PROPERTIES OF BIOLOGICAL COPPER.
MOLYBDENUM AND NICKEL COMPOUNDS

BY

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DECLARATION

Unless stated, the work presented in this thesis was wholly the original work of the author. The author has not submitted this thesis for any other degree of the Council of National Academic Awards, nor a University. The work presented in this thesis was performed by the author at the School of Chemistry, Leicester Polytechnic during the period September 1982 - September 1985.

David E. Pratt
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ABSTRACT

Properties of Biological Copper, Molybdenum and Nickel Compounds

David E. Pratt

The kinetics of the Cu(II)albumin and Ni(II)albumin exchange reactions with the amino acids cysteine, histidine and glycine have been examined by spectroscopic means (UV/VIS, ESR, CD). The results suggest that rapid equilibrium may not occur at blood plasma levels, and that indeed for Ni(II)albumin the equilibrium rates are such that no effective metal ion exchange can occur with the amino acid pool. This may explain the differing metabolic activities of Cu and Ni. At physiological pH, Ni was shown to exist in two binding configurations at the N-terminal end of albumin, the known square planar form, and an octahedral configuration; Cu has only one configuration at physiological pH.

The reaction of Cu(II) ions with MoS$_4^{2-}$ produces an insoluble Cu-containing product, this offers a possible explanation of how Mo can cause Cu deficiency in ruminants. The reaction was observed to go to completion at a 1.5:1 Cu : MoS$_4^{2-}$ ratio. Product precipitates were collected and studied by a number of techniques. The results suggested the presence of two products; one isostructural with the known (NH$_4$)$_2$Cu$_2$MoV$_2$S$_4$, and the other with a stoichiometry Cu$_x$Mo$_y$S$_4$O$_x$ (x = 2-3). Addition of MoS$_4^{2-}$ to Cu(II)albumin at the 10$^{-4}$ molar level resulted in the slow reduction of Cu(II); evidence was obtained for the formation of an (albumin-) Cu$^+$-Mo-S complex. Replacing WS$_4^{2-}$ for MoS$_4^{2-}$ resulted in similar reactions, although evidence for the reduction of W(VI) was not as clear cut as for Mo(VI). The redox properties of MoS$_4^{2-}$, MoOS$_3^{2-}$ and WS$_4^{2-}$ were studied by cyclic voltammetry.

A computer program was written to interface a microcomputer with a semi-automatic pH potentiometric titration apparatus.
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ABBREVIATIONS

Amino Acids (AA):

Ala = Alanine
Arg = Arginine
Asn = Asparagine
Asp = Aspartic Acid
Cys = Cysteine
Gln = Glutamine
Glu = Glutamic Acid
Gly = Glycine
His = Histidine
Ile = Isoleucine
Leu = Leucine
Lys = Lysine
Met = Methionine
Pen = Penicillamine
Phe = Phenylalanine
Ser = Serine
Thr = Threonine
Trp = Tryptophan
Tyr = Tyrosine
Val = Valine
Cys-Cys = Cystine
Pro = Proline

Peptides and Proteins:

GHL = Gly-L-His-L-Lys
BSA = Bovine Serum Albumin
HSA = Human Serum Albumin
DSA = Dog Serum Albumin
RSA = Rat Serum Albumin
Cp = Caeruloplasmin
Organic Reagents:

BAL = 2,3-dimercaptopropanol
trien = Triethylenetetramine
DTNB (Ellman's Reagent) = 5,5'-dithiobis
(2-nitrobenzoic acid)
DTPA = Diethylenetriamine pentaacetic acid
NTA = Nitriloacetic acid
MOPS = 3-(N-morpholino)propane sulphonic acid
PBN = Phenyl-N-t-butylnitrone
DPPH = 2,2-diphenylpicrylhydrazyl
DMF = Dimethylformamide
MeCN = Acetonitrile
CHAPTER 1

THE INTRODUCTION

1.1 Metal Ions in Human Health

Within man there are several aqueous phases, each separated from the environment. It is within these aqueous phases that reactions essential to life are carried on, and for which the continued existence of an organism depends. Inorganic cations and anions play a vital role in the homeostasis within these fluids. Trace amounts of a number of metal ions, including copper and molybdenum, are essential to the activity of many enzymes. Trace metals are also present as metal complexes in other biological molecules such as haemoglobin, ferritin, metallothionein and vitamin $B_{12}$.

An element is considered essential if it is present in all healthy tissues, has a fairly constant concentration range between different animals, and when excluded from a body produces reproducible physiological abnormalities which are reversible upon readmittance of the element [1]. Complete absence will lead to death. The essential elements are shown in table 1(i). Although these cations and anions are indispensable, excessive amounts of them are toxic, so that it is important that their concentrations are regulated, either by mechanisms existing within the
Table 1(i) The essential and beneficial elements in man*.

<table>
<thead>
<tr>
<th>Category</th>
<th>Elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trace metal ions</td>
<td>Fe, Cu, Mn, Zn, Co, Mo</td>
</tr>
<tr>
<td>Bulk metal ions</td>
<td>Na, K, Mg, Ca</td>
</tr>
<tr>
<td>Non-metallic ions</td>
<td>H, B, C, N, O, F, Si, P, S, Cl, Se, I</td>
</tr>
<tr>
<td>Beneficial metal</td>
<td>Cr, Sn, V, Ni</td>
</tr>
</tbody>
</table>

* Adapted from [2].
animal, or by externally imposed controls. Other metal ions which are highly toxic, but do not appear to serve any useful biological function, include the environmental pollutants, lead, cadmium and mercury.

The plentiful inorganic materials that surround man have been used for centuries as therapeutic agents. Over 3000 years ago a decoction of rust in wine was claimed to have cured the sexual impotence of Iphycleus of Thessaly, and in the 4th century B.C., Hippocrates recommended the use of metallic salts for medicinal purposes.

A further area of complexity arises. The body is capable of regulating the intake and excretion of the elements such that the metal ions remain at a beneficial level, however this balance may be disrupted. Deficiencies or toxicities can be exacerbated by other elements. Interactions between the elements may be described as either positive or negative, where a positive interaction is one in which the presence of an element is essential for the normal metabolic function of a second element, and a negative interaction is one in which the presence of an excess of one (or more) elements antagonises the normal action of a second. An excessive intake of zinc, for example, is known to induce copper deficiency in man [3], and in ruminants it has been shown that pastures high in molybdate may induce copper deficiency [4].
1.2 Copper Balance in Human Subjects

A number of disease states have been recognised which are associated with elevated levels of serum copper, table 1(ii). Elevated serum levels usually result from depletion in one or more organs. This gives a clear indication of the importance of copper to human health. Typical effects of copper deficiency (depletion in the most susceptible organs; brain, liver and kidneys), include growth retardation, diarrhoea, defects in the structure and pigmentation of hair, and in more severe deficiency, structural defects in the connective matrix of cartilage, tendons, blood vessels and skeleton.

The recommended daily allowance for a healthy adult human is 2 mg of copper and 50 µg/kg for infants. Normal daily intake is of the order 2-4 mg relative to the 75 mg total body copper [6]. Copper is found predominantly in the brain, liver and muscle, table 1(iii); the variability of estimates may reflect either imprecision in the measurements or lack of sensitivity. Copper concentration in organs of humans vary with age [7], most significant are the changes found to occur during the fetal and neonatal periods. The high concentration of copper found in the newborn liver (325 µg/g, age 0-3 months; 24 µg/g, age 3 months - 1 year) may be due to deficiency in breast milk, 0.05 mg/100 g. Copper is never found free in the organs or
<table>
<thead>
<tr>
<th>Diseases of humans in which there are elevated levels of serum copper*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic poisoning</td>
</tr>
<tr>
<td>Hyperthyroidism</td>
</tr>
<tr>
<td>Central nervous system disorders</td>
</tr>
<tr>
<td>Leukaemia</td>
</tr>
<tr>
<td>Collagen diseases</td>
</tr>
<tr>
<td>Liver diseases</td>
</tr>
<tr>
<td>Diabetes</td>
</tr>
<tr>
<td>Malaria</td>
</tr>
<tr>
<td>Hepatolenticular degeneration</td>
</tr>
<tr>
<td>Sickle cell anaemia</td>
</tr>
<tr>
<td>Iron deficiency anaemia</td>
</tr>
<tr>
<td>Schizophrenia</td>
</tr>
</tbody>
</table>

* Taken from [5]
Table 1(iii) The distribution of copper in an adult human*.

<table>
<thead>
<tr>
<th>Area</th>
<th>Cu/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>5.40-11.25</td>
</tr>
<tr>
<td>Liver</td>
<td>7.07-28.70</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.83-1.11</td>
</tr>
<tr>
<td>Heart</td>
<td>0.83-1.52</td>
</tr>
<tr>
<td>Muscle</td>
<td>18.0 -43.2</td>
</tr>
<tr>
<td>Skin</td>
<td>1.12-2.10</td>
</tr>
<tr>
<td>Digestive</td>
<td>1.13-2.55</td>
</tr>
<tr>
<td>Blood</td>
<td>0.72-1.25</td>
</tr>
<tr>
<td>Bile</td>
<td>0.06-2.05</td>
</tr>
<tr>
<td>Urine</td>
<td>18 ± 7 µg day⁻¹</td>
</tr>
</tbody>
</table>

*reference [8]
fluids of the body because of its extremely toxic nature, but rather as a complex, bound to proteins or smaller organic compounds.

High concentrations of copper are found in chocolate, nuts, oysters, other shell foods and dried fruits, whereas low concentrations appear in milk and milk products, eggs, fresh fruit, meat and vegetables, table 1(iv).

1.3 Copper Metalloenzymes and Proteins

Copper is found to be a vital constituent of copper-containing enzymes, which lose this activity when the metal ion is removed. Copper proteins and enzymes perform diverse functions, these include the transport of oxygen and electrons, catalysts in oxidation-reduction reactions, and protection against toxic oxygen radicals. These have been reviewed recently [5], functions of some of which are shown in table 1(v).

1.4 Copper Metabolism
1.4 (i) Introduction

In order that a full understanding of the functions of copper within the body are to be understood, detailed knowledge of the metabolism is required. This includes events such as absorption, transportation, intracellular
Table 1(iv) Dietary sources of copper, [9].

<table>
<thead>
<tr>
<th>Food Item</th>
<th>Samples</th>
<th>Copper* (mg/100g)</th>
<th>Food Item</th>
<th>Samples</th>
<th>Copper** (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oysters</td>
<td>430</td>
<td>17.1</td>
<td>Eggs</td>
<td>42</td>
<td>0.10</td>
</tr>
<tr>
<td>Calf's liver</td>
<td>64</td>
<td>7.9</td>
<td>Apples</td>
<td>69</td>
<td>0.09</td>
</tr>
<tr>
<td>Beef liver</td>
<td>59</td>
<td>2.8</td>
<td>Frankfurters</td>
<td>2</td>
<td>0.08</td>
</tr>
<tr>
<td>Sunflower seeds</td>
<td>2</td>
<td>1.8</td>
<td>Fresh figs</td>
<td>5</td>
<td>0.07</td>
</tr>
<tr>
<td>Gelatin</td>
<td>13</td>
<td>1.8</td>
<td>Oranges</td>
<td>31</td>
<td>0.06</td>
</tr>
<tr>
<td>Lobster</td>
<td>7</td>
<td>1.7</td>
<td>Ice cream</td>
<td>63</td>
<td>0.05</td>
</tr>
<tr>
<td>Walnuts</td>
<td>5</td>
<td>1.4</td>
<td>Human milk</td>
<td>14</td>
<td>0.05</td>
</tr>
<tr>
<td>Molasses</td>
<td>64</td>
<td>1.4</td>
<td>Butter</td>
<td>1638</td>
<td>0.03</td>
</tr>
<tr>
<td>Chocolate</td>
<td>19</td>
<td>1.0</td>
<td>Cottage cheese</td>
<td>1</td>
<td>0.02</td>
</tr>
</tbody>
</table>

* "High" copper content (>1.0 mg Cu/100g)

** "Low" copper content (<1.0 mg Cu/100g)
Table 1(v) Some copper metalloenzymes and proteins [5].

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caeruloplasmin</td>
<td>Copper transport, oxidation</td>
</tr>
<tr>
<td>Cytochrome C oxidase</td>
<td>Terminal oxidase</td>
</tr>
<tr>
<td>Dopamine-β-hydroxylase</td>
<td>Noradrenaline synthesis</td>
</tr>
<tr>
<td>Haemocyanin</td>
<td>Oxygen transport</td>
</tr>
<tr>
<td>Laccase</td>
<td>Terminal oxidase</td>
</tr>
<tr>
<td>Metallothionein</td>
<td>Copper storage</td>
</tr>
<tr>
<td>Plastocyanin</td>
<td>Electron transfer</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>Superoxide radical destruction</td>
</tr>
<tr>
<td>Tyrosinase</td>
<td>Amino acid metabolism</td>
</tr>
</tbody>
</table>
storage and excretion, and incorporation into enzymes that require copper to function, figure 1(i).

1.4 (ii) Copper Absorption

Recent reviews on copper metabolism [5,6] suggest that the precise mode of absorption of the metal ion is uncertain. It is believed that the acidic environment within the stomach is conducive for removing copper present in proteins within food. Absorption proceeds with reduced intensity through the intestines. It has also been suggested that small peptides and amino acids either released from the mucosal cells or pancreatic secretions, or present in serum, may facilitate the movement of copper into the mucosal cells. It remains unclear whether the oxidation state of the copper ion is important at this stage. The cysteine rich metallothionein is also believed to be involved in copper metabolism, although its precise function is uncertain.

1.4 (iii) Copper Transport

Following the introduction and passage of transition metal ions such as copper, zinc, cadmium and nickel into the gastrointestinal tract of man and other animals, absorption across the gut mucosa occurs, leading to the introduction of these metal ions in blood. These metal
Figure 1(i) Schematic outline of the metabolism of a typical trace element.
ions do not appear free, but are complexed to various ligands, and transported to the tissues for use.

In 1953 Gubler et al [10] isolated and identified the blue copper protein in serum, caeruloplasmin, accounting for 96% of the total circulating copper. However, this was found to be in a non-exchangeable form. Further studies were to follow by Bearn and Kunkel [11], who established that after absorption copper appears in the bloodstream bound to an $\alpha_1$-globulin, albumin, and to a lesser extent the amino acids. In vivo studies showed that upon orally administering $^{64}$Cu to rats and bleeding after 15 minutes, 4.5 hours and 24 hours, the copper was initially bound wholly to the albumin fraction, but afterwards the radioactive fraction was predominantly associated with an $\alpha_1$-globulin, which was identified as the protein caeruloplasmin observed by Gubler et al [10].

This view has remained very much unchanged, although recently results from Chilvers et al [12] have suggested that the serum copper was found in three fractions; caeruloplasmin bound (85-95%); albumin (5-15%); and a fraction (<3%) which showed properties similar to a copper-thionein like protein. No amino acid bound fraction was observed.

Other workers have produced corroboratory evidence in
support of the earlier work. Henkin [13] showed that the major ligands for copper in plasma were caeruloplasmin, which binds approximately 90% of the circulating copper, albumin which binds 9% and the amino acid fraction, ca. 1%, which were interrelated as shown in 1(i).

MACROMOLECULAR LIGANDS

MICROMOLECULAR LIGANDS

\[
\begin{align*}
\text{Caeruloplasmin} & \rightleftharpoons \text{Albumin} \rightleftharpoons \text{Amino Acids} \\
90\% & 9\% & 1\%
\end{align*}
\]

Several groups of workers have attempted to elaborate on this scheme. Sarkar et al [14] suggested that a ternary species of the form albumin-Cu(II)-amino acid is in equilibrium with the amino acid fraction which is then transported across the membrane, scheme 1(ii).

\[
\begin{align*}
\text{Cu(II) + amino acid} & \rightleftharpoons \text{Cu(II)} - \text{amino acid} \\
\text{albumin-Cu(II)-amino acid} & \rightleftharpoons \text{albumin-Cu(II)+amino acid}
\end{align*}
\]

Scheme 1(ii)

It is known that metal ions may mediate the binding of small molecules to proteins [15]. The formation of
intermediary ternary species may therefore play an important role in the exchange and transport of trace metals [14].

Further mechanisms have been proposed to account for the circulating copper such as that by Pickart [16]. The proposed scheme is shown in figure 1(ii). The mechanism requires the tripeptide Gly-L-His-L-Lys (GHL) as an intermediate. Experiments performed with cultured hepatoma cells suggested that the exchangeable albumin-bound copper is in equilibrium with the GHL fraction, which in turn transfers the copper through the membrane to cells, negating the need for the copper-amino acid fraction in this role. Pickart postulated a hypothetical transport pathway for copper that suggests a relationship between caeruloplasmin, albumin, histidine and GHL. The precise mechanism of how the metal ion traverses the cell wall remains unclear.

Further experiments with hepatoma cells [17] showed the existence of selective copper binding sites on the cell membrane. Two possible mechanisms may exist to transport the copper into the cell. Either a temporary ternary complex is formed consisting of albumin, copper and a compound on the membrane, after which the albumin diffuses away to recirculate leaving copper behind, or the entire copper-albumin complex is internalized as a whole,
Figure 1(ii) The copper transport mechanism proposed by Pickart [16],
Further detailed examination of each of these fractions is required. The caeruloplasmin fraction is generally considered to be the non-exchangeable form of copper, the protein acting possibly in a transport/storage capacity. The albumin and amino acid fractions are generally considered the exchangeable forms of copper. Since part of the work in this thesis is aimed at elucidating the nature of this interaction further elaboration is necessary.

Albumin is the major protein component in blood plasma, and is known to serve a number of physiological roles. Albumins are single chain proteins of about 585 amino acid residues. Human serum albumin has a relative molecular mass of about 66,000, although there are slight variations between species. The protein comprises seventeen disulphide bonds creating a series of sequential loops. Albumin is believed to have evolved from three smaller proteins each containing three loops in a long-short-long arrangement. The composition of even pure albumin may show slight variations. Albumin contains a solitary thiol group at the thirty-fourth position, which may undergo reaction to form a disulphide bond. The amino acid sequences are known for a number of albumins from different species, including man, dog, rat and bovine.
Figure 1(iii) Model of copper binding to the cell membrane [17].

- Cu
- Tightly bound Cu
- albumin
The sequence of the first twenty-four amino acids are shown in figure 1(iv). Albumin is known to transport fatty acids, bilirubin, steroids, amino acids and various hormones, as well as having specific binding sites for a number of metals.

Shearer et al [21] characterized, by limited peptic digestion of bovine serum albumin, the first twenty-four residues, and showed them to contain three histidine residues at position 3, 9 and 18. The same group [22] also showed that the primary binding site in the peptide for Cu(II) involved the NH₂-terminal group. On addition of one equivalent of Cu(II), the terminal amino group and the histidine residue 3 were protected, the reactivity of histidine residue 18 was also reduced. The tetrapeptide L-Asp-L-Thr-L-His-L-Lys was obtained which corresponded to the first four residues of bovine serum albumin, and formed an equimolar complex with Cu(II).

Human serum albumin was shown to contain one specific copper(II) binding site [23] involving the N-terminal sequence L-Asp-L-Ala-L-His, the atoms ligated to copper are the backbone peptide nitrogen atoms of two residues, the terminal amino group, and an imidazole nitrogen of the histidine residue. Attempts by Sarkar and his co-workers have been made to mimic the binding site with model peptides such as Gly-Gly-L-His-NHMe [24]. Addition of one
Figure 1(iv) The amino acid sequences for the N-terminal (1-24), amino acid sequences of bovine, rat, human and dog serum albumins [20].

equivalent of copper(II) was observed to form a single species over a wide range of pH, the square planar species in figure 1(v) was believed to be present. Spectroscopically this species resembled that observed with the Cu(II)-human serum albumin complex, which would imply that the coordination geometry was similar. Detailed studies were undertaken with the peptide Gly-Gly-L-His to examine its suitability as a chelator in the treatment of diseases such as Wilson's disease (hepatolenticular degeneration)[25], a genetic defect characterized by copper(II) deposits within the body. In a severe haemolytic crisis copper(II) deposits must be rapidly removed.

Further detailed analyses have been performed on the copper(II) binding site of the peptide (1-24) of human serum albumin employing $^{13}$C and $^1$H NMR spectroscopy [26]. The results of these experiments suggest that the specific binding site is at the NH$_2$-terminal nitrogen, two intervening deprotonated peptide nitrogens, and the imidazole nitrogen in a square planar environment, with the carboxyl group of the aspartic acid unit forming a fifth coordinating group. The proposed structure is shown in figure 1(vi).

Electron spin resonance (ESR) spectroscopy has been employed in studying the environment about the copper(II)
Figure 1(v) The structure of the 1:1, Cu(II)(Gly-Gly-L-His) complex at physiological pH [24].

Figure 1(vi) The metal ion binding site in human serum albumin [26].
atom in the human serum albumin complex [27]. At 77K the ESR spectrum of the copper(II) complex, figure 1(vii), exhibited only one form of ESR signal between pH 6.5 and pH 11, no intermediate forms were detected. The presence of an equally spaced nine-line superhyperfine structure with spacing ~15G indicated considerable covalent binding between copper(II) and four nitrogen atoms derived from the protein. The ESR parameters for this system are shown in table 1(vi).

Unlike human serum albumin, dog serum albumin fails to exhibit the characteristics of a specific first binding site for copper(II) [28]. At high pH the site involving the α-amino group is preferred over the other possible loci. The peptide (1-24) of dog serum albumin was obtained by limited peptic hydrolysis of whole albumin. The first equivalent of copper(II) bound to the peptide was found to be distributed between at least two sites indicating non-specific binding, which may be attributed to the absence of the histidine-3 residue [20].

Approximately 1% of the circulating copper in plasma is associated with the micromolecular fraction, principally containing the amino acids [13]. The concentration of amino acids in plasma are shown in table 1(vii). One proposal is that the amino acid fraction may be involved in the mediation of the metal ion across the plasma membrane,
Figure 1(vii) The ESR spectra of human serum albumin (0.4 mM) in the presence of 1 eq. of $^{65}$Cu(II)Cl$_2$ in 0.15 moldm$^{-3}$ NaCl, pH 7 at 77K [27].
Table 1(vi) The ESR data of Cu(II) ion for its 1:1 complexes with human and dog serum albumin, Gly-Gly-L-His-NHMe, and L-Asp-L-Ala-L-His-NHMe, at 77K [27].

<table>
<thead>
<tr>
<th>Ligand Compound</th>
<th>pH</th>
<th>$g\parallel$</th>
<th>$g_m$</th>
<th>$A_{\parallel}$ (gauss)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog serum albumin</td>
<td>6.5</td>
<td>2.256</td>
<td>2.059</td>
<td>163</td>
</tr>
<tr>
<td>Dog serum albumin</td>
<td>9.5</td>
<td>2.183</td>
<td>2.051</td>
<td>203</td>
</tr>
<tr>
<td>Human serum albumin</td>
<td>6.5</td>
<td>2.166</td>
<td>2.051</td>
<td>214</td>
</tr>
<tr>
<td>Human serum albumin</td>
<td>9.2</td>
<td>2.169</td>
<td>2.049</td>
<td>214</td>
</tr>
<tr>
<td>Gly-Gly-L-His-NHMe</td>
<td>6.4</td>
<td>2.17</td>
<td>2.051</td>
<td>211</td>
</tr>
<tr>
<td>Gly-Gly-L-His-NHMe</td>
<td>11.2</td>
<td>2.163</td>
<td>2.051</td>
<td>209</td>
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<tr>
<td>L-Asp-L-Ala-L-His-NHMe</td>
<td>6.5</td>
<td>2.167</td>
<td>2.050</td>
<td>211</td>
</tr>
<tr>
<td>L-Asp-L-Ala-L-His-NHMe</td>
<td>11.1</td>
<td>2.166</td>
<td>2.049</td>
<td>209</td>
</tr>
<tr>
<td>Ligand</td>
<td>Concentration mol dm$^{-3}$</td>
<td>Ligand</td>
<td>Concentration mol dm$^{-3}$</td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>-----------------------------</td>
<td>------------</td>
<td>-----------------------------</td>
<td></td>
</tr>
<tr>
<td>Alanate</td>
<td>$3.70 \times 10^{-4}$</td>
<td>Serinate</td>
<td>$1.22 \times 10^{-4}$</td>
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</tr>
<tr>
<td>Aminobutyrate</td>
<td>$2.40 \times 10^{-5}$</td>
<td>Threoninate</td>
<td>$1.50 \times 10^{-4}$</td>
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<tr>
<td>Arginate</td>
<td>$9.50 \times 10^{-5}$</td>
<td>Tryptophanate</td>
<td>$1.00 \times 10^{-5}$</td>
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<tr>
<td>Asparaginate</td>
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<td>Tyrosinate</td>
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<tr>
<td>Aspartate</td>
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<td>Valinate</td>
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<td></td>
</tr>
<tr>
<td>Citrullinate</td>
<td>$2.70 \times 10^{-5}$</td>
<td>Histamine</td>
<td>$3.00 \times 10^{-8}$</td>
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</tr>
<tr>
<td>Cysteinate</td>
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<td>Carbonate</td>
<td>$2.45 \times 10^{-2}$</td>
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</tr>
<tr>
<td>Cystinate</td>
<td>$4.00 \times 10^{-5}$</td>
<td>Phosphate</td>
<td>$3.81 \times 10^{-4}$</td>
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</tr>
<tr>
<td>Glutamininate</td>
<td>$5.21 \times 10^{-4}$</td>
<td>Silicate</td>
<td>$1.38 \times 10^{-4}$</td>
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<tr>
<td>Glutamate</td>
<td>$4.80 \times 10^{-5}$</td>
<td>Sulphate</td>
<td>$2.11 \times 10^{-4}$</td>
<td></td>
</tr>
<tr>
<td>Glycinate</td>
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<td>Thiocyanate</td>
<td>$1.40 \times 10^{-5}$</td>
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</tr>
<tr>
<td>Histidinate</td>
<td>$8.50 \times 10^{-5}$</td>
<td>Ammonia</td>
<td>$2.40 \times 10^{-5}$</td>
<td></td>
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<tr>
<td>Hydroxy-proline</td>
<td>$7.00 \times 10^{-6}$</td>
<td>Citrate</td>
<td>$1.13 \times 10^{-4}$</td>
<td></td>
</tr>
<tr>
<td>Isoleucinate</td>
<td>$6.50 \times 10^{-5}$</td>
<td>Lactate</td>
<td>$1.82 \times 10^{-3}$</td>
<td></td>
</tr>
<tr>
<td>Leucinate</td>
<td>$1.24 \times 10^{-4}$</td>
<td>Malate</td>
<td>$3.50 \times 10^{-5}$</td>
<td></td>
</tr>
<tr>
<td>Lysinate</td>
<td>$1.78 \times 10^{-4}$</td>
<td>Oxalate</td>
<td>$1.20 \times 10^{-5}$</td>
<td></td>
</tr>
<tr>
<td>Methionate</td>
<td>$2.90 \times 10^{-5}$</td>
<td>Pyruvate</td>
<td>$9.50 \times 10^{-5}$</td>
<td></td>
</tr>
<tr>
<td>Ornithinate</td>
<td>$5.80 \times 10^{-5}$</td>
<td>Salicylate</td>
<td>$5.00 \times 10^{-6}$</td>
<td></td>
</tr>
<tr>
<td>Phenylalanate</td>
<td>$6.40 \times 10^{-5}$</td>
<td>Succinate</td>
<td>$4.20 \times 10^{-5}$</td>
<td></td>
</tr>
<tr>
<td>Prolinate</td>
<td>$2.11 \times 10^{-4}$</td>
<td>Ascorbate</td>
<td>$4.30 \times 10^{-5}$</td>
<td></td>
</tr>
</tbody>
</table>
and thus its interrelationship with the transport form of the metal ion (the metal albumin protein complex) is important. The amino acid complexes are typically either binary or ternary species, \( \text{Cu(II})(\text{AA}_1)_2 \) or \( \text{Cu(II)}(\text{AA}_1)(\text{AA}_2) \). Understanding the interactions between metal ions and ligands in biofluids, such as blood plasma, is not straightforward. Due to the very low concentrations of the species formed, and the intricate distribution of the equilibria involved, no feasible experimental approach can be found. Hence computer simulations must be performed in order to elucidate the distribution of the metal ions within the biofluids [29]. However, the value of the calculations are dependent on a number of factors, the most important of which is the determination of stability constants for each of the metal ion-amino acid complexes formed in plasma. Stability constants for ternary species of the form metal ion-amino acid\(_1\)-amino acid\(_2\) may have to be estimated [30], because they cannot be determined easily experimentally. The calculations also assume that equilibrium conditions prevail in blood plasma. One such computer program is ECCLES (Evaluation of Constituent Concentrations in Large Equilibrium Systems). This program calculates the concentrations of metal-ligand complexes at different pH values and requires as input data the stability constants of each equilibrium reaction together with the total or free concentrations of each component. The results of two such calculations [29,31]
are shown in table 1(viii). The differing distributions of Cu(II) within the micromolecular weight fraction of plasma is largely due to the differing stability constants employed in the computations. From these results it would appear that the major micromolecular weight species present is the binary Cu(II)(L-His)\(_2\) species.

Stopped-flow kinetic studies have been performed on the Cu(II) ion binding to bovine serum albumin [32], and on the Cu(II) exchange reactions of L-histidine with human serum albumin [33]. A mechanism has been proposed to account for the observations, scheme 1(iii).

\[
\begin{align*}
\text{Cu(II)(L-His)\(_2\) + albumin} & \xrightarrow{k_1} \text{L-His + L-His - Cu(II)-albumin} \\
\text{Cu(II)albumin + L-His} & \xrightarrow{k_2} \text{L-His - Cu(II) - albumin}
\end{align*}
\]

Scheme 1(iii)

where \(k_{\text{ra}}\) and \(k_{\text{ra}}\) refer to a structural rearrangement reaction. Pseudo-first order rate constants for the exchange of Cu(II) from L-histidine to albumin and albumin to L-histidine were found to be 0.67 s\(^{-1}\) and 0.04 s\(^{-1}\) respectively at pH 7.53 in 0.1 moldm\(^{-3}\) N-ethylmorpholine-HCl buffer. The rate determining step being ascribed to the formation of the mixed ternary species.
Table 1(viii) Computer distribution of Cu(II) amongst the low molecular weight (lmw) species in serum.

<table>
<thead>
<tr>
<th>Complex Species</th>
<th>Percentage total lmw</th>
<th>Complex Species</th>
<th>Percentage total lmw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(His)$_2$</td>
<td>19</td>
<td>Cu-His-Cys$^-$</td>
<td>21</td>
</tr>
<tr>
<td>Cu-His-Thr</td>
<td>18</td>
<td>Cu-His-Cys-H</td>
<td>17</td>
</tr>
<tr>
<td>Cu-His-Ser</td>
<td>9</td>
<td>Cu(His)$_2$</td>
<td>11</td>
</tr>
<tr>
<td>Cu-His-Ala</td>
<td>6</td>
<td>Cu-His-Thr</td>
<td>8</td>
</tr>
<tr>
<td>Cu-His-Gly</td>
<td>5</td>
<td>Cu-His-Val</td>
<td>5</td>
</tr>
<tr>
<td>Cu-His-Lys-H$^+$</td>
<td>5</td>
<td>Cu-His-Lys-H$^+$</td>
<td>5</td>
</tr>
<tr>
<td>Cu-His-Val</td>
<td>5</td>
<td>Cu-His-Ala</td>
<td>4</td>
</tr>
<tr>
<td>Cu-His-Gln</td>
<td>5</td>
<td>Cu-His-Ser</td>
<td>4</td>
</tr>
<tr>
<td>Cu-His-Glu</td>
<td>4</td>
<td>Cu-His-Phe</td>
<td>3</td>
</tr>
<tr>
<td>Cu-His-Leu</td>
<td>3</td>
<td>Cu-His-Gly</td>
<td>3</td>
</tr>
</tbody>
</table>
Kinetic studies have also been performed on the reaction of 1:1 Cu(II):Gly-Gly-L-His solutions with the nucleophiles ethylenediaminetetraacetate (EDTA), L-histidine, and triethylenetetramine (trien). The transfer of copper(II) from complexes of serum albumin, (human and bovine), Gly-Gly-L-His, Gly-Gly-L-His-Gly, and L-Asp-L-Ala-L-His-L-Lys to trien exhibit parallel behaviour; a proton-assisted nucleophilic pathway at physiological pH [34,35].

