and ethyl 4-nitrophenyl phosphate (2.3)=ENPP at 75°C in the pH range 5.8 to 8.3[8]. The reaction occurs by coordination of the diester to the $Cu(bipy)^{2+}$ moiety followed by intramolecular attack of the cis-coordinated hydroxide at the phosphorus centre. The rate enhancement is 10^3-10^4 fold. With the ester ENPP saturation and Michaelis-Menton kinetics were observed (K_M=4.7x10^{-2}M). The rate constant for attack of the metal bound hydroxide on the complexed diester is estimated to be



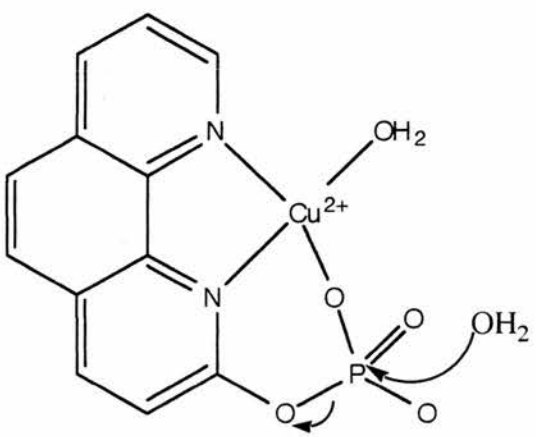
(2.2) = BNPP



(2.3) = ENPP

$5.6x10^{-4}s^{-1}$ at 75°C.

Divalent metal ion catalysis of the hydrolysis of 2-(1,10-phenanthrolyl)phosphate has also been studied[6].The metal ions Cu^{2+},Ni^{2+},Co^{2+} and Zn^{2+} all catalyse the reaction but only Cu^{2+} has a large effect. Intramolecular attack by Cu^{2+} bound hydroxide is prevented by steric restraints and this may be an important factor in the comparatively low rate enhancements observed. Copper facilitates hydrolysis by a Lewis acid effect but does not allow an intramolecular reaction.



[Scheme 2.5]

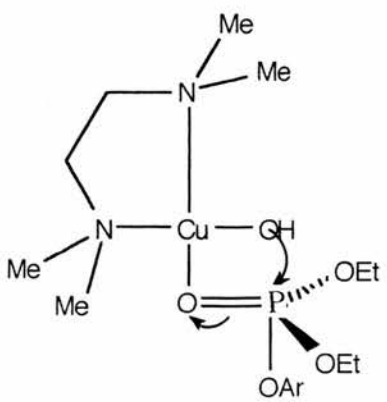
The reaction proceeds via attack of H_2O on the electrophilic phosphorus centre in the complex. The advantage of the copper complex is its enhanced solubility at pHs higher than that at which copper hydroxide would normally precipitate.

Copper in aqueous solutions encourages the hydrolysis of the P(V) triesters due to the presence of high concentrations of coordinated hydroxide ions at near neutral pH. Thus a nucleophilic species is close to the active phosphorus, an environment which is analogous to that of the active site of the enzyme system.

Recently Hay and Govan[8] have demonstrated the catalytic ability of a variety of copper amine complexes on the hydrolysis of 2,4-dinitrophenyldiethyl phosphate (DNPDEP) under neutral and mildly basic conditions.The pH-rate profiles indicate that the catalytically active species results from the removal of the relatively acidic proton from the complex, suggesting that $[CuL(OH)]^+$, or the kinetic equivalent CuL^{2+} and a free hydroxide ion, are the active species in the reaction.

The mechanistic possibilities for the involvement of the $CuL(OH)^+$ species in the hydrolysis are:

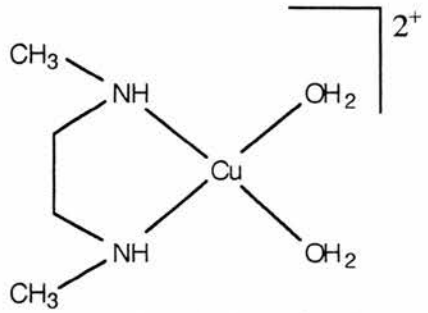
1. General base catalysis.
2. Nucleophilic attack on the phosphorus atom by a metal bound hydroxide.
3. Electrophilic activation of the phosphorus P=O by the metal centre towards attack by free hydroxide.
4. A hybrid type mechanism where the metal centre delivers a coordinated hydroxide ion to the phosphorus centre of the DNPDEP molecule while simultaneously drawing the electron density from the phosphorus atom by interacting with the phosphoryl oxygen, [2.4].



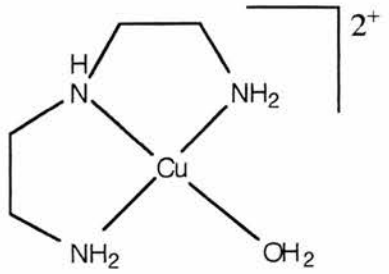
[2.4]

The high values of the second order rate constant (k_m) for the reaction with the metal complex suggests that a hybrid mechanism operates. For the hydrolysis of DNPDEP catalysed by $[Cu(dpa)(OH)OH_2)]^+$, (dpa = dipyridylamine), k_m=10.85 dm^3 mol^{-1} s^{-1} at 35°C, some 20 times greater than the k_{OH} value of 0.58dm^{-3} mol^{-1} s^{-1} for base hydrolysis. As the free hydroxide ion is more than 1 million times stronger as a base than $[Cu(dpa)(OH)OH_2)]^{2+}$ the possibility of general base catalysis occuring is considered unlikely. Lewis acid facilitation of nucleophilic reactions at P(V) centres has been observed in the iridium (III) pentamine[10] moiety complexed to the P=O group of trimethyl phosphate. The second order rate constant for hydroxide ion attack increases 400 fold.
The large increase in rate can be attributed to the bifunctional role of the copper(II) complex with electrophilic activation of the coordinated phosphate ester and the provision of a cis hydroxide nucleophile in a position to allow intramolecular nucleophilic attack at the phosphorus centre.

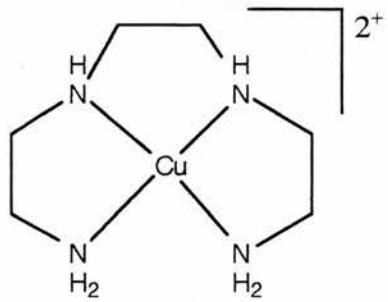
In support of the suggested mechanism, Hay and Norman showed that copper(II) complexes with a single coordination site available e.g. $[Cu(diethylenetriamine)]^{2+}$ do not promote the hydrolysis of DNPDEP as effectively as the bidentate cis hydroxy-aqua complexes. These observations are in agreement with those by other workers[9,10,11] who investigated the effects of copper complexes on the hydrolysis of Sarin and found that the half life of hydrolysis was 3.5min, 25min and 65min for complexes with N',N'dimethylethylenediamine, diethylenetriamine and triethylenetetramine respectively, at 25°C and pH7 using 1mM catalyst concentrations.



N',N'-Dimethylethylenediamine -Cu(II)complex



Diethylenetriamine-Cu(II)complex



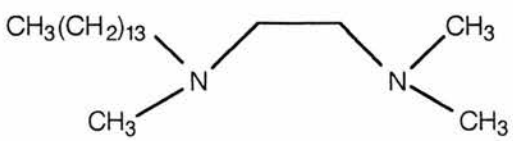
Triethylenetetramine-Cu(II) complex

Important requirements for the active copper (II) complexes can be summarised under the headings:

1. Availability of cis aqua sites on complex, facilitating the push-pull mechanism.
2. The use of bulky ligands to inhibit dimer formation e.g. N,N-dimethyl-ethylenediamine.
3. A pKa of the bound water molecule close to 7.

Heterogeneous catalysis by copper complexes

Copper in metallomicelle systems has also been found to catalyse the hydrolysis of various phosphate esters. The ligand A2 was developed by Menger and co workers[12] and the copper complex has high catalytic activity in the hydrolysis of 4-nitrophenyl diphenyl phosphate (PNPDPP).



A2

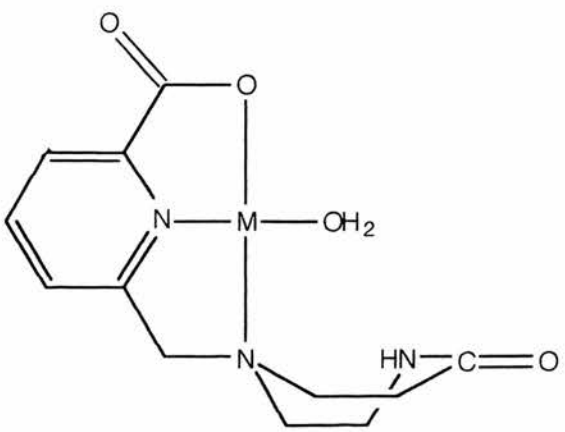
The amino groups were deliberately made tertiary to preclude possible irreversible reactions between nitrogen and the substrate when dissolved in water above the critical micelle

concentration ($1.8x10^{-4}M$, determined tensiometrically). A2 forms metallomicelles having a Stern region[12] filled with cupric ion. In the hydrolysis of PNPDPP the copper complex of A2 was found to be an extremely potent catalyst with $k_{obs}=4.1x10^{-2}s^{-1}$ using 1.5mM A2 (25°C at pH6.0). The catalysed reaction is greater than 10^5 times faster than the rate expected in the absence of any catalyst at pH6.0 ,and 200 times faster than if catalysed by an equivalent concentration of the tetramethylethylene diamine copper complex. The observed 10^5 rate increase probably arises from multiple effects:

a) PNPDPP is bound to the micelle surface[14] where the OH of $[Cu(L)(OH)]^+$ is located. Hence attack on the PNPDPP phosphorus stimulates a rapid intramolecular reaction.

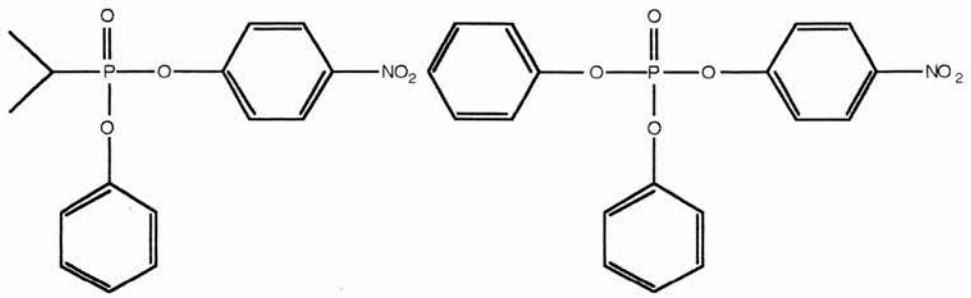
b) The water bound to micellar copper has very low pKa. The pH insensitivity of the rate of hydrolysis between 6.0 and 8.3 indicates that the pKa of the metal bound hydroxide must be about 3 pK units lower than that of 9 estimated by Allison and Angelici[14]. This is not an unprecedented case of such an environmentally reduced pKa. Groves and Dias[15] found that the tridentate copper complex [2.5] possesses an extraordinarily low pKa of 7.6, presumably resulting from its position in the highly cationic Stern region. Moreover the low dielectric constant (approximately equal to 36) known to exist at micelle surfaces[16] would augment the release of the proton from $Cu\text{-}OH_2$.

c) The copper (II) polarises the P=O, electrophilic catalysis being greater for that than found for monomeric copper due to the cationic nature of the Stern region of the micelle.



[2.5]

Menger also studied the effects of solid supported copper (II) complexes[17] on the hydrolysis of 4-nitrophenyl isopropyl phosphate (NPIPP)[2.6] and PNPDPP[2.7].



NPIPP
[2.6]

PNPDPP
[2.7]

Menger synthesised four types of polymer loaded complexes, Fig. 2.1.

1) Metal directly loaded onto the polymer;

2) Metal between polymer and 14 membered carbon aliphatic chain;

3) Polymer connected to 6 membered aliphatic chain, in turn connected to copper;

4) Polymer connected to 6 membered carbon chain which is connected to the copper ion in turn connected to 14 membered chain system;

Representations of the four polymer types investigated by Menger.

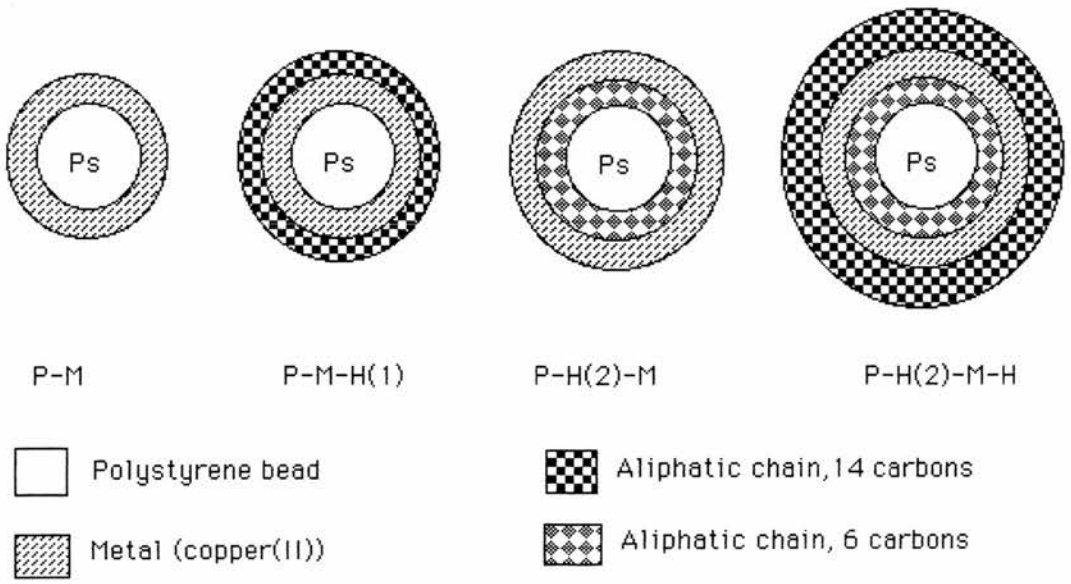


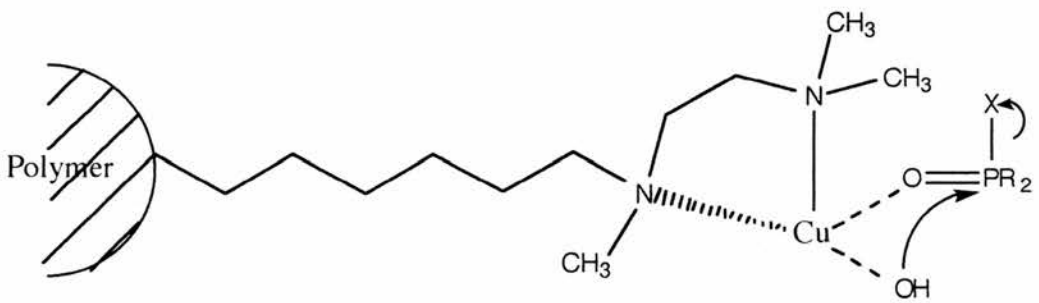
Figure 2.1

All polymer types demonstrated some sort of catalysis of the hydrolysis of the simulants. The best results were obtained using a high copper loading of the P-H(2)M type adapted polystyrene, which achieved the unofficial criteria according to McKay[18] of destruction of

PNPDPP in a cigarette break ($t_{1/2}$ = 2.7 min, 25°C, pH 8).

Overall by comparison of the effect of equal amounts of copper on both types of catalyst it can be seen that the more successful system in terms of rate enhancement was that involving the micellised copper surfactant which demonstrated a 12 fold rate enhancement compared with the equivalent concentration (if fully dissolved) of the P-H(2)M polymer based copper.
The main advantages of polymer catalysts are:
1) The catalyst can be easily removed by filtration;
2) The polymers could be more easily adapted to self decontaminating coatings for surfaces of equipment and protective clothing than the micelle systems.

The mechanism proposed for the solid state catalysis of phosphate esters is analogous to that of the micelle systems ie a hybrid type mechanism, intramolecular attack of copper bound OH^- and simultaneous electrophilic interaction of the copper with P=O group.



[Figure 2.2]

So far the majority of the work has been conducted using polystyrene polymers however other polymers are easily available such as silica and also Chelex 100 a chelating resin which has the advantage of being commercially available.
The hydrophobicity of the polystyrene backbone and the necessity for the ease of application of polymers to aqueous reaction media leads to the investigation of other types of polymer supported complexes.
The formation of chelating resins which exhibit a high selectivity for transition metal ions especially copper can be achieved through introduction of an amine to a silica back bone[20] thus making available resins for use in metal ion chromatography, recovery of the trace metal ions, waste water treatment and in this case heterogeneous catalysts for the hydrolysis of phosphate and carboxylic esters.

2.2. SIMULANTS

In place of the nerve agents themselves simulants are used in these studies as they are less toxic and safer to handle

The simulants are less reactive towards hydrolysis than the nerve agents so that catalysts which are active against the simulants frequently show high activity towards the nerve agents.

DNPDEP was selected as the principle substrate for this study because:

1. It is an easily handled simulant of the more dangerous nerve agents,
2. Considerable work has already been carried out on DNPDEP therefore a large body of data is available for studies of the relative activities of the catalysts,
3. The hydrolysis of DNPDEP can be monitored spectrophotometrically,
4. DNPDEP is water soluble. Many other simulants are only soluble in organic solvents.

2.2.1 SYNTHESIS

a) Preparation of 2,4-dinitrophenyl diethylphosphate.

Diethylchlorophosphate (63.0g,0.366 mol) was added dropwise to a stirred suspension of dry sodium 2,4-dinitrophenoxide (75.4g, 0.366 mol) in 450 ml of dry toluene. On completion of the addition the mixture was refluxed for five hours, cooled, washed with ice water (150 ml), cold 5% sodium carbonate (150 ml) and ice water (150 ml). The toluene layer was dried over $MgSO_4$ and concentrated in vacuo to give crude DNPDEP in a 60% yield. The crude product was kept in the deep freeze and aliquots purified immediately prior to use as described below. Anal. Calcd. for $C_{10}H_{13}N_2O_8P$: C,37.51; H, 4.09; N,7.74. Found: C, 36.88; H,4.06; N, 8.23%. ^{13}C NMR; ($CDCl_3$, TMS) : 16.3; 65.8; 121.6 (CH); 123.6 (CH); 129.1 (CH); 140.7 (C-X); 143.8 (C-X); 148.4 (C-X). IR; 1540 (NO_2); 1350 (P=O); 1032 (P-O-alkyl); 1270 (P-O-aryl) cm^{-1}.

b) Purification of phosphate esters prior to use:

Immediately prior to use 1.0g of ester was dissolved in 20ml of ice cold chloroform, and the solution shaken in a separating funnel with 25ml of ice cold 5% $NaHCO_3$, 25ml of ice cold

water, 25ml of ice cold 0.1M HCl and 2 x 25ml aliquots of ice cold water. The organic layer was kept over Na_2SO_4 for several hours and finally the solvent was removed at 40°C under vacuum. The phosphate residue was then used for kinetic experiments.

CAUTION: The ester exhibits **strong anticholinesterase activity**, great care should be employed it its handling and use. Glassware etc should be decontaminated with solutions of hypochlorite (bleach).

2.3 COPPER LOADED POLYMERS

2.3.1 Preparation of copper loaded polystyrene

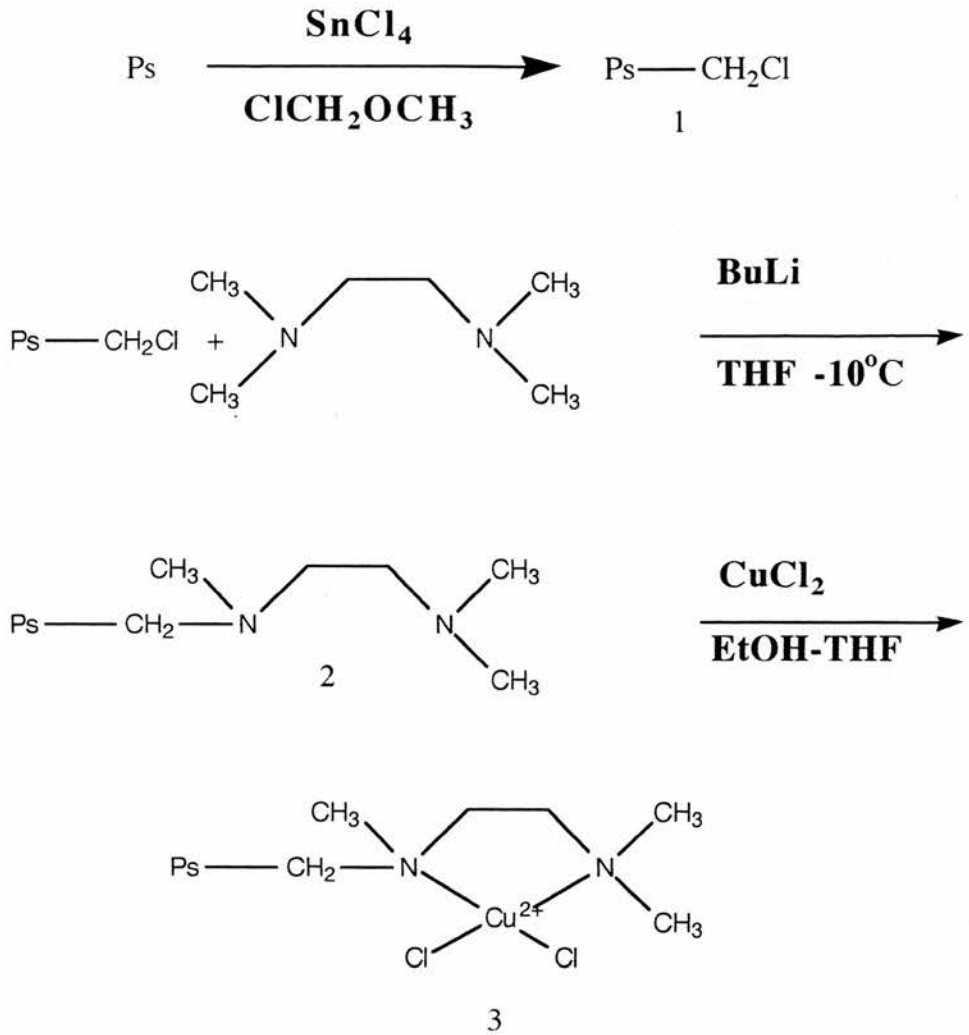
Merrifield's[20] preparation of chloromethylated polystyrene and Menger's[17] procedure for the amination of polystyrene were employed.

Merrifield used the chloromethylation of gelatinous polystyrene beads cross linked with 1% divinyl benzene, as the initial stage in the formation of his solid supports for peptide synthesis. His work, originating in 1959, on the synthesis of peptides achieved by this method led to the award of the 1984 Nobel prize for chemistry.

The general synthetic method is outlined in Scheme 2.6.

a) Chloromethylation of polystyrene.

Polystyrene resin beads (S-X beads supplied by Bio-Rad) (10g) previously copolymerised with 2% divinyl benzene were swelled in dichloromethane by stirring at 25°C for 1 hour in a 3-necked round bottomed flask. The mixture was then cooled to 0°C. A cold solution of $SnCl_4$ (0.8ml) in chloromethyl methyl ether (20ml) was added slowly from a dropping funnel whilst stirring. EXTREME HAZARD: The chloromethyl methyl ether used for the chlormethylation of the resin is an **extremely potent carcinogen** causing cancer in rats at 0.1 p.p.m. There is a high possibility that the impurity bis-(chloromethyl)ether (LTEL=0.001 p.p.m.) may be present, also a carcinogen and cross linking alkylating agent. This ether was used following the most stringent safety guidelines due to its high volatility. All items in contact with the reacting mixture were decontaminated with acetone followed by 5 hours in a 5M ammonia bath. Since the time of my work a supplier of the Merrifield chloromethylated resin has been identified (Sigma).The suspension was stirred at 0°C for a further 30 minutes.The mixture was filtered on a fritted glass funnel and washed with 1000 cm^3 of a solution of 3 parts dioxane and 1 part 3M HCl allowing time for each of the solvents to penetrate the beads fully,the beads were further washed with dioxane, water and methanol in turn, again, allowing time for each of the solvents to soak into the beads. The beads were dried overnight under a high vacuum over $CaCl_2$.



(Ps=polystyrene)

[Scheme 2.6]

b) Amination of polystyrene.

Butyllithium (25.5ml, 1.6M in hexane, 0.408 mol) was added slowly with stirring at -5°C to N,N,N'-trimethylethylene diamine (4.12g in 100ml THF, 0.04 mol) under N_2. Chloromethylated resin (9.8g) that had been swelled in anhydrous THF (200ml) for 1 hour was then added to the amine solution and the reaction mixture was stirred continuously at -15°C for 6 hours. The progress of the reaction was monitored via the disappearance of the C-Cl IR band near 705cm^{-1}.

Upon completion of the reaction the mixture was poured into 500ml of methanol and filtered.

The resin was washed repeatedly with THF / methanol in an ultra sound bath. After 24 hours drying under reduced pressure light yellow beads (ca.4g) were obtained.The modified Volhard[21] method and elemental analysis confirmed the absence of chlorine in the resin.

c) Copper loading

The beads (0.5g) were swelled in THF (25ml) for 1 hour and were subsequently mixed with $CuCl_2$ (0.14g, 1.04mmol, in 4 ml EtOH) with stirring. After 1 hour the resin was isolated by filtration, washed a THF / EtOH mixture in an ultra sound bath and dried in vacuo for 1 day. Beautiful bright green beads were produced, ca.0.6g.

Degree of chloromethylation.

The modified Volhard chloride analysis[21] was performed as follows.

Approximately 200mg of the chloromethylated polystyrene was heated at 100°C in a test-tube for 2 hours . The mixture once cooled was transferred into a small Erlenmeyer flask with 30cm^3 of 50% acetic acid and 5 cm^3 of concentrated HNO_3. With the addition of standard (0.1N) $AgNO_3$ whilst stirring a white precipitate formed, indicating the presence of chloride in the polymer.The above reaction conditions for chloromethylation can be controlled by changing the amount of $SnCl_4$ used, or the time and temperature of the reaction.

Combustion and chloride analysis indicated 9.23% loading of chlorine onto the polystyrene.

Analysis

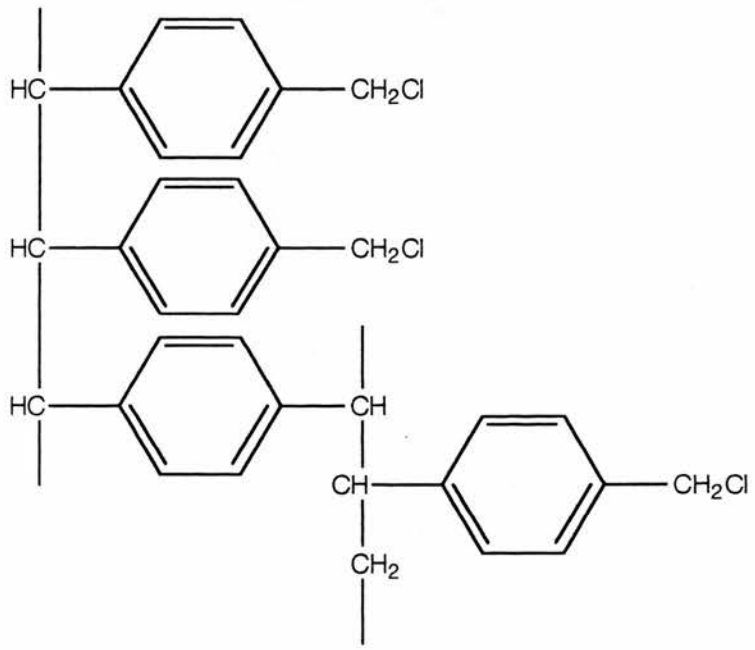
Combustion analysis of the functionalised polystyrene resin gave the following results: C,86.8; H,9.0; N,5.1% (total 100.9%) which is within experimental error for the analysis. Chlorine was absent from the sample. The weight of the polymer containing one mole of the ligand can be calculated by considering that the nitrogen is associated with the trimethyl ethylenediamine moiety. On the basis of the nitrogen analysis, it can be shown that this weight is 555.6g and that this amount of the polymer should bind one mole of copper (65.55g). One gram of the aminated polymer can bind 0.118g copper and so the maximum % of bound copper in the fully copper loaded polymer was expected to be 10.55%. The loading of copper onto the polymer was found to be 9.01%.

Characterisation.

Solid state IR studies of all intermediates and the aminated product showed that both chloromethylation and amination of the polystyrene were completed successfully.

Figure 2.3 shows the IR Spectrum (KBr disc) with peaks at 1491, 1601 (polystyrene), and 694 (s,υ[C-Cl]) cm^{-1} for Ps-CH_2Cl.See [2.8].

Fugure 2.4 shows IR Spectrum (KBr disc) of the aminated polystyrene with absorbances at 1600, 1490, and 1500cm^{-1}.See [2.9].



