THE FUNCTIONALISATION OF WOOL BY TRIS(HYDROXYMETHYL)PHOSPHINE FOR METAL ION RECOVERY

THE UNIVERSITY OF
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Te Whare Wānanga o Waikato

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Abstract

Tris(hydroxymethyl)phosphine (THP) was prepared by the addition of a stoichiometric amount of base to tetrakis(hydroxymethyl)phosphonium chloride (THPC). Freshly prepared THP was successfully immobilised onto wool through a Mannich-type condensation reaction between a hydroxymethyl group and an amine on the wool surface, forming stable >P-CH_2-N< coupling links. The immobilisation of THP to wool stabilised the THP, which resulted in the decreased oxidation of THP to tris(hydroxymethyl)phosphine oxide (THPO).

The presence of immobilised phosphine groups was determined colorimetrically by reaction with Ni^{2+} ions, which produced a bright orange nickel-phosphine complex, as well as quantitatively, by measuring nickel uptake using ICP-MS. Immobilised THP-wool showed proportional binding for varying concentrations of metal solution. Decreasing or increasing the concentration of the metal solution resulted in a corresponding proportional response of metal binding. Following immobilisation onto wool, oxidation of the system by 6% H_2O_2 resulted in a reduced binding of 24% for Cu, 25% for Co, 27% for Ni, and 93% for Cd relative to unoxidised THP immobilised onto wool.

Additional modification of the THP-wool systems via reaction with amino acids and other related compounds overall did not appear to enhance the metal binding capacity relative to the unmodified THP-wool system. The only modified THP-wool system that showed either retention or an increase in metal binding capacity for all metals analysed was that of 2-aminopyridine, followed by oxidation with H_2O_2

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THE UNIVERSITY OF WAIKATO
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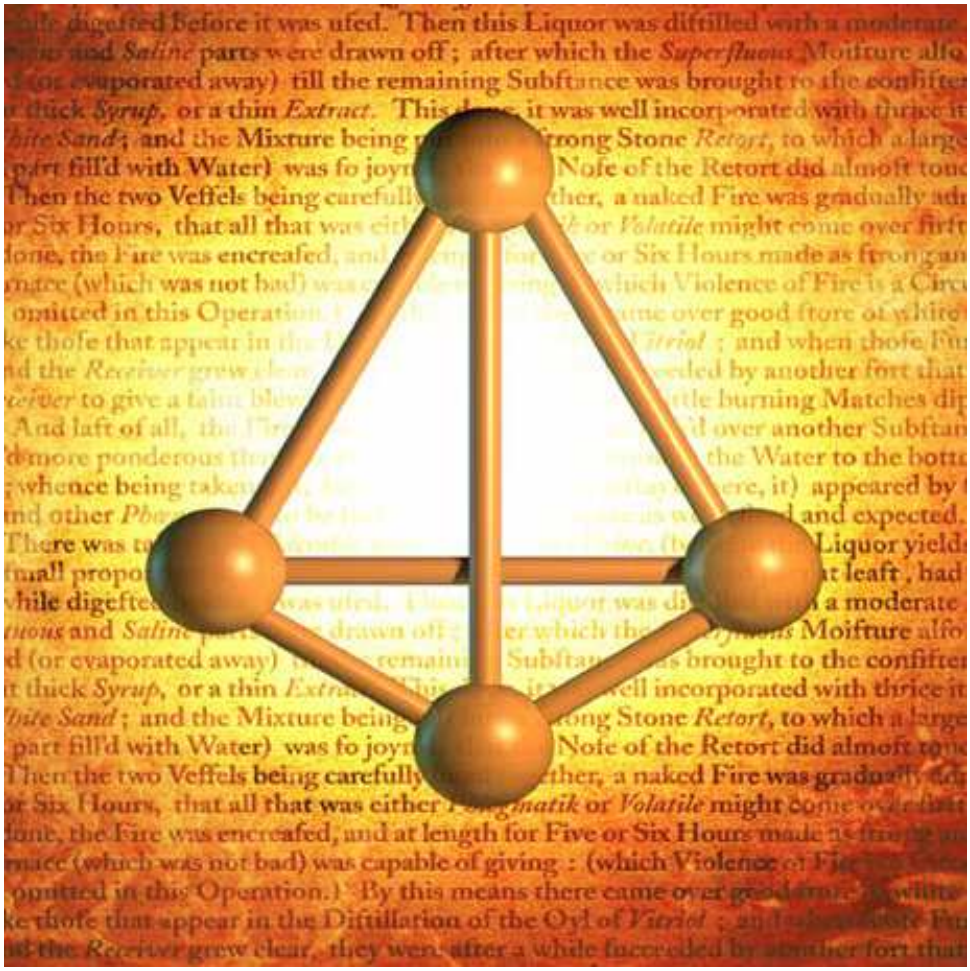
THP	tris(hydroxymethyl)phosphine
THPC	tetrakis(hydroxymethyl)phosphonium chloride
THPO	tris(hydroxymethyl)phosphine oxide
HMP	hydroxymethyl phosphine
bhpe	bis[bis(hydroxymethyl)phosphino]ethane
EtOH	ethanol
MeOH	methanol
NMR	nuclear magnetic resonance
SS	solid state
IR	infrared
SEM	scanning electron microscope
MS	mass spectrometry
ES/MS	electrospray mass spectrometry
EDAX	energy dispersive x-ray analysis
FF	flammable fabrics
FR	flame retardant



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Chapter 1: Introduction

1.1 Prologue

This project entails the investigation into the ability of wool-bound tris(hydroxymethyl)phosphine (THP), and modified versions thereof, to bind metals for their extraction out of an aqueous solution.

The following introduction has the aim of being a sound overview of the topic area which is relevant to this research. Thus, it seeks to provide background to the main areas of the thesis:

1. Synthesis of hydroxymethyl phosphines, and their subsequent reactions
2. Uses for hydroxymethyl phosphines, both in research and in industry,
3. Chemistry of wool

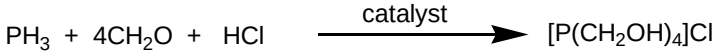
The aims of this section are given in Section 1.6.

1.2 Chemistry of hydroxymethyl phosphines

1.2.1. Synthesis

1.2.1.1 Tetrakis(hydroxymethyl)phosphonium chloride (THPC)

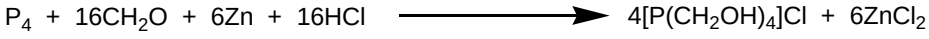
THPC is a precursor to THP (Section 1.2.1.2). The synthesis of THPC is primarily via the reaction of phosphine with formaldehyde and hydrochloric acid, as shown in Scheme 1-1. Whilst this method has undergone some slight modifications over the years, to specialise for certain end uses, it is largely the same as the one that Hoffman employed in 1921.[1]



Scheme 1-1: Conversion of phosphine gas to THPC

Phosphine (PH_3) however, is an extremely hazardous chemical compound which is known to pose serious health hazards.[2] Also in its pure form, phosphine gas is spontaneously inflammable at room temperature, with this instability being attributed to traces of diphosphine (P_2H_4) and also P_4.[3, 4] When utilising formaldehyde, hemiacetal formation can result, giving species with P-CH_2O-CH_2OH groups,[5, 6] though these species react as P-CH_2OH groups.

THPC can also be prepared by the reaction of yellow phosphorus with formaldehyde, hydrochloric acid and an electropositive metal such as zinc as shown in Scheme 1-2.[7]



Scheme 1-2: Formation of THPC via yellow phosphorus route

THPC (Figure 1-1 [a]) is predominantly marketed as an 80% aqueous solution. It is a colourless crystalline compound, which is very soluble in water and the lower aliphatic alcohols and insoluble in most of the common organic solvents.[8] As a crystalline solid it is highly hydroscopic.

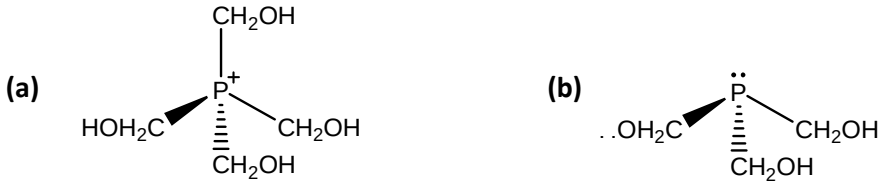
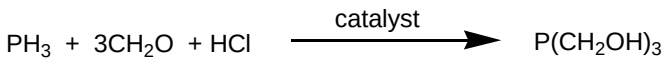


Figure 1-1: Structures of (a) THPC and (b) THP

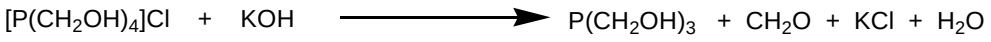
1.2.1.2 Tris(hydroxymethyl)phosphine (THP)

THP (Figure 1-1 [b]) was first prepared in 1958 by Reuter and Orthner.[9]



Scheme 1-3: Conversion of phosphine gas to THP

Scheme 1-3 shows that like THPC (shown in Scheme 1-1), THP can also be synthesised from phosphine (PH_3).

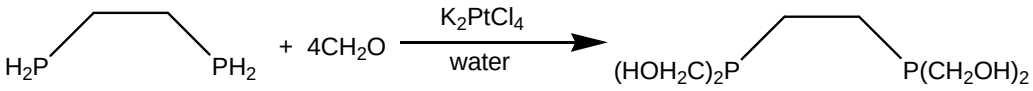


Scheme 1-4: THPC to THP

A far more convenient synthesis of THP is by the reaction of THPC with base (Scheme 1-4). This results in facile P-C bond cleavage at room temperature. Bases can include tertiary amines or stoichiometric OH^-. If excess OH^- is used this leads to the catalytic decomposition of the THP to the oxide, $(HOCH_2)_3P=O$ (THPO).[10]

1.2.1.3 Other hydroxymethyl phosphines (HMPs)

There are a wide range of HMPs that are utilised as ligands to metals. For example, one of these is the bidentate ligand bis[bis(hydroxymethyl)phosphino]ethane (bhpe)[11], which is formed (Scheme 1-5)[12] in a similar manner to many other HMPs, by reaction of the primary phosphine with formaldehyde.



Scheme 1-5: Formation of bis[bis(hydroxymethyl)phosphino]ethane (bhpe)

Numerous other hydroxymethyl phosphines are known and can be utilised as ligands for metal centres. These are of the type R_2PCH_2OH or $RP(CH_2OH)_2$, where R is an alkyl or aryl group. These can utilise the reactivity of Figure 1-2 [d] to substitute R groups either adding or removing hydroxymethyl groups to form

these derivatives. However the main route is that of RPH_2 undergoing the same chemistry as seen in Scheme 1-1.

1.2.2. Reactivity of hydroxymethyl phosphines

Hydroxymethyl phosphines (HMPs) display a wide variety of reactivity, of which some important uses can be obtained (Figure 1-2).

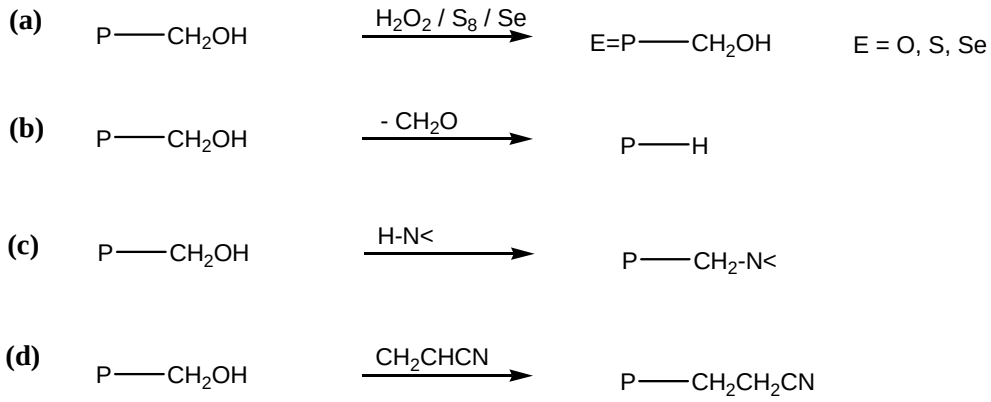
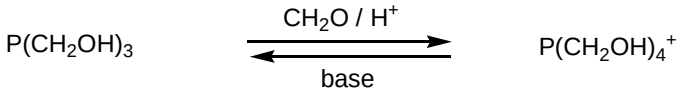


Figure 1-2: Selected reactions of hydroxymethyl phosphines

The formation of the P-C bond is quite reversible[13], as demonstrated with the ease of reaction, to and from, THP and THPC (Scheme 1-6). As illustrated in Figure 1-2 [b], the P-C bond can also be converted back to the P-H bond, with removal of formaldehyde.



Scheme 1-6: Reversibility of THP to THPC

HMPs react with amino-containing compounds very rapidly via a Mannich-type condensation reaction (Figure 1-2 [c]), yielding an extremely stable >P-CH_2-N< linkage.[14] This reaction allows for the possibility of cross-linkages and polymer formations.

Figure 1-2 [d] provides the opportunity for the formation of alkyl phosphines by addition of the P-CH_2OH group to an unsaturated compound with elimination of CH_2O. The resulting ligands, e.g. $P(CH_2CJ_2CN)_3$, are also of interest as ligands for metal centres.

HMPs, other than salts, are not known to react directly with alcohols.[14]

1.3 Immobilised Phosphines

Phosphines, such as triphenylphosphine (PPh_3), are extensively used in coordination and organometallic chemistry. Phosphorus based ligands such as PPh_3 are used in a variety of metal complexes, showing good binding to a range of transition metals. One such example is Wilkinson's catalyst,[15] $RhCl(PPh_3)_3$, which catalyses the hydrogenation of alkenes. Another example is Vaska's complex,[16] $IrCl(CO)(PPh_3)_2$. The list of complexes of phosphine ligands is substantial.

Hydroxymethyl phosphines also have a good ability to bind with a wide variety of transition metals overall, from the early (e.g. Re(V)) through to the late (e.g. Rh(I), Pd(II), Pt(II), Ag(I) and Au(I)), producing a wide variety of water-soluble transition metal complexes.[17, 18] In the literature, this has been one of the key features of interest for HMPs.

There has been considerable interest in the immobilisation of phosphines onto insoluble supports (e.g. silica, polystyrene). This facilitates the simple recovery of metal-phosphine based catalysts from reaction mixtures. A number of reviews cover this topic[19], and support (immobilised) phosphines are commercially available.

1.4 Applications of THP and related HMPs

Areas of use for hydroxymethyl phosphines, and in particular THP, which are going to be highlighted in this discussion, are:

1. Catalysis
2. Flame retardancy
3. Enzyme immobilisation
4. Biocide treatment, and bleaching and stabilising pulps
5. Medical

1.4.1. Catalysis

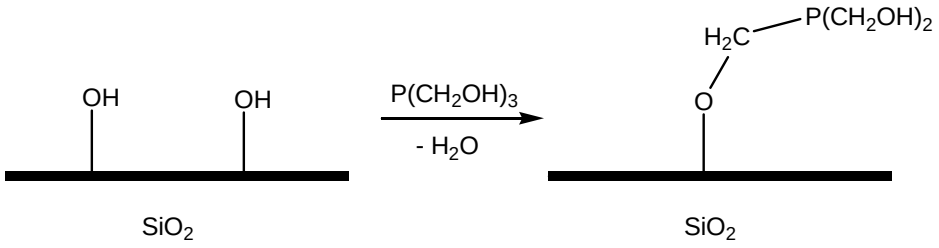
Catalysis is an extremely important chemical process where the rate of a chemical reaction is increased by the presence of a catalyst. This catalyst is not consumed in the reaction itself. Catalysis is used in over 90% of industrial chemical production worldwide, with processes such as the Monsanto[20] process and the Cativa[21] process, both well known for the production of acetic acid by the carbonylation of methanol.