A further drawback to the computer simulated plasma model is its inability to handle the well known redox reaction that occurs between Cu(II) and L-cysteine;

\[
4\text{CysH} + 2\text{Cu(II)} \rightarrow 2/n[\text{Cu(I)Cys}]_n + \text{Cys-Cys} + 4\text{H}^+ \quad 1(iv)
\]

Cysteine is present in plasma at a concentration of \(23 \times 10^{-6}\) mole dm\(^{-3}\), and may compete for the available exchangeable copper present. A number of workers have examined this reaction [36-38]. Under alkaline conditions Cavalini et al [36] identified a stable intermediate as a copper(II): L-cysteine, 1:2 complex. Hanaki and Kamide [37] showed that hydrogen peroxide was evolved as an intermediate from oxygen, and the existence of a transient copper(II):L-cysteine 1:1 complex, within the pH range 6.5 to 7.8. The mechanism of copper ion autoxidation of cysteine has been examined in some detail by
Zwart et al [38]. The reaction under alkaline conditions was found to be zero order with respect to cysteine, 0.5 with respect to oxygen and at low metal ion concentrations, first order with respect to copper.

Several investigators have reported the reduction of Cu(II) by biological membranes of human red cell [39], or mitochondria [40], in which the thiol groups are reputed to be involved in various fundamental processes such as oxidative phosphorylation [41]. The reaction of cysteine with caeruloplasmin copper has also been examined [42,43], and it was shown that a one-electron transfer from cysteine to caeruloplasmin occurred resulting in the specific reduction of a type 1b Cu(II) ion. It is uncertain whether the oxidation state of the copper is important when transfer across biological membranes occurs.

Part of the work in this thesis is aimed at examining the reactivity of the Cu(II)-albumin 1:1 complex with the amino acids L-histidine and L-cysteine.

1.5 Inborn Errors of Copper Metabolism

Copper deficiency and toxicity are more frequently encountered in veterinary medicine than in human medicine. However, abnormal copper metabolism has been established in two human genetically transmitted diseases, i.e. Wilson's disease and Menkes' syndrome, which arise from pathological
alterations in the homeostasis of copper metabolism. In most disease states where abnormal copper metabolism is involved, damage to one or more of the regulatory mechanisms occurs, and copper imbalance appears as a secondary manifestation of the disease. Elevated plasma copper and caeruloplasmin levels are generally the result of liver diseases which impair biliary excretion.

1.5 (i) Wilson's Disease

The disease was first accurately described by Wilson in 1912, although the first case was probably diagnosed in 1861 [6]. Wilson's disease is associated with severe, potentially lethal, degenerative changes in the brain, together with severe liver pathology which can also be fatal. Clinical symptoms generally vary with respect to the relative severity of hepatic and neurologic manifestations. The symptoms of Wilson's disease include the development of greenish-brown Kayser-Fleischer rings at the margin of the cornea, impaired renal functions, cataracts, haemolytic anaemia, bone lesions and behavioural abnormalities. Wilson's disease follows the pattern; (i) defective copper clearance is present at birth, (ii) copper gradually accumulates in the liver, (iii) eventually excess hepatic copper spills into the blood as freely exchangeable copper, and (iv) copper accumulates in several tissues, accumulation in the liver and brain being particularly
toxic. If untreated, the disease is fatal.

Adequate control of the excess copper may be undertaken by employing chelation therapy. Chelating agents must be water soluble, resistant to metabolic degeneration and retain adequate chelating ability at physiological pH values. The agents and their metal chelates must be readily excreted by the kidney and they must react rapidly and relatively specifically with the metal ions to be removed. They must also not be appreciably toxic at therapeutic concentrations.

Walshe demonstrated that penicillamine (PEN) was effective in the treatment of Wilson's disease [52-54]. Until then sufferers were treated with 2,3-dimercaptopropanol (BAL) with only marginal benefit. Some patients may develop intolerance to treatment with PEN, and in these cases the chelator trien is prescribed.

1.5 (ii) Menkes' Syndrome

The condition was first described in 1962 as a syndrome characterized by pili torti, retarded growth, progressive cerebral degeneration leading to early death, and affecting males only [55].

It was observed that clinical similarities existed
between the disease and nutritional copper deficiency that is well known to occur in sheep. Orally administered copper sulphate fails to amend the low copper status in blood and tissue, however, intravenously injected copper is metabolised normally. It was suggested that in Menkes' disease a defect exists in the gastrointestinal absorption mechanism of copper. The copper appears to become trapped in a number of tissues, particularly the kidney, which leads to a deficiency in others. Mutated brindled mice have the same defect as that underlying the human disorder and represent an excellent model. Studies by Dehez et al [56] showed that in the brindled mice a number of tissues accumulated copper; the intestinal walls, salivary glands and lymph nodes, and suggested that because all the affected tissues contain epitheloid cells that a genetic defect in these cells is responsible for the disease.

1.6 Zinc and Nickel Metabolism
1.6 (i) Transport of Zinc and Nickel in Plasma

Zinc was found to exhibit a generally similar distribution to copper in human serum [13]. Approximately 32% was found bound to a macromolecular species, \( \alpha_2 \)-macroglobulin, and was believed to be in an exchangeable form, 66% was found bound to the protein albumin, and the remaining 2% was found to be bound to the micromolecular
fraction, the amino acids. Chilvers et al [12] showed that 80-90% of zinc was bound to albumin, 10-20% to an $\alpha_2$-macroglobulin, and the remainder (<3%), was associated with a retinol-binding protein complex, and a low relative molecular mass fraction, probably the amino acids.

Rabbit serum was observed to contain three Ni(II) binding fractions, ultrafilterable Ni(II), 16%; albumin bound Ni(II), 40%; and the remainder an $\alpha_2$-macroglobulin bound fraction [44-45]. In human serum 95.7% of Ni(II) was found associated with albumin, 4.2% was bound to low molecular mass components, and 0.1% associated with a high molecular mass fraction, as yet unidentified. In the low molecular mass fraction the nickel is predominantly bound to the amino acid L-histidine, ca 72%, the remaining amino acids were found not too complex to any appreciable extent. Equilibria between the albumin bound, and the L-histidine bound Ni(II) could facilitate the transport of Ni(II) between blood and tissues [46].

The physiological role of nickel(II) is not as yet established. The diverse clinical manifestations of nickel toxicology have been reviewed by Sunderman [47]. It has been shown that both copper(II) and nickel(II) have the same primary binding site on human serum albumin, although the copper(II) binding site is much stronger, so that copper(II) will displace nickel(II) from the protein [48].
The two metal ions exhibit very similar distribution in blood plasma (as may be expected from their similar coordination properties), but thereafter their metabolic routes differ. This work examines the kinetic behaviour of the two albumin complexes.

Dog serum albumin again proves to be an anomaly. The affinity of dog serum albumin for copper has been shown to be poorer than for serum albumins from other species, however, the affinity for zinc was found to be similar, suggesting that the NH₂-terminal on the proteins was not the primary zinc ion binding site [49].

A circular dichroism study with dog serum albumin showed it to bind six or more Cu(II) and Ni(II) ions with affinities that were lower than for human and bovine serum albumin [50]. This study also showed that L-histidine residues were not involved in copper(II) binding to dog serum albumin. The absence of a specific nickel(II) binding site on dog serum albumin has also been clearly illustrated [51].

1.7 Molybdenum as a Biochemical Antagonist of Copper

Molybdenum induced copper deficiency is fairly widespread amongst ruminants, only a small increase in dietary molybdenum uptake is required to induce chemical
changes indicative of copper deficiency. The disease results in growth failure, skeletal damage and degenerative changes in nervous tissues, although its potency varies markedly and its effects are often difficult to predict. Extensive efforts are being made to understand the mechanism of action within ruminants. Differences exist in the *modus operandi* in monogastrics and ruminants which has led to an interest in comparative behaviour between the two groups.

Most instances of molybdenum induced copper deficiency arise when the molybdenum content in soils is sufficient to elevate the molybdenum content of forage to 2 mg/kg or more, a situation which arises in approximately one-twelfth of the cultivated land of the U.K., extensive areas of Australia, U.S.S.R. and the Americas [57]. Molybdenum is probably absorbed into biological systems as the molybdate anion, and within ruminants, sulphide derivatives of the molybdate may then play a role in the antagonism towards copper. The molybdate ion, MoO$_4^{2-}$, has no inhibitory effect on copper metabolism, however the thiosubstituted derivatives are effective antagonists, particularly the tetrathiomolybdate, MoS$_4^{2-}$ ion, which strongly inhibits copper absorption. Its systemic effects extend to a depression of caeruloplasmin and cytochrome oxidase activities as well as typical clinical manifestations which are reversible by treatment with copper [58].
The formation of the thiomolybdate ions, $\text{MoO}_4^{2-}S_x^{4-x}$, ($x = 0, 1, 2, 3$) from molybdate and sulphide salts in aqueous media have been studied under conditions which simulate the fluid phase of the rumen [59]. These species are formed in a stepwise manner as shown below:

$$[\text{MoO}_4^{2-}] \rightarrow [\text{MoO}_3S]^{2-} \rightarrow [\text{MoO}_2S_2]^{2-} \rightarrow [\text{MoOS}_3]^{2-} \rightarrow [\text{MoS}_4]^{2-}$$

The extent and rate of formation of these species within the rumen is dependent on the S:Mo ratio, and the solution pH. Formation of the tetrathiomolybdate species is favoured by a high S:Mo ratio and a pH of 6.0. At a pH 6.5-7.0 its formation is relatively slow; for example, at an S:Mo ratio of 330:1, pH 7 and at 39°C, over 48 hours were required for the formation of the fully thiosubstituted derivative. At lower levels, however, only the di- and trithiomolybdate derivatives are likely to be present [59].

Spectroscopic evidence for the production of tetrathiomolybdate in rumen cultures derived from molybdate and potential sources of sulphide has been obtained by Mills and his co-workers [60]. Addition of Cu(II) to a solution of ammonium tetrathiomolybdate in strained rumen contents resulted in large changes in the electronic absorption spectrum of this compound. At a Cu:Mo ratio of 1:1 the absorption maxima were totally eliminated, which is in agreement with the work of Clarke and Laurie [59], who
demonstrated that the addition of a solution of Cu(L-His)₂ to (NH₄)₂MoS₄ resulted in the total elimination of the absorption maxima at a 1:1 Cu(II):MoS₄²⁻ ratio. In the latter study a fine red-brown precipitate appeared which was removed by centrifugation but was not characterized.

Evidence for the generation of tetrathiomolybdate in the rumen cannot be obtained directly because of the low levels of molybdenum that are ingested; the concentrations of any thiomolybdates formed would be less than the detectable limits of electronic spectroscopy.

In view of the possibility that other metals (e.g. W) can combine with sulphide to form thioanions and may also be able to induce copper deficiency in animals, a study has been made on the effect of ammonium tetrathiotungstate [(NH₄)₂WS₄] on the absorption and distribution of copper in rats. The rats were found to develop symptoms consistent with copper deficiency [61].

The administration of tetrathiomolybdate to rats, and to a lesser extent tri- and dithiomolybdates, causes dramatic changes in the copper distribution within plasma. In particular, tetrathiomolybdate administration to rats results in an increase in the proportion of plasma copper retained in the albumin fraction [62].
Addition of tetrathiomolybdate to the Cu(II)albumin 1:1 complex leads to a decrease in the thiomolybdate absorptions and the appearance of a new peak at 500 nm. Similar behaviour was observed on addition of trithiomolybdate to the Cu(II)albumin complex. This may suggest the formation of Cu-S bonds. Unlike the reaction with Cu(L-His)$_2$ no fine suspensions of red-brown solids were observed. The stoichiometry of the products and their properties have not been clearly delineated [59].

Experiments performed by Mason and his group [63] indicated that the thiomolybdates bind to metal free albumin in vivo, and are relatively stable until displaced in some manner.

The inhibition of caeruloplasmin activity, the copper-containing transport/storage protein, by thiomolybdate has been examined [64]. Reaction with thiomolybdates caused the reduction of Cu(II) to Cu(I), as determined by the loss of ESR signal. Thiomolybdates were also found to inhibit the activity of other copper-containing enzymes, namely ascorbate oxidase, cytochrome oxidase, superoxide dismutase and tyrosinase. Chidambaram et al [64] came to the conclusion that a thiomolybdate-caeruloplasmin complex could account for their observations, l(vi).
\[
\text{Mo(VI)S}_4^{2-} + \text{Cu(II)Cp} \rightarrow \text{Mo(VI)Cp} \quad \text{(vi)}
\]

This would then markedly reduce the activity of the plasma copper enzyme.

An overall scheme for the generation of Cu-MoS$_4^{2-}$ species is shown in figure 1(viii), and the intention of part of this project is to examine the insoluble products generated from this interaction, and the species formed by the interaction of MoS$_4^{2-}$ with the Cu(II)albumin complex.

1.8 Molybdenum Enzymes

Molybdenum is known to be a necessary co-factor for five enzymatically catalysed redox reactions: nitrogen and nitrate reduction and purine, aldehyde and sulphite oxidation. A summary of the known molybdenum enzymes is shown in table 1(ix).

Molybdenum enzymes have been classified into two groups according to the molybdenum containing co-factors. Nitrogenase, the enzyme which is responsible for the conversion of dinitrogen to ammonia, contains a unique iron-molybdenum-sulphur cluster "FeMoco", whereas the other well-characterized Mo enzymes contain a mononuclear oxomolybdenum site, "Moco". Zumft [66] showed the nitrogenase co-factor to consist of units of thiomolybdates
Figure 1(viii) Proposed scheme for the Cu-Mo-S interaction in ruminants [65].

Food uptake: $\text{Cu}^{n+}$, $\text{SO}_4^{2-}$, and S-amino acids $\text{MoO}_4^{2-}$

In the rumen: $\text{CuS}$, $\text{MoS}_4^{2-}$

Cu-Mo-interaction in the intestine

Resorption

$\text{Cu-MoS}_4$-complex

In the tissue: Protrains

Protein-Cu-MoS$_4$ complex

Result - Clinical and biochemical deficiency of copper.
Table 1(ix) The molybdenum containing enzymes [67].

<table>
<thead>
<tr>
<th>ENZYME</th>
<th>REACTION CATALYSED</th>
<th>PROSTHETIC GROUPS</th>
<th>PROTEIN SUBUNITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogenase (C. pasteurianum)</td>
<td>( \text{N}_2 + 6\text{H}^+ + 6\text{e}^- \rightarrow 2\text{NH}_3 )</td>
<td>( 2(2[\text{Fe}_4\text{S}_4], \text{FeMo-co}) )</td>
<td>( 2 \times 50,000; 2 \times 60,000 )</td>
</tr>
<tr>
<td>Xanthine oxidase (bovine milk)</td>
<td>( \text{RH} + \text{H}_2\text{O} \rightarrow \text{ROH} + 2\text{H}^+ + 2\text{e}^- )</td>
<td>( 2(2[\text{Fe}_2\text{S}_2]) )</td>
<td>( 2 \times 150,000 )</td>
</tr>
<tr>
<td>Sulfite oxidase (chicken liver)</td>
<td>( \text{SO}_3^- + \text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + 2\text{e}^- + 2\text{H}^+ )</td>
<td>( 2(\text{Fe-PPIX,Mo-co}) )</td>
<td>( 2 \times 55,000 )</td>
</tr>
<tr>
<td>Nitrate reductase (E. coli)</td>
<td>( \text{NO}_3^- + 2\text{e}^- + 2\text{H}^+ \rightarrow \text{NO}_2^- + \text{H}_2\text{O} )</td>
<td>( 4 \text{Fe}_4\text{S}_4 )</td>
<td>( 1 \times 142,000; 1 \times 60,000 )</td>
</tr>
<tr>
<td>Nitrate reductase (Chlorella vulgaris)</td>
<td>( \text{NO}_3^- + 2\text{e}^- + 2\text{H}^+ \rightarrow \text{NO}_2^- + \text{H}_2\text{O} )</td>
<td>( 4(\text{FAD,FepPIX}) )</td>
<td>( 4 \times 90,000 )</td>
</tr>
<tr>
<td>CO(_2) reductase (C. pasteurianum)</td>
<td>( \text{HCOO}^- \rightarrow \text{CO}_2 + \text{H}^+ + 2\text{e}^- )</td>
<td>(Mo-co)</td>
<td></td>
</tr>
</tbody>
</table>
as determined by their characteristic electronic absorption spectrum.

Most information on the study of molybdenum atoms in nitrogenase and the other proteins has been obtained from the application of X-ray Absorption Spectroscopy, and EXAFS (Extended X-ray Absorption Fine Structure)\[67,68\].

1.9 Coordination Chemistry of Tetrathiomolybdate(VI)
1.9 (i) Introduction

Tetrathiomolybdate, $\text{MoS}_4^{2-}$, is a component of both the molybdenum-containing enzymes and the Cu-Mo antagonism. Research groups are actively engaged in the generation of new synthetic analogues which may mimic the behaviour of the molybdenum containing fractions in each case, and a number of reviews have appeared illustrating the present level of knowledge \[65,69,70\].

1.9 (ii) Copper-Tetrathiomolybdate Systems

It was one hundred years ago when the thiomolybdates were first prepared \[71\]. Since then Mellor \[72\] and, more recently, McDonald \emph{et al} \[73\] have reviewed these preparations. The usual method of preparing the thiomolybdate salts is by passing hydrogen sulphide through alkaline solutions of molybdate salts. The solutions
during these reactions become first yellow $[\text{MoO}_2\text{S}_2]^{2-}$, then orange $[\text{MoOS}_3]^{2-}$ and finally red $[\text{MoS}_4]^{2-}$. The analogous thiotungstate salts were first prepared by Mellor [72], and these preparations have also been reviewed by McDonald et al [73]. In contrast to the thiomolybdates, exhaustive bubbling through the tungsten solutions is required, approximately 6 hours for tetrathiotungstate preparation. All the thiometalate salts are highly coloured, the UV/VIS absorption spectral data of the thiomolybdate and thiotungstate salts are shown in table 1(x), along with the infra-red spectral data. These thiometalates show characteristic M-S and M-O stretching frequencies (M=Mo,W).

The reaction of thiomolybdates with copper has attracted attention because of its biological importance. The reactions of copper and other transition metal ions with thiomolybdate anions were first noted by Mellor [72], and compounds of the type $\text{CuMoS}_4$, $\text{Ag}_2\text{MoS}_4$, $\text{BaMoS}_4$ and $\text{CaMoS}_4$ reported. Bok and Boeyens reported the formation of copper(I) thiomolybdate upon mixing solutions of copper(I) chloride and ammonium tetrathiomolybdate. The compound was obtained as a finely divided powder which gave a few diffuse halos in its x-ray powder diffraction pattern. The chemical analyses were consistent with the formation of $\text{Cu}_2\text{MoS}_4$[74].

Clarke and Doyle [75] prepared some solid
Table 1(x) Spectral data for thiomolybdates and thiotungstates [72].

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>INFRARED(^a)</th>
<th>UV/VISIBLE(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\nu_{\text{M-S}})</td>
<td>(\nu_{\text{M=O}})</td>
</tr>
<tr>
<td>((\text{Et}_4\text{N})_2\text{MoS}_4)</td>
<td>470</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>865</td>
</tr>
<tr>
<td>((\text{Et}_4\text{N})_2\text{MoO}_3)</td>
<td>470</td>
<td>865</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>((\text{Et}_4\text{N})_2\text{MoO}_2\text{S}_2)</td>
<td>460</td>
<td>860, 840</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>((\text{Et}_4\text{N})_2\text{W}_4)</td>
<td>458</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>((\text{Et}_4\text{N})_2\text{WOS}_3)</td>
<td>450</td>
<td>885</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>((\text{Et}_4\text{N})_2\text{WO}_2\text{S}_2)</td>
<td>445</td>
<td>885, 845</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>((\text{NH}_4)_2\text{MoS}_4)</td>
<td>480</td>
<td>-</td>
</tr>
<tr>
<td>((\text{NH}_4)_2\text{MoO}_3)</td>
<td>485</td>
<td>835</td>
</tr>
<tr>
<td>(\text{Cs}_2\text{MoO}_3)</td>
<td>480</td>
<td>855</td>
</tr>
<tr>
<td>((\text{NH}_4)_2\text{MoO}_2\text{S}_2)</td>
<td>490</td>
<td>830, 792</td>
</tr>
<tr>
<td>((\text{NH}_4)_2\text{WS}_4)</td>
<td>460</td>
<td>-</td>
</tr>
<tr>
<td>((\text{NH}_4)_2\text{WOS}_3)</td>
<td>470</td>
<td>860</td>
</tr>
<tr>
<td>((\text{NH}_4)_2\text{WO}_2\text{S}_2)</td>
<td>470</td>
<td>850, 795</td>
</tr>
</tbody>
</table>

\(^a\)Spectra taken as KBr pellets; values in wavenumbers, ±5 cm\(^{-1}\).  
\(^b\)Peak positions in nm with molar absorptivities in parentheses.  
\(^c\)In H\(_2\)O.  
\(^d\)In MeCN.  
\(^e\)Peak positions and molar absorptivities (in H\(_2\)O) identical within experimental error to the \([\text{NEt}_4]^+\) salts.
tetrathiomolybdate complexes by mixing aqueous solutions of ammonium tetrathiomolybdate with cobalt, nickel, copper and zinc sulphate solutions. The mole ratio of Cu:Mo:S for the copper tetrathiomolybdate product was 1:0.96:3.91, the sample exhibited no characteristic absorption in the range 4000-400 cm\(^{-1}\), and gave a powder diffraction photograph which resembled that for copper sulphide. These observations led Clarke and Doyle to the conclusion that the so-called metal thiomolybdates were mixtures of sulphides of copper and molybdenum. An unreported observation during this precipitation was the drop in pH to 2.0 [76].

Muller and Menge obtained an impure product, copper tetrathiomolybdate, from the reaction of copper(II) chloride and ammonium tetrathiomolybdate. They also reported the preparation of Cu\(_2\)WS\(_4\), and observed the reduction of copper(II) to copper(I) by the tetrathiotungstate ion [77].

A great deal of the recent chemistry on these systems has centred on the generation of new Cu(I)-Mo(VI)-S systems. The copper(I) ammonium tetrathiomolybdate has been well characterized [76]. This was prepared by the addition of copper(II) sulphate to ammonium paramolybdate in an ammoniacal solution saturated with hydrogen sulphide. The infra-red spectrum reveals a characteristic band at
460 cm\(^{-1}\), and the structure, determined from powder diffraction measurements, was found to consist of chains of CuS\(_4\) and MoS\(_4\) tetrahedra connected via edges, figure 1(ix). The corresponding tungsten analogue was found to be isostructural.

A large number of polynuclear Cu(I)-Mo(VI)-S systems have been obtained, and the chemistry of these compounds has recently been reviewed by Sarkar and Mishra [66]. The synthesis of a number of copper(I)-thiomolybdate(VI) compounds are shown in figure 1(x). These polymeric compounds do not however take into account the protein interaction, the interaction of thiomolybdates with the Cu(II)albumin complex appears to be an important component of the antagonism, and hence warrants further investigation.

A number of polynuclear molybdenum and copper sulphides have been prepared which may have biological significance as models of molybdenum containing enzymes. Muller and his co-workers have been actively engaged in developing new polynuclear molybdenum-sulphur complexes [80-83], figure 1(xi). The dimeric Mo(V) species [Mo\(_2\)S\(_8\)]\(^{2-}\) [80] and [Mo\(_2\)O\(_2\)S\(_6\)]\(^{2-}\) [81] are isostructural. The Mo(IV) cluster, [Mo\(_3\)S\(_{13}\)]\(^{2-}\) has been prepared by the addition of ammonium polysulphide to a mixture of ammonium paramolybdate and NH\(_2\)OH-HCl. Like [Mo\(_2\)(S\(_2\))\(_6\)]\(^{2-}\) [83] and
Figure 1(iii)

(a) A unit cell projection of the structure of CuNH₄MoS₄ along the [001] direction. Sulphur atoms are located at the vertices of the tetrahedra.

(b) A projection of the structure of CuNH₄MoS₄ along the [110] direction [75].
Figure 1(x) The Synthesis and Interconversion Reactions of Copper(I)-Thiomolybdate(VI) Complexes [77].
Figure 1(xi) Polynuclear Mo-S compounds.
I; $[\text{Mo}_2 \text{V}(S_2)_6]^{2-}$, II; $[\text{Mo}_2 \text{V}(S_2)_2S_4]^{2-}$, III; $[\text{Mo}_2 \text{V}_0_{2}S_2(S_2)_2]^{2-}$
IV; $[\text{Mo}^{IV}_3S(S_2)_6]^{2-}$, V; $[\text{Mo}^{III}_4(\text{NO})_4S_3(S_2)_5]^{2-}$

I

II

III

IV

V
the cluster contains both terminal and bridging disulphide groups. The Mo(III) cluster 
\[\text{[Mo}_4\text{(NO)}_4\text{S}_3\text{(S}_2\text{)}_5^4^-\text{]}\] has also been prepared. The interrelationship of a number of polymeric molybdenum sulphur complexes is shown in figure 1(xii).

Muller's group has also been engaged in the generation of new polymeric copper-sulphur complexes. The complex (PPh$_4$)$_2$[Cu$_6$S$_{17}$] was prepared by the addition of an ethanolic polysulphide solution to Cu(acac)$_2$. The product contains polysulphide (S$_4^{2-}$ and S$_5^{2-}$) ions, copper is present in the +1 oxidation state [84]. Other copper(I) sulphur aggregates included the mixed crystal Cu(I) compound (PPh$_4$)$_2$[Cu$_4$(S$_4$)(S$_2$)$_2$]$_{0.4}$[Cu$_4$(S$_5$)$_3$]$_{0.6}$ [85], figure 1(xiii).

Tungsten behaves chemically in a similar manner to molybdenum; both metals exhibit a wide variety of stereochemistries in addition to the variety of oxidation states, consequently substitution with tungsten is fairly common.

Trinuclear species of the type [W$_3$S$_9$]$^{2-}$ and [W$_3$OS$_8$]$^{2-}$ are both prepared from WS$_4^{2-}$ [86]. Complex anions of the type [M(WS$_4$)$_2$]$^{2-}$ and [M(MoS$_4$)$_2$]$^{2-}$ have been reported [87], (M=transition metal). The bis(tetrathiotungstate)nickelate(II) ion, Ni(WS$_4$)$_2^{2-}$, is
Figure 1(xii) Scheme for the generation of a number of polynuclear molybdenum-sulphur complexes.

\[ \text{[Mo}^{IV} \text{S}(S_2)_2]^{2-} \]

\[ \text{[Mo}^{VI} \text{O}_n \text{S}_{4-n}]^{2-} \]

\[ \text{[Mo}^{VII} \text{O}_{24}]^{6-} \]

\[ \text{[Mo}^{IV} \text{O}_2 \text{S}_2(S_2)_2]^{2-} \]

\[ \text{[Mo}^{IV} \text{S}(S_2)_3(SCH}_2\text{CH}_2\text{S})_3]^{2-} \]

\[ \text{[Mo}^{IV} \text{S}_4(SCH}_2\text{CH}_2\text{S})_3]^{2-} \]

\[ \text{[NH}_4\text{]}_2 \text{S}_x \]

\[ \text{NH}_2\text{OH-HCl /}[\text{NH}_4][\text{SCN}] \]

\[ \text{S}^{-} \]
Figure 1(xiii) Polynuclear copper-sulphur aggregates. 
I $[\text{Cu}_6\text{S}_{17}]^{2-}$; II $[\text{Cu}_4\text{S}_x]^{2-}$ [83,84].
prepared by the addition of aqueous solutions of Ni(II) salts and \((\text{NH}_4)_2\text{WS}_4\). The structure is shown below in 1(vii).

\[
\begin{array}{c}
\text{S} \\
\text{W} \\
\text{S} \\
\text{Ni(II)} \\
\text{S} \\
\text{W} \\
\text{S} \\
\end{array}
\]

1(vii)

1.9 (iii) Iron-thiomolybdate Compounds

Attempts to prepare molybdenum-iron-sulphur aggregates resembling the "FeMoco" co-factor have led to the generation of a number of synthetic analogues exhibiting similar spectral characteristics to the native protein.

A number of complexes containing an FeMoS\textsubscript{4} core have been prepared, including \[((\text{PhS})_2\text{FeMoS}_4)_2\] \textsuperscript{2-} [88], and \[[\text{Et}_3\text{N}]_3[\text{Fe(MoS}_4)_2]\] [89]. Compounds containing Fe\textsubscript{3}MoS\textsubscript{4} cubes have also been prepared, the complex \[[\text{Et}_3\text{N}]_4[\text{Fe}_6\text{Mo}_2\text{S}_8(\text{SCH}_2\text{CH}_2\text{OH})_9]\] was prepared by Christou et al [90], by the method outlined in scheme 1(viii), where \(R = \text{SCH}_2\text{CH}_2\text{OH}\) and \(R' = \text{Et}\). Similar complexes have also been prepared by Wolff et al [91], including \[[\text{Mo}_2\text{Fe}_6\text{S}_8(\text{SEt})_9]_3\] \textsuperscript{3-} and \[[\text{Mo}_2\text{Fe}_6\text{S}_9(\text{SEt})_8_3]_3\] \textsuperscript{3-}.

The chemistry of Fe-Mo-S complexes and their possible relevance as analogues for structural features in the molybdenum site of nitrogenase has been reviewed by Coucouvanis [92].
6Fe Cl₃(−) + MeOH → 6[Fe(SR)₃]ₙ + 18NaCl + 6NaSR

N₂

24NaSR

2(NH₄)₂MoS₄

(NH₄)₃[Fe₆Mo₂S₈(SR)₉] + 4 SSR + NH₄SR

R'NBr (excess)

(R₄N)₃[Fe₆Mo₂S₈(SR)₉]

Scheme 1(viii)

1.10 Potentiometric Titrations

In section 1.4(iii) it was pointed out that a small variation in the value of a stability constant can lead to a wide disparity in the distribution of metal ion species in plasma. Accurate determination of values of formation constants is obviously of critical importance. Potentiometric titrations have been employed within this school in the past for determination of stability constants of the nickel(II)-triethylenetetramine, nickel(II)-D-penicillamine [93] and copper(II)-penicillamine disulphide systems [94]. The measurement of the potential of an appropriate indicator electrode has been used as a method of detecting the equivalence point in a variety of titrations. When a potentiometric titration is performed interest is focussed upon changes in the EMF of the electrochemical cell as a
titrant of precisely known concentration is added to a solution of the test element. The method can be applied to any titrimetric reaction for which an indicator electrode is available to follow the activity of one of the substances involved.

The chief advantages of potentiometric titrations are their applicability to turbid, fluorescent, opaque or coloured solutions. The equipment necessary to carry out a potentiometric titration is shown in figure 1(xiv).

The critical problem in a titration is the recognition of the point at which the quantities of reacting species are present in equivalent amounts - the equivalence point. The titration curve can be followed point by point, plotting as ordinate successive values of the cell EMF versus the correspondingly added volume. Additions of titrant should be the smallest accurately measurable increments that provide an adequate density of points. Over most of the titration the EMF varies gradually, but near the end points the cell EMF changes abruptly as the logarithm of the concentration undergoes a rapid variation. The problem therefore is to detect the sharp change in cell EMF that occurs in the vicinity of an end point.

When performed manually a potentiometric titration is a tedious and time-consuming operation. Automatic
equipment for performing and recording the titration curves provides a logical solution to the problem. An automatic titrater enables an operator to perform other tasks while the instrument delivers the requisite titrant and stops the delivery at a preset end point, or perhaps continues beyond the end point when the entire curve is traced.

Semi-automatic titration apparatus available within this school, and used in most laboratories, consists of a piston burette (Radiometer ABU12) capable of delivering $2.5 \text{cm}^3 \pm 2.5 \mu\text{l}$ to a titration vessel (Radiometer TTA60) where efficient mixing is obtained through a mechanical stirrer incorporated in the titration cell (Radiometer TTA60), see figure 1(xiv). A Radiometer PHM64 pH meter is available, fitted with K4040 calomel and G2040C glass electrodes. This apparatus has been used in previously reported work [93,94,95]. However, performing the operation is a strictly manual affair and can be very time consuming. On manual collection of the data analyses were carried out using the programs CALIB 3, to determine the end point, and the formation constants were determined by applying the methods of Sarkar and Kruck [95], applying the four computer programs PLOT 3, GUESS 3, LEASK 3 and BETACALC 3.

The aim of the work described herein was to interface a titration assembly with a microcomputer to remove some of
Figure 1(xiv) Equipment for potentiometric titrations.

To potentiometer

Reference and indicator electrodes

Buret (titrant)

Solution (titrate)

Stirring bar

Magnetic stirrer
the laborious nature of the work, and in so doing reduce the risk of errors due to a manual operator, in particular in the initial data handling. The data could then be analysed in the normal manner with the aforementioned computer programs.
CHAPTER 2
INSTRUMENTAL METHODS

2.1 Introduction

A number of instrumental methods have been employed during the programme of research outlined in this thesis. Briefly, it is intended to outline each technique together with its limitations. The instrumental methods used are summarized in table 2(i).