[2.8]

Figure 2.3 IR spectrum (KBr mull) for chloromethylated polystyrene.

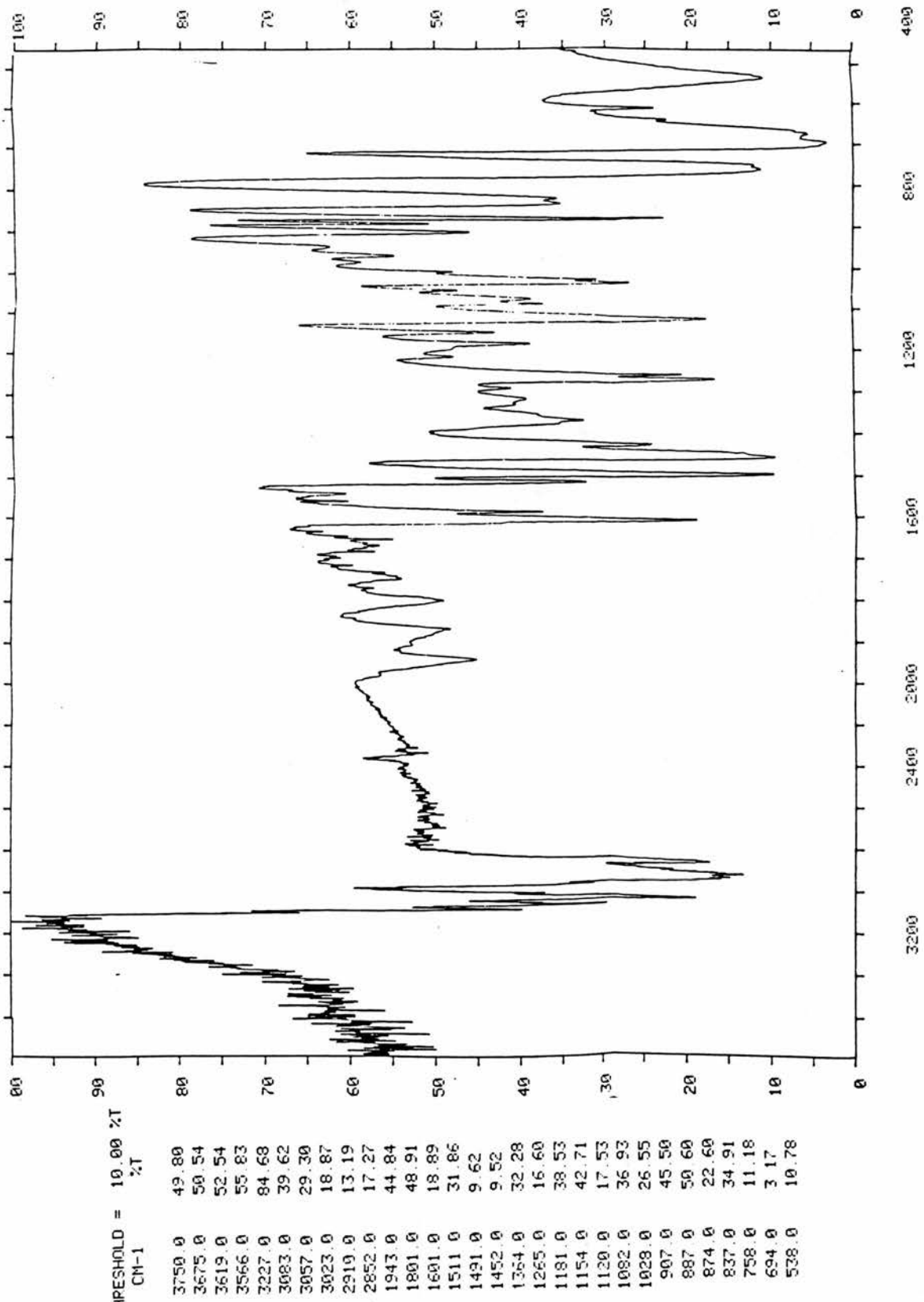
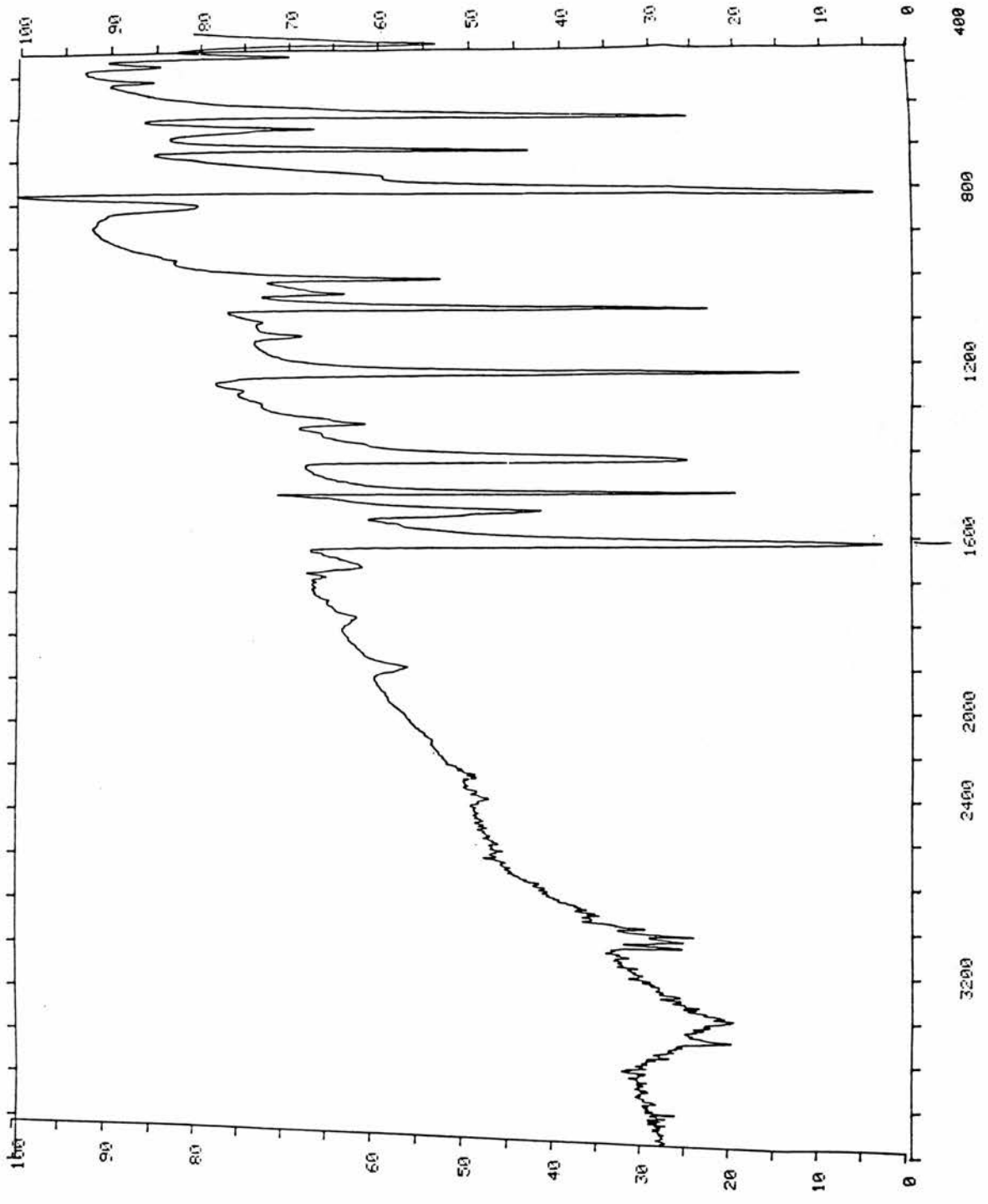
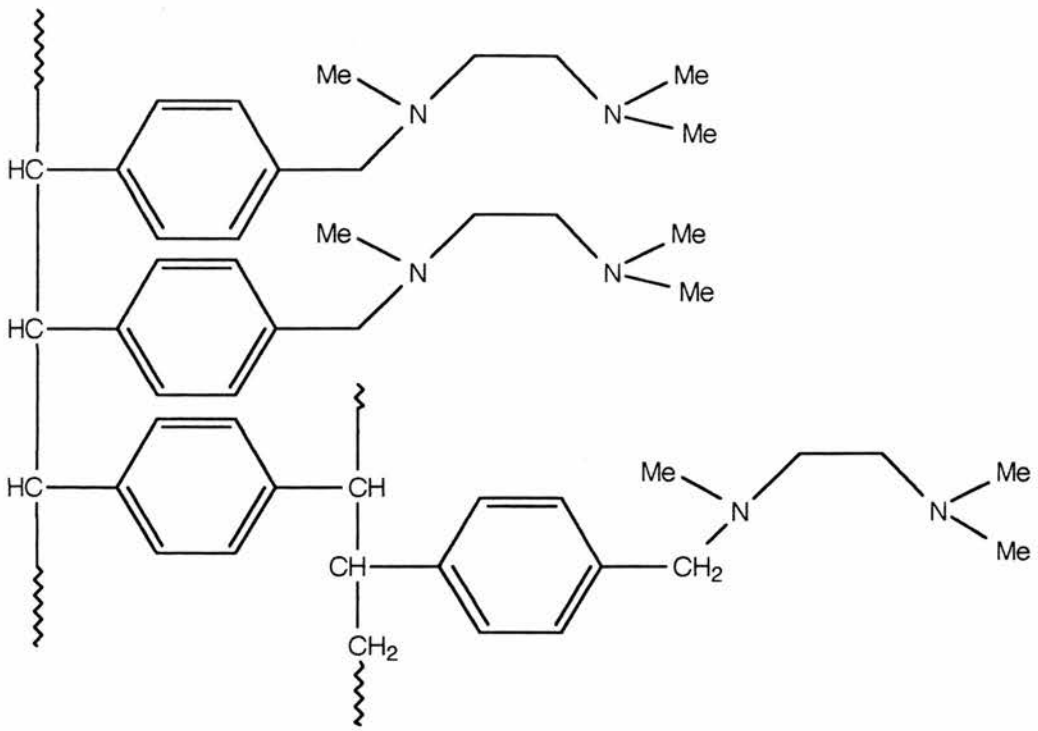


Figure 2.4 IR spectrum (KBr mull) for aminated polystyrene





[2.9]

2.3.2 Preparation of copper loaded silica gel.

The general procedure was adapted from the method developed by Lindoy and co workers[19] for the attatchment of nitrogen macrocycles to silica gel. The experimental method is outlined in Scheme 2.7.

General: All chemicals employed were reagent grade and were used without further purification. The porous silica gel, Nucleosil-100-5 (bead size 5μm, pore size 100A) was purchased from the Macherey-Nagel Company in Germany. The microanalysis was carried out by Ms. Sylvia Smith and the solid state ^{1}H and ^{13}C n.m.r. studies by Dr. F.G.Riddell.

a) Silation reaction.

Silica gel (2g) was suspended in xylene (16ml) (isomeric mixture) containing distilled water (0.4ml). The mixture was stirred gently for 4 hours with a mechanical stirrer. (3-Chloropropyl)trimethoxy silane (2.2ml) was added and then the suspension was stirred for 6 hours at 80°C in an inert atmosphere (N_2). Following removal of the solvents by vacuum

distillation the product (4.91g) was dried under a high vacuum.

b) End capping of unreacted silanol groups.

A suspension of the silated Si gel from above in 4 times its weight of chlorotrimethyl silane was refluxed for 2 hours under nitrogen. The mixture was then allowed to stand overnight at room temperature. The excess reactant was removed by rotary evaporation and then the product (2) was washed with distilled water, to remove traces of HCl and dried under vacuum.

c) Amine attachment.

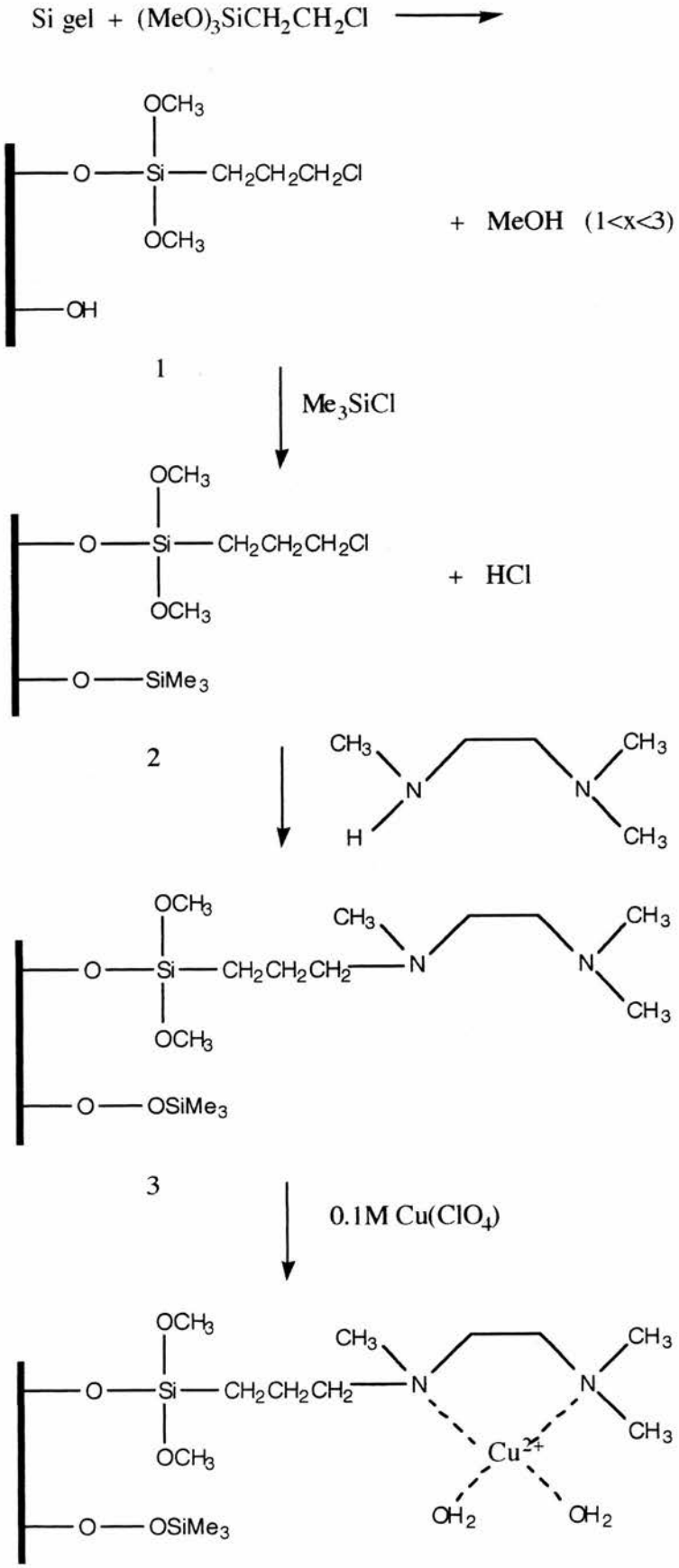
The modified silica gel (2.4184g) was added to a solution of N,N,N'-trimethyl ethylene diamine (0.986g, 9.7mmol) in xylene (8.04ml). The mixture was placed in an ultrasonic bath for 30 minutes. The solution was then refluxed for 15 hours (without stirring) at 150°C. The product was filtered off and repeatedly washed with hot methanol and then dried under a high vacuum.

d) Copper loading.

Following the guidelines set out in the literature the aminated silica gel was mixed with sufficient 0.1M copper perchlorate solution (perchlorate is only weakly coordinating to copper) to saturate all the amine sites and the suspension was placed in an ultrasonic bath for 1 hour to allow for complete wetting and loading (the literature infers that loading time is proportional to the swelling rate and that 10 minutes should be sufficient). The pale blue copper loaded silica gel was removed by filtration, washed with a minimum of distilled water and submitted for combustion analysis.

Analysis

CHN analysis of the functionalised silica gel gave the following results: C,11.76; H,2.26; N,2.6 %. The nitrogen percentage correlates to 0.9285mmol of attached amine per gram of functionalised silica gel. One mole of N, N, N'-trimethyl ethylenediamine is therefore associated with 1077g of the aminated silica gel. Thus, under the best loading conditions 65.55g copper can be incorporated into 1077g of the modified silica gel, the maximum weight percentage of copper which may be incorporated is 65.55/1142.55 x 100 = 5.74%. The actual copper loading was found to be 0.64%.

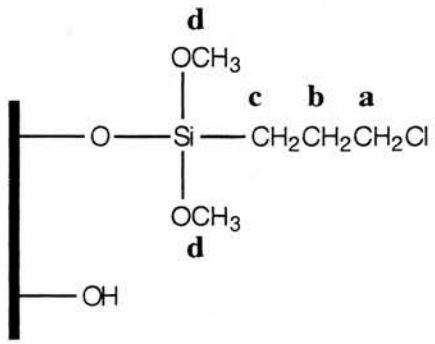

Si gel + (MeO)3SiCH2CH2Cl
OCH3
O
Si
CH2CH2CH2Cl
OCH3
OH
+ MeOH (1<x<3)
1
Me3SiCl
OCH3
O
Si
CH2CH2CH2Cl
OCH3
O
SiMe3
+ HCl
2
CH3
N
H
N
CH3
CH3
CH3
OCH3
O
Si
CH2CH2CH2
N
N
CH3
CH3
OCH3
O
OSiMe3
3
0.1M Cu(ClO4)
Cu2+
OH2
OH2

[Scheme 2.7]

Characterisation.

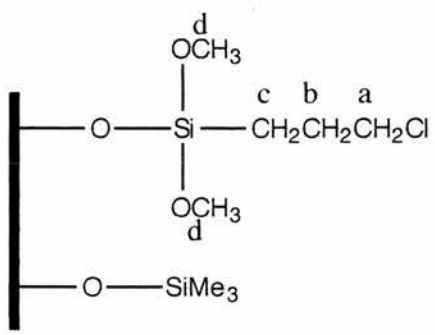
The solid state n.m.r. (^{1}H and ^{13}C) studies proved very useful in the characterisation of the silica gel through its various stages of functionalisation.

Figure 2.5 shows the ^{13}C n.m.r. spectrum of the silica gel intermediate (1) prior to end capping. Signals occurring at δ 26.85 ($SiCH_2\underline{C}H_2$), 47.50 ($\underline{C}H_2Cl$) and 51.00 ($SiO\underline{C}H_3$).



[2.10]

Figure 2.6 shows the solid state ^{13}C n.m.r. spectrum of the final end capped silica gel intermediate [2.11]. (Note the presence of the $SiCH_3$ signal which is absent in Figure 2.5) signals at δ2.18 ($Si\underline{C}H_3$), 10.66 ($Si\underline{C}H_2$), 26.98 ($Si\underline{C}H_2CH_2$), 47.46 ($\underline{C}H_2Cl$) and 50.76 ($SiO\underline{C}H_3$).



[2.11]

Figure 2.5 ^{13}C solid state n.m.r. spectrum for silica gel prior to end capping.

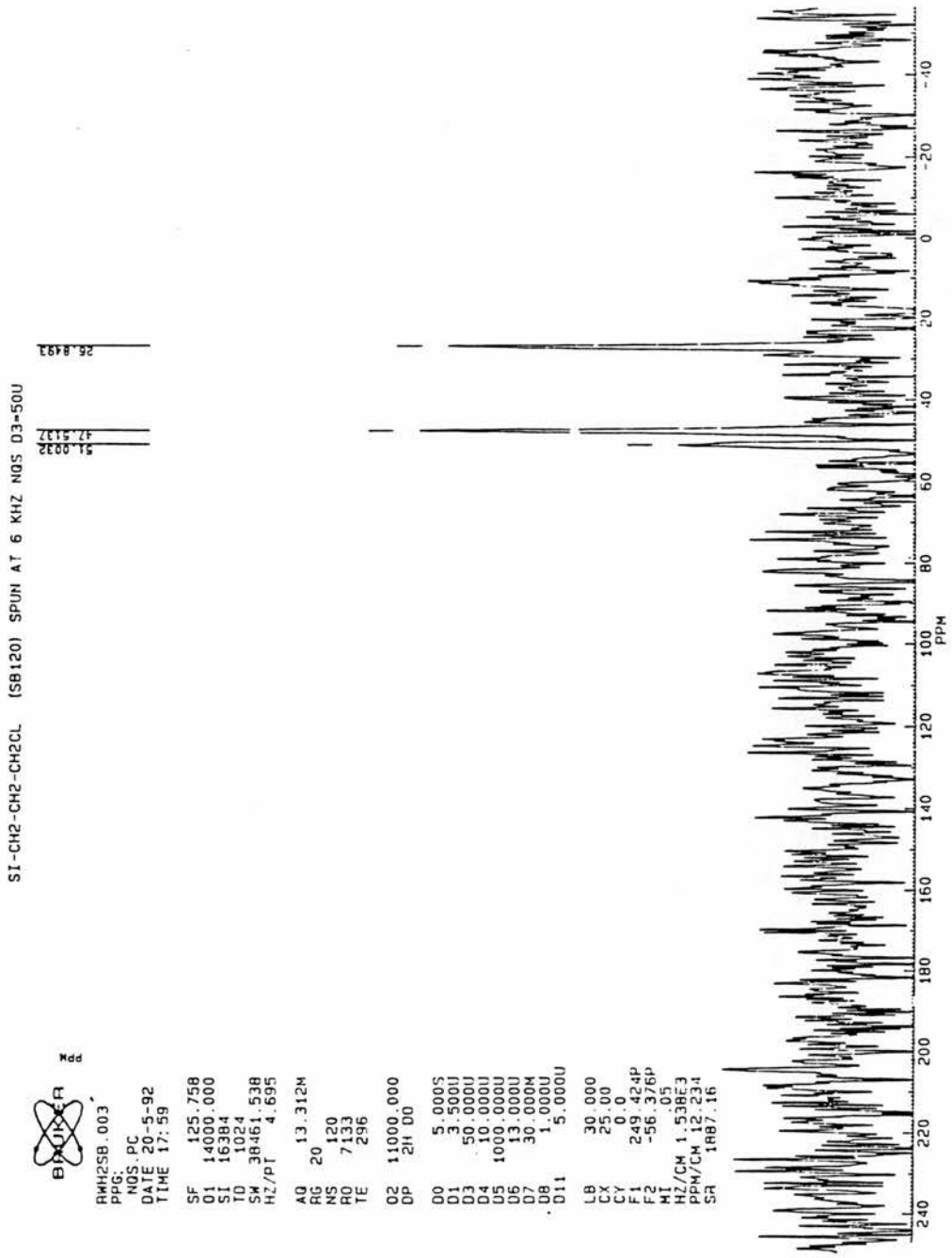
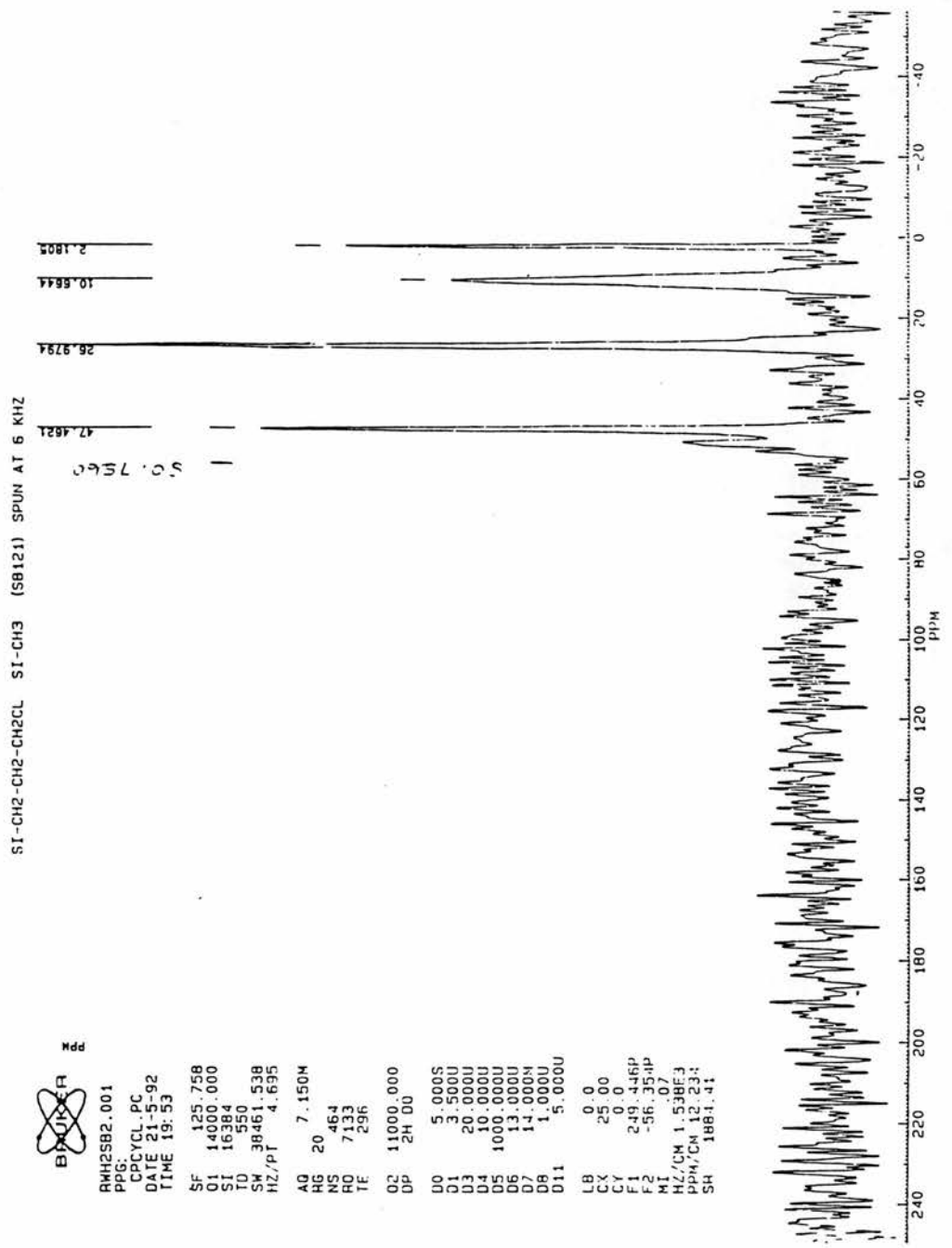


Figure 2.6 ^{13}C solid state n.m.r. spectrum for silica gel following end capping with trimethyl groups.

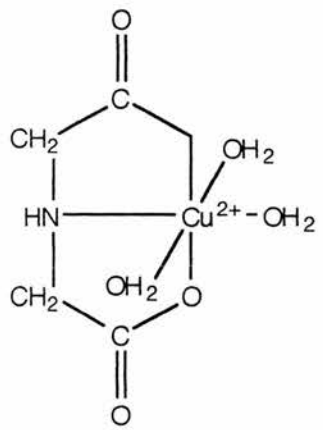


2.3.3 Preparation of CHELEX 100 resin loaded copper.

The copper loaded resin was prepared[22] using first the batch method to swell the beads followed by a column method for the addition of copper. Chelex 100 (5.0g) was added to 0.1N HCl (100ml, 0.1M) and the mixture stirred for 1 hour. The slurry thus formed was poured into a chromatography column allowed to settle and rinsed with several bed volumes of distilled and deionized H_2O to give the hydrogen form of the Chelex resin. Copper (II) sulphate (0.1M) solution was added to the column and the excess copper removed by rinsing with distilled, deionized H_2O until the eluent ran clear. The column was emptied and the contents dried in vacuo over P_2O_5, the bright blue copper loaded Chelex 100 beads (loading=3.23+/-0.02 % from combustion analysis) were stored in an airtight jar until required for kinetic experiments.

2.3.3.1Preparation of [Cu(IMDA)(H_2O)3].