Owing to THP's ability to bind to numerous transition metals, this makes it very worthwhile for investigations into its ability to act as a ligand for catalysts. Displacement of a bound ligand (e.g. PPh_3) by THP provides one route to the synthesis of catalytically active analogues of PPh_3 catalysts. In one such case initial thoughts concluded that simple displacement would occur via the reaction shown in Scheme 1-7, however it has been concluded that it was actually forming $Ru(THP)_2[PH(CH_2OH)_2]_2Cl_2$. Whilst two THP ligands bind normally, two others are actually modified, losing formaldehyde to form $PH(CH_2OH)_2$, demonstrating the reversibility of the P-C bond even further. This complex shows good potential for the hydrogenation and hydrogenolysis of lignin. [22]

Scheme 1-7: Displacement of PPh_3 ligands by THP in $RuCl_2(PPh_3)_3$

The addition of PH_3 to formaldehyde to give THP is itself catalysed by a range of platinum compounds. Both $[PtCl_2(THP)_2]$ and $[Pt(THP)_4].H_2O$ can be utilised for this catalysis, along with $Na_2[PtCl_6]$ or $K_2[PtCl_4]$. Investigations[6] into these Pt catalysts for this process found that $[Pt(THP)_4].H_2O$ is in fact in an equilibrium with the hydridoplatinum complex $[PtH(THP)_4]^+OH^-$. Other THP complexes are also known to catalyse this reaction, including $[Pd(THP)_4]$ and $[Ni(THP)_4]$.[23, 24]

$Ir_4(CO)_{12}$ is a good example of how THP that is immobilised onto a SiO_2 surface (Scheme 1-8), provides a good support surface for iridium carbonyl clusters to be stabilised. The THP ligands substitute CO to form $Ir_4(CO_{10})(THP/SiO_2)$ which is an active catalyst for the hydroformylation of ethane and partial oxidation of propene.[25]



Scheme 1-8: Immobilisation of THP onto a SiO_2 surface

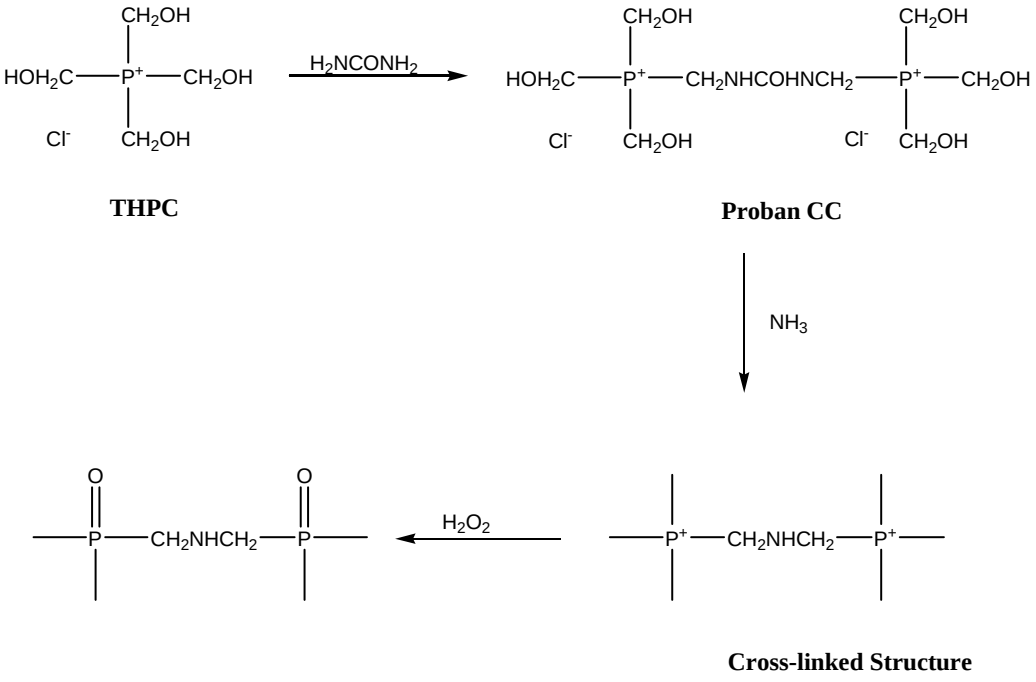
1.4.2. Flame Retardancy (FR)

In 1953 the Flammable Fabrics (FF) Act was passed in the USA. Following this, as well as an amendment in 1967 by the U.S. Congress, was a greatly accelerated research effort on cellulosics and other textiles. Research towards flame-proofing of cellulosics had in fact been underway for a number of years prior to the FF Act being passed, however this previous work was very much dwarfed in

comparison to the size and scale of research following the passing of the act. The main aims of the act were to improve and set stringent safety standards[26] in particular on children's sleepwear, as well as standards on other apparel fabrics.

FR treatment, in essence, is primarily focussed around increasing the amount of oxygen or heat required for combustion of a particular treated item to take place. Cotton, in particular, if ignited with sufficient oxygen present and heat input, will burn like most organic polymers. The FF Act aimed to have the apparel fabrics treated to a stage that once the flame or heat source is removed, then the fabric would be self-extinguishing. The background of FR is well covered in a review by Vail *et al.*[14]

The Proban™ process (developed by Rhodia, formerly Albright and Wilson) is one of two main flame-retardant treatments for textiles. This process utilises THPC and the reactivity of hydroxymethyl phosphines to amines (Figure 1-2 [c]).



THPC

Proban CC

Cross-linked Structure

Scheme 1-9: The Proban process

This process (Scheme 1-9)[27] forms an insoluble polymer in fibre voids and the interstices of the cotton yarn. The insoluble Proban polymer is held mechanically to the cellulose fibres and yarns, and does not chemically bond to the surface. The applied finish displays a susceptibility to hypochlorite bleach, which reduces the flame retardancy level.

The other main treatment is the Pyrovatex™ process (Ciba). Pyrovatex (Figure 1-3) is added to trimethylol melamine and the cellulose which is being treated. Unlike Proban, Pyrovatex does chemically bond to the cellulose. This process does not utilise hydroxymethyl phosphines, but does contain an organophosphorus compound.

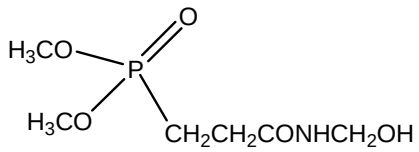


Figure 1-3: Pyrovatex

1.4.3. Enzyme Immobilisation

The immobilisation of enzymes is primarily focussed around cost efficiency. The cost of the enzyme is reduced significantly if the enzyme can be retained due to immobilisation, hence allowing significant re-use of the enzyme until the activity is diminished. There are many other advantages with enzyme immobilisation, such as improved stability and longevity, however a detailed discussion is outside the scope of this review. The main disadvantages of enzyme immobilisation are focussed around loss of activity, physical change due to immobilisation resulting in loss of potentially important functional groups, mass transfer, and prolonged operation.

Immobilisation of enzymes began with Nelson and Griffin in 1916[28], whereby

they monitored the activity of invertase when immobilised onto charcoal or aluminium hydroxide, finding that the activity was not diminished. Research on enzyme immobilisation continues to attract great interest.[29]

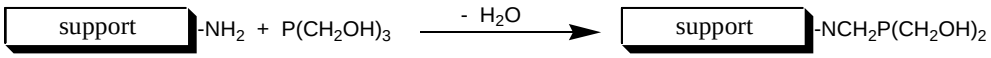
There are five different main methods for immobilising enzymes:

1. Adsorption
2. Covalent cross-linking
3. Encapsulation in a porous matrix
4. Entrapment in a polymer
5. Covalent binding

The first four methods have significant issues, for example adsorption results in relatively weakly bound enzymes and desorption may occur when temperature, substrate ionic strength or concentration change. Covalent binding does however often place great stress on the enzyme, which then frequently results in loss of activity. However it is the most applicable and applied technique for immobilisation of enzymes.[30]

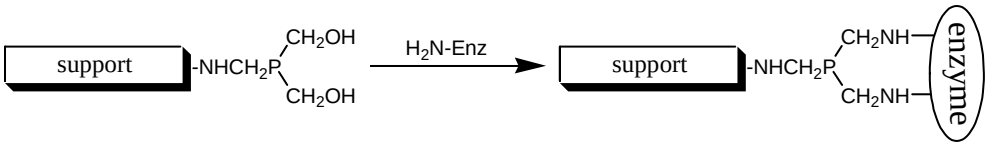
For covalent attachment of enzymes to a support matrix, it is important that only those functional groups of the enzyme that are not essential for the enzyme's catalytic processes, to interact and bind to the support matrix. The first published covalent immobilisation by Grubhofer and Schleith in 1953[31] attached pepsin and α-amylase to diazotised polyaminostyrene. Numerous coupling reagents, e.g. glutaraldehyde, have been applied to the immobilisation of enzymes since then, utilising groups such as amino ($-NH_2$), carboxyl ($-COO^-$), hydroxyl (-OH), indole, imidazole, phenolic, thiol (-SH) and threonine groups.

Recently, THP, a new coupling agent, was proposed for the covalent immobilisation of enzymes.[32] As mentioned previously in section 1.2.1.2, THP is synthesised by treatment of THPC with base. Due to THPC being manufactured on a mass scale for other industrial uses, it is both cheap and readily available.



Scheme 1-10: Mannich-type condensation reaction of THP onto an amino-containing support

Due to the reactivity demonstrated in (Figure 1-2 [c]), this allows amino-containing supports to immobilise THP onto the support (Scheme 1-10). Following this the two remaining hydroxymethyl groups are available to immobilise the enzyme (Scheme 1-11). This has been shown via a number of studies[32-36] to be useful for enzyme immobilisation. An example is shown in a recent publication by Cheng[35] which showed that when β-fructofuranosidase is immobilized to THP, the immobilized enzyme is both more thermally stable and also has a higher recyclobility factor, in comparison to when the enzyme was bound to glutaraldehyde or as the free enzyme.



Scheme 1-11: Mannich-type condensation reaction as used for the immobilisation of enzymes by THP

THP generation creates a nucleophilic phosphorus atom, resulting in more reactive hydroxymethyl groups compared to THPC. This leads to the one draw-back of THP, being the fact that THP is only moderately air-stable, oxidising to THPO in air. This has the effect of lessening the nucleophilicity of the central phosphorus atom, thereby reducing the reactivity of the hydroxymethyl groups. THPO is therefore unreactive towards amine groups under ambient conditions, allowing the reactivity of P-CH_2OH groups to be 'turned off' by (irreversible) oxidation to P(O)-CH_2OH groups. Modifications to the THP to try to find a completely air-stable analogue have taken place, with this proving successful in the case of ferrocenylmethylbis(hydroxymethyl)phosphine, [$FcCH_2P(CH_2OH)_2$], a completely air-stable, crystalline compound.[37]

1.4.4. Other uses

1.4.4.1 Biocide and Bleaching Pulp

THP and THP salts (e.g. THPC) are widely patented for uses such as a biocide and for industrial uses such as bleaching pulp. When these are reacted with various nitrogen-containing compounds they form a stable solid which utilises a P-C-N bond.[38, 39] Some oil fields and cooling water towers employ the biocidal use of THP and THP salt systems.

THP and THPC are both patented for use as a bleaching and brightness stabilisation technique for lignocellulosic materials.[40] The method employs use of THP and/or THPC to kill the catalase-producing bacteria and to destroy the enzyme that is found in the added pulping liquors, which are used in the bleaching of pulps by hydrogen peroxide.[40, 41]

1.4.4.2 Medical

THP as a ligand is very comparable in many respects to other phosphorus based ligands such as triphenylphosphine (PPh_3). A large advantage for THP being used as a ligand over other phosphorus based ligands in metal complexes for medicinal use, is the fact that the hydroxymethyl groups provide high solubility in water, which is ideal for drugs.

For example, THP has been utilised as a ligand on Cu(I), forming $[Cu(THP)_4][PF_6]$.[42] This Cu(I) complex produces a much higher *in vitro* antitumor activity compared to its reference drug cisplatin. Other hydroxymethyl phosphines have also been utilised, such as bhpe forming $[Cu(bhpe)_2][PF_6]$ (Figure 1-4). The work on this complex followed that of Pillarsetty[43] *et al*. who produced the $[Au(THP)_4]Cl$ complex, which too showed good antitumor capacity, following previous Au complex work.[44]

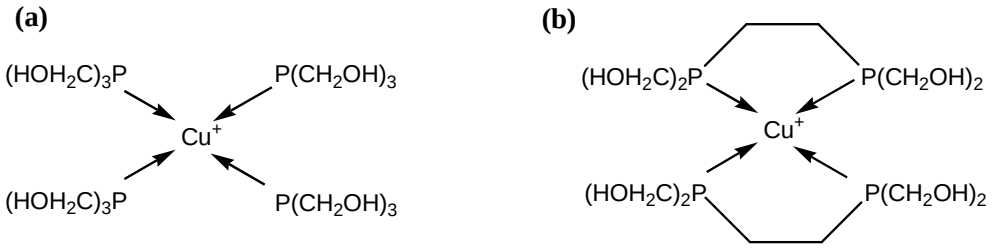


Figure 1-4: (a) $[Cu(THP)_4]^+$ (b) $[Cu(bhpe)_2]^+$

Katti *et al.* have been extensively researching in this area.[3, 17, 43-45] Of particular interest has been their work utilising rhenium with THP and other similar HMP ligands. ^{188}Re has been utilised as a therapeutic radioisotope, utilising the solubility of HMPs.[46]

1.5 Wool

Wool has for centuries played a vital part in society, namely in keeping people well insulated. It is non-conducting and is very durable for a wide range of uses by humans.

Wool contains a myriad of functional groups on its surface, primarily due to the fact that wool is a large protein made up of a number of amino acids. Wool contains a high sulfur content, due to the amino acid cystine (Figure 1-5).[47]

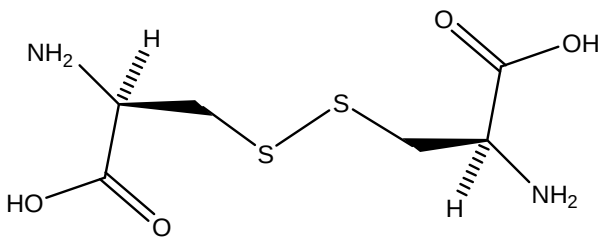


Figure 1-5: Cystine

Cystine is just one of many amino acids which are part of the protein keratin, of

which forms the basis of wool.[48, 49] Keratin exists in either the α or β form, with wool mainly consisting of keratin in the α form. The β form is dominant in other structures that grow from skin, such as nails. Another prominent amino acid in keratin is alanine, which also features similar functional groups to cystine, but excluding sulfur.