A number of kinetic experiments were performed (Chapter 3) which were followed principally by UV/VIS spectroscopy. However, corroboratory evidence was obtained by applying ESR, CD and radiochemical techniques.

The second half of this thesis describes the attempted characterization of a series of solid materials. The principal technique employed for analysing the amount of inorganic material at the trace level was Atomic Absorption spectroscopy; sodium analyses were performed by Flame Photometry. The technique of Cyclic Voltammetry was used to examine the oxidation reduction potential of the central metal ion in a number of complexes. Infra-red spectroscopy and X-ray Powder Diffraction were both employed in a purely qualitative manner whilst Thermogravimetric Analysis provides a quantitative measurement of any weight change.
Table 2(i) Instrumental techniques used.

<table>
<thead>
<tr>
<th>Instrumental Method</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultraviolet-visible Spectroscopy (UV-VIS)</td>
<td>✓</td>
</tr>
<tr>
<td>Electron Spin Resonance Spectroscopy (ESR)</td>
<td>✓</td>
</tr>
<tr>
<td>Atomic Absorption Spectroscopy (AA)</td>
<td>✓</td>
</tr>
<tr>
<td>Flame Photometry (FP)</td>
<td>✓</td>
</tr>
<tr>
<td>Circular Dichroism Spectroscopy (CD)</td>
<td>✓</td>
</tr>
<tr>
<td>Infra-red Spectroscopy (IR)</td>
<td>✓</td>
</tr>
<tr>
<td>Radiochemistry</td>
<td>✓</td>
</tr>
<tr>
<td>Cyclic Voltammetry (CV)</td>
<td>✓</td>
</tr>
<tr>
<td>X-ray Powder Diffraction (XRD)</td>
<td>✓</td>
</tr>
<tr>
<td>Thermogravimetric Analysis (TGA)</td>
<td>✓</td>
</tr>
<tr>
<td>X-ray Photoelectron Spectroscopy (XPS)</td>
<td>✓</td>
</tr>
</tbody>
</table>
associated with a thermal transition, since TGA directly records any loss in weight on heating with time, or temperature. In X-ray Photoelectron spectroscopy, a monochromatic beam of x-rays is used to eject electrons from either the core shell or the valence shell of an atom giving information about electronic structure through data on binding energies. XPS can be applied both qualitatively and quantitatively. However, this is only a surface technique and does not necessarily give a realistic comparison with the properties of the bulk. XPS is often referred to as ESCA (Electron Spectroscopy for Chemical Analysis), in its application to the analysis of surfaces.

2.2 Ultraviolet-Visible Spectroscopy (UV/VIS)

The instrumental methodology of UV/VIS is well documented [96]. The instrument employed was a Perkin-Elmer 555 Spectrometer, which used a holographic-blazed grating to produce monochromatic light. Typical operational parameters are shown in table 2(ii).

Matched cuvettes were not used because the 555 has an inbuilt background correction routine. Stray light is less than 0.008% at 220 nm, and at 340 and 370 nm the figure is below 0.001%. The wavelength accuracy is ±0.5 nm, repeatability ±0.2 nm, and the photometric accuracy ±0.005 at 1 absorbance unit, repeatability ±0.002 at 1 absorbance
Table 2(ii) Ultraviolet-visible spectrometry parameters.

Wavelength range .................. 190-860 nm

Bandpass .......................... 2 nm

Scanning speeds ................... 60-240 nm/min

Recorder format ................... 20 nm/cm

Photometric absorption ranges..... -0.3 - 3

Response times .................... 0.2, 0.5, 1, 2, 4, 7 s
The baseline drift is <0.005 absorbance unit per hour.

2.3 Electron Spin Resonance Spectroscopy (ESR)

The principles of ESR have been outlined by Symons [97], and its application to transition metal complexes reviewed by Goodman and Raynor [98]. In a simplified picture unpaired electrons can be considered to behave as minute magnets which are randomly orientated. In the presence of a strong applied magnetic field, these magnets reorientate into one or other of two allowed positions or energy levels (spin quantum numbers $m_S = +\frac{1}{2}$, and $m_S = -\frac{1}{2}$), as shown in figure 2(i). The difference in energy ($\Delta E$) between these two energy levels is proportional to the strength of the applied magnetic field ($H$).

$$\Delta E = g\beta H \quad 2(i)$$

where $g$ is the spectroscopic splitting factor, and $\beta$ is the Bohr magneton. Therefore, for a given strength of applied magnetic field, quanta of energy can only be absorbed by the unpaired electrons when electromagnetic radiation is supplied at a frequency $\nu$, where $\nu$ is determined by the equation,

$$\Delta E = h\nu \quad 2(ii)$$
I Unpaired electrons in the two allowed positions when under the influence of a strong magnetic field.

II $\Delta E$ increases proportionally with increase of strength of the applied magnetic field $H$.

III An ESR absorption spectrum shown as a plot of intensity of absorption against applied magnetic field strength.
and h is Planck's constant.
Thus

$$hv = g\beta H$$

For an applied magnetic field of 3570 Gauss, $\nu$ is equivalent to a wavelength of approximately 3 cm, i.e. is in the microwave region of the electromagnetic spectrum. An ESR absorption experiment therefore consists of measuring the number of multiples of $\Delta \nu$ absorbed.

An isolated electron gives rise to electron spin angular momentum (S). However, the additional phenomena of orbital angular momentum (L), and nuclear spin angular momentum (I), can contribute to the resultant electron magnetic moment. The spectroscopic splitting factor (g) is a measure of the coupling of the spin and orbital angular moments. In many solids the coupling of the orbital and electron spin momenta is broken down almost completely by the strong internal electric fields. ESR then measures the free spins of the unpaired electrons. For completely free electrons $g = 2.0023$.

ESR spectra, in this work, were recorded on a Bruker ER200D. Samples were placed in 4 mm quartz ESR tubes, in either liquid or powder forms, and frozen in liquid nitrogen, 77K. For qualitative measurements no attempts were made to ensure sample tubes were placed
within the cavity in identical positions. However, for quantitative experiments greater efforts were made to ensure the reproducible positioning of the ESR tube in the cavity. This was accomplished by using a nylon rod stopper carefully positioned at the bottom of the cavity. Typical instrumental parameters are shown in table 2(iii).

2.4 Atomic Absorption Spectroscopy (AA)

Introducing a fine spray of a sample solution into an appropriate flame, a fraction or all of the metallic ions in the droplets are eventually converted into free atoms. Once formed, the free atoms may be detected and determined quantitatively by Atomic Absorption spectroscopy. The unexcited atoms are capable of absorbing radiation from an external source when the radiation corresponds exactly to the energy required for a transition of the test element from its ground state to an upper excited electronic state. Unabsorbed radiation passes through a monochromater, that isolates the exciting spectral line, and into a photodetector.

The instrument employed was an Instrumentation Laboratory, IL151 model. AA was used for the quantitative determination of both copper and molybdenum, parameters for the instrumental set are shown in table 2(iv).

An air/acetylene fuel mixture produces a flame of
Table 2(iii) Instrumental parameters used in ESR spectroscopy.

Temperature ................. 120 - 298K

Microwave power ............. 1.7 - 3.8 mW

Field modulation ............ 2.5 - 8.0 G

Gain .......................... $5 \times 10^4$ - $1 \times 10^6$

Time constant ............... 1 - 5s

Mid range* .................... 3000 - 3300 G

Scan range .................... 500 - 2000 G

Scan time ..................... 100 - 500s

(* for half-field spectra mid range 1500 - 1650 G)
Table 2(iv) Instrumental parameters in AA analyses.

<table>
<thead>
<tr>
<th></th>
<th>Molybdenum</th>
<th>Copper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light source</td>
<td>Hollow cathode</td>
<td>Hollow cathode</td>
</tr>
<tr>
<td>Lamp current</td>
<td>8 mA</td>
<td>3 mA</td>
</tr>
<tr>
<td>Wavelength</td>
<td>313.3 nm</td>
<td>324.7 nm</td>
</tr>
<tr>
<td>Bandpass</td>
<td>0.5 nm</td>
<td>1 nm</td>
</tr>
<tr>
<td>Burner head</td>
<td>Nitrous oxide</td>
<td>Boling</td>
</tr>
<tr>
<td>Flame</td>
<td>Nitrous oxide/Acetylene</td>
<td>Air/Acetylene</td>
</tr>
</tbody>
</table>
2400°C, whereas the nitrous oxide/acetylene fuel mixture produces a flame of 2800°C. A hotter flame prevents the generation of stable metal oxides which may be produced at the lower temperature, and block the burner head. Instrumental error for copper analyses were ±0.04 ppm, whereas for molybdenum they were ±0.3 ppm.

2.5 Flame Photometry

Flame Photometry is similar to AA in that the flame evaporates solvent from the droplets of particles leaving a cloud of small particles of the solid compound; in Flame Photometry the atoms are excited in this flame and emit radiation which is then detected. The technique is a principle method for analysis of the alkaline metals, such as lithium and sodium. The number of excited atoms in the flame is maximized, which then relax emitting electromagnetic radiation. Sodium analyses were performed with a Corning 400 flame photometer; samples were prepared in the range 0-10 ppm.

2.6 Circular Dichroism Spectroscopy (CD)

Certain UV/VIS electronic absorption transitions of optically active (chiral) compounds exhibit what is known as the 'Cotton Effect'. That is, they exhibit differential absorption for right ($E_r$) and left ($E_l$) circularly
polarised light. This differential absorption forms the basis of circular dichroism spectroscopy. A typical (CD) spectrum, and its relation to other related spectroscopic techniques, is shown in figure 2(ii). Circular Dichroism spectra were recorded on a C.N.R.S.-Roussel-Jouan Dichrographe. III. The instrumental parameters are shown in table 2(v).

The relationship between the measured spectrum and $\Delta E$ is given by the expression

$$\Delta E = \frac{\text{peak height (mm)} \times \text{sensitivity (dm}^2\text{mol}^{-1})}{[m] \times 0.1 \times \text{pathlength (cm)}}$$

More detailed theoretical and practical considerations can be found elsewhere [99,100].

2.7 Infra-red Spectroscopy (IR)

Interpretation of the infrared spectra on the basis of characteristic frequencies is not always sufficient to permit positive identification of an unknown, but certainly the type or class of compound can be deduced. Spectra are usually compared with those from similar types of compounds. Infra-red spectroscopy was employed in this work for a comparison of synthesised compounds with those in the literature in a qualitative manner. A
Figure 2(ii) The relationship between UV/VIS (I), CD (II) and optical rotatory dispersion, ORD (III).
Table 2(v) Parameters employed in the Circular Dichroism spectrometry experiments.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>307 - 607 nm</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>$5 \times 10^{-6}$</td>
</tr>
<tr>
<td>Pathlength</td>
<td>0.5 - 2 cm</td>
</tr>
<tr>
<td>Scanning speed</td>
<td>60 nm/min</td>
</tr>
<tr>
<td>Recorder format</td>
<td>20 nm/cm</td>
</tr>
</tbody>
</table>
Perkin-Elmer 683 Spectrometer was used to record IR spectra, samples were prepared as potassium bromide discs, typical instrumental parameters are shown in table 2(vi).

2.8 Radiochemistry

The introduction of a radioactive-labelled material into a sample system allows for a rapid and economical method of tracing the behaviour of a specific component. The radionuclide used in these studies was the beta-emitter $^{63}$Ni with a half-life of 92 years. Low energy beta-emitters, such as $^3$H, $^{14}$C and $^{63}$Ni are commonly assayed by liquid scintillation counting.

$^{63}$Ni(II) ($\beta^-, 0.067$MeV) was obtained in the form of $^{63}$NiCl$_2$ in 0.1 mol dm$^{-3}$ HCl (specific activity in $\text{ldm}^3\text{cm}^{-3}$) from Amersham International. Samples of the product of reaction were dissolved in liquid scintillation cocktail 'W Scintrain' from BDH. Activities were measured with a Packard Tri-carb liquid scintillation counter to a total 10,000 counts, or for five minutes.

The long half-life of the radionuclide precluded any errors in counting due to radioactive decay. Total counts were of the order of 10,000 to minimise the statistical error, a random error of 1% at the 68.3% confidence level is then present in the result.
Table 2(vi) Instrumental parameters employed in recording of Infra-red spectra.

Chart expansion ............... 0.5

Slit width ..................... 3, 6

Noise filter .................... 1

Scan time ............ (Noise filter x Slit width) mins

Wavelength range ............ 200 - 4000 cm\(^{-1}\)
2.9 Cyclic Voltammetry (CV)

An introduction to the principles of Cyclic Voltammetry may be found in a number of references [101-103]. In the CV experiment a voltage applied to the "working electrode" is scanned linearly from an initial value, $E_i$, to a predetermined limit, $E_j$. The instrument may then cycle back to the initial value, $E_i$, or some other predetermined limit. The current response is plotted as a function of the applied potential, and the result is the current-voltage curve or voltammogram. The voltammogram for a reversible redox system is shown in figure 2(iii). The current depends on two steps in the overall process, the movement of electroactive material to the electrode surface, and the electron transfer process. Initially, the electron transfer process is rate-determining, but as the concentration of material at the electrode surface is depleted, it is the mass transport process that becomes rate determining. The voltammogram reaches a maximum, and then decays. On reaching $E_j$ the potential is switched, and the scan begins in the reverse direction. In a truly reversible redox system, the product is oxidised back to starting material; an inverse peak is obtained on the reverse sweep.

Mass transport of the electroactive species only occurs by diffusion, and thus to ensure conductivity a
Figure 2(iii) Cyclic voltammogram for a reversible redox process.

Mass transport step becomes rate determining.
Maximum current.
Concentration gradient, and mass transport continues to decrease, causing the current to decay.
$E_j$ switching potential.
Reduced form is oxidized back on reverse scan.
0.1 mol dm\(^{-3}\) solution of background electrolyte is used. It is impossible to measure absolute single electrode potentials, hence the working electrode always refers to a non-polarizable reference electrode, such as a saturated calomel electrode (SCE). Most instruments use a three-electrode cell in which a counter or auxiliary electrode provides the current that is needed at the working electrode, virtually no current flows through the reference electrode and its potential remains constant. The three-electrode system minimizes voltages due to ohmic loss through the solution by placing the reference electrode close to the working electrode surface.

In the CV experiments reported in this thesis, a three-electrode system was employed with a platinum or vitreous carbon button working electrode, a platinum wire auxiliary electrode and a saturated calomel electrode (SCE) as the reference. Potentials were also referenced to the ferrocene/ferricinium system. The instrument used consisted of a Ministat Potentiostat, a Hi-Tec Waveform Generator and a Bryans X-Y Recorder. The CV experiment is largely of qualitative use, although in some instances evaluation of quantitative parameters, such as the number of electrons transferred in a redox reaction is possible.
2.10 X-ray Powder Diffraction (XRD)

A powder diffraction photograph gives a finger print of a crystalline compound, and the unit cells of crystalline components of a mixture can be identified individually. No two substances have absolutely identical diffraction patterns when considering both the angle and intensity of all diffracted beams.

To obtain a powder diffraction photograph the samples were ground using an agate pestle and mortar. The resultant powder was then mixed with diluted (with toluene) cowgum. This mixture was then mounted onto the tip of a fine glass spike, in the form of a spherical globule. The sample was then aligned in the x-ray camera and exposed to nickel-filtered copper radiation for two hours. Experimental conditions are shown in table 2(vii).

X-ray data thus obtained was then compared to known x-ray data of other compounds mainly via the Joint Committee on Powder Diffraction Standards file (JCPDS).

2.11 Thermogravimetric Analysis (TGA)

A thermogravimetric balance records the loss of weight with time, or temperature, resulting from decomposition or other chemical change. The sample is continuously weighed as it is heated to elevated temperatures.
Table 2(vii) Instrumental and experimental conditions used to obtain X-ray Diffraction photographs.

Sample mount type ........... Cow gum spike

Generator .................. Philips PW1010

Camera ..................... Debye-Scherrer (114.83 mm dia)

Radiation .................. Copper Kα

Filter ....................... Nickel

Wavelength .................. 1.5418 Å

Running conditions ........ 40 kV, 16 mA

Exposure ..................... 2 hours

Film ........................ Kodak 'Direct' X-ray film
Measurements were performed on an automatic Stanton-Redcroft TG750 TGA balance. Approximately 9 mg were added to the balance, and the sample heated from ambient to 1000°C. The heating rate, recording of sample weight change and the temperature are all performed automatically.

2.12 X-Ray Photoelectron Spectroscopy (XPS)

XPS is concerned with the measurement of core electron binding energies. A molecule or atom is bombarded with a source of high energy x-rays which causes the emission from sample atoms of inner-shell electrons. All electrons whose binding energies are less than the energy of the exciting x-rays are ejected. The kinetic energy, $E_k$, of these photoelectrons are then measured by an energy analyzer. The core-electron binding energies, $E_b$, can be computed via the equation

$$E_b = hv - E_k - \Theta$$

where $hv$ is the energy of the monochromatic exciting radiation and $\Theta$ is the spectrometer 'work function'. The energy of the ejected electron, $E_k$, is thus characteristic of the atom involved and its chemical environment. The 'work function' varies between spectrometers, hence binding energies are quoted relative to known standards.
Binding energies of core electrons are affected by the valence electrons and therefore by the chemical environment of the atom. When the atomic arrangement surrounding the atom ejecting a photoelectron is changed, it alters the charged environment at the atomic site, and a characteristic shift in the binding energies of the core electrons will be induced. Any parameter that affects the electron density about the atom is expected to result in a chemical shift in electron binding energy. This includes such parameters as oxidation state, ligand electronegativity or coordination.

In order to make assignments it is necessary to have a catalogue of spectra or correlation charts of chemical shifts of atoms in similar environments.

XPS requires a monochromatic radiation source of sufficient energy to eject an electron from the sample. A device must then be present to collect the emitted electrons, count them, and carefully measure their kinetic energy. XPS work is mostly performed using either MgK\(\alpha\) (1253.6 eV) or AlK\(\alpha\) (1486.6 eV) radiation.

Quantitative analysis is also possible in XPS. The intensity of a given peak can be expected to be proportional to the concentration or absolute amount of the species giving rise to the peak. However, XPS is only a
surface technique. X-rays may penetrate over 100 nm, but not all the ejected photoelectrons may escape and reach the detector; some are reabsorbed by the solid. A quantitative analysis may not be representative of bulk properties.

A background of the theory, techniques and applications of XPS can be found in the series edited by Brundle and Baker [104]. X-ray Photoelectron spectra were recorded on a Vacuum Generators (VG) machine using AlK$\alpha$ radiation on finely divided powders, at the Loughborough University of Technology; by the Loughborough University Consultancy Service.
CHAPTER 3
AND NICKEL(II)
THE COPPER(II)ALBUMIN EXCHANGE REACTIONS
WITH THE AMINO ACIDS

3.1 Introduction

It is now fairly well established that within blood plasma the protein serum albumin has a strong specific binding site for Cu(II) and Ni(II). These complexes would appear to serve an important transport function for the metal ions in serum. The nature of the binding site and the thermodynamics of binding have been thoroughly studied [105]. Within plasma, naturally occurring copper is present in three fractions; caeruloplasmin, albumin and the amino acid components: added nickel also binds to three fractions; albumin, the amino acids and a fraction associated with a higher molecular weight protein. It has generally been assumed that a facile equilibrium exists between the albumin and the amino acid fraction, the amino acid fraction then transferring the metal ion through the plasma membrane.

3.1 (i) Copper(II) Exchange Reactions

Bearn and Kunkel established that addition of $^{64}$Cu to rats resulted in the distribution of copper between albumin and caeruloplasmin [11]. Fifteen minutes
post-administration the $^{64}$Cu was mainly associated with the albumin bound fraction, and after 24 hours >70% was associated with albumin, at 6 hours a significant proportion was associated with caeruloplasmin fraction. In contrast, in Wilson's disease patients after 6 hours the $^{64}$Cu was still in the albumin fraction. Neumann and Sass-Kortsak claimed that Bearn and Kunkel had shown that the Cu(II)-albumin fraction was in rapid equilibrium with tissue copper [106]. They [11] certainly established that an equilibrium between the Cu(II)-albumin and the Cu(II)-amino acid fractions can exist, however, no rate of equilibration was established. Sarkar and Kruck also suggested that the albumin bound fraction of serum copper was in rapid equilibrium with that in the tissues [107]. Albumin-bound copper has a plasma half-life of approximately 10 minutes whereupon it is absorbed by the liver, plasma caeruloplasmin-bound copper has a half-life of about 13 hours in the rat. Tissue uptake of plasma albumin derived copper is maximal at 1 hour, whereas maximum tissue levels derived from plasma caeruloplasmin occurs at about 40 hours [6].

Although the stopped-flow kinetics of Cu(II) ion binding with bovine serum albumin have been studied, these experiments were not performed under physiological conditions. Within the pH range examined, 4-6.4, a single reaction process was observed and attributed to chelation
of the Cu(II) ion at the primary binding site on the protein [32]. Lau and Sarkar have examined the exchange reactions of copper(II) from L-histidine to human serum albumin and Gly-Gly-L-His [33]. Exchange rates, at 20°C and pH 7.53, from L-histidine to albumin and peptide were determined as 0.67s\(^{-1}\) and 0.42s\(^{-1}\) respectively, and from albumin and peptide to L-histidine as 0.04s\(^{-1}\) and 0.07s\(^{-1}\) respectively. In reactions involving albumin the rates increased with increasing Cu(II)(L-His)\(_2\) concentration, reaching a maximum at a 30 fold excess. A saturation phenomenon was also observed for the reverse reaction. Margerum and co-workers have examined the kinetics of the Cu(II)Gly-Gly-L-His exchange reactions with trien in some detail [34]. The overall scheme for the reaction is detailed below.

\[
\text{Cu(H}_2\text{Gly-Gly-L-His)}^- + H_n\text{trien}^{n+} + (2+p-n)H^+ \\
\rightarrow \quad \text{Cu(trien)}^{2+} + Hp(\text{Gly-Gly-L-His})^{p-1} \quad 3(1)
\]

Between pH 6.4 and 8.3 the rate of formation of Cu(trien)\(^{2+}\), at constant pH, was found to be first order with respect to trien concentration,

\[
d[\text{Cu(trien)}^{2+}] / dt = k[\text{Cu(H}_2\text{Gly-Gly-L-His)}^-][\text{trien}] \quad 3(ii)
\]

which holds true at low trien concentrations. However, at
At high trien levels the rate of formation of \( \text{Cu(trien)}^{2+} \) was found to be pH dependent and first order with respect to hydrogen ion concentration between pH 6.4 and 8.3.

\[
\frac{d[\text{Cu(trien)}^{2+}]}{dt} = k_H[H^+][\text{Cu(H-2Gly-Gly-L-His)}^-]  \quad 3(\text{iv})
\]

The predominant contribution to the rate at pH<8.5 was determined as the proton-assisted pathway, whereas at pH>8.5 it is a nucleophilic pathway with attack by \( \text{Htrien}^+ \) and trien that dominates. The proposed mechanism is given below,

\[
\begin{align*}
\text{Cu(H-2L)}^- + H^+ & \underset{k_H}{\overset{k_{-H}}{\rightleftharpoons}} \text{Cu(H-1L)} \\
\text{Cu(H-1L)} + \text{H}_2\text{trien}^{2+} & \overset{k_{H_2T}}{\rightarrow} \text{Cu(trien)}^{2+} + \text{HL} \\
\text{Cu(H-2L)}^- + \text{trien} & \overset{k_N}{\rightarrow} \text{Cu(trien)}^{2+}
\end{align*}
\]

87
Wong and Margerum also stated that L-histidine exchange with Cu(II)Gly-Gly-L-His occurs in a similar manner, i.e. at physiological pH a proton-assisted nucleophilic pathway would be the major route. Exchange reactions of copper(II) complexes of human and bovine serum albumin with trien were said to follow a similar mechanism, although no data was presented in support of these conclusions [35].

The effect of L-cysteine on the exchangeable copper in blood plasma has been examined by Laurie and Mohammed [108]. Cysteine is known to reduce Cu(II) to Cu(I) by the reaction:

\[
4\text{CysH} + 2\text{Cu(II)} \rightarrow 2/n[\text{Cu(I)Cys}]_n + \text{Cys-Cys} + 4\text{H}^+ \quad (ix)
\]

At pH 7.4, an ionic background of 0.15 moldm\(^{-3}\), L-cysteine and Cu(II) concentrations of 23x10\(^{-6}\) and 1x10\(^{-6}\) moldm\(^{-3}\) respectively, total oxidation of L-cysteine was observed in ca 7 minutes. The following scheme was proposed to account for their observations:

\[
\text{Cu(H}_2\text{L})^- + \text{H}_2\text{O} \xrightarrow{k_d} \left[\text{Cu( H}_2\text{O} \right]^{n}_{\text{trien}} \rightarrow \text{[Cu(trien)]}^{2+} (viii)
\]

where L = Gly-Gly-L-His.
The re-oxidation of Cu(I) to Cu(II) is fast, the crucial factors in the kinetics of the reaction are the Cys/Cu and O₂/Cu ratios.

The mechanism of the copper ion catalysed autoxidation of L-cysteine in alkaline medium has been examined in some detail [36-38]. In the studies by Zwart et al., [38], the order with respect to L-cysteine was approximately zero, and with respect to oxygen 0.5. The order with respect to copper was found to be variable, and was composed of a combination of first and second order terms; the following kinetic expression for the copper catalysed autoxidation of L-cysteine in alkaline medium was proposed;

\[ r_{O_2} = k_1 [O_2]^{0.5} [Cu] + k_{11} [O_2]^{0.5} [Cu]^2 \]  

The oxidation of cysteine, cysteine methyl ester, and penicillamine by copper(II)-2,9-dimethyl-1,10-phenanthroline complexes have been studied [109]. The order with respect to Cu(II) was found to be 1, in the pH range 4-7, in contrast to the second order behaviour of weaker Cu(II) reductants such as glutathione.
3.1 (ii) Nickel(II) Exchange Reactions

Although a thorough search of the literature has been undertaken, no accounts of kinetic studies of nickel(II) exchange reactions between albumin and the amino acids have been found. The thermodynamics of nickel(II) binding to the protein human serum albumin have been examined [48]. The log(association constant) for the binary, Ni(II)HSA, and ternary, Ni(II)HSA-L-His, complexes have been determined as 9.57 and 16.23 respectively. Not unexpectedly, the values are lower than for the corresponding copper(II) systems, 16.18 and 21.86 respectively [112].

3.2 Experimental

Bovine serum albumin was obtained from Fluka (fraction V, puriss); L-histidine was from BDH (biochemical); L-cysteine and glycine from Koch-Light (pure); copper chloride dihydrate, potassium dihydrogen orthophosphate and sodium hydroxide were obtained from BDH (AnalaR); N-ethylmorpholine was purchased from Aldrich and distilled prior to use, collecting the fraction at 138-139°C; sodium tetraborate decahydrate (borax) was obtained from BDH (General Purpose) and recrystallised from warm distilled water prior to use; sodium chloride was obtained from Fisons (Analytical Reagent).
All water was distilled and deionized (Elgastat, Elgacan cartridges C114) prior to use, to a resistivity of greater than 3 Mohm cm$^{-1}$. For studies at pH 7.4, solutions were prepared either in an N-ethylmorpholine buffer, adjusted with cvs HCl(BDH), or a potassium dihydrogen orthophosphate buffer, adjusted with cvs NaOH(BDH); variable pH studies were performed in unbuffered solutions. For anaerobic studies either oxygen-free N$_2$, or Ar, was passed through the solutions. All L-cysteine experiments were performed under anaerobic conditions unless otherwise stated, solutions were prepared immediately prior to use. All plasma model solutions contained 0.15 moldm$^{-3}$ NaCl. Kinetic experiments were performed at least in triplicate, except for CD and ESR studies. All experiments were performed in thermostated cell blocks ($\pm 0.1^\circ$C). Instrumental parameters are outlined in Chapter 2.

3.3 The Metal(II)-Bovine Serum Albumin Complexes

3.3. (i) Examination of the pH Dependence of the UV/VIS and ESR Spectra of Cu(II)BSA

ESR spectra of the Cu(II)BSA 1:1 complex were recorded in the pH range 7.45-10.25 (concentration of Cu(II)BSA, $5 \times 10^{-4}$ moldm$^{-3}$), figure 3(i). Slight variations were observed between the calculated parameters, $g_m$, $g_\parallel$ and $A_\perp$, table 3(i). This may be due to; (i) the large expansion (1000 Gauss) over which the spectra were
Figure 3(i) ESR spectrum of Cu(II)BSA recorded at pH 7.45; concentration of CuII, $5 \times 10^{-4}$ moldm$^{-3}$; BSA, $5.8 \times 10^{-4}$ moldm$^{-3}$. 
Table 3(i) ESR spectral data obtained for the Cu(II)BSA 1:1 complex at various pH values; solutions frozen in liquid nitrogen, 77K (concentration of Cu(II)BSA, $5 \times 10^{-4}$ mol dm$^{-3}$).

<table>
<thead>
<tr>
<th>pH</th>
<th>Relative Intensity</th>
<th>$g_{ll}$</th>
<th>$A_g$ (Gauss)</th>
<th>$g_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.45</td>
<td>1</td>
<td>2.174</td>
<td>213</td>
<td>2.025</td>
</tr>
<tr>
<td>8.35</td>
<td>1.08</td>
<td>2.174</td>
<td>213</td>
<td>2.028</td>
</tr>
<tr>
<td>9.40</td>
<td>1.14</td>
<td>2.174</td>
<td>213</td>
<td>2.031</td>
</tr>
<tr>
<td>10.25</td>
<td>1.16</td>
<td>2.174</td>
<td>213</td>
<td>2.031</td>
</tr>
<tr>
<td>6.5(*)</td>
<td>-</td>
<td>2.166</td>
<td>214</td>
<td>2.051</td>
</tr>
<tr>
<td>9.5(*)</td>
<td>-</td>
<td>2.166</td>
<td>214</td>
<td>2.049</td>
</tr>
</tbody>
</table>

* from the work of Rakhit and Sarkar [27].

** Calibrated against 2,2-diphenylpicrylhydrazyl reference.
recorded; (ii) imprecision in the positioning of the DPPH marker; (iii) imprecision in the field centre marker. The results are in agreement with those of Rakhit and Sarkar [28], who showed that, within the pH range 6.5-11, the ESR spectrum of the Cu(II)HSA 1:1 complex does not alter. The nine-line superhyperfine structure observed by Rakhit and Sarkar is not clearly resolved in our spectrum.

The UV/VIS spectrum recorded over the same pH range showed no change in the magnitude of the absorbance at 525 nm, figure 3(ii). The extinction coefficient for the Cu(II)BSA 1:1 complex was determined to be 110 dm$^3$mol$^{-1}$cm$^{-1}$, c.f. Cu(II)HSA, 101 dm$^3$mol$^{-1}$cm$^{-1}$ [110]. Lau and Sarkar have reported an alkaline pH dependent UV/VIS spectrum of Cu(II)HSA [111]. This was not observed in this work, the results are consistent with only one type of Cu(II)BSA 1:1 complex in the pH range 7.4-10.0, which is probably the square planar complex postulated by Sarkar [110].

3.3 (ii) Examination of the pH Dependence of the UV/VIS and CD Spectra of Ni(II)BSA

The UV/VIS and CD spectra of Ni(II)BSA were found to be pH dependent in the range 6.5-9.3. In the observed pH range the UV/VIS spectra consists of a maximum at 420 nm and shoulders at 340 and 450-480 nm, figure 3(iii). Martin
Figure 3(ii) UV/VIS spectrum recorded for Cu(II)BSA; pH 7.45 - 10.25; concentration of Cu(II), 4.95x10^{-4} \text{ mol dm}^{-3}; E = 110 \text{ dm}^{3}\text{mol}^{-1}\text{cm}^{-1}.
Figure 3(iii) UV/VIS spectrum of Ni(II)BSA recorded as a function of pH; concentration of Ni(II)BSA, $5 \times 10^{-4}$ moldm$^{-3}$; pH 6.5, I; pH 7.6, II; pH 8.5, III; pH 9.3, IV.
showed for Ni(II)BSA that the absorbance reached a maximum at pH 9.5, which coincided with the full development of the yellow colour of the solution [112]. The result is also in agreement with that of Glennon and Sarkar, who showed a simple pH dependence for the Ni(II)HSA 1:1 complex [48].