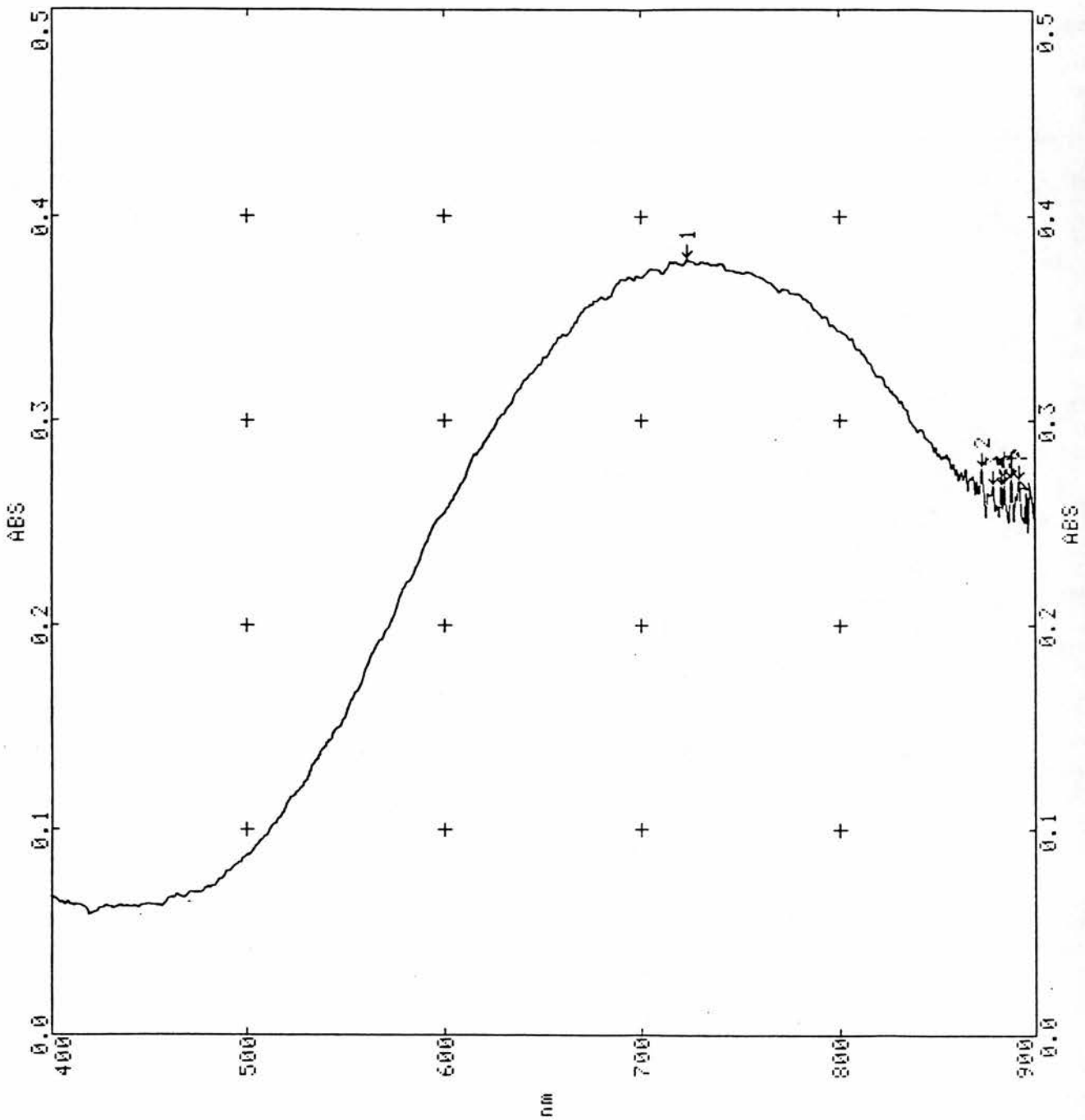
The copper complex of iminodiacetic acid, [Cu(IMDA)(H_2O)3], [2.12], was prepared as described below.



[2.12]

Iminodiacetic acid (94g, 0.03mol) was dissolved in water (ca.30ml) and solid basic copper(II) carbonate (excess) added. The mixture was heated on a water bath for 30 mins and the excess carbonate filtered off. The volume of solvent was reduced under vacuum until crystallisation of the copper complex took place. The complex was filtered off and dried over P_2O_5 in vacuo.

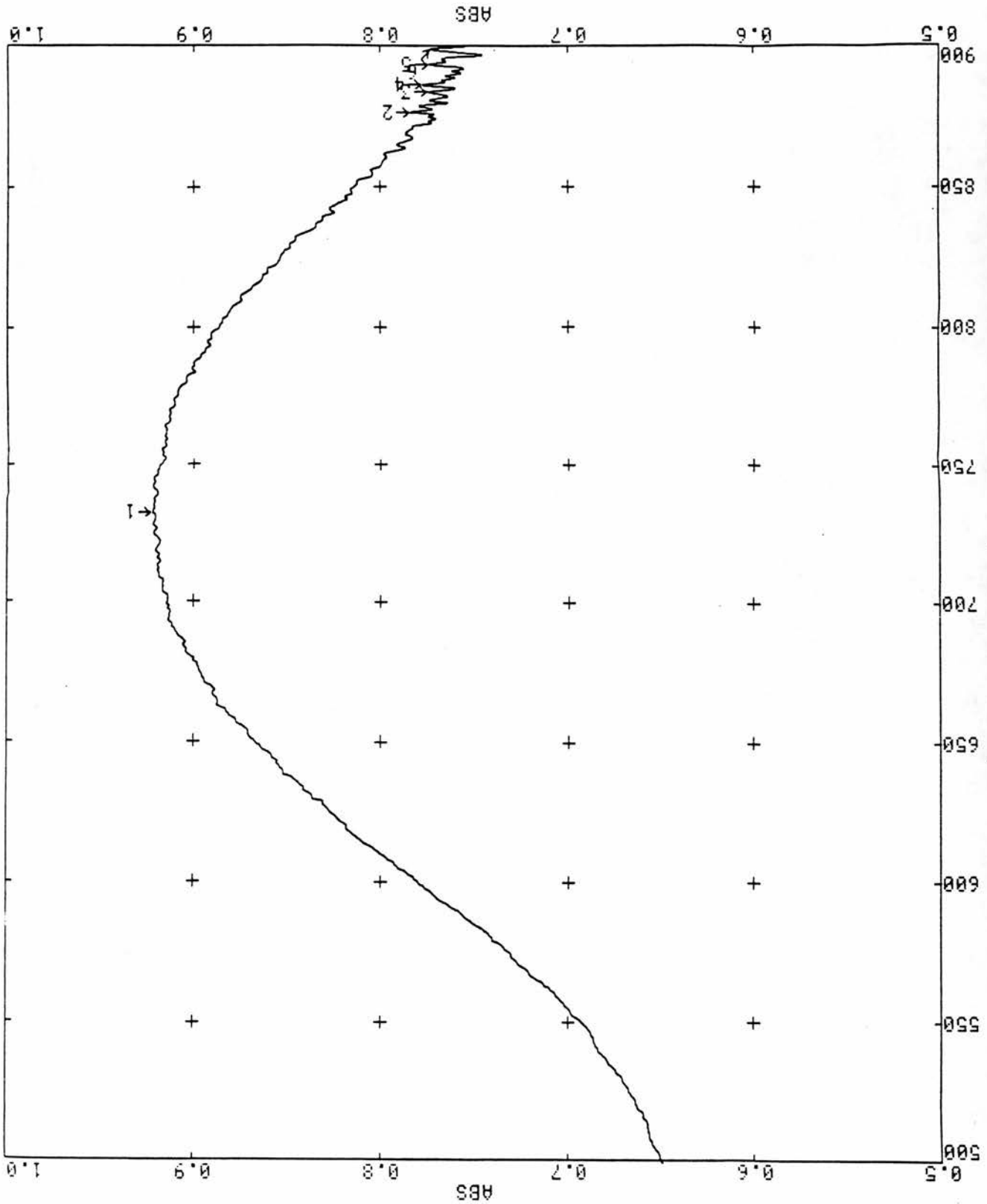
Figure 2.7 Solid state absorption spectrum (nujol mull) of copper (II) loaded Chelex 100 resin.



3 1 λ400.0-900.0 B2.0 SS2000 ABS ~

	1
λ	724.0
ABS	0.379

Figure 2.8 Solid state absorption spectrum (nujol mull) of [Cu(IMDA)(H_2O)3].



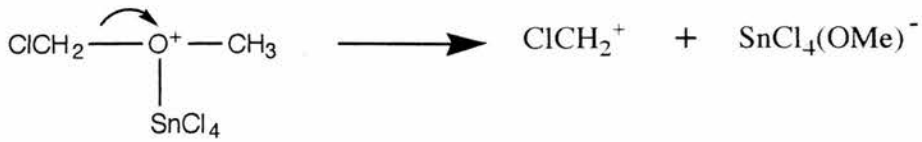
1

λ 732.3

ABS 0.922

2.4 DISCUSSION OF SYNTHESIS

Polystyrene is chloromethylated by reaction of 2% divinyl benzene cross linked polystyrene with chloromethylmethyl ether in the presence of tin (IV) chloride.Tin (IV) chloride presumably generates the electrophile CH_2Cl^+ from chloromethylmethyl ether by the process:



[Scheme 2.9]

Subsequently alkylation of the polystyrene occurs by electrophilic attack of $ClCH_2^+$. The resulting chloromethylated polystyrene, which takes the para form due to steric restrictions, is subject to nucleophilic attack by the trimethyl amine ion $N(CH_3)_2(CH_2)_2N(CH_3)^-$ formed by the reaction of butyllithium with trimethylethylenediamine.

Combustion analysis and the IR spectra (Figures 2.3, 2.4) confirm that polystyrene loaded with trimethylethylene diamine complex of copper can be prepared by the relatively straight forward schemes set out by Merrifield[20] and Menger[19]. The copper loaded polystyrene contains ca. 10.55% by weight of copper.The chloromethylation stage must be replaced by a safer procedure in order to prevent the use of chloromethylmethyl ether in the laboratory. It has since been found that chloromethylated polystyrene can be purchased from Sigma which greatly simplifies the procedure.

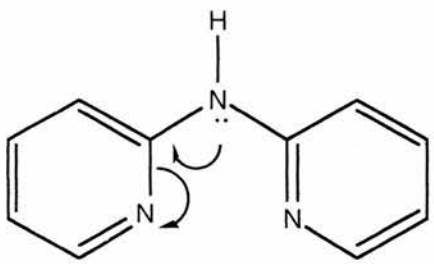
The Lindoy procedure[19] allows the relatively straightforward synthesis of aminated silica gel. Silation of the silanol groups was effected with (3-chloropropyl)trimethoxysilane which reacts with the terminal hydroxyl groups of the silica gel displacing MeOH. The reaction can substitute 1, 2, or 3 of the methoxy groups on the silica back bone.In order to prevent the unreacted silanol groups from complexing directly with metal ions it was necessary to mask these groups. The second stage reaction with chlorotrimethyl silane allows the replacement of the silanol groups with trimethylsilane groups. In addition, this step serves the purpose of increasing the stability of the polymer backbone towards hydrolysis.

Heating the modified silica gel precursor in a solution with the amine gave the aminated silica gel by nucleophilic substitution of the chlorine by the secondary nitrogen. The aminated silica

gel contained 0.9285 mmol of amine per gram of functionalized silica gel which correlates well with the literature data on the attatchment of macrocycles.

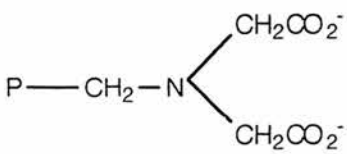
The maximum possible copper uptake for this aminated silica gel to all the amine sites is 5.94%. Lindoy et al found the maximum uptake of a variety of metals including copper was less than that required to fill all the available sites, (ca. 71% of sites by copper at saturation). The actual copper take up onto trimethylethylenediamine modified silica gel was found to be 0.64% (combustion analysis), somewhat less than calculated indicating 10.77% of all amine sites taken up.

Attempts were made using various conditions and solvents to incorporate 2,2'-dipyridyldiamine onto silica gel, however, these were unsuccessful. Analytical data showing the absence of nitrogen in the final product. The lack of success can be attributed to the reduced basicity and nucleophilicity of the secondary nitrogen resulting from delocalisation of the lone pair onto the pyridine rings caused by the strongly electron withdrawing pyridine nitrogen atoms.



[2.13]

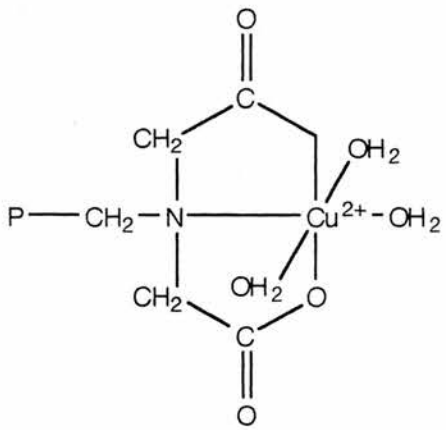
Chelex 100 resin is commercially available from Bio-Rad and is thus an excellent starting point for studies into the properties and potential catalytic activity of copper bound polymers. The resin has a high preference for copper, iron and other heavy metals. Chelex 100 resin is a styrene-divinyl benzene co-polymer containing imminodiacetate functional groups and is structurally classified as a weak acid cation exchanger by virtue of its carboxylic acid groups. The general structure is shown below:



P is the polymer back bone.

Hoek and Reejik[23] studied the structural features of copper (II), cobalt (II) and nickel (II) complexes of Chelex 100 and concluded that the iminodiacetate functional group in the resin

coordinated as a tridentate chelating ligand to these metal ions (also see Figures 2.7 and 2.8). The coordination polyhedra is then completed by either H_2O molecules or oxygen from the neighbouring carboxylates of iminodiacetate groups resulting in MO_5N chromophore. They suggested that large amounts of MO_4N_2 or MO_6 chromophores are highly unlikely on the basis of the crystal field parameters for the cobalt and nickel complexes.



[2.14]

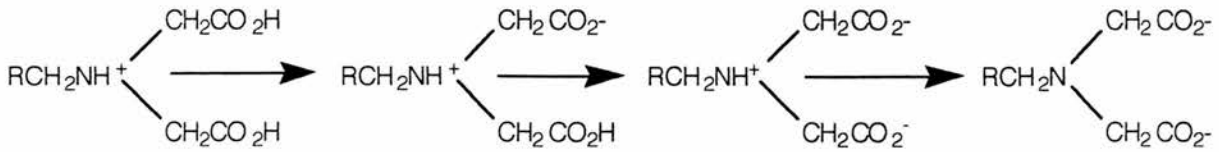
Some d-d spectral data for the copper (II) complexes are shown in Table 2.1

Table 2.1 Visible spectra of copper (II) complexes of Chelex-100 resin.

Complex	Band Maxima (cm^{-1})	Band Maxima (nm)
$CuCl_2$ Chelex 100 (50% - 70% loading)	14,200	704
$CuCl_2$ Chelex 100 (0.5% - 30% loading)	14,500	689
$Cu(ClO_4)_2$ Chelex 100 (50% - 70% loading)	14,500	689

Commercial resins such as Chelex 100 and Dowex A-1 have been used for the selective separation of metals (particularly alkaline earths from alkali metals) as a function of pH.

Various zwitterionic forms are produced as the pH changes and are summarised below:



pH 2.21 pH 3.99 pH 7.41 pH 12.30

[Scheme 2.10]

The copper (II) form of Chelex 100 was prepared and proved useful in developing techniques for the characterisation of copper bound polymers.The loading of the resin is a function of pH, and is very low below pH 2, increasing rapidly from pH 2 to 4, and reaching a maximum at pH > 4.

Chelex 100 undergoes large volume changes swelling ca.100% in going from the hydrogen to monovalent salt form. The resin volume in water with different ions is Na 1.00,H^+ 0.45, Cu^{2+} 0.60, Fe^{2+} 0.45, Zn^{2+} 0.55 and the volumes also change depending on the solvent medium employed e.g. for the sodium form the volume changes from 1.00 in water to 0.47 in acetone, 0.70 in methanol, 0.45 in ethanol, 0.48 in isopropanol and 0.96 in ethyl acetate. The solid state visible spectrum of copper (II) complexes of solid supported ligands is an useful method for characterisation. The solid state spectrum of copper (II) loaded Chelex -100 resin is shown in Fig. 2.7. The λ_{max} at 724nm is consistent with an NO_5 chromophore. The complex [CuIMDA$(H_2O)_3$] Fig. 2.8 has λmax 732 nm corresponding closely to that of Chelex-100 bound copper confirming that the copper is indeed chelating to the iminodiacetic ion in Chelex-100.

Although the commercial availability of Chelex 100 is an advantage, the copper complex is charge neutral and would be expected to display low Lewis acidity, and hence the emphasis for future kinetic work was placed on the silica and polystyrene supported copper complexes for the catalysis of phosphate ester hydrolysis.

2.5 KINETICS

2.5.1. The effect of copper loaded polystyrene on the hydrolysis of DNPDEP.

Preliminary studies on the kinetics of the base and solid supported metal complex catalysed hydrolysis of DNPDEP were carried out spectrophotometrically. A Perkin-Elmer Lambda 5 spectrophotometer was used to monitor the release of the 2,4-dinitrophenoxide ion at 400nm. Figures 2.9 and 2.10 show typical interval scan spectra depicting the hydrolysis of DNPDEP in basic solution. The extinction coefficient of the product 2,4-dinitrophenoxide at 360nm is $1.13 \times 10^{4} M^{-1} cm^{-1}$.

KINETICS

The following kinetic run is typical of that used throughout the work:

A thermostated beaker (50ml) containing a solution of the TAPS (tris(hydroxymethyl) methylaminopropane sulphonic acid) (25ml, 0.1M, pH 8.5, 0.1M$NaClO_4$) and copper loaded polystyrene (15mg, corresponding to mM Cu^{2+} if the polymer had dissolved) was sealed and stirred for 1 hour maintained at the required temperature by a circulating water thermostat. The reaction was initiated by the addition of DNPDEP (dissolved in spectroscopic grade acetonitrile) (8µl, 0.171M, giving a final concentration of $5.472 \times 10^{-5} M$). The stirring was continued until thorough mixing of the contents had occured the stirring was then stopped and the polymer allowed to settle (40-50 secs). A sample (3ml) was removed and transferred to the spectrophotometer cell where the absorbance at 360nm was recorded. The sample was then returned to the beaker, which was resealed and stirring resumed until the next sample was required. Plots of $\ln(A_{\omega}-A_t)$ versus time were linear over the periods of time studied.

Figure 2.9 Interval scan absorption spectrum for DNPDEP in 0.1M NaOH at 25°C. Time interval 20s.

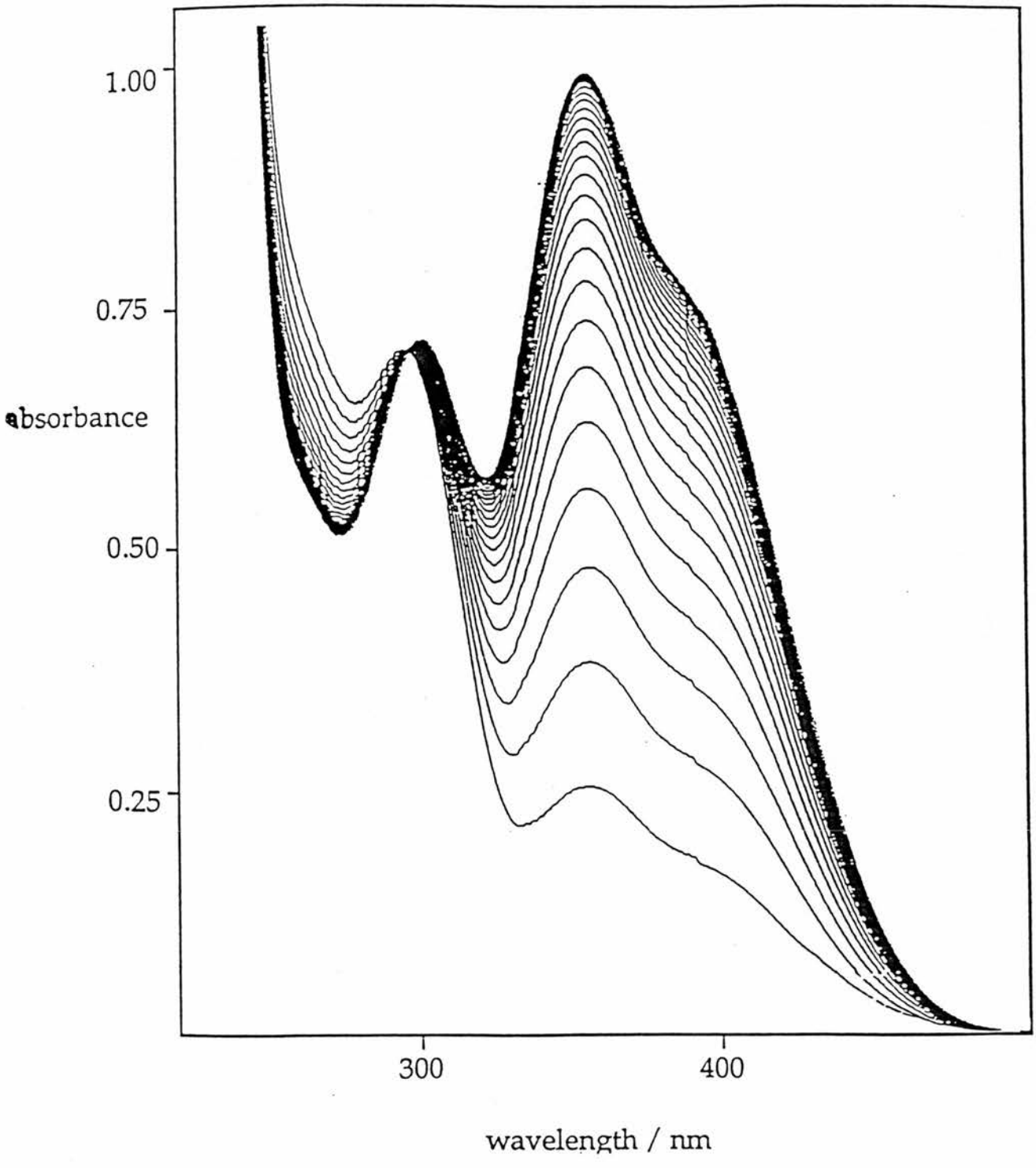
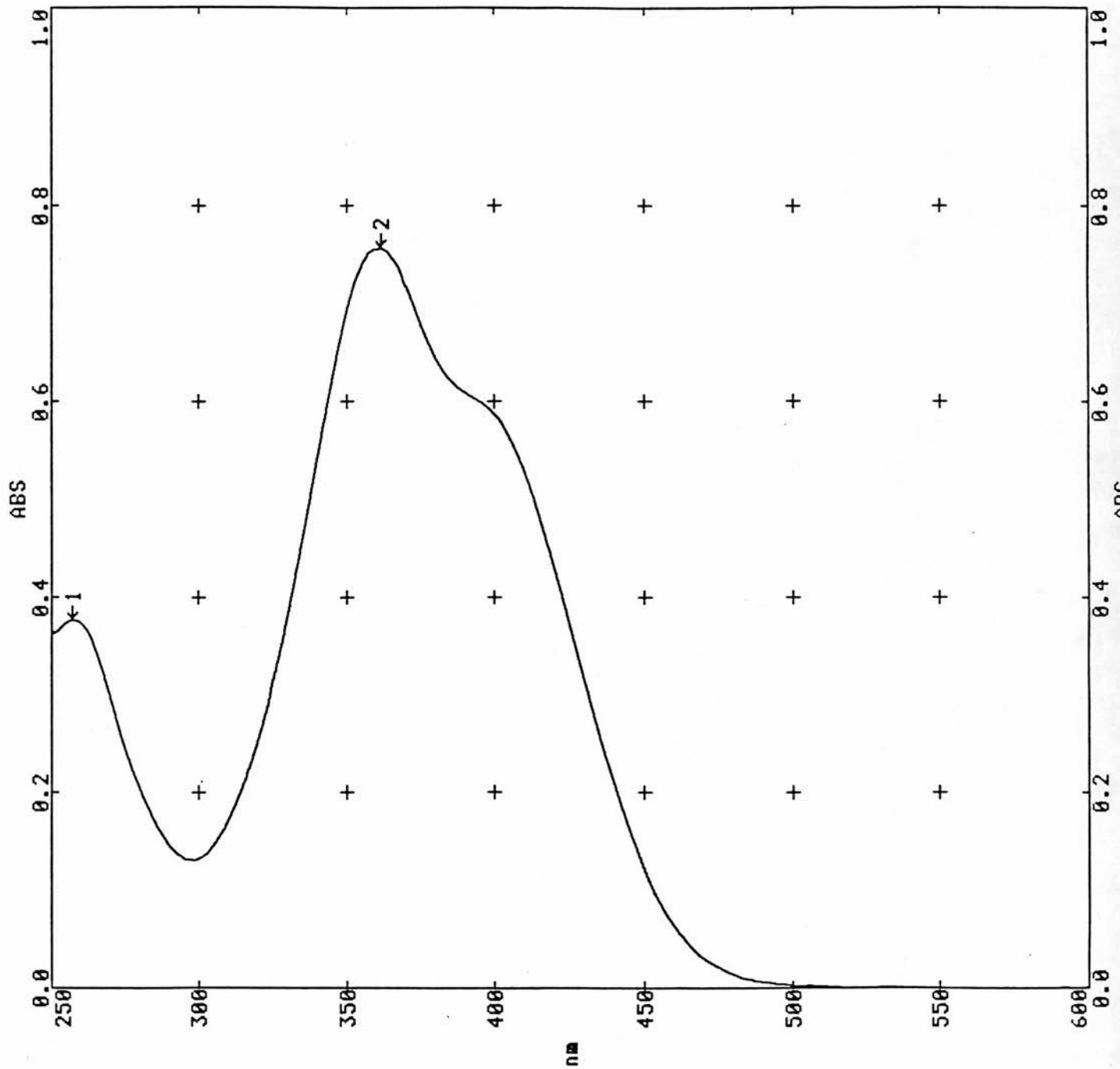


Figure 2.10 Absorption spectrum for 2,4-dinitrophenol , 5.28×10^{-5} mol dm^{-3} in basic solution.



	1	2
λ	257.1	361.4
ABS	0.376	0.755

2.6 RESULTS

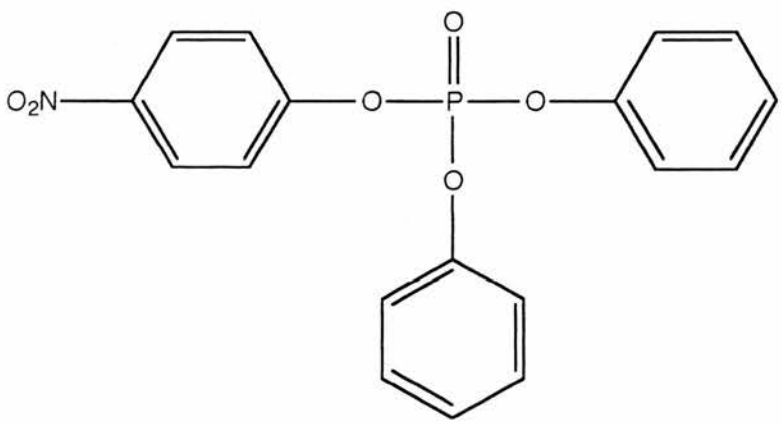
2.6.1Simulant selection.

Previous investigations[12, 16] have used the readily prepared simulant 4-nitrophenyl diphenylphosphate (PNPDPP) [2.14] which was found to be unsuitable for a variety of reasons including

a) Insolubility in aqueous solutions necessitating the use of mixed solvents, complicating the analysis of kinetics data.

b) Appreciable hydrolysis of phenoxide concurrently with the hydrolysis of 4-nitrophenoxide.

c) 4-nitrophenol has a pK of 7.1 so that in acidic solutions (pH less than 5) the readily observed anion is not formed.



PNPDPP

[2.14]

DNPDEP was chosen as it overcomes many of these difficulties and a large body of data is available from previous work with which to compare results. The ethyl substituents enhance the water solubility considerably and are much poorer leaving groups than 2,4-dinitrophenol. The lower pK_a of 2,4-dinitrophenoxide enables reactions to be monitored at pHs above 4.1. Also the pK_a of 2,4-dinitrophenol (ca.4.1) is similar to that of HF (ca.3.5) and as the rates of hydrolysis of phosphate esters are dependent on the pK_a of the leaving group the dinitrophenoxide can be considered a good analogue for the more hazardous fluoro phosphate esters.

2.6.2 The hydrolysis of DNPDEP in the absence and presence of copper loaded polystyrene.

The rate expression for the hydrolysis of DNPDEP with all species present remaining constant is:

$$\text{Rate} = k_{obs}[\text{Ester}]$$

For base hydrolysis:

$$k_{obs} = k_{OH}[OH^-] + k_o$$

For catalysed hydrolysis:

$$k_{obs} = k_{cat}[\text{cat}] + k_{OH}[OH^-] + k_o$$

Where k_{obs} is the experimentally determined first order rate constant.

k_o is spontaneous first order reaction rate with water.

k_{OH} is the second order rate constant for the reaction with hydroxide ion.

k_{cat} is the overall constant governing the effect of the catalyst (normally second order).

Since the comparison for these purposes is between the observed hydrolysis with and without catalyst, all other species remaining constant the rate constants can be redefined:

$$\text{Rate} = k_{obs}[\text{ester}]$$

Hence the observed rates can be compared simply by using the initial rate method:

$$\text{Rate} = \frac{\Delta\text{concentration}}{\text{Time}}$$

$$k_{obs} = \frac{\text{Rate}}{\text{average concentration of ester}}$$

$$t_{1/2} = \frac{\ln 2}{k_{obs}}$$

Table 2.2 Data for the hydrolysis of DNPDEP in the absence of copper loaded polystyrene polymer at 56°C, pH8.5, I=0.1M($NaClO_4$).