Chemical modification of the surface of wool has been carried out for a large number of years. Some of these modifications of the wool surface attempt to achieve the following:

1. To increase flame retardation of the wool (as discussed earlier)
2. To straighten wool
3. Colouration
4. Shrink resistance

The availability of numerous amino acid derived functional groups gives the ability to immobilise other species to the surface. For the research described in this thesis, we have employed a method similar to that used for flame retardation, whereby THP is bound to wool for investigations of metal ion recovery. Due to wool's functional groups, in particular amine and carboxylate, it does already possess some metal ion binding ability.[50]

Following the dramatic price spike for wool in World War II, there have been two small spikes in price, one being the commodity boom of the late 1970's and the other being the wool boom of the late 1980's.[51] However since this time, there has been a general decline in the real wool price, inflation adjusted, to historic lows. Wool is now a very cheap and easily accessible renewable resource, ideal for research, especially in these environmentally aware times.

1.6 Aims

The overall aim of this project was to investigate the ability of wool-bound THP, and modified systems thereof, to bind metals for their extraction out of an aqueous solution.

Each chapter contains a brief introduction. Chapter 2 is a small chapter which covers the experimental techniques.

Chapter 3 describes preliminary experiments. This includes investigations into areas such as the stability of THP, when bound or non-bound to wool, what conditions are important for the binding of THP to wool, as well as the factors affecting the wool-THP system's metal ion recovery. The experimental of each investigation is detailed separately within each area in this chapter.

Taking into account the findings in Chapter 3, these were to shape and model the experimental and to aid the understanding of findings discussed in Chapter 4. Chapter 4 investigates the modification of the wool-THP system via reaction with varying amino-acids and the effect of these modifications on the ability to bind metals from aqueous solution.

The final chapter, Chapter 5, is the conclusion of the research. This chapter also includes recommendations for future work in the topic area.

Chapter 2: Experimental Techniques

This chapter covers the general experimental techniques employed in this research, with details of both solvents and materials used, together with instrument details. It also describes the preparation of tris(hydroxymethyl)phosphine (THP).

2.1 General experimental techniques

All work was conducted under atmospheric conditions, due to the complexity of handling large numbers of samples in each batch of work. Nitrogen was utilised for storage of NMR samples overnight when minimisation of sample oxidation was required.

After reaction with THP or further reaction with amino acids and/or metals, the samples were dried on paper towels on the bench; this method allowed for effective drying of large numbers of samples at the same time. Samples were batch weighed in one sitting to reduce any variance between samples. This ensured that atmospheric conditions, primarily humidity, would have a minimal effect on the results, as humidity would be consistent within the batch. Humidity can affect sample results by varying the weight of the samples, due to moisture content in the samples.

2.2 Solvents

Deionised water was used throughout this research and was generated via reverse osmosis followed by deionisation; the resistivity always exceeded 16 Mohm cm^{-1}.

Both drum grade methanol and ethanol (95%) were used without further purification.

2.3 Materials

80% (w/w) aqueous tetrakis(hydroxymethyl)phosphonium chloride (THPC) was obtained from Albright and Wilson Ltd. UK (now Rhodia).

Amino acid samples were purchased through Sigma-Aldrich and were reagent grade or higher and used without further purification.

Wool samples were standard white knitting wool of 8 ply thickness. The brand was Cleckheaton. Lots 742587, 746458 and 741432 were used.

2.4 Instrumental techniques

2.4.1. Nuclear magnetic resonance (NMR) spectroscopy

^{31}P NMR solution spectroscopy was performed on a Bruker Avance 300 MHz instrument, with D_2O as the lock solvent. All spectra were recorded in 5 mm glass tubes. ^{31}P spectra were recorded proton-decoupled.

^{31}P NMR solid-state spectroscopy was performed on a Bruker Avance DRX 200 MHz instrument, with MAS spinning speed of 5 KHz. 10,000 scans were acquired for all samples, with continuous wave ^{1}H decoupling used. All spectra were recorded in a 4 mm ZrO_2 tube with a Kel-F cap. A 4 mm MAS broadband multinuclear probe (Bruker) was used for all spectra. Samples were analysed at Scion Research, Rotorua.

2.4.2. Electrospray Mass Spectrometry (ES/MS)

Mass spectra were obtained on a VG Platform II Electrospray Mass Spectrometer. The solvent used was 1:1 methanol – water.

2.4.3. Scanning Electron Microscope (SEM)

Microscopic analysis was conducted on a Hitachi S-4700 Field Energising (FE)

Scanning Electron Microscope (SEM). Imaging was conducted at 5 kV except for EDAX which was conducted at 20 kV. Samples were platinum coated using a Hitachi E-1030 ion spotter coater.

2.4.4. Fourier Transform Infra-Red Spectroscopy (FT-IR)

Spectra were obtained using a Perkin Elmer Spectrum Spotlight FT-IR Microscope, employing a liquid nitrogen cooled MCT detector with 4 cm^{-1} resolution. Samples were analysed in reflectance mode using a gold mirror as a background. Spectrum software and Spectrum Image software was used.

2.4.5. Inductively Coupled Plasma (ICP) Mass Spectrometry

Analysis was conducted on a PerkinElmer SCIEX ICP-MS ELAN DRC II, using software ELAN v3.3. Samples were introduced via a CETAC ASX-520 autosampler, holding a maximum of 240 samples. It features a SeaSpray nebuliser and a baffled Quartz cyclonic spray chamber. A sample flow of 1 mL/min was utilised. The nebuliser gas flow was 0.92 L/min.

All samples were acidified using 2% HNO_3, unless otherwise noted. All results were multiplied by a factor of 1.02, to correct for the dilution effect of acidification.

2.5 Preparation of starting materials

2.5.1. Synthesis of tris(hydroxymethyl)phosphine (THP)

THP solution was synthesised from tetrakis(hydroxymethyl)phosphonium chloride (THPC) and a stoichiometric amount of KOH, according to (Scheme 1-1). [10, 34]

$$[P(CH_2OH)_4]Cl + KOH \longrightarrow P(CH_2OH)_3 + CH_2O + KCl + H_2O$$

Scheme 2-1: The reaction of THPC to THP

THPC (3 g, 80% w/w aqueous solution) was weighed out into a clean flask and diluted to 30 mL with deionised water. A freshly-prepared solution of KOH (0.707 g) in 10 mL deionised water was added dropwise to the stirred THPC. This immediately generated a solution of tris(hydroxymethyl)phosphine (1.56 g in 40 mL). The THP solution was used on all occasions as soon as possible after generating, to minimise air oxidation.

Chapter 3: Preliminary investigations

3.1 Introduction

This chapter describes preliminary work carried out to investigate the behaviour of THP, when both bound and non-bound to wool, and also to look into varying methods for both qualitative and quantitative investigations into THP systems.

Overall, the investigations carried out here shaped the reaction conditions for the quantification of metal binding for the THP-wool systems described in Chapter 4.

3.2 Instruments

A variety of instruments were utilised, or attempted to be utilised, throughout this project. This section takes a brief look into each instrument and the problems or solutions that each instrument provided.

3.2.1. Scanning Electron Microscope (SEM)

Previous work which utilised the immobilisation of THP on aminopropyl silica showed that the SEM was a powerful method for looking at immobilised *Escherichia coli. (E. coli)*.[52] For that study it proved to be a very useful for investigating the morphology of the system, so a similar application for SEM was sought for this research.

1 g of wool (6 pieces) was placed in a freshly prepared solution of THP (0.31 mol/L, 200 mL) for 1 hr. These samples were then removed, and washed in 200 mL of water for 5 minutes. Three pieces were then added to a freshly prepared saturated solution of Ni^{2+} for 1hr, then washed in water (200 mL) for 5

minutes. Samples were then left overnight to dry in air and then analysed by SEM.

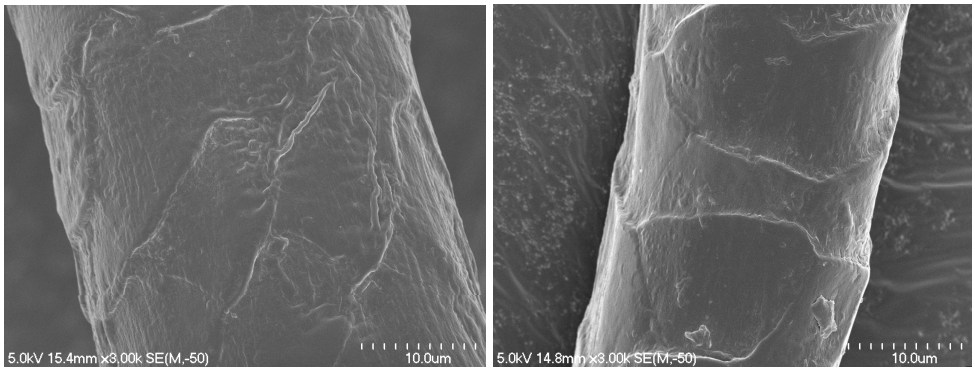


Figure 3-1: SEM images showing on left (a) native wool and on right (b) THP functionalised wool

Samples that were analysed were: (a) native wool, (b) THP functionalised wool and (c) THP functionalised wool that was then reacted with a high concentration of nickel solution. The morphology of the wool appeared not to visibly change (Figure 3-1).

EDAX analysis has also been attempted to determine elemental composition. This analysis was not able to be conducted on the samples due to the wool being very 3-dimensional to analyse, resulting in large variations. The ideal sample for EDAX analysis is for a sample that has very little to no 3-dimensional characteristics to it.

3.2.2. Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR is a powerful investigative method for identification of chemical changes to a species. ^{31}P is able to be analysed via NMR. Since THP and corresponding hydroxylmethyl phosphines contain phosphorus, this means that when samples are reacted and modified successfully a chemical shift would be expected to reflect the chemical reaction that has taken place.

For this two methods were utilised, solution ^{31}P solution state NMR and ^{31}P solid state (MAS) NMR.

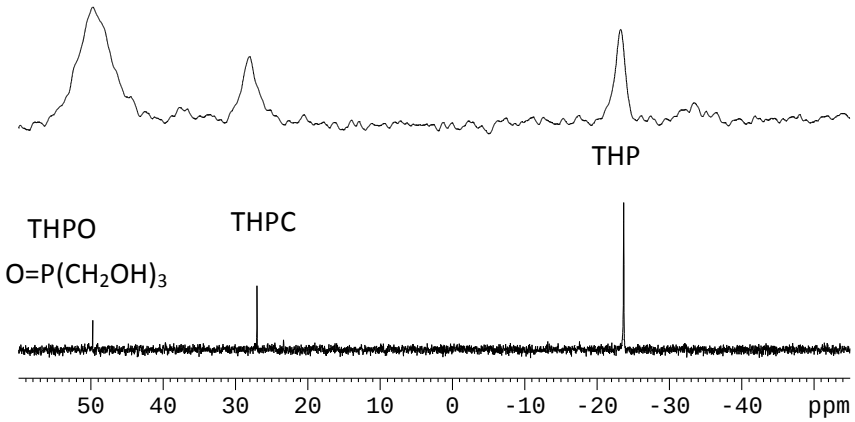


Figure 3-2: ^{31}P Solid state (MAS) NMR of THP-wool, top, and ^{31}P solution state NMR of THP below

A freshly prepared solution of THP was produced (0.31 mol/L, 80 mL). This was analysed using solution state ^{31}P NMR. A portion of this THP solution (40 mL) was then reacted with 0.5 g of wool for 1 hr with the sample being washed for 1 minute, in 20 mL of water. The THP-wool was then analysed after 3 days by solid state ^{31}P NMR.

Peaks in the solid-state NMR correspond to those peaks seen in solution-state NMR for a sample of THP (Figure 3-2). Presumably this is due to residual THP solution present which had not washed off properly.

3.2.3. Infrared Spectroscopy (IR)

Once bound to wool, analysis utilising a microscope FT-IR was unsuccessful as there was no notable variation in the spectra between the different samples. A P=O absorption would be expected around the 1300 - 1140 cm^{-1} region[53] for a THP-wool system oxidised with 6% H_2O_2, but this is not seen, likely due to the

fact that the phosphorus loading is low(ca 1%) so it is difficult to distinguish, in the presence of other absorptions in the same region of the spectrum.

3.2.4. Elemental Analysis

A wool (0.5 g) sample was reacted with a freshly prepared THP solution (0.31 mol/L, 30 mL) for 1 hr. Following this, it was then washed in water (200 mL) for 5 minutes. Both the THP-wool and native wool were subjected to micro-elemental analysis to determine the amount of phosphorus in the samples.

A sample of native unmodified wool was found to contain phosphorus below detection limits, of 0.1% by weight. Analysis of THP functionalised wool found it contained 1.11% by weight.

3.2.5. Atomic Absorption

Attempts were made to analyse samples of THP modified wool, after reaction to nickel solutions. Limited literature[54] could be found on analysis of wool for nickel, but there were some literature that focused on metal analysis from hair[55-57]. Many of these papers focussed on utilising a Graphite Furnace AA, which was not available. Other specialised analytical equipment was also not available, for example, a closed-vessel microwave digestion system.

The main problems for utilising the Atomic Absorption Spectrometer (AAS) were the fact that the instrument had varying detection limits and lacked the ability to analyse all metals. The majority of papers worked around the requirement to acid digest the samples to extract the metals for analysis. The ashing and then acid digestion of samples was impractical for such large numbers of samples. Initial investigations showed large variability between extraction from differing acids, and acid strengths.

Analysis of the solution both before and after wool samples had been suspended in the solutions was the logical way to overcome digestion problems. Utilising a known amount of solution, of a known concentration, one could then quantify the metal binding of the wool systems.

Utilising the AAS for this meant that analysis of the solutions would be difficult due to its higher detection limits compared to the inductively coupled plasma mass spectrometer (ICP-MS). It was decided that due to the ease of analysis, the low detection limits and the wider range of metals that could be analysed for the ICP-MS, that this was the preferred method.

3.2.6. Inductively Coupled Plasma (ICP) Analysis

The ICP has the ability to analyse almost all metals, therefore having a wide scalability. A range of metals (Hg, Ag, Ni, Co, Cu, Cd) were screened for analysis. The metals chosen are all softer metals with an affinity for soft ligands, such as phosphines, so were seen to provide a good overview for the screening of THP modified wool and further modified systems. Detailed analysis of the data proved in Section 4.4.1 that the ICP was a method capable of accurately analysing most metals.

3.2.6.1 Mercury Analysis

Mercury was to be one of six metals to be screened to compare the metal binding efficiencies of the wool when bound to THP systems. Mercury is a metal that is extremely problematic in the environment, hence if

After deciding to utilise ICP-MS for analysis, mercury was one metal could be successfully analysed using ICP-MS. The analysis of mercury on the ICP-MS required that instead of samples having 2% conc. HNO_3 by volume, samples would have by volume 1% conc. HCl and 1% conc. HNO_3, resulting in 2% by volume acidification like the other samples.

Analysis of mercury using ICP-MS is normally done at extreme trace level, in the very low ppb range. The initial results utilising mercury showed a great amount of variation, which was unviable given the need for accurate results. In one instance, the same sample analysed as a triplicate produced results with over 75% variation.

To try to overcome this variation, additional flushes were conducted in between samples being analysed – as well as additional HCl flushes. However none of these measures proved fruitful, so no further studies using mercury were carried out.

3.3 Stability of THP

3.3.1. NMR analysis

As part of this study, it was desirable to undertake a study of the air-stability of THP; this was necessary in order to develop an immobilisation protocol which would minimise loss of THP by oxidation to its oxide THPO, $OP(CH_2OH)_3$.