For square planar Ni(II) complexes only two of the three d-d electronic transitions are magnetic dipole-allowed [113], hence only two Cotton Effects are expected in the visible region. For Ni(II)BSA a positive Cotton Effect was observed at 412 nm, and a negative effect at 477 nm, figure 3(iv). Martin and co-workers had previously shown the complex to exhibit a negative effect at 475 nm, and a positive effect at 420 nm, in a solution of pH 10.5 [114].

These results are not unexpected since it is well known from peptide studies that whilst Cu(II) can effectively deprotonate coordinating peptide groups at neutral pH, Ni(II) only does so at higher pH values because of its weaker thermodynamic binding [115]. At high pH the Ni(II) may be bound wholly in a square planar environment, however, at lower pH values only a proportion is coordinated in this manner, e.g. at pH 7.4 ca 70%.

Further addition of albumin at pH 7.4 failed to alter either the UV/VIS or CD spectra, which implies that all the Ni(II) is coordinated to the protein. The most probable
Figure 3(iv) CD spectrum of Ni(II)BSA recorded as a function of pH; concentration of Ni(II)BSA, 5x10^{-4} moldm^{-3}; pH 6.9, I; pH 7.5, II; pH 8.1, III; pH 9.0, IV; pH 9.5, V.
explanation is that the Ni(II) is bound at lower pH values
to the N-terminal end of the protein in an octahedral form
which, as expected, is spectroscopically silent at the
concentrations used. This would suggest that the following
pH dependent equilibrium is present in solution.

\[
\begin{align*}
\text{[Ni(II)BSA]} & \rightleftharpoons \text{[Ni(II)BSA]}^{+nH^+} \\
\text{octahedral} & \rightleftharpoons \text{square planar}
\end{align*}
\] 3(xii)

3.4 The Metal(II)-Bovine Serum Albumin Exchange
Reactions
3.4 (i) Cu(II)BSA Exchange with L-Histidine

The progress of this reaction was followed by
monitoring the reduction in the absorbance of the Cu(II)BSA
chromophore at 525 nm. The kinetic data thus obtained was
treated in the following manner. Consider the reaction;

\[
\text{Cu(II)BSA} + \text{L-His} \rightarrow \text{Products}
\] 3(xiii)

\[
\text{Rate} = -\frac{d[\text{Cu(II)BSA}]}{dt} = k[\text{Cu(II)BSA}]^a[\text{L-His}]^b[X]^c[Y]^d... 
\] 3(xiv)

If the initial concentration of L-His is varied, and the
initial concentrations of the remaining components are
constant,
\[
\frac{\text{Rate}_1}{\text{Rate}_2} = \frac{[\text{L-His}]_1^b}{[\text{L-His}]_2^b} \quad 3(\text{xv})
\]

or

\[
\log\left(\frac{\text{Rate}_1}{\text{Rate}_2}\right) = b \log\left(\frac{[\text{L-His}]_1}{[\text{L-His}]_2}\right) \quad 3(\text{xvi})
\]

where \(\text{Rate}_1\) is the initial rate at an initial concentration \([\text{L-His}]_1\). Thus a plot of \(\log(\text{Rate}_1/\text{Rate}_2)\) versus \(\log([\text{L-His}]_1/[\text{L-His}]_2)\) will produce a straight line of gradient \(b\) through the origin. In this manner the orders with respect to each component may be evaluated.

Addition of an excess of \(\text{L-His}\) to \(\text{Cu(II)BSA}\) at \(37^\circ\text{C}\) results in a decrease of the absorbance band at \(525\ \text{nm}\) of \(\text{Cu(II)BSA}\), and an increase in absorption at \(\text{ca} \ 620-630\ \text{nm}\), figure 3(v). This is in accord with the equilibration reaction:

\[
\text{Cu(II)BSA} + n\text{L-His} \rightleftharpoons \text{Cu(II)(L-His)}_2 + (n-2)\text{L-His} + \text{BSA} \quad 3(\text{xvii})
\]

The reaction profiles obtained at \(37^\circ\text{C}\) are shown in figures 3(vi) to 3(ix). The new equilibrium was obtained in \(\text{ca} \ 60\text{s}\), and the final spectrum in all cases was consistent with the presence of only \(\text{Cu(II)BSA}\) and \(\text{Cu(II)(L-His)}_2\) as the absorbing species. Initial rates of reaction were
Figure 3(v) Final UV/VIS spectra of Cu(II)BSA plus L-His, pH 7.5, 37°C: initial concentration of Cu(II)BSA, 4.82x10^{-4} moldm^{-3}.

Cu(II)BSA : L-His; 4.82 : 0, C0; 4.82 : 8.19, C1; 4.82 : 16.38, C2; 4.82 : 32.76, C3; 4.82 : 65.5, C4; 4.82 : 81.90, C5.
Figure 3(vi) The effect of L-His addition on the Cu(II)BSA UV/VIS spectrum, pH 7.4, 37°C: initial concentration of Cu(II)BSA, $4.82 \times 10^{-4}$ moldm$^{-3}$; glycine, $3.0 \times 10^{-3}$ moldm$^{-3}$. Cu(II)BSA : L-His; 4.82 : 8.5, C6; 4.82 : 42.5, C7; 4.82 : 85.0, C8.
Figure 3(vii) The effect of L-His addition on the Cu(II)BSA UV/VIS spectrum, pH 7.4, 37°C: initial concentration of Cu(II)BSA; $3.856 \times 10^{-4}$ moldm$^{-3}$; glycine, $3.0 \times 10^{-3}$ moldm$^{-3}$.

Cu(II)BSA : L-His; 3.856 : 8.5, C9; 3.856 : 42.5, C10; 3.856 : 85.0, C11.
Figure 3(viii) The effect of L-His addition on the Cu(II)BSA UV/VIS spectrum, pH 7.4, 37°C: initial concentration of 
Cu(II)BSA, $2.892 \times 10^{-4} \text{ moldm}^{-3}$; glycine, $3.0 \times 10^{-3} \text{ m o l d m}^{-3}$. 
Cu(II)BSA : L-His; 2.892 : 8.5, C12; 2.892 : 42.5, C13; 
2.892 : 85.0, C14.
Figure 3(ix) The effect of L-His addition on the Cu(II)BSA UV/VIS spectrum, pH 7.4, 37°C: initial concentration of Cu(II)BSA, $4.82 \times 10^{-4}$ mol dm$^{-3}$; glycine absent. Cu(II)BSA : L-His; 4.82 : 17.0, C15; 4.82 : 25.5, C16; 4.82 : 34.0, C17.
calculated, tables 3(ii) and 3(iii). Applying the procedure outlined earlier, the order with respect to L-His was determined to be ca 1, in the presence or absence of the amino acid glycine. The order with respect to Cu(II)BSA was highly variable, and may suggest that it is not a simple reaction, or more likely, it is due to a large experimental scatter.

The reaction profiles at 10°C are shown in figure 3(x), the new equilibrium was obtained in ca 3 minutes, the final spectrum again was consistent with only two absorbing species, Cu(II)BSA and Cu(II)(L-His)$_2$. The rate of formation of Cu(II)(L-His)$_2$ was followed by monitoring the change in absorbance at 720 nm, figure 3(xi). The absorbance was found to increase rapidly on addition of L-His, followed by a slight decrease before equilibration. This may be due to the formation of a transient intermediate, which absorbs strongly at ca 720 nm, during the course of the reaction.

The order with respect to L-His is ca 1, the rate of formation of Cu(II)(L-His)$_2$ is dependent upon the Cu(II) : L-His ratio. This result would appear to agree with that of Margerum and co-workers [34], however, the full rate law was not completely evaluated in this work.
Table 3(ii) Kinetic parameters obtained for the Cu(II)BSA exchange reactions with L-His, at 37°C (C6-C17), or at 10°C (C18-C22).

Rates calculated by; (i) calculated gradient through initial points;

(ii) gradient determined from curve.

<table>
<thead>
<tr>
<th>System</th>
<th>Concentration of Cu(II)/10⁶moldm⁻³</th>
<th>Concentration of L-His/10⁶moldm⁻³</th>
<th>Initial Rate /s⁻¹</th>
<th>log ([L-His]₁)</th>
<th>log(Rate₁/Rate₂)</th>
<th>Order with respect to L-His</th>
</tr>
</thead>
<tbody>
<tr>
<td>C6</td>
<td>4.82</td>
<td>85.00</td>
<td>2.4 (4.8)</td>
<td>0.00</td>
<td>0.00 ( 0.00)</td>
<td></td>
</tr>
<tr>
<td>C7</td>
<td>4.82</td>
<td>42.50</td>
<td>2.5 (2.3)</td>
<td>-0.30</td>
<td>-0.03 (-0.32)</td>
<td>1.0 (1.1)</td>
</tr>
<tr>
<td>C8</td>
<td>4.82</td>
<td>8.50</td>
<td>0.3 (0.4)</td>
<td>-1.00</td>
<td>-0.94 (-1.13)</td>
<td></td>
</tr>
<tr>
<td>C9</td>
<td>3.856</td>
<td>85.00</td>
<td>3.9 (7.1)</td>
<td>0.00</td>
<td>0.00 ( 0.00)</td>
<td></td>
</tr>
<tr>
<td>C10</td>
<td>3.856</td>
<td>42.50</td>
<td>3.0 (3.9)</td>
<td>-0.30</td>
<td>-0.11 (-0.27)</td>
<td>1.0 (1.2)</td>
</tr>
<tr>
<td>C11</td>
<td>3.856</td>
<td>8.50</td>
<td>0.4 (0.5)</td>
<td>-1.00</td>
<td>-0.94 (-1.16)</td>
<td></td>
</tr>
<tr>
<td>C12</td>
<td>2.892</td>
<td>85.00</td>
<td>3.8 (10.5)</td>
<td>0.00</td>
<td>0.00 ( 0.00)</td>
<td></td>
</tr>
<tr>
<td>C13</td>
<td>2.892</td>
<td>42.50</td>
<td>3.3 (6.3)</td>
<td>-0.30</td>
<td>-0.07 (-0.23)</td>
<td>0.8 (1.2)</td>
</tr>
<tr>
<td>C14</td>
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<td>8.50</td>
<td>0.7 (0.8)</td>
<td>-1.00</td>
<td>-0.76 (-1.12)</td>
<td></td>
</tr>
<tr>
<td>C15</td>
<td>4.82</td>
<td>34.00</td>
<td>2.6 (11.1)</td>
<td>0.00</td>
<td>0.00 ( 0.00)</td>
<td></td>
</tr>
<tr>
<td>C16</td>
<td>4.82</td>
<td>25.50</td>
<td>1.9 (5.0)</td>
<td>-0.12</td>
<td>-0.13 (-0.35)</td>
<td>1.2 (2.2)</td>
</tr>
<tr>
<td>C17</td>
<td>4.82</td>
<td>17.00</td>
<td>1.1 (2.4)</td>
<td>-0.30</td>
<td>-0.37 (-0.66)</td>
<td></td>
</tr>
<tr>
<td>C18</td>
<td>4.82</td>
<td>64.30</td>
<td>2.3 (5.3)</td>
<td>0.00</td>
<td>0.00 ( 0.00)</td>
<td></td>
</tr>
<tr>
<td>C19</td>
<td>4.82</td>
<td>48.20</td>
<td>2.1 (3.2)</td>
<td>-0.13</td>
<td>-0.03 (-0.21)</td>
<td></td>
</tr>
<tr>
<td>C20</td>
<td>4.82</td>
<td>34.00</td>
<td>1.6 (3.2)</td>
<td>-0.28</td>
<td>-0.15 (-0.21)</td>
<td>1.6 (1.4)</td>
</tr>
<tr>
<td>C21</td>
<td>4.82</td>
<td>25.50</td>
<td>1.4 (1.6)</td>
<td>-0.40</td>
<td>-0.21 (-0.51)</td>
<td></td>
</tr>
<tr>
<td>C22</td>
<td>4.82</td>
<td>17.00</td>
<td>1.0 (0.7)</td>
<td>-0.58</td>
<td>-0.35 (-0.87)</td>
<td></td>
</tr>
</tbody>
</table>
Table 3(iii) Kinetic parameters for the Cu(II)BSA exchange reaction with L-His, at 37°C.

<table>
<thead>
<tr>
<th>System</th>
<th>Concentration of Cu(II) /10^4 moldm^{-3}</th>
<th>Concentration of L-His /10^4 moldm^{-3}</th>
<th>Initial Rate /s^{-1}</th>
<th>log ([Cu(II)]<em>{1} / [Cu(II)]</em>{2})</th>
<th>log(Rate_{1} / Rate_{2})</th>
<th>Order with respect to Cu(II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C6</td>
<td>4.82</td>
<td>85.00</td>
<td>2.4 (4.8)</td>
<td>0.00</td>
<td>0.00 (0.00)</td>
<td></td>
</tr>
<tr>
<td>C9</td>
<td>3.856</td>
<td>85.00</td>
<td>3.9 (7.1)</td>
<td>-0.10</td>
<td>0.21 (0.18)</td>
<td>-0.9 (-1.5)</td>
</tr>
<tr>
<td>C12</td>
<td>2.892</td>
<td>85.00</td>
<td>3.8 (10.5)</td>
<td>-0.22</td>
<td>0.21 (0.34)</td>
<td></td>
</tr>
<tr>
<td>C7</td>
<td>4.82</td>
<td>42.50</td>
<td>2.5 (2.3)</td>
<td>0.00</td>
<td>0.00 (0.00)</td>
<td></td>
</tr>
<tr>
<td>C10</td>
<td>3.856</td>
<td>42.50</td>
<td>3.0 (3.9)</td>
<td>-0.10</td>
<td>0.08 (0.23)</td>
<td>-0.5 (-2.0)</td>
</tr>
<tr>
<td>C13</td>
<td>2.892</td>
<td>42.50</td>
<td>3.3 (6.3)</td>
<td>-0.22</td>
<td>0.12 (0.44)</td>
<td></td>
</tr>
<tr>
<td>C8</td>
<td>4.82</td>
<td>8.50</td>
<td>0.3 (0.4)</td>
<td>0.00</td>
<td>0.00 (0.00)</td>
<td></td>
</tr>
<tr>
<td>C11</td>
<td>3.856</td>
<td>8.50</td>
<td>0.4 (0.5)</td>
<td>-0.10</td>
<td>0.21 (0.15)</td>
<td>-1.8 (-1.6)</td>
</tr>
<tr>
<td>C12</td>
<td>2.892</td>
<td>8.50</td>
<td>0.7 (0.8)</td>
<td>-0.22</td>
<td>0.39 (0.35)</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3(x) The effect of L-His addition on the Cu(II)BSA UV/VIS spectrum, pH 7.4, 10°C: initial concentration of Cu(II)BSA, $4.82 \times 10^{-4}$ moldm$^{-3}$; glycine absent.

Cu(II)BSA : L-His; $4.82 : 17.0$, C18; $4.82 : 25.5$, C19; $4.82 : 34.0$, C20; $4.82 : 48.2$, C21; $4.82 : 64.3$, C22.
Figure 3(xi) The effect of L-His addition on the Cu(II)BSA IV/VIS spectrum, pH 7.4, 37°C: initial concentration of Cu(II)BSA, $4.82 \times 10^{-4}$ moldm$^{-3}$; L-His, $85.0 \times 10^{-4}$ moldm$^{-3}$. 
3.4 (ii) Computer Simulations of Copper(II)-Albumin Equilibria

The computer program ECCLES [29] was employed to determine the concentrations of Cu(II)BSA and Cu(II)(L-His)_2 present at equilibrium in some of the systems examined in 3.4(i). The stability constants employed in these calculations are given in table 3(iv). The values of the L-histidine complexes were taken from Freeman and Martin [116]; for the Cu(II)BSA 1:1 complexes the log(stability constant) was assumed to be approximately equal to the log(formation constant) of Cu(II)HSA, 16.18 (pH 7.5, ionic strength 0.16 moldm^{-3}, and 6°C) [111]. Ionic strength corrections were performed using the Davies' equation,

\[ -\log f = 0.5 z f^{2/3} \left[ \frac{I^{2/3}}{1 + I^{2/3}} \right] - 0.3I \quad 3(\text{xviii}) \]

where \( z \) = charge on the species, and \( I \) = ionic strength of the solution. The results of a series of computations are given in table 3(v). The implied distribution is clearly not in good agreement with the results obtained from experimental observations. This discrepancy may be due to errors in the values of one or more of the stability constants employed in the calculation, most likely the...
Table 3(iv) Stability constant data for the computer simulation experiments.

<table>
<thead>
<tr>
<th>Species</th>
<th>Log (Stability Constant)</th>
<th>Log (Stability Constant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu H⁺ L-His</td>
<td>I = 0.1 moldm⁻³ (25°C)</td>
<td>I = 0.15 moldm⁻³ (25°C)</td>
</tr>
<tr>
<td>0 1 1</td>
<td>9.11</td>
<td>9.086</td>
</tr>
<tr>
<td>0 2 1</td>
<td>15.15</td>
<td>15.126</td>
</tr>
<tr>
<td>0 3 1</td>
<td>16.92</td>
<td>16.92</td>
</tr>
<tr>
<td>1 1 1</td>
<td>14.06</td>
<td>13.988</td>
</tr>
<tr>
<td>1 0 1</td>
<td>10.13</td>
<td>10.082</td>
</tr>
<tr>
<td>1 -1 1</td>
<td>2.1</td>
<td>2.028</td>
</tr>
<tr>
<td>1 2 2</td>
<td>26.8</td>
<td>26.752</td>
</tr>
<tr>
<td>1 1 2</td>
<td>23.64</td>
<td>25.568</td>
</tr>
<tr>
<td>1 0 2</td>
<td>18.10</td>
<td>18.028</td>
</tr>
<tr>
<td>2 -2 2</td>
<td>8.01</td>
<td>7.866</td>
</tr>
</tbody>
</table>
Table 3(v) Results of the computer simulation model; experimental values in brackets.

<table>
<thead>
<tr>
<th>System of L-His /10^4 moldm^{-3}</th>
<th>Concn.</th>
<th>Percentage of Constituents at Equilibrium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cu(II)BSA</td>
</tr>
<tr>
<td>8.19</td>
<td>99.8</td>
<td>0.2</td>
</tr>
<tr>
<td>C1</td>
<td>16.38</td>
<td>99.1 (74)</td>
</tr>
<tr>
<td></td>
<td>24.57</td>
<td>98.1</td>
</tr>
<tr>
<td>C2</td>
<td>32.76</td>
<td>96.9 (58)</td>
</tr>
<tr>
<td></td>
<td>40.95</td>
<td>95.5</td>
</tr>
<tr>
<td>C3</td>
<td>49.14</td>
<td>94.1 (53)</td>
</tr>
<tr>
<td></td>
<td>57.33</td>
<td>92.6</td>
</tr>
<tr>
<td>C4</td>
<td>65.52</td>
<td>91.0 (50)</td>
</tr>
<tr>
<td></td>
<td>73.71</td>
<td>89.5</td>
</tr>
<tr>
<td>C5</td>
<td>81.90</td>
<td>88.0 (56)</td>
</tr>
</tbody>
</table>
Cu(II)BSA value appears to have been overestimated.

3.4 (iii) The Cu(II)BSA Reaction with L-Cysteine

Reactions were performed at either 10, 25 or 37°C, and monitored by the reduction in absorbance at 525 nm, or the reduction in the ESR spectrum of Cu(II). The initial rates of reaction were calculated for each system, tables 3(vi) and 3(vii). The initial rate of reaction appeared to be dependent upon the L-Cys : Cu(II) ratio which is in agreement with the findings of Laurie and Mohammed [108]. At 25°C several minutes were required for equilibration to occur, only a residual absorbance remained at 525 nm. Reaction profiles are given in figures 3(xii) and 3(xiii). Addition of excess albumin failed to have any effect on the initial rate of reaction. The ESR data obtained during the course of the reaction were consistent with only one type of Cu(II) species being present, table 3(viii). Typical reaction profiles obtained at 37°C are shown in figures 3(xiv) and 3(xv). Equilibration again took several minutes. In order to obtain more reproducible data over the initial period of the reaction, the temperature was reduced to 10°C, figures 3(xvi) and 3(xvii). The order with respect to each component was calculated, tables 3(vi) and 3(vii). The results obtained varied markedly between different systems, and they were clearly not possible to evaluate with a high degree of certainty.
<table>
<thead>
<tr>
<th>System</th>
<th>Concentration of Cu(II) /10^4 moldm^{-3}</th>
<th>Concentration of L-Cys /10^4 moldm^{-3}</th>
<th>Initial Rate /s^{-1}</th>
<th>log ([L-Cys]_1 /[L-Cys]_2)</th>
<th>log (Rate_1 /Rate_2)</th>
<th>Order with respect to [L-Cys]</th>
</tr>
</thead>
<tbody>
<tr>
<td>C23</td>
<td>5.00</td>
<td>10.0</td>
<td>0.4(0.5)</td>
<td>0.00</td>
<td>0.00( 0.00)</td>
<td>-0.1(0.0)</td>
</tr>
<tr>
<td>C26</td>
<td>5.00</td>
<td>20.0</td>
<td>0.2(0.5)</td>
<td>0.30</td>
<td>0.00( 0.00)</td>
<td>-0.07( 0.00)</td>
</tr>
<tr>
<td>C27</td>
<td>5.00</td>
<td>30.0</td>
<td>0.2(0.5)</td>
<td>-0.48</td>
<td>-0.48( -0.48)</td>
<td>-0.1(0.0)</td>
</tr>
<tr>
<td>C32</td>
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<td>9.17</td>
<td>1.4(4.0)</td>
<td>0.00</td>
<td>0.00( 0.00)</td>
<td>-0.1(0.0)</td>
</tr>
<tr>
<td>C31</td>
<td>4.82</td>
<td>8.50</td>
<td>1.6(3.0)</td>
<td>-0.03</td>
<td>0.01(-0.37)</td>
<td>2.0(1.7)</td>
</tr>
<tr>
<td>C33</td>
<td>4.82</td>
<td>6.88</td>
<td>1.5(2.6)</td>
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<td>0.04(-0.18)</td>
<td>2.0(1.7)</td>
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<tr>
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<td>4.82</td>
<td>4.59</td>
<td>0.4(1.2)</td>
<td>-0.30</td>
<td>-0.53(-0.53)</td>
<td>-1.10(-1.15)</td>
</tr>
<tr>
<td>C35</td>
<td>4.82</td>
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<td>-0.60</td>
<td>-1.10(-1.15)</td>
<td>-1.10(-1.15)</td>
</tr>
<tr>
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<td>45.90</td>
<td>2.6(3.2)</td>
<td>0.00</td>
<td>0.00( 0.00)</td>
<td>-0.22(-0.27)</td>
</tr>
<tr>
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<td>22.90</td>
<td>1.6(1.7)</td>
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<td>-0.77(-0.68)</td>
<td>1.1(1.0)</td>
</tr>
<tr>
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<td>9.17</td>
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<td>-0.95(-0.82)</td>
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<tr>
<td>C39</td>
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<td>6.88</td>
<td>0.3(0.5)</td>
<td>-0.82</td>
<td>-1.02(-0.97)</td>
<td>1.1(1.0)</td>
</tr>
<tr>
<td>C40</td>
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<td>4.59</td>
<td>0.3(0.4)</td>
<td>-1.00</td>
<td>-1.02(-0.97)</td>
<td>1.1(1.0)</td>
</tr>
<tr>
<td>System</td>
<td>Concentration of Cu(II) /10^4 moldm⁻³</td>
<td>Concentration of L-Cys /10^4 moldm⁻³</td>
<td>Initial Rate /s⁻¹</td>
<td>log([Cu(II)]₁ / [Cu(II)]₂)</td>
<td>log(Rate₁ / Rate₂)</td>
<td>Order with respect to [Cu(II)]</td>
</tr>
<tr>
<td>--------</td>
<td>--------------------------------------</td>
<td>--------------------------------------</td>
<td>-------------------</td>
<td>---------------------------</td>
<td>-------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>C23</td>
<td>5.00</td>
<td>10.00</td>
<td>0.4(0.5)</td>
<td>0.00</td>
<td>0.00(0.00)</td>
<td>-0.7(-0.8)</td>
</tr>
<tr>
<td>C24</td>
<td>2.50</td>
<td>10.00</td>
<td>0.8(1.0)</td>
<td>-0.30</td>
<td>0.29(0.30)</td>
<td></td>
</tr>
<tr>
<td>C25</td>
<td>1.25</td>
<td>10.00</td>
<td>1.1(1.6)</td>
<td>-0.60</td>
<td>0.44(0.50)</td>
<td></td>
</tr>
<tr>
<td>C31</td>
<td>4.82</td>
<td>8.50</td>
<td>1.6(2.9)</td>
<td>0.00</td>
<td>0.00(0.00)</td>
<td>0.00(0.00)</td>
</tr>
<tr>
<td>C30</td>
<td>3.856</td>
<td>8.50</td>
<td>1.8(2.2)</td>
<td>-0.10</td>
<td>0.09(0.11)</td>
<td>0.0(-1.1)</td>
</tr>
<tr>
<td>C29</td>
<td>2.892</td>
<td>8.50</td>
<td>1.4(1.7)</td>
<td>-0.22</td>
<td>0.06(0.24)</td>
<td></td>
</tr>
<tr>
<td>C44</td>
<td>4.82</td>
<td>8.50</td>
<td>0.3(0.5)</td>
<td>0.00</td>
<td>0.00(0.00)</td>
<td></td>
</tr>
<tr>
<td>C43</td>
<td>3.856</td>
<td>8.50</td>
<td>0.3(0.5)</td>
<td>-0.10</td>
<td>0.06(0.00)</td>
<td></td>
</tr>
<tr>
<td>C42</td>
<td>2.892</td>
<td>8.50</td>
<td>0.2(0.5)</td>
<td>-0.22</td>
<td>-0.05(0.08)</td>
<td>-0.2(-0.4)</td>
</tr>
<tr>
<td>C41</td>
<td>1.928</td>
<td>8.50</td>
<td>0.3(0.6)</td>
<td>-0.40</td>
<td>0.10(0.15)</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3(xii) The effect of L-Cys addition on the Cu(II)BSA UV/VIS spectrum, pH 7.4, 25°C: initial concentration of Cu(II)BSA, $5.0 \times 10^{-4}$ moldm$^{-3}$, glycine absent.

Cu(II) : BSA : L-Cys; 5.0 : 5.8 : 20.0, C26(+);
5.0 : 5.8 : 30.0, C27 (o);
5.0 : 11.6 : 10.0, C28 (△).
Figure 3(xiii) The effect of L-Cys addition on the Cu(II)BSA UV/VIS spectrum, pH 7.4, 25°C: initial concentration of L-Cys, $1.0 \times 10^{-3}$ moldm$^{-3}$; glycine absent. Cu(II)BSA : L-Cys; 5.00 : 10.0, C23; 2.50 : 10.0, C24; 1.25 : 10.0, C25.
Table 3(viii) ESR data for the Cu(II)BSA reaction with L-His, 25°C and pH 7.4 (C23), 37°C and pH 7.4 (C45) and at 37°C and pH 9.3 (C48).

<table>
<thead>
<tr>
<th>System</th>
<th>Time (s)</th>
<th>Relative Cu(II)BSA Concentration</th>
<th>(g_\parallel)</th>
<th>(A_\parallel) (Gauss)</th>
<th>(g_m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C23</td>
<td>0</td>
<td>100</td>
<td>2.169</td>
<td>215</td>
<td>2.025</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>ca 90</td>
<td>2.171</td>
<td>214</td>
<td>2.025</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>ca 80</td>
<td>2.162</td>
<td>214</td>
<td>2.025</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>ca 55</td>
<td>2.170</td>
<td>216</td>
<td>2.025</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>ca 45</td>
<td>2.167</td>
<td>215</td>
<td>2.023</td>
</tr>
<tr>
<td>C45</td>
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<td>ca 100</td>
<td>2.170</td>
<td>213</td>
<td>2.024</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>ca 30</td>
<td>*</td>
<td>*</td>
<td>2.022</td>
</tr>
<tr>
<td></td>
<td>135</td>
<td>ca 20</td>
<td>*</td>
<td>*</td>
<td>2.022</td>
</tr>
<tr>
<td>C48</td>
<td>0</td>
<td>100</td>
<td>2.171</td>
<td>214</td>
<td>2.026</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>ca 80</td>
<td>2.171</td>
<td>214</td>
<td>2.026</td>
</tr>
<tr>
<td></td>
<td>135</td>
<td>ca 70</td>
<td>2.171</td>
<td>214</td>
<td>2.026</td>
</tr>
</tbody>
</table>

* too weak to measure accurately.
Figure 3(xiv) The effect of L-Cys addition on the Cu(II)BSA UV/VIS spectrum, pH 7.4, 37°C: initial concentration of Cu(II)BSA, $4.82 \times 10^{-4}$ moldm$^{-3}$; glycine absent.

Cu(II)BSA : L-Cys; 4.82 : 4.59, C32; 4.82 : 6.88, C33; 4.82 : 9.17, C34.
Figure 3(xv) The effect of L-Cys addition on the Cu(II)BSA UV/VIS spectrum, pH 7.4, 37°C: initial concentration of L-Cys, $8.5 \times 10^{-4}$ moldm$^{-3}$; glycine absent.

L-Cys : Cu(II)BSA; $8.5 : 2.892$, C29; $8.5 : 3.856$, C30; $8.5 : 4.82$, C31: initial concentration of L-Cys, $2.3 \times 10^{-4}$ moldm$^{-3}$; $2.3 : 4.82$, C35.
Figure 3(xvi) The effect of L-Cys addition on the Cu(II)BSA UV/VIS spectrum, pH 7.4, 10°C: initial concentration of Cu(II)BSA, $4.82 \times 10^{-4}$ moldm$^{-3}$, glycine absent.

Cu(II)BSA : L-Cys; 4.82 : 45.9, C36; 4.82 : 22.9, C37; 4.82 : 9.17, C38; 4.82 : 6.88, C39; 4.82 : 4.59, C40.
Figure 3(xvii) The effect of L-Cys addition on the Cu(II)BSA UV/VIS spectrum, pH 7.4, 10°C: initial concentration of L-Cys, 8.5 x 10^{-4} moldm^{-3}; glycine absent. Cu(II)BSA : L-Cys; 1.928 : 8.5, C41; 2.892 : 8.5, C42; 3.856 : 8.5, C43; 4.82 : 8.5, C44.
When the reaction was performed at pH 9.3, a slight reduction in the initial rate was observed compared with pH 7.4, figures 3(xviii) and 3(xix). A proton-aided pathway may thus occur which is reduced at the higher pH, alternatively, this may reflect increasing negative charge on the protein as the pH is raised.

3.4 (iv) The Cu(II)BSA Exchange Reaction with L-His/L-Cys Mixtures

The details of the kinetics are given in table 3(ix), and reaction profiles are shown in figures 3(xx) and 3(xxi). The initial rate of reaction was dependent upon the Cu(II) : L-Cys ratio. In general equilibration was attained in ca 1-2 minutes. The final spectrum exhibited no evidence for any Cu(II)(L-His)$_2$ product. The amino acid L-His may play an intermediary role, but the product is probably Cu(I) from cysteine reduction in all cases.

3.4 (v) The Cu(II)BSA Reaction with Glycine

Glycine (Gly) was used in a number of experiments to represent the remaining amino acids found in plasma, ca $3 \times 10^{-3}$moldm$^{-3}$. In order to prepare plasma like solutions all the amino acids should be incorporated, and any effects noted. Addition of Gly to Cu(II)BSA resulted in a slight increase in absorption at 525 nm, which may be
Figure 3(xviii) The effect of L-Cys addition on the Cu(II)BSA UV/VIS spectrum, pH 7.4, 37°C: initial concentration of Cu(II)BSA; $4.82 \times 10^{-4}$ moldm$^{-3}$; glycine absent. Cu(II)BSA : L-Cys; 4.82 : 2.29, C45; 4.82 : 4.59, C46; 4.82 : 11.5, C47.
Figure 3(xix) The effect of L-Cys addition on the Cu(II)BSA UV/VIS spectrum, pH 9.3, 37°C: initial concentration of Cu(II)BSA; $4.82 \times 10^{-4}$ moldm$^{-3}$; glycine absent.

Cu(II)BSA : L-Cys; 4.82 : 2.29, C48; 4.82 : 4.59, C49; 4.82 : 11.5, C50.
Table 3(ix) Results obtained for the Cu(II)BSA reaction with L-His/L-Cys mixtures, pH 7.4, 37°C.

(Concentrations of NaCl, 0.1 moldm$^{-3}$, KH$_2$PO$_4$, 0.15 moldm$^{-3}$.)