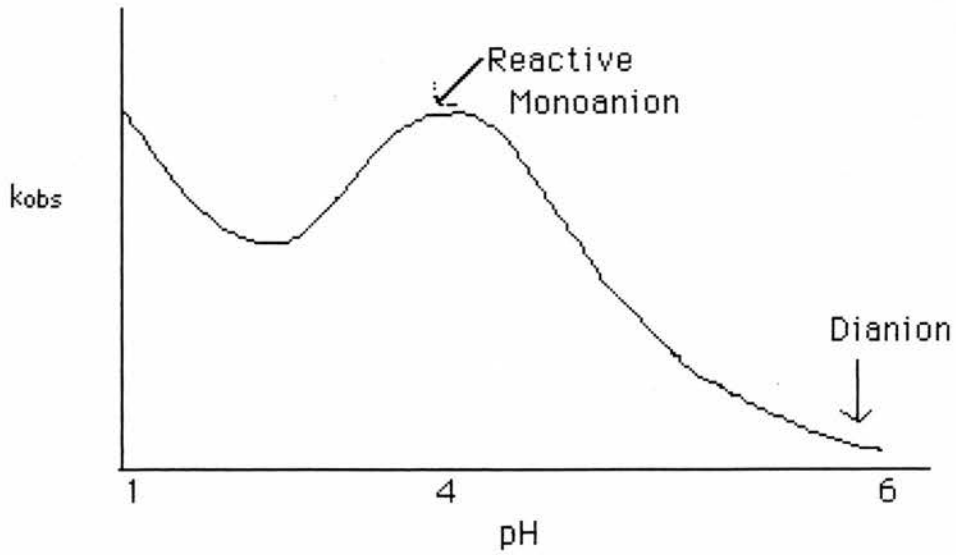
Rate	5.4×10^{-9} mmol s^{-1}
k_{obs}	$1.68 \times 10^{-6} s^{-1}$
$t_{1/2}$	6876.5min

Table 2.3 Data for the hydrolysis of DNPDEP in the presence of 15mg copper loaded polystyrene at 56°C, pH8.5, I=0.1M($NaClO_4$).

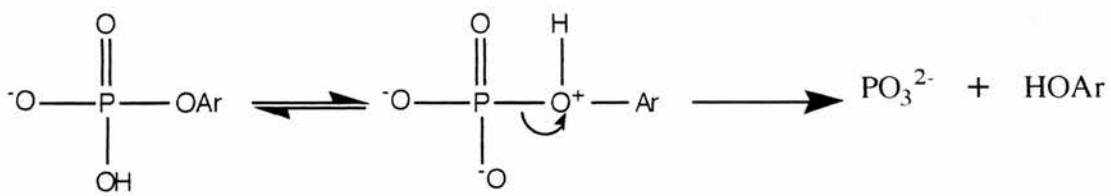
Rate	1.31×10^{-8} mmol s^{-1}
kobs	$4.43 \times 10^{-6} s^{-1}$
$t_{1/2}$	2607min

2.6.3 Preliminary studies of the copper II based Chelex 100 promoted hydrolysis of4-nitro-phenylphosphate.

Some preliminary studies were carried out on the effects of copper (II) loaded Chelex 100 on the hydrolysis of 4-nitrophenylphosphate. Phosphate monoesters exhibit a 'roller coaster' pH-rate profile of the type shown diagramatically below:



The monoanion is the reactive species in the hydrolysis, while the increase in rate at ca. pH 1 is due to general acid catalysis. The high reactivity of the monoanion can be rationalised in terms of the reactions:



The dianion produced at pH 6 is very unreactive to water and base hydrolysis. Attack of hydroxide ion on a dianionic species will not be favoured electrostatically and hence base hydrolysis is not favoured.

2.7 DISCUSSION

Due to time limitations it was only possible to carry out a limited number of experiments on the effects of copper loaded polystyrene on the hydrolysis of DNPDEP. A small rate enhancement (ca. 2.5 fold) was observed, Table 2.3, at pH 8.5. Futher experiments involving variation of the pH and the polymer concentration are required before any mechanistic conclusions regarding the reaction can be defined.

2.8 REFERENCES

1. P. Hendry and A.M. Sargeson, Progress In Inorganic Chemistry,
2. J.E. Coleman and P. Gettins, Adv. Enzymol., 55, 381 (1983).
3. V. Scheller-Krattiger and H. Sigel, Inorg. Chem., 2, 698 (1986).
4. S.H. Gellman, R. Petter and R. Breslow, J. Am. Chem. Soc.,108, 2388, (1986).
5. J.R. Morrow and W.C. Trogler, Inorg. Chem., 27, 3387 (1988).
6. T.H. Fife and M.P. Pujari, J. Am. Chem. Soc., 110, 7790 (1988).
7. J.R. Morrow and W.C. Trogler, Inorg. Chem., 27, 3387 (1988).
8. N. Govan, Ph.D. Thesis, University of St. Andrews (1991).
9. R.L. Gustafson and A.E. Martell, J. Am. Chem. Soc., 84, 2309 (1962).
10. T. Wagner-Jauregg, B.E. Hackley, T.A. Lies, O.O. Owens and R. Roper, J. Am. Chem. Soc., 77, 922 (1955).
11. J. Epstein and D.H. Rosenblatt, J. Am. Chem. Soc., 80, 3596 (1958).
12. F.M. Menger, L.H. Gan, E. Johnson and D.H. Hurst, J. Am. Chem. Soc., 109, 2800 (1987).
13. J.C. Erikson and G. Gillberg, J. Acta. Chem. Scand., 20, 2019 (1966).
14. J.W. Allison and R.J. Angelici, J. Inorg. Chem. Soc., 10, 2233 (1971).
15. J.T. Groves and R.M. Dias, J. Am. Chem. Soc., 101, 1033 (1979).
16. P. Mukergee and A. Ray, J. Phys. Chem., 70, 2144 (1966).
17. F.M. Menger and T. Tsuno, J. Am. Chem. Soc., 111, 4903 (1989).
20. From ref. 17. "frequently voiced but unpublished doctrine in circles concerned with nerve agent decontamination."
21. V. Dudler, L.F. Lindoy, D. Salin and C.W. Schlaepfer, Aust. J. chem., 40, 1557, (1987).
22. J.M. Stewart and J.D. Young, Solid Phase Peptide Synthesis, W.H. Freeman & Co., San Fransisco (1969).
23. P.B. Hawk, B.L. Oser and W.H. Sommerson, Practical Physiological Chemistry, Blakiston, 13th Edition, 955 (1954).
24. Bio-Rad Bulletin 2020 Chelex 100™.
25. F.S.K. Sanhi and J. Reedijk, Coord. Chem. Revs., vol.59, 1 (1984).

CHAPTER THREE

3.1 INTRODUCTION

The hydrolysis of Carboxylate Esters.

Investigations into the base, water and metal ion promoted hydrolysis of carboxylic esters have in the past yielded interesting results. Certain metalloenzymes are known to catalyse the hydrolysis of amino acid and carboxylic esters. In order to elucidate the mechanism of these catalysts it is of interest to study the effects of metal ions action on ester hydrolysis.

Zinc is an essential element to all forms of life[1,2]. In 1939, following its detection in 1928, Carbonic Anhydrase was shown to contain a zinc (II) coordinated to three histidine residues at its active site.

Carbonic Anhydrase catalyses a range of reactions including the hydration of carbon dioxide and aldehydes,

$$CO_2(aq) + H_2O \rightleftharpoons H_2CO_3$$

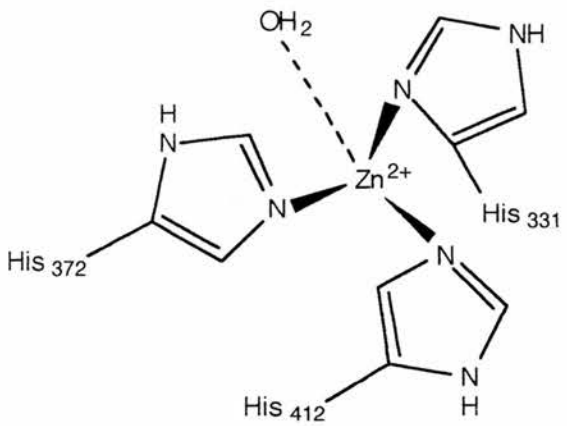
and the the hydrolysis of carboxylic, sulphonic and carbonic esters. CO_2 hydration is the biologically important reaction necessary for respiration and intracellular CO_2-HCO_3^- equilibration.

In the absence of the metalloenzyme the reaction mechanism is believed to involve the attack of oxygen (OH_2 or OH^-) on the electrophilic carbon. The rate in the presence of CA at physiological pH for the hydration of CO_2 is reported to be 10^9 fold that of the reaction in its absence. The enzyme has a turnover number of 10^6, that is, one mole of the enzyme can hydrate 10^6 moles of CO_2 per second at 37°C. Turn over is defined as equivalents of substrate converted per second.

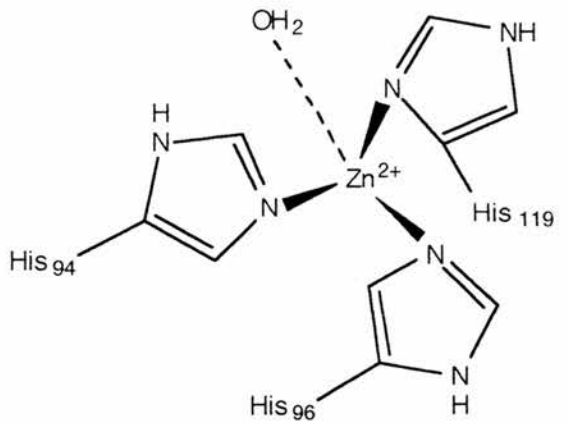
Carboxypeptidase A[2] catalyses the hydrolysis of the terminal peptide bond at the carboxyl end of proteins and peptide chains. It is also effective towards terminal ester linkages. The enzyme is found in the pancreas of most mammals, including humans, and its structure has been deduced by X-ray crystallography,. The zinc ion is coordinated by two histidine nitrogen atoms and an oxygen from the carboxyl side chain of a glutamate residue.

Some 80 zinc enzymes have now been characterised. In these enzymes the zinc ion is considered to serve as a primary catalytic centre for bringing substrate and nucleophile together through formation of a coordination complex and to activate the substrate carbonyl group

toward the action of the nucleophile in carboxypeptidase A, or to activate the water molecule in the reversible hydration of CO_2 in carbonic anhydrase. In the related alkaline phosphatases[3] the zinc ion functions analogously by activating the serine hydroxyl group and by electrophilic activation of the P=O bond.

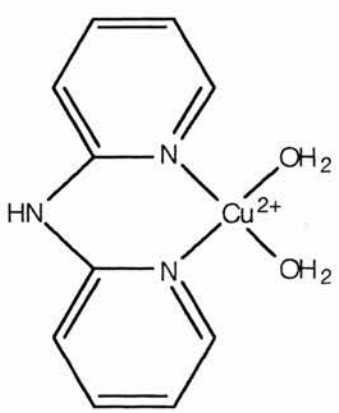


[3.1] Carboxypeptidase active centre



[3.2] Carbonic Anhydrase active centre

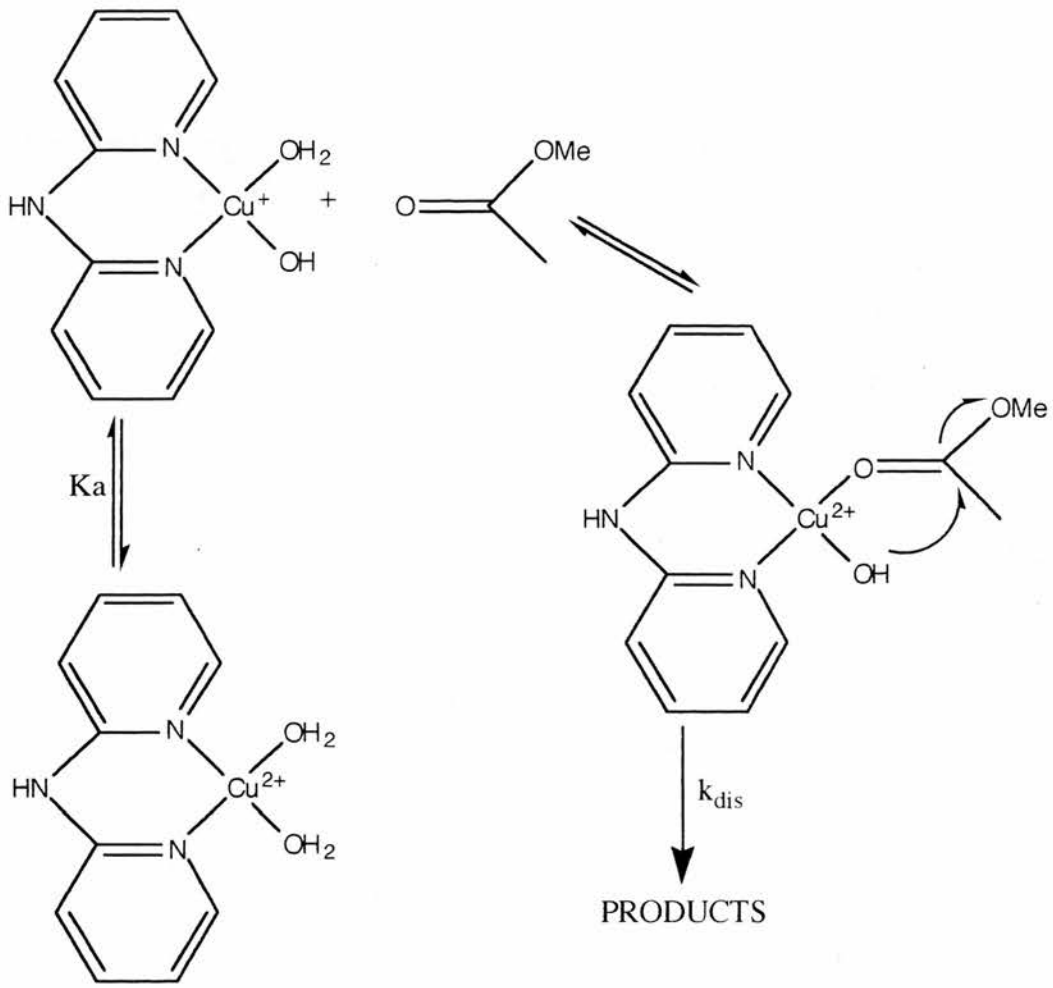
Only a few zinc (II) complexes have been reported as models of these hydrolytic metalloenzymes. Until recently the most successful examples of model complexes have involved cobalt (III) or copper (II)[4,5] complexes. Copper has been chosen in a variety of studies as it is normally the most effective Lewis acid catalyst of the M^{2+} cations in the first transition series. Copper (II) ions catalyse the hydrolysis of 4-nitrophenylglycinate and significant rate enhancements are observed[6]. Recently Chin et al[5] showed that the copper (II) dipyridylamine complex [3.3] is a highly efficient catalyst for the hydrolysis of methyl acetate.



[3.3]

The pKa of the copper coordinated water is 7.2 at 25°C, and the pH rate profile indicates that

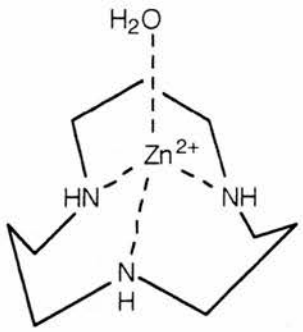
catalysis involves the coordination of the ester to copper, followed by intramolecular metal hydroxide attack on the coordinated ester. Formation or breakdown of the tetrahedral intermediate is believed to be the rate determining step.



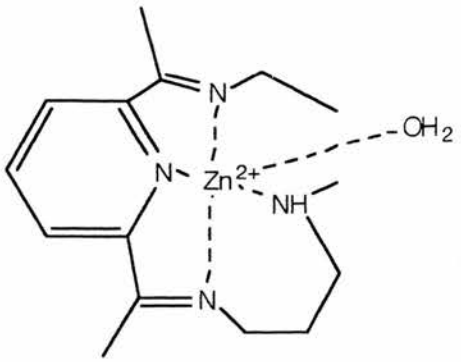
[Scheme 3.1]

Zinc complexes have recently been synthesised as catalysts and mimics of the function of CA[7]. The zinc complex of 1,5,9-triazacyclododecane ([12]aneN3) (pKa 7.3 at 25°C, [3.3]) has shown promising results in the hydration of acetaldehyde. This is the first zinc complex with a pKa similar to that of the water bound to the zinc in CA where the pKa is ca. 7.5 (at 25°C). The maximum rate constant k_{cat} of $200M^{-1}s^{-1}$ is 1/7 the value of bovine CA ($1.4x10^3M^{-1}s^{-1}$). The pH rate profile reveals an inflection at about pH 8 and 0°C (the pKa of the complex is 7.9 at 0°C) suggesting that the Zn-OH species plays a critical role in the catalytic activity acting as a nucleophile towards CO_2. The Zn-OH distance is extremely short

1.94Å, indicating an extremely strong affinity for OH^-.

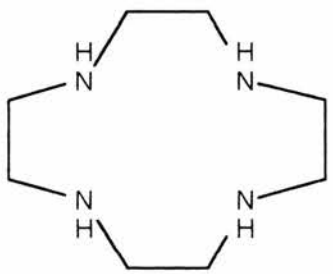


[3.3]

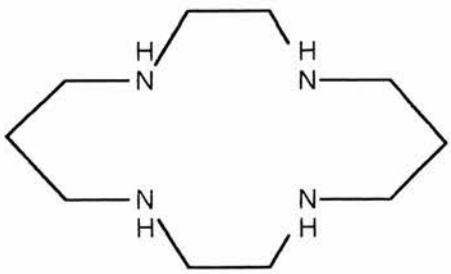


[3.4]

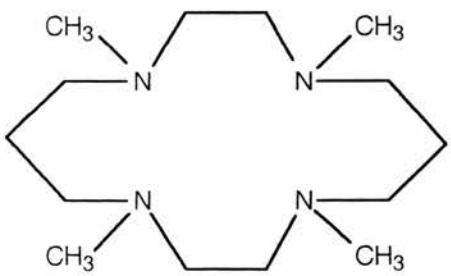
Currently this is the best model for CA. A further model[1,8] [3.4] has a pKa of 8.7, and its activity is attributed to the low pKa for the deprotonation of the water molecule bound at the 5th coordination site. The low pKa is thought to be caused by strong binding to the ligated water as a result of the ligand being sterically forced to adopt a cis-position on zinc thus leaving an open site for water binding. In the [12]aneN4 [3.5] and [14]aneN4 [3.6] zinc complexes, the [14]N4 ligand coordinates around the trans position of the zinc and leaves the cis sites for weaker binding to water, thus increasing the pKa of the complex.



pKa = 8.02+/-0.03[a]
[3.5]



pKa = 9.77+/-0.05[a]
[3.6]

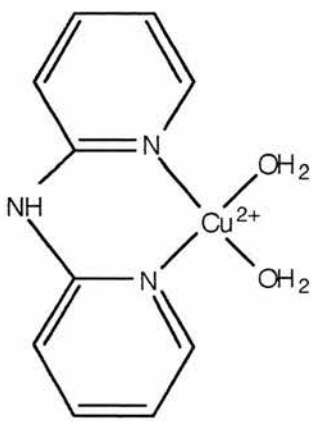


pKa = 8.36[a]
[3.7]

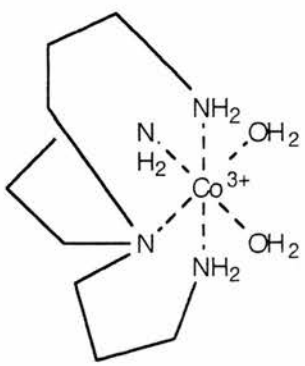
a 25°C.

Substituting the hydrogens by methyl groups in [14]aneN4 [3.7] causes the pKa of the zinc complex to be reduced. Steric hinderance by the methyl groups prevents the ligand adopting an equatorial position around zinc. As a result, a strong binding site for water is available in the cis-position and the pKa for the complex is 8.36 at 25°C.

The zinc complex of 1,5,9-triazacyclododecane is very effective in the hydration of acetaldehyde at 0°C, and the zinc (II) complex also catalyses the hydrolysis of unactivated esters such as methyl acetate under mild conditions (pH 7 and 25°C). Comparing the effect of copper (II) [3.8] and cobalt (II) [3.9] complexes[7] with the 1,5,9-triazacyclododecane zinc (II) complex on the hydrolysis of methyl acetate it has been established that the catalytic turnover with the zinc (II) complex is 60 min ([CH_3COOCH_3] = 1M, pH8, 25°C) compared to 23min with Cu (II) complex ([CH_3COOCH_3] =1M, pH7, 25°C) and 34min with the Co (II) complex ([CH_3COOCH_3] = 1M, pH7.6, 25°C).

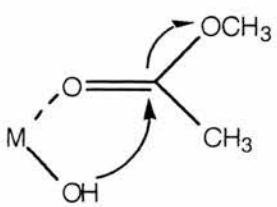


[3.8]



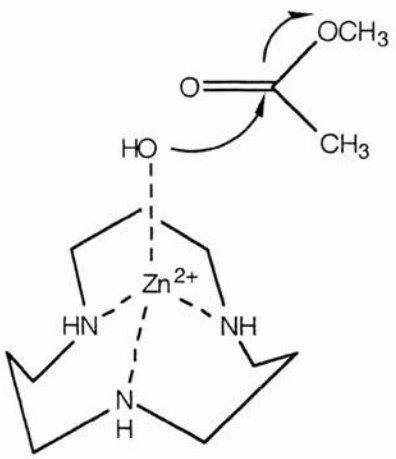
[3.9]

It is proposed that the hydrolysis involving the copper and cobalt complexes occurs by coordination of the ester to a labile vacant site on the copper, followed by intramolecular attack of the hydroxide (M-OH), on the carbonyl carbon of the ester:

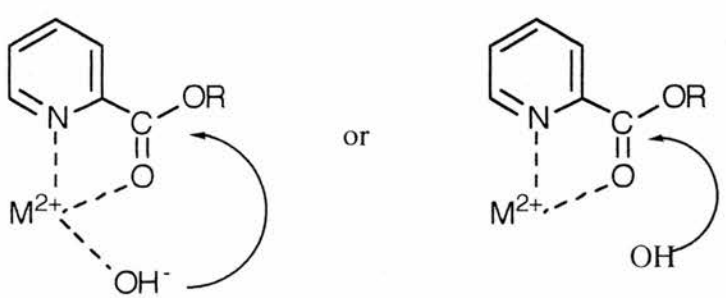


If the zinc complex is compared with a Co (III) complex in which no site is available for prior ester coordination, e.g. ($(NH_3)_5Co$-OH) it was found that the zinc bound OH is one order of

magnitude more active as an intermolecular nucleophile than cobalt (III) bound OH.

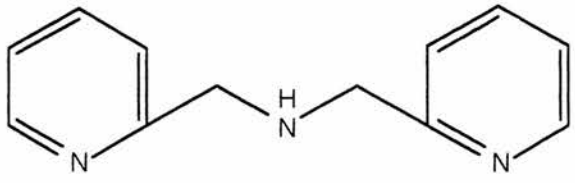


The hydrolysis of various picolinate esters in the presence of Cu (II) and Ni (II) was investigated by Fife and Przytas[8] over a large range of pH.The work was performed in an attempt to elucidate the mechanism of the zinc (II) metalloenzyme carboxypeptidase A in the hydrolysis of peptides and O-acyl derivatives of α-carboxylic acids. They found that the divalent ions of copper and nickel in solution had pronounced effects on the rates of both OH^- and H_2O catalysed hydrolysis reactions. Rate enhancements of the orders of 10^2-10^5 fold were observed in hydroxide ion catalysed reactions in the presence of 0.01M Ni^{2+} (a non saturating concentration), and of 10^4-10^5 fold at concentrations of 0.001M Cu^{2+} (5.8×10^4 fold rate enhancement in the case of 4-nitrophenyl picolinate). In the pH independent H_2O catalysed reactions the rate increases were 10 to 200 fold. It was found that the leaving group had no great effect on the rate of hydrolysis, suggesting that the rate determining step in the copper promoted hydrolysis was likely to be the nucleophilic attack of the hydroxyl ion rather than the break down of the tetrahedral intermediate into products. Hydrolysis took place via metal coordinated hydroxide ion attack at the carbonyl group of the ester or attack by external OH^- ions on the metal complex.



Scheme [3.2]

The chelation effect in binding the metal ion onto the pyridinium nitrogen was shown to be important in the enhancement of rates. For the hydrolysis of 4-nitrophenyl isonicotinate where chelation is not possible there is no significant catalytic effect with divalent metal ions. The work of Fife et al with copper (II) was carried out in the pH range of 3-7. Low pH's were used in order to prevent the precipitation of hydroxo complexes which would occur at a higher pH. The hydrolysis of amino acid esters[9] has also been shown to be a metal ion catalysed[10] process. Initial investigations into the effect of copper II complexes on the hydrolysis of methyl glycinate were carried out by Angelici et al[11] using the copper II complex of bis (2-pyridylmethyl) amine [3.10] $[Cu(BPA)(H_2O)_2]^{2+}$.



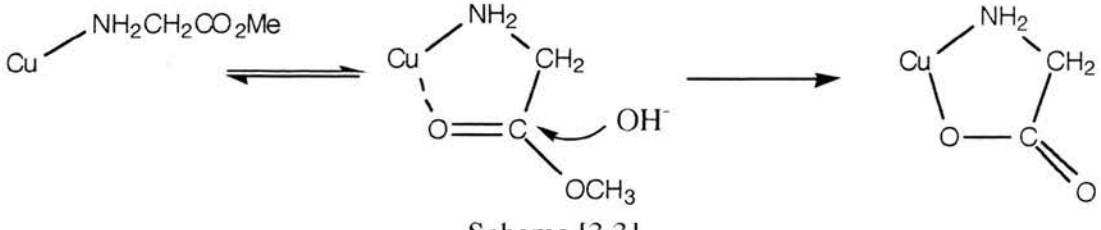
[3.10]

The hydrolysis in the presence of $Cu(BPA)^{2+}$ proceeds in the following steps.

$$Cu(L)^{2+} + MeGly \underset{}{\overset{K_x}{\rightleftharpoons}} Cu(L)(MeGly)^{2+} \xrightarrow[OH^-]{k_{OH}} Cu(L)(Gly)^{+}$$

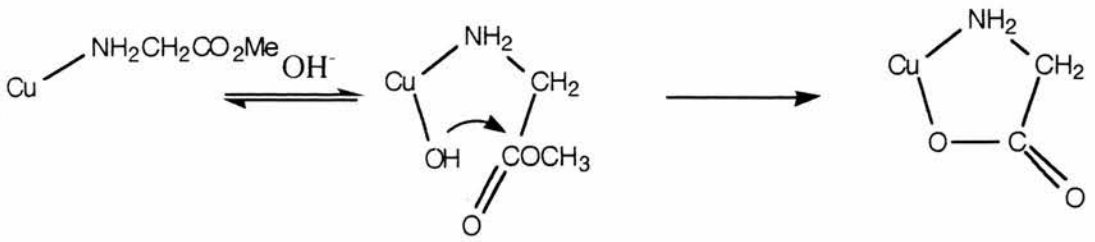
K_X is the complex-substrate binding equilibrium constant.
k_{OH} is the rate constant for the hydrolysis of methylglycine.

Under the conditions of the study the MeGly ester was found to be bound almost entirely as $Cu(BPA)\text{-}(MeGly)^{2+}$ therefore the observed rates represent the second step only. The first order dependence on the concentration of OH^- leads to two possible mechanisms, one involving an initial rapidly established equilibrium in which ester group coordination occurs and then rate determining attack of OH^-;



Scheme [3.3]

the other involving the rapid equilibrium formation of the Cu-OH complex followed by intramolecular OH^- attack.

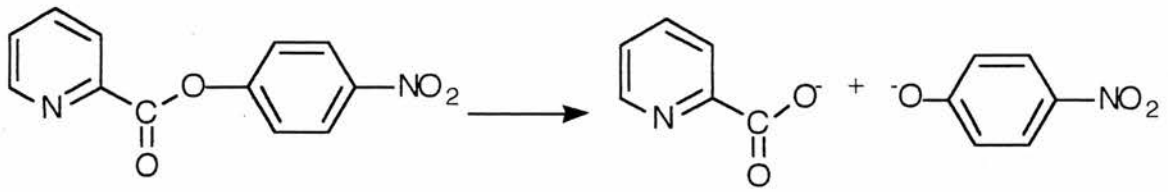


Scheme [3.4]

Although elaborate studies by Buckingham, Foster and Sargeson[12] have shown both pathways to be important in the hydrolysis involving the inert complex cis-$Co(en)_2(Br)(NH_2\text{-}CH_2CO_2\text{-}^{i}\text{-}Pr)$ it has not been possible to establish unequivocally whether one or the other or both mechanisms are involved in the observed hydrolysis reactions of amino acid esters catalysed by labile Cu^{2+} complexes.