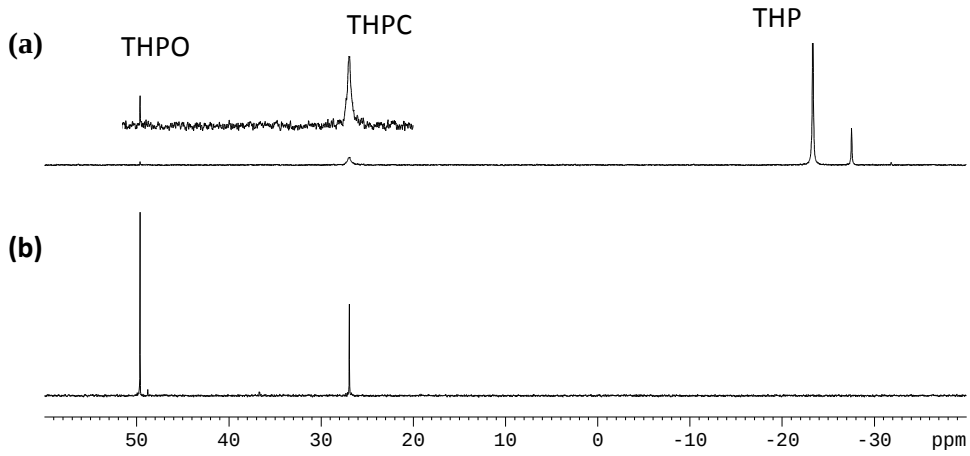


Figure 3-3: ^{31}P NMR of THP after a period of time of (a) 1 hr and (b) 5 days.

A freshly prepared sample of THP (0.31 mol/L, 40 mL) was prepared, and

analysed by ^{31}P NMR in a D_2O/H_2O solution. This solution was left exposed to air and was reanalysed after 5 days.

Figure 3-3 shows that when non-bound tris(hydroxymethyl)phosphine (THP) is exposed to air it will readily undergo oxidation to form tris(hydroxymethyl)phosphine oxide (THPO). After a period of 5 days all THP in aqueous solution is found to convert to THPO, with some also converting back to its precursor tetrakis(hydroxymethyl)phosphine chloride (THPC). The peak observed in (a) at -28 ppm is a hemiacetal (Figure 3-4).[5, 6]

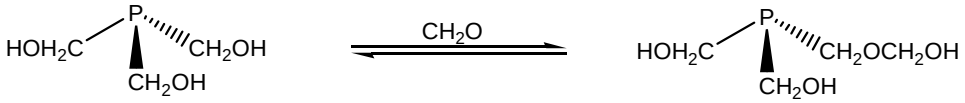


Figure 3-4: Hemiacetal formation between P-CH_2OH groups and CH_2O

3.3.2. Nickel immobilisation to THP modified wool over time

The presence of immobilised THP can be colorimetrically determined by reaction with Ni^{2+} ions. Pale green octahedral Ni^{2+}, when converted to square-planar Ni^{2+}, changes colour to a deeper orange. Softer ligands such as sulfur and phosphorus prefer to form 4-coordinate square planar species.[58] Therefore any change in colour observed is therefore likely to be due to the presence of reactive THP groups bound to the surface of the wool, having not oxidized. THPO does not have the ability to form stable nickel complexes.

Quantifying the amount of nickel binding, via ICP-MS, enabled the level of immobilisation of the THP onto the wool support to be established. ^{31}P solution NMR of THP showed a relatively rapid (5 days) degradation to THPO which results in no THP being left in solution. Thus, by utilising the nickel solution, we can determine if immobilising THP to the surface of the wool increases its stability, via its nickel binding capacity.

3.3.2.1 Method

Tris(hydroxymethyl)phosphine (THP) was prepared in accordance with the standard procedure. 400 mL of THP solution (0.31 mol/L) was prepared. Just over 8 g of wool consisting of 60 pieces of wool cut to a length of around 20 – 25 cm, was reacted with the freshly prepared THP solution for exactly 3 hrs.

Following the reaction, the wool was then washed in water, and dried overnight. Three pieces of wool were then reacted with a nickel solution (0.15 mol/L, 20 mL) for 1 hr. The remaining solution was then removed for ICP-MS analysis, then acidified with 2% conc. HNO_3.

Samples were analysed every day for the first week, then once a week thereafter. The monitoring occurred for five weeks. Triplicates were used initially as well as at the one week and three week stage, to monitor the variation.

3.3.2.2 Results and Discussion

Colour was a good basic determination as to if the nickel content that was being immobilised onto the wool was changing. Visually one was unable to see any difference. The ICP-MS results also concluded that over the 35-day period, the immobilisation of nickel by the THP modified wool system did not vary, sitting constantly around 3.0 mmol Ni / g of wool throughout the analysis period (Figure 3-5). This indicates that by being bound to the wool, the phosphorus is stabilised towards oxidation, thus retaining its ability to bind to nickel. This seems at odds with solid-state NMR, though it does concur with FT-IR, where no P=O absorption could be ascertained.

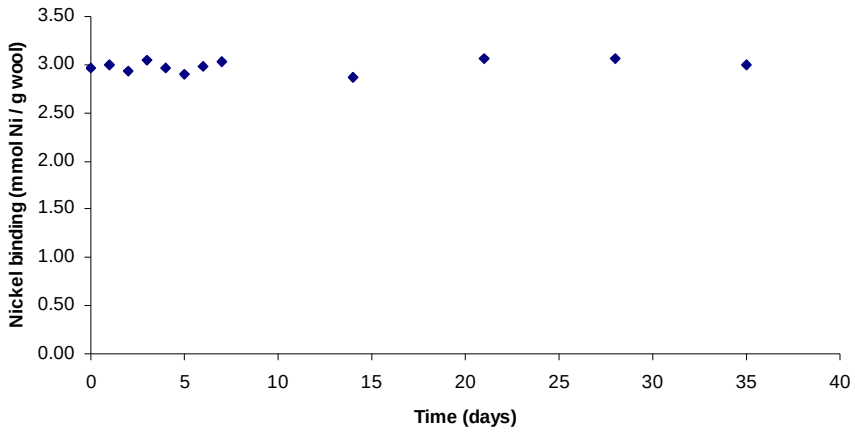


Figure 3-5: The relationship between the exposure time of THP modified wool in air and the subsequent metal binding

The variation both at the beginning (day 1) and end (day 45) showed little change, with a coefficient of variation of 3.5 %.

3.3.2.3 Conclusion

The immobilisation of THP onto wool stabilises it sufficiently, so that over a period of 45 days no distinguishable difference occurred in the nickel binding rate out of aqueous solution.

3.4 Optimisation of binding conditions

3.4.1. Time in metal solution

It is important to understand how long the wool and modified wool systems should be placed in the metal solution, to ensure that there is sufficient metal loading, whilst ensuring practicality is achieved.

3.4.1.1 Method

A fresh solution of THP (400 mL, 0.31 mol/L) was prepared in accordance with

the standard procedure (2.5.1). Just over 7 g of wool consisting of 54 pieces of wool of approximate length 20-25 cm, were reacted with the freshly prepared THP solution for 1 hr. The wool samples were then washed with water (200 mL) for 5 minutes. Samples were then left overnight (12 hrs) to dry.

Samples were then split and reacted in nickel solution (20 mL, 2.98 mmol) for varying lengths of time as shown in Figure 3-6, after which the samples were removed and dried. The remaining solution was then pipetted for ICP analysis, then acidified with 2% conc. HNO_3.

3.4.1.2 Results and Discussion

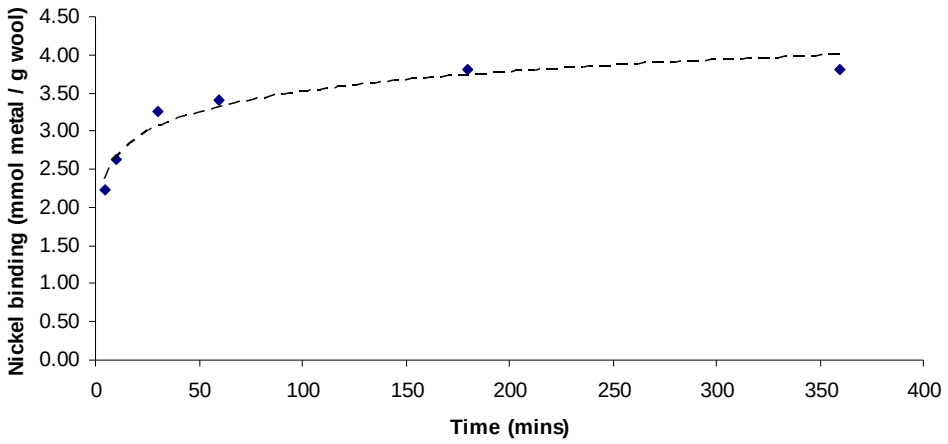


Figure 3-6: The relationship between exposure time of THP modified wool in nickel solution and the subsequent metal binding

Results (Figure 3-6) show an enhancement of metal binding practically until the 3 hr mark. The difference between having the THP modified wool sit in the metal solution for 3 hrs or 6 hrs is essentially negligible.

Reacting the THP modified wool in the metal solution for only 1 hr leads to a decrease in metal binding of only 12% compared to having it in the metal solution for 3 hrs.

Subsequent experiments used a nickel solution exposure time of 1 hr.

3.4.2. Concentration of THP solution

Variation of the concentration of the THP solution was investigated for the effects that this has on the THP-wool metal binding, again monitored using Ni^{2+} binding.

3.4.2.1 Method

A series of THP solutions of different concentrations were prepared. 8 solutions of THPC (3 g, 80% w/w solution, 0.0128 mol), diluted to 30 mL, were created. Added to these were fresh KOH solutions (10 mL), of varying concentrations (Table 3-1), generating varying concentrations of THP solution.

Table 3-1: Table illustrating the varying concentrations of THP generated

	KOH	[KOH]	[THP]
	g	*mol/L*	*mol/L*
A	0.1095	0.195	0.049
B	0.2110	0.376	0.094
C	0.3061	0.546	0.136
D	0.4363	0.778	0.194
E	0.5045	0.899	0.225
F	0.6531	1.164	0.291
G	0.7341	1.265	0.316
H	0	0	0

9 pieces of wool (1.4 - 1.6 g), of approximate length 20 – 25 cm were placed in each freshly prepared THP solution for exactly 1 hr. Samples were held submerged for the about a minute to ensure that the wool would be wet with the THP solution. The wool samples were then washed with water (200 mL), for

5 minutes. Samples were then left overnight (12 hrs) to dry.

Samples were then split, for triplicate analysis, and reacted in nickel solution (20 mL, 3.13 mmol, 0.157 mol/L) for 3 hrs, after which the samples were removed and dried. The remaining solution was then removed by pipette for ICP-MS analysis, following acidification with 2% conc. HNO_3.

Nickel concentrations were subtracted from the internal standard **H**, which contained no KOH, so subsequently no THP was generated. These were then converted to provide the metal binding per gram of wool.

3.4.2.2 Results and Discussion

Table 3-2: Effect of THP concentration and nickel binding

	[THP]	Ni
	mol/L	*mmol metal /g wool*
A	0.049	0.04
B	0.094	0.16
C	0.136	0.55
D	0.194	2.70
E	0.225	4.06
F	0.291	5.23
G	0.316	5.34

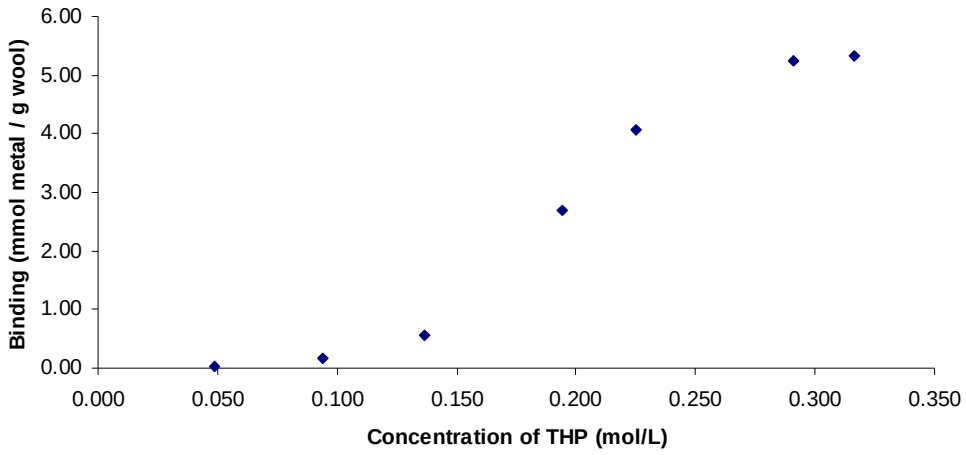


Figure 3-7: Comparison between THP concentration and metal binding of nickel

Figure 3-7 demonstrates that at THP concentrations of around 0.3 mol/L, the relationship between binding and THP concentration plateaus. Hence, if there is slight variation in the amount of KOH on a small scale, this will not disproportionately effect the binding of the THP-wool system.

3.4.2.3 Conclusion

It appears that THP concentrations ≥ 0.3 mol/L produce a uniform THP loading. It is important to note that the more wool will need more THP. For use of 5-7 g of wool it is recommended to utilise THP (0.31 mol/L), with 0.063 moles of THP (5x normal scale). This equates to 0.01 moles THP per gram of wool. This will ensure an excess of THP for the wool being bound in for 1 hr.

3.4.3. Solvation Effects

For wool that had been soaked in ethanol, it was found that if being analysed within the first 2 hrs of this soaking, that when the wool came to being reacted in a metal solution that it would readily sink and be completely covered. However, when left overnight for 12 hrs in air, this effect was not present, and the samples

behaved in a similar manner as those that had been soaked in water for the same period.

One amino acid to be used in Chapter 4 is p-aminobenzoic acid, which is only slightly soluble in water[59]. It was important to analyse the metal binding for THP-wool modified with this amino acid, as it has previously been utilised for metal recovery after immobilisation onto a gel-paper system.[60]

To investigate if ethanol increased or decreased the metal binding ability of the modified wool system, after reaction with THP samples were split and reacted with 2-aminopyridine which had been dissolved in (a) water and (b) ethanol. The unmodified THP wool system was also compared, via soaking for 1 hr in (a) water and (b) ethanol.

Following the soaking, samples were left to dry overnight. They were then analysed in solutions of Cu, Cd, Co and Ni. The remaining solution was then analysed with ICP-MS.

Results showed around 2% variation for all four metals analysed, being Cu, Cd, Co and Ni. A slight increase was shown for copper and a slight decrease for Cd, Co and Ni. However, being only 2% variation between the two solvents, shows that within experimental errors that the difference is negligible.

It therefore appears that utilisation of ethanol does not increase or decrease the metal binding rates if an amino acid has been bound whilst in an ethanol solution.

3.4.4. Pickup rates with variable metal concentrations

It is important to understand the role of concentration of the metal solution that the THP wool systems are reacting with. The aim of this experiment was to

determine if the wool system became saturated and therefore had a reduced metal binding ability, or if it picked up a portion of the available metal and therefore as the metal concentration increased so to does the metal binding proportionately.

3.4.4.1 Method

Fresh THP (400 mL, 0.31 mol/L) was prepared in accordance with the standard procedure (2.5.1). Just under 8 g of wool consisting of 54 pieces of wool of approximate length 20-25 cm, was reacted with the freshly prepared THP solution for 1 hr. The wool samples were then washed with water (200 mL), for 5 minutes. Samples were then left overnight (12 hrs) to dry.

These wool samples were then reacted as triplicates (3 pieces of wool) with varying Ni^{2+} concentrations (Table 3-3) for 1 hr, after which the samples were removed and dried. The remaining solution was then removed for analysis by ICP-MS following acidification with 2% conc. HNO_3.