<table>
<thead>
<tr>
<th>System</th>
<th>Concentration of Cu(II) $/10^4$ moldm$^{-3}$</th>
<th>Concentration of L-His $/10^4$ moldm$^{-3}$</th>
<th>Concentration of L-Cys $/10^4$ moldm$^{-3}$</th>
<th>Initial Rate $/s^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C51</td>
<td>4.82</td>
<td>8.50</td>
<td>23.00</td>
<td>4.0(6.9)</td>
</tr>
<tr>
<td>C52</td>
<td>4.82</td>
<td>5.10</td>
<td>13.80</td>
<td>2.7(4.1)</td>
</tr>
<tr>
<td>C53</td>
<td>4.82</td>
<td>3.40</td>
<td>9.20</td>
<td>1.4(1.7)</td>
</tr>
<tr>
<td>C54</td>
<td>4.82</td>
<td>34.00</td>
<td>9.17</td>
<td>1.6(2.6)</td>
</tr>
<tr>
<td>C55</td>
<td>4.82</td>
<td>25.50</td>
<td>6.88</td>
<td>1.5(1.7)</td>
</tr>
<tr>
<td>C56</td>
<td>4.82</td>
<td>17.00</td>
<td>4.59</td>
<td>0.5(1.3)</td>
</tr>
</tbody>
</table>
Figure 3(xx) The effect of L-His/L-Cys addition on the Cu(II)BSA UV/VIS spectrum, pH 7.4, 37°C: initial concentration of Cu(II)BSA, 4.82 x 10^{-4} mol dm^{-3}; glycine absent.
Cu(II)BSA : L-His : L-Cys; 4.82 : 8.5 : 23.0, C51; 4.82 : 5.1 : 13.8, C52; 4.82 : 3.4 : 9.2, C53.
Figure 3(xxi) The effect of L-His/L-Cys addition on the Cu(II)BSA UV/VIS spectrum, pH 7.4, 37°C: initial concentration of Cu(II)BSA, $4.82 \times 10^{-4}$ moldm$^{-3}$; glycine absent. Cu(II)BSA : L-His : L-Cys; 4.82 : 34.0 : 9.17, C54; 4.82 : 25.5 : 6.88, C55; 4.82 : 17.0 : 4.59, C56.
attributable to growth of a small amount of Cu(II)(Gly)$_2$, figure 3(xxii). This was only observed, however, after ca 18 hours, whereas in the presence of L-His or L-Cys reactions were complete in a few minutes.

3.4 (vi) The Ni(II)BSA Reaction with L-Histidine

The progress of the reactions were followed by monitoring the reduction in absorbance at 420 nm in the UV/VIS spectrum of Ni(II)BSA, or of the negative Cotton Effect at 477 nm in the CD spectrum. Addition of excess L-His to Ni(II)BSA at pH 7.4 and 9.3 resulted in the slow decrease of the Ni(II)BSA spectrum. At pH 7.4 complete conversion to Ni(II)(L-His)$_2$ took ca 2 hours, typical reaction profiles are given in figure 3(xxiii). The initial rates of reaction, and order with respect to each component were calculated, table 3(x). At pH 7.4 the order with respect to L-His was ca zero. Addition of L-His to Ni(II)BSA resulted in the collapse of the CD spectrum, and the residual weak spectrum was that expected for Ni(II)(L-His)$_2$, figure 3(xxiv). This is in agreement with the reaction,

\[
\text{Ni(II)BSA} + n(L-\text{His}) \rightarrow \text{Ni(II)}(L-\text{His})_2 + (n-2)(L-\text{His}) + \text{BSA} \quad 3(\text{xix})
\]

At pH 9.3 the reaction with L-His was considerably
Figure 3(xxii) The UV/VIS spectrum of Cu(II)BSA plus glycine: initial concentration of Cu(II)BSA, $4.82 \times 10^{-4}$ moldm$^{-3}$; UV/VIS spectrum of Cu(II)BSA, I; UV/VIS spectrum of Cu(II)(Gly)$_2$, $4.82 \times 10^{-4}$ moldm$^{-3}$, II; Cu(II)BSA : Gly, 1 : 2, recorded after 18 hours, III; Cu(II)BSA + Cu(II)(Gly)$_2$, calculated additive spectrum, 1 : 1, IV.
Figure 3(xxiii) The effect of L-His addition on the Ni(II)BSA UV/VIS spectrum, pH 7.4, 37°C: initial concentration of Ni(II)BSA, $4.82 \times 10^{-4}$ moldm$^{-3}$; glycine absent. Ni(II)BSA : L-His; 4.82 : 81.90, N1 (∨); 4.82 : 65.52, N2 (●); 4.82 : 49.14, N3 (▲); 4.82 : 32.76, N4 (○); 4.82 : 16.38, N5 (x).
Table 3(x) Kinetic data for the Ni(II)BSA exchange reaction with L-His, 37°C.

(Concentration of NaCl, 0.15 moldm$^{-3}$; KH$_2$PO$_4$ (pH 7.4), 0.1 moldm$^{-3}$ (N1-N5); Borax (pH 9.3), 0.1 moldm$^{-3}$ (N6-N10).)

<table>
<thead>
<tr>
<th>System</th>
<th>Initial concentration of Ni(II) /10$^4$ moldm$^{-3}$</th>
<th>Initial concentration of L-His /10$^4$ moldm$^{-3}$</th>
<th>Initial Rate /s$^{-1}$</th>
<th>$\log ([L\text{-His}]_1 /[L\text{-His}]_2)$</th>
<th>$\log (\text{Rate}_1 /\text{Rate}_2)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>4.82</td>
<td>81.90</td>
<td>0.038</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>N2</td>
<td>4.82</td>
<td>65.52</td>
<td>0.047</td>
<td>-0.10</td>
<td>0.09</td>
</tr>
<tr>
<td>N3</td>
<td>4.82</td>
<td>49.14</td>
<td>0.027</td>
<td>-0.22</td>
<td>-0.12</td>
</tr>
<tr>
<td>N4</td>
<td>4.82</td>
<td>32.76</td>
<td>0.038</td>
<td>-0.40</td>
<td>0.00</td>
</tr>
<tr>
<td>N5</td>
<td>4.82</td>
<td>16.38</td>
<td>0.036</td>
<td>-0.70</td>
<td>-0.02</td>
</tr>
<tr>
<td>N6</td>
<td>4.82</td>
<td>81.90</td>
<td>$8.1 \times 10^{-4}$</td>
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<td>0.00</td>
</tr>
<tr>
<td>N7</td>
<td>4.82</td>
<td>73.71</td>
<td>$7.5 \times 10^{-4}$</td>
<td>-0.05</td>
<td>-0.03</td>
</tr>
<tr>
<td>N8</td>
<td>4.82</td>
<td>65.52</td>
<td>$6.1 \times 10^{-4}$</td>
<td>-0.10</td>
<td>-0.12</td>
</tr>
<tr>
<td>N9</td>
<td>4.82</td>
<td>57.33</td>
<td>$6.3 \times 10^{-4}$</td>
<td>-0.15</td>
<td>-0.11</td>
</tr>
<tr>
<td>N10</td>
<td>4.82</td>
<td>49.14</td>
<td>$5.8 \times 10^{-4}$</td>
<td>-0.22</td>
<td>-0.14</td>
</tr>
</tbody>
</table>

Order: $0.1$, $0.8$
Figure 3(xxiv) Final CD spectrum of Ni(II)BSA plus L-His, 1 : 2, I; Ni(II)(L-His)$_2$, II; initial concentration of Ni(II), $4.82 \times 10^{-4}$ moll$^{-1}$m$^{-3}$.
slower, requiring greater than 24 hours for equilibration to be attained, figure 3(xxv). The extent of the reaction was less than at pH 7.4, changes in the CD spectrum again paralleled the UV/VIS changes. The order with respect to L-His was ca 1. Orders with respect to Ni(II) were not calculated.

3.4 (vii) The Ni(II)BSA Exchange Reaction with L-Cys

When L-Cys was added to Ni(II)BSA at pH 7.4 and 25°C, under anaerobic conditions, a decrease in the absorbance occurred at 420 nm, with an increase at ca 465 nm, figure 3(xxvi). The latter peak can be attributed to Ni(II)(L-Cys)₂ formation, figure 3(xxvii). At least three hours were required for this new equilibrium to be obtained. After ca 14 hours the absorbance shifted back to 420 nm, which is probably due to the bleeding of air back into the solution oxidizing the L-Cys present. An intense charge transfer band at ca 330 nm was also seen to develop during the course of the reaction. Addition of less than the stoichiometric ratio resulted in an increase in absorbance at 420 nm, together with the increased charge transfer band, no absorbance developed at 465 nm. The results are consistent with the following overall reaction,
Figure 3(xxv) The effect of L-His addition on the Ni(II)BSA UV/VIS spectrum, pH 9.3, 37°C: initial concentration of Ni(II)BSA, 4.82 x 10^{-4} moldm^{-3}; glycine absent.
Ni(II)BSA : L-His; 4.82 : 81.90, N6(x);
4.82 : 73.71, N7(ο); 4.82 : 65.52, N8(△);
4.82 : 57.33, N9 (▽); 4.82 : 49.14, N10(+).
Figure 3(xxvi) The effect of L-Cys addition on the Ni(II)BSA UV/VIS spectrum, pH 7.4, 25°C: initial concentration of Ni(II)BSA, $5.0 \times 10^{-4}$ moldm$^{-3}$; L-Cys, $3.0 \times 10^{-3}$ moldm$^{-3}$; glycine absent. Spectra recorded at 0s, I; 60s, II; 3 hours, III; 14 hours, IV.
Figure 3(xxvii) UV/VIS spectrum of Ni(II)(L-Cys)$_2$, pH 7.4, 25°C: concentration of Ni(II)(L-Cys)$_2$ 4.0 x 10$^{-4}$ moldm$^{-3}$. 
Ni(II)BSA + n(L-Cys) $\xrightarrow{\text{Air}}$ Ni(II)(L-Cys)$_2$ + (n-2)(L-Cys) + BSA

Scheme 3(xx)

3.5 The Influence of L-Cysteine on the Exchangeable Copper in Blood Plasma

Laurie and Mohammed earlier examined this reaction at the physiological temperature, 37°C [108], it was intended to follow the reaction at the lower temperatures employed in a number of earlier kinetic runs, 10°C. Sulphydryl content may be determined by using Ellman's colorimetric method [117]. The organic reagent 5,5'-dithiobis(2-nitrobenzoic acid), Ellman's reagent or DTNB, reacts with thiol groups to produce a highly coloured anion ($E_{\text{max}} = 13,600 \text{ dm}^3\text{mol}^{-1}\text{cm}^{-1}$ at 412 nm). Since the early applications by Ellman, several groups have used DTNB to determine sulphydryl content of albumin, and L-cysteine containing solutions [118,119]. The high extinction coefficient of the chromophore allows for accurate determination of sulphydryl content at the physiological level.
3.5 (i) Experimental

5,5'-dithiobis(2-nitrobenzoic acid) was obtained from Fluka (puriss), all other reagents were as described in 3.2. Experiments were performed under aerobic conditions, although L-cysteine solutions were prepared under anaerobic conditions prior to use. The concentrations of components used in these models are given in table 3(xi). All experiments were performed at 10°C.

3.5 (ii) Results and Discussion

Addition of L-Cys to Cu(II)(L-His)₂ at elevated levels, ca 5x10⁻⁴ moldm⁻³, resulted in the immediate completion of the redox reaction upon mixing. However, at lower concentrations, figure 3(xxviii), 7 minutes was required for the reaction to go to completion. Addition of L-Cys to Ni(II)(L-His)₂ at elevated levels resulted in only a slight change in the UV/VIS spectrum after ca 3 hours, however, no absorption was observed at 465 nm indicative of Ni(II)(L-Cys)₂. The facile nature of the Cu(II) reaction may be due to the precipitation of L-cystine acting as a driving force.

Various attempts were made to monitor the {Cu(II)-BSA-L-Cys} system with Ellman's reagent. Addition of the reagent to BSA(5.8 x 10⁻⁴ moldm⁻³) led to an
Table 3(xi) Concentrations of reactants used in plasma models, and in plasma itself.

(Cu(II), Zn(II), L-His, L-Cys, L-Ala, $10^6$ moldm$^{-3}$; KH$_2$PO$_4$, NaCl, moldm$^{-3}$)

<table>
<thead>
<tr>
<th>Component</th>
<th>System</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>Blood Plasma*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(II)</td>
<td></td>
<td>1.0</td>
<td>-</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Zn(II)</td>
<td></td>
<td>-</td>
<td>-</td>
<td>16.0</td>
<td>16.0</td>
</tr>
<tr>
<td>L-His</td>
<td></td>
<td>85.0</td>
<td>85.0</td>
<td>85.0</td>
<td>85.0</td>
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<td>L-Cys</td>
<td></td>
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<td>23.0</td>
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</tr>
<tr>
<td>L-Ala</td>
<td></td>
<td>-</td>
<td>-</td>
<td>30.0</td>
<td>28.0</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
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<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>NaCl</td>
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<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>7.4</td>
<td>7.4</td>
<td>7.4</td>
<td>7.4</td>
</tr>
</tbody>
</table>

* Taken from reference[29].
Figure 3(xxviii) The effect of L-cysteine on the 'exchangeable' copper in blood plasma model; aerobic conditions (concentrations) in table 3(xi).
increase in absorbance at 412 nm, until ultimately after ca 15 minutes it was too large to monitor. Ghiggeri et al have examined this time dependence, and also observed a maximum value being attained after ca 15 minutes, a gradual decrease then occurred [119]. When modified bovine serum albumin was used, i.e. bovine serum albumin in which the L-cysteine residue had been converted to L-cystine (Miles Laboratories Inc.), the absorbance reached a maximum of ca 0.25. The same authors also demonstrated that addition of the reagent to L-cysteine resulted in the peak absorbance being attained virtually immediately, and then gradually decreasing with time. If the modified protein exhibited similar behaviour to the native protein then it may be possible to follow reactions at the physiological levels. However, several experiments aimed at examining its suitability, most notably the ESR spectrum of the Cu(II): protein 1:1 complex, showed that the Cu(II) binding site had been affected.

3.6 Computer Simulation of Metal Ion Equilibria in Biofluids; Low Molecular Weight Complex Distribution of Nickel

It is required to find the species concentrations of the components of a multicomponent metal ligand system at equilibrium. Luccassen and Sarkar showed that the major Ni species, present in the low molecular weight fraction, of
blood plasma, is bound to the amino acid L-histidine [46]. However, the stability constants for the Ni(II) : L-Cys, 1:1 and 1:2 complexes, are greater than the corresponding L-His complexes. An investigation of the distribution between the amino acid complexes of Ni(II), and albumin, at physiological levels was undertaken employing the computer program ECCLES [29]. Stability constant data was taken from various sources [120,121]. The values for the amino acid ternary species were approximated by using the relationship:

$$\log K_{MAB} = \frac{1}{2}[\log K_{MA_2} + \log K_{MB_2}] + 0.5 \log K_D$$

where $MA_2$ and $MB_2$ are binary complexes, and $MAB$ is the ternary complex, $K_D = 4$ (statistical factor) [30].

The distribution of Ni(II) amongst the low molecular weight amino acid species is given in table 3(xii). All stability constants were corrected to an ionic strength of 0.15 moldm$^{-3}$, at 25°C. The Ni(II) concentration was varied over the range $10^{-6}$-$10^{-9}$ moldm$^{-3}$, no appreciable difference was observed in the distribution between species. The major Ni(II) binding component was calculated to be Ni(L-His)$_2$, which is in agreement with the observations of Luccassen and Sarkar [46].

The stability constants for the Ni(II)HSA binary and
Table 3(xii) The distribution of Ni(II) between the amino acid components of blood; remaining species <0.1%.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Percentage Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni(II)(L-His)$_2$</td>
<td>76.2</td>
</tr>
<tr>
<td>Ni(II)(L-His)(L-Cys)$^-$</td>
<td>14.7</td>
</tr>
<tr>
<td>Ni(II)(L-His)$^+$</td>
<td>5.5</td>
</tr>
<tr>
<td>Ni(II)(L-His)(L-Gln)</td>
<td>1.3</td>
</tr>
<tr>
<td>Ni(II)(L-Cys)$_2^{2-}$</td>
<td>0.7</td>
</tr>
<tr>
<td>Ni(II)(L-His)(Gly)</td>
<td>0.4</td>
</tr>
<tr>
<td>Ni(II)(L-His)(Ala)</td>
<td>0.3</td>
</tr>
<tr>
<td>Ni(II)(L-His)(L-Val)</td>
<td>0.3</td>
</tr>
<tr>
<td>Ni(II)(L-Cys)</td>
<td>0.3</td>
</tr>
<tr>
<td>Ni(II)(L-Cys)(L-Gln)</td>
<td>0.1</td>
</tr>
</tbody>
</table>
[Ni(II)HSA-L-His] ternary species were taken from the work of Glennon and Sarkar [48]. Computations were performed with and without the presence of the ternary species, table 3(xiii). If the ternary species [Ni(II)HSA-L-His] existed in the quantity suggested by these calculations, it would have been expected that the earlier spectroscopic studies would have revealed its presence as a stable intermediate. Luccassen and Sarkar found that 95.7% of the Ni(II) added to plasma was associated with albumin, 4.2% was bound to low molecular mass components, ca 72% of which was bound to L-histidine [46].

3.7 The Distribution of $^{63}$Ni between BSA and L-Histidine

3.7 (i) Experimental

All chemicals were used as described in 3.2. $^{63}$Ni(II) was obtained from Amersham International. Separations of low and high molecular weight Ni(II) binding components were performed by ultrafiltration (Amicon, XM10 membranes, approximately 60 psi applied N$_2$ pressure), or by gel filtration on Sephadex G-75 (Pharmacia Fine Chemicals). The final concentration of components in the plasma model solutions were; Ni(II), 1x10$^{-6}$ moldm$^{-3}$; BSA, 5.8x10$^{-4}$ moldm$^{-3}$; L-His, 8.5x10$^{-5}$ moldm$^{-3}$; Gly, 1.0x10$^{-3}$ moldm$^{-3}$; KH$_2$PO$_4$, 0.1 moldm$^{-3}$ (pH 7.4); NaCl, 0.15 moldm$^{-3}$. All equilibration reactions were performed at 37°C in a thermostated cell block, samples were
Table 3(xiii) The distribution of Ni(II) between plasma binding components; including ternary species, A; excluding the ternary species, B; remaining species <0.1%.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Percentage Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td></td>
</tr>
<tr>
<td>Ni(II)BSA-L-His</td>
<td>67.8</td>
</tr>
<tr>
<td>Ni(II)BSA</td>
<td>31.4</td>
</tr>
<tr>
<td>Ni(II)(L-His)$_2$</td>
<td>0.6</td>
</tr>
<tr>
<td>Ni(II)(L-His)(L-Cys)$^-$</td>
<td>0.1</td>
</tr>
<tr>
<td>Ni(II) (L-His)$^+$</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>B</strong></td>
<td></td>
</tr>
<tr>
<td>Ni(II)BSA</td>
<td>97.4</td>
</tr>
<tr>
<td>Ni(II)(L-His)$_2$</td>
<td>2.0</td>
</tr>
<tr>
<td>Ni(II)(L-His)(L-Cys)$^-$</td>
<td>0.4</td>
</tr>
<tr>
<td>Ni(II)(L-His)$^+$</td>
<td>0.1</td>
</tr>
</tbody>
</table>
periodically removed, separated, and the activity assayed.

3.7 (ii) Results and Discussion

Two experiments were performed in which L-His was added to \(^{63}\text{Ni(II)}\)BSA and \(^{63}\text{Ni(II)}\) was added to BSA/L-His mixtures. Samples were withdrawn periodically and the activity assayed, table 3(xiv). It was also noted that in the absence of any amino acids considerable activity was still found in the ultrafiltrate. It was apparent from the results obtained that the components could not be adequately separated using ultrafiltration.

When an equilibrium mixture was separated by gel filtration two maxima were observed, attributable to \(^{63}\text{Ni(II)}\)BSA and the \(^{63}\text{Ni(II)}\)-amino acid fractions, figure 3(xxix). Neither technique allowed for the rapid enough assaying of the fractions to be of use for kinetic applications.

3.8 Spin Labelling of the Albumin Protein Sulphydryl Group
3.8 (i) Introduction

Graceffa applied a spin-trap to protein sulphydryl groups by trapping a sulphur radical generated by the addition of Ce\(^{4+}\) [122]. The radical RS\(^-\) once generated was trapped with phenyl-N-t-butyl nitron.
Table 3(xiv) The percentage of total activity in the filtrate (low molecular mass components) in the Ni(II) plasma experiments.

<table>
<thead>
<tr>
<th>Time /min</th>
<th>(i) Ni(II)BSA + L-His</th>
<th>(i) Ni(II)BSA + L-His</th>
<th>(ii) Ni(II) + L-His/BSA</th>
<th>(iii) Ni(II)BSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.8</td>
<td>28.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28.0</td>
<td>23.1</td>
<td>29.1</td>
<td>28.3</td>
<td>19.0</td>
</tr>
<tr>
<td>56.1</td>
<td>20.7</td>
<td>30.3</td>
<td>24.6</td>
<td>27.3</td>
</tr>
<tr>
<td>88.3</td>
<td>25.8</td>
<td></td>
<td>20.3</td>
<td></td>
</tr>
<tr>
<td>116.7</td>
<td></td>
<td>50.8</td>
<td>16.8</td>
<td>13.3</td>
</tr>
<tr>
<td>149.0</td>
<td>26.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>179.5</td>
<td>25.3</td>
<td></td>
<td>11.8</td>
<td>12.5</td>
</tr>
<tr>
<td>205.0</td>
<td></td>
<td></td>
<td>14.2</td>
<td></td>
</tr>
<tr>
<td>224.0</td>
<td>19.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>262.5</td>
<td>23.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>72 hours</td>
<td>11.0</td>
<td>4.5</td>
<td>13.2</td>
<td>4.3</td>
</tr>
</tbody>
</table>
Figure 3(xxix) Separation of equilibrium mixtures by gel filtration; Ni(II)BSA plus L-His (I); Ni(II)ß3Aý (II).
The intention of the experiments reported here was to spin-trap cysteine and bovine serum albumin using Graceffa's technique, with the possibility of extending this to some of the systems examined earlier.

3.8 (ii) Experimental

Bovine serum albumin and L-cysteine were used as earlier, (3.2); diethylenetriamine pentaacetic acid (DTPA) and nitriloacetic acid (NTA) were purchased from BDH (General Purpose); ammonium cerium(IV) nitrate was obtained from BDH (AnalaR); 3-(N-morpholino)propanesulphonic acid (MOPS) from BDH (biochemical), and phenyl-N-t-butylnitrone (PBN) from Aldrich.

The procedure followed was that outlined by Graceffa [122]. A Ce$^{4+}$-NTA solution was prepared by the addition of 1 volume of 0.5 moldm$^{-3}$ Ce(NH$_4$)$_2$(NO$_3$)$_6$ to
9 volumes of 0.1 molar \( NTA \), 0.5 molar \( MOPS \), pH 7.0, with vigorous stirring on a Vortex mixer. The sulphhydryl (RSH) solution contained either albumin, 25 mg cm\(^{-3}\), or L-Cysteine, \( 0.53 \times 10^{-3} \) molar\(^{-3}\), in \( 29 \times 10^{-3} \) molar\(^{-3}\) PBN, \( 1.1 \) molar\(^{-3}\) DTPA, \( 26 \times 10^{-3} \) molar\(^{-3}\) MOPS, pH 7.0. One volume of Ce\(^{4+}\)-NTA solution was mixed with 19 volumes of RSH solution containing the spin-trap and the ESR spectrum recorded. All solutions were prepared under anaerobic conditions.

3.8 (iii) Results and Discussion

The resultant ESR spectra obtained for the spin-trapped BSA and L-Cys are given in figure 3(xxx). The spectrum of nitroxide spin-labelled BSA shows an immobilized label and is in agreement with Graceffa's observations \( 2A_{zz} = 62G \). However, the labelled L-Cys did not produce a particularly well resolved spectrum, even though the most stringent precautions were undertaken to exclude air from the system, and the spectrum was recorded immediately after addition of Ce\(^{4+}\).

3.9 Conclusions

These results clearly show that Cu(II)BSA undergoes rapid ligand exchange with the amino acids L-Cys and L-His, but not Gly, at the \( 10^{-4} \) molar\(^{-3}\) Cu level. The major low
Figure 3(xxx) ESR spectra of spin-trapped BSA(I), and L-cysteine (II), both recorded at room temperature.

(Gains, $2 \times 10^6$ (I), $6.3 \times 10^6$ (II).)
molecular weight fraction in blood plasma is generally considered to be the Cu(II)(L-His)$_2$ complex, however, these results would suggest that the redox reaction with L-cysteine may be more significant.

The Ni(II)BSA ligand exchange reactions with the amino acids L-Cys and L-His are much slower than the corresponding Cu(II) reactions. At pH 7.4 approximately 2 hours are required for equilibration in the exchange reaction with L-His, and at pH 9.3 ca 24 hours are required for equilibration. L-His bound Ni(II) was not found to react at an appreciable rate with L-Cys, even at the $10^{-4}$ moldm$^{-3}$ Ni level. The computer simulations would suggest that this would hold at physiological levels, Ni(II)(L-His)$_2$ is the predominant low molecular weight species.

If the reaction is first order with respect to L-His this would suggest an associative type mechanism in which the intermediate ternary species Cu(II)BSA-L-His or Ni(II)BSA-L-His are formed. Intermediates of this type have been previously reported as thermodynamically stable species, however, evidence from these studies suggest that these species are only formed transiently. The results obtained for the exchange reactions with L-Cys were much less conclusive, and no evidence for the formation of intermediate ternary species was obtained. Where the order
with respect to L-His was zero, in the Ni(II)BSA exchange reaction at pH 9.3, this would suggest a more dissociative type mechanism. A proposed scheme for the reaction with L-His is outlined in 3(xxii).

\[
\begin{align*}
\text{M(BSA) } &\xrightleftharpoons[k_d']{k_d} \text{M}^{2+} + \text{BSA} \\
\text{L-His} &\xleftarrow{k_a} \text{M(BSA-L-His)} \xrightarrow{k_1} \text{M(L-His)} \\
\text{M(BSA-L-His)} &\xrightarrow{k_3,\text{BSA}} \text{M(L-His)_2} \\
\end{align*}
\]

Scheme 3(xxii), (M = Ni, Cu)

It was not possible to carry out measurements of ligand exchange reactions at the concentrations found physiologically [29], i.e. Cu, 1x10^{-6} moldm^{-3}; albumin, 5.8x10^{-4} moldm^{-3}; L-histidine, 8.5x10^{-5} moldm^{-3}. It is however, possible to obtain some idea of the reaction rates of Cu(II)BSA at this level from these, and previously reported results [108]. At 10°C the reaction Cu(II)BSA plus L-Cys is >20 times slower than the analogous
Cu(II)(L-His)$_2$ plus L-Cys reaction. This factor of ca 20 would mean that the reaction Cu(II)BSA plus L-Cys would require ca 2 hours for completion, reactions with Cu(II)HSA would probably be very similar. These reactions are relatively slow compared to blood-Cu turnover rates in healthy humans.

The Ni(II)BSA reactions were much slower than the corresponding Cu(II)BSA exchange reactions, despite the similarities in co-ordination chemistry and blood distribution. The results presented here suggest that at the likely physiological concentration of Ni(II) in blood (ca $10^{-6}$ moldm$^{-3}$) the dissociation rate of Ni(II)albumin would be much too slow to be of physiological significance.
CHAPTER 4

NATURE OF THE REACTION BETWEEN COPPER(II) AND THIOMOLYBDATES, AND THE PRODUCTS FORMED

4.1 Introduction

The antagonism between copper and molybdenum in ruminants has been fairly well established (see Introductory Chapter). Thiomolybdates play a central role in the widespread molybdenosis that afflicts ruminants. The formation of 'copper thiomolybdates' within the rumen is generally believed to lead to a biologically unavailable form of copper, which leads to hypocuprosis.

Addition of a solution of \((\text{NH}_4)_2\text{MoS}_4\) to \(\text{Cu}_2\text{Cl}_2\) gives a black precipitate which has been characterized by Bok and Boeyens as \(\text{Cu}_2\text{MoS}_4\) [74]. Clark and Doyle prepared a "copper tetrathiomolybdate" by the addition of copper sulphate to ammonium tetrathiomolybdate. The product was not, however, fully characterized [75]. There is considerable interest in the interaction of thiomolybdates with copper containing enzymes. Chidambaram et al, for example, noted the effect of thiomolybdates on a number of copper enzymes, and found them to inhibit the oxidase activity of caeruloplasmin by forming an irreversible inhibitor-enzyme complex [64].
Tetrathiomolybdate in this respect was found to be more active than oxythiomolybdates. Nederbragt et al observed that dietary Mo administration changed the distribution of intravenously injected $^{64}$Cu; a reduced rate of $^{64}$Cu clearance being the most prominent feature of this change in both ruminants and monogastric animals [123]. This behaviour was ascribed to the formation of a Cu-Mo-S complex, probably bound to plasma albumin. The absence of any ESR signal in concentrated Mo-plasma containing fractions suggested that the tightly bound copper was in the +1 oxidation state.

Mason and his co-workers observed the co-accumulation of Cu and MoS$_4^{2-}$ on albumin [63], but did not observe any evidence of an irreversible chemical interaction. However, Mo did increase the proportion of Cu associated with albumin, and its metabolism was altered. It has recently been observed that MoS$_4^{2-}$ has six primary binding sites on bovine serum albumin [124].

A number of groups have performed electro-chemical studies on complexes which contain MoS$_4^{2-}$ units [125-128]. Cyclic Voltammetry provides a relatively simple method for deriving a number of electrochemical parameters, such as the redox potential.

The intention of the work reported in this chapter was
to examine the products of the reaction of tetrathiomolybdate with aqueous copper(II), and with the copper(II)-albumin 1:1 complex, in an attempt to resolve some of the controversy covering this area. The electrochemical properties of the tetrathiomolybdate ion have also been examined.

4.2 Preparation of Tetrathiomolybdate Salts

Ammonium tetrathiomolybdate was prepared by the method of Mellor [72]. Ammonium paramolybdate, (BDH, AnalAr) 5g was dissolved in 15cm³ distilled water, and ammonia (BDH, General Purposes) 50cm³, added, a vigorous stream of hydrogen sulphide (BDH) was bubbled through the solution which changed colour, turning yellow then red. After 20 minutes, crystals, red with a green shimmer, of the product formed and these were filtered, washed with methanol and dried in a dessicator under a vacuum.

Tetraethylammonium tetrathiomolybdate was prepared by the method of McDonald et al [73]. Ammonium tetrathiomolybdate, 5g, was dissolved in 10% tetraethylammonium hydroxide (Fluka, pract) in distilled water, 60cm³, and the solution subjected to pumping to remove ammonia. The reaction mixture was filtered into propan-1-ol (BDH, General Purpose) 100cm³, the precipitated product was isolated by filtration, washed with propan-1-ol.
and diethyl ether (BDH, General Purpose) and dried in a dessicator under vacuum.

Sodium tetrathiomolybdate was prepared by the method of Laurie et al. [129]. Ammonium tetrathiomolybdate, 5g, was dissolved in ice-cold sodium hydroxide solution, (BDH, General Purpose) 1.7g in 20cm$^3$ distilled water, and warmed to 40-50°C whilst pumping to remove any ammonia present. The red solution was filtered, and the filtrate left to evaporate to dryness. The red solid was extracted with acetone, 60cm$^3$, and diethyl ether, 50cm$^3$, added to the filtrate. Red crystals of the product formed, these were filtered, washed with ether and dried in a vacuum dessicator.

Copper(I) ammonium tetrathiomolybdate was prepared by the method of Redman [130]. A solution of ammonium hydrogen sulphide was prepared by saturating a solution of concentrated ammonium hydroxide with hydrogen sulphide. To 75cm$^3$ of this stirred solution was added a solution consisting of 37.5cm$^3$ of ammonia, 37.5cm$^3$ of distilled water, 10g of ammonium paramolybdate and 7.5g of copper(II) sulphate. A black precipitate appeared which re-dissolved almost immediately to form a deep-red solution. The solution was boiled for 2-4 minutes, during which time a crystalline precipitate formed. This was removed by filtration and washed with ammonia, water and ethanol.
product was dried in air, and stored in a vacuum dessicator.