3.2 BASE AND COPPER (II) COMPLEX CATALYSED HYDROLYSIS OF 4-NITROPHENYL PICOLINATE.

4-Nitrophenyl picolinate is a useful substrate for studying the catalytic effects of metal complexes on the hydrolysis of carboxylic esters. There are advantages in using it as a screening agent to perfect experimental procedures prior to work with nerve agent simulants. It enables simple comparison of the catalytic effects of divalent metal complexes on carboxylic and phosphate esters. There are strong indications from previous work that such divalent metal complexes are wide spectrum catalysts, catalysing a range of reactions including phosphate ester hydrolysis and the hydrolysis of active esters such as p-nitrophenyl acetate. p-Nitrophenyl picolinate has the added advantage of being readily synthesised, water soluble, and its hydrolysis can readily be followed by spectrophotometric monitoring of the release of the p-nitrophenoxide ion.



λmax=400nm

3.2.1 SYNTHESIS

Preparation of 4-nitrophenyl picolinate.

The ester was prepared by stirring 0.01mole quantities of picolinic acid, 4-nitrophenol and dicyclohexylcarbodiimide in 100cm^3 of chloroform or methylene chloride for 16 hours. The solution was filtered to remove the dicyclohexylurea which precipitated and the solvent was removed by rotary evaporation. The ester was treated with decolourising charcoal in methanol and recrystallised from methanol, m.p. 145-147°C (decomp.) (lit. 144-146°C)[14].

2,2-Dipyridylamine (Aldrich) was recrystallised twice from toluene, m.p. 84°C ($C_{10}H_9N_3$ M=171).

Stock solutions of copper (II) sulphate (0.006M), tetramethylethylenediamine (TMEN) (0.0063M) and dipyridylamine (DPA) (0.0063M) were made at constant ionic strength I=0.1M

(KCl).

1:1 mixtures by volume of the copper solution and the ligand were used to prepare solutions of the copper chelate (0.003M) as desired.Solutions were freshly prepared before the kinetic studies as on standing for several days hydroxo complexes began to precipitate.

3.2.2 MATERIALS AND METHODS

All reagents used were the purest available. Convol ampules were used for the preparation of standard sodium hydroxide solutions. The interval scan spectra were determined with a Philips Pu 8720 UV-vis spectrophotometer. pH was measured on the Radiometer Titralab system standardised prior to use with the appropriate Radiometer buffers. The experiments which follow involve the use of a stock solution of p-nitrophenyl picolinate at 0.01M made up in HPLC grade acetonitrile which was dried over 3A molecular sieves immediately prior to use. All potentiometric titrations were performed on the Radiometer Titralab previously calibrated with the appropriate Radiometer buffer solutions and the data obtained was processed using the "Superquad" program.

3.2.3 KINETICS

The base and metal complex catalysed hydrolysis of 4-nitrophenyl picolinate was monitored spectrophotometrically by observation of the increase in absorbance at 400nm against time due to the release of the p-nitrophenoxide ion. Reactant disappearance can be followed at 280nm (pH1 to 6), and product appearance at 330nm (pH4 to 6) and 400nm (pH7 to 8).

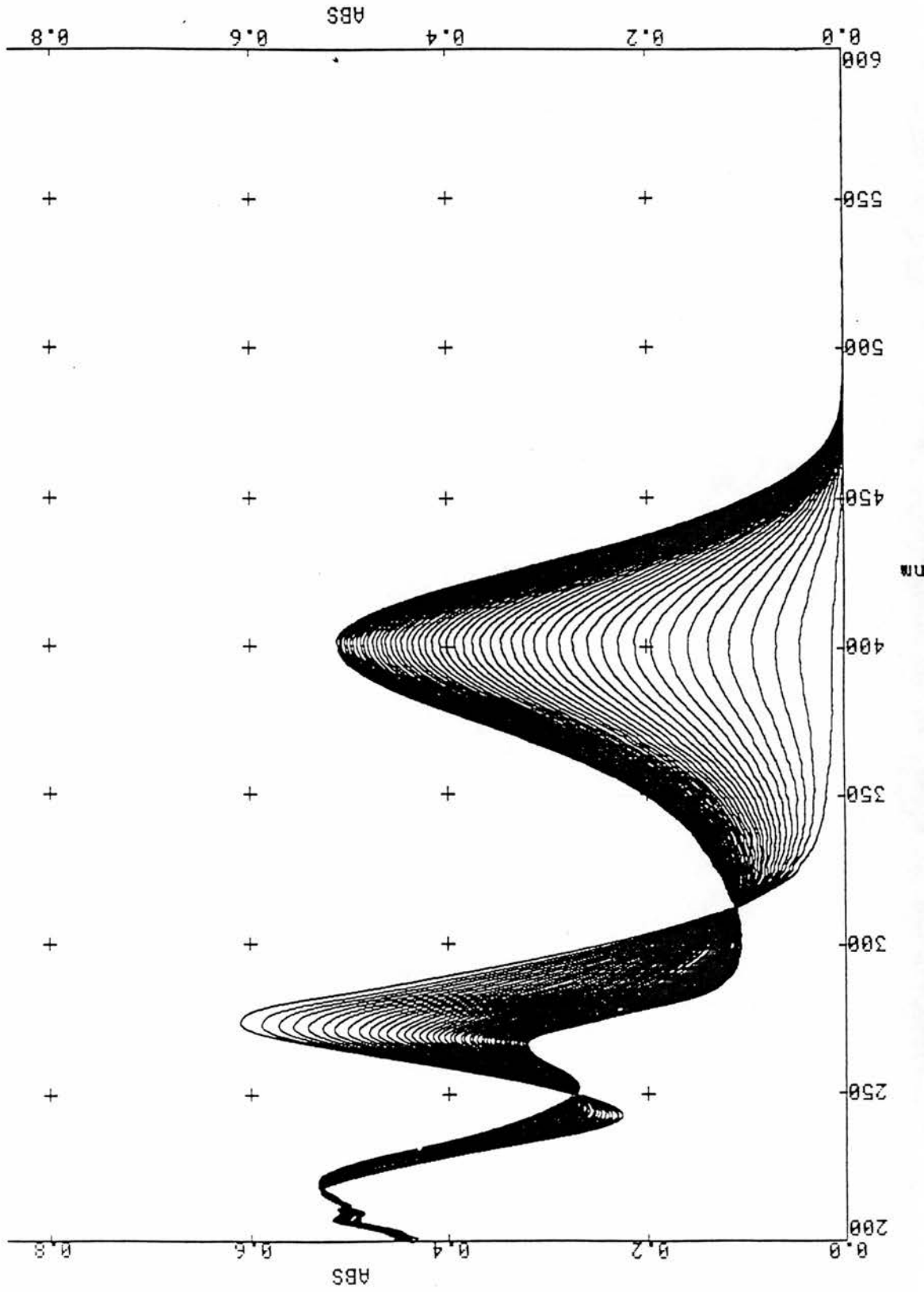
a) Determination of the rate constants for the base hydrolysis of p-nitrophenyl picolinate.

The system was maintained at the desired temperature +/- 0.1°C by circulating water via a thermostat. Reactions were initiated by injecting 10μL ($1x10^{-7}$mol) of the stock solution (0.01M), of p-nitrophenyl picolinate into a 1cm quartz cuvette containing 3ml of the Tris buffer (I=0.1 M KCl) at the desired pH (7 to 9) previously equilibriated at the desired temperature. Thesolution was rapidly shaken and the absorbance at 400nm monitored for at least 5 half lives. All kinetic runs were carried out in duplicate and the mean rate constants are reported. A typical interval scan spectrum is shown in Figure 3.1

b) Determination of the rate constants for the copper chelate catalysed hydrolysis of p-nitrophenyl picolinate.

Reactions were initiated by syringing 5μl of the p-nitrophenyl picolinate solution into the 1cm cuvette containing 3ml of an aqueous mixture of the appropriate buffer and the chelate solution previously equilibriated at the required temperature. Release of the p-nitrophenoxide ion was monitored at 400nm. The variation of k_{obs} with copper chelate concentration was determined for both complexes. The conditions adopted were 24.8+/-0.1°C, pH8.03 for CuTMEN and 30.1+/-0.1°C, pH7.92 for CuDPA both with a constant ionic strength of 0.1M KCl. The reactions were followed for at least five half lives. All experiments were run in at least duplicate and the tabulated data shows the mean values of the rate constants obtained.

Figure 3.1 Interval scan absorption spectrum for p-nitrophenyl picolinate in Tris buffer pH7.6, 24°C, I = 0.1moldm^{-3}. Time interval 1min 45s.



3.2.4 RESULTS

a) Base hydrolysis

Since hydrogen ion terms are negligible at the pH's used (pH7 to 9) the equation belowreflects the kinetics with high accuracy.

$$k_{obs} = k_o + k_{OH}[OH^-] + k_{nuc}[nuc^-]$$

k_{obs} = experimentally determined rate constant.

k_o = spontaneous rate constant for water (1st order).

k_{OH}= rate constant for hydroxide ion (2nd order).

k_{nuc} = rate constant for any other nucleophiles present (2nd order).

Water and hydroxide ion concentrations are significant at the pH values employed in this study and the absence of other species enables reduction of the rate equation to:

$$k_{obs} = k_o + k_{OH}[OH^-]$$

At 30°C and I=0.1mol dm^{-3} (KCl), $k_o = 7.35 \times 10^{-4} s^{-1}$ ($k_{H2O} = k_o/55.5 = 1.32 \times 10^{-5} dm^3\ mol^{-1}\ s^{-1}$) and $k_{OH} = 180 dm^3\ mol^{-1}\ s^{-1}$. The activation parameters for the base hydrolysis of the ester are $\Delta H^* = 14.6 kJ\ mol^{-1}$ and $\Delta S^*_{298} = -154 J\ K^{-1}\ mol^{-1}$.

The kinetic results are summarised in Table 3.1, Figures 3.2 and3.3 and the rate constants leading to the activation parameters in Table 3.2.

The hydroxide ion concentration was deduced from the pH using the appropriate values for pK_w and the activity coefficient derived from the Davies equation[15]. Plots of $\ln(A_\infty - At)$ versus time were linear for at least 5 half lives.

Table 3.1 Rate constants for the hydrolysis of p-nitrophenyl picolinate at various temperatures and I = 0.1mol dm^{-3} (KCl).

pH	$10^6[OH^-]$ /mol dm^{-3}	10^4kobs /s^{-1}
30°C		
8.00	1.91	9.80
8.20	3.02	13.10
8.42	5.02	16.50
8.58	7.25	20.80
8.79	11.76	29.70
8.95	17.00	37.10
$k_O = 7.35x10^{-4}s^{-1}$; $k_{OH} = 180+/-7\ dm^3\ mol^{-1}\ s^{-1}$		
36°C		
7.44	0.79	10.17
7.78	1.73	12.00
8.00	2.88	14.20
8.42	7.57	23.80
8.58	10.94	31.01
$k_O = 8.36x10^{-4}s^{-1}$; $k_{OH} = 206+/-2\ dm^3\ mol^{-1}\ s^{-1}$		
41°C		
7.78	2.42	13.01
8.00	4.02	16.83
8.20	6.36	22.21
8.42	10.56	31.66
$k_O = 7.58x10^{-4}s^{-1}$; $k_{OH} = 229+/-2\ dm^3\ mol^{-1}\ s^{-1}$		

Figure 3.2 shows a typical plot of kobs versus the hydroxide ion concentration for the hydrolysis of 4-nitrophenyl picolinate in the pH range 8.00-8.95 at 30°C. The plot is linear with positive intercept, indicating a first order dependence on the hydroxide ion concentration and the existence of a substantial water reaction. Least squares analysis of the results gives $k_{obs} = 7.35x10^{-4} + 180.3[OH^-]$ with a correlation coefficient of 0.997. The value of $k_{H2O} = k_o/55.5 = 7.35x10^{-4}/55.5 = 1.32x10^{-5} dm^3\ mol^{-1}\ s^{-1}$ at 30°C and I = 0.1mol dm^{-3}. The relative effects of hydroxide ion and water in the hydrolysis reaction are given by the ratio $k_{OH}/k_{H2O} = 1.37x10^7$, a value which is similar to those previously observed in ester hydrolyses of this type.

The rate constants $k_o = 7.35x10^{-4} s^{-1}$ and $k_{OH} = 180 dm^3\ mol^{-1}\ s^{-1}$ were used to prepare the pH-rate profile, figure 3.3, for the p-nitrophenyl picolinate at 30°C and I = 0.1mol dm^{-3}. At pH8.0, the half life of the ester is 10.7min and at pH 9.0 the half life is 2.75min.

Figure 3.2 Hydrolysis of p-nitrophenyl picolinate in the pH range 8 to 8.95 at 30°C. Least squares analysis give $k_O = 7.35 \times 10^{-4} s^{-1}$ and $k_{OH} = 180.3 +/- 7\ dm^3 mol^{-1}\ s^{-1}$.

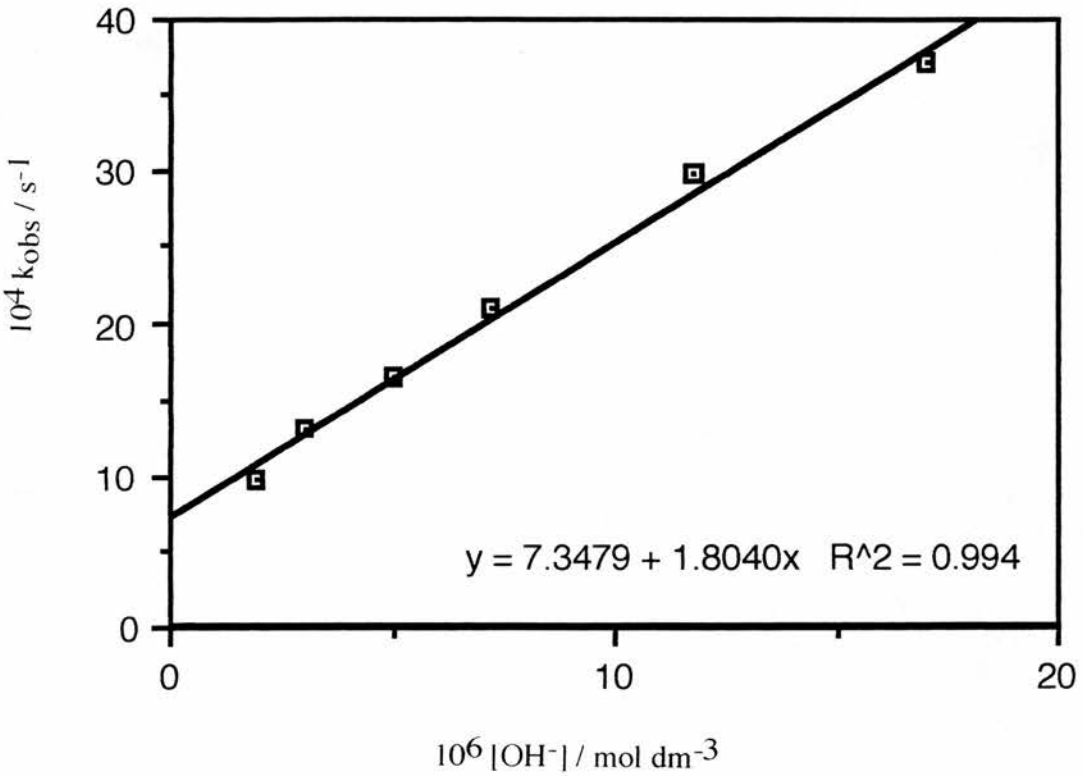
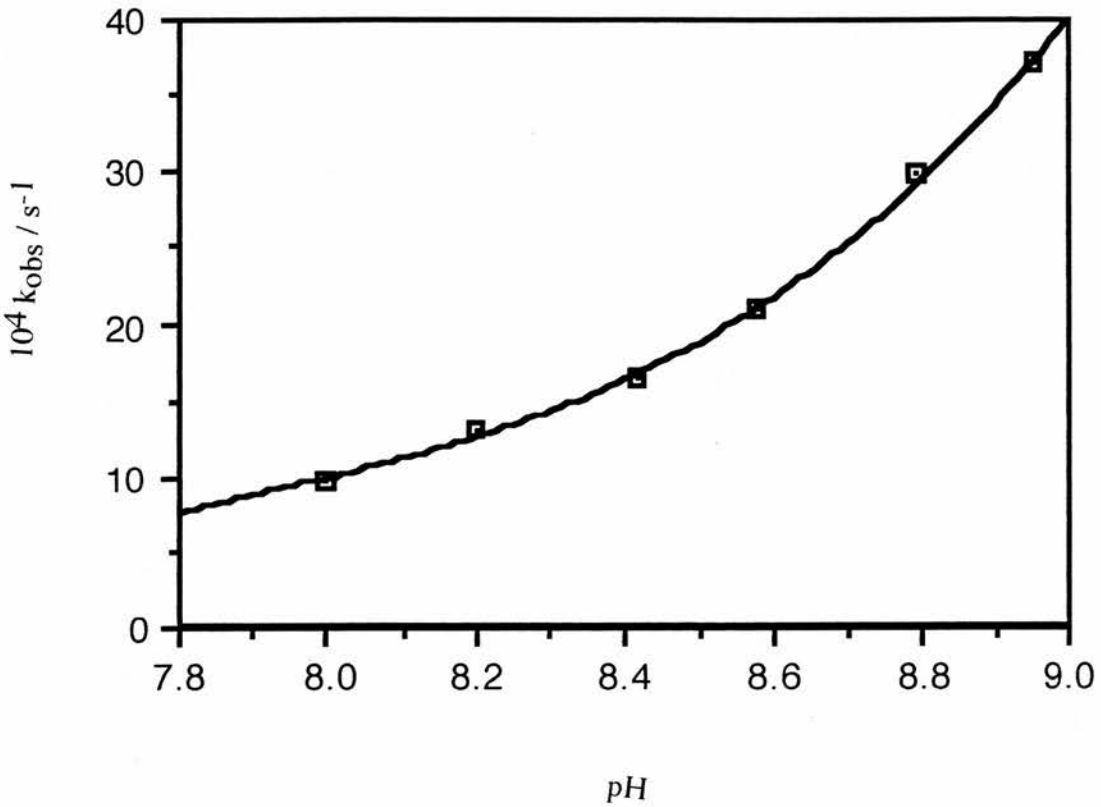


Figure 3.3 pH-rate profile for p-nitrophenyl picolinate at 30°C.



Activation parameters for the base hydrolysis.

The data in Table 3.1 is of adequate quality to enable the calculation of the thermodynamic parameters of the hydrolysis of p-nitrophenyl picolinate.

Using the expression

$$A + B \underset{}{\overset{k_c^*}{\rightleftharpoons}} X^* \rightleftharpoons \text{Products}$$

where X is in equilibrium with A and B the rate of the reaction can be determined as the product of [X*] at equilibrium, and the specific rate of its decomposition.

From the transition state theory[16].

$$\text{specific rate of decomposition} = kT/h$$

Where k = Bolzmann constant,

T = Absolute temperature,

h = Planks constant.

Hence,

$$V = \frac{kT}{h}[X^*]eqm = \frac{kTK^*c[A][B]}{h}$$

hence experimentally determined second order rate constant, k, is

$$k = \frac{KTk^*c}{h}$$

From simple thermodynamics:

$$\Delta G^* = -RT\ln k^*c = \Delta H - T\Delta S$$

hence

$$k = \frac{kT}{h}.\exp -\frac{\Delta G^*}{RT} = \frac{kT}{h}.\exp -\frac{\Delta H^*}{RT}\exp\frac{\Delta S^*}{R}$$

therefore

$$\ln\frac{k}{T} = \ln\frac{k}{h} + \frac{\Delta S^*}{R} - \frac{\Delta H^*}{RT}$$

and a plot of $\ln \frac{k}{T}$ versus $\frac{1}{T}$ should be linear with slope $= \frac{-\Delta H^*}{R}$ and intercept $= \ln \frac{K}{h} + \frac{\Delta S^*}{R}$

and since ln K/h = 23.76 the value of ΔS^* is easily be deduced.

Table 3.2 Activation parameters for the base hydrolysis of 4-nitrophenyl picolinate

Temp / °C	k_{OH} / $dm^3mol^{-1}s^{-1}$	$k_{OH(calc)}$[(b)] / $dm^3mol^{-1}s^{-1}$
30	180	177
36	206	202
41	229	225

$\Delta H^* = 14.55\ kJmol^{-1}$; $\Delta S^* = -154JK^{-1}mol^{-1}$

(b) Values of k_{OH} calculated from the above activation parameters. At 25°C k_{OH} = $158dm^3mol^{-1}s^{-1}$ and at 50°C $k_{OH} = 270dm^3mol^{-1}s^{-1}$.

b) $[Cu(TMEN)(OH_2)_2]^{2+}$ catalysed hydrolysis.

The effect of $[Cu(TMEN)(OH_2)_2]^{2+}$ on the hydrolysis of 4-nitrophenylpicolinate was studied at pH8.03 and 24.8°C. The results obtained are summarised in Table 3.3

Table 3.3 The $[Cu(TMEN)(OH_2)_2]^{2+}$ catalysed hydrolysis of 4-nitrophenyl picolinate.

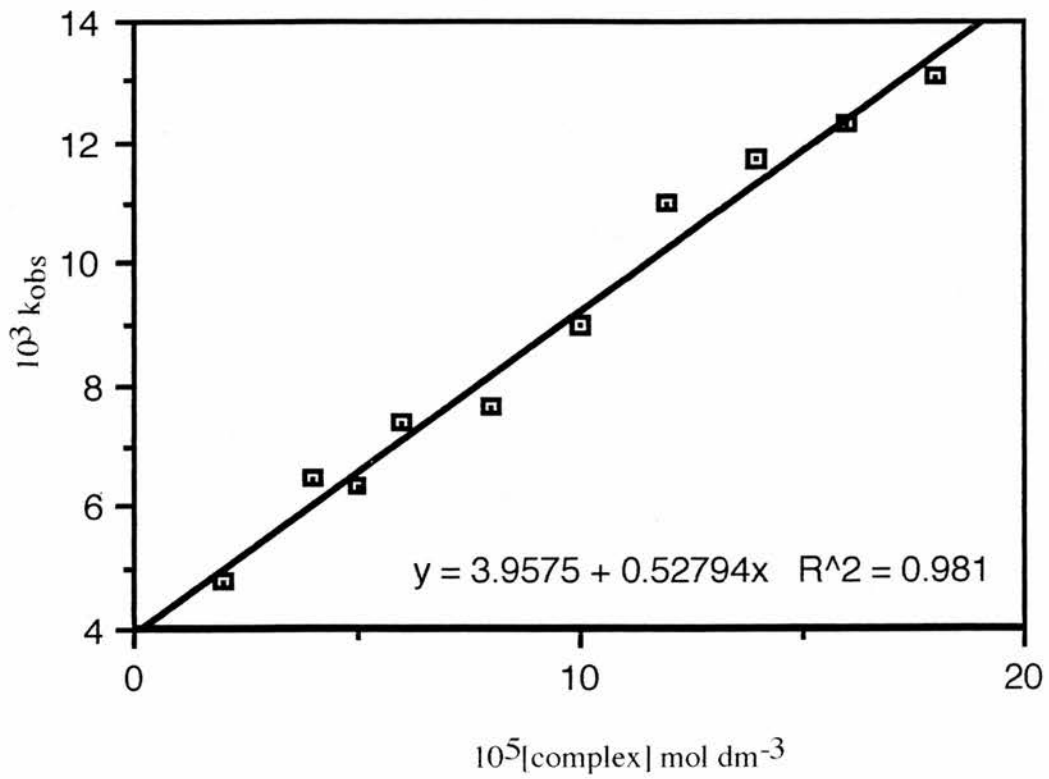
10^5[complex] /moldm^{-3}	10^3kobs /s^{-1}	10^5[complex] /moldm^{-3}	10^3kobs /s^{-1}
2.00	4.76	10.00	8.96
4.00	6.46	12.00	11.00
5.00	6.38	14.00	11.70
6.00	7.40	16.00	12.30
8.00	7.67	18.00	13.10

A plot of kobs versus the complex concentration, figure 3.4, is linear with positive intercept. The positive intercept of $3.96 \times 10^{-3} s^{-1}$ corresponds to the uncatalysed rate in the absence of the metal complex. Saturation kinetics are not observed due to the low concentrations of metal complex employed in the study (10^{-4} to 10^{-5}moldm^{-3}). The slope of the plot gives the catalytic rate constant for the metal complex at pH8.03, $k_{cat} = 52.8 dm^3 mol^{-1} s^{-1}$. Using a metal complex concentration of 10^{-4}moldm^{-3} results in an observed rate of $k_{obs} = 9.24 s^{-1}$ and a half life of 1.2 minutes effectively doubling the rate of the reaction.

The pK values of $[Cu(TMEN)(OH_2)_2]^{2+}$ are $pK_1 = 7.35$ and $pK_2 => 10$ (ionisation of water molecules) at 24.8°C. It is known from previous studies of pH-dependence of the activity of the metal complex that the hydroxoaqua complex is the active species in the reaction.

Figure 3.4 The $[Cu(TMEN)(H_2O)_2]^{2+}$ hydrolysis of p-nitrophenyl picolinate at 24.8°C and pH8.03.

The slope gives the catalytic rate constant $k_{cat} = 52.8 dm^3 mol^{-1} s^{-1}$.



c) $[Cu(DPA)(OH_2)_2]^{2+}$ catalysed hydrolysis.

The effect of $[Cu(DPA)(OH_2)_2]^{2+}$ on the hydrolysis of 4-nitrophenylpicolinate was studied at pH7.92 and 30.1+/- 0.1°C The results obtained are summarised in Table 3.4.

Table 3.4 The $[Cu(DPA)(OH_2)_2]^{2+}$ catalysed hydrolysis of 4-nitrophenyl picolinate.

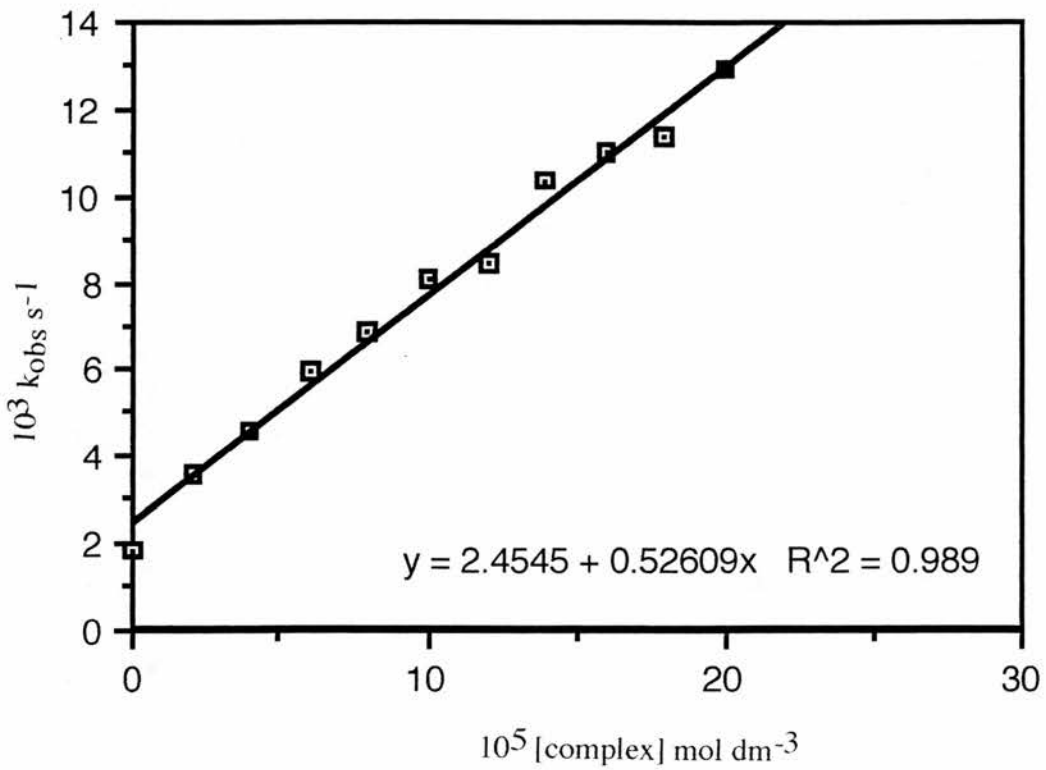
10^5[complex] /moldm^{-3}	10^3kobs /s^{-1}	10^5[complex] /moldm^{-3}	10^3kobs /s^{-1}
2.00	3.61	12.00	8.39
4.00	4.55	14.00	10.33
6.00	5.95	16.00	10.98
8.00	6.89	18.00	11.32
10.00	8.04	20.00	12.94

A plot of kobs versus the complex concentration is linear with positive intercept, Figure 3.5. The positive intercept of $2.45 \times 10^{-3} s^{-1}$ corresponds to the uncatalysed rate in the absence of the metal complex. Saturation kinetics are not observed due to the low concentrations of metal complex employed in the study (10^{-4} to 10^{-5}moldm^{-3}). The slope of the plot gives the catalytic rate constant for the metal complex at pH7.92, $k_{cat} = 52.6 dm^3 mol^{-1} s^{-1}$. Using a metal complex concentration of 16×10^{-3}moldm^{-3} effectively increases the rate of reaction more than four fold to kobs = $10.92 \times 10^{-3} s^{-1}$ giving a half life of 1.05 minutes.