Table 3-3: Varying nickel concentrations and the subsequent nickel binding by the THP-wool system

	$[Ni^{2+}]$ *mol/L*	Ni binding *mmol Ni /g wool*
A	0.0199	0.43
B	0.0374	0.84
C	0.0797	1.74
D	0.0982	1.98
E	0.2066	4.29
F	0.4161	8.07

3.4.4.2 Results

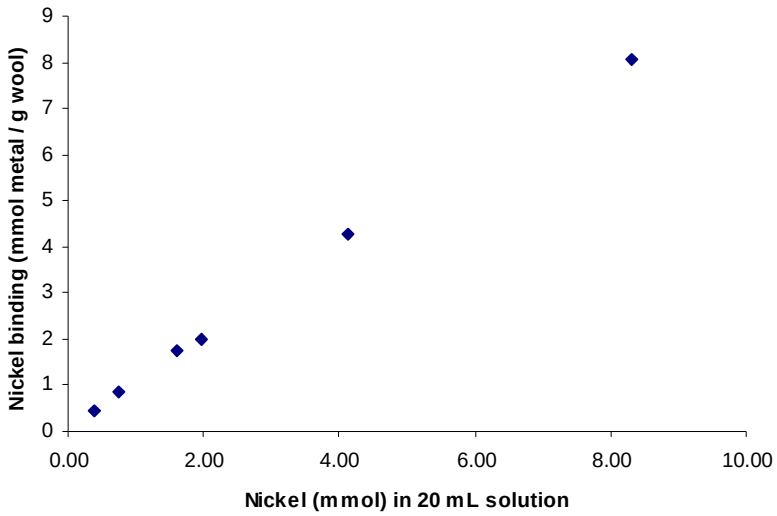


Figure 3-8: Linear response between the availability of nickel to its binding

Figure 3-8 shows a linear response between the availability of metal ions and the binding rate of the THP-wool system to these. The R^2 factor for the line of best fit was 0.9988. This shows that saturation has not yet been reached over the range of available metal in this experiment, as otherwise a plateau effect would be expected.

3.4.4.3 Conclusion

The linear relationship between the concentration available and the actual metal being bound shows that the metal binding produces a proportional response to the concentration. This is important for the amino acid work (Chapter 4), as it shows that as long as the metal concentration is within the 0.02 – 0.4 mol/L range, it will likely show a proportional linear relationship.

Chapter 4: Amino Acid Modified Systems

4.1 Introduction

Following on from the understanding obtained in the preliminary results, an attempt was made to investigate whether modification of the THP-wool system with amino acids and related compounds would either increase or decrease the ability to bind to specific metals.

For this analysis, five metals were to be screened. These were silver, copper, cadmium, cobalt and nickel. Both cobalt and nickel have the advantage that once bound they provided colouration to the wool systems.

A few systems were also oxidised to see what affect the oxidation played in metal binding.

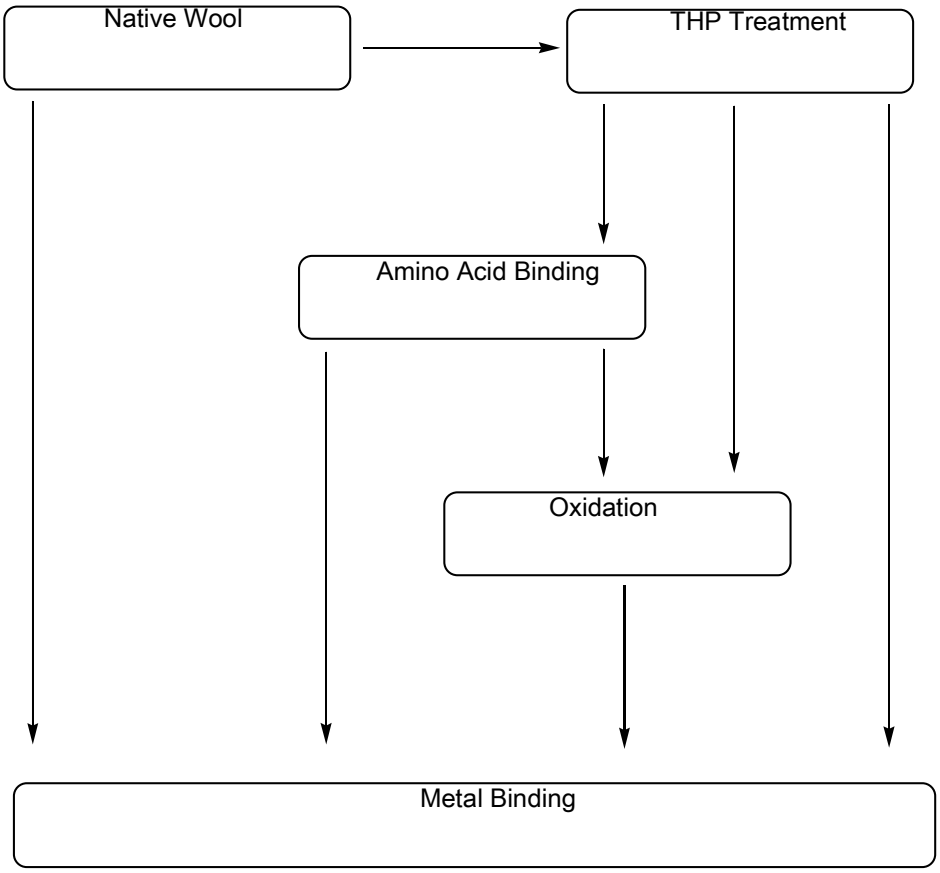
4.2 Experimental

4.2.1. Preparation of samples

A number of potential pathways for modification prior to metal analysis was employed. This is illustrated in Scheme 4-1.

4.2.1.1 THP treatment

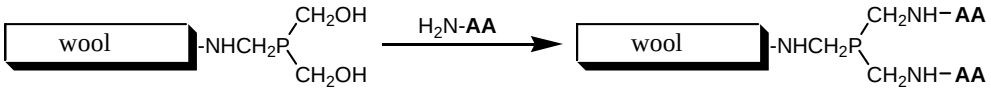
For each system being analysed 36 pieces of wool (5.12 – 6.2 g) of approximate length 20 – 25 cm, were placed in a freshly prepared THP solution (0.31 mol/L, 200 mL) for exactly 1 hr. Samples were held submerged for about the first minute to ensure that the wool would be wet, to aim for even THP immobilisation onto its surface. The wool samples were then washed with water (200 mL), for 5 minutes, to remove the unbound THP.



Scheme 4-1: Overview of sample preparation

4.2.1.2 Amino acid binding

Samples were placed into a 200 mL solution, containing approximately 0.01 mol of the dissolved amino acid (0.05 mol/L) for 1 hr. Structures of amino acids and related compounds are shown in Figure 4-1. *p*-Aminobenzoic acid (PABA) was dissolved in ethanol. The wool samples were then washed in water (200 mL), for 5 minutes, to remove the excess amino acid that had not bound. PABA modified THP-wool was washed in ethanol (95%, 200 mL). The modification with an amino acid or related compound onto THP-wool is illustrated in Scheme 4-2.



Scheme 4-2: Modification with an amino acid or related compound (AA), onto THP-wool

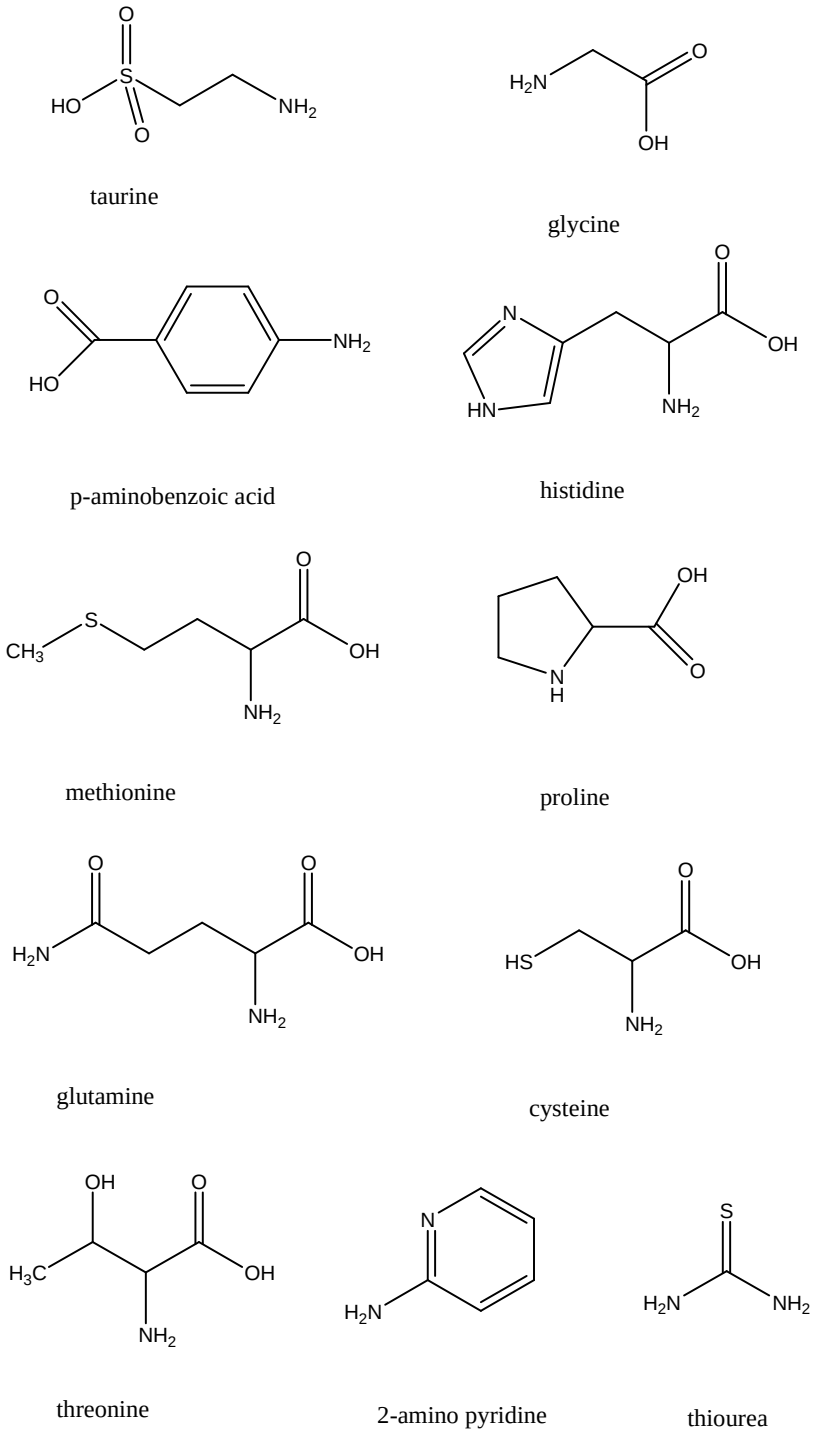


Figure 4-1: Structures of all the used amino acids, and related compounds

4.2.1.3 Oxididation

If a THP-wool system, or modified versions thereof, was to be oxidised, they were then placed in a solution of hydrogen peroxide (6%, 200 mL) for 5 minutes. Samples were then washed in water (200 mL) for a further 5 minutes.

4.2.1.4 Metal binding

Prior to metal binding, wool samples (prepared following reactions detailed in Sections 4.2.1.1 - 4.2.1.3) were left out on the bench to dry overnight for 12 hrs.

20 mL of each metal stock solution (Table 4-5) was then pipetted into 50 mL beakers; each metal analysis was done in duplicate. For each wool system, after 12 hrs of drying overnight, three pieces of wool were placed in each beaker and left for exactly 1 hr. After the samples were removed, 10 mL of the remaining solution was then pipetted out for ICP analysis. These were acidified with 2 % conc. HNO_3.

The wool samples were then dried in air, until they were consistent relative to one another in the varying atmospheric conditions (humidity levels). This was shown to take 6 days from when the last samples were created. The samples were left for two weeks then individually weighed as a batch.

4.2.2. ICP analysis

All five metals being analysed were checked against external standards (50 ppb, 1000 ppb). Silver results were excluded due to high variation (Section 4.4.1). Standards contained 2% by volume of conc. HNO_3 acid, the same as the samples submitted.

4.2.3. NMR Spectroscopy

For solution NMR, a large batch of THP was prepared in accordance with the standard synthesis (Section 2.5.1). Each amino acid was added on a 3:1 molar ratio to THP. Samples were purged with nitrogen and analysed immediately.

For solid-state NMR, one large batch of THP (0.31 mol/L, 400 mL) was reacted with 72 pieces of wool (*ca*. 12 g). These THP-wool samples were then reacted with each dissolved amino acid (0.05 mol/L, 20 mL) individually. All samples were then analysed as soon as possible, being 3 days after preparation.

4.3 Results

Table 4-1: Comparison of binding of metals (mmol / g wool) by wool and modified systems

	Cu	Cd	Co	Ni
	mmol metal / g wool			
native	2.96	0.00	0.50	1.10
unmodified THP	**4.55**	**2.39**	**6.22**	**6.36**
oxidised unmodified THP	3.44	0.15	4.64	4.65
taurine	2.38	0.09	0.25	0.52
glycine	1.91	0.31	0.85	1.02
p-aminobenzoic acid	3.44	0.06	2.31	3.14
histidine	0.61	0.31	0.22	0.02
methionine	1.74	0.15	1.15	1.32
proline	4.04	0.58	5.69	5.46
oxidised proline	4.95	0.54	6.24	5.73
glutamine	4.55	0.73	5.98	5.88
cysteine	3.73	0.08	0.08	0.22
threonine	2.89	0.67	3.88	4.59
2-aminopyridine	4.11	2.42	6.77	6.36
oxidised 2-aminopyridine	5.34	2.60	6.77	6.36
thiourea	5.46	2.30	6.93	6.56
oxidised thiourea	2.46	0.05	3.05	2.75

Results obtained in Table 4-1 are better represented as a percentage comparison relative to the unoxidised and unmodified THP-wool, as shown in Table 4-2. This gives a direct ability to see whether reaction with an amino acid has either increased or decreased the metal binding. Included for comparison is native wool.

Table 4-2: Recovery of metals for systems compared to unoxidised and unmodified THP-wool, represented as a percentage

	Cu	Cd	Co	Ni
		%		
native	65	0	8	17
oxidised unmodified THP	76	6	75	73
taurine	52	4	4	8
glycine	42	13	14	16
p-aminobenzoic acid	75	2	37	49
histidine	13	13	3	0
methionine	38	6	18	21
proline	89	24	92	86
oxidised proline	109	23	100	90
glutamine	100	31	96	93
cysteine	82	3	1	3
threonine	64	28	62	72
2-aminopyridine	90	101	109	100
oxidised 2-aminopyridine	117	109	109	100
thiourea	120	96	112	103
oxidised thiourea	54	2	49	43

4.4 Discussion

4.4.1. Statistics

Whilst ICP analysis of each metal was conducted as duplicates for each amino acid, this was extended further for blanks to measure the variation of the ICP analysis itself. Each metal had eight replicates of stock solution added to the analysis batch, to allow for the statistical determination of the variation of the instrument for each metal. The stock solution results were also utilised for subtraction to determine the actual metal pickup of each of the wool systems. Due to the nature of the comparison of results to THP, the plain THP modified wool system was analysed as a batch of six to minimise errors.