4.3. Determination of the Stoichiometry of the Reaction between Cu(II) and MoS$_4^{2-}$

Mixtures were prepared of (NH$_4$)$_2$MoS$_4$ and Cu(II)(Gly)$_2$ of ca $1 \times 10^{-2}$ moldm$^{-3}$, in 0.1 moldm$^{-3}$ phosphate buffer, pH 7.4, in the presence, and absence, of an ionic background of 0.15 moldm$^{-3}$ sodium chloride. Reactions were initiated by the addition of MoS$_4^{2-}$ to Cu(II), and diluted such that the final concentration was ca $1 \times 10^{-4}$ moldm$^{-3}$. In this manner the final ratio of components was varied. The mixtures were carefully centrifuged to remove any solid products that had formed, and the UV/VIS spectra of the supernatent recorded. The spectra that were obtained are shown in figures 4(i) and 4(ii). It is clear that the Cu(II) : MoS$_4^{2-}$ stoichiometric ratio, for complete reduction of either the Cu(II)(Gly)$_2$ spectrum, or that of MoS$_4^{2-}$ is ca 1.5:1. This contradicts the earlier observations of Clarke and Laurie [131], who suggested that complete reaction occurred at a 1:1 ratio.

4.4. Preparation and Characterization of 'Copper Tetrathiomolybdates'

4.4 (i) Experimental

'Copper tetrathiomolybdates' were prepared by the
Figure 4(i) UV/VIS spectra of mixtures of 
\((\text{NH}_4)_2\text{MoS}_4, 1 \times 10^{-4} \text{ mol dm}^{-3}\) and Cu(II)(Gly)$_2$.

Mo : Cu, 

- - - - 1:0 ; - - - - 1:0.8 ; - - - - 1:0.96; 
- - - - 1:1.12; - - - - 1:1.28; - - - - 1:1.44; 
- - - - - - - - 1:1.60
Figure 4(ii). UV/VIS spectra of mixtures of 

\[ \text{Cu(II)(Gly)}_2, \; 3.84 \times 10^{-3} \text{ mol dm}^{-3} \] 

and \( (\text{NH}_4)_2\text{MoS}_4 \).

\[ \text{Cu : Mo, 1:0, I; 1:0.16 II; 1:0.31 III; 1:0.47 IV; 1:0.63 V; 1:0.78 VI; 1:0.94 VII} \]
mixing of freshly prepared aqueous solutions of MoS$_4^{2-}$ and Cu(II), in the absence of buffer or sodium chloride. The precipitated products were separated by filtration, washed with ethanol, ether, and dried in a warm oven or over silica gel. Products were prepared at various Cu(II) : MoS$_4^{2-}$ ratios as indicated in table 4(i).

Elemental analyses were performed as follows; copper and molybdenum were determined by Atomic Absorption spectroscopy; copper, molybdenum, sulphur, carbon, hydrogen and nitrogen analyses were obtained from the University of Manchester, Microanalytical Department; carbon, hydrogen, sulphur, and nitrogen analyses were also obtained from C,H,N Analysis Ltd., Leicester; sodium analyses were performed by Flame Photometry.

Samples for Atomic Absorption spectroscopy were digested in the following manner. To approximately 0.1g of the solid, accurately weighed, was added 5cm$^3$ of bromine, followed by 5cm$^3$ of carbon tetrachloride. After 20 minutes, concentrated nitric acid, 15cm$^3$, was added. The beaker was left standing with occasional swirling for ca 2 hours before heating on a water bath to remove bromine, and then evaporating the contents to dryness. Hydrochloric acid, ca 10cm$^3$(conc) was added and the resultant solution evaporated to dryness. Hydrochloric acid, 3cm$^3$, and nitric acid, ca 50cm$^3$(2 moldm$^{-3}$), were then
Table 4(i) Reactant ratios at which the 'copper tetrathiomolybdates' were obtained.

<table>
<thead>
<tr>
<th>Reactants</th>
<th>Ratio 1:1</th>
<th>1.5:1</th>
<th>2:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(II)SO₄ : (NH₄)₂MoS₄</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Cu(II)SO₄ : (NEt₄)₂MoS₄</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu(II)SO₄ : Na₂Mo₄ O₃.5H₂O</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
added and the resultant solution boiled for 10 minutes. The contents of the beaker and washings were made up to 100cm³ with nitric acid (2 moldm⁻³). Solutions were further diluted to give ranges of 1.0-4.0 ppm for copper analyses, 10.0-40.0 ppm for molybdenum analyses, and 1.0-10.0 ppm for sodium analyses.

The products were characterized by a variety of spectroscopic techniques: Ultraviolet-visible Spectroscopy; Electron Spin Resonance Spectroscopy; Infra-Red Spectroscopy; Thermogravimetric Analysis; X-ray Diffraction; and X-ray Photoelectron Spectroscopy; all of which are described in Chapter 2.

4.4 (ii) Results and Discussion

The reaction of Cu(II) and MoS₄²⁻ was virtually instantaneous. The analyses showed the products to be composed principally of Cu, Mo and S, although small amounts of C, H and N were detected. A small unidentified proportion was assumed to be oxygen. The Mo analyses performed by AA at Leicester were found to be highly variable, which may be due to either a matrix effect, or incomplete digestion of the samples. The Cu analyses were found to be highly reproducible, ±0.5% in most instances. The results of the analyses are given in table 4(ii).
Table 4(4) Elemental analyses of the copper tetrathiomolybdate solids. Estimated from differences in percentage compositions, obtained by XRF analyses performed at Manchester University; not analysed; e: H. N. Analysis Ltd.; f: flame Photometry; values in parenthesis are calculated values.

<table>
<thead>
<tr>
<th>Reactants</th>
<th>Cu</th>
<th>Mo</th>
<th>S</th>
<th>N</th>
<th>H</th>
<th>Na</th>
<th>O</th>
</tr>
</thead>
<tbody>
<tr>
<td>CuO:Cu(II)(NN₄)₂MoS₄</td>
<td>21.7</td>
<td>25.2</td>
<td>35.2</td>
<td>22.8</td>
<td>33.2</td>
<td>24.8</td>
<td>33.2</td>
</tr>
<tr>
<td>CuO:Cu(II):Na₂MoO₄</td>
<td>21.5</td>
<td>25.5</td>
<td>35.5</td>
<td>22.8</td>
<td>33.2</td>
<td>24.8</td>
<td>33.2</td>
</tr>
<tr>
<td>CuO:Cu(II):Na₂MoO₄</td>
<td>19.9</td>
<td>24.8</td>
<td>33.2</td>
<td>24.8</td>
<td>33.2</td>
<td>24.8</td>
<td>33.2</td>
</tr>
<tr>
<td>CuO:Cu(II):MoO₃</td>
<td>16.5</td>
<td>24.8</td>
<td>33.2</td>
<td>24.8</td>
<td>33.2</td>
<td>24.8</td>
<td>33.2</td>
</tr>
<tr>
<td>CuO:Cu(II):MoO₃</td>
<td>16.5</td>
<td>24.8</td>
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<td>CuO:Cu(II):MoO₃</td>
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</tr>
<tr>
<td>CuO:Cu(II):MoO₃</td>
<td>16.5</td>
<td>24.8</td>
<td>33.2</td>
<td>24.8</td>
<td>33.2</td>
<td>24.8</td>
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<tr>
<td>CuO:Cu(II):MoO₃</td>
<td>16.5</td>
<td>24.8</td>
<td>33.2</td>
<td>24.8</td>
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</tr>
<tr>
<td>CuO:Cu(II):MoO₃</td>
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<td>24.8</td>
<td>33.2</td>
<td>24.8</td>
<td>33.2</td>
<td>24.8</td>
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<tr>
<td>CuO:Cu(II):MoO₃</td>
<td>16.5</td>
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<td>33.2</td>
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<td>33.2</td>
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<tr>
<td>CuO:Cu(II):MoO₃</td>
<td>16.5</td>
<td>24.8</td>
<td>33.2</td>
<td>24.8</td>
<td>33.2</td>
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</tr>
<tr>
<td>CuO:Cu(II):MoO₃</td>
<td>16.5</td>
<td>24.8</td>
<td>33.2</td>
<td>24.8</td>
<td>33.2</td>
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<td>33.2</td>
</tr>
<tr>
<td>CuO:Cu(II):MoO₃</td>
<td>16.5</td>
<td>24.8</td>
<td>33.2</td>
<td>24.8</td>
<td>33.2</td>
<td>24.8</td>
<td>33.2</td>
</tr>
<tr>
<td>CuO:Cu(II):MoO₃</td>
<td>16.5</td>
<td>24.8</td>
<td>33.2</td>
<td>24.8</td>
<td>33.2</td>
<td>24.8</td>
<td>33.2</td>
</tr>
</tbody>
</table>
This work showed that complete reduction of the Cu(II)(Gly)$_2$ and (NH$_4$)$_2$MoS$_4$ absorbances occurred at a ratio Cu(II) : MoS$_4^{2-}$ of 1.5:1, however, the elemental analyses showed the product to consist of Cu:Mo in a 1:1 ratio. The analyses also imply the presence of {MoS$_4$} units in the products. Addition of 1.5 moles of Cu(II) to 1 mole of MoS$_4^{2-}$ was found to precipitate ca 1 mole of Cu in the product, i.e. the black solid is virtually formed in a 100% yield. The analyses also show the presence of small amounts of other cations, i.e. NH$_4^+$, Na$^+$ or NEt$_4^+$, in the product.

The results of the X-ray Powder Diffraction measurements are given in table 4(iii). The CuMo(1) product is seen to have a component isostructural with the known compound CuNH$_4$MoS$_4$, and to exhibit lines which are found in the CuMo(1.5) product. Thus CuMo(1) appears to be composed of a mixture of CuNH$_4$MoS$_4$ and CuMo(1.5). CuMo(1.5) and NaCuMo are isostructural, and very similar in line pattern to Bok and Boeyens Cu$_2$MoS$_4$ [74]. The CuMo(2) exhibits a different pattern again, which could be attributed to CuMo(1.5) and some other unidentified compound. CuMo(1.5), CuMo(2) and NaCuMo exhibited diffuse patterns, typical of amorphous compounds, as is to be expected from their rapid precipitation from solution.

The Infra-red spectrum of the CuMo(1.5) product is
Table 4(iii): XRD results for the 'copper tetrathiomolybdates', d-values, $10^{-10}$ m ($I/I_0$); s = sharp, d = diffuse.

<table>
<thead>
<tr>
<th></th>
<th>CuMo(1)</th>
<th>CuMo(1.5)</th>
<th>CuMo(2)</th>
<th>NaCuMo</th>
<th>CuNH₄MoS₄</th>
<th>Cu₂MoS₄</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5.67(100s)</td>
<td>5.67(100s)</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>5.29(10s)</td>
<td>5.3(100d)</td>
<td>5.4</td>
<td>5.3(100d)</td>
<td>5.47(87d)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.82(100d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.00(15s)</td>
<td></td>
<td></td>
<td></td>
<td>4.01(25s)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.77(11d)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.37(10d)</td>
</tr>
<tr>
<td></td>
<td>2.98(25s)</td>
<td>3.1(10d)</td>
<td>3.04(10d)</td>
<td>3.1(10d)</td>
<td>2.99(30s)</td>
<td>3.19(63d)</td>
</tr>
<tr>
<td></td>
<td>2.84(5s)</td>
<td></td>
<td></td>
<td></td>
<td>2.83(12s)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.72(5d)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.52(1s)</td>
<td></td>
<td></td>
<td></td>
<td>2.53(8s)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.43(5s)</td>
<td></td>
<td></td>
<td></td>
<td>2.44(18s)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.35(5d)</td>
<td>2.38(1d)</td>
<td>2.40(10d)</td>
<td>2.38(32d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.25(5s)</td>
<td></td>
<td></td>
<td></td>
<td>2.24(20s)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.84(10s)</td>
<td>1.89(15d)</td>
<td>1.89(3d)</td>
<td>1.90(10d)</td>
<td>1.85(20s)</td>
<td>1.87(100d)</td>
</tr>
<tr>
<td></td>
<td>1.79(1s)</td>
<td>1.76(3d)</td>
<td></td>
<td></td>
<td>1.79(12s)</td>
<td></td>
</tr>
</tbody>
</table>
shown in figure 4(iii). The spectral data for a number of the 'copper tetrathiomolybdates' are given in table 4(iv). The presence of the band at ca 460 cm\(^{-1}\) can be assigned to a \(\nu(Mo-S)\) mode; the band at ca 1400 cm\(^{-1}\) is due to \(\nu(N-H)\), (absent in the sodium product); the band at ca 910 cm\(^{-1}\) compares favourably with the data in table 4(v) for Mo-O containing systems. It is not possible to conclude from the Infra-red spectra whether the Mo atom is in the +6 or +5 oxidation-state. The \(\nu(Mo-O)\) and \(\nu(Mo-S)\) absorbances appear to vary little with oxidation state.

The 'copper tetrathiomolybdates' were found to exhibit slight solubility only in DMSO or DMF solvents. The CuMo(1) product was the slightly more soluble, and the UV/VIS spectrum of this solution compares favourably with that of \(\text{CuNH}_4\text{MoS}_4\), figure 4(iv). When CuMo(1.5) was dissolved in hot refluxing DMF the resultant green solution gave a broad band at ca 580 nm, and on cooling the solution sulphur was precipitated, suggesting that decomposition had occurred.

The Thermogravimetric Analyses gave a fairly complex pattern of increases and decreases in weight on heating up to 1000\(^\circ\)C. Total weight losses for the 'copper tetrathiomolybdates' were in the range 27-32\%. Binnie et al claimed that at 400\(^\circ\)C \(\text{CuNH}_4\text{MoS}_4\) is oxidized to \(\text{CuMoO}_4\) [76], the expected weight loss would be 26.9\%. At
Figure 4(iii) Infra red spectrum, recorded as a KBr disc, of CuMo(1.5)
Table 4(iv) Infra-red spectral results, recorded as KBr discs, for 'copper tetrathiomolybdates' (cm\(^{-1}\)).

<table>
<thead>
<tr>
<th></th>
<th>CuMo(1)</th>
<th>CuMo(1.5)</th>
<th>CuMo(2)</th>
<th>NaCuMo</th>
<th>CuNH(_4)MoS(_4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>410(w)</td>
<td>410(w)</td>
<td>410(w)</td>
<td>405(w)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>460(sh)</td>
<td>455(sh)</td>
<td>455(sh)</td>
<td>450(sh)</td>
<td>460(sh)</td>
<td></td>
</tr>
<tr>
<td>490(w)</td>
<td>490(w)</td>
<td>495(w)</td>
<td>490(w)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>620(w)</td>
<td>620(w)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>910(m)</td>
<td>910(m)</td>
<td>910(m)</td>
<td>905(m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1010(vw)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1120(w)</td>
<td>1110(m)</td>
<td>1110(m)</td>
<td>1120(m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1400(m)</td>
<td>1400(m)</td>
<td>1400(w)</td>
<td></td>
<td>1390(m)</td>
<td></td>
</tr>
<tr>
<td>1605(w)</td>
<td>1610(w)</td>
<td>1615(w)</td>
<td>1605(m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3150(w)</td>
<td>3150(w)</td>
<td>3200(w)</td>
<td></td>
<td>3100(w)</td>
<td></td>
</tr>
<tr>
<td>3500(w)</td>
<td>3500(w)</td>
<td>3500(w)</td>
<td>3500(w)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Table 4(v) Infra-red spectral data for Mo-O containing systems.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\nu$(MoS) /cm$^{-1}$</th>
<th>$\nu$(MoO) /cm$^{-1}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>(NH$_4$)$<em>2$Mo$</em>{VI}$O$_3$S$_3$</td>
<td>485</td>
<td>835</td>
<td>73</td>
</tr>
<tr>
<td>(NH$_4$)$<em>2$Mo$</em>{VI}$O$_2$S$_2$</td>
<td>490</td>
<td>830, 792</td>
<td>73</td>
</tr>
<tr>
<td>(NEt$<em>4$)[Mo$</em>{VI}$O(SPh$_4$)]</td>
<td>934$^a$</td>
<td></td>
<td>132</td>
</tr>
<tr>
<td>(AsPh$<em>4$)[Mo$</em>{VI}$O(SPh$_4$)]</td>
<td>940$^a$</td>
<td></td>
<td>132</td>
</tr>
<tr>
<td>(NEt$<em>4$)[Mo$</em>{VI}$O(Sp-MeC$_6$H$_4$)$_4$]</td>
<td>932$^a$</td>
<td></td>
<td>132</td>
</tr>
<tr>
<td>[Cu$_3$(PPh$_3$)$<em>3$ClMo$</em>{VI}$O$_3$S$_3$]</td>
<td>451, 444</td>
<td>923, 908</td>
<td>65</td>
</tr>
<tr>
<td>[Cu$_3$(AsPh$_3$)$<em>3$ClMo$</em>{VI}$O$_3$S$_3$]</td>
<td>910</td>
<td></td>
<td>65</td>
</tr>
<tr>
<td>[NEt$_4$]$<em>2$Mo$</em>{V}$O$_2$(μS$_2$)</td>
<td>473</td>
<td>947</td>
<td>133</td>
</tr>
<tr>
<td>-[(S$_2$C$_2$(CO$_2$CH$_3$)$_2$)$_2$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[NEt$_4$]$<em>2$[Mo$</em>{V}$O$_2$S$_2$(S$_2$)$_2$]$_b$</td>
<td>460</td>
<td>950</td>
<td>81</td>
</tr>
<tr>
<td>K$<em>2$[Mo$</em>{IV}$O(C$_2$H$_4$(S$_2$)$_2$)$_2$]$_2$EtOH</td>
<td>355</td>
<td>918s</td>
<td>134</td>
</tr>
<tr>
<td>K$<em>2$[Mo$</em>{IV}$O(MeC$_6$H$_3$(S$_2$)$_2$)$_3$]$_3$EtOH</td>
<td>350</td>
<td>903s</td>
<td>134</td>
</tr>
</tbody>
</table>

$^a$, recorded as nujol mull; $^b$, $\nu$(S-S) 510 cm$^{-1}$.  

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Figure 4 (iv) UV/VIS spectra of CuNH₄MoS₄(Ⅰ) and CuMo(Ⅰ)(Ⅱ) in DMF.
440°C, a weight loss of ca. 25% was observed in this study. The powder patterns of the products, after heating to 1000°C, showed some resemblance to that of the non-stoichiometric compound Cu$_{3.85}$Mo$_{3012}$ of Katz et al [135], table 4(vi). However, the weight losses recorded are too large for this to be the sole product in these studies.

The X-ray Photoelectron spectrum for CuMo(1.5) is given in figure 4(v). The results obtained for a number of the 'copper tetrathiomolybdates' are given in table 4(vii). The spectra show the presence of Cu(I) on the surface, as indicated by the lack of satellite lines. The latter arise from a "shake up" transition as a result of an inner-shell vacancy induced by photoionization [136], and are characteristic of Cu in oxidation state II. The Mo binding energy was the same as that for the Mo(V) dimer reported by Rittner et al [81], this contains a \{MoOS$_4$\} centre. Oxygen was also observed to be present to an extent greater than expected from surface contamination. From the single peaks obtained it appears that Mo, S and O are present in a single chemical state, although it is not clear what oxidation state the atoms are in. The spectrum of CuMo(1.5) shows considerable electron delocalization.

ESR signals were not observed in dilute solutions of 'copper tetrathiomolybdates' in DMF, however, signals were
Table 4(vi) XRD results of the residues from heating 'copper tetrathiomolybdates', d-values 10⁻¹⁰ m (I/I₀).

<table>
<thead>
<tr>
<th></th>
<th>CuMo(1)</th>
<th>CuMo(1.5)</th>
<th>CuMo(2)</th>
<th>CuNH₄MoS₄</th>
<th>Cu₃₈₅Mo₃O₁₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>d-values 10⁻¹⁰m</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>8.49(15)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.27(20)</td>
<td>7.25(15)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.05(12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>3.83(10)</td>
<td>3.71(10)</td>
<td></td>
<td></td>
<td></td>
<td>3.64(w)</td>
</tr>
<tr>
<td>3.59(15)</td>
<td>3.61(w)</td>
<td></td>
<td></td>
<td></td>
<td>3.51(10)</td>
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<td>3.42(100)</td>
<td>3.41(100)</td>
<td>3.40(100)</td>
<td>3.41(100)</td>
<td>3.42(vs)</td>
<td>3.32(60)</td>
</tr>
<tr>
<td>3.32(60)</td>
<td>3.31(70)</td>
<td>3.31(70)</td>
<td>3.34(20)</td>
<td>3.34(w)</td>
<td>3.31(70)</td>
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<td>3.08(20)</td>
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<tr>
<td>2.65(40)</td>
<td>2.64(60)</td>
<td>2.63(65)</td>
<td>2.64(50)</td>
<td>2.65(vs)</td>
<td>2.65(40)</td>
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<td>2.51(50)</td>
<td>2.51(60)</td>
<td>2.52(30)</td>
<td>2.53(w)</td>
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</tr>
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<td></td>
<td></td>
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<td>2.51(s)</td>
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<td>2.45(50)</td>
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<td>2.47(w)</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td>1.72(20)</td>
</tr>
</tbody>
</table>
Figure 4(v) XPS spectrum of CuMo(1:5).

(Binding Energy (BE) = $h\nu(A1K_{\alpha}, 1486.8$ eV) - Kinetic energy (kE))
Table 4(vii) Results obtained from the X-ray Photoelectron Spectroscopy study of 'copper tetrathiomolybdates'; binding energies (eV); resolution ± 0.2 eV.

<table>
<thead>
<tr>
<th>Sample</th>
<th>S(2p)</th>
<th>Mo(3d$_{5/2}$)</th>
<th>N(1s)</th>
<th>Cu(2p$_{3/2}$)</th>
</tr>
</thead>
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<tr>
<td>$(\text{NH}_4)_2\text{Mo}^{VI}\text{S}_4$</td>
<td>162.3</td>
<td>229.9</td>
<td>402.1</td>
<td></td>
</tr>
<tr>
<td>Cu$^+$(NH$_4$)Mo$^{VI}\text{S}_4$</td>
<td>161.6</td>
<td>229.7</td>
<td>402.0</td>
<td>932.5</td>
</tr>
<tr>
<td>CuMo(1.5)$^d$</td>
<td>163.2</td>
<td>230.5</td>
<td></td>
<td>934.4</td>
</tr>
<tr>
<td>CuMo(1.5)$^e$</td>
<td>163.1</td>
<td>230.5</td>
<td></td>
<td>934.5</td>
</tr>
<tr>
<td>$[\text{Mo}_2\text{O}_2\text{S}_2(\text{S}_2)^2^-]$</td>
<td></td>
<td>230.5$^a$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cs$_2$Mo$^{VI}\text{S}_4$</td>
<td>164.5$^b$</td>
<td></td>
<td></td>
<td>934.1$^c$</td>
</tr>
<tr>
<td>Cu$^+$CN</td>
<td></td>
<td></td>
<td></td>
<td>934.1$^c$</td>
</tr>
</tbody>
</table>

$^a$from reference 81; $^b$from reference 137; $^c$from reference 138; $^d,e$ samples prepared at different times.

$^f$Binding energies with respect to C(1s); 285.0 eV.
obtained for the solids at liquid nitrogen temperature. The spectra obtained are presented in figure 4(vi) to 4(ix). Both $^{95}$Mo and $^{97}$Mo have nuclear spin $I=5/2$ and their natural abundances are 15.8% and 9.6% respectively. Isotopic, fluid, spectra would generate an ESR signal consisting of a large central line of $I=0$, ca 75%, and six smaller lines due to $^{95}$Mo and $^{97}$Mo, if Mo(V) was present. However, although attempts were made to dissolve the 'copper tetrathiomolybdates', this approach was unsuccessful. Spectra were therefore recorded with the powders, producing anisotropic spectra. For a species with non-axial symmetry three principal $g$ values, $g_x$, $g_y$ and $g_z$ would be expected. The hyperfine due to $I=5/2$ would lead to a line broadening of features for these magnetically concentrated solids rather than well resolved spectra. The NaCuMo spectrum is the better resolved, and the parameters $g_x$, $g_y$ and $g_z$ are more easily obtained. The values obtained for each of these samples are given in table 4(viii). If the samples were not thoroughly washed an ESR signal due to a Cu(II) species was occasionally observed, figure 4(viii). The hyperfine coupling constant, $A_H$ (Cu), and $g_H$ (Cu), are consistent with a tetrahedrally coordinated Cu(II) species [139]. The signal due to Mo(V) was found to be due to only one type of paramagnetic species by power saturating the sample. Attempts to obtain ESR signals from the dimeric Mo(V) compounds, $\text{[Mo}_2^{\text{V}}\text{O}_2\text{S}_2(\text{S}_2)_2]^{2-}$ and $\text{[Mo}_2^{\text{V}}\text{S}_4(\text{S}_2)_2]^{2-}$, were unsuccessful as
Figure 4(vi) ESR spectrum of solid CuMo(1); recorded at 120K.
Figure 4 (vii) ESR spectrum recorded of solid CuMo(1.5); recorded at 120K.
Figure 4(viii) ESR spectrum of solid CuMo(2), recorded at 120K.
Figure 4 (ix) ESR spectrum of solid NaCuMo, recorded at 120K.
Table 4(viii) ESR parameters for the 'copper tetrathiomolybdates'.

<table>
<thead>
<tr>
<th>'Copper Tetrathiomolybdate'</th>
<th>$g_x$</th>
<th>$g_y$</th>
<th>$g_z$</th>
<th>$g_{zz}(Cu)$</th>
<th>$A_{zz}(Cu)$ (Gauss)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CuMo(1)</td>
<td>2.03</td>
<td>2.01</td>
<td>1.98</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CuMo(1.5)</td>
<td>2.03</td>
<td>2.01</td>
<td>1.90</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CuMo(2)</td>
<td>*</td>
<td>2.01</td>
<td>1.99</td>
<td>2.41</td>
<td>128</td>
</tr>
<tr>
<td>NaCuMo</td>
<td>2.03</td>
<td>2.01</td>
<td>1.95</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* not well resolved.
expected from two Mo(V) centres undergoing electron exchange.

4.5 - The Copper(Albumin)-Tetrathiomolybdate Interaction

4.5 (i) - Experimental

The reagents used are as described in sections 4.2 and 3.2. All the experiments were performed in 0.1 mol dm\(^{-3}\) potassium dihydrogen orthophosphate buffer, pH 7.4, 0.15 mol dm\(^{-3}\) sodium chloride. Electron Spin Resonance spectra were obtained by freezing solutions in liquid nitrogen. Spectra were also obtained of the product by precipitating the material using trichloroacetic acid, TCA, and recording the spectra of the resultant powder. The UV/VIS experiments were performed at 25°C in a thermostated cell block. To obtain TCA-insoluble material a 10% TCA (BDH, General Purpose) solution was added to the albumin mixtures in a 1:1 volume-ratio. The precipitated material was further washed with 5% TCA after separation by centrifugation.

4.5 (ii) - Results and Discussion

Addition of MoS\(_4^{2-}\) to albumin resulted in accelerated hydrolysis, such that after ca 24 hours only 20% of the original tetrathiomolybdate species was left. Under the same conditions in the absence of albumin aqueous solutions of ammonium tetrathiomolybdate were stable for up to 1 day.
at pH ca 7.0.

Addition of MoS$_4^{2-}$ to Cu(II)BSA solutions resulted in the reduction of tetrathiomolybdate absorbances at 466 and 316 nm, and the appearance of new peaks at 500 and 350 nm; figure 4(x). Very little change was observed after ca 24 hours. Several systems were examined in which the Cu(II) concentrations were varied, the final spectra obtained after 24 hours are given in figure 4(xi). The final spectra show the development of the new absorbance at 500 nm, although this is less pronounced at a Cu(II) : MoS$_4^{2-}$ 0.5:1 ratio. Similar results were obtained at elevated Cu(II) levels, ca 5 x 10$^{-4}$ moldm$^{-3}$, figure 4(xii). Again, a shift was observed in the thiomolybdate absorbance from 466 to 500 nm. The final spectra for a number of systems are given in figure 4(xiii). The magnitude of the final absorbance at 500 nm appears to be proportional to the initial concentration of MoS$_4^{2-}$.

ESR spectra were obtained of the Cu(II)BSA-MoS$_4^{2-}$ systems at various stages of the reaction. The relative intensities of the Cu(II) signal are given in table 4(ix). Although it was very difficult to obtain accurate intensity measurements, even though the most stringent conditions were employed, it is clear from these results that after 24 hours at a Cu:Mo ratio of 1:1 no signal is observed, but
Figure 4(x) The Cu(II)BSA-MoS$_4^{2-}$ interaction:

Initial concentration of Cu(II) = MoS$_4^{2-}$ = 1 x 10$^{-4}$ moldm$^{-3}$; concentration of BSA, 5.8 x 10$^{-4}$ moldm$^{-3}$.

Spectra recorded at 1 min, 11 mins; 1 hour; 24 hours.
Figure 4 (xi) The Cu(II)BSA-MoS$_4^{2-}$ interaction; spectra recorded after ca 24 hours.

Initial concentration of BSA, $5.8 \times 10^{-4}$ mol dm$^{-3}$; MoS$_4^{2-}$, $1 \times 10^{-4}$ mol dm$^{-3}$.

MoS$_4^{2-}$ : Cu ; 1:0.5 ; 1:1 ; 1:1.5 ; 1:2
Figure 4(xii) The Cu(II)BSA-MoS$_4^{2-}$ interaction;
Concentration of Cu(II) = MoS$_4^{2-}$ = 5 x 10$^{-4}$ mol dm$^{-3}$;
BSA, 5.8 x 10$^{-4}$ mol dm$^{-3}$.

Spectra recorded at 1 min, I; 6 min, II; 11 min, III;
1 hour, IV; 3 hours, V; 5 hours, VI; 96 hours, VII.
Figure 4 (xiii) The Cu(II)BSA-MoS$_4^{2-}$ interaction; spectra recorded after ca 96 hours.

Concentration of Cu(II), $5 \times 10^{-4}$ moldm$^{-3}$; BSA $5.8 \times 10^{-4}$ moldm$^{-3}$.

Cu(II) : MoS$_4^{2-}$, 1:0.2 (I); 1:0.4 (II); 1:0.6 (III); 1:0.8 (IV); 1:1.0 (V); 1:1.2 (VI); 1:1.4 (VII); 1:1.6 (VIII).
Table 4(ix) The Cu(II)BSA-MoS$_4^{2-}$ interaction; relative Cu(II) concentration determined by ESR.

<table>
<thead>
<tr>
<th>Time/hours</th>
<th>Initial concentration of Cu(II)BSA ($\times 10^4$ mol dm$^{-3}$)</th>
<th>Initial concentration of MoS$_4^{2-}$ ($\times 10^4$ mol dm$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>80</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>24</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>25</td>
<td>20</td>
</tr>
</tbody>
</table>


at a Cu:Mo ratio of 1:0.6 a signal is still present. This suggests that the Cu(II)BSA : MoS$_4^{2-}$ stoichiometry is 1:1. No insoluble product is formed. The ESR spectra of the TCA precipitated solids gave very weak signals at ca $g=2$, which may be due to a Mo(V) species. However, this was not highly reproducible.

4.6 Electrochemical Reductions of Tetrathiomolybdate

4.6 (i) Experimental

The apparatus was used as described in 2.8. All measurements were made in ca 0.1 moldm$^{-3}$ solutions of tetrabutylammonium tetrafluoroborate (Sigma, Laboratory Reagent) in de-aerated solvents, using a vitreous carbon, or platinum disc, working electrode, and a platinum wire auxiliary electrode. Measurements were referenced with respect to a standard calomel electrode (SCE), as well as to an internal ferrocene-ferricinium reaction couple. Solution concentrations were ca $5 \times 10^{-3}$ moldm$^{-3}$. All solvents were thoroughly dried and distilled prior to use. Dimethylformamide (DMF), and acetonitrile (MeCN), were obtained from BDH (General purpose); tetraethylammonium tetrathiomolybdate was prepared by the method outlined by McDonald et al [73].
Initial experiments involved a platinum working electrode. The Cyclic Voltammogram of \((\text{NET}_4)_2\text{MoS}_4\), figure 4(xiv), shows an irreversible electron reduction at -2.46V versus SCE, however, when a vitreous carbon electrode is used a reversible one electron reduction is observed at -2.50V, figure 4(xv). This difference is probably due to further reactions occurring at the Pt electrode surface, which would not occur at the inert carbon electrode surface. The results obtained from these experiments are given in table 4(x). With acetonitrile as solvent a single reduction was again observed. A number of smaller irreversible reductions were also observed which may be due to solvent or sulphur reduction/oxidation reactions.

Cyclic Voltammograms of trithiomolybdate, and tetrathiotungstate recorded over the same range did not show any reductions. Other groups have observed that on increasing oxy-substitution of Mo-S systems leads to the cathodic reduction potential being reduced [125,140].