The first pK value of $[Cu(DPA)(OH_2)_2]^{2+}$ is pK1 = 6.9+/- 0.08. It is known from previous studies of pH-dependence of the activity of the metal complex that the hydroxoaqua complex is the active species in the reaction.

Figure 3.5 The $[Cu(DPA)(H_2O)_2]^{2+}$ hydrolysis of p-nitrophenyl picolinate at 30.1°C and pH7.92.

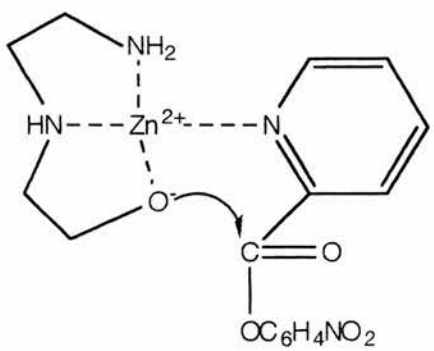
The slope gives the catalytic rate constant $k_{cat} = 52.6 dm^3 mol^{-1} s^{-1}$.



3.2.5 DISCUSSION

The mechanisms by which divalent metal ions effect increases in the rates of hydrolyses of esters are extremely important in developing our knowledge about the functions of enzymes such as carbonic anhydrase and carboxypeptidase A. It is clear from our data that the copper (II) complexes studied increase the rate of hydrolysis of 4-nitrophenyl picolinate under the conditions used. The observed rate of hydrolysis in the absence of copper (II) complex at 30°C, pH8.00 is $9.8 \times 10^{-4} s^{-1}$, this value is more than tripled by the presence of 2×10^{-5}M of copper DPA complex (k_{cat} is $52.6 dm^3 mol^{-1} s^{-1}$) at 30.1°C, pH7.92 and increased more than four fold by the presence of 2×10^{-5}M (copper-TMEN) (k_{cat} is $52.8 dm^3 mol^{-1} s^{-1}$ at 24.8°C and pH8.03). Extrapolation of data to zero catalyst concentration is greater than the value for base hydrolysis, infering free Cu^{2+} ion promoted catalysis.

Previous workers[17] have noted that in the presence of the zinc (II) complex of N-hydroxyethylethylenediamine [3.11] an increase in the rate of hydrolysis of 4-nitrophenyl picolinate is observed. The rate enhancement presumably arises through formation of a ternary complex. This ternary complex perturbs the pKa (estimated to be 8.4) of the hydroxyethyl group hence providing a higher concentration of the effective nucleophile. In addition the complex acts as a template to orient the substrate for intramolecular attack of the ionized hydroxymethyl group on the carbonyl carbon of the ester.

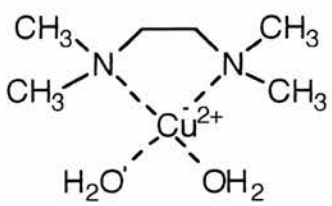


[3.11]

Fife et al[8] followed these investigations with work on the hydrolysis of picolinate esters in the presence of copper (II) and nickel (II) both showing substantial effects. The rate enhancement was shown to be a result of chelation, the metal ion binding to the pyridine nitrogen and the carbonyl oxygen leading to significant Lewis acid catalysis.

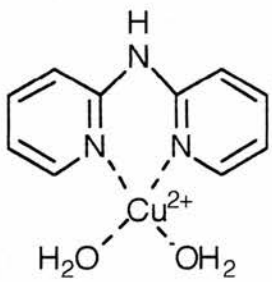
The investigations into metal chelate catalysis of the hydrolysis of phosphate triesters such as

dinitrophenyl diethyl phosphate[9] and Sarin[18] established that these complexes display activities which decrease with increasing negative charge on the ligand and also as the number of coordination sites of the metal that are satisfied by the ligand increase. For reasonable stability of copper complexes in dilute solution it is necessary to have at least a bidentate ligand coordinated to the metal.The most effective complexes are 1:1 complexes of copper and the bidentate ligand with two vacant coordination sites. A summary of the effectiveness of various copper (II) complexes on the hydrolysis of Sarin is shown in Scheme 3.6. In using two bulky bidentate ligands in our work, tetramethyethylene diamine and dipyridyl amine,



$[Cu(tmen)(OH_2)_2]^{2+}$

[3.12]



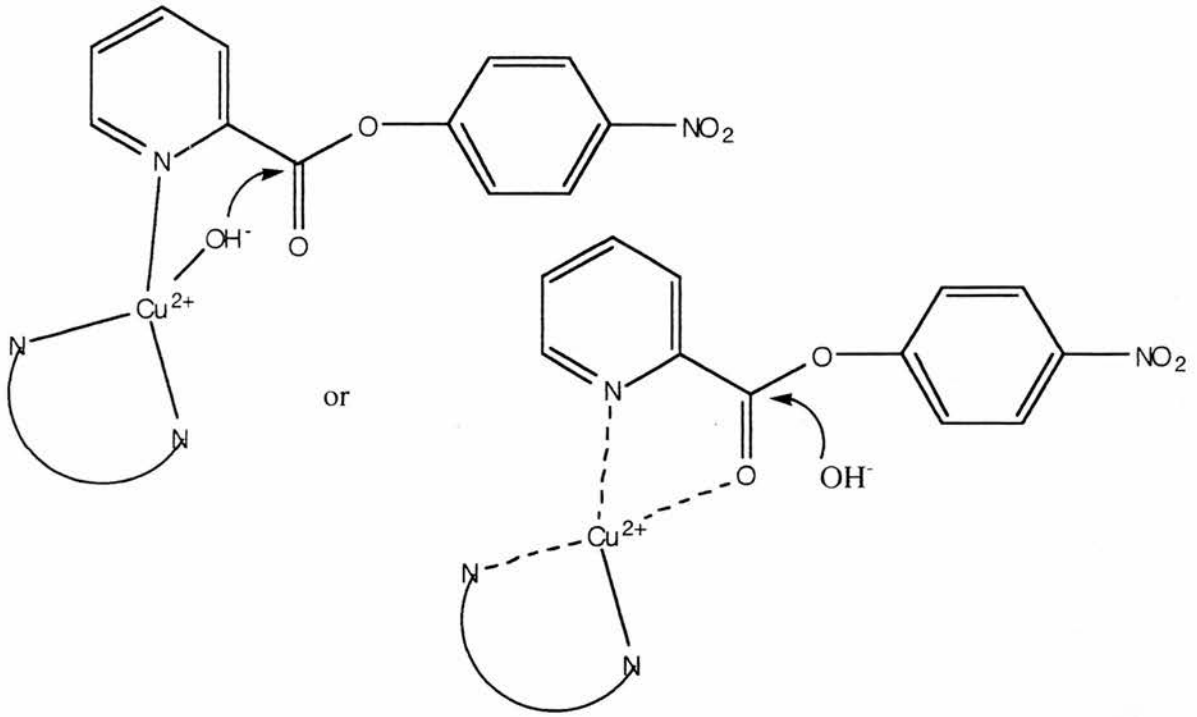
$[Cu(dpa)(OH_2)_2]^{2+}$

[3.13]

the formation of bis-complexes was prevented whilst providing two equatorial sites for the coordination of the water molecules. Jahn-Teller tetragonal distortion weakening the interaction at the axial sites in the d^9 metal ion.

Possible mechanisms which can be considered for the Cu(tmen) and Cu(dpa) catalysed hydrolysis are:

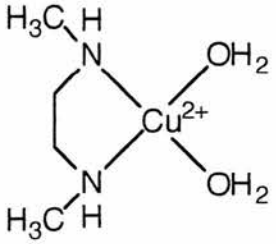
1. Formation of a ternary complex by coordination of the pyridine nitrogen by copper followed by intramolecular attack by coordinated hydroxide ion.
2. Formation of a ternary complex involving the pyridine nitrogen and the carbonyl oxygen of the substrate followed by attack of external hydroxide. (Scheme 3.5).



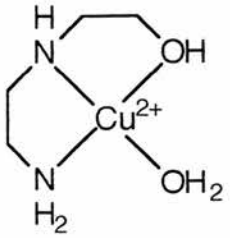
Intramolecular hydroxide attack "External hydroxide and Lewis acid catalysis

Scheme 3.5

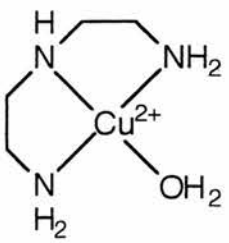
Figure 3.6 shows a typical distribution[13] curve for the copper(II)-TMEN system. It can be seen that at pH 8.03 there are two species increasing in concentration, the monohydroxo chelate $[CuL(OH)]^{+}$ and its dimer $[Cu_2(L)_2(OH)_2]^{2+}$. At this pH there is only a very low concentration of the dihydroxo complex. Formation of the inactive dihydroxo - bridged dimer will be a function of the concentration of the metal complex. Higher concentrations favour dimer formation.



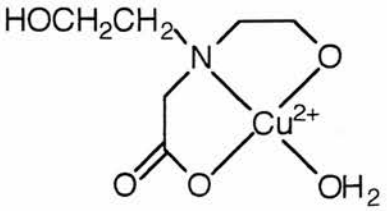
N',N'-Diethylenediamine-Cu(II) complex; $t_{.5}$=3.5min



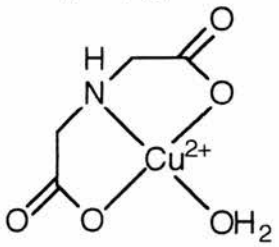
N-Hydroxyethylethylenediamine Cu(II) complex; $t_{.5}$=15 min



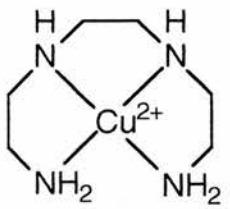
Diethylenetriamine - Cu(II) complex; $t_{.5}$=25min



N,N-Dihydroxyethylglycino-Cu(II) complex; $t_{.5}$=25min



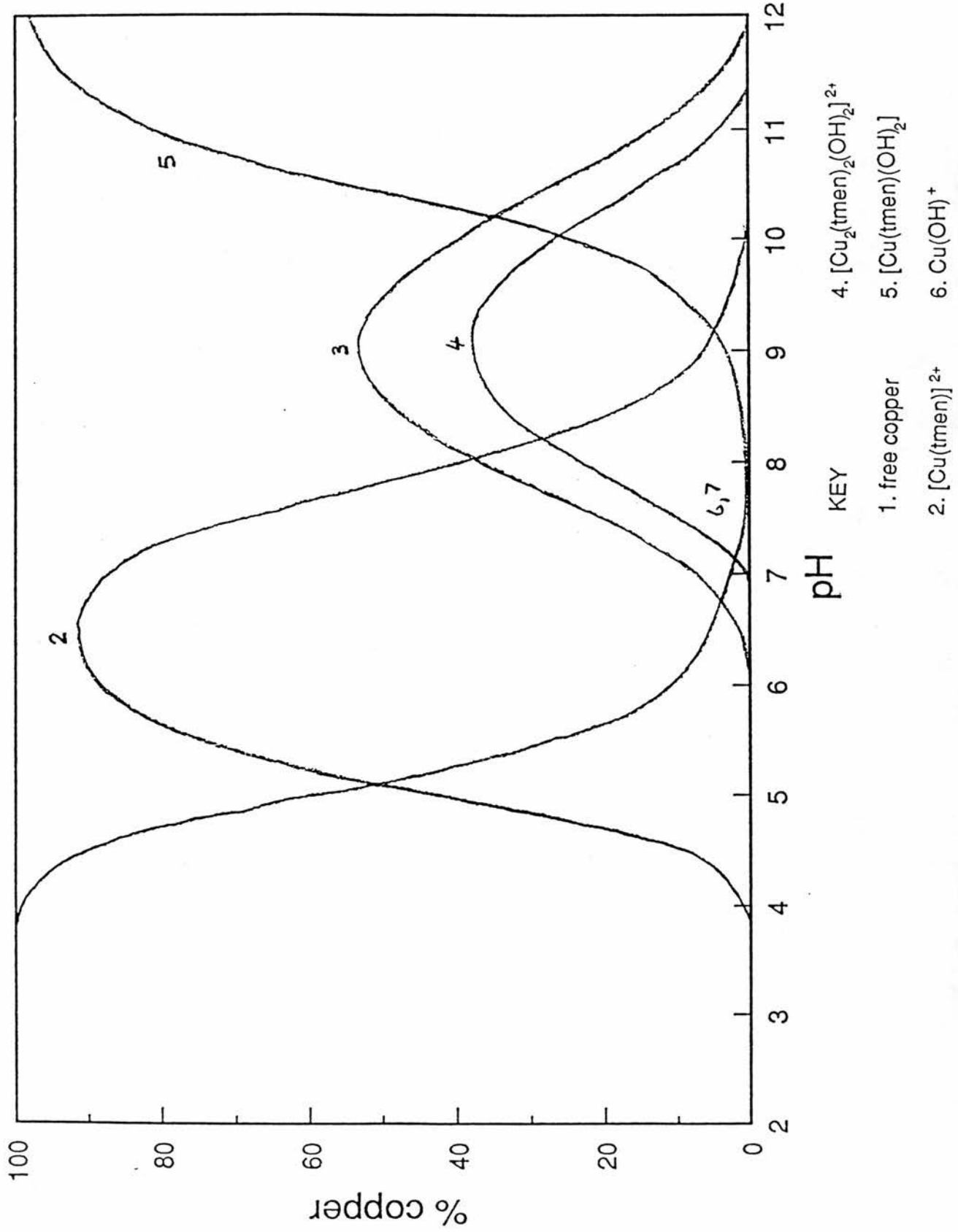
N-Hydroxyethyliminodiacetato-Cu(II) complex; $t_{.5}$=57min



Triethylenetriamine-Cu(II) complex;$t_{.5}$=65min

Scheme 3.6 Half lives for the hydrolysis of Sarin[18] by copper(II) chelate complexes at 25°C and pH 7.0. [Sarin] = [complex] = 10^{-3}M.

Figure 3.6 Species distribution profile, expressed as a percentage of total copper against pH for the copper-TMEN system. $[Cu^{2+}] = [TMEN] = 2.5mM$.



3.3 COPPER CHELATE PROMOTED HYDROLYSIS OF ETHYL GLYCINATE

3.3.1 SYNTHESIS

Ethyl glycinate.HCl.

This compound was prepared by refluxing absolute ethanol, thionyl chloride and the amino acid using standard methods[19]. The compound was twice recrystallised from ethanol by addition of ether m.p. 142 - 143°C (lit[19] 142 - 143°C).

3.3.2 MATERIALS

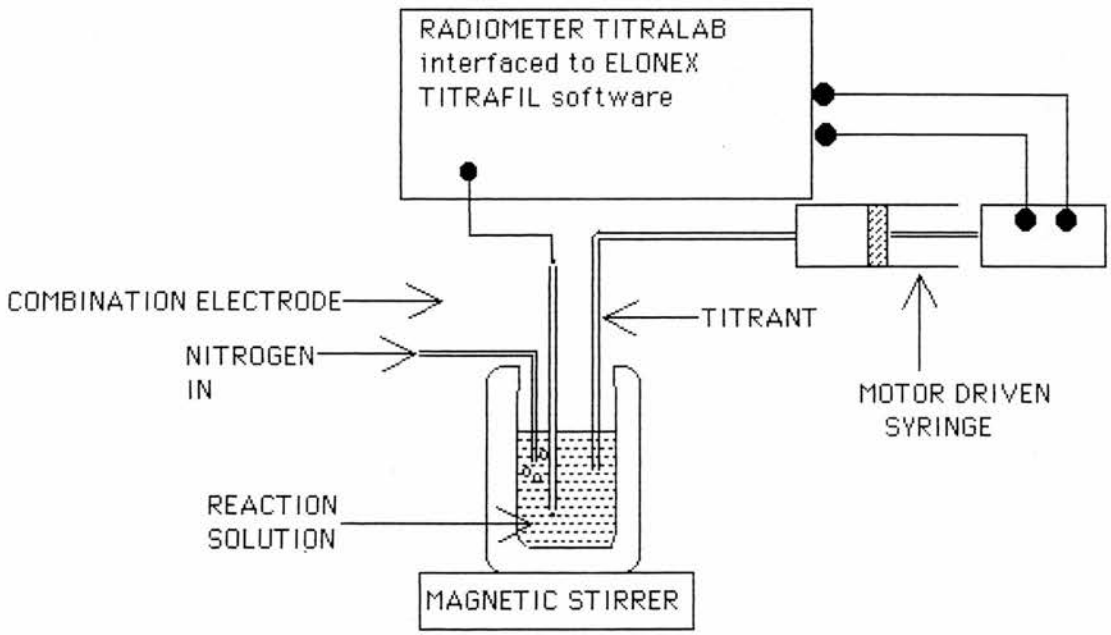
All reagents used were the purest available. Convol ampules were used for the preparation of standard sodium hydroxide solutions. pH values and kinetic measurements were determined on the Radiometer Titralab system, which was standardised prior to use with the appropriate Radiometer buffers .

3.3.3 KINETICS

The apparatus used in these experiments is shown schematically in Figure 3.7.
The effect of divalent metal ion complexes was studied using the pH-stat technique (there is no UV-visible chromophore in the ethyl group).The reaction medium (15ml total) containing glycine ethyl ester HCl ($5x10^{-4}$mol dm^{-3} total concentration) was added to the reaction vessel. The mixture was maintained at the desired temperature 25+/-0.1°C in a double walled glass beaker with constant stirring. The pH of the solution was then adjusted (between pH6 and 7) using concentrated HCl. The reaction was initiated by injecting the chelate solution into the vessel, final concentration $5.5x10^{-3}$moldm^{-3} (more than ten fold excess over the amino acid ester). Nitrogen gas was bubbled through the solutions during all runs. The variation of k_{obs} with hydroxide ion concentration was determined for both complexes. The pH-stat apparatus consisted of an Orion Research Combined pH electrode type 8103sc which controlled the

Radiometer Titralab system autoburette delivering the titrating alkali (0.005M NaOH), to the reaction vessel thus maintaining the initial pH throughout the reaction. Approximately 2.5 ml base was consumed during the reaction. The Titralab sytem was interfaced with an Elonex software programme, Titrafile, onto which all data was stored. All runs were carried out in duplicate, the data reported are the average values. Monitoring was carried out for at least 5 half lives.

Figure 3.7



3.3.4 RESULTS

a) $[Cu(TMEN)(OH_2)_2]^{2+}$ catalysed hydrolysis.

The effect of $[Cu(TMEN)(OH_2)_2]^{2+}$ on the hydrolysis of ethyl glycinate was studied in the pH range 5.32 and 7.31 at 25°C I = 0.1moldm^{-3} (KNO_3). The rate constants obtained using a complex concentration of 5.5x10^{-3}mol dm^{-3} and excess ester are shown in Table 3.5.

A plot of k_{obs} versus the hydroxide ion concentration is linear with negligible intercept, Figure 3.8. The reaction displays a first order dependence on the hydroxide ion concentration and $k_{OH}=k_{obs}/[OH^-]=4.7+/-0.5x10^3dm^3mol^{-1}s^{-1}$ at 25°C and I=0.1moldm^{-3} (KNO_3). Since

k_{OH} for the base hydrolysis of ethyl glycinate is 39.1$dm^3mol^{-1}s^{-1}$ at 25°C[9], the rate enhancement is $1.2x10^2$ fold under the experimental conditions used.

Table 3.5 The $[Cu(TMEN)(OH_2)_2]^{2+}$ catalysed hydrolysis of ethyl glycinate using a complex concentration of $5.5x10^{-3}$mol dm^{-3} and ester concentration of $5x10^{-4}$mol dm^{-3}

pH	$10^8[OH^-]$ moldm^{-3}	10^4k_{obs} s^{-1}	$10^{-3}k_{OH}$ /$dm^3mol^{-1}s^{-1}$
5.319	0.272	0.099	3.6
5.966	1.207	0.613	5.0
6.161	1.89	1.177	6.2
6.354	2.95	1.453	4.9
6.822	8.667	3.198	3.7
7.309	26.6	14.22	5.3

b) $[Cu(dpa)(OH_2)_2]^{2+}$ catalysed hydrolysis.

The effect of $[Cu(dpa)(OH_2)_2]^{2+}$ on the hydrolysis of ethyl glycinate was studied between pH6.02 and 7.32 at 25°C I = 0.1moldm^{-3} (KNO_3). The rate constants obtained are summarised in Table 3.6

A plot of k_{obs} versus the hydroxide ion concentration is linear with negligible intercept, Figure 3.9. The reaction displays a first order dependence on the hydroxide ion concentrationand $k_{OH}=k_{obs}/[OH^-]=4.4+/-2x10^3dm^3mol^{-1}s^{-1}$ at 25°C and I=0.1moldm^{-3} (KNO_3). Since

k_{OH} for the base hydrolysis of ethyl glycinate is $39.1 dm^3 mol^{-1} s^{-1}$ at 25°C[9], the rate enhancement is 1.13×10^3 fold under the experimental conditions used.

Table 3.6 The $[Cu(dpa)(OH_2)_2]^{2+}$ catalysed hydrolysis of ethyl glycinate using a complex concentration of 5.5×10^{-3} mol dm^{-3} and ester concentration of 5×10^{-4} mol dm^{-3}

pH	$10^8[OH^-]$ mol dm^{-3}	$10^4 k_{obs}$ [a] s^{-1}	$10^3 k_{OH}$ dm^3 mol^{-1} s^{-1}
6.015	1.35	0.362	2.7
6.236	2.25	0.844	3.75
6.296	2.582	1.04	4.0
6.404	3.31	1.313	4.0
6.479	3.93	1.39	3.5
6.681	6.26	3.889	6.2
7.322	27.4	19.04	6.9

Figure 3.8 The $[Cu(TMEN)(OH_2)_2]^{2+}$ catalysed hydrolysis of ethyl glycinate in the pH range 5.319 to 7.309 at 25°C. The value of $k_{OH} = 4.7+/-0.5x10^3\ dm^3mol^{-1}s^{-1}$.

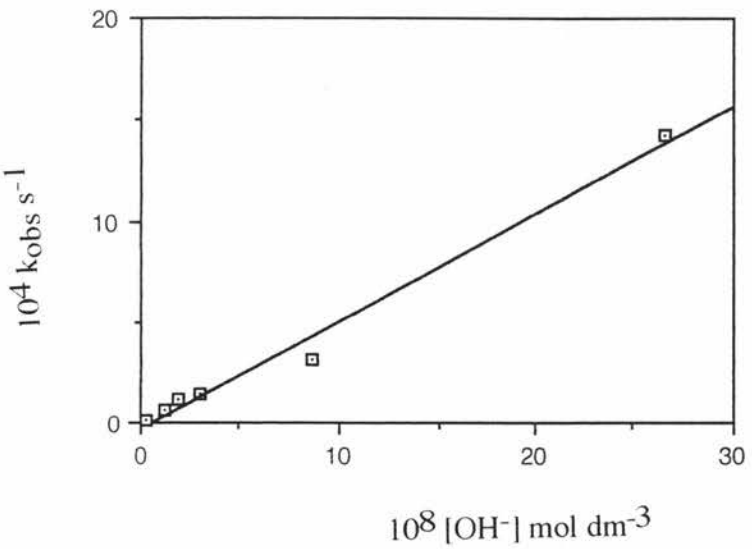
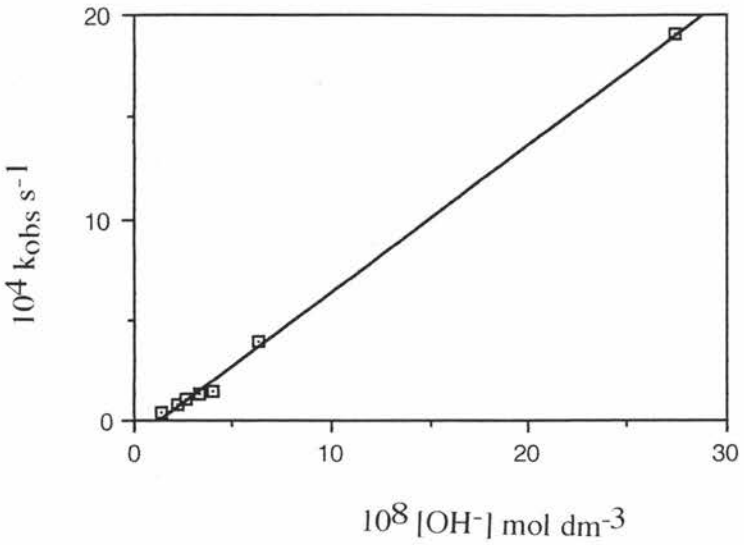
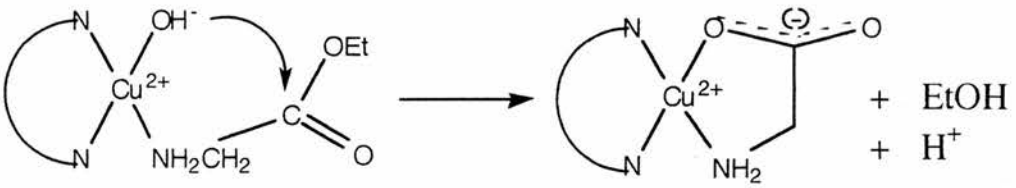


Figure 3.9 The $[Cu(DPA)(OH_2)_2]^{2+}$ catalysed hydrolysis of ethyl glycinate in the pH range 6.015 to 7.322 at 25°C. The value of $k_{OH} = 4.4+/-2x10^3\ dm^3mol^{-1}s^{-1}$.



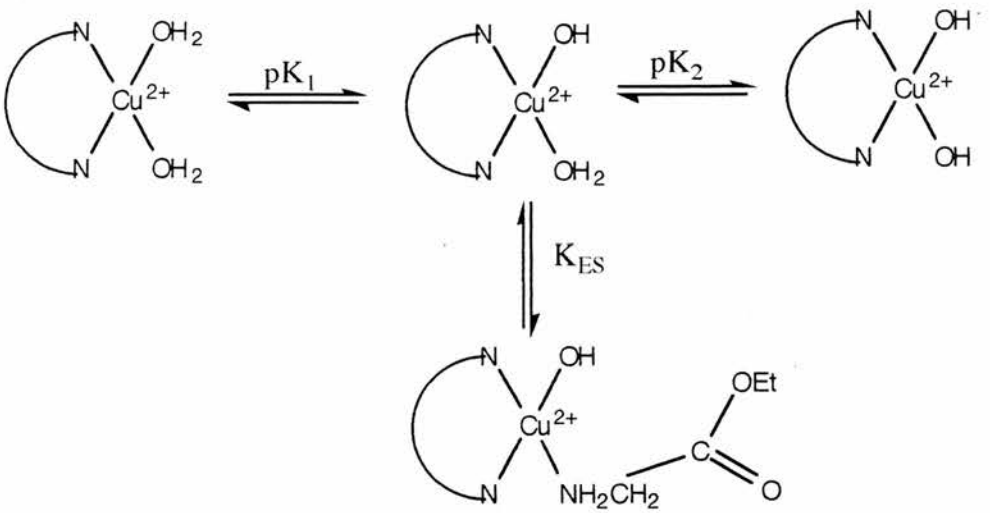
3.3.5 DISCUSSION

The kinetic results obtained confirm that $[Cu(TMEN)(OH_2)_2]^{2+}$ and $[Cu(DPA)(OH_2)_2]^{2+}$ catalyse the hydrolysis of ethylglycinate leading to rate enhancements of ca. 10^2 fold. It is probable that the mechanism involves intramolecular attack by coordinated hydroxide as shown in Scheme 3.7. However, to confirm this mechanism



Scheme 3.7

it would be necessary to study the pH dependence over a wide pH range. The overall scheme is summarised in Scheme 3.8.



Scheme 3.8

At high pH the catalytically inactive dihydroxy complex will form which should lead to a rate decrease at high hydroxide ion concentrations.