The set of eight replicates was to analyse the variation of the actual ICP analysis, ensuring that each metal had a good reproducibility factor. This is reported as a value which is a coefficient of variation (cv).[61] In simple terms the cv is the standard deviation divided by mean and is represented as a percentage, as shown in Equation 4-1.

$$cv = 100\frac{s}{m}$$

Equation 4-1: Determination of the coefficient of variation (cv), which is represented as a %

Considering that the coefficient of variation is dependant on the standard deviation, then it is important that the correct standard deviation calculation is utilised. There are two separate types of standard deviation calculation, with the difference being the determination of the degrees of freedom. The standard use for software such as excel, is that the standard deviation is calculated with the equation shown in Equation 4-2.

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(x_i - m\right)^2}$$

Equation 4-2: Univariate probability distribution standard deviation (univariate)

The univariate standard deviation calculation, as shown in Equation 4-2, is utilised when calculating the standard deviation for a large pool of samples, where only a select few are actually being analysed. An example would be an election poll, whereby it is almost impossible to quiz everyone on who they are voting for and therefore not all the population is being sampled. Because of this, there is one more degree of freedom in the calculation.

However, with the analysis of the eight metal samples of the stock solution, all eight of the population are being analysed. Therefore, we are able to use the population probability distribution standard deviation calculation, as shown in Equation 4-3.

$$s = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}\left(x_i - m\right)^2}$$

Equation 4-3: Population probability distribution standard deviation (population)

By utilising the population standard deviation calculation, as shown in Equation 4-3, this results in an always slightly smaller value, when compared to the univariate standard deviation. When *N* is very large, this effect is near negligible. However, in this study of the amino acids and with the limit of 240 samples in the ICP-MS auto-sampler, this required a limit of eight for each stock solution which in turn results in a 6.9% difference between the two standard deviations. These differences are shown in Table 4-3.

Table 4-3: A direct comparison of standard deviations computed from the two varying methods, per 20 mL of metal stock solution

Standard Deviation method	Ag	Cu	Cd	Co	Ni
			mmols metal		
Univariate (Equation 4-2)	0.161	0.130	0.066	0.183	0.099
Population (Equation 4-3)	0.151	0.121	0.062	0.171	0.093

The exclusion limit to be applied for the cv for analysis of the metal stock solutions by ICP was set to 5 %. Therefore for all results exceeding 5 % in size, were to be reanalysed and if the result couldn't be brought to within the 5 % exclusion limit then these were excluded.

Table 4-4: Coefficient of variations (cv) shown for each metal, with the two different standard deviations utilised, following the reanalysis of silver (Ag)

Method of Standard Deviation utilised	Ag	Cu	Cd	Co	Ni
			%		
Univariate (Equation 4-2)	90.02	3.49	1.75	4.59	2.65
Population (Equation 4-3)	84.20	3.27	1.64	4.30	2.48

The initial analysis of silver resulted in a cv of 120 %. The silver samples were reanalysed as a 100-fold dilution, with an increased flush time between samples as well as an HCl flush between every third sample. However, following this change in method the results still greatly exceeded the 5 % limit as shown in Table 4-4. The other four metals were within the 5 % range for the cv, so therefore were acceptable.

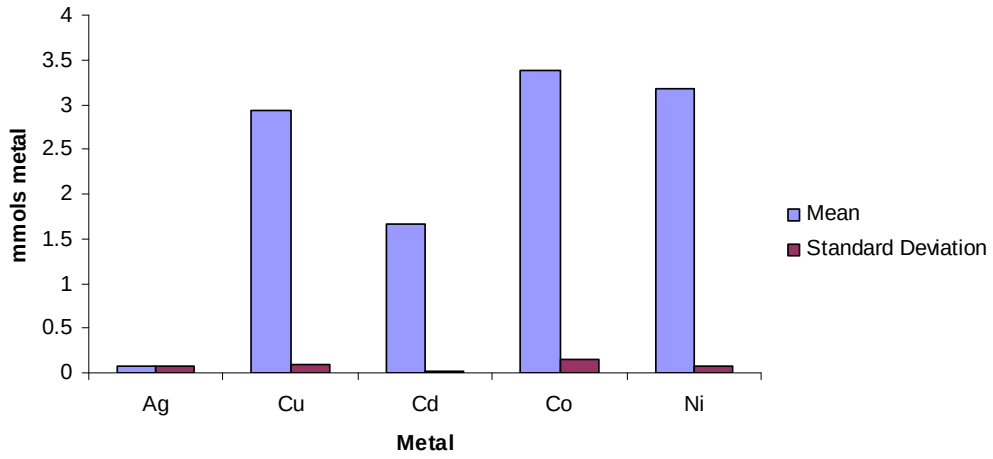


Figure 4-2: Graph showing a visual representation of the mean and the size of the standard deviation, utilising the population standard deviation

The variation for the silver analysis is best represented in visual form and is shown this way in Figure 4-2, which compares the size of the mean to that of the calculated standard deviation. This illustrates that the silver results are disproportionate to its standard deviation. The standard deviation for each metal in exact terms, is relatively consistent. However the results when compared to the mean, which the cv achieves, show that silver analysis is unreliable.

Another method of exclusion was utilised for practical reasons.[62] Considering that for each wool system the results were obtained as duplicates, a comparison of the variation to the mean (Equation 4-4) ensured that the duplicates were reportable.

$$v = 100\frac{(x_i - m)}{m}$$

Equation 4-4: Determination of the variation to the mean, represented as a percentage

Hence, utilisation of an exclusion factor of 20 % was applied, as long as the results were > 1 mmol metal / g wool. The practical reason for the 1 mmol metal / g wool limit was that the variation increases when the mean is close to zero, so as a percentage the variation was disproportionately unbalanced in size. For the purpose of this analysis, it was impractical to have a large number of samples within the set just to minimise error, hence the limit of 1 mmol metal / g wool, was the only appropriate limit that could be applied.

For the purpose of analysis of near zero values the following rules were asserted, as recommended in a discussion[63] document on the topic:

1. Negative readings were used in the determination of means and deviations, unaltered.
2. After subtraction of the number of moles of metal bound, which results in a mean for metal binding being negative, this was then set to zero for reporting. Only silver data required this for the amino acid section, however the silver batch was excluded.

4.4.2. NMR

When amino acids, and related compounds, are reacted with the THP-wool system (Section 6.2) these only show the peak at 50 ppm in solid-state (MAS) ^{31}P NMR. Thiourea showed indications of a broad peak at -24 ppm, corresponding to the THP peak in solution state, however no conclusions could be made due to the large baseline distortion that takes place often in solid state (MAS) NMR, which can tend to hide peaks once a baseline calibration has taken place. It is possible that the peak observed is free THP, but could also be bound >P-CH_2-N< species, as peaks are broad.

^{31}P solution state NMR however showed good potential to determine the nature of reaction taking place when amino acids and related compounds are reacted with THP (Section 6.1). This provides a good model, as if modification is being shown in solution by NMR, then it would be expected that modification would

occur on the surface of the THP-wool system as well.

4.4.3. Stock solutions

All stock solutions were targeted towards 4 ppm, having been diluted down from 1000 ppm solutions. Each sample Table 4-5 shows the number of mmols metal in each 20 mL, which shows how much metal was available prior to attempted binding by the wool systems.

Table 4-5: Number of mmols of each metal in 20 mL of stock solution

	Cu	Cd	Co	Ni
		mmols metal		
Stock solution	2.93	1.67	3.38	3.18

After analysis of the metal solutions by ICP following attempted binding, the number of mmoles of metal extracted was determined a conversion calculation. The concentration after binding was subtracted from the stock solution concentration, then converted to an actual amount of mmol metal that was removed for that volume.

4.4.4. Comparison of metal binding by metal

4.4.4.1 Copper

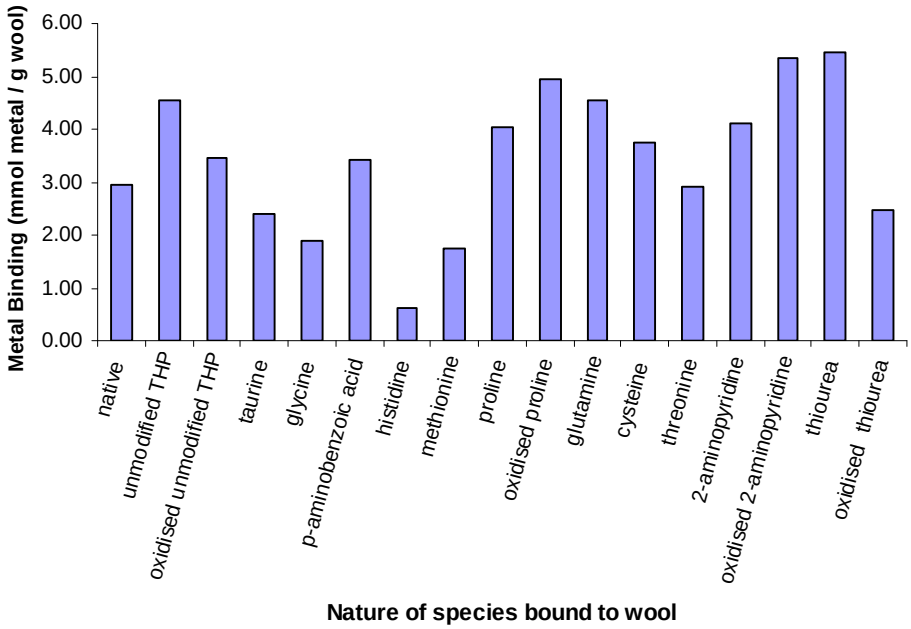


Figure 4-3: Comparison between copper binding and the wool system

Copper(II) in water will readily give the aqua ion $[Cu(H_2O)_6]^{2+}$. Addition of ligands to the aqua ion leads to the formation of complexes by displacement of water.[58] Copper(II) is known to be a very robust cation for binding to, with a particular affinity for amino groups.

Figure 4-3 demonstrates that native wool without any modification shows a good binding potential for copper. Cystine (Figure 1-5) is one of the major amino acid's that is natively incorporated into wool, and has readily available a number of amines for binding to nickel.

Thiourea, when unoxidised, is the only modified system, which has a higher copper binding than the unmodified THP system. Solution state NMR (Section

6.1) of thiourea, when reacted with THP, indicate that unlike others it does not cause a chemical shift to any original THP peaks. This indicates that thiourea isn't actually binding to the THP, the only amino acid or related compound to not show signs of binding. However, THP-thiourea reacted materials are known from literature.[14]

The oxidised 2-aminopyridine system significantly enhances the copper binding capacity, compared to the unoxidised 2-aminopyridine. It is possible that a pyridine oxide group is being formed for this to occur.

4.4.4.2 Cadmium

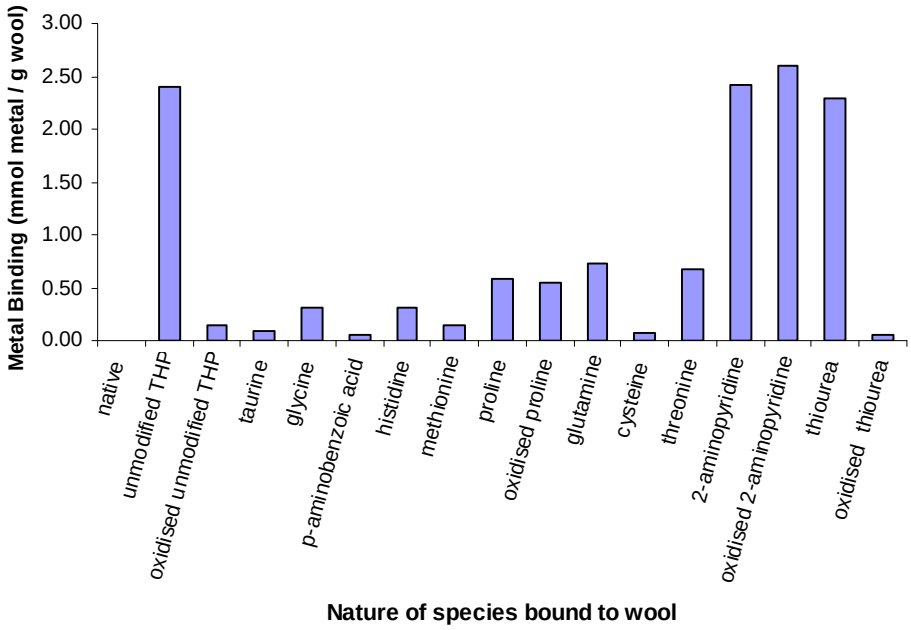


Figure 4-4: Comparison between cadmium binding and the wool system

The only systems which showed good metal binding potential was for the non-acidic systems of unmodified THP, 2-aminopyridine, oxidised

2-aminopyridine and thiourea. Carboxylic acid and sulfonic acid functional groups present in the other ligands tended to suppress cadmium binding potential. Cadmium extraction in acidic conditions has previously been shown to be difficult.[64] Interestingly there is a sudden decline in metal binding ability following oxidation of the thiourea, most likely due to the oxidation of the sulfur resulting in a decreased pH, and potential loss of thiourea sulfur, which would be expected to be a good ligand d towards soft metal ions such as Cd^{2+}.

Cadmium forms numerous complexes with amine ligands. Pyridines and pyrazoles are also good ligands.[58]

Native wool also demonstrates no detectable binding towards cadmium.

4.4.4.3 Cobalt

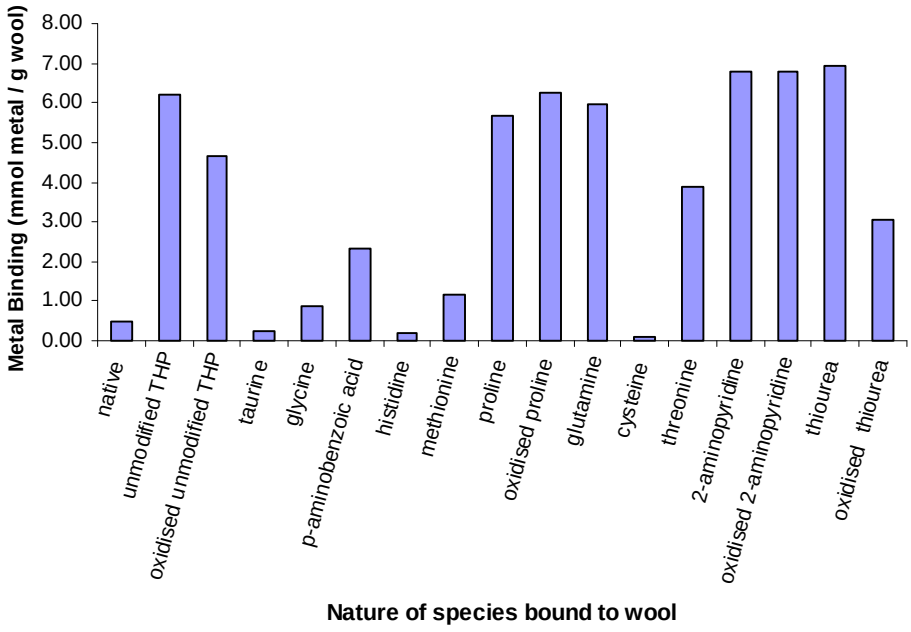


Figure 4-5: Comparison between cobalt binding and the wool system

As is the case with the binding of cadmium, the systems for 2-aminopyridine and thiourea both show good binding for cobalt. The systems for proline and glutamine also showed good potential, with recovery rates of 90 % of that of the unmodified THP system.