4.7 Conclusions

Mixing of solutions of Cu(II) and MoS\(_4^{2-}\) leads to the immediate precipitation of red-brown/black solids. Complete reaction was found to occur at a 1.5:1 Cu:Mo
Figure 4(xiv) Cyclic Voltammograms of (NEt₄)₂MoS₄ and Ferrocene in solution of 0.1 mol dm⁻³ [NBu₄]BF₄ in DMF at room temperature, at a vitreous carbon electrode; scan rate 100 mv/sec ((NEt₄)₂MoS₄), 300 mv/sec (Ferrocene).
Figure 4(xv) Cyclic Voltammogram of \((\text{NET}_4)_2\text{MoS}_4\) in solution of 0.1 moldm\(^{-3}\) \([\text{NBu}_4]\text{BF}_4\) in DMF at room temperature, at a platinum disc electrode; scan rate 100 mV/sec.
<table>
<thead>
<tr>
<th>Solvent</th>
<th>$E^\circ$(reversible)$^c$ /V$^d$</th>
<th>Irreversible waves $E_c$(redn).</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMF$^a$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMF$^b$</td>
<td>-2.50</td>
<td></td>
</tr>
<tr>
<td>MeCN$^a$</td>
<td></td>
<td>-2.55</td>
</tr>
</tbody>
</table>

$^a$ platinum disc electrode

$^b$ vitreous carbon disc electrode

$^c$ $E^\circ = (E_c + E_a)/2$; $E_c$ = potential on cathodic sweep;
   $E_a$ = potential on anodic sweep.

$^d$ versus SCE; for ferrocene - ferricinium subtract 0.48V
ratio, although the solid products only contain 1:1 Cu:Mo.
Formation of the solids is near-stoichiometric.

Addition of Cu(II) to (NH₄)₂MoS₄ at a 1:1 ratio gives
the known compound Cu⁺NH₄⁺MoVİS₄ and a second compound.
This latter compound is more prevalent at higher Cu:Mo
ratios were the amount of Cu⁺(M⁺)MoVİS₄ decreases
(M⁺=NH₄⁺, Na⁺, NEt₄⁺). The second compound appears to have
the composition Cu⁺MoVS₄Oₓ (x=2-3), where oxygen is
determined to be present from the analytical differences
from 100% composition. It is uncertain as to whether this
product contains small amounts of other monovalent cations
such as NH₄⁺. The environment about the Mo(V) centre, from
XPS results, is very similar to that surrounding the Mo
atom in [Mo₂V₂O₂(S₂)₂]²⁻ [81], i.e. an {MoVOS₄} type
centre. Reduction of Mo(VI) to Mo(V) occurred readily on
addition of Cu(II), however, the electrochemical studies on
MoS₄²⁻ showed that only at large cathodic potentials was
any reduction of the anion observed. This makes the
reduction by Cu(II) a very surprising step. Earlier,
Clarke and Laurie had suggested that the reaction of Cu(II)
and MoS₄²⁻ in aqueous solutions leads to the formation of
Cu₂⁺MoVİS₄ [131]. However, their reactions reflected a 1:1
Cu:Mo stoichiometry, which was clearly not evident in the
present study. Their erroneous conclusion is due to poor
spectra resulting from the presence of a fine suspension in
their solutions (evident in figure 1 of Clarke and Laurie's
A large number of compounds have been reported as models for the Cu-Mo antagonism [65,78], see Introductory Chapter, all of these contain Cu(I)-Mo(VI) states. The results of this work clearly suggest that the formation of a Cu(I)-Mo(V) product may be more significant.

In this reaction both Cu(II) and Mo(VI) are reduced, however, it is not clear what has been oxidized. It is known that metal-sulphur compounds can undergo reaction with both nucleophiles (Nu) or electrophiles (E); e.g.

\[
\text{Mo}^r - \text{S} + \text{Nu} \rightarrow \text{Mo}^{r-2} + \text{NuS} \quad 4(i)
\]

\[
\text{Mo}^r - \text{S} + \text{E} \rightarrow \text{Mo}^r + \text{ES}^2- \quad 4(ii)
\]

either a two electron reduction of the Mo centre, or the abstraction of \(S^2-\) with no net change in charge of the Mo centre results. An intramolecular redox process may also occur:

\[
\text{Mo}^r (S^2-) \rightarrow \text{Mo}^{r-2}(S^0) \quad 4(iii)
\]

\[
\text{Mo}^r (S^2-)_2 \rightarrow \text{Mo}^{r-2}(S_2^2-)
\]

The Mo(V) dimer prepared by Stiefele and co-workers [80],
\( \text{Mo}_2\text{S}_8^{2-} \), see structure figure 1(xiii) was prepared by the addition of an external oxidant to \( \text{MoS}_4^{2-} \) inducing a four-electron oxidation, i.e. conversion of four \( S^2^- \) ligands to two \( S_2^{2-} \) ligands. This induced internal redox mechanism also explains the formation of \([\text{Mo}^{\text{IV}}(\text{S}_4)^2]^{2-}\), from the reaction of \( \text{Mo}^{\text{VI}}\text{S}_4^{2-} \) with \( S_8 \) [141]. The Mo(IV) product results from an internal electron transfer from \( S^2^- \) to Mo(VI) induced by \( S_8 \). Sulphur, like molybdenum, may exist in a number of oxidation states; sulphide may be oxidized according to the following:

\[
\begin{align*}
8S^{2-} & \overset{8e^-}{\leftrightarrow} 4S_2^{2-} & 4e^- \\
& \overset{4e^-}{\leftrightarrow} 2S_4^{2-} & \overset{4e^-}{\leftrightarrow} S_8
\end{align*}
\]

Muller et al [142] have examined the \( \text{M}^{2+}/\text{M}'\text{S}_4^{2-}/\text{H}_2\text{O} \) systems (\( \text{M}=\text{Fe}, \text{Co}, \text{Ni}, \text{Pd}, \text{Pt}, \text{Zn}, \text{Cd}; \text{M}'=\text{Mo}, \text{W} \)). In aqueous solutions products were of the type \([\text{M}(\text{S}_4)^2]^{2-}\), no net change in the oxidation states of the metal or sulphur centres were observed. As described in the Introductory Chapter, Christou et al [90] have prepared a product containing Fe(II) and Mo(V), \((R'_4\text{N})_3[\text{Fe}_6\text{Mo}_2\text{S}_8(\text{SR})_9]\); starting from \((\text{NH}_4)_2\text{Mo}^{\text{VI}}\text{S}_4 \) and Fe(III)(SR)_3 (abbreviations are described in Chapter 1). Therefore, the reduction of both Cu(II) and Mo(V) centres has some precedents. The work reported here shows reduction occurring at both metal centres, however, the results of these experiments do not give any indication of the oxidation state of the sulphur atoms in the product.
It is probable that sulphides have been oxidized to disulphide, thus:

\[

cu(II) + e^- \rightarrow cu(I) \\
\text{Mo(VI)} + e^- \rightarrow \text{Mo(V)} \\
2S^{2-} \rightarrow S_2^{2-} + 2e^- \\
\]

Scheme 4(vi)

Unfortunately, the role of oxygen is not clearly delineated, and if the stoichiometry is \( \text{Cu}^{I}\text{Mo}^{V}\text{S}_2(\text{S}_2)\text{O} \), then there is an excess of anionic charge. Both elemental analyses and TGA rule out oxygen being present as \( \text{H}_2\text{O} \) or \( \text{OH}^- \).

Addition of \( \text{MoS}_4^{2-} \) to \( \text{Cu(II)}\text{BSA} \) at the \( 10^{-4} \) moldm\(^{-3} \) level is seen to result in the slow reduction of \( \text{Cu(II)} \); reaction is complete after 24 hours. At the same levels the UV/VIS spectrum of \( \text{MoS}_4^{2-} \) changes; initially \( \text{MoS}_4^{2-} \) maxima are present at 316 and 466nm, after 24 hours an absorbance maximum is present at 500nm, and a shoulder at \( \text{ca} 340 \text{nm} \). In contrast to reactions with \( \text{Cu(II)}\text{SO}_4\text{5H}_2\text{O} \) no precipitate was observed.

ESR spectra of the TCA-precipitated \{\( \text{Cu(BSA)}-\text{MoS}_4^{2-} \)\} product only exhibited a very weak, and non-reproducible signal. It was not clear whether this was a residual
Cu(II) signal or due to Mo(V). TCA-insoluble Cu is often used as a measure of plasma Cu distribution in vivo.

The accelerated hydrolysis of Mo$S_4^{2-}$ by albumin was a competing reaction in these experiments, and consequently it was not possible to determine from the UV/VIS experiments the stoichiometric ratio. The final UV/VIS spectra, figure 4(xi), shows a similarity to that obtained for the CuMo(1) product examined in 4.4.

The results obtained here clearly provide evidence for an (Albumin-)Cu$^I$-Mo-S complex, the reaction shows similarities to those reported for the 'copper tetrathiomolybdates' discussed earlier in this section.

The thiomolybdates, but not molybdate, when added to the diet of rats, results in the appearance in plasma of a Cu and Mo-containing fraction [60]. It has been suggested [123] that the formation of a Cu-Mo-S aggregate with albumin bound Cu, leads to a reduction in absorption of Cu. Mason and co-workers [63] have also suggested that the Cu and thiomolybdates that form in the rumen co-accumulate on albumin, but do not undergo any irreversible chemical interaction. The proportion of plasma Cu associated with albumin was found to increase. The results obtained from the present study are in agreement with these observations. It is evident that a Cu(I)-Mo-S(-protein) product may
account for some of the reduced absorption of Cu in the diet of rats (monogastrics) or ruminants, supplemented with tetrathiomolybdate.

Future investigations should be aimed at a study of the EXAFS spectrum of the 'copper tetrathiomolybdates'; EXAFS would give an insight into the environment about the metal centres in greater detail, together with oxidation states and bond lengths. The monoatomic nature of the sulphur ligands makes this a particularly suitable compound for study by EXAFS. However, this technique is not readily applicable to mixtures, impurities may lead to problems in interpretation of the results.
CHAPTER 5

NATURE OF THE REACTION BETWEEN COPPER(II) AND
THIOTUNGSTATES, AND THE PRODUCTS FORMED

5.1 Introduction

Several reviews have appeared which show that the thiotungstate salts undergo similar reactions to their thiomolybdate analogues [65,69,70]. Indeed, it has been shown that the supplementation of rat diets with ammonium tetrathiotungstate induces similar systemic changes in copper distribution to those which occur in the tetrathiomolybdate supplemented rat [58,61]. Tungstate, WO$_4^{2-}$, was not found to exhibit any inhibitory effect.

It was intended, in the work described here, to investigate the products formed by the interaction between WS$_4^{2-}$ and Cu(II), and the Cu(II)BSA 1:1 complex, i.e. the analogous reactions to those examined in Chapter 4.

5.2 Preparation of Tetrathiotungstate Salts

Ammonium tetrathiotungstate was prepared by the method of Mellor [72]. Tungstic acid was prepared by the addition of HCl(conc) to a saturated solution of sodium tungstate.
Tungstic acid (10g) was dissolved in conc. ammonium hydroxide (100cm$^3$) and distilled water (20cm$^3$). Hydrogen sulphide was bubbled through the solution, maintained at ca 55°C, until after approximately 6 hours the solution spectrum was that expected for WS$_4^{2-}$ ion (absorption maxima at 391 and 276nm [73]).

Crystals of the product precipitated out after 2-3 days; these were isolated by filtration, washed with cold water and ethanol, and finally dried in a dessicator under vacuum.

Tetraethylammonium tetrathiotungstate was prepared by the method of McDonald et al. [73]. Ammonium tetrathiotungstate (3.5g) was dissolved in 10% tetraethylammonium hydroxide in distilled water (28cm$^3$) and 30cm$^3$ of additional water. The reaction mixture was subjected to pumping for 2 hours to remove the ammonia, gentle warming was applied if necessary. The product was isolated by filtration after addition to propan-1-ol, washed with propan-1-ol and diethyl ether, and finally dried in a vacuum dessicator.

5.3 Determination of the Stoichiometry of the Reaction Between Cu(II) and WS$_4^{2-}$

On mixing solutions of ammonium tetrathiotungstate (or
tetraethylammonium tetrathiotungstate) with Cu(II) or Cu(II)(Gly)_2 a reduction occurred in the WS_4^{2-} absorption as shown in figure 5(i). The changes were similar to those observed for MoS_4^{2-}. In the WS_4^{2-} reactions some precipitation was observed, this was much finer when the ammonium salt was used rather than the tetraethylammonium salt. Complete reduction of the WS_4^{2-} absorption at 391 and 276 nm was observed at a 1.5:1 Cu(II) : WS_4^{2-} level. All reactions were performed at pH 7.4, with, and without, 0.15 mol dm^{-3} sodium chloride as the background electrolyte. These observations are in agreement with the results obtained in 4.3 for MoS_4^{2-}.

5.4 Preparation and Characterization of 'Copper Tetrathiotungstates'

5.4 (i) Experimental

'Copper tetrathiotungstates' were freshly prepared by the addition of solutions of CuSO_4 with either (NH_4)_2WS_4 or (NEt_4)_2WS_4. Samples were separated by filtration, the orange/brown solids were washed with ethanol and diethyl ether, and finally dried in either a warm oven or in a vacuum dessicator. Samples were prepared at Cu(II) : WS_4^{2-} ratios of 1:1, 1.5:1 and 2:1.

Elemental analyses were performed as described in 4.4(i). The products were characterized by a variety of
Figure 5(i) UV/VIS spectra of mixtures of $(\text{NEt}_4)_2\text{WS}_4$, $1 \times 10^{-4}$ moldm$^{-3}$, and Cu(II)(Gly)$_2$ \n
$\text{WS}_4^{2-}$ : Cu(II) $1 : 0$ (I); $1 : 1$ (II); $1 : 1.2$ (III); $1 : 1.5$ (IV); $1 : 1.8$ (V); $1 : 2.5$ (VI).
spectroscopic techniques; Ultraviolet-visible Spectroscopy; Electron Spin Resonance Spectroscopy; Infra-red Spectroscopy; Thermogravimetric Analysis and X-ray Diffraction; as outlined in Chapter 2.

5.4 (ii) Results and Discussion

The elemental analyses obtained for the 'copper tetrathiotungstates' are given in table 5(i). The products were found to be composed principally of Cu, W and S, although evidence was also present to suggest the presence of small amounts of other cations, i.e. NH\textsubscript{4}\textsuperscript{+} or NEt\textsubscript{4}\textsuperscript{+} in the products. No evidence was obtained, from the analyses, for oxygen in the samples. The analyses would suggest the presence of \{WS\textsubscript{4}\} units in the products.

The results of the X-ray Powder Diffraction measurements are given in table 5(ii). The CuW(1) product is seen to have a component isostructural with the known CuNH\textsubscript{4}WS\textsubscript{4}, and to exhibit lines which are found in the CuW(1.5) and CuW(2) products. CuW(1.5) and CuW(2) showed very close resemblance, although an additional weak feature is present in CuW(1.5). NEtCuW was found to exhibit a totally different pattern, which may be due to the influence of the larger cation distorting the structure. Except for CuW(1) and CuNH\textsubscript{4}WS\textsubscript{4}, all lines were diffuse owing to the amorphous nature of the products.
Table 5(1) Elemental analyses of the copper tetraphthiotungstate solids.

- obtained by AA;
- analyses performed at Manchester University;
- estimated from differences in percentage composition;
- not analysed;
- greater than 100%; values in parenthesis are calculated values.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Cu</th>
<th>W</th>
<th>S</th>
<th>C</th>
<th>H</th>
<th>N</th>
<th>O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(II):(NH₄)₂WS₄</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu₇W(1)</td>
<td>1:1</td>
<td>22.6ᵃ</td>
<td>43.7ᵇ</td>
<td>27.7ᵇ</td>
<td>0.0ᵇ</td>
<td>1.6ᵇ</td>
<td>4.1ᵇ</td>
</tr>
<tr>
<td>Cu₇W(1.5)</td>
<td>1.5:1</td>
<td>24.1ᵃ</td>
<td>39.3ᵇ</td>
<td>30.7ᵇ</td>
<td>d</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>Cu₇W(2)</td>
<td>2:1</td>
<td>25.3ᵃ</td>
<td>d</td>
<td>d</td>
<td>d</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>Cu(II):(NET₄)₂WS₄</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NETCu₇</td>
<td>1.5:1</td>
<td>11.9ᵃ</td>
<td>40.9ᵇ</td>
<td>27.6ᵇ</td>
<td>17.0ᵇ</td>
<td>3.65ᵇ</td>
<td>2.3ᵇ</td>
</tr>
<tr>
<td>(NH₄)₂WS₄</td>
<td></td>
<td>52.00ᵇ</td>
<td>37.20ᵇ</td>
<td>0.00ᵇ</td>
<td>2.25ᵇ</td>
<td>8.00ᵇ</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(52.87)</td>
<td>(36.78)</td>
<td>(2.30)</td>
<td>(8.05)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5(ii) XRD results for the 'copper tetrathiotungstates', 
d-values, \(10^{-10} \text{m} \ (I/I_0)\); s = sharp, d = diffuse.

<table>
<thead>
<tr>
<th>CuW(1)</th>
<th>CuW(1.5)</th>
<th>CuW(2)</th>
<th>CuNH₄WS₄</th>
<th>NEtCuW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>[77]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.81(100d)</td>
</tr>
<tr>
<td>5.6(100s)</td>
<td>5.6(100s)</td>
<td>5.3(100d)</td>
<td>5.3(100d)</td>
<td>7.49(80d)</td>
</tr>
<tr>
<td>4.3(5s)</td>
<td>4.34(2d)</td>
<td>4.11(3d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.0(40s)</td>
<td>3.8(15d)</td>
<td>3.8(10d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.1(5s)</td>
<td>3.1(15d)</td>
<td>3.1(10d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.00(20s)</td>
<td></td>
<td>2.99(55s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.84(15s)</td>
<td></td>
<td>2.83(35s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.65(5d)</td>
<td>2.60(1d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.55(10s)</td>
<td></td>
<td>2.54(35s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.45(20s)</td>
<td></td>
<td>2.45(70s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.40(5s)</td>
<td>2.40(30d)</td>
<td>2.40(25d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.25(18s)</td>
<td></td>
<td>2.25(70s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.00(1s)</td>
<td></td>
<td>2.00(9s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.97(10s)</td>
<td></td>
<td>1.96(35s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.90(8s)</td>
<td>1.89(30d)</td>
<td>1.89(25d)</td>
<td>1.89(20s)</td>
<td></td>
</tr>
<tr>
<td>1.86(50s)</td>
<td></td>
<td>1.86(80s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.80(10s)</td>
<td>1.80(10d)</td>
<td></td>
<td>1.79(35s)</td>
<td></td>
</tr>
<tr>
<td>1.59(8s)</td>
<td></td>
<td>1.62(30s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.58(5s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The Infrared spectrum obtained for CuW(l) is given in figure 5(ii) and the results obtained for a number of "copper tetrathiotungstates" are given in table 5(iii). The band at ca 440 cm\(^{-1}\) can be assigned to \(\nu(W-S)\); the band at ca 1400 cm\(^{-1}\) is due to \(\nu(N-H)\); the band at ca 910 cm\(^{-1}\) could be assigned to a \(\nu(W-O)\) absorption; table 5(iv). The spectra of CuW(l) was significantly different to CuW(1.5) and CuW(2); only one \(\nu(W-S)\) band was present in CuW(l) whereas three bands were present in the other products.

The "copper tetrathiotungstates" were all fairly soluble in solvents such as ethanol and DMF. The UV/VIS spectrum of CuW(l) dissolved in DMF gave absorptions at ca 422 and 316 nm; NEtCuW dissolved in DMF exhibited bands at 408 and 286 nm, figure 5(iii).

The Thermogravimetric Analyses give a fairly complex pattern of increases and decreases in weight; weight losses over the range 0 - 1,000°C were, for the CuW(l), CuW(1.5) and CuW(2), ca 35, 20 and 19% respectively.

Electron Spin Resonance Spectra were obtained for the "copper-tetrathiotungstates"; spectra were recorded of the powders at 120 K. Reproducibility was, however, very difficult to achieve. If a W(V) species is present a strong central absorption feature should be present, due to \(^{182}\text{W}, ^{184}\text{W}, \text{ and } ^{186}\text{W, } I=0 (85.7%)\), and two weaker features.
Figure 5(ii) Infra red spectrum, recorded as a KBr disc, of CuW (1).
Table 5(iii) Infra-red spectral results, recorded as KBr discs, for 'copper tetrathiotungstates' (cm$^{-1}$).

<table>
<thead>
<tr>
<th></th>
<th>CuW(1)</th>
<th>CuW(1.5)</th>
<th>CuW(2)</th>
<th>(NH$_4$)$_2$WS$_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>410 (w)</td>
<td>410 (w)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>445 (sh)</td>
<td>440 (sh)</td>
<td>440 (sh)</td>
<td>460 (sh)</td>
<td></td>
</tr>
<tr>
<td>480 (w)</td>
<td>480 (w)</td>
<td>482 (m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>618 (w)</td>
<td>615 (vw)</td>
<td></td>
<td></td>
<td>800 (w)</td>
</tr>
<tr>
<td>900 (vw)</td>
<td>915 (m)</td>
<td>910 (m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1115 (m)</td>
<td>1115 (w)</td>
<td></td>
<td></td>
<td>1210 (vw)</td>
</tr>
<tr>
<td>1400 (m)</td>
<td>1400 (m)</td>
<td>1410 (w)</td>
<td>1400 (m)</td>
<td></td>
</tr>
<tr>
<td>1600 (m)</td>
<td>1600 (w)</td>
<td>1600 (w)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3200 (w)</td>
<td>3200 (w)</td>
<td>3200 (w)</td>
<td>3200 (w)</td>
<td></td>
</tr>
<tr>
<td>3500 (w)</td>
<td>3500 (w)</td>
<td>3500 (w)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5(iv) Infra-red spectral data (cm$^{-1}$) for W-O containing systems.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\nu$(W-S)</th>
<th>$\nu$(W-O)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Cu$_3$(PPh$_3$)$_3$ClWVIOS$_3$]</td>
<td>436,430</td>
<td>928,922</td>
<td>65</td>
</tr>
<tr>
<td>[Cu$_3$(AsPh$_3$)$_3$ClWVIOS$_3$]</td>
<td></td>
<td>920</td>
<td>65</td>
</tr>
<tr>
<td>(NEt$_4$)$_2$WVIOS$_3$</td>
<td></td>
<td>885</td>
<td>73</td>
</tr>
<tr>
<td>(NEt$_4$)$_2$WVI$_2$OS$_2$</td>
<td></td>
<td>885,845</td>
<td>73</td>
</tr>
</tbody>
</table>
Figure 5(iii) UV/VIS spectra recorded for CuW(1); I and II (Gain x 0.2), and NEtCuW; III.
due to $^{183}$W, $I = \frac{1}{2}$ (14.3%), assuming an isotropic spectra was obtained. If the W(V) atom is in the centre of low symmetry where $g_x \neq g_y \neq g_z$, three strong features should be present each surrounded by two weaker features. The spectra that were obtained are given in figures 5(iv) to 5(viii). In some cases, figure 5(vi), the signals clearly show the presence of tetrahedral Cu(II) being present, $g_\| (Cu) = 2.51$, $A_\| (Cu) = 123G$. The other ESR signals cannot be attributed to any definite species, i.e. Cu(II) or W(V) etc.

5.5 The Copper(Albumin)-Tetrathiotungstate Interaction

5.5 (i) Experimental

Reagents were used as described in 3.2 and 5.2. All experiments were performed in 0.1 m$\text{oldm}^{-3}$ potassium dihydrogen orthophosphate buffer, pH 7.4, 0.15 m$\text{oldm}^{-3}$ sodium chloride. Electron Spin Resonance spectra were obtained by freezing solutions in liquid nitrogen, and of TCA-precipitated products. The UV/VIS experiments were recorded at 25°C in a thermostated cell block.

5.5 (ii) Results and Discussion

The hydrolysis of tetrathiotungstate in the presence of albumin was monitored. At pH 7.4 and 0.15 m$\text{oldm}^{-3}$ sodium chloride a 20% reduction was observed, at the
Figure 5(iv) ESR spectrum of solid CuW (1); recorded at 120K.
Figure 5(v) ESR spectrum of solid CuW (1.5), recorded at 120K.
Figure 5(vi) ESR spectrum of solid CuW (1.5); recorded at 120K.
Figure 5(vii) ESR spectrum of solid CuW (2); recorded at 120K.
Figure 5(viii) ESR spectrum of solid NEtCuW; recorded at 120K.
In the absence of albumin, solutions of tetrathiotungstate were stable for several days.

Addition of WS$_4^{2-}$ to Cu(II)BSA resulted in the reduction in absorbance at 391nm and a shift to ca 420nm after approximately 24 hours, figure 5(ix). A number of systems were examined at different Cu(II) : WS$_4^{2-}$ initial ratios. The final spectra recorded after 24 hours for these systems are given in figure 5(x). At 0.5:1, Cu(II) : WS$_4^{2-}$ level, the final spectrum shows a significant absorbance at 400nm which may be due to unreacted WS$_4^{2-}$. At higher ratios, 1:1, 1.5:1 and 2:1, the final spectra showed a maximum at ca 420nm. At the elevated levels, 5 x 10$^{-4}$ moldm$^{-3}$ WS$_4^{2-}$, the evidence was less conclusive, but would suggest that the stoichiometric ratio for complete reaction was Cu(II)BSA : WS$_4^{2-}$ 1:1, see figures 5(xi) and 5(xii).

The results of the ESR experiments are given in table 5(v). Spectra were recorded at various time intervals, the relative intensities of the Cu(II)BSA signals were calculated. The results are also consistent with the product being a 1:1 Cu(II)BSA : WS$_4^{2-}$ adduct. The TCA-precipitated protein failed to exhibit any ESR signal at 120K. This is consistent with copper being in the +1 oxidation state.
Figure 5(ix) The Cu(II)BSA-WS$_4^{2-}$ interaction;

Initial concentration of Cu(II)=WS$_4^{2-}$=1x10$^{-4}$ moldm$^{-3}$, BSA 5.8x10$^{-4}$ moldm$^{-3}$, pH 7.4, 0.15 moldm$^{-3}$ sodium chloride.

Spectra recorded at 1 min (I), 6 min (II), 11 min (III), 1 hour (IV), 3 hours (V), 5 hours (VI), 24 hours (VII).
Figure 5(x) The Cu(II)BSA-WS$_4^{2-}$ interaction; spectra recorded after ca 24 hours.

Initial concentration of BSA, 5.8x10$^{-4}$ moldm$^{-3}$; WS$_4^{2-}$, 1x10$^{-4}$ moldm$^{-3}$.

WS$_4^{2-}$ : Cu ; 1 : 0.5 (I); 1 : 1 (II);

1 : 1.5 (III); 1 : 2 (IV).
Figure 5(xi) The Cu(II)BSA-WS$_4^{2-}$ interaction.

Concentration of Cu(II)-WS$_4^{2-}$ = $5 \times 10^{-4}$ moldm$^{-3}$, BSA, $5.8 \times 10^{-4}$ moldm$^{-3}$.

Spectra recorded at 11 min, (I); 1 hour, (II); 3 hours, (III); 5 hours, (IV); 96 hours, (V).
Figure 5(ii) The Cu(II)BSA-WS$_4^{2-}$ interaction; spectra recorded after ca 24 hours.

Concentration of Cu(II), 5x10$^{-4}$ moldm$^{-3}$; BSA, 5.8x10$^{-4}$ moldm$^{-3}$. Cu(II) : WS$_4^{2-}$, 1 : 0.6 (I); 1 : 0.8 (II); 1 : 1 (III); 1 : 1.2 (IV).
Table 5(v) The Cu(II)BSA-WS$_4^{2-}$ interaction; relative Cu(II) concentrations determined by ESR.

<table>
<thead>
<tr>
<th>Time/hours</th>
<th>Initial concentration of Cu(II)BSA/10$^4$ moldm$^{-3}$</th>
<th>Initial concentration of WS$_4^{2-}$/10$^4$ moldm$^{-3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100 100 100</td>
<td>3 5 7</td>
</tr>
<tr>
<td>1</td>
<td>25 10 0</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>15 0 0</td>
<td></td>
</tr>
</tbody>
</table>
5.6 Conclusions

The similarity in chemistry of inorganic sulphur compounds of molybdenum and tungsten is now well established [69, 70]. A number of Cu-M-S (M=Mo, W) adducts have been prepared to mimic the complexes formed in the rumen as a result of the molybdenum and tungsten induced copper deficiency [65]. Coucouvanis has reviewed the chemistry of Fe-M-S complexes derived from MS₄²⁻ anions (M=Mo, W) [92]. It would therefore be expected that the products from the interaction of Cu(II) and WS₄²⁻ would exhibit similar chemistry to their MoS₄²⁻ analogues.

The stoichiometry for reaction of Cu(II) and WS₄²⁻ was found to be ca 1.5:1, determined by the total reduction in the WS₄²⁻ UV/VIS absorbance. The 'copper-tetrathiotungstates' were found to form immediately on addition of aqueous solutions of Cu(II) and WS₄²⁻. The brown products were found to be composed principally of Cu, W and S; although a significant quantity of other cations, NH₄⁺ and NEt₄⁺, were also present.

The addition of Cu(II) to (NH₄)₂WS₄ in a 1:1 ratio leads to the formation of CuNH₄WS₄ and another product designated CuW(1.5). At a 1.5:1 Cu : W ratio a little CuNH₄WS₄ is formed, together with another product which was not fully characterized, CuW(1.5). The analyses do not
suggest any oxygen is present in the samples, however, the Infra-red spectra exhibit a band at ca 900 cm\(^{-1}\) which could be attributed to \(\nu(W-O)\).

The results obtained are in agreement with the observations in Chapter 4 for the 'copper tetrathiomolybdates', although evidence for \(W(V)\) species was not conclusive, and the 'copper tetrathiotungstates' do not appear to contain any oxygen. Evidence for reduction of \(W(VI)\) is not as clear-cut as for \(Mo(VI)\). Results from the Cyclic Voltammetry experiments showed that reduction of \(WS_4^{2-}\) requires a higher electrode potential than does \(MoS_4^{2-}\), so an internal redox mechanism may not occur in the tungsten case.

It has been suggested, by Mills and co-workers [61], that (NH\(_4\))\(_2WS_4^{2-}\) supplemented rats, who consequently develop symptoms associated with copper deficiency, do so because \(WS_4^{2-}\) reacts by the same pathway as \(MoS_4^{2-}\) supplemented rats. The formation of a \(\{Cu(II)BSA-WS_4^{2-}\}\) complex may thus reduce absorption of Cu(II) from plasma.

The results of this work suggest that the reaction of Cu(II)BSA with \(WS_4^{2-}\) leads to the formation of a 1:1 adduct. Hydrolysis of \(WS_4^{2-}\) in the presence of albumin, pH 7.4, was markedly slower than for \(MoS_4^{2-}\). The observations are again in agreement with the analogous
MoS$_4^{2-}$ systems, however, the study in this chapter was less rigorous, and is less conclusive.
6.1 Introduction

The chief argument for the automation of a repetitive process is that it frees the operator from a sometimes tedious and time-consuming job, allowing him/her to pursue other tasks. For the accurate determination of stability constants, the importance of which was outlined in the Introductory Chapter, pH potentiometric titrations are usually performed. This time-consuming operation requires the collection of a large number of data points, for further analysis, and presents an ideal case for a fully computer-controlled system. Error incurred by manual operation may be reduced in significance. A computer-controlled system allows for the collection of far more data points than could be obtained manually. Commercially available semi-automatic potentiometric titration apparatus has been used by a number of operators [93,94,95]. The intention of this chapter was to describe the steps involved in interfacing a microcomputer with a semi-automatic pH titration apparatus. The objectives were as follows: to fully automate the addition of titrant to the titration vessel; to collect the results.
of addition, i.e. EMF or pH versus volume of titrant added; from the change in EMF/pH produced to allow the computer to calculate and add an appropriate amount of titrant; to collect and store as many data points as possible using a disc for future access and processing; present the operator with a visual record of the progress of the titration and of the final titration curve.

6.2 The Apparatus

6.2 (i) The Titration Assembly

The semi-automatic titration apparatus available within this school, and used in most laboratories, consisted of a piston burette (Radiometer ABU 12) capable of delivering 2.5cm³ ± 2.5μL coupled to a titration vessel (Radiometer TTA 60) where efficient mixing was obtained through a mechanical stirrer incorporated in the titration cell. A Radiometer PHM 64 pH meter was available, fitted with K4040 calomel and G2040C glass electrodes.

6.2 (ii) The Microcomputer Assembly

Available within the school are a number of Commodore-64 microcomputers with 64K RAM. The assembly consisted of the microcomputer, a Commodore 1541 disc-drive, a Commodore MPS 801 printer, and a Kaga monochrome monitor. The interface, constructed by
D.A. Armitage [143], connected the computer with the burette and pH meter, figure 6(i).