3.4 REFERENCES

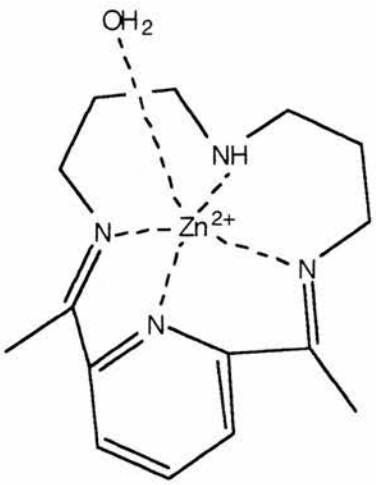
1. P. Woolley, Nature (London), 258, 677 (1975).
2. F.A. Cotton and G. Wilkinson, "Advanced Inorganic Chemistry", Chapt. 30, 5th ed., Wiley, New York (1988).
3. E. Kimura and T. Koike, Comments Inorg. Chem., 11, 285 (1991). and E. Kimura and T. Koike, J. Am. Chem. Soc., 113, 8935 (1991).
4. J. Chin and M. Banaszczyk, J. Am. Chem. Soc., 111, 2724 (1989).
5. J. Chin and V. Jubian, J. Chem. Soc. Chem. Commun., 839 (1989).
6. R.W. Hay and A.K. Basak, J. Chem. Soc., Dalton Trans., 39 (1986).
7. E. Kimura, T. Koike, T. Shiota, M. Shiro and M. Kodama, J. Am. Chem. Soc., 112, 5805 (1990).
8. T.H. Fife and T.J. Przystas, J. Am. Chem. Soc., 107, 1041 (1985).
9. I.J. Grant and R.W. Hay, Aust. J. Chem., 18, 1189 (1965).
10. R.W. Hay, L.J. Porter and P.J. Morris, Aust. J. Chem., 19, 1197 (1966).
11. R. Nakon, P.R. Rechani, R.J. Angelici, J. Am. Chem. Soc., 96, 2117 (1974).
12. D.A. Buckingham, D.M. Foster and A.M. Sargeson, J. Am. Chem. Soc., 91, 4102 (1969).
13. N. Govan, Ph.D. Thesis, University of St. Andrews (1991).
14. D.S. Sigman and C.T.Jorgensen, J. Am. Chem. Soc., 94, 1724 (1972).
15. C.W. Davies, J. Chem. Soc., 2093 (1938).
16. A.A. Frost, "Kinetics and Mechanism", Chapt. 5, Wiley.
17. K. Ogino, K. Shindo, T. Minami, W. Tagaki and T. Eiki, Chem. Soc. Japan, 56, 1101, (1983).
18. T. Wagner-Jauregg, B.E. Hackley, T.A. Lies, O.O. Owens and R. Roper, J. Am. Chem. Soc., 77, 922 (1955).
19. C.S. Marvel, Org. Synth.,Coll. Vol. II, Blatt.

APPENDIX ONE

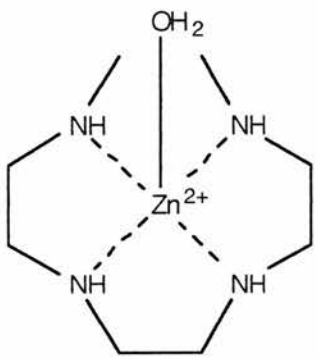
MACROCYCLIC LIGAND SYNTHESIS

INTRODUCTION

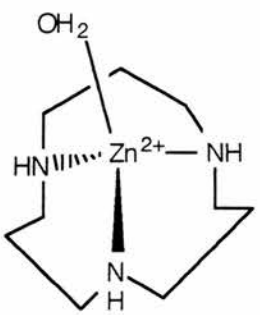
Investigations into the roles of divalent metal ions such as zinc in the active centres of metalloenzymes has led to the extensive study and synthesis of many different types of metal complexes in an attempt to mimic these enzymes. Kimura and Koike[1] have shown that the zinc II complex of [12]aneN3 is an excellent catalyst for the hydrolysis of carboxylic esters, phosphate esters and the hydration of CO_2. The complex has a close structural resemblance to the active site zinc of the enzyme carboxypeptidase with three nitrogen donors and a pK for the water molecule similar to that of the enzyme.



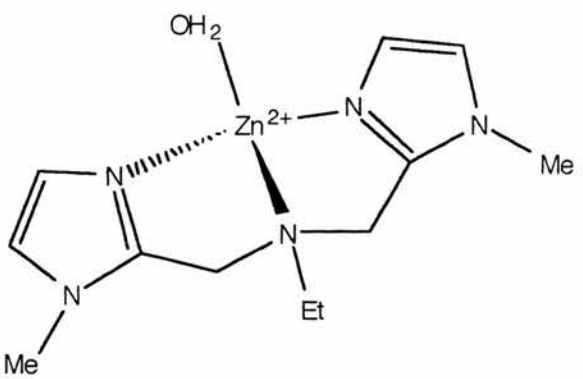
pKa(H2O) = 8.7



Zn II Cyclen
pKa(H2O) = 8.0



Zn II-[12]ane N3
pKa(H2O) = 7.3



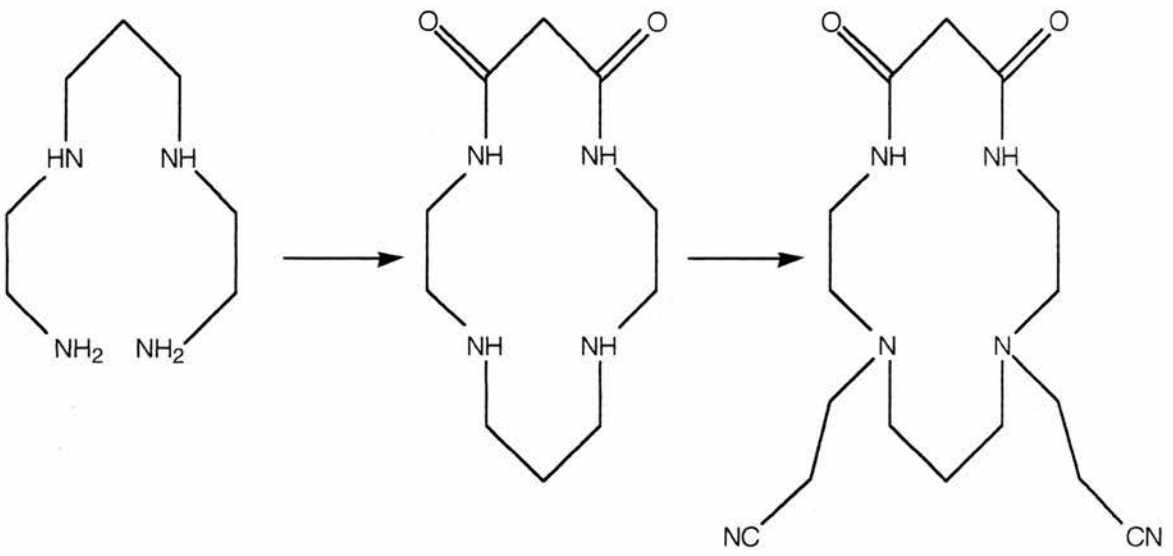
pKa(H2O) = 8.3

[A.1.1]

Structure of zinc macrocyclic complexes and the pK values of associated water molecules.

SYNTHESIS

1. Preparation of N,N'-Bis(2-cyanoethyl)-5,7-dioxo-1,4,8,11-tetraazacyclotetradecane.



N,N'-Bis(2-cyanoethyl)-5,7-dioxo-1,4,8,11-tetraazacyclotetradecane was prepared by a modified literature route[2]. 1,9-Diamino-3,7-diazanonane(7.3g,0.045mol) was dissolved in ethanol (1000cm^3) and the solution cooled to ca. 0°C in an ice bath. Diethyl malonate $CH_2(CO_2Et)_2$ (8.0g, 0.05mol) was added to the constantly stirred solution. Following 7 days of stirring at room temperature a pale pink colour formed. The volume was reduced to 25cm^3 in vacuo and white needle like crystals (ca 4.3g) formed on standing overnight. Following recrystallisation from EtOH the ligand had m.p.164-166°C (Lit.[1] 176-177°C). Spectral evidence was in agreement with previous literature data.

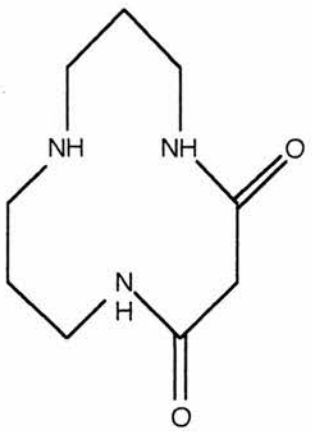
A solution of 5,7-dioxo-1,4,8,11-tetraazacyclodecane (1.04g, 3.29mmol)in acrylonitrile CH_2CHCN (40ml) was refluxed for 8 hours and the excess of solution removed by rotary evaporation. The white sticky solid thus formed was recrystallised from hot ethanol, ethylene chloride (9:1) mixture yielding colourless crystals (0.96g, 62.9% yield). Apparatus in contact with acrylonitrile was carefully decontaminated by rinsing thoroughly with acetone into waste solvent storage.

m.p. 144-147°C (lit. value 135°C might be due to Michael retro addition). CNH : (Found:

C,56.47; H,8.09; N,24.84%. $C_{16}H_{27}N_6O_2$. Calcd.: C,57.4; H,7.78; N,25.1%).

I.r.: 3308[sυ(N-H)], 2928[s,υ[CH2], 2246[s,υ(CN)], 1650[s,str,υ(C=O)] and 1356[s,υ(COC-H)] cm^{-1}.

2. Preparation of 1,5,9-triazacyclododecane-2,4-dione.



The preparation of this ligand was carried out following a procedure from the literature[3]. A solution of diethy malonate (16.02g, 0.125mol) and 1,7-diamino-4-azanonane (13.1g, 0.1mol) in ethanol (1200cm^3) was boiled under reflux for 5 days. The solvent was removed and the remaining yellow oil (ca.16.15g) quickly solidified. It was purified by chromatography on silica gel. The chromatography column was 3-4 inches in diameter, 15-20 inches in length. The following set of eluants were employed:

a) 40% CH_2Cl_2; 59%MeOH; 1% 0.88 NH_3.

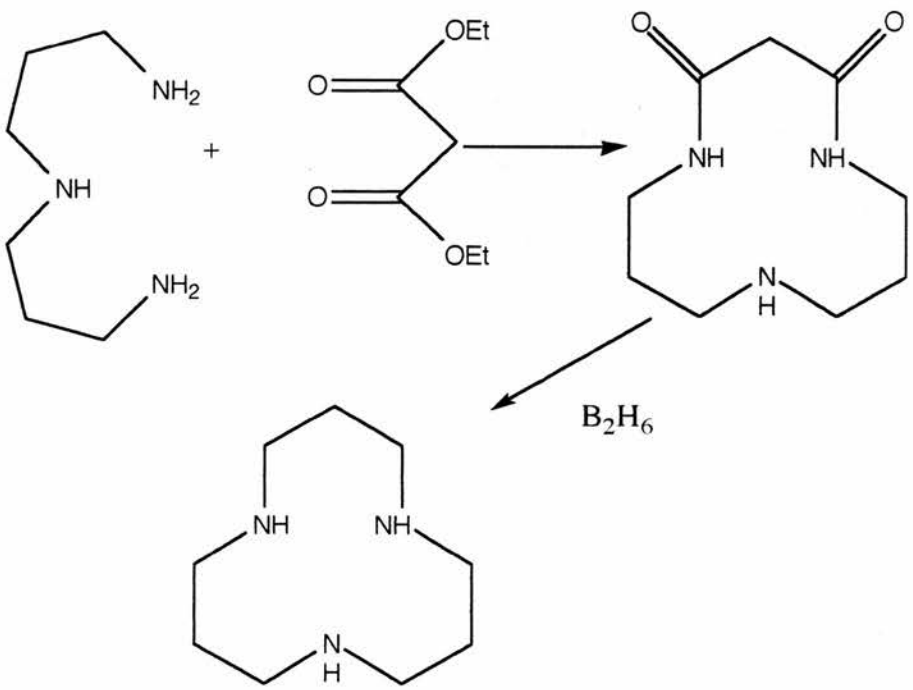
b) 40% CH_2Cl_2; 57%MeOH; 3% 0.88 NH_3.

c) 40% CH_2Cl_2; 55%MeOH; 5% 0.88 NH_3.

The column of 0.125-0.15mm and 0.2-0.5mm grade silica was prepared with solution (a). The crude product was applied to the column dissolved in the minimum of solution (a), polymeric impurities were removed on elution of solutions (a) and (b) and the product retrieved with solution (c) and obtained, following removal of solvents as a pure colourless solid. m.p. 152-154°C. (R_F =0.7 with 40% CH_2Cl_2, 59%MeOH; and 1% 0.88 NH_3). Yield ca 2.8g (14%).

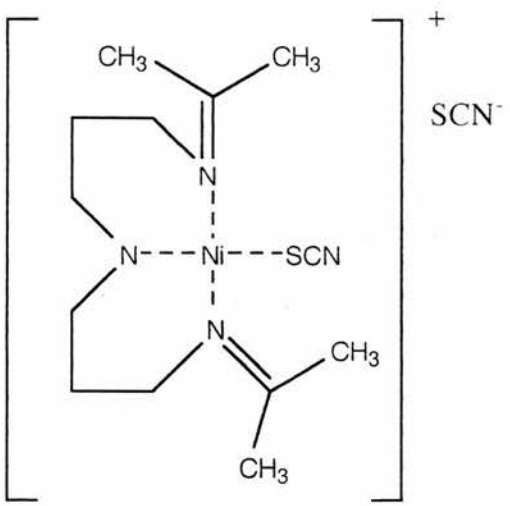
CNH : (Found: C, 49.0: H, 8.75: N, 20.9. Calc. for C9H17N3O2 : C, 49.2; H, 8.55; N, 21,1%). ^1H n.m.r. δ_H ($CDCl_3$), 8.56 (2H, br, t, NHCO), 3.40 (4H, dt, CH_2NHCO), 3.13 (2H, s, CH_2CO), 2.76 (4H, t, NCH_2CH_2), 1.85 (1H, br, s, NH) and 1.66 (4H, quint.,

$CH_2CH_2CH_2$) I.r.:3294 [s, υ(CH2)], 1652[s, υ(C=O), 1558 [s, υ(N-H)], 1436 [s,υ(=CH_2)], 1296[str, υ(C-O)], 728 cm^{-1}[w, υ(=CH_2 rocking)]. (The infrared spectrum is shown in figure A.1.1.) m/z 200 (M^++1) and 199 (M^+)



Scheme [A.1.1] Synthesis of Dioxo[12]aneN3 and [12]aneN3.

3. Preparation of thiocyano-(3,3'-di-N-isopropylene) dipropylamine nickel (II) thiocyanate. (Nidpt2AcSCN)SCN[4]



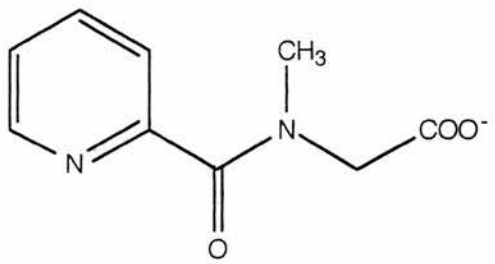
To a blue solution of dipropylene triamine (20g, 0.152mol) and nickel chloride hexahydrate (18.06g, 0.076mol) in methanol, acetone was added until a white precipitate began to form. Excess methanol was then added to dissolve the precipitate and the reaction mixture was refluxed for 2 hours. Ammonium thiocyanate (11.57g, 0.152mol) was added to the resulting blue green mixture whilst stirring. Once homogeneous the mixture was left over the weekend at 30°C. Following the removal of the solvent in vacuo the brown red mixture was cooled in an ice-salt mixture. The violet crystals thus formed were filtered, washed in ice cold methanol and dried. (17.15g, 58.5% yield). CHN: (Found: C,48.83; H,7.21; N,20.52%. Calc.: C,43.6; H,6.4; N,17.96%). I.r.: 3540, 3480(m, υ[NH]), 3416(s, υ[(CH3)2C=O]), 3228(m, υ[C-NH-C]), 2960(s, υ[-CH3]), 2936(s, υ[-CH2-]), 2095(m, υ[-N=C=S]), 1707(s, υ[CO]), 1654(s, υ[C=N]) cm^{-1}. U.V.-vis: λmax 560.1nm, υ2=578, υ3=378nm. (Lit.[4]υ2=599, υ3=385nm).

4. Preparation of picolinyl sarcosine

The synthesis of picolinyl sarcosine was carried out as a possible substrate for amide hydrolysis[4].

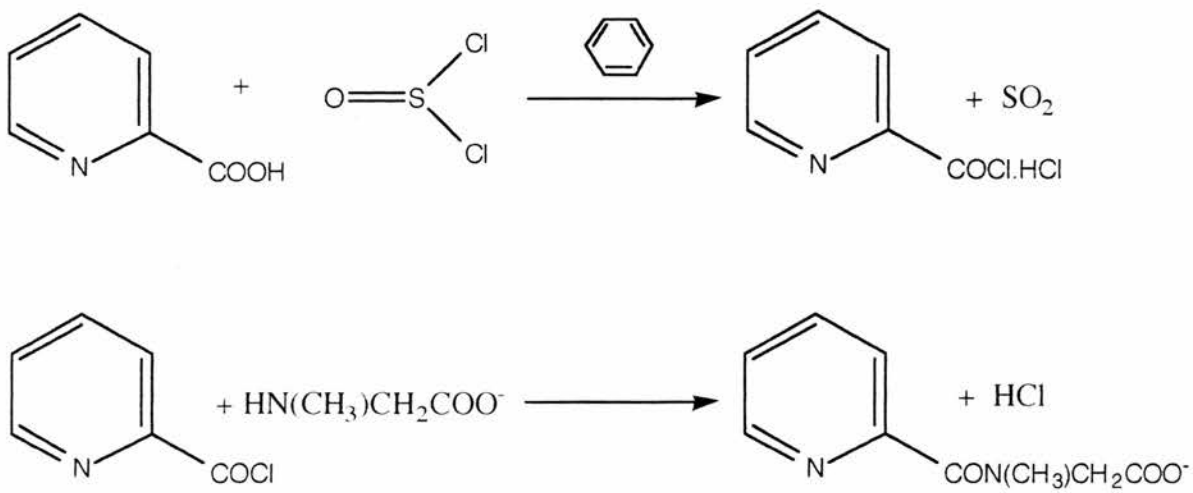
Picolinyl sarcosine was produced by first preparing picolinyl chloride hydrochloride from the

refluxing of benzene, thionyl chloride and picolinic acid, evaporation to dryness, recrystallisation from hexane and the addition of acid chloride portionwise to a rapidly mixing alkali solution of sarcosine until TLC testing indicated complete acylation. The pH was then adjusted to pH 4.5 and repeat crystallisation produced picolinyl sarcosine as a creamy white solid.



A mixture of picolinic acid (10g, 0.08mol) and a slight excess of thionyl chloride (11.897g, 0.1mol) in benzene (40ml) were boiled under reflux until the production of hydrogen chloride gas ceased. Evaporation to dryness followed by recrystallisation from hexane produced the pale yellow, needle like, crystals of the acid chloride. Sarcosine (1.336g, 0.015mol) was dissolved in 1 equivalent of 1M NaOH(15cm^3) to this solution an excess of the acid chloride was added portion wise whilst maintaining the pH of the solution at pH10.5 by dropwise addition of 4M NaOH. Once acid chloride addition was completed as indicated by t.l.c. as the acylation of the sarcosine, (about 2 equivalents required) the pH was lowered to 4.5 and the solvent removed on a rotary evaporator. Repeated, mixed recrystallisation with H_2O/EtOH produced the required N-picolinyl sarcosine.

I.r.: 3427(b, υ[H-bond OH]), 2957(s, υ[C=O]), 1613(m, υ[NC=O]), 1568(s, u[CH=]), 1391(s, υ[CO_2^-]), 1117(s, υ[C-OH])cm^{-1}.

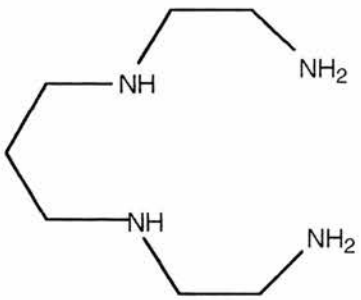


Scheme A.1.2 Synthesis of picolinyl sarcosine

5. Preparation of 2,3,2-triethylenetetramine.

A large number of complexes of tetraaza macrocyclic ligands are known. These ligands have from 12 to 16 atoms in the ring the most common being 14 membered rings, the simplest of which is 1,4,8,11-tetraazacylotetradecane. Ligands of this type have been useful in binding to copper and zinc, investigations into their effect on the hydrolysis of phosphate and carboxylic esters could prove very worthwhile.

2,3,2-Tet was prepared as a precurser for macrocyclic ligands following a method published in the literature[5].



Ethylenediamine (599.98g, 9.9996mol) was placed in a 3-necked flask and cooled in an ice water mixture. Dibromopropane (168.24g, 0.833mol) was added slowly dropwise while vigorously mechanically stirring. Heat was evolved throughout the addition (about 1 hour). Following this the solution was heated on a steam bath for 1 hour and then concentrated to 1/3 of the volume on a rotary evaporator. The resulting orange oil, returned to the original vessel, was mixed with pulverised potassium hydroxide pellets (124.95g, 2.227mol) and the mixture heated with stirring on a steam bath for 2 hours.

Solids were removed by filtration from the mixture once it had cooled to room temperature. The solid portion was washed with great care using ether, removing all the absorbed product. The ether washings and the filtrate, combined, were reduced once more to a viscous oil under low pressure. Extraction and decantation with ether finally removed the products from traces of solid formation and following the repetition of removal of solvent the product was purified by vacuum distillation.

^{1}H n.m.r. in D_2O (ppm down field from TMS): δ1.65(q, CH_2CHCH_2), 2.63(t, 2-CH_2NH_2), 2.6(t, 2NH-CH_2-CH_2-NH_2), 2.45(t, $NHCH_2CH2CH_2NH$). I.r.: 3355(s, υ[N-H]), 2808(b, υ[C-H stretch]), 1602(m, υ[-NH_3^+]), 1461(m, υ[CH def.]).

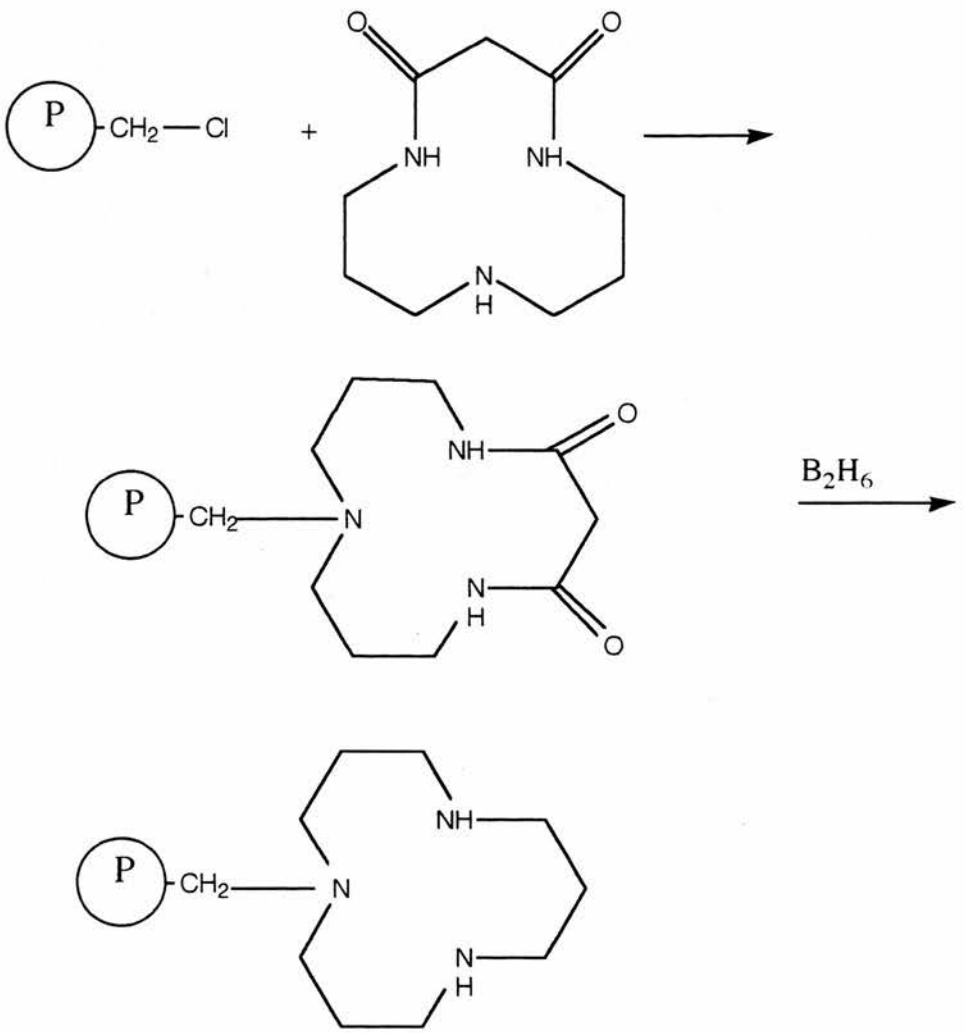
Figure A.1.1 Infrared spectrum (KBr disc) of Dioxo[12]aneN$_3$.

6. USES OF MACROCYCLES.

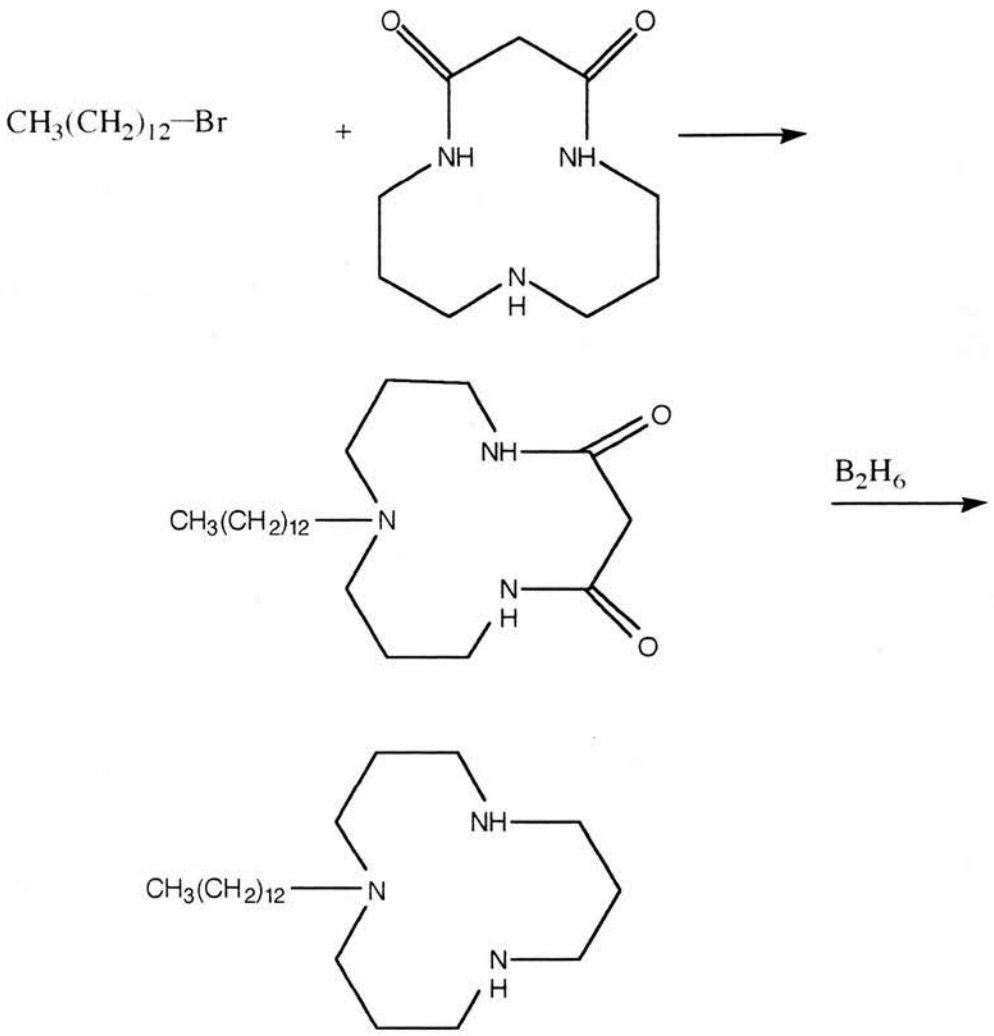
These macrocycles may also be incorporated onto polymer backbones and metallomicelles, possible synthetic schemes are shown below.

a) Functionalisation

A modified verion of Menger's synthesis of functionalised polystyrene (Chapter two).



b) Metallomicelle synthesis:



REFERENCES

1. E. Kimura and T. Koike, Comments Inorg. Chem. ,11, 285 (1991); E. Kimura, T. Koike, T. Shiota and Y. Iitaka, Inorg. Chem., 29, 4621 (1990); E. Kimura, Tshiota, T. Koike, M. Shiro and M. Kodama, J. Am. Chem. Soc., 112, 5805 (1990).
2. R.W. Hay and P.R. Norman, Trans. Met. Chem., 5, 232 (1980).
3. I.M. Helps, D. Parker, K.J. Jankowski, J. Chapman and P.E. Nicholson, J. Chem. Soc., Perkin Trans., 2079 (1989).
4. R.W. Renfrew, R.S. Jamison and D.C. Weatherburn, Inorg. Chem., 18, 1584 (1979).
5. K.V. Reddy, S-J. Jin, P.K. Arora, D.S. Sfeir, S.C.F. Malonary, F.L. Urbach and L.M. Sayre, J. Am. Chem. Soc., 112, 2332 (1990).
6. E.K. Barefield, F. Wagner, A.W. Herlinger and A.R. Dahl, Inorg. Synth., XVI, 220.

APPENDIX TWO

SOLUTION CHEMISTRY

1.MATERIALS AND METHODS.

Potentiometric titrations were carried out using a Radiometer Titralab system interfaced with an Apple IIe computer. Temperature was maintained as required +/-0.1°C and each titration was performed on a solution volume 25cm^3 adjusted to an ionic strength of 0.1 mol dm^{-3} with $NaNO_3$. All titrations were carried out in an inert atmosphere (N_2 was bubbled continuously into the solutions). Measurements were made with an Orion Research Combined pH electrode type 8103sc. The electrode system was standardised prior to each titration in terms of hydrogen ion activities and all equilibrium constants are expressed in mixed concentration and activity units. The results were processed on a Zenith P.C. using the Superquad program.

2. Ionisation Constants of Trimen.

As the ligand N,N,N'-trimethylethylenediamine is central to much of the solid support polymer work, the pK values for the ligand were determined.The ligand dihydrobromide was prepared by dropwise addition of concentrated hydrobromic acid to a cooled solution of the amine in ethanol (ice-bath). The precipitated dihydrobromide was thoroughly dried in vacuo over silica gel.The potentiometric titration was carried out at 25°C and I = 0.1mol dm^{-3} (KNO_3) using standard sodium hydroxide solution as titrant. The titration data was analysed using the SUPERQUAD program. Typical final output is summarised in table A.2.1, giving log $\beta 1$ = 9.78+/-0.03 and log $\beta 2$ = 16.30+/-0.04. The pK values for the equilibria:

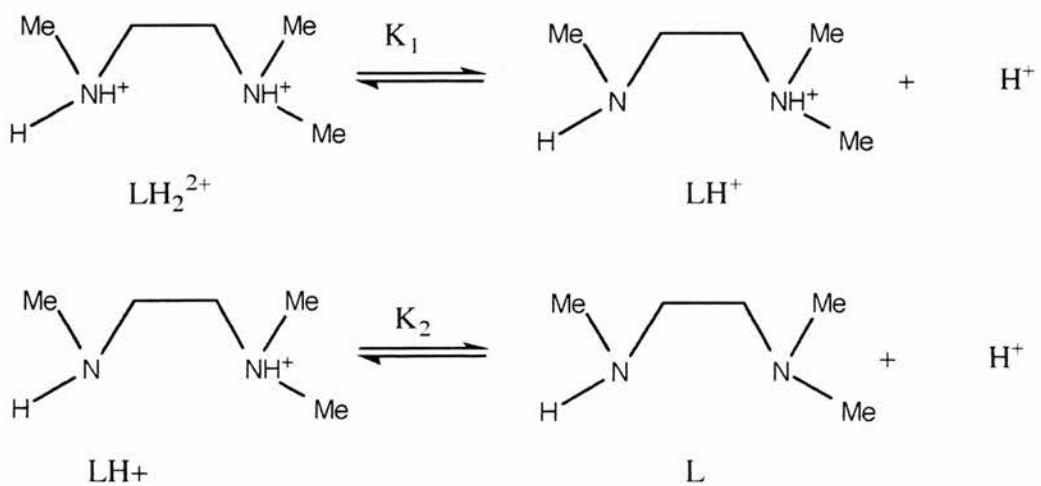


Table A.2.1 Typical Final output from Superquad.

```
SUPERQUAD.II - For the IBM-PC

   SUS    (.0005 M,   ),I=.1m KNO3

   MAXIT   IPRIN   MODE     TOL        ACCM          RELAC
    25       3       1   0.10E-03   0.10E-74   0.298023E-07

    Reactant 1-     S875
    Reactant 2-     H+

The Temperature of Solution(s) is 25.00 Degrees Centigrade

          Formation        Log     Refinement    Stoichiometric
          Constants       Betas       Keys        Coefficients

   A      5.0119E  9     9.7000        1          1   1
   B      2.5119E 16    16.4000        1          1   2
   C      0.1738E-13   -13.7600        0          0  -1
```

Estimated Constants

```
 2 Formation Constants to be refined

 2 Special Parameters to be refined

                                  Curve     Value
    Tot mMoles        H+            1    2.5000E-01
    Added Conc        H+            1   -1.0000E-01

    SUS    (.0005 M,   ),I=.1m KNO3
  5 Iterations
Refinement Terminated Successfully
  Chi-Squared = 21.11
  Chi Squared should be less than 12.60 at the 95 percent confidence le
  Sigma =     3.8684

                     Value       Rel Std Dev     Log Beta Std Deviation

Beta A Refined    5.96361E  9      0.0574         9.77551    0.02586     1  1
Beta B Refined    1.99611E 16      0.0889        16.30018    0.04042     1  2
Beta C Constant   0.17378E-13                   -13.76000                0 -1

                                Curve Initial Value    Final Value      Std De

 Tot mMoles          H+           1        0.25000       0.24804        0.0010
 Added Conc          H+           1       -0.10000      -0.10615       -0.0007

Correlation Matrix - Parameters ordered as above

      1    2    3
 2 0.82
 3 0.08 0.49
 4-0.46-0.10 0.74

 Residuals Plots - Units of SD     3.8684
```

Refined Constants

are pK_1 = 9.78+/-0.03 and pK_2 = 6.52 +/-0.04 at 25°C and I = 0.1moldm^{-3}. These values may be compared with the quoted pK's for ethylenediamine[1], pK_1 = 9.87 and pK_2 = 7.23 and I = 0.1moldm^{-3}.

The pK values obtained were used to construct the speciation curves shown in Figure A.2.1.

2. Ionisation constants of 1,7-diamino-4-azaheptane (dipropylenetriamine).

The ionisation constants of 1,7-diamino-4-azaheptane (dipropylenetriamine = dpt), the amine involved in the synthesis of [12]aneN3, were also determined by titration of the ligand hydrobromide at 25°C and I = 0.1moldm^{-3} (KNO_3).

The refined β values are summarised below:

$\log \beta_1$ = 10.70+/-0.006

$\log \beta_2$ = 20.43+/-0.01

$\log \beta_3$ = 28.23+/-0.01

giving pK_1 = 10.70, pK_2 = 9.72 and pK_3 = 7.81 relating to the equilibria

$$LH_3^{3+} \rightleftharpoons LH_2^{2+} + H^+ \; ; \; K_1$$

$$LH_2^{2+} \rightleftharpoons LH^+ + H^+ \; ; \; K_2$$

$$LH^+ \rightleftharpoons L + H^+ \; ; \; K_3$$

These constants may be compared with the only values quoted in the literature[2] pK_1 = 10.75; pK_2 = 9.60; pK_3 = 7.71 at 25°C and I = 0.1moldm^{-3} (KNO_3). The speciation curve is shown in figure A.2.2.

3. Solution chemistry of dioxo[12]aneN3 with copper (II) and Nickel (II).

The solution chemistry of dioxo[12]aneN3 with copper (II) and nickel (II) was also investigated. Figures A.2.3 and A.2.4 show potentiometric titrations of the ligand alone and in the presence of half an equivalent of the metal ion (ligand to metal ratio = 2:1). Significant

complex formation is observed.

4. Ionisation constants of N,N'-bis(2-cyanoethyl)-5,7-dioxo-1,4,8,11-tetraazacyclotetradecane

Potentiometric titrations of the ligand in the presence and absence of copper (II) and nickel (II) perchlorates were carried out[3]. The temperature was maintained at 25+/-0.1°C I = 0.1 moldm^{-3} ($NaNO_3$).The formation constants are defined according to equation (1) where m,l and h are the stoichiometric coefficients for the metal, ligand and proton respectively.

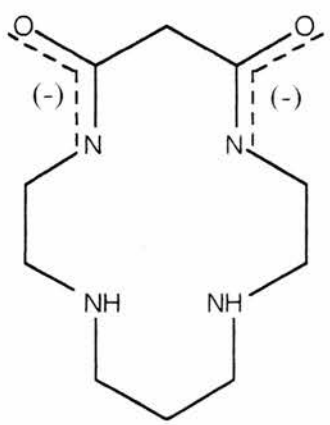
Equation 1

$$\beta_{mlh} = \frac{[MmLlHh]}{[M]m[L]l[H]h} \qquad (1)$$

Typical concentrations of the metal and ligand in a 1:1 ratio were in the range of $1x10^{-3}$-$3x10^{-3}$ mol dm^{-3}. The results were processed on a Zenith P.C. using the Superquad program.

Results and Discussion

N,N'-bis(2-cyanoethyl)-5,7-dioxo-1,4,8,11-tetraazacyclotetradecane (L) is easily prepared in good yield by refluxing dioxocyclam with acrylonitrile. The appearance of the strong band at 2248cm^{-1} υ(C=N) in the infrared confirms the cyanoethylation of the two sec-NH groups. Dioxo cyclam readily forms neutral complexeswith copper(II) and nickel(II) by deprotonation of the amide nitrogens resulting in the dianion (A2.1).



(A2.1)

It is expected that the basicity of the macrocycle would be reduced by alkylation of the two sec-NH donors with electron withdrawing 2-cyanoethyl groups. Potentiometric titration at 25°C and I=0.1mol dm^{-3}, in the presence of two equivalents of acid gave the pK values as pK_1=3.05, pK_2=5.94 relating to the equilibria.

$$LH_2^{2+} \rightleftharpoons LH^+ + H^+ \; ; \; K_1$$

$$LH^+ \rightleftharpoons L + H^+ \; ; \; K_2$$

The results previously obtained by Hay and Hassan for the pK values for dioxocyclam pK_1=5.87 and pK_2=9.62 confirm that a substantial reduction in the basicity of the cyanoethyl derivative was indicated.

The K_1/K_2 ratio is quite large for L and also for dioxocyclam and this is expected to be due to the electrostatic repulsion caused in LH_2^{2+}. In the more flexible ethylene diamine when a similar titration is carried out the pK values are: pK_1 = 7.28 and pK_2 = 9.98.

The interaction of Copper II with the ligand was also studied by titration of solutions of the macrocycle and copper II perchlorate (molar ratio L/Cu II =1:1) containing excess nitric acid.

The titration data can be rationalised in terms of the equilibria

$$Cu^{2+} + L \rightleftharpoons [CuLH^{-1}] + H^+ \qquad \log\beta_{11-1}=-3.43$$

$$Cu^{2+} + L \rightleftharpoons [CuLH^{-2}] + 2H^+ \qquad \log\beta_{11-2}=-9.18$$

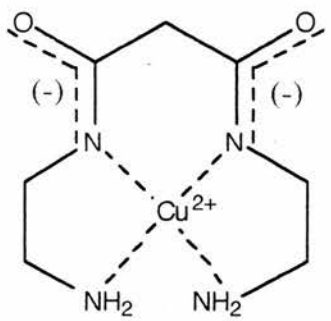
Where $\log \beta_{lmh}$ are the formation constants (l-ligand, m-metal, h-proton). When h is negative deprotonation of the complex occurs. The species $[CuLH^{-2}]$ begins to form at pH 5.2 and is some 80% abundant at pH6.6

The pKa for the ionisation

$$[CuLH_{-1}]^+ = [CuLH_{-2}] + H^+$$

can be readily derived as pKa = log β_{11-1} - logβ_{11-2} = 5.75 at 25°C.

The blue complex [CuLH$_{-2}$].2H$_2$O was characterised in the solid state. Conductivity measurements showed that the complex was a non electrolyte. It is paramagnetic with μ_{eff}=1.92B.M. at 18°C. The aqueous solution spectrum of λmax = 521nm (ε=107dm^{-3} mol^{-1}cm^{-1}) may be compared to that of dioxocyclam =506nm(ε=86dm^{-3}mol^{-1}cm^{-1}) for the copper complex and 516nm (ε=63) for the copper complex of N, N'- di(2-aminoethyl)-malo diamide.



The absence of absorption at υ(N-H) in the i.r. spectrum of the metal complex confirmed the deprotonation of the secondary nitrogen atoms, also exhibited in the nickel complex.
Titration data can be rationalised by the equilibrium

$$Ni^{2+} + L = [NiLH_{-2}] + 2H^{+} \qquad \log\beta_{11-2} = -14.45$$

Deprotonation of the amide sites occurs with increasing pH to give the square planar complex [NiLH$_{-2}$] λmax= 472nm (ε=81cm^{3}mol^{-1}cm^{-1}) due to $^1A_{1g}$ - E_g transition of the singlet ground state nickel ion.
The resulting orange complex [NiLH$_{-2}$]Ni[OH$_2$]$_2$[ClO$_4$]$_2$ i.r. spectrum has bands due to υ(OH) = 3360 cm^{-1}, υ(C=-N) (2200cm^{-1}) and ionic perchlorate (1100, 620 cm^{-1})(3).
The nitrile stretching vibration is lower than that for the free ligand indicating some interaction with the second nickel ion, by coordination with the nitrile and the octahedral coordination sphere completed by 2 H$_2$O molecules and in the solid state 2 ClO$_4^-$ ions. Being paramagnetic μeff=2.9B.M. at 18°Cis consistent with one nickel centre being diamagnetic and is a 2:1

electrolyte in water (Vm=195 $\Lambda^{-1}cm^2$ mol^{-1}) also the visible spectrum has weak bands attributable to the presence of an octahedral nickel(II) species. Hence it can be seen that the alkylation of the sec-NH donors of dioxocyclam has indeed greatly changed the properties of the ligands and complex.

Figure A.2.1 The Speciation Curve for the protonation of N,N,N',-trimethylenethylenediamine.

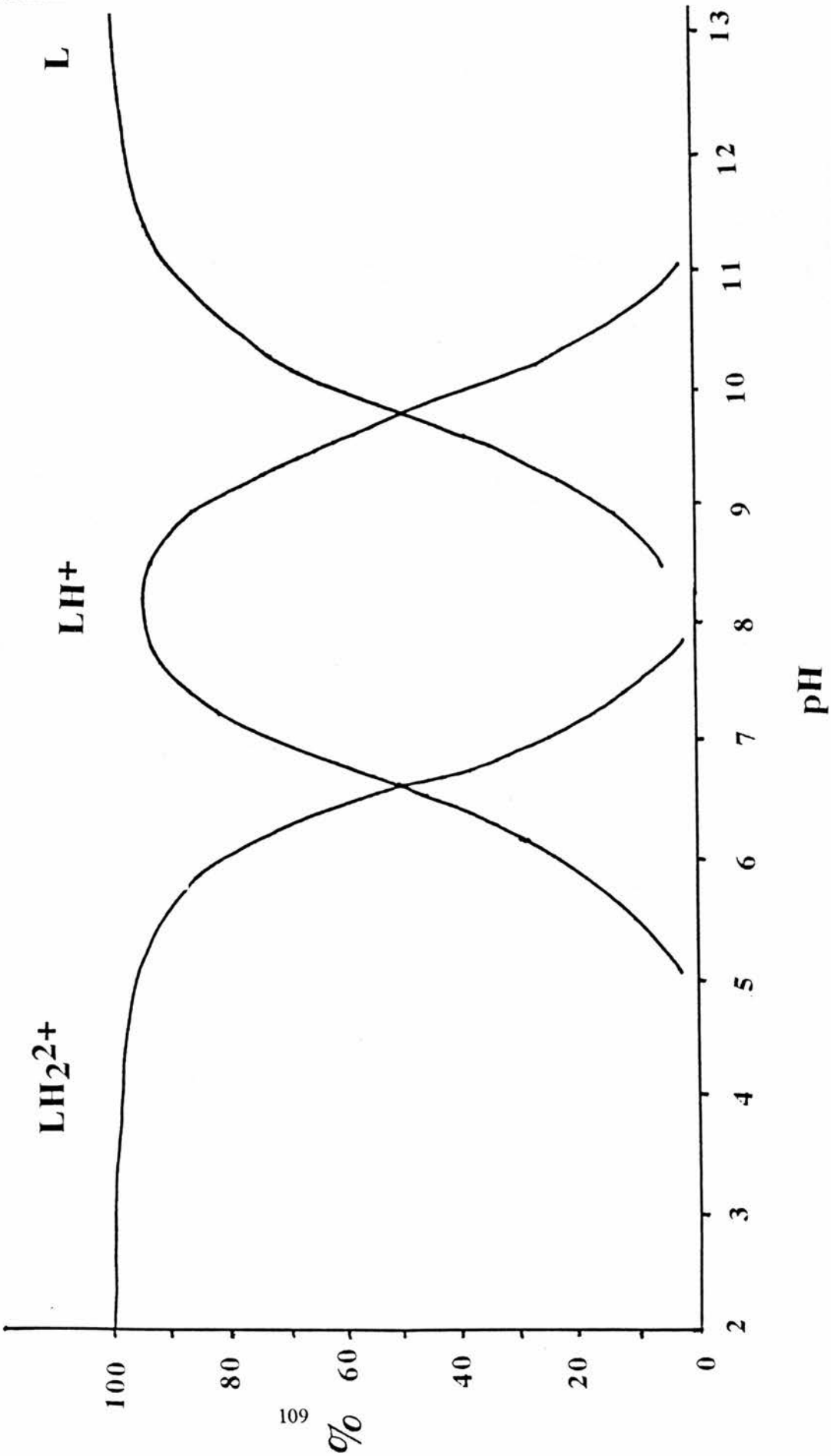


Figure A.2.2 The Speciation Curve for the protonation of 1,7-diamino-4-azaheptane.

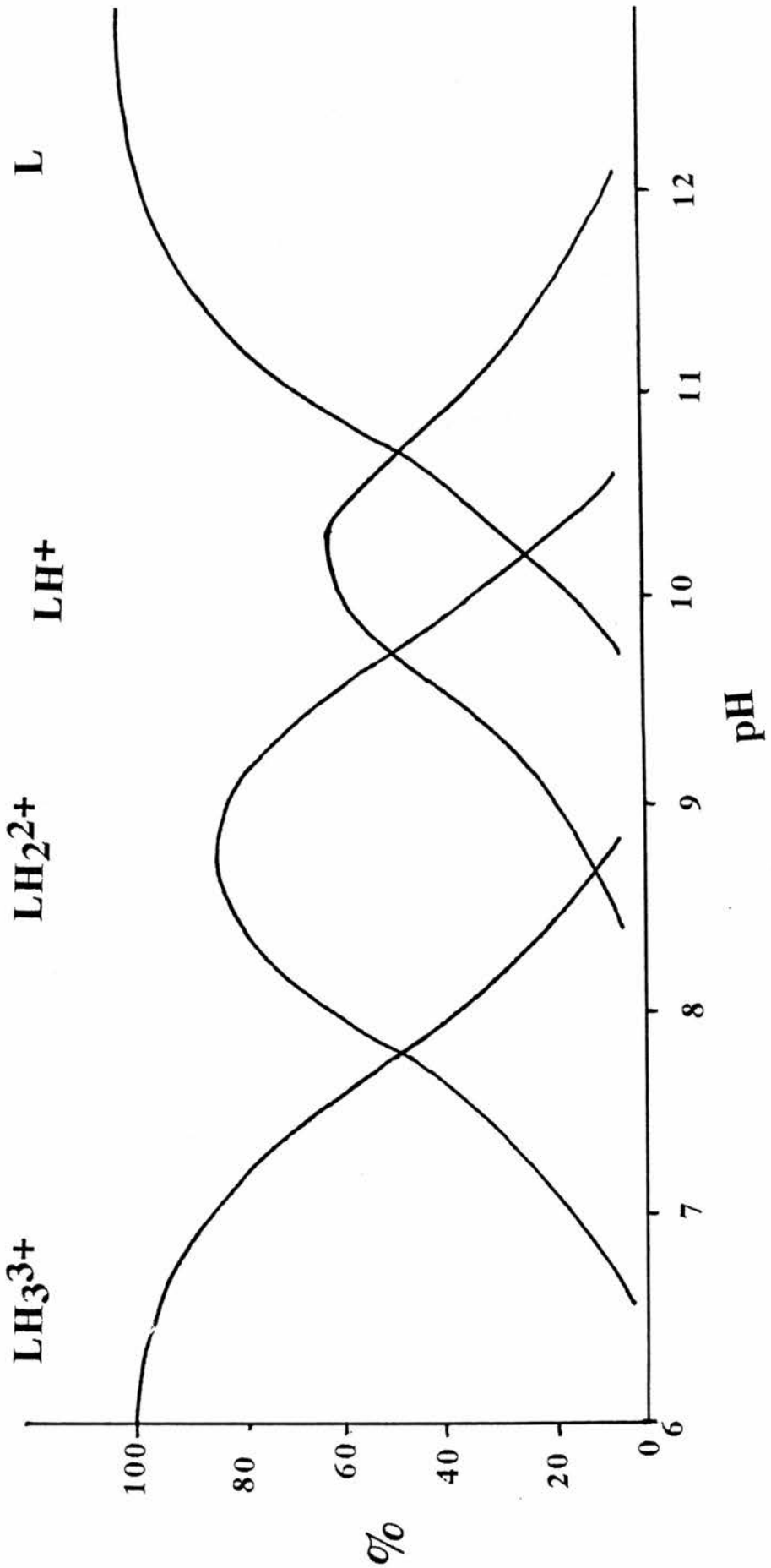


Figure A.2.3 Potentiometric Titration of dioxo[12]aneN3 alone and with Nickel II.

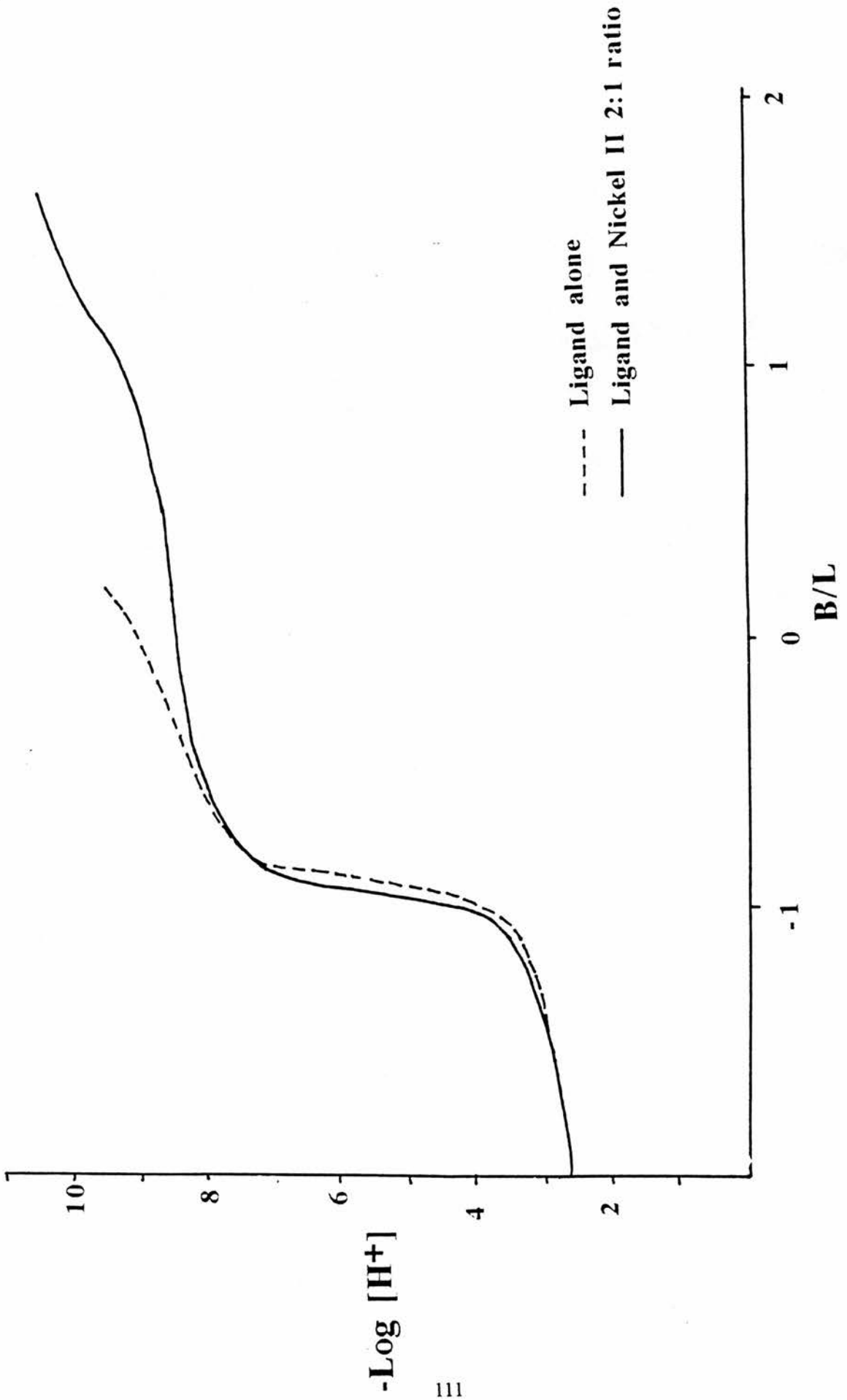
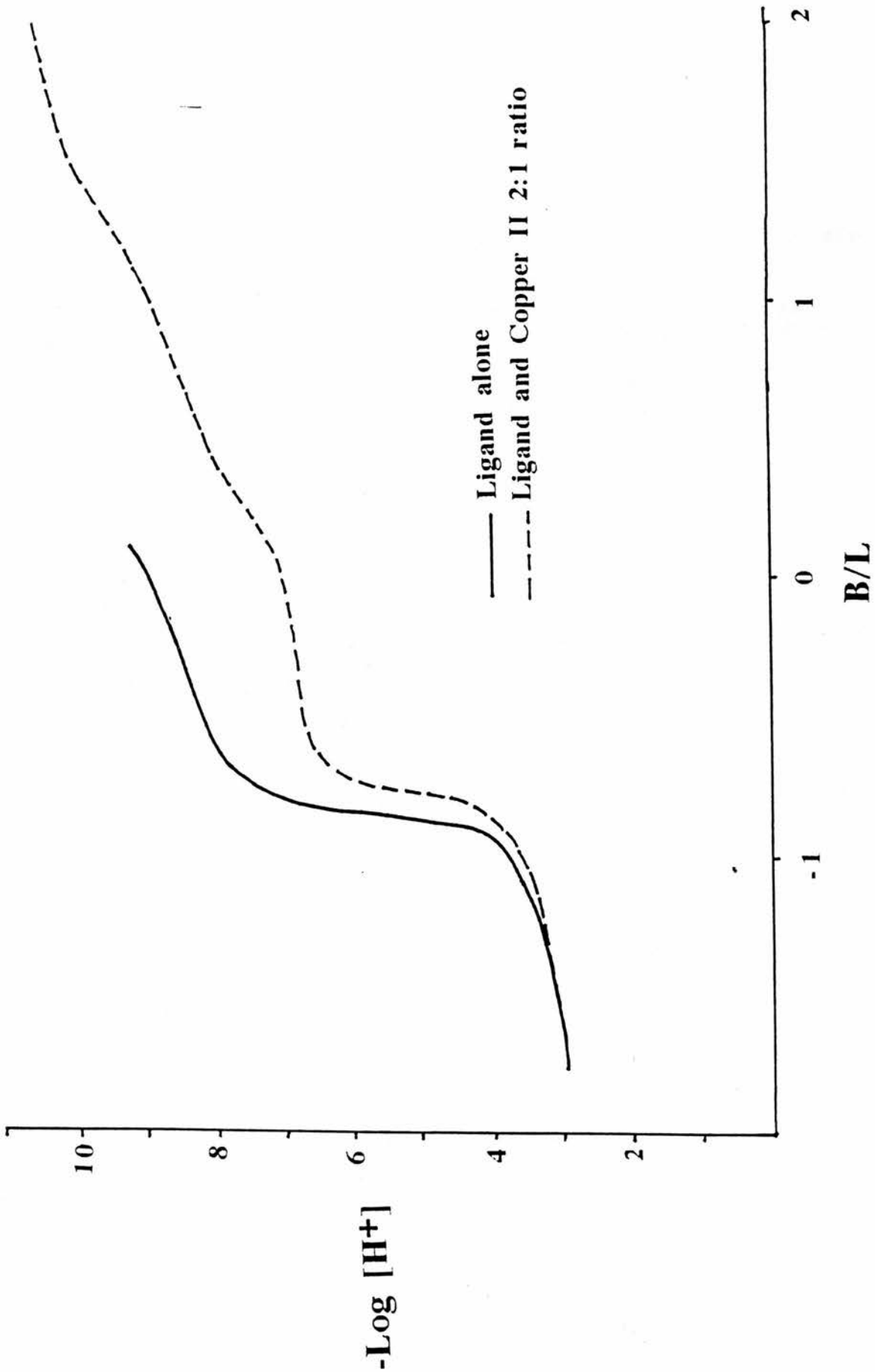


Figure A.2.4 Potentiometric Titration of dioxo[12]aneN3 alone and with copper II.



REFERENCES

1. R.G. Lancaste and A.E. Martell quoted in "Stability Constants", Special Publication No17, The Chemical Society, London 1964.
2. T. Arishima, K. Hamada and S. Takamoto, Nippon Kagoku Kashi, 6, 1119 (1973).
3. R.W. Hay, M. Tofazzal, H. Tarafder, M.M. Hassan and S.E. Blatchford, Trans. Met. Chem., 18, 55 (1993).