Heavily suppressed binding was observed on all sulfur containing systems, other than that of thiourea. Again, this can be explained by the suspected non-binding of the thiourea and hence the result for this system is around par with that of the unmodified THP.

The systems for taurine, histidine and cysteine all showed significantly suppressed cobalt binding, with recovery rates of less than 5 % of that of the unmodified THP system.

4.4.4.4 Nickel

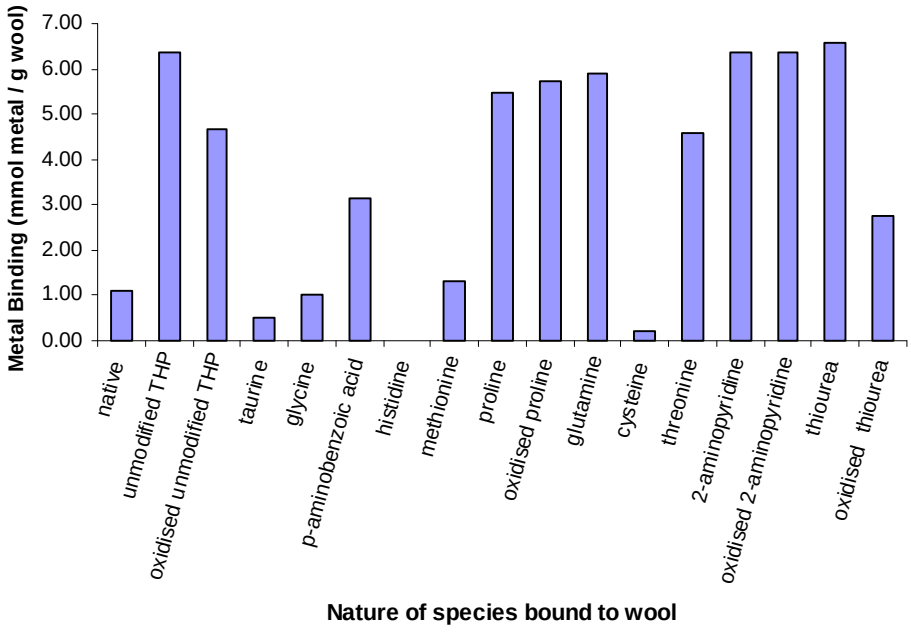


Figure 4-6: Comparison between nickel binding and the wool system

Like copper, cadmium and cobalt, the oxidised system of 2-aminopyridine showed a high binding ability.

Systems which featured sulfur based functional groups tended to observe a suppressed binding rate comparative to other modified systems. The exception to this was again thiourea.

For the p-aminobenzoic acid system, colorimetrically the wool appeared to be bound to a higher content of nickel compared to the remaining systems. However, as seen, the results from ICP-MS concluded that it had in fact not enhanced binding.

4.4.5. Selectivity of systems

Graphs for all systems illustrating the metal binding of the system compared to THP can be found in Section 6.3. These comparisons illustrate clearly the metal binding ability of the system for each metal, relative to other metals analysed.

Along with native wool, both taurine and cysteine show an ability to predominantly favour binding of copper out of solution. Cysteine in particular shows a much greater ratio of copper binding, relative to cadmium, cobalt and nickel, as shown in Figure 4-7. This selectivity towards binding copper has the theoretical ability to produce filter-type applications, whereby only copper is bound out of solution. This may be the case as cysteine is the only SH compound that was analysed, and Cu^{2+} will form CuSR when reacted with RSH.

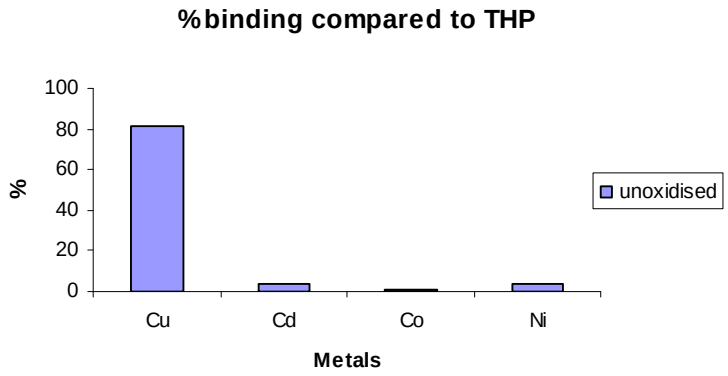


Figure 4-7: Metal binding ability for the cysteine system

Selective binding towards either cadmium, cobalt or nickel was not observed by any system that was utilised. However, numerous systems were able to retain their binding ability towards copper, cobalt and nickel, thus selectively decreasing their metal binding ability for cadmium. This low metal binding ability for cadmium is predominantly associated with the presence of a carboxylic acid group, or another acidic functional group such as sulfonic acid on taurine.[65]

Chapter 5: Conclusions

In this research it has been shown that THP can be successfully immobilised onto a wool support.

THP in solution will undergo oxidation to form THPO. THPO removes or decreases metal binding in most cases, unless metal binding is occurring through the P=O system. By immobilising THP onto wool it has been shown to stabilise and therefore decrease the rate of oxidation of the THP. The presence of immobilised THP was determined colorimetrically by reaction with Ni^{2+} ions, which produced a bright orange nickel-phosphine complex, as well as quantitatively, by measuring nickel uptake using ICP-MS.

Additional modification of the THP-wool systems via reaction with amino acids and other related compounds overall did not appear to enhance the metal binding capacity relative to the unmodified THP-wool system. The only modified THP-wool system that showed either retention or an increase in metal binding capacity for all metals analysed was that of 2-aminopyridine, which following immobilisation onto the THP-wool system was then oxidised. Considering that the system is already oxidised, no decrease in metal binding would be expected due to oxidation from the air, so this system could have some viable ability to be used as a filter exposed to air.

The results showed that the unmodified THP-wool system is a very robust and very productive system. THP immobilisation onto wool is a cost effective method of functionalisation, due to the wide availability of THP precursors. As well as this it has shown itself to be a very effective functionalisation method for metal ion recovery out of solution by wool.

This study has endeavoured to provide a model study for metal binding of THP

modified wool systems, but utilisation of a larger number of metals would be of interest. By utilising metal ions with significantly varying chemistry towards varying functional groups, would enable a greater understanding of the potential for selectivity of the THP modified wool systems. The binding of THP to wool potentially allows the subsequent grafting of any metal binding group, or other functionalisation, through THP-amine chemistry discussed in this thesis.

It would be very interesting to investigate the metal binding ability of the THP wool system, when there are a wide range of coexistent metals in the one solution. This would identify if the system has preferential binding towards particular metals. If the THP wool system was to be utilised as a filter for environmental waters, then it would be fair to assume that these environmental waters would contain coexistent metals.

Chapter 6: Appendices

6.1 Solution State NMR spectra

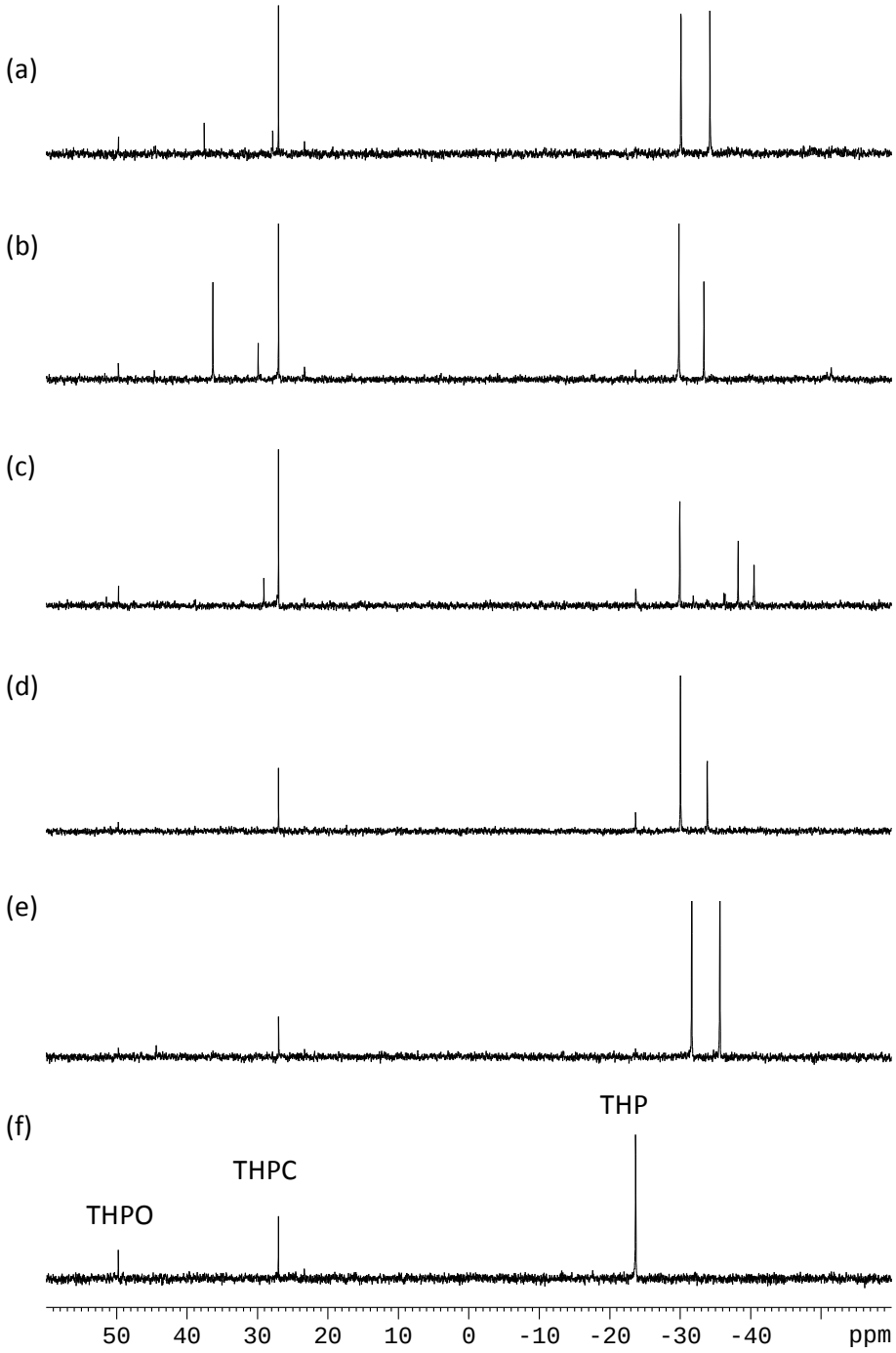


Figure 6-1: ^{31}P solution state NMR of THP added to (a) taurine (b) glycine (c) histidine (d) methionine (e) proline, with (f) unmodified THP for comparison. Lock solvent D_2O.

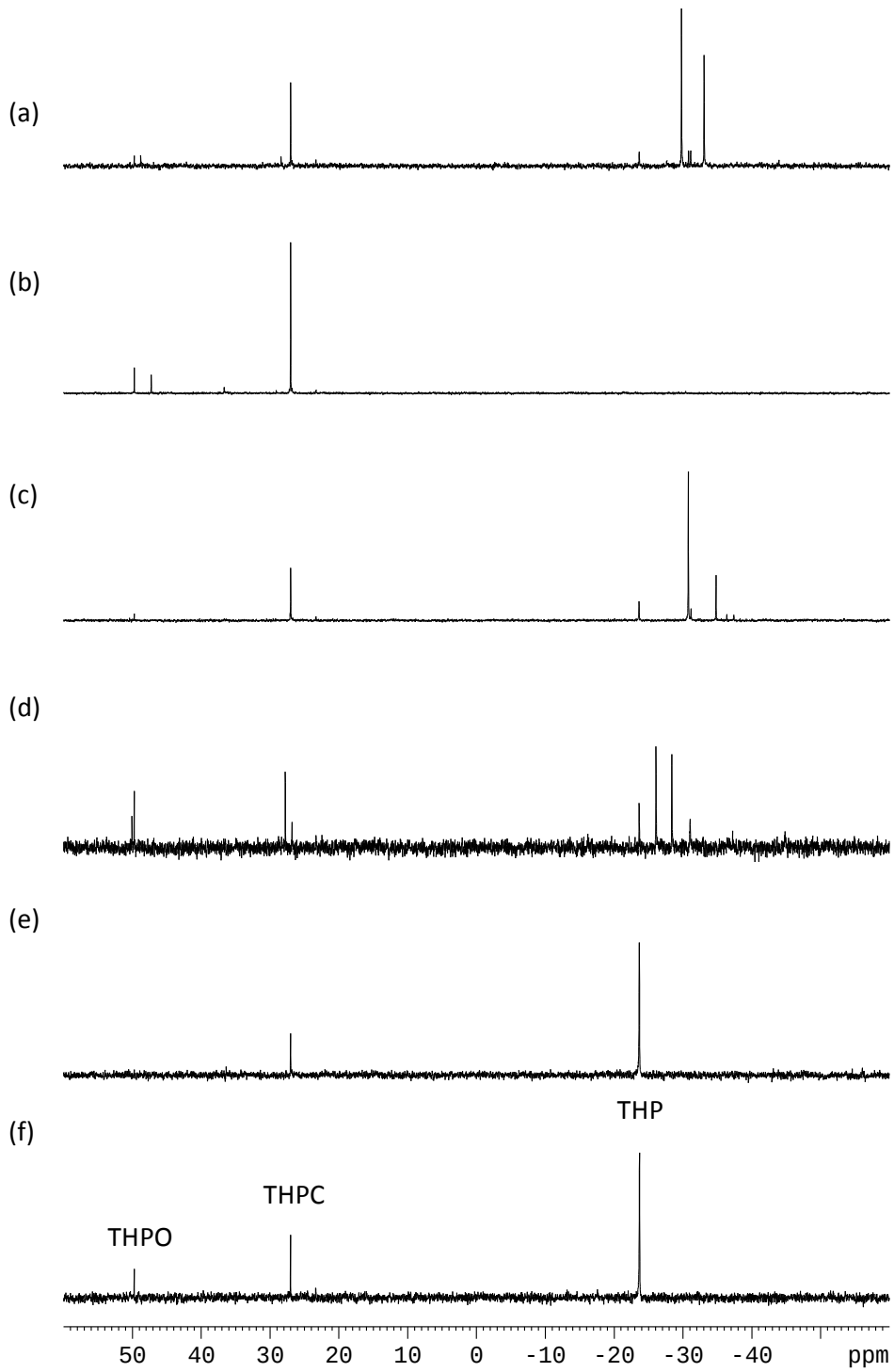


Figure 6-2: ^{31}P solution state NMR of THP added to (a) glutamine (b) cysteine (c) threonine (d) 2-aminopyridine (e) thiourea, with (f) unmodified THP for comparison. Lock solvent D_2O.

6.2 Solid state NMR spectra

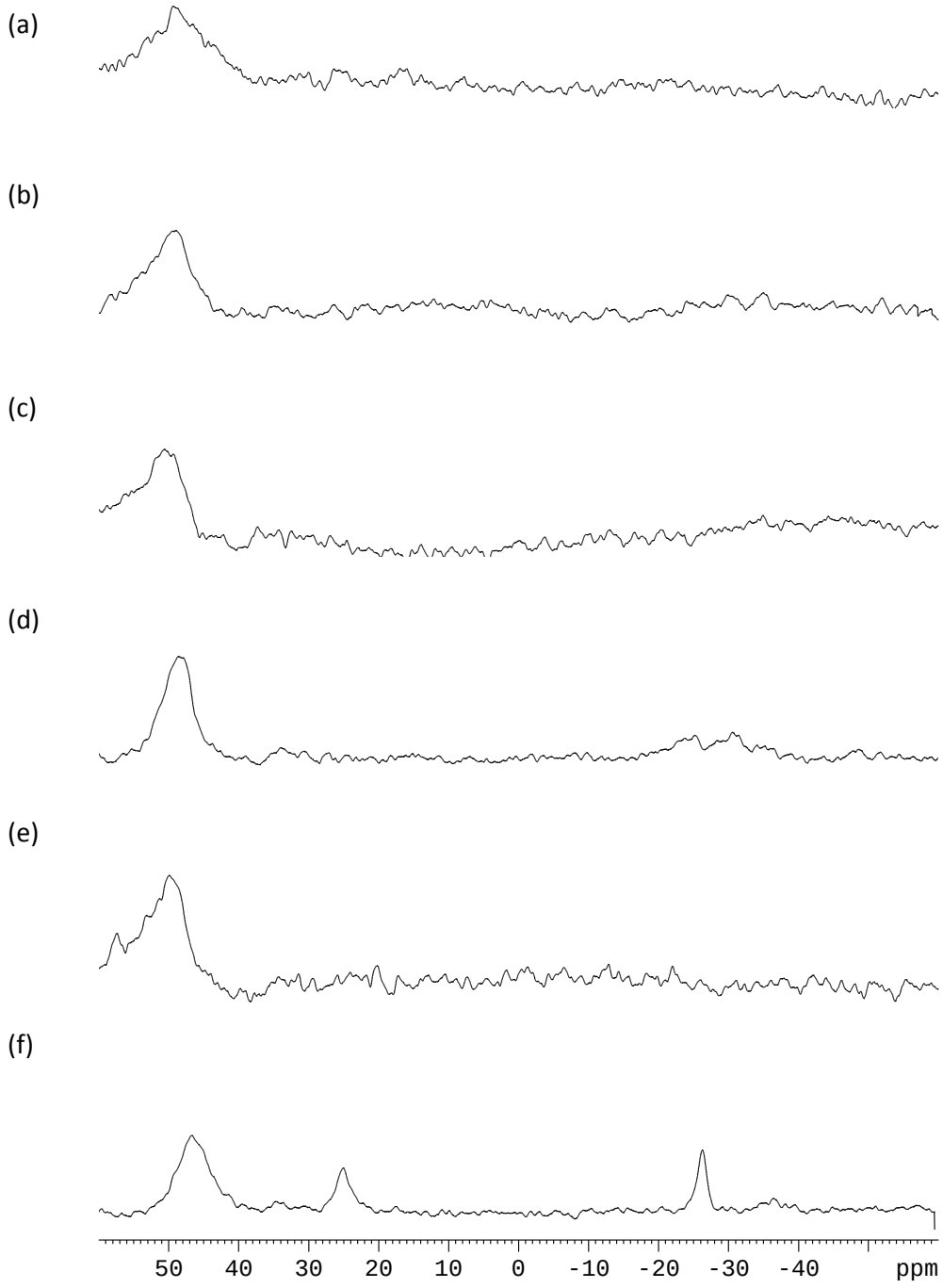


Figure 6-3: ^{31}P solid state (MAS) NMR of THP-wool reacted with (b) 2-aminopyridine (c) methionine (d) thiourea (e) proline, with both (a) oxidised, unmodified THP-wool and (f) unmodified THP-wool for comparison

6.3 Graphs illustrating binding of each system compared to THP

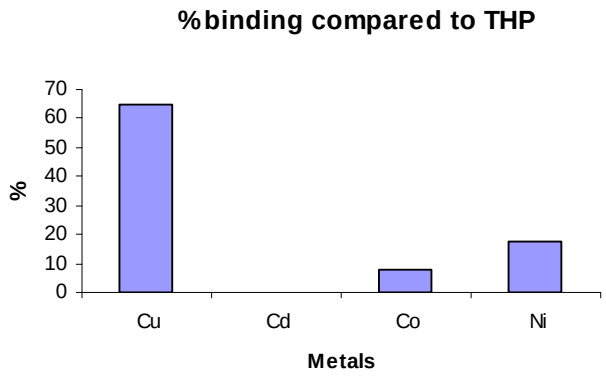


Figure 6-4: Metal binding ability for native wool

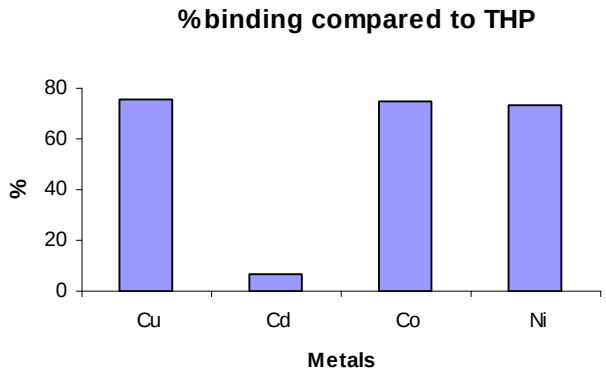


Figure 6-5: Metal binding ability for the oxidized THP system

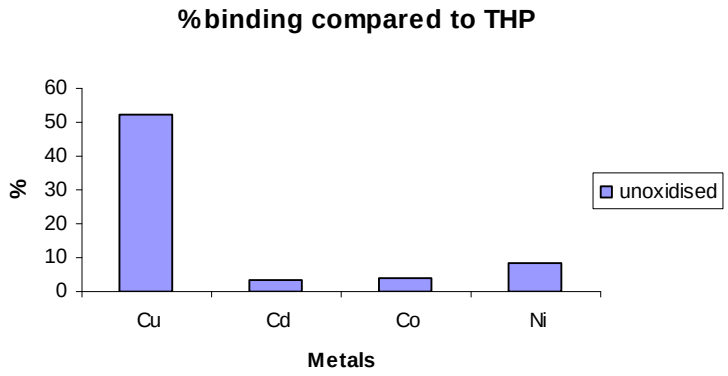


Figure 6-6: Metal binding ability for the taurine system

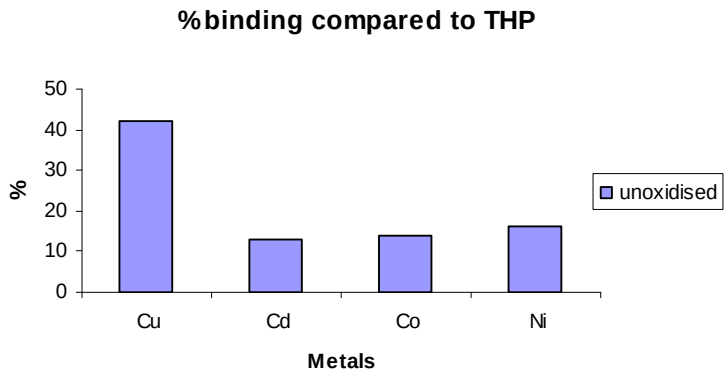


Figure 6-7: Metal binding ability for the glycine system

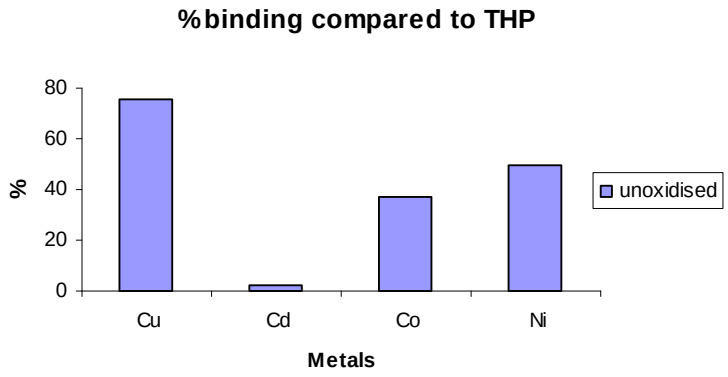


Figure 6-8: Metal binding ability for the p-aminobenzoic acid system

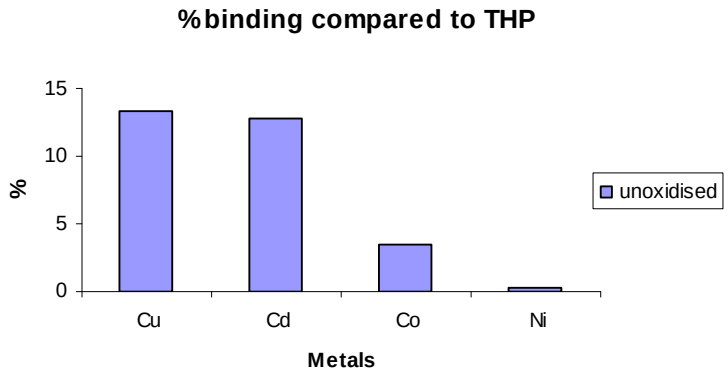


Figure 6-9: Metal binding ability for the histidine system

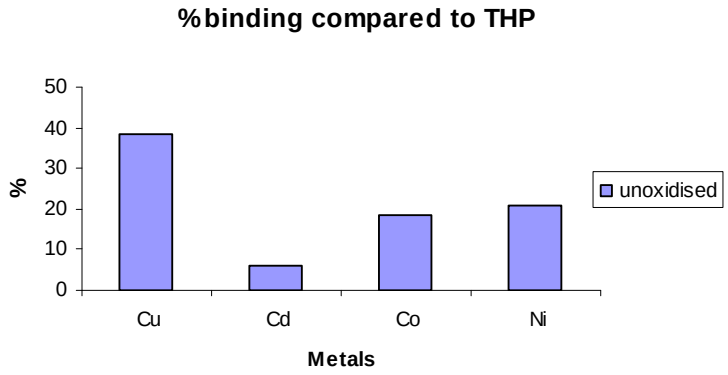


Figure 6-10: Metal binding ability for the methionine system

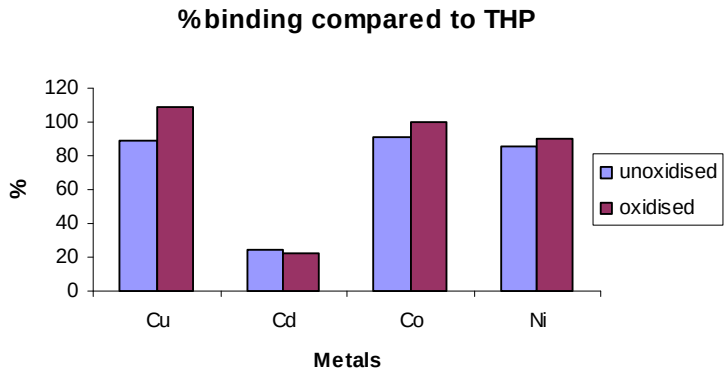


Figure 6-11: Metal binding ability for the proline system

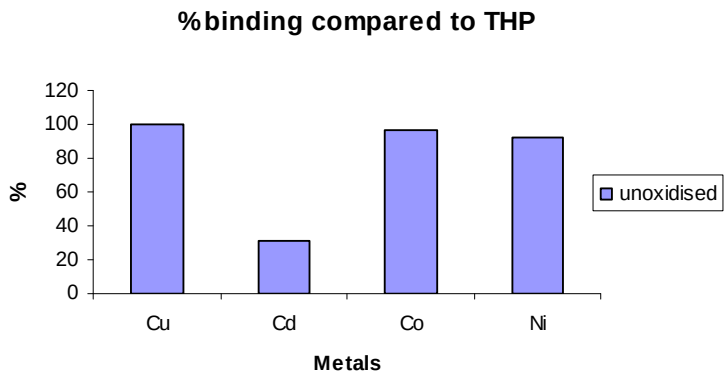


Figure 6-12: Metal binding ability for the glutamine system

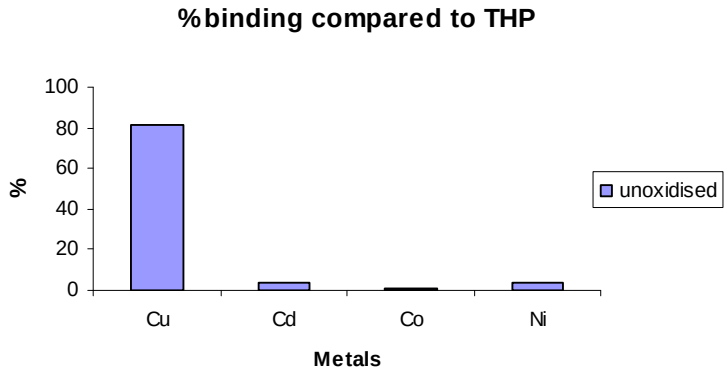


Figure 6-13: Metal binding ability for the cysteine system

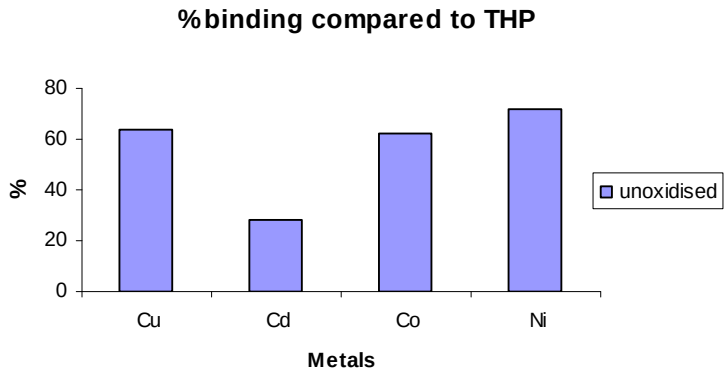


Figure 6-14: Metal binding ability for the threonine system

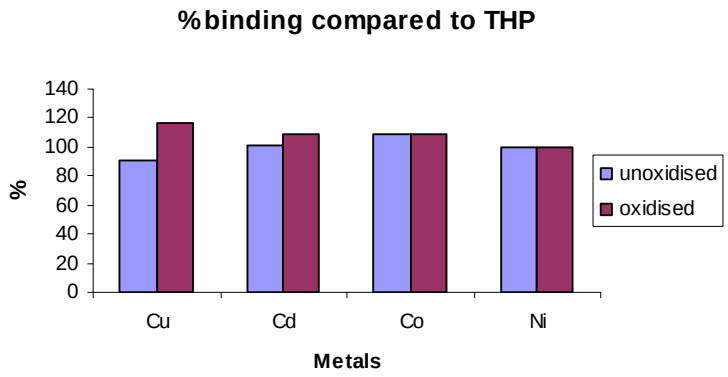


Figure 6-15: 2-aminopyridine

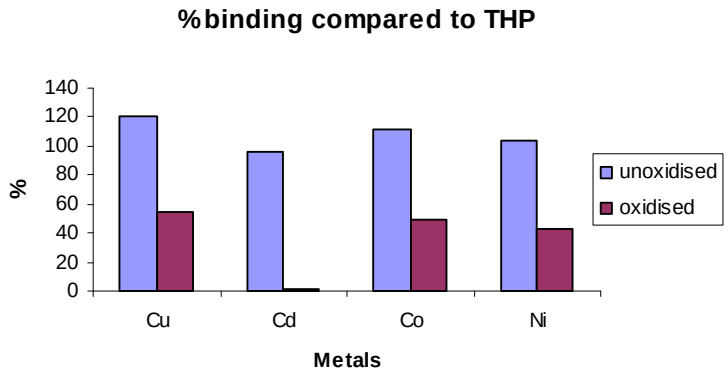


Figure 6-16: Thiourea

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