6.3 The Computer Program

The program is written in ANSI standard BASIC, using an extended interpreter known as MAT 64 [144]. The flow diagram, and the actual program are given in figure 6(ii) and list 6(i) respectively. On commencing a new titration the experiment is initialized by inputting the date, run number, nature of the reactants, and any special (experimental) conditions (program lines 90-130). At this stage the approximate total volume of titrant to be added may be input, this may be updated during the titration. This is necessary because the program will not cease at equilibration or any other end point. The option is also present to examine previously collected data stored on disc (program lines 81-86).

The computer program commences by reading the initial EMF from the pH meter (EMF was chosen rather than pH because this has been used in previous work in this school [93,94]). This is performed by the routine in program lines 61-72, which defines the procedure to transfer the digital reading from the pH meter to the computer memory, using the computer's userport. The pH meter screen is controlled by lines PA2 and FLAG, which
Figure 6(i) Hardware block diagram.
Figure 6(ii) Flow chart for the procedure outlined in the execution of the computer program.

SYSTEM INITIALIZATION
READ INITIAL EMF

IS EMF STABLE
YES
ADD TITRANT
READ EMF

IS NEW EMF STABLE
YES
CALCULATE \( \Delta E_{\text{MF}} / \Delta \text{VOL} \)
FOR NEW TITRANT ADDITION
ADD CALCULATED VOLUME OF TITRANT

IS VOLUME > 2.3cm\(^3\) OR INPUT VOLUME
YES
REFILL
NO

ABORT

CONTINUE TITRATION

DATA OUTPUT
GRAPH TABLE STORE ON DISC
List 6(i) The Computer Program

```
1 REM PPPP 0000 TTTT EEE N N TTTT 5555
2 REM P . P 0 0 TT E . NN N TT 5
3 REM PPPP 0 0 TT EEE N65N TT 5555
4 REM P 0 0 TT E N NN TT 5
5 REM P 0000 TT EEE N N TT 5555
6 REM
7 REM PROGRAM TO PERFORM POTENTIOMETRIC
8 REM TITRATIONS
9 REM.
10 PRINT"SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS
100 PRINT "INPUT THE RUN NUMBER: ": INPUT RU$
110 PRINT "INPUT THE NATURE OF THE REACTANTS: ": INPUT SY$
120 PRINT "INPUT ANY SPECIAL CONDITIONS"
130 PRINT "(BACKGROUND ELECTROLYTE ETC.)": INPUT SP$
140 PRINT "IS THE BURETTE READY?"
141 PRINT AT (5,1); "PRESS SPACE TO CONTINUE"
142 GET A$: IF A$="": THEN 155: ELSE 142
155 FOR I=1 TO S: EF; (I)=0: LO(I)=O: VO(I)=0: B(I)=0: NEXT I
160 PRINT AT (7,1); "INPUT APPROX. VOL. OF TITRE READ."": INPUT MO
170 REPEAT K=K+1
180 IF K>1 THEN GOSUB 5000
190 S=S+1
200 GOSUB 3000
230 IF K=1 THEN PRINT "THE EMF OF THE INITIAL SOLUTION: ": EK(K)
240 GOTO 340
250 PRINT "THE EMF OF THE SOLUTION IS: ": EK(K)
260 PRINT "THE PREVIOUS EMF WAS: ": EK(K-1)
270 PRINT "TOTAL VOLUME OF TITRANT ADDED IS: ": LO(K)/100
340 UNTIL K=500
2988 REM
2989 REM
2990 REM EMF INPUT ROUTINE'
X991 REM
2992 REM
3000 N=0
3010 IF N=100 THEN 3160
3020 N=N+1
3030 X=0: PRINT ""
3040 REPEAT X=X+1'
3050 E(X)=FNP'H
3055 PRINT AT (2*X+1,5); USING "E(#) _ ####. #". X, E(X)
3056 IF K=1 AND E(X)=0.0 THEN PRINT AT (15,1)"CHECK PH METRE SCALE": ELSE 3070
3070 FOR 1=1 TO 700. NEXT 1
3090 UNTIL X=5
3100 EV(N)=(E(1)+E(2)+E(3)+E(4)+E(5))/5
3110 IF N>2 THEN 3120: ELSE 3010
3120 IF EV(N)>=EV(N-1)-0.5 AND EV(N)<=EV(N-1)+0.5 THEN 3130: ELSE 3010
3130 IF EV(N)>=EV(N-2)-0.5 AND EV(N)<=EV(N-2)+0.5 THEN 3140: ELSE 3010
3140 EK(K)=EV(N)
3141 IF LO(K)<=100*(2.3+L*2.5) THEN 3142: ELSE 3146
3142 IF LO(K)<=100*MO THEN 3152: ELSE PRINT AT (15,1); "VOL. A
DO YOU WISH TO CONTINUE: YES/NO:

IF C$ = G$ THEN 3150: ELSE IF C$ = N$ THEN 6000: ELSE 3143
3145 L = L + 1
3146 PRINT AT (17, 1); "BURETTE IS VIRTUALLY EMPTY: DO YOU WISH TO PROCEED: YES/NO"
3147 INPUT L$
3148 IF L$ = G$ THEN L = L + 1: GOTO 3152
3149 IF L$ = N$ THEN 6000: ELSE 3146
3150 PRINT "INPUT NEW VOLUME TO BE ADDED": INPUT MN
3151 MO = (LO(K) / 100) + MN
3152 RETURN
3160 PRINT (15, 1); "FLUCTUATING EMF: DO YOU WISH TO PROCEED: YES/NO": INPUT H$
3170 IF H$ = G$ THEN 3000: ELSE IF H$ = N$ THEN 6000: ELSE 3160
4988 REM
4989 REM
4990 REM VOLUME ADDITION ROUTINE
4991 REM AND CALCULATION
4992 REM
4993 REM
5000 IF K = 2 THEN VO(K) = 10: GOTO 5025
5005 IF SGN(EK(K-1)) <> SGN(EK(K-2)) THEN VO(K) = 1: GOTO 5025
5010 VO(K) = INT((VO(K-1) - (100 * (EK(K-1) - EK(K-2)) / 376)) + 0.5)
5015 IF VO(K) < 1 THEN VO(K) = 1
5025 GOSUB 20000
5030 IF K = 2 THEN LO(K) = VO(K): ELSE LO(K) = VO(K) + LO(K-1)
5040 RETURN
5989 REM
5990 REM DATA OUTPUT PRESENTATION
5991 REM
5992 REM
6000 PRINT "DO YOU REQUIRE A DATA OUTPUT YES/NO": INPUT O$
6001 REM
6010 IF O$ = G$ THEN 6020: ELSE IF O$ = N$ THEN END: ELSE 6000
6020 PRINT "WHICH OF THE FOLLOWING DO YOU REQUIRE?"
6030 PRINT "GRAPH: VISUAL DISPLAY (1)"
6040 PRINT "DATA TABLE: VISUAL DISPLAY (2)"
6050 PRINT "GRAPH: HARD COPY (3)"
6060 PRINT "DATA TABLE: HARD COPY (4)"
6063 PRINT "WRITE TO FILE (5)"
6067 PRINT "READ FROM FILE (6)"
6068 PRINT "CONTINUE TITRATION (7)"
6070 PRINT "END PROG. (8)"
6080 PRINT "INPUT CODE NUMBER (1-8):": INPUT E$
6090 I = VAL(E$)
6100 ON I+1 GOTO 6020, 7000, 8000, 9000, 10000, 7500, 7700, 3150, 20
008: GOTO 6020
6989 REM
6990 REM GRAPHICAL DISPLAY OF DATA ON
6991 REM SCREEN
6992 REM
7000 GOSUB 7002: TEXT: GOTO 6020
7002 GOSUB 21000
7003 GRAPHICS CLR
7004 PRINT AT (1,1): USING 7400. SY$
7005 PRINT AT (22,1): USING 7400,"VOL./ML"
7006 A$="EMF/MV"
7007 FOR I=1 TO LEN(A$): PRINT AT(I+6,10); MID#'(A$, I, 1): NEXTI
7008 PRINT AT (3,1); LEFT$(DA$,8)
7009 PRINT AT (5,1); LEFT$(RU$,8)
7010 PRINT AT (3,33); "EMF(1)"
7011 PRINT AT (4,33); USING "###.##", EK(1)
7012 PRINT AT (6,33); USING "EMF(###)" , S
7013 PRINT AT (7,33); USING "###.##", EK(S)
7014 PRINT AT (9,33); "VOL(1)"
7015 PRINT AT (10,33); USING"#.#", (LO(1)/100)
7016 PRINT AT (12,33); USING "###", S
7017 PRINT AT (13,33); USING "#.#", (LO(S)/100)
7019 PLOT (85,40) TO (235,40) TO (235,39) TO (84,39)
7020 PLOT (84,39) TO (84,190) TO (85,190) TO (85,40)
7030 R=1
7040 PLOT (85+150*LO(R)/LO(S), 40+150*((EK(R)-LE)/(HE-LE)))
7050 REPEAT R=R+1
7060 LINE TO (85+150*LO(R)/LO(S), 40+150*((EK(R)-LE)/(HE-LE))
7070 UNTIL R=S
7080 PRINT AT (25,1): USING 7400,"PRESS SPACE TO CONTINUE"
7090 GET A$; IF A$=" " THEN 7091: ELSE 7090
7091 RETURN
7400 IMAGE: 'CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
7495 REM
7496 REM
7497 REM SAVE PROGRAM ONTO DISC ROUTINE
7498 REM
7499 REM
7500 PRINT"DO YOU WISH TO RENAME THE FILE": INPUT TE$
7501 IF G$=TE$ THEN 7502:ELSE 7508
7502 PRINT"INPUT NEW NAME": INPUT RE$
7503 RU$=RE$
7508 PRINT"I'M WRITING THE FILE "; DA$; RU$
7509 OPEN 1,0,1,DA$+RU$",SEQ,WRITE"
7510 PRINT#1,S
7520 FOR R=1 TO S
7530 PRINT#1, EK(R)
7535 LO(R) = LO(R) / 100
7540 PRINT#1, LO(R)
7545 LO(R) = LO(R) * 100
7550 NEXT R
7551 PRINT#1, DA$
7552 PRINT#1, RU$
7553 PRINT#1, SY$
7554 PRINT#1, SP$
7560 CLOSE 1: GOTO 6020
7695 REM
7696 REM
7697 REM READ DATA FROM DISC
7698 REM
7699 REM
7700 FOR I = 1 TO S: EK(I) = 0: LO(I) = 0: VO(I) = 0: NEXT I
7702 RU$ = "": DA$ = "": SY$ = "": SP$ = ""
7703 PRINT" INPUT FILE NAME": INPUT FM$
7705 PRINT" I'M READING FILE "; FM$
7710 OPEN 1,8,0,FM$+, SEQ, READ"
7720 INPUT#1, S
7730 FOR R = 1 TO S
7740 INPUT#1, EK(R)
7745 INPUT#1, LO(R)
7750 LO(R) = LO(R) * 100
7760 NEXT R
7761 INPUT#1, DA$
7762 INPUT#1, RU$
7763 INPUT#1, SY$
7764 INPUT#1, SP$
7770 CLOSE 1: GOTO 6020
7984 REM
7985 REM
7986 REM REPRESENTATION OF THE RESULTS
7987 REM ON THE SCREEN IN TABULAR FORM
7988 REM
7989 REM
8000 R = -1
8005 V = 0
8007 IF R >= S THEN 6020
8010 PRINT" EMF VOLUME EMF VOLUME"
8020 PRINT" /MV /ML /MV /ML"
8030 PRINT"CCCCCCCCCCCCCCCC CCCCCCCCCCCCCCCCC"
8040 IMAGE: ####. # #. ## ####. # #. ##
8050 REPEAT R = R + 2
8070 V = V + 1
8080 PRINT AT (6 + (2*V), 4); USING 8040, EK(R), LO(R) / 100, EK(R+1)
"LO(R+1)/100"
8090 FOR I=1 TO 1000: NEXT I
8100 IF V=8 THEN 8120
8110 UNTIL R=S OR R=S+1
8120 PRINT AT (25,8): "PRESS SPACE TO CONTINUE"
8130 GET A$: IF A$=" "THEN 8005: ELSE 8130
8985 REM
8986 REM
8987 REM GRAHPICAL DISPLAY OF RESULTS
8988 REM ON THE PRINTER
8989 REM
8990 REM
9000 GOSUB 7002
9010 PRINT AT (25,1); ""
: CALL DUMP: GOTO 6020
9020 REM
9021 REM
9022 REM TABULAR REPRESENTATION OF THE
9023 REM RESULTS ON THE PRINTER
9024 REM
9025 REM
10000 OPEN 1.4
10010 PRINT#1, Chr$(14)"SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS"
10020 PRINT#1
10030 PRINT#1, "POTENTIOMETRIC TITRATIONS"
10040 PRINT#1
10050 PRINT#1,"AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA"
10060 PRINT#1
10070 PRINT#1
10080 PRINT#1
10090 PRINT#1
10100 PRINT#1," EMF VOLUME EMF VOLUME"
10110 PRINT#1," /MV /ML /MV /ML"
10120 PRINT#1
10130 IMAGE: ####.# #.## ####.# #.##
10140 FOR R=1 TO S STEP 2
10150 PRINT#1, USING 10130,EK(R),LO(R)/100,EK(R+1),LO(R+1)/100
10160 NEXT R
10170 PRINT#1, CHR$(15)
10180 PRINT#1,"THE DATE:"
10190 PRINT#1, DA$
10200 PRINT#1
10210 PRINT#1,"RUN NUMBER:"
10220 PRINT#1, RU$
10230 PRINT#1
10240 PRINT#1,"THE SYSTEM UNDER EXAMINATION:"

240
10250 PRINT#1,SY$ 
10260 PRINT#1 
10270 PRINT#1,"SPECIAL INSTRUCTIONS" 
10280 PRINT#1,SP$ 
10290 PRINT#1 
10300 PRINT#1,"ML=MILLILITRES: MV=MILLIVOLTS" 
10310 PRINT#1 
10320 PRINT#1 
10330 PRINT#1,CHR$ (14) "SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS
21130 LE=B(1)
21131 HE=B(S)
21132 GOTO 21120
21133 REM
21134 REM
21135 REM PROGRAM TO DUMP SCREEN IMAGE
21136 REM ONTO THE PRINTER
21137 REM (COURTESY D.A.A.)
21138 REM
21139 REM
60000 DEF DUMP; A$, BT, B, C, D, P, Q, R, S, T: TEXT CLR
60010 PRINT AT (1,10); "SCREEN DUMP ROUTINE"
60020 PRINT AT (3,10); "FOR HI-RES GRAPHICS"
60030 PRINT AT (5,5); "ENTER PRINTER TYPE"
60040 PRINT AT (8,7); "EPSON FX80........ 1"
60045 PRINT AT (9,7); "SHINWA CP80A....... 2"
60050 PRINT AT (9,7); "CBM MPS-801...... 3"
60060 PRINT AT (11,5); "ENTER 1 - 2 - 3"
60070 REPEAT GET A$: UNTIL A$<"": P=VAL(A$): IF P<1 OR P>3 THEN
60080 PRINT AT (11,7+4*P); "": A$; "": IF P=3 THEN P=4: GOTO 6016
60090 PRINT AT (13,5); "ENLARGEMENT RATIO"
60100 PRINT AT (15,7); "1: 1............ A"
60110 PRINT AT (16,7); "2: 1 ............ B"
60120 PRINT AT (18,5); "ENTER A OR B"
60130 REPEAT GET A$: UNTIL A$<"": Q=ASC(A$)-64: IF 0<1 OR Q>2
60140 PRINT AT (18,6+5*Q); °; A$; ""
60150 IF P=1 THEN B$=CHR$(27)+"*"+CHR$(4): P=7: ELSE B$=CHR$(2)
60160 PRINT AT (20,8); "AUTOMATIC LINE FEED (Y/N)"
60170 GET A$: IF A$="Y" THEN S=1: ELSE IF A$="N" THEN S=129: ELSE
60180 PRINT AT (22,5); "CHECK PRINTER IS ON A NEW FORM"
60190 PRINT AT (24,7); "PRESS SPACE BAR TO CONTINUE": REPEAT
60200 DATA 160.2,169.8,145,45,200,152,145,45,96
60210 DATA 32,138,173,32,247.183,32.138,158,169,252,32,126,1
60220 DATA 162,15,74,8,44,0.203.48,14,126.3,203,40,8
60230 DATA 126,3,203,40,126.3,203,202,16,228,136,16,221,169,
60240 DATA 3,76,157,156
60250 IF P=4 THEN 60500
60260 FOR C=16 TO 71: READ R: POKE 51712+C, R: NEXT C
60300 OPEN S, P
60310 PRINT#S, CHR$(27); "A"; CHR$(8);: REM SET LINE SPACING
60320 POKE $CA03,8*Q: SYS($CA00): POKE $CA23,8*Q-1: POKE $CB00, 128/0
60325 POKE $CA1F, 11-4*0
60330 FOR R=0 TO 24: FOR D=0 TO 2*Q STEP 4
60340 PRINT#S, B$: CHR$(64*O); CHR$(O);
60350 FOR C=0 TO 39
60360 SYS($CA10): E000+8*(40*R+C)+D
60370 PRINT#S, A$;
60380 NEXT C
60390 PRINT#S, CHR$(27); "2";
60400 CLOSE S
60430 GOTO 60700
60500 DATA 162, 7, 169, 1, 157, 3, 203, 202, 16, 250, 96
60510 DATA 32, 138, 173, 32, 247, 183, 32, 138, 158, 169, 252, 32, 126, 15, 8, 160, 0
60520 DATA 177, 20, 162, 7, 74, 62, 3, 203, 202, 16, 49, 169, 3, 76, 157, 156
60530 RESTORE 60500
60540 FOR C=16 TO 58: READ R: POKE 51712+C, R: NEXT C
60550 OPEN S, P
60560 SYS($CA00)
60570 FOR R=0 TO 195 STEP 7: PRINT#S, CHR$(B);
60580 FOR C=0 TO 39: SYS($CA10)
60590 FOR B=6 TO 0 STEP -1
60600 SYS($CA1B): E000+8*(C+5* ((R+B) AND 248))+( (R+B) AND 7)
60610 NEXT B: PRINT#S, A$: NEXT C: PRINT#S: NEXT R
60620 PRINT#S, CHR$(B);: FOR C=0 TO 39: SYS($CA10): FOR B=3 TO 0 STEP -1
60630 SYS($CA1B): E000+8*(C+5* ((R+B) AND 248))+( (R+B) AND 7)
60640 NEXT B
60650 FOR B=0 TO 7: POKE $CB03+B, (PEEK($CB03+B) AND 15) OR 128: NEXT B
60660 PRINT#S, A$: NEXT C
60670 PRINT#S
60680 PRINT#S
60690 PRINT#S, CHR$(15): CLOSE S
60700: RETURN
inhibit the display update, and indicates when the display has been updated respectively. Userport lines PB0 to PB3 are used for the data input, and PB4 to PB6 select the signal information (mV range, sign and decimal point, and digits). Line PB7 is left to control the burette motor.

The information is transferred as a sign followed by $4\frac{1}{2}$ binary coded decimal (BCD) digits, and the range in digital serial format. The data is assembled in a string, N$, and is transformed into a number at the end of the routine.

The stability of the EMF is checked by the routine given in program lines 3000-3170. The average EMF of the first five values is stored in the computer memory, and compared with the average values of the next two successive groups of five values. If the average EMFs are within the range ±0.5mV, stability is deemed to have been attained. If, however, this requirement is not met, then the average of the next set is read, and compared with the previous two averages. If these satisfy the criteria for stability then this routine terminates. If not, the routine continues until stability is reached. The ±0.5mV range may be adjusted depending upon the accuracy with which the EMF is required; the time between each reading may also be altered. The entire operation may be aborted if the EMF continues to fluctuate. The EMF value which is finally stored in the computer memory is the average of the last five values displayed upon the screen.
The volume of titrant to then be added is determined by the routine in program lines 5000-5040. The initial volume of titrant added in all cases is 0.1cm³, however this may be altered. Subsequent volumes are determined by comparing the change in EMF for the volume of titrant added.

The mechanics of the addition process is controlled by program lines 20000-20007. The burette is turned on for a length of time sufficient to deliver 0.01cm³; multiples of this volume are added until the total calculated value has been added. To overcome motor/gearbox inertia, a delay is inserted between each addition.

The EMF is then obtained at the new volume in a similar way to that described for the initial reading. These values are stored in the computer memory until an output of the results is required. The titration will cease if the total volume added is greater than the volume input in the initialization procedure, or at 2.3cm³ when the burette is virtually empty. It is possible to make further additions by refilling the burette.

When an output of the results is required options are available for either graphical, or tabular presentation, using either screen or hard copy. An option for storing the results on disc for further analysis at a later date is
also provided. Finally, the options are present to either end the titration completely, or to continue the operation.

6.4 Acid-Base Titrations

Titrations were performed using the described system with sodium hydroxide as the titrant and hydrochloric acid in the titration vessel. Both solutions were obtained from BDH(cvs); sodium hydroxide was used as received, approximately 2 moldm\(^{-3}\), and hydrochloric acid 0.1 moldm\(^{-3}\). Solutions were flushed with argon prior to use; argon was continually bubbled through the solution during the titration. The results obtained from two such titrations are given in figures 6(iii) and 6(iv), and tables 6(i) and 6(ii). The time required to perform these titrations was of the order 2.5-3 hours. In both these cases the end point was between 1.13 and 1.14 cm\(^3\). This discrepancy from the expected result, 1.25 cm\(^3\), is probably due to either inaccuracy in the preparation of the solutions, or some other intrinsic part of the mechanics of the operation, rather than a failure in the computer program itself.

6.5 Conclusions

The system could be improved by more efficient programming, although the titrations clearly illustrate that the devised program produces results which are within
Figure 6(iii) Graphical representation of results from 1st acid-base titration.
Figure 6(iv) Graphical representation of results from 2nd acid-base titration.

HCL + NaOH

13 AUG 8
2

EMF(1)
-314.4

EMF(121)
314.0

VOL(1)
0.00

VOL(121)
2.01
Table 6(i) Tabular Representation of Results from 2nd Acid-Base Titration

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THE DATE: 13 AUG 85
RUN NUMBER: 2
THE SYSTEM UNDER EXAMINATION: HCl + NaOH
SPECIAL INSTRUCTIONS: NONE
ML=MILLILITRES; MV=MILLIVOLTS
Table 6(ii) Tabular Representation of Results from 1st Acid-Base Titration

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THE DATE: 13 AUG 85

RUN NUMBER: 1

THE SYSTEM UNDER EXAMINATION: HCL + NaOH

SPECIAL INSTRUCTIONS NONE

ML=MILLILITRES; MV=MILLIVOLTS
acceptable limits. Adjustment of various parameters within the program itself, depending upon the reactants under investigation, could be refined to optimize speed of operation and accuracy.
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APPENDIX
Preparation and Properties of the Sodium Salt of Tetraethyl-4-thiomolybdate (VI), \( \text{Na}_2\text{MoS}_4 \cdot 3.5\text{H}_2\text{O} \)

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School of Chemistry, Leicester Polytechnic, P. O. Box 143, Leicester LE1 9BH, U. K.

Received August 1, 1984

The thiomolybdate anions \( \text{MoO}_4\text{S}_2\text{X}_x \), \( x = 0-3 \), are considered as active intermediates in the Cu-Mo antagonism that afflicts ruminants [1-3]. \( \text{MoS}_4^{2-} \) is also involved in the formation of the active site of nitrogenase [4]. These simple tetrahedral anions are of interest spectroscopically and their chemical properties are well documented [5, 6]. More recently (\( \text{NH}_4 \))\(_2\text{MoS}_4 \) has been shown to be an effective antidote against certain metal ions [7]. In this study replacement of \( \text{NH}_4^+ \) by \( \text{Na}^+ \) reduced the toxicity (LD50 value) of the salt, this is of importance to current clinical trials of these salts. Although \( \text{Na}_2\text{MoS}_4 \) is commercially available it is invariably of low purity and mostly contains insoluble \( \text{MoS}_2 \) and \( \text{MoS}_3 \). There is no published method for the preparation of the \( \text{Na}^+ \) salt.

Interestingly, the use of large cations to obtain solid salts has led to the conclusion that these are necessary to stabilise the thiomolybdate ions [6]. We herein report a method of preparing the \( \text{Na}^+ \) salt of the \( \text{MoS}_4^{2-} \) anion together with its characterisation.

Experimental

**Apparatus and Materials**

IR spectra were obtained as KBr discs with a Perkin-Elmer 683 spectrophotometer. UV/visible spectra of aqueous solutions were recorded on a Perkin-Elmer 555 spectrophotometer. X-ray powder diffraction data were obtained using Ni-filtered Cu-Kα radiation from a Philips PW101 diffraction generator and either a Debye-Scherrer or a Gandolfi camera. Thermogravimetric analyses were made with an automatic Stanton-Redcroft TG750 TGA balance.

Reagents were of 'AnalaR' grade or equivalent, except for acetone and diethyl ether which were of 'standard Laboratory Reagent' grade. \( \text{H}_2\text{S} \) of 99.6% purity was obtained from BDH Ltd. All water was distilled and deionised before use.

**Preparations**

\( (\text{NH}_4)_{2}\text{MoS}_4 \)

It was prepared by the method outlined by Mellor [9]. Ammonium paramolybdate (5 g) was dissolved in water (15 cm\(^3\)) and ammonia (0.88, 50 cm\(^3\)) added. A vigorous stream of \( \text{H}_2\text{S} \) was bubbled through the solution for ca. 20 min. The dark red solution on cooling to room temperature deposited red crystals of the product. The crystals were filtered, washed with ethanol and dried in vacuo. Yield ca. 80%.

\( \text{Na}_2\text{MoS}_4 \cdot 3.5\text{H}_2\text{O} \)

\( (\text{NH}_4)_{2}\text{MoS}_4 \) (5 g) was dissolved in an ice-cold aqueous NaOH solution (1.7 g, 20 cm\(^3\)). \( \text{NH}_3 \) and \( \text{H}_2\text{O} \) were then slowly removed by vacuum pumping for 3-4 h. The solid red residue was extracted with acetone (60 cm\(^3\)) and then diethyl ether (150 cm\(^3\)) added to the filtrate. Red crystals of the product formed, these were filtered, washed with ether (3 \( \times \) 10 cm\(^3\)) and then dried in vacuo. Yield 3.5 g (55%). Analytical data from two different preparations are given in Table I and spectroscopic properties in Table II.

Results and Discussion

**Preparations**

The starting material (\( \text{NH}_4 \))\(_2\text{MoS}_4 \) is the easiest of the tetraethyl-4-thiomolybdate salts to prepare in high
yield and of good purity, our preparation followed the simpler procedure recommended earlier by Mellor [9] rather than that recently published by McDonald et al. [10]. The recommendation of heating to 50–60 °C in the latter procedure is not necessary since the reaction is exothermic. For obtaining the sodium salt from (NH₄)₂MoS₄ we followed the method suggested by McDonald et al. [10] for the synthesis of tetraalkylammonium salts, the reaction can be summarized as:

\[(NH_4)_2MoS_4(\text{o}) + 2NaOH(\text{o}) \rightarrow Na_2MoS_4(\text{o}) + 2H_2O + 2NH_3\dagger\]

The product, obtained as the 3.5 H₂O hydrate, is extremely water soluble and, in contrast to the K⁺ and NH₄⁺ salts, is also soluble in polar organic solvents. As with the other salts it must be stored over a dessicant otherwise decomposition to black insoluble sulphides occurs.

Whilst the K⁺ salt can be prepared with care [3] directly from molybdate, this is not a suitable route for obtaining the Na⁺ salt. The high basicity of the reaction medium resulted in decomposition during solvent removal. Neither could the Na⁺ salt be precipitated by adding large excesses of organic solvents, it is too highly soluble. Attempts made at lower basicity were also unsuccessful, reaction proceeding mainly to the MoS₃²⁻ stage.

**Characterisation**

The spectroscopic properties of the MoS₄²⁻ ion are well documented [5, 6, 11]. Tables I and II show the Na⁺ salt to have identical properties to that of the NH₄⁺ salt. The 480 cm⁻¹ band in the IR spectrum is assigned to ν(Mo–S). The other bands can be attributed to the presence of H₂O, this was confirmed by TGA which showed a weight loss over the range 80–160 °C corresponding to 3.5 (±0.1) mol H₂O. The X-ray powder diffraction patterns of the NH₄⁺ and K⁺ salts are identical and show them to have the same structure as β-K₂SO₄.[12]. The Na⁺ salt is seen from Table III to have a different crystal structure.

The differences in behaviour presumably reflect the influence of the smaller Na⁺ ion. The suggestion [6] that the larger cations are necessary to stabilise the MoS₄²⁻ ion is in fact a reflection of the differences in solubilities of these salts rather than any differences in thermodynamic stability.

**Acknowledgements**

DEP is a recipient of a Leicestershire Education Authority research assistantship. X-ray powder diffraction measurements were kindly made by Mr. K. Andrews. The UV/visible spectrometer was purchased from a Medical Research Council Grant.

**References**


duction of neutral proteinases had been previously stimulated by the ionophore A23187 failed to become «hyperstimulated» in the presence of Co²⁺ (data not shown).

As the concentrations of Co²⁺ needed to produce these effects are found in patients with prosthetic joints [3,4], the cellular reactions we describe here deserve further scrutiny.

REFERENCES


THE STRUCTURE AND REACTIVITY OF Ni(II)-ALBUMIN PROTEIN COMPLEX

There is much concern regarding the toxic effects of ingested nickel, particularly among workers in nickel refineries [1,2]. For this reason the biochemical studies undertaken by SARKAR and his co-workers [3,4] in relation to ingested Ni are of particular importance. They have shown that the main Ni(II)-binding constituents in human blood are the amino acid histidine (His) and the protein albumin. This distribution resembles that for Cu(II) except the albumin has a much stronger affinity [4] for Cu(II) than Ni(II). Nevertheless, these studies have established that the two metal ions bind to the same site in the albumin, and this, from Cu(II) studies, is known to involve the N-terminal amino acid residues. The proposed near square-planar amino N, deprotonated peptide N atoms, and a histidine N is depicted in Fig. 1.

The ability of Ni(II) to cause peptide N deprotonation is known to be less than that of Cu(II), and a structure such as that in Fig. 1 would require a high pH for its formation. In keeping with this the UV/Vis absorption spectrum of 1:1 Ni(II):albumin shows an intense absorption band at 420 nm, characteristic of a square-planar environment, which reaches a maximum absorption at pH>9 [4].

We have examined the bovine albumin binding of Ni(II) by means of UV/Vis absorption and CD spectroscopy. In agreement with GLENNON and SARKAR [4] the square-planar type spectra reach a maximum at pH>9. The spectra, shown in Fig. 2, reveal that at the pH of blood (7.4) 70% of the Ni is bound as in Fig. 1. The remaining 30% must be octahedrally co-ordinated since, under the conditions used, this would be spectroscopically silent. The rapidity of interconversion between the two forms as the pH is altered suggests that the octahedral site must also be at the N-terminal end of the protein chain.

We have also found that the rate of ligand exchange for Ni(albumin), as in

\[ \text{Ni(albumin)} + 2\text{HisO} \rightarrow \text{Ni(HisO)}_2 + \text{albumin} \]
is very slow, requiring >90 min to achieve equilibrium at 37°C and pH 7.4 (0.15 M NaCl solution), the octahedral species reacting more rapidly (via a dissociative mechanism) than the square-planar form which reacts associatively. The difference in kinetic behaviour between Ni(albumin) and Cu(albumin) could be an important factor in their different metabolic properties [4].

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The use of acetylsalicylic acid, aspirin, to relieve pain, reduce fever plus a wide variety of other ailments is well known. However, the theories to explain the effects of aspirin are vague. One theory postulates that the body under stress will have a two fold or greater increase of copper ions in the bloodstream with a loss of essential copper from the organs. The role of the aspirin is the formation of a copper chelate which facilitates the return of copper to the deficient cells [1]. The chemistry of the coordination of aspirin with metal ions is therefore necessary to have a complete understanding of the therapeutic role of aspirin. Copper(II)aspirinate has been prepared and structural studies of the solid [2] report a polymeric material of units of \([\text{Cu(C}_6\text{H}_2\text{O}_4\text{)}_2]_2\) with the carboxylic group acting as a bridging ligand between two Cu(II) ions as well as Cu-Cu bonding. The aspirin complex in the solid state does not exhibit chelation. There are no other studies that have been reported on the interaction of aspirin with copper; in fact, very little has been reported on the interaction of aspirin with metal ions.

We wish to report the results for the determination of the stability constants for the iron(II)-aspirin system.

The aspirin as ligand is monobasic: