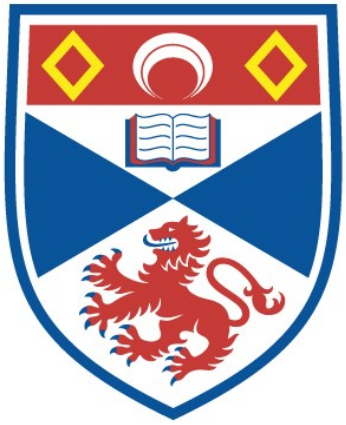
SOME STUDIES ON THE FORMATION AND INTERCONVERSION OF IRON-SULFUR-NITROSYL COMPLEXES

Debra Forrest Blyth

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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SOME STUDIES ON THE FORMATION AND INTERCONVERSION OF IRON-SULFUR-NITROSYL COMPLEXES

DEBRA F. BLYTH

A THESIS PRESENTED TO THE UNIVERSITY OF ST. ANDREWS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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DEDICATION

To Mum, Dad and Carolyn

SUMMARY

Chapter One provides an introduction into the area of iron-sulfur-nitrosyl chemistry as well as two short reviews on iron-sulfur containing amino complexes and on the direct formation of metal-nitrosyl complexes from the action of nitrite or nitric oxide.

Chapter Two describes the formation of $Na[Fe_4S_3(NO)_7]$ and $Fe_2(SMe)_2(NO)_4$ from the amino acids cysteine and methionine respectively with iron(II)/(III) salts and nitrite/nitrate, as well as the effect of pH on these reactions.

Chapter Three provides an account of the effect of nitrite on preformed iron-sulfur containing amino acid complexes.

Chapter Four contains a study of the conversion of $Fe_4S_4(NO)_4$ to $Na[Fe_4S_3(NO)_7]$ using FTIR and ESR spectroscopy.

Chapter Five provides a short summary of the conclusions found in the above three chapters.

ABSTRACT

Studies on the effect of substituting iron(III) for iron(II) or nitrate for nitrite in the reaction of cysteine with iron(II) salts and nitrite with and without sodium ascorbate present demonstrated the formation of the tetranuclear $Na[Fe_4S_3(NO)_7]$ complex when iron(III) is substituted for iron(II), albeit in smaller yield; but no iron-sulfur-nitrosyl clusters were detected when nitrite was replaced with nitrate. Similarly in the case of reaction of methionine with iron(II) salts and nitrite in the presence of sodium ascorbate, the dinuclear complex $Fe_2(SMe)_2(NO)_4$ is formed in reduced yield when iron(III) is substituted for iron(II) and no iron-sulfur-nitrosyl complexes are detected when nitrite is replaced with nitrate.

The effect of pH on the reactions of cysteine and methionine with iron(II) salts and nitrate, with and without sodium ascorbate present in the case of cysteine, were studied. The cysteine reactions showed that in the presence of sodium ascorbate, the yield of $Na[Fe_4S_3(NO)_7]$ fell slightly as pH was decreased due to formation of the less soluble ascorbic acid from acidification of sodium ascorbate. At very low pH both cysteine reactions with and without sodium ascorbate present did not yield $Na[Fe_4S_3(NO)_7]$ due to the formation of nitric oxide from nitrite, which would rapidly oxidise to give nitrogen dioxide, and to the instability of the $Na[Fe_4S_3(NO)_7]$ complex itself at very low pH. The dinuclear complex $Fe_2(SMe)_2(NO)_4$ is very stable to conditions of low pH but its formation at very acidic conditions was hindered, again due to the formation of nitric oxide from nitrite.

A range of iron-sulfur containing amino acid complexes of the general type $[Fe_3O(\text{amino acid})_6(H_2O)_3]X_7$, were synthesised and their reactions with nitrite studied. In the presence of sodium ascorbate, when the amino acid is methylcysteine, both $[Fe_4S_3(NO)_7]^-$ and $Fe_2(SMe)_2(NO)_4$ were isolated after reaction with nitrite. However no identified iron-sulfur-nitrosyl complexes could be detected when the amino acid was methionine. In the absence of sodium ascorbate both the methylcysteine and methionine complexes yielded a range of unidentified complexes which infra-red spectroscopy demonstrated absorbed strongly in the nitrosyl stretching region and it was postulated that the species observed were inorganic nitrosyl complexes.

FTIR analysis of $Fe_4S_4(NO)_4$ in the polar coordinating solvents THF, DMF and DMSO demonstrated conversion to $[Fe_4S_3(NO)_7]^-$. No such conversion was observed using the solvents diethyl ether or dichloromethane. An intermediate species was

observed in THF and DMSO using FTIR spectroscopy which was postulated to be a $[Fe(solvent)_6][Fe_4S_4(NO)_4]_2$ complex. Another peak observed in the latter stages of the conversion was believed to be that of a dinuclear $Fe(SR)_2(NO)_4$ type complex. ESR analysis of $Fe_4S_4(NO)_4$ in THF and DMSO however demonstrated formation of a mononuclear species of type $[Fe(NO)_2(X)_2]^{n+}$. Thus in the dilute solutions required for ESR analysis, mononuclear species of the type $[Fe_2(NO)_2(X)_2]^{n+}$ are favoured but in the more concentrated solutions needed for FTIR analysis polynuclear species of the type $[Fe_4S_4(NO)_4]^-$ are predominant.

ACKNOWLEDGEMENTS

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Introduction

1.1 Sources of Nitrite and Nitrate in the Diet

Both nitrite and nitrate are present in food and water consumed by humans. The main sources of nitrate and nitrite in the diet are vegetables and cured meat products. Drinking water can also be a major source of nitrate but levels found vary regionally. This section deals with each nitrite/nitrate source separately.

Origins of Nitrite and Nitrate

In the conventional nitrogen cycle[1] (see Figure 1.1), nitrate is taken up by plants, incorporated into organic substances and later transferred to animals and man.

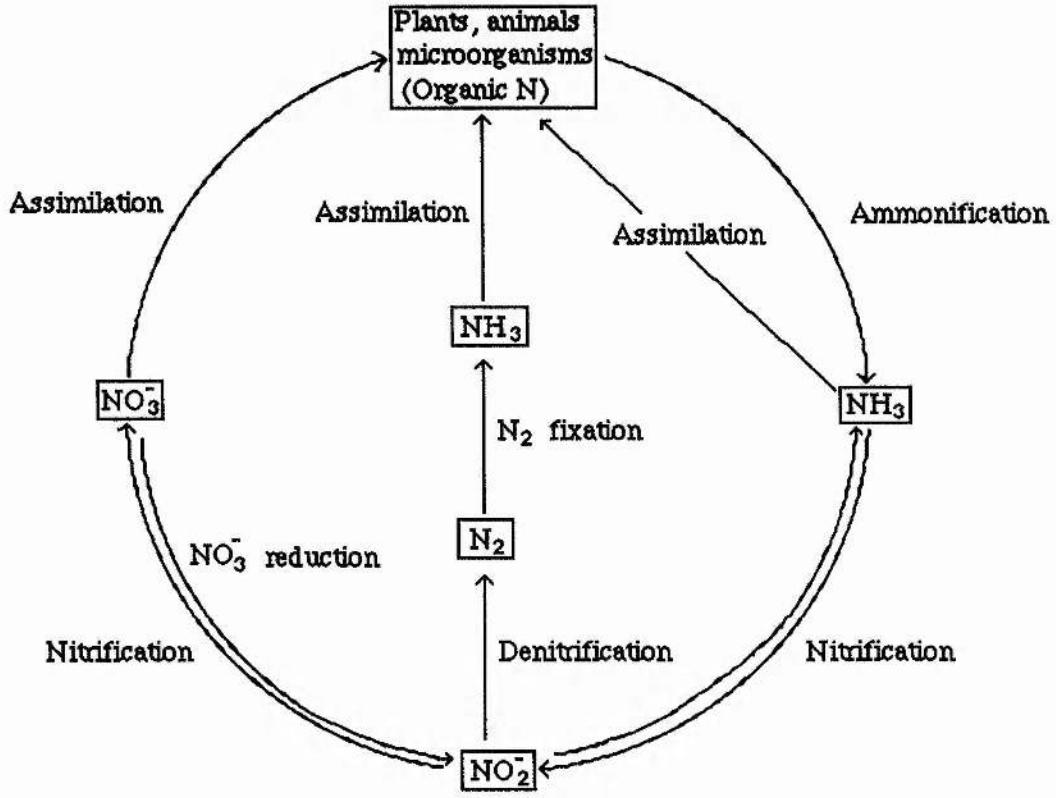


Figure 1.1; A generalised N cycle showing the major processes involved.

Plants synthesise all their protein and other organic nitrogen compounds from inorganic nitrogen taken up as nitrate or ammonia. Most comes from the soil, although, in legumes, bacteria in root nodules use atmospheric nitrogen to make nitrate. If extra nitrate is available to the plant it may take it up and if synthetic processes are unable to

use it all, it may be retained. Whereas the nitrate content of vegetable products is a result of their cultivation and the fixation of atmospheric nitrogen, the presence of nitrate (and nitrite) in certain animal products (some meats and cheeses) is due to human addition and in fermented meat products, to the production processes.

Soils contain[1] about 10 000 kg of organic nitrogen per hectare which is slowly mineralised by biological processes, the final stages being to ammonium, nitrite and nitrate. The conversion of ammonium to nitrite in soils is more rapid, except at very low temperature but the conversion of nitrite to nitrate is so rapid that nitrite is rarely detectable in soils. Nitrogen is added to the soil in organic forms in crop residues and manures and in synthetic forms (ammonium, nitrate and urea) in fertilisers. Fixation of atmospheric nitrogen by legumes adds about 150 kg/ha per year of nitrogen to the soil where these crops are grown[2] to which about 2 kg/ha per year are added by free-living nitrogen-fixing organisms[2]. Plant roots take up nitrogen as either ammonium or nitrate. Since other forms are rapidly converted into nitrate at temperatures sufficient for plant growth, nitrate is the form in which most nitrogen is taken up. In some species nitrate is reduced in the root, but in some common crop species the principal site of nitrate reduction is in the leaves. Crops which are well supplied with nitrogen will often accumulate high concentrations of nitrate in their leaves The nitrate content of plants is controlled in part by an enzyme, nitrate reductase, which is present in soil and requires a molybdenum co-factor. Plant nitrate levels appear to be much higher in areas of molybdenum deficiency than in other areas, although information on the extent of this is not available. The use of fertiliser nitrogen has increased in recent years and it is probable that this has resulted in an increase in nitrate concentrations in some crops (especially leaf crops). However, if more nitrogen is added than the crop can use then can lead to an increased loss of nitrogen from the soil by volatisation (as ammonia, nitrous oxide and by leaching).

Ammonium is bound by negatively-charged soil particles and so has low mobility and is resistant to leaching. Nitrate remains in the soil solution, so it is mobile and can be leached below the depth to which roots penetrate, ultimately passing into groundwater. In addition, nitrate may enter surface water by run-off. Most of the leaching loss of nitrate occurs during the winter months with greatest loss from arable land where mineralisation continues in the autumn, when crop uptake is small or absent. There is very little leaching loss from low intensity grassland, but there is some evidence of loss from intensively-grazed systems. There is also a leaching loss following the ploughing of old grassland, due to the rapid initial breakdown of accumulated organic matter[3,4].

Nitrate and Nitrite in Vegetables

Nitrate concentrations in vegetables vary enormously ranging from about 1 to 10 000 mg/kg fresh weight. The literature on the nitrate content of vegetables has been authoritatively reviewed by Corre and Breimer[5] who grouped fresh vegetables into five classes (see Table 1.1). In contrast, nitrite occurs in healthy living plants at low concentrations, typically levels of 1-2 mg/kg nitrite may be found in fresh vegetables and 10 mg/kg is rarely exceeded[5]. The range of nitrate concentrations observed amongst different samples of the same variety of vegetable is sometimes very large, for example, nitrate concentrations in beetroot from various retail outlets were shown in the same investigations[6] to vary between 630 and 6800 mg/kg.

Nitrate is likely to accumulate in plant tissues wherever the amount of nitrate available in the soil substantially exceeds the plant's requirement. Consequently the nitrate contents of vegetables tends to increase in proportion to the amount of nitrogen applied, irrespective of effects on crop yield[7]. Nitrate is increased by applications of both organic and inorganic forms of nitrogen. Thus not only are there variations in the nitrate contents of vegetables caused by factors inherent in plants, but the nitrate contents are also affected by the conditions under which the plants are grown and harvested, for example, carrots grown during the winter in a greenhouse contained more nitrate than those which matured in summer[8] possibly due to the lower light intensity in winter. Similarly beets cultivated under artificial lighting contained more nitrate when grown with 8 hour periods of illumination than with 20 hour periods of illumination[9]. There is some evidence[10] to show that by increasing the irrigation of growing plants their nitrate content at harvest are reduced. Nitrate concentrations in snap beans, carrots and celery decreased with increasing maturity[11].

Storage of vegetables appears to have unpredictable effects on the content of nitrate. Studies in Japan found that the nitrate concentration in carrots did not change during storage[12], whereas storage of potatoes under a low partial pressure of oxygen caused the nitrate concentration to decrease and that of nitrite to increase[13]. The same workers[13] found that in gamma-irradiated tubers nitrate concentrations increased immediately after irradiation, but concentrations of both ions decreased during subsequent storage. Work in New Zealand[6] with a variety of vegetables indicated that there were no consistent changes in their nitrate and nitrite concentrations during storage. Cooking of vegetables tends to reduce their nitrate contents by about 20 per cent[6,11] and in most cases reduced nitrite contents to less than 0.2 mg/kg[6].

<200 mg/kg	<500 mg/kg	<1000 mg/kg
asparagus	broccoli	cabbage (white & red)
chicory	cauliflower	carrot
garden bean	cucumber	curly kale
grean pea	egg plant	french bean
mushroom	gherkin	parsley (root)
potato	melon	
sweet pepper	onion	
sweet potato	scorzonera	
tomato	turnip	

<2500 mg/kg	>2500 mg/kg
cabbage (oxheart)	beetroot
celeriac	celery
endive	chervil
garden cress	lamb's lettuce
leek	lettuce
parsley (leaves)	purslane
rhubarb	radish and black radish
	spinach
	turnip tops

Table 1.1; Typical nitrate content of various vegetables[5]

Nitrate and Nitrite in Cured Meat Products

Nitrite and nitrate are added to cured meat products such as porks, hams, salamis and so on, in order to prevent the growth of bacteria such as *Clostridium botulinum* as well as to provide the characteristic pink colour observed in many of these products. Botulism is a potentially fatal form of food poisoning, caused by the anaerobic bacterium *Clostridium botulinum*. The most potent of the toxins produced by this bacterium is *C. botulinum* toxin type A; it is a protein with a molecular weight of about 1 million a.m.u., and is probably the most toxic single substance known: 1 mg will kill *ca.* 20 million mice and a few grams would kill the entire UK human population.

The use of salt to preserve or 'cure' meat is a long established practice, deriving originally from the need to ensure a continuing supply of meat during periods when fresh meat was not available. The salt employed in these days was generally impure and nearly always contained some saltpetre (sodium nitrate). With time a better understanding of the role played by saltpetre has resulted in the realisation that nitrite rather than nitrate is the principle active ingredient in curing salt mixtures. However, since nitrate is at least partially converted to nitrite by microbiological and biochemical processes occuring in meat, nitrate is still an ingredient in some commercial curing processes. There are regulations[14] controlling the addition of sodium and potassium nitrites and nitrates to food products which state that for cured meats the permitted upper limit for nitrate is 0.05% by weight as sodium nitrate (E251) and for nitrite, 0.02% by weight as sodium nitrite (E250).

Nitrate in Dairy Products

Liquid milk generally does not contain more than 1 to 5 ppm of nitrate and the level of nitrate in the diet of dairy cows appears to have only a very small effect on the nitrate concentration in the milk they produce[15]. Some cheeses may contain modest levels of nitrate and/or nitrite. Although not used in cheese manufactured in the United Kingdom, nitrate has been used in cheesemaking in continental Europe for about 150 years. It is used, particularly in some cheeses which are salted in brine, to control the production of gas and undesirable flavours by coliforms and anaerobes during ripening, a practice recognised in international standards for cheeses such as Edam, Gouda and Friese[16]. Such cheeses are imported into the United Kingdom. English-type cheeses (Cheddar, Cheshire and Leicester) contain relatively low levels of nitrate, which is almost certainly derived from the natural nitrate content of milk.

Nitrate in Drinking Water

The levels of nitrate in drinking water supplies have recently attracted considerable political and media interest, largely due to the failure of some UK water authorities to meet the requirements of the EEC directive of 1985 which specifies a maximum level of 50 ppm nitrate.

Whenever the levels of nitrate in water supplies are high the press has tended to blame current agricultural practices, in particular the alleged use of too much nitrogenous fertilizer. However nitrate concentrations in rivers in the hard rock areas of Scotland and Wales typically contain less than 2 ppm nitrate, whereas levels above 50 ppm are found in certain rivers in the lowlands and south eastern England. The highest nitrate levels occur in waters drawn from the deep chalk aquifers underlying the eastern counties of England. Long term studies at Rothamstead and elsewhere have shown conclusively that very little if any of the applied fertilizer nitrogen finds its way into water courses as nitrate[3,4]. Rather, the nitrate in the drinking water in the South-East, which is drawn from deep chalk aquifers with a turnover time of 40-50 years, results from the changes in the pattern of agriculture in the region, during and immediately after the Second World War, from traditional mixed farming to intensive cereal growing which saw the ploughing up of much ancient pasture land. It is the accumulated nitrogen in these pastures which has appeared and will continue to appear for decades to come as nitrate in the aquifers.

Nitrite is usually undetectable in drinking water leaving the treatment works. However when ammonia is present naturally or is added deliberately during treatment to convert the free residual chlorine to chloramine, samples in the distribution system sometimes contain concentrations in the range 0.1 to 0.3 ppm nitrite.

For some people beer may be a major source of nitrate, particularly if it has been brewed from water having a relatively high level of nitrate - four pints per day of beer of average nitrate content are sufficient almost to double the average daily intake of nitrate[17].

1.2 Biological Fate of Nitrite/Nitrate

When nitrate is consumed, about half is excreted directly and without change: of the remainder, about 20% is reduced by oral microflora to nitrite, which then finds its way to the stomach. No other metabolic fate for nitrate has been identified and ingested nitrate appears to take no part in any reactions in the body, other than reduction to nitrite[18].

There are three main fates for ingested nitrite: reaction with amino acids can give N-nitroso compounds; reactions with metalloporphyrins (as found in haemoglobin or myoglobin) can give nitrosometalloporphyrins; and reactions with iron-sulfur proteins can give iron-sulfur-nitrosyl complexes. Each of these fates of nitrite will be discussed separately in this section.

Nitrite and Amino Acids

Nitroso compounds are divided into two classes, nitrosamines and nitrosamides. The former are N-nitroso derivatives of secondary amines, while the latter are derivatives of substituted amines and related compounds. N-nitrosamines are relatively stable compounds under most conditions[19] found in foods and do not decompose during food processing or preparation. However, nitrosamides are much less stable and are unlikely to survive common cooking procedures[20].

The reaction of nitrite with amines proceeds via a series of steps (see Figure 1.2). Nitrite cannot react directly with amines, but first must be converted to nitrous anhydride (N_2O_3). As the first two equations in Figure 1.2 show, this reaction is favoured by acidic conditions. However, if the conditions are too acidic, the amine will be protonated and not able to react with the nitrous anhydride. This means that nitrosation occurs most rapidly at some optimum pH, for the most common amines in foods, this optimum is pH 2-4.

$$2(NO_2^-) \xrightleftharpoons{2H^+} 2(HNO_2)$$

$$2(HNO_2) \xrightleftharpoons{\text{fast}} N_2O_3 + H_2O$$

$$N_2O_3 + R_2NH \xrightarrow{\text{slow}} R_2NN{=}O + HNO_2$$

Figure 1.2; Steps in the nitrosation of secondary amines at moderate acidities

The formation of nitrosocompounds is a cause for concern, because over 90% of the more than 800 nitroso compounds which have been tested in laboratory animals have caused cancer[21]. While no known case of human cancer has been shown to result from exposure to nitroso compounds, much indirect evidence indicates that humans would be susceptible. The concern that human foods might contain nitroso compounds stems from the discovery in the early 1960s that domestic animals fed fish meal preserved with high levels of sodium nitrite were dying of liver failure. The nitrosamine, N-nitrosodimethylamine was determined to be the cause of the liver failure and resulted from the reaction between dimethylamine contained in the fish and the added nitrite[22,23]. Extensive surveys have shown other nitrosamines already present in foods, in particular, malt beverages and cured meats, primarily fried bacon[24]. However ascorbic acid (vitamin C), erythorbic acid (isoascorbic acid), which is inactive as vitamin C, and tocopherol (vitamin E) all inhibit nitrosation.

N-Nitroso compounds can be formed very readily at stomach pH from nitrite and amino acids or peptides containing nitrogenous side chains. The reaction[25,26] of some amino acid derivatives with acidified nitrite are summarised in Figure 1.3. Proline (1) readily undergoes N-nitrosation to give N-nitrosoproline, which although is apparantly not itself a carcinogen, can decarboxylate to give N-nitrosopyrollidine which is powerfully carcinogenic. For all, except proline, N-acetylation protects the amino groups leaving only the side chain as a reactive site, as in peptides and proteins. Thus arginine (2) and tryptophan (3) form derivatives which contain N-nitroso groups in the side chain. Under forcing conditions, methionine (4) gives N-nitrosation at the α-nitrogen, but cysteine (5) undergoes S-nitrosation. However, like most other nitrosothiols, the cysteine derivative is unstable; the S-N bond is weak and nitric oxide, one of the products of homolysis is a stable radical.

$$RSNO \rightarrow RS^{\bullet} + NO^{\bullet}$$

Thus the nitrosothiol derivative is likely to be formed readily when peptides and proteins containing the cysteinyl residue are nitrosated, but it is also readily destroyed. Evidence[26] suggests that heterolytic and homolytic mechanisms may compete in this decomposition. The heterolytic mechanism could lead to N-nitrosamines.

$$R_2NH + RS\text{-}N{=}O \rightarrow R_2NNO + RSH$$

The homolytic process would generate nitric oxide which may be a possible route to nitrosylmyoglobin in the curing process[27] (see Nitrite and Metalloporphyrins).

In general at physiological pH only side chain nitrosations are likely to occur as N-nitrosation of the nitrogen of a peptide link requires more drastic nitrosation conditions than are likely to be found in normal healthy people[25]. However, studies[28,29] have shown that smokers contain three times as much thiocyanate in their saliva than non smokers. Thiocyanate in the gastric juice can act as a catalyst to the nitrosation reaction, thus enhancing the formation of N-nitroso compounds from diet derived nitrite *in vivo*.

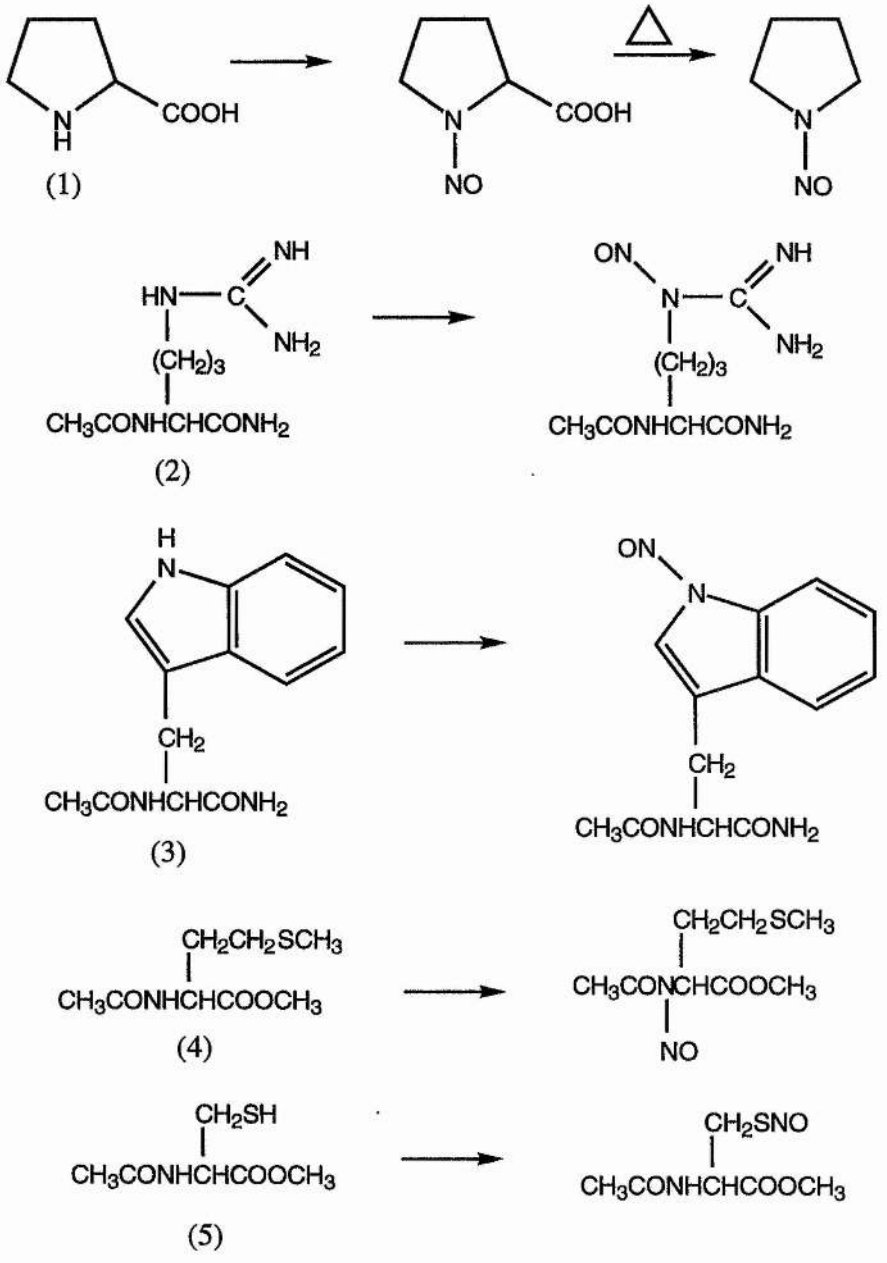


Figure 1.3; Nitrosation of amino acid derivatives

Nitrite and Metalloporphyrins

The second type of reaction which nitrite undergoes in the body involves metalloporphyrins, in particular the iron porphyrins found in haemoglobin, the oxygen carrying protein of the blood, and myoglobin, the oxygen storage protein of muscle tissue.

It is the reaction of nitrite with myoglobin which produces the characteristic pink colour observed in hams and other pork products[27]. In the resting protein, the iron is coordinated by five nitrogen atoms, four from the porphyrin ring and the fifth from an amino acid side chain of the protein. Nitrosylation produces six-coordinated iron and subsequent heating, which leads to the breakage of the iron-imizadole bond leaves a five-coordinated iron-nitrosyl complex[30] physically trapped within a matrix of denatured globin as shown diagrammatically in Figure 1.4[30]. Reactions of nitrous acid with α-amino-acid side chains[31] (see section on Nitrates and Amino Compounds) may also contribute to denaturation.

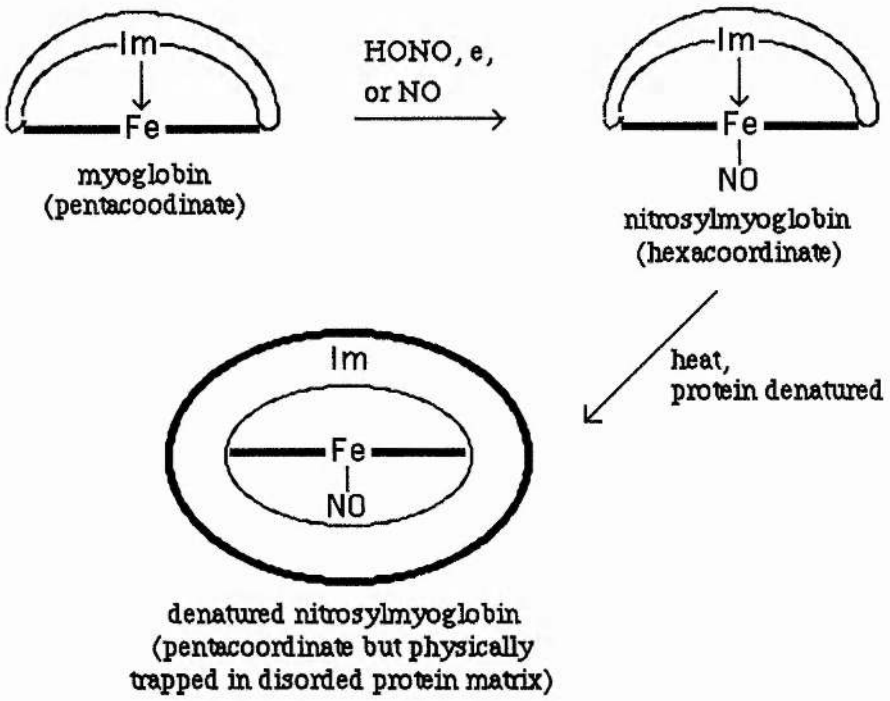


Figure 1.4; Diagrammatic representation of chemical modifications of myoglobin during the curing process

Occasionally, meat which has been incorrectly cured produces green pigments in a reaction known as 'nitrite greening'. The green colouration has been shown[32,33] to be due to reaction of acidified nitrite at the vinyl groups of porphyrin rings (see Figure 1.5).

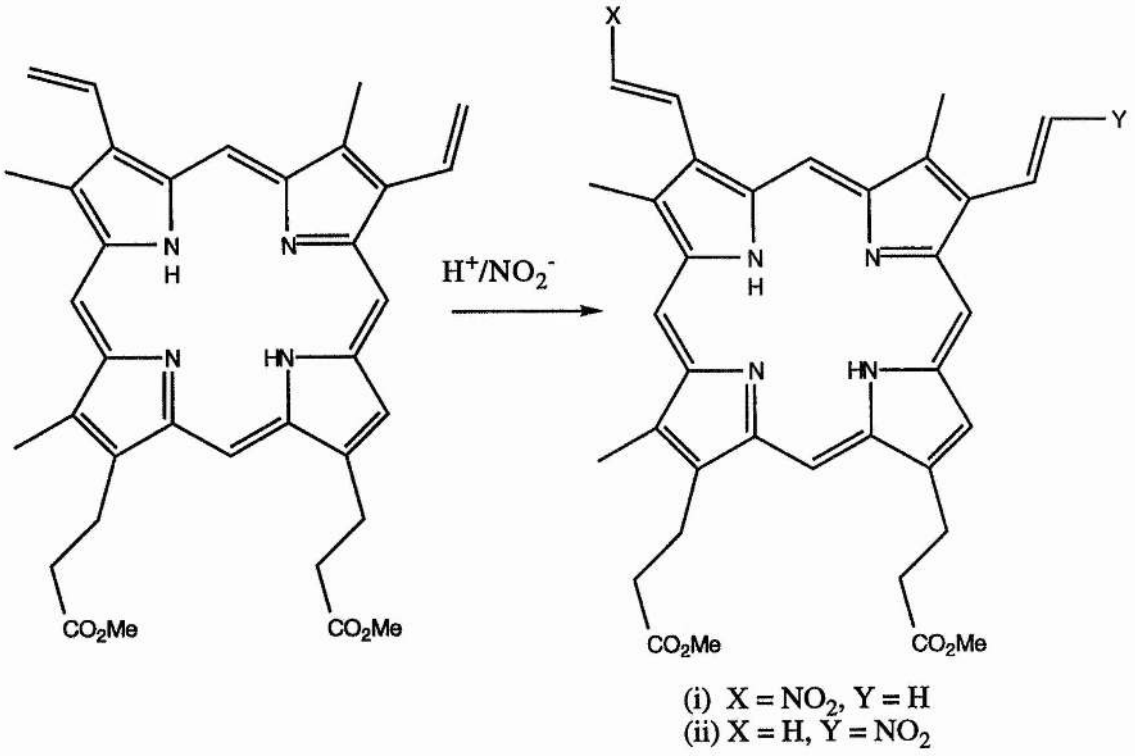


Figure 1.5; Nitrosation at the vinyl groups of a porphyrin ring

In new-born children, most of the haemoglobin is the so-called foetal haemoglobin, or haemoglobin F, and during the next year or so of life this is gradually replaced by adult haemoglobin[25]. However, foetal haemoglobin is much more susceptible to nitrosylation then normal haemoglobin: if some nitrosylation occurs, haemoglobin is converted to methaemoglobin where the oxygen-carrying capacity of blood is proportionally diminished[34]. When the conversion to methaemoglobin exceeds 10% in the blood, the condition is termed methaemoglobinaemia which is potentially fatal at elevated concentrations. Because of this high reactivity of foetal haemoglobin towards nitrite, food products aimed specifically at babies and infants may not contain added nitrite.

The acute infantile methaemoglobinaemia associated with the use of water for preparation of milk for bottle feeding is associated only with the use of well-water and was thus termed well-water methaemoglobinaemia. This is however a rare condition, the incidence of which has decreased in Western Europe in the past twenty years and is now virtually non-existent[34]. Nitrate itself does not cause methaemoglobinaemia; it has to be reduced to nitrite to induce the condition. Sometimes methaemoglobinaemia may occur with a low nitrate intake and this is thought to be associated with enhanced endogenous nitrate synthesis due to enteritis. The great majority of cases however of well-water methaemoglobinaemia occurred when nitrate levels exceed 100 ppm. In

those cases where methaemoglobinaemia was associated with water containing less than 100 ppm nitrate, the infants showed signs of gastroenteritis and the water was of poor hygenic quality, in most cases heavily contaminated with bacterial Coliforms and in one particular case, with remains of decaying animals[34]. There is no evidence that methaemoglobinaemia is caused by bacterially sound water supplies at concentrations up to 100 ppm nitrate.

Nitrite and Iron-Sulfur Proteins

Many of the enzymes which catalyse oxidation reactions, as in the oxidation of carbohydrates or fatty acids for energy liberation, or reduction reactions as in the fixation by plants of carbon dioxide into carbohydrates or of nitrogen into ammonia and thence to amino acids, contain iron-sulfur centres. There are several types of such centres including [2Fe-2S] and [4Fe-4S] types (see Figure 1.6). Because of the range of important biological processes mediated by these iron-sulfur centres in both plants and animals, they are very widely distributed in foodstuffs as well as in bacteria, including *Clostridium botulinum.*

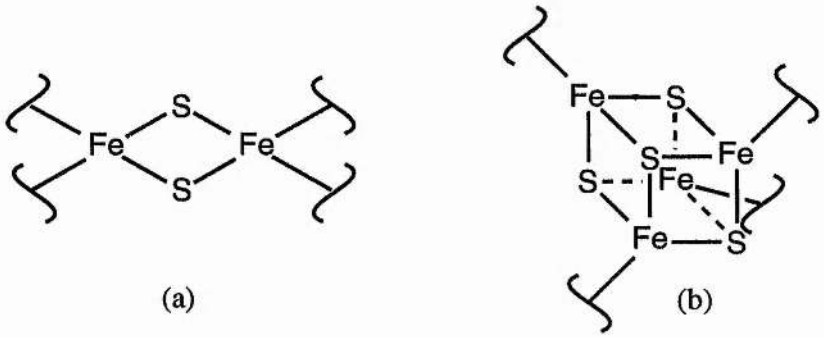


Figure 1.6; The active site of the (a) [2Fe-2S] and (b) [4Fe-4S] proteins

When such iron-sulfur centres react with nitrite, there are three types of possible product, containing $[Fe(SR)_2(NO)_2]^-$, $Fe_2(SR)_2(NO)_4$ and $[Fe_4S_3(NO)_7]^-$ respectively. These three types of iron-sulfur-nitrosyl complexes are readily interconverted under mild conditions[35], so that any biochemical process which gives access to one type, in general, provides access to all three. In fact, these iron-sulfur-nitrosyl complexes are formed extremely readily from a wide range of biochemical sulfur or iron sources with nitrite, under the action either of heat or microwave radiation. Recent work[36] has demonstrated that in the presence of iron(II) salts, nitrite reacts with cysteine, and sources of cysteine including hydrolysed caseins under a range of experimental conditions to give the anti-microbial iron-sulfur-nitrosyl salt, $Na[Fe_4S_3(NO)_7]$ (see Section 1.4). The yield of $Na[Fe_4S_3(NO)_7]$ is increased when sodium ascorbate is also present. The conditions under which these reactions occur are similar to that employed

in the curing process, where sodium nitrite and sodium ascorbate are added to the meat before heating to high temperature. The very facile formation of the tetramer complex $Na[Fe_4S_3(NO)_7]$ has also been shown[36] with a very wide range of sulfur sources thus indicating that no preformed $\{Fe_4S_4\}$ cluster is necessary for the formation of the black anion. The transfer is a self-assembly reaction producing the tetranuclear anion from simple precursors.

However, it has also been shown[37], that similar reactions carried out in the presence of methionine yields the dinuclear iron-nitrosyl complex, $Fe_2(SMe)_2(NO)_4$. The CH_3S group is incorporated intact from methionine into the red methyl ester; this has been proven[37] using methionine-methyl-d_3 in place of methionine, producing $Fe_2(SCD_3)_2(NO)_4$. That the methionine is also the source of sulfur was demonstrated[37] using iron(II) chloride rather than iron(II) sulfate. However, the red methyl ester promotes the tumorigenic activity of environmental carcinogens such as polycyclic aromatic hydrocarbons and N-nitrosamines and is therefore a cause for concern.

The formation of tetranuclear $[Fe_4S_3(NO)_7]^-$ from cysteine and its derivatives[36] and of dinuclear $Fe_2(SMe)_2(NO)_4$ from derivatives of methionine[37] requires cleavage of C-S bonds. Ghadami and Hill have recently shown[38] that such C-S bond cleavage in cystine derivatives is extremely easy under conditions similar to those employed above (see Figure 1.7). A similar cleavage of methionine derivatives would yield MeSH, long known[39] to provide $Fe_2(SMe)_2(NO)_4$ in the presence of iron(II) and either nitrite or NO.

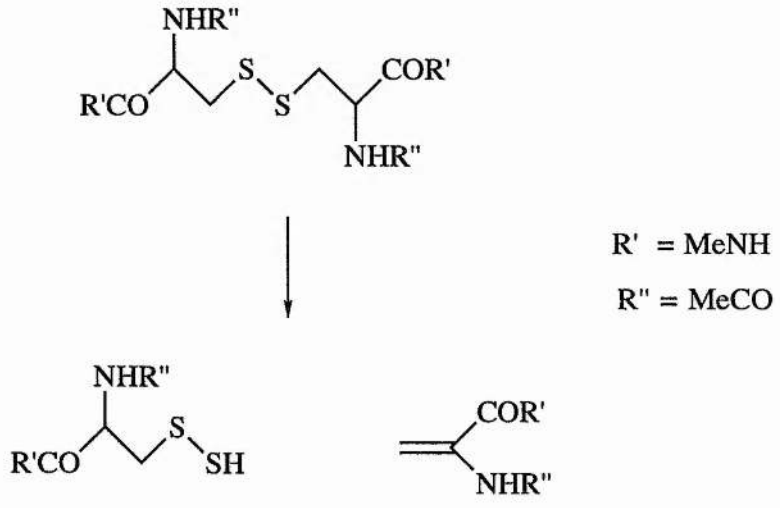


Figure 1.7; Cleavage of C-S bonds in cysteine derivatives

1.3 Chemical Properties and Spectroscopy of Iron-Sulfur-Nitrosyl Complexes

History

Roussin[40] synthesised the first iron-sulfur-nitrosyl cluster in 1858 by addition of iron(II) sulfate to a mixture of sodium nitrite and ammonium sulfide, and suggested that his black complex was related to the nitroprusside ion, $[Fe(CN)_5NO]^{2-}$, which had been described by Playfair[41] only eight years earlier as both complexes had been shown to contain nitric oxide. He also claimed to have interconverted nitroprusside and his new salt, an observation confirmed much later by Glidewell et al[42]. The correct empirical formula was determined using elemental analysis by Pavel[43] in 1882 and the structure of $[Fe_4S_3(NO)_7]^-$ has since been characterised using X-ray crystallography for the caesium[44], tetraphenylarsonium[45] and the trimethylsulfonium[46] salts (see Figure 1.8).

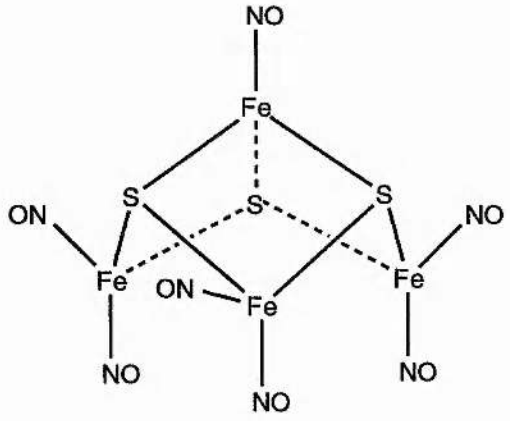


Figure 1.8; The anion of Roussin's black salt

Roussin observed that reaction of $[Fe_4S_3(NO)_7]^-$ with strong alkali yielded a red solution which deposited a red crystalline solid[40] on cooling. The structure of 'Roussin's red salt' was determined only recently as the tetramethylammonium salt[47] of $[Fe_2S_2(NO)_4]^{2-}$ (see Figure 1.9).

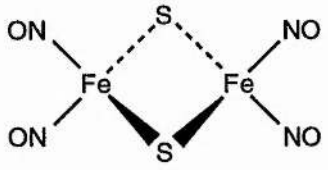


Figure 1.9; The anion of Roussin's red salt

The first organic derivative, $Fe(SEt)_2(NO)_4$, of $[Fe_2S_2(NO)_4]^{2-}$ was prepared by Pavel[43] using an ethyl halide and he again established its correct empirical formula. Subsequent molecular weight determinations on a range of organic derivatives which had been prepared by reaction of thiols and iron(II) salts with nitric oxide[39] showed that these compounds contain dimeric formulas of the type $Fe_2(SR)_2(NO)_4$. Later work[48] confirmed the dimeric nature of $Fe_2(SEt)_2(NO)_4$ and X-ray structure determination showed[49] these complexes to be similar to $[Fe_2S_2(NO)_4]^{2-}$. The diamagnetic nature of both $Fe_2(SR)_2(NO)_4$ and salts containg $[Fe_2S_2(NO)_4]^{2-}$ or $[Fe_4S_3(NO)_7]^-$ was first shown in 1931 by Cambi and Szegö [50].

The stoichiometrically related thiosulfato complex $[Fe_2(S_2O_3)_2(NO)_4]^{2-}$ was first reported by Hofmann and Wiede[39] in 1895 and subsequently confirmed by Manchot[51] and Brauer[52]. The early preparative methods were based on $Fe_2I_2(NO)_4$; but the complex can be prepared more conveniently by reaction of iron(II) salts and thiosulfate with either nitric oxide or sodium nitrite[53]. The crystal structure has been determined only very recently by Lambert[53] and discloses the planar nature of the Fe_2S_2 ring with the pendant SO_3 groups on opposite sides of the ring giving a *trans* conformation, imparting C_{2h} symmetry to the dianion. The same structure is observed for the neutral Roussin esters in the solid state. The thiosulfato anion is diamagnetic and has approximately linear nitrosyl ligands.

The cubane type cluster, $Fe_4S_4(NO)_4$, (see Figure 1.10) was first prepared by Chu, Dahl and Gall[54,55] in 1974 by refluxing $Hg[Fe(CO)_3NO]_2$ and elemental sulfur in toluene. Alternatively, $Na[Fe_4S_3(NO)_7]$ can be refluxed with sulfur under the same conditions to give $Fe_4S_4(NO)_4$ in slightly smaller yields[56]. Conversion of the Fe_4S_3 centre to the Fe_4S_4 centre may proceed either by the addition of one sulfur atom to the existing framework or by fragmentation perhaps to mononuclear intermediates, followed by subsequent reformation of the iron-sulfur skeleton. The mechanistic picture becomes even more complex with the observation that the chemical nature of the counter-ion also seems to have an important role to play, as $(PPh_3NPPh_3)[Fe_4S_3(NO)_7]$ is not converted to the cubane even after refluxing for seven days in toluene[57] and by the observation[58] that reflux of $Na[Fe_4S_3(NO)_7]$ alone provides some $Fe_4S_4(NO)_4$.

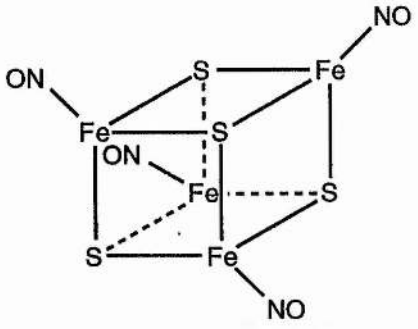


Figure 1.10; Cubane type cluster, $Fe_4S_4(NO)_4$

The triphenylphosphine derivative of 'Roussin's black anion', $[Fe_4S_3(NO)_4(PPh_3)_3]$ was prepared[59] by Scott & Holm in 1993 by refluxing cubane with triphenylphosphine in dichloromethane. The structure is analogous to that of 'Roussin's black anion', where the three equitorial nitrosyl ligands on the basal iron atoms have been replaced with PPh_3 groups. Treatment of $[Fe_4S_3(NO)_4(PPh_3)_3]$ with excess triphenylphosphine in dimethylformamide gave the first prismatic iron-sulfur-nitrosyl complex, $[Fe_6S_6(NO)_6]^{2-}$ as the $[Fe(DMF)_6]^{2+}$ salt (see Figure 1.11).

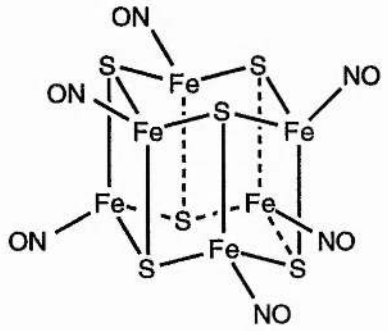


Figure 1.11; Prismane type structure, $[Fe_6S_6(NO)_6]^{2-}$

X-Ray Crystal Structures

The first X-ray studies carried out on iron-sulfur-nitrosyl complexes were made primarily to end speculation and to determine precisely their constituent atoms. The structure of Roussin's black anion $[Fe_4S_3(NO)_7]^-$ (see Figure 1.12) has been determined for the caesium salt[44], the tetraphenylarsonium salt[45] and the trimethylsulfonium salt[46]. In all three samples thus studied the anion shows almost identical dimensions and the same approximate C_{3v} symmetry. The anion consists of a flattened Fe_4 tetrahedron, three faces of which are triply bridged by sulfur atoms. The unique apical iron atom is coordinated to one NO^+ ligand and each basal iron atom is coordinated to one axial (ie approximately parallel to the symmetry axis) and one equatorial (ie approximately perpendicular to the symmetry axis) NO^+ ligand. The

average Fe_a-Fe_b distance is 2.700Å and the Fe_b-Fe_b distance is 3.570Å. The average Fe_a-S bond length is 2.206Å and the average Fe_b-S bond length is 2.258Å. All of the Fe-N-O groups are approximately linear.

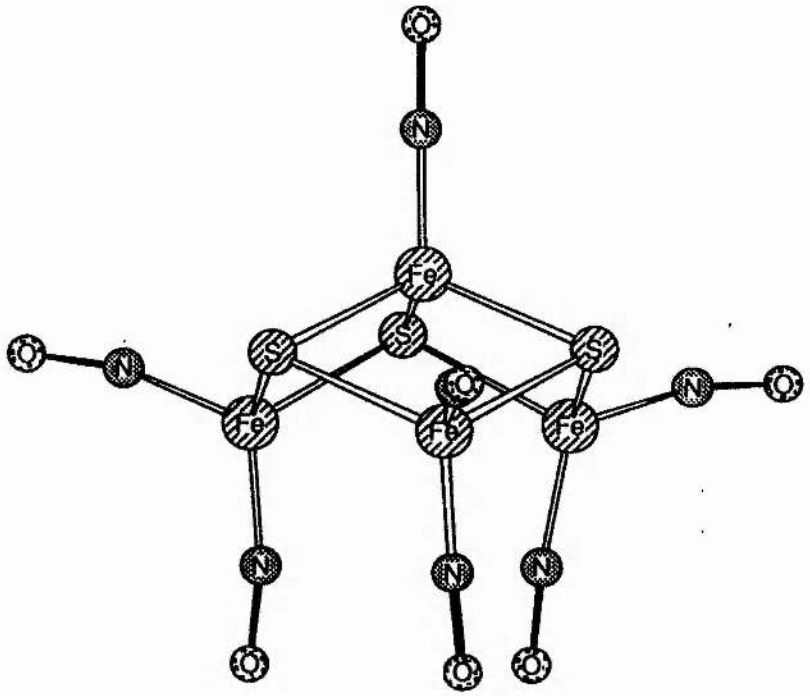


Figure 1.12; Structure of Roussin's black anion, $[Fe_4S_3(NO)_7]^-$

The crystal structure of Roussin's red anion $[Fe_2S_2(NO)_4]^{2-}$ (see Figure 1.13) has been determined only recently as the tetramethylammonium salt[47] which contains two crystallographically distinct anions in the asymmetric unit. Each anion exhibits approximate D_{2h} symmetry, although only C_i crystallographic symmetry. Thus the Fe_2S_2 fragment is planar, with an average Fe-S distance of 2.244Å, which is very similar to the Fe_b-S distance observed in $[Fe_4S_3(NO)_7]^-$. Likewise the Fe-Fe distance of 2.714Å in $[Fe_2S_2(NO)_4]^{2-}$ is very similar to the Fe_a-Fe_b distance in $[Fe_4S_3(NO)_7]^-$. Again the Fe-N-O groups are approximately linear. If the sulfur atoms of the dianion have R groups attached to give the neutral molecule $Fe_2(SR)_2(NO)_4$, then the molecular symmetry may become C_{2v} or C_{2h} depending on the nature of the R group. The X-ray structural analysis carried out on a range of $Fe_2(SR)_2(NO)_4$ complexes (R = Me[60], Et[49], *n*-C_5H_{11}[60], Me_3C[60], $HgCH_3$[61]) all exhibit only C_{2h} symmetry in the solid state, with no sign of the C_{2v} syn isomers.

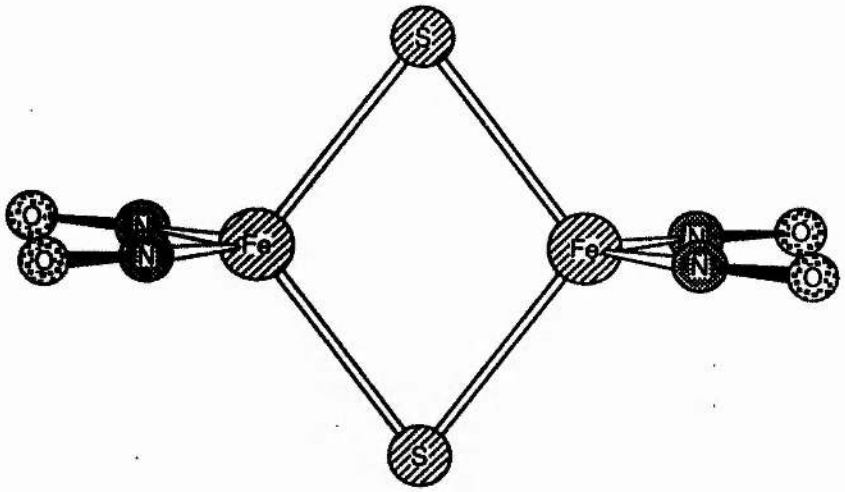


Figure 1.13; Structure of Roussin's red anion, $[Fe_2S_2(NO)_4]^{2-}$

Crystallographic studies[54] carried out on the neutral cubane type cluster $Fe_4S_4(NO)_4$ indicate that the iron atoms form an almost perfect tetrahedron. Each triangular iron face is triply bridged by a sulfur atom (see Figure 1.14). The Fe-Fe distances of 2.649Å are slightly shorter than those found in $[Fe_4S_3(NO)_7]^-$ and $Fe_2(SR)_2(NO)_4$ and the twelve chemically equivalent Fe-S bond lengths vary from only 2.208 to 2.224Å, with a mean value of 2.217Å. The S-S distance is longer than the Fe-Fe distance so as to drastically distort the structure from being a cube to being more a pair of intersecting tetrahedra. The Fe-S-NO groups are all approximately linear.

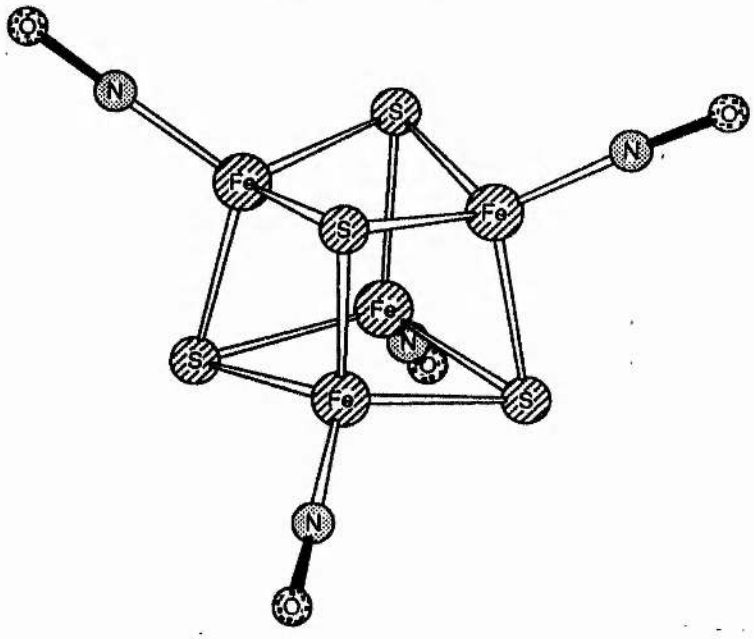


Figure 1.14; Structure of the cubane-type $Fe_4S_4(NO)_4$

The most recent iron-sulfur-nitrosyl complex to be synthesised and characterised is the $[Fe_6S_6(NO)_6]^{2-}$ dianion[59] which X-ray determination shows to consist of a triangular iron antiprism where each iron atom is triply bridged by a sulphur atom (see Figure 1.15). The mean Fe-S bond length is 2.221Å and the Fe-N-O groups are all approximately linear.

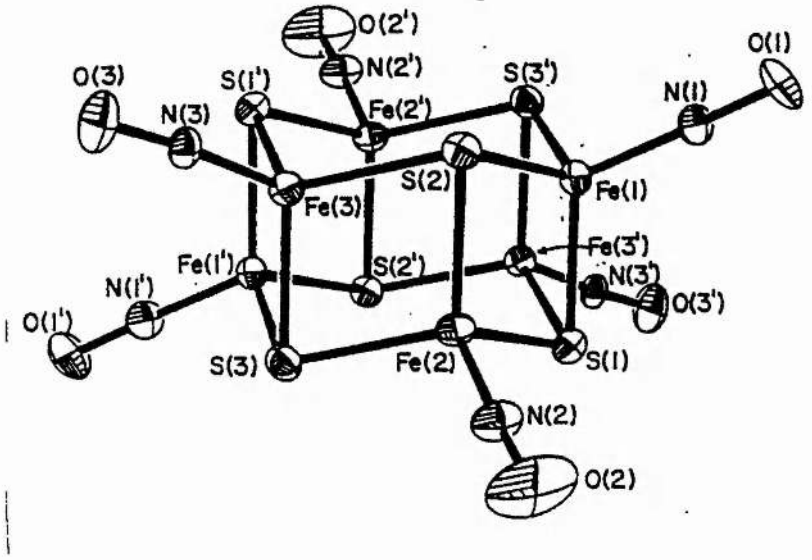
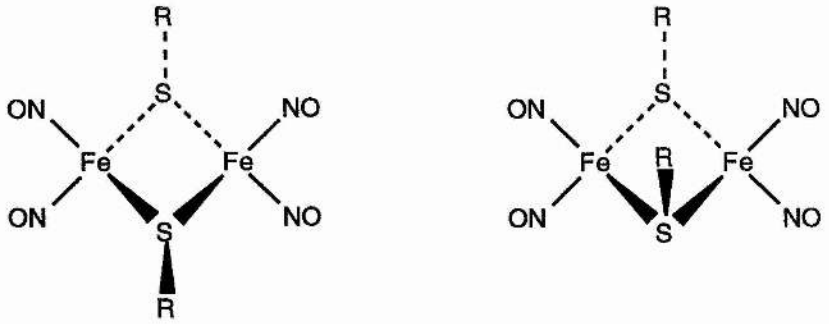


Figure 1.15; Structure of the prismane-type $[Fe_6S_6(NO)_6]^{2-}$

NMR Spectroscopy

Glidewell and Johnson[62] carried out ^1H NMR studies on solutions of $Fe_2(SR)_2(NO)_4$ complexes in non coordinating solvents for a wide range of organic substituents R (R = Me, Et, *n*-Pr, *n*-Bu, *i*-Bu, *t*-Bu, Ph and $PhCH_2$). For all complexes, they found the presence of two isomeric forms: these spectra have been interpreted in terms of the syn (in which the R groups are found on the same side of the ring) and anti (in which the R groups are on opposite sides of the ring) forms (see Figure 1.16), although the ^1H NMR spectra do not establish these forms. For all substituents except R = *t*-Bu[63], the isomers are present in virtually equal abundance, which suggests that they have approximately the same energy. When R = *t*-Bu, the isomer ratio is 1:3 in favour of the syn isomer which has been rigorously established by ^{15}N NMR (see below). The existence of two forms has been confirmed by ^{13}C NMR in a number of cases[56,62].

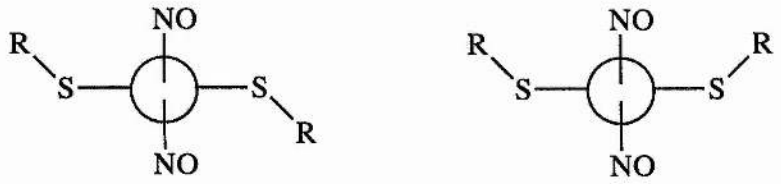


Anti Isomer, C_{2h} Syn Isomer, C_{2v}

Figure 1.16; Isomeric forms of $Fe_2(SR)_2(NO)_4$

Both naturally occurring isotopes of nitrogen, ^{14}N (99.635% abundance) and ^{15}N (0.365% abundance) have non-zero spin and are therefore capable of being investigated by NMR. ^{15}N has nuclear spin I = 1/2 and is capable of producing sharp, well defined peaks for NMR. However, due to the low natural abundance of ^{15}N, for study by ^{15}N NMR, samples must be enriched to 99% in ^{15}N. This is done by preparing the complexes using isotopically enriched starting materials such as $Na^{15}NO_2$. Enrichment allows spectra to be accumulated in a realistic time scale. In contrast, ^{14}N has nuclear spin I = 1 and consequently possesses a quadrupole moment. This leads to line broadening due to shortened relaxation time and therefore no resolvable coupling between nuclei and consequently no line splitting, so less chemical information is yielded. If the nucleus is surrounded by a highly symmetrical arrangement of substituents however, line broadening is diminished.

Every complex of the type $Fe_2(SR)_2(NO)_4$, in non coordinating solvents gives a ^{15}N NMR spectrum consisting of a singlet and a pair of doublets[63,64,65] which correspond to the anti and syn isomers respectively. All the nitrosyl ligands are equivalent in the C_{2h} anti isomer so only a singlet is observed. In the syn isomer, although the two irons are equivalent and the two R groups are equivalent, the two nitrosyl ligands in each $Fe(NO)_2$ fragment are non equivalent in C_{2v} symmetry (see Figure 1.17) and so the coupling $^2J(^{15}NFe^{15}N)$ between the ^{15}N nuclei gives the observed pair of doublets in an AX pattern. ^{15}N NMR spectroscopy also confirms that the Fe-N-O groups of $Fe_2(SR)_2(NO)_4$ are linear; bent NO ligands are characteristically deshielded by up to 450 ppm relative to linear NO ligands[66,67,68].



(a) Anti isomer (b) Syn isomer

Figure 1.17; Newman projections of syn and anti isomers of $Fe_2(SR)_2(NO)_4$.

As expected the ^{15}N spectrum of $[Fe_2S_2(NO)_4]^{2-}$ consists of a singlet as all the nitrosyl ligands are equivalent[61]. If however, recording of the spectrum is attempted in dichloromethane solution, only the $[Fe_4S_3(NO)_7]^-$ spectrum is observed[62,65].

The C_{3v} structure of Roussin's black anion, $[Fe_4S_3(NO)_7]^-$ found[44,45] by X-ray analysis has three distinct nitrosyl environments in the ratio 1:3:3. The unique apical nitrosyl ligand is observed as a singlet with relative intensity one, while the two basal nitrosyl ligands which can be described as axial and equatorial from each of the three $Fe(NO)_2$ fragments consists of a pair of doublets, each of relative intensity three, forming an AX system. The ^{15}N chemical shifts are characteristic of linear Fe-N-O groups. The ^{14}N NMR spectrum of Roussin's black anion however only shows the presence of three broad singlets in the ratio 1:3:3 due to the line broadening obscuring any coupling (see Figure 1.18).

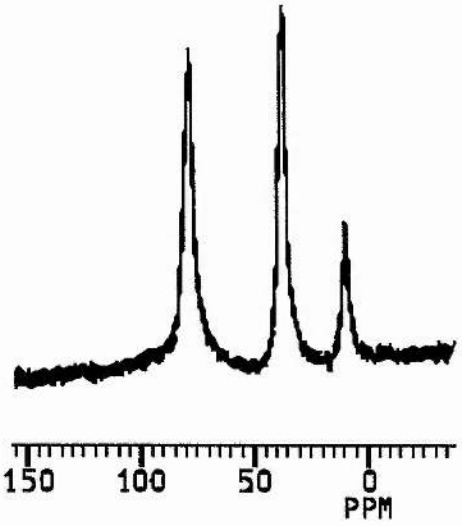


Figure 1.18; ^{14}N NMR spectrum of $[Fe_4S_3(NO)_7]^-$

Chemical Reactivity

Reactions with Nucleophiles

Although the ^1H NMR spectra of solutions of $Fe_2(SR)_2(NO)_4$ in a range of non-coordinating solvents showed sharp peaks demonstrating the presence of two isomeric forms[69] (see Figure 1.17), only very broad, poorly resolved ^1H resonances were observed for solutions of $Fe_2(SMe)_2(NO)_4$ in the polar solvents DMF and DMSO. Examination of the $Fe_2(SMe)_2(NO)_4$ and $Fe(SPr\text{-}i)_2(NO)_4$ complexes using ESR[35] confirmed the presence of two paramagnetic species, $[Fe(NO)_2(L)_2]^+$ (L = solvent) in which both RS^- groups have been displaced, and $Fe(NO)_2(SR)L$, in which one SR group remains bound to the iron. Typically for these complexes, a five line spectrum corresponding to a $[Fe(NO)_2]^+$ fragment is obtained and in general, π-acceptor solvents form complexes of the type $[Fe(NO)_2(L)_2]^+$, while solvents with little or no π-bonding capacity as ligands form neutral solvocomplexes of the type $Fe(NO)_2(SR)(L)$. Solutions of alkyl esters in DMF or DMA yields both possible products.

By the use of a stronger nucleophile, RS^-, again in the solvent DMF, the retention of both RS^- groups in a mononuclear complex can be obtained[35]. Alternatively, reacting $Fe_2(SMe)_2(NO)_4$ with RSH in a basic solution also yields $[Fe(NO)_2(SR)_2]^-$.

$$Fe_2(SR)_2(NO)_4 + 2RSH \xrightarrow{\text{base}} 2[Fe(SR)_2(NO)_2]^-$$

Addition of a different thiol R'SH to a dilute solution of $Fe_2(SR)_2(NO)_4$ in base, or addition of an excess of $R'S^-$ to a DMF solution of $Fe_2(SR)_2(NO)_4$ always results in the ligand-exchanged complex $[Fe(NO)_2(SR')_2]^-$.

$$Fe_2(SR)_2(NO)_4 + 2R'SH \xrightarrow{\text{base}} 2[Fe(SR')_2(NO)_2]^-$$

The observation that many reactions of $Fe_2(SR)_2(NO)_4$ with nucleophiles will cleave it into mononuclear dinitrosyl complexes led to a whole range of similar reactions of $Fe_2(SR)_2(NO)_4$ with other nucleophiles. The nucleophiles, Y^-, so far investigated[35,64,70,71] include $Y = Br^-$, I^-, NCO^-, NCS^-, NO_2^-, and $[MoS_4]^{2-}$ which yields the dimetallic complex[71] $[Fe(NO)_2(S_2MoS_2)]^-$ (See Figure 1.19). In each case the incoming nucleophile binds directly to the iron, unlike reactions with nitroprusside[72,73], where incoming nucleophiles bind to the nitrogen atom of the nitrosyl ligand[65,74-81]. A possible exception is the sulfite anion, SO_3^{2-}, which may bind to the oxygen at the nitrosyl ligand[82-84],although evidence for this is not conclusive. Empirical calculations have been described[75,85,86] which indicate that metal-nitrosyl complexes having $\nu(NO) > 1880\ cm^{-1}$ undergo addition of nucleophiles

to the nitrogen ligand; that complexes having 1880 cm^{-1} > ν(NO) > 1800 cm^{-1} undergo no reactions with nucleophiles; and that complexes having 1800 cm^{-1} > ν(NO) undergo addition of nucleophiles to the metal. The examples of nitroprusside and alkyl ester reactions fall neatly into these categories; ν(NO) for $[Fe(CN)_5(NO)]^{2-}$ is 1938 cm^{-1} and ν(NO) for $Fe_2(SR)_2(NO)_4$ is always below 1800 cm^{-1}.

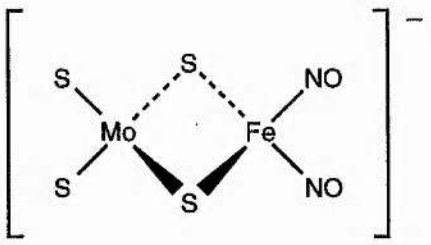


Figure 1.19; $[Fe(NO)_2(S_2MoS_2)]^-$

Although aqueous solutions of Roussin's black anion, $[Fe_4S_3(NO)_7]^-$ give no ESR spectra at neutral pH, it was observed that raising the pH gave a weak 3-line spectrum indicative of a mononuclear nitrosyl species[87]. However in DMF solution, a complex ESR spectrum[35] indicative of at least three paramagnetic species is observed, which are presumed to contain solvocomplexes. In the presence of RS^- however, a new spectrum is observed in DMF solution, indicating the presence of $[Fe(NO)_2(SR)_2]^-$ together with the mononitrosyl species, $[Fe(NO)(SR)_3]^-$.

$$[Fe_4S_3(NO)_7]^- \xrightarrow{9RS^-} 3[Fe(SR)_2(NO)_2]^- + [Fe(SR)_3(NO)]^- + 3S^-$$

Since the formal oxidation states of iron in $[Fe(NO)_2(SR)_2]^-$ and $[Fe(NO)(SR)_3]^-$ are Fe(-I) and Fe(I) respectively, it is likely that $[Fe(NO)_2(SR)_2]^-$ arises from the basal iron atoms in $[Fe_4S_3(NO)_7]^-$ and that $[Fe(NO)(SR)_3]^-$ arises from the apical iron.

The ESR spectrum of a mixture of $[Fe_4S_3(NO)_7]^-$ and $[Me_2NCS_2]^-$ in DMF showed the presence of $Fe(NO)(S_2CNMe_2)_2$ as the sole paramagnetic complex detectable[35]. This mononitrosyl complex was isolated in 88% yield based on total iron indicating that not only the apical Fe(NO) group, but also the basal $Fe(NO)_2$ groups in $[Fe_4S_3(NO)_7]^-$ are incorporated into the product. In contrast, complete conversion of all the iron-nitrosyl fragments in $[Fe_4S_3(NO)_7]^-$ to $Fe(NO)_2$ groups is effected by reaction of $Na[Fe_4S_3(NO)_7].H_2O$ with sodium nitrite in DMF, yielding $[Fe(SH)_2(NO)_2]^-$, but no mononitrosyl complexes. With $[MoS_4]^{2-}$ in DMF solution, $[Fe_4S_3(NO)_7]^-$ gives three paramagnetic products[71], $[Fe(NO)_2(SH)_2]^-$, $[Fe(NO)(SH)_3]^-$ and $[Fe(NO)(S_2MoS_2)_2]^{2-}$. From reactions such as these, the conclusion[35] has been drawn that mononitrosyliron fragments $\{Fe(NO)\}^7$ (where 7 is equal to the number of d electrons on the metal plus the number of NO electrons in excess of those on NO^+) are

favoured in the presence of chelating ligands such as $[Me_2NCS_2]^-$ or $[MoS_4]^{2-}$ but the dinitrosyliron fragments $\{Fe(NO)_2\}^9$ are favoured by the nonchelating ligands RS^-.

The formation of mononuclear complexes[35] from $Fe_4S_4(NO)_4$ is broadly similar to formation from $[Fe_4S_3(NO)_7]^-$. In DMF solution, $Fe_4S_4(NO)_4$ alone yields a weak ESR spectrum indicative of the presence of at least three paramagnetic centres. Addition of RS^- yields the mononitrosyl complex $[Fe(SR)_3NO]^-$ which converts to give the corresponding dinitrosyl complex $[Fe(SR)_2(NO)_2]^-$.

$$Fe_4S_4(NO)_4 \xrightarrow{12RS^-} 4[Fe(SR)_3NO]^- + 4S^{2-}$$

$$2[Fe(SR)_3NO]^- \rightleftharpoons [Fe(SR)_2(NO)_2]^- + [Fe(SR)_4]^-$$

Dissolved mononuclear complexes can be encouraged to dimerise by the use of a non-polar solvent, thus on addition of a large volume of benzene or toluene, the green $[Fe(SR)_2(NO)_2]^-$ is converted to the red $Fe_2(SR)_2(NO)_4$.

$$2[Fe(SR)_2(NO)_2]^- \underset{\text{solvent}}{\overset{\text{non-polar}}{\rightleftharpoons}} 2RS^- + Fe_2(SR)_2(NO)_4$$

This process is effectively the reverse of what happens when $Fe_2(SR)_2(NO)_4$ is placed in DMF in the presence of RS^-. However in the presence of the chelating ligand $[Me_2NCS_2]^-$ the mononitrosyliron complex $Fe(NO)(S_2CNMe)_2$ is formed exclusively from $Fe_4S_4(NO)_4$.

Reactions with Electrophiles

The most important reactions of electrophiles with iron-sulfur complexes involves $[Fe_2S_2(NO)_4]^{2-}$, acting as the nucleophile, and molecular halides, RX (R = Me[43,88,89], Et[43,89], C_3H_5 (allyl)[89], $PhCH_2$[89,90], $HC{\equiv}CCH_2$, Me_3SiCH_2 and $CH_3C(O)CH_2$[89]). In all cases, the product obtained was $Fe_2(SR)_2(NO)_4$. Organometallic halides are also good electrophiles and thus by use of Ph_3SnCl, Me_3SnBr, PhHgCl[89] or CH_3HgCl[61], the corresponding products of type $Fe_2(SMR_x)_2(NO)_4$ were obtained as reddish crystalline solids.

Attempts at reactions[89] with inorganic *gem*-dihalides, Me_2SnCl_2 and Ph_2PbCl_2, have so far proved unsuccessful, probably due to the long S···S nonbonded distance in the dianion which does not allow small metal centres to chelate efficiently. The size of the metal is also a limitation in the reaction of *cis* dihalide complexes with $[Fe_2S_2(NO)_4]^{2-}$, in that although reaction[91] with *cis*$[(Ph_3P)_2PtCl_2]$ provides $[Fe_2\{S_2Pt(PPh_3)_2\}(NO)_4]$ in high yield, attempts[89] to prepare analogous complexes

containing the smaller homologues nickel and palladium failed. In contrast to the formation of the platinum complex, which is simply a nucleophilic displacement of chloride by the sulfur in $[Fe_2S_2(NO)_4]^{2-}$, reaction[89] with the cobalt gem halides, $[\eta^5\text{-}C_5R_5Co(CO)I_2]$ (R = H or Me) yielded the novel trinuclear cluster $[Fe(\mu_3\text{-}S)_2\{Co(\eta^5\text{-}C_5R_5)_2\}(NO)_2]$ (see Figure 1.20). For successful reaction of $[Fe_2S_2(NO)_4]^{2-}$ at transition metal centres, a strongly electrophilic metal is required; thus while no reaction occured with $[\eta^5\text{-}C_5H_5Fe(CO)_2Br]$, the more electrophilic $[\eta^5\text{-}C_5H_5Fe(CO)_2(THF)]BF_4$ reacted to give $[Fe_2\{SFe(CO)_2(\eta^5\text{-}C_5H_5)\}_2(NO)_4]$ in high yield. The proton as an electrophile[88], such as with CF_3COOH or HBF_4, yields $Fe_2(SH)_2(NO)_4$ which decomposes to give $[Fe_4S_3(NO)_7]^-$.

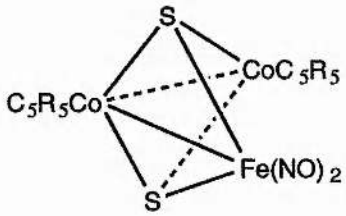


Figure 1.20; $[Fe(\mu_3\text{-}S)_2\{Co(\eta^5\text{-}C_5R_5)_2\}(NO)_2]$ (R = H or Me)

Powerful electrophiles are required for reaction with the tetranuclear $[Fe_4S_3(NO)_7]^-$; thus while there is no reaction with weakly electrophilic reagents such as halogenoalkanes, reaction does occur[46] with the arenediazonium tetrafluoroborates, $p\text{-}XC_6H_4N_2^+BF_4^-$ (X = H, Me, F, Cl, MeO, MeCO, CN or NO_2) to yield the corresponding $Fe_2(SR)_2(NO)_4$. Alkylation[46] of $Na[Fe_4S_3(NO)_7]$ with $R_3O^+BF_4^-$ (R = Me or Et) yields $Fe_2(SR)_2(NO)_4$; hence the sulfur of $[Fe_4S_3(NO)_7]^-$ is again presumably acting as the nucleophilic centre towards R_3O^+, with further steps to change the nuclearity.

Redox Reactions

Roussin[40] carried out the first redox reactions with iron-sulfur-nitrosyl complexes when he claimed to have interconverted his black salt and nitroprusside. More than 100 years later his work was confirmed[42] by reacting an aqueous solution of $[Fe_4S_3(NO)_7]^-$ with excess CN^- to give essentially quantitative conversion to $[Fe(CN)_5NO]^{2-}$, which over a period of time proceeds further to give $Fe(CN)_6^{4-}$. The nitroprusside ion can be converted back to $[Fe_4S_3(NO)_7]^-$ by treatment with H_2S in hot solution. Furthermore, Roussin's black and red salts are themselves interconvertible[40,52]; treatment of $[Fe_4S_3(NO)_7]^-$ with base yields $[Fe_2S_2(NO)_4]^{2-}$ but under acidic conditions, the reverse reaction occurs.

Cyclic voltammetry studies[54] of $Fe_4S_4(NO)_4$ show two reversible, one-electron waves, indicating the existence of both the monoanion and the dianion. The monoanion, $[Fe_4S_4(NO)_4]^-$, was isolated by reduction of the neutral parent with potassium benzophenone in the presence of 2,2,2-cryptand which acts as a macrocyclic inclusion ligand. In like manner[92], cyclic voltammetry indicates a one-electron reduction of $[Fe_4S_2(NO)_4(NCMe_3)_2]$, and by use of sodium amalgam as reductant, the monoanion, $[Fe_4S_2(NO)_4(NCMe_3)_2]^-$ was isolated as the $[Ph_3PNPPh_3]^+$ salt.

Solutions of the neutral complexes, $Fe_2(SR)_2(NO)_4$ (R = Me, Et, *n*-Pr, *i*-Pr, *n*-Bu, *s*-Bu, *t*-Bu and CH_2COOCH_3) in THF all undergo reversible reduction using cyclic voltammetry to give the mono and dianions[93]. The paramagnetic complexes $[Fe_2(SR)_2(NO)_4]^-$ can also be prepared by chemical reduction of neutral $Fe_2(SR)_2(NO)_4$ with sodium or $Li(BHEt_3)$. In every case a green solution exhibiting a symmetric nine-line ESR spectrum was observed. The dianion $[Fe_2S_2(NO)_4]^{2-}$ also exhibits[93] two reversible one-electron reductions, but for $[Fe_2(S_2O_3)_2(NO)_4]^{2-}$ only the first electron reduction was reversible; subsequent further reduction was wholly irreversible. For the tetranuclear complex, $[Fe_4S_3(NO)_7]^-$, three reversible one-electron reductions to yield $[Fe_4S_3(NO)_7]^{x-}$ (x = 2, 3 and 4) occur, but the oxidation reactions are complex and irreversible[93].

Not only can nitrosyl groups be transferred to preformed iron-sulfur structures[42], but they can be transferred from these structures to other atoms. The ready nitrosation of a range of secondary amines by $Fe_2(SMe)_2(NO)_4$ has been shown for a range of secondary amines[94,95]. Studies[58,96] on morpholine and pyrrolidine show that although solutions of these amines underwent slow nitrosylation by $Fe_2(SMe)_2(NO)_4$ in air, under anaerobic conditions solutions of the amines in coordinating solvents were necessary for nitrosylation to occur, indicating that the rate of nitrosyl transfer may be higher where solvocomplexes are present. Attempted nitrosation of secondary amines by the tetranuclear cluster $[Fe_4S_3(NO)_7]^-$ has so far proved unsuccessful[58].

1.4 Biological Role of Iron-Sulfur-Nitrosyl Complexes

Iron-sulfur-nitrosyl complexes can act as anti-microbial agents, carcinogens or as vasodilators. Each of these three possible biological roles will be discussed separately in this section.

Antimicrobial Activity

It has been known for many years that $K[Fe_4S_3(NO)_7]$ exhibits antibacterial action against a range of microorganisms, including both aerobic and anaerobic types[97-99] and that its antiseptic action[100] provides a good disinfectant of contaminated water.

The mode of action by which nitrite inhibits the development of *Clostridium* species and other food spoilage organisms in preserved foodstuffs has been the subject of investigation for a number of years[100,101]. A clue as to the anti-*Clostridial* action of nitrite comes from the Perigo effect[102] - the greater, but almost pH independent inhibition of growth found after heating in a laboratory medium, even with no residual nitrite present. This is believed to be due to formation from nitrite to more powerful inhibitors often termed Perigo type factors (PTFs). While a number of chemical species have been suggested as candidates for PTFs, including S-nitrosocysteine, Roussin's black anion $[Fe_4S_3(NO)_7]^-$, Roussin's red anion $[Fe_2S_2(NO)_4]^{2-}$ and the paramagnetic cysteinato complex (see Figure 1.21), none of these have yet been proved to be a PTF.

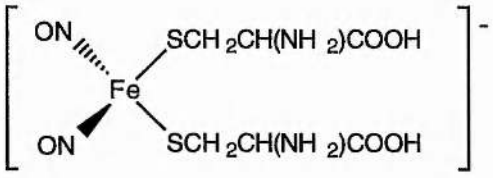


Figure 1.21; Cysteinato complex

Work supporting the case of $[Fe_4S_3(NO)_7]^-$ as a PTF has shown[103] that reactions of aqueous nitrite with synthetic models for both the [2Fe-2S] and [4Fe-4S] centres of redox proteins (see Figure 1.22) readily gave the complex $[Fe(SH)_2(NO)_2]^-$, identifiable by ESR spectroscopy, as an intermediate in the formation of the tetra-iron complex $[Fe_4S_3(NO)_7]^-$. The complex $[Fe_4S_3(NO)_7]^-$ was shown[104-106] to inhibit *Clostridium* species and to be formed[106] when nitrite was autoclaved with a test medium containing iron(II) sulfate and cysteine. It was also shown[106] that neither S-nitrosocysteine nor $[Fe_2S_2(NO)_4]^{2-}$ could be produced under the same conditions which produced $[Fe_4S_3(NO)_7]^-$. A complex isolated from a culture medium of acid-hydrolysed casein, ascorbate and nitrite was shown[106] chemically and

spectroscopically to have a very close resemblance to an alkali metal salt of $[Fe_4S_3(NO)_7]^-$ although its identity was not proven.

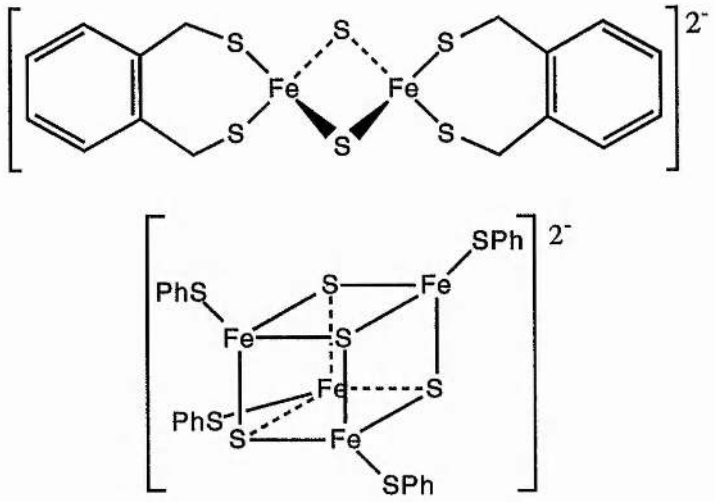


Figure 1.22; Synthetic models for [2Fe-2S] and [4Fe-4S] iron-sulfur centres

Although these observations show that $[Fe_4S_3(NO)_7]^-$ both acts as a *Clostridial* inhibitor and can be isolated from nitrite-treated proteins, it does not mean that the intact anion is the active species. Indeed complexes of the type $[Fe(SR)_2(NO)_2]^-$ which can be formed from the action of nitrite upon preformed iron-sulfur clusters[103,107] and from intact[35] $[Fe_4S_3(NO)_7]^-$ which may well be the active species. Further supporting this view, it has been found that both[108] $[Fe_4S_3(NO)_7]^-$ and the paramagnetic cysteinato complex, $[Fe(NO)_2(SCH_2CHNH_2COOH)_2]$ were inhibitors of *Clostridium sporogenes* in culture medium.

The very ready formation of $[Fe(SR)_2(NO)_2]^-$ species both *in vivo* and *in vitro*[107,109-113] and the central role that such complexes play in the reaction chemistry of iron-sulfur-nitrosyl complexes suggests that the antimicrobial action of nitrite depends not only on the disruption of respiration (by destruction of the natural iron-sulfur centres of redox proteins[107]) but also specifically upon the formation of $[Fe(SR)_2(NO)_2]^-$ type species, whose exact role is, as yet, undefined.

Cancerous States

Iron-centred paramagnetic complexes of the type $[Fe(NO)_2X_2]^{x+}$ formed by reactions between iron salts and nitric oxide in the presence of anionic ligands exhibited a g value of 2.03 in the ESR spectrum[87]. Further work in this area extended the range of anionic ligands to include halides and pseudohalides[114], alcohols and alkoxides[115], mercaptides[116,117] and mercaptopurines and mercaptopyrimidines[118]. Similar paramagnetic iron-nitrosyl complexes have been identified in extracts of the livers of

rats fed on known chemical carcinogens and nitrate solution. ESR signals at g = 2.035 from rat liver extracts were observed within 5 to 45 days after feeding rats with the carcinogen, aminoacetylfluorine[109]; liver tumours did not actually appear for 4 to 12 months. The tumours themselves were ESR silent and the signal at 2.035 did not appear in the absence of nitrate. However, the identification of the g = 2.03 species as being of general type $[Fe(NO)_2X_2]^{x+}$ was far from definitive. However these assignments are supported by the definitive characterisation[35] of $[Fe(NO)_2(SR)_2]^-$ from $Fe_2(SMe)_2(NO)_4$ and the demonstration of such g = 2.03 complexes from synthetic and natural $[Fe_2S_2(SR)_4]^{2-}$ and $[Fe_4S_4(SR)_4]^{2-}$ complexes under mild conditions[35,103]. Thus the paramagnetic monoiron complexes are readily formed *in vivo*[107,109-113] and are associated with cancerous states in laboratory animals, although this association does not in itself imply any causal relationship.

A study[119] of the geographical variation in the occurrence throughout China of a wide variety of cancers indicated a particularly high, but very localised, level of oesophageal cancer in the Linxian valley in northern China[119,120]. Investigation of the local diet demonstrated the high consumption of pickled vegetables, prepared by storage underwater for several months, before eating them along with their covering of white fungus, *Geotrichum candidum*. The water supply in the region was found to be rather high in nitrate and nitrite. Extracts of these preserved vegetables were found to induce stomach cancers in mice and rats[121], and over half of Linxian vegetables preserved in this way contained the dinuclear $Fe_2(SMe)_2(NO)_4$ species[122]. It is interesting to note that in urban Beijing, vegetables preserved in the same way contained much less $Fe_2(SMe)_2(NO)_4$ and the incidence of oesophageal cancer is correspondingly low[122]. When employed alone, the complex $Fe_2(SMe)_2(NO)_4$ displays only weak mutagenic properties[121,123-125], but it acts as a nitrosating agent of secondary amines to form N-nitrosamines[96,126].

Studies[127] using parsley, *Petroselinum crispum*, contaminated with *Geotrichum candidum* in the presence of nitrite led to the formation and extraction of $Fe_2(SMe)_2(NO)_4$. However, the presence of *Geotrichum candidum* does not appear to be significant, as samples containing nitrite gave $Fe_2(SMe)_2(NO)_4$ whether or not inoculated with the culture: on the other hand, in the absence of added nitrite no $Fe_2(SMe)_2(NO)_4$ was detected even in the presence of *Geotrichum candidum*. It is thus likely that formation of $Fe_2(SMe)_2(NO)_4$ observed depends upon nitrosylation of the iron-sulfur clusters in the parsley ferredoxins for which a biosynthetic route has been proposed[57] (see Figure 1.23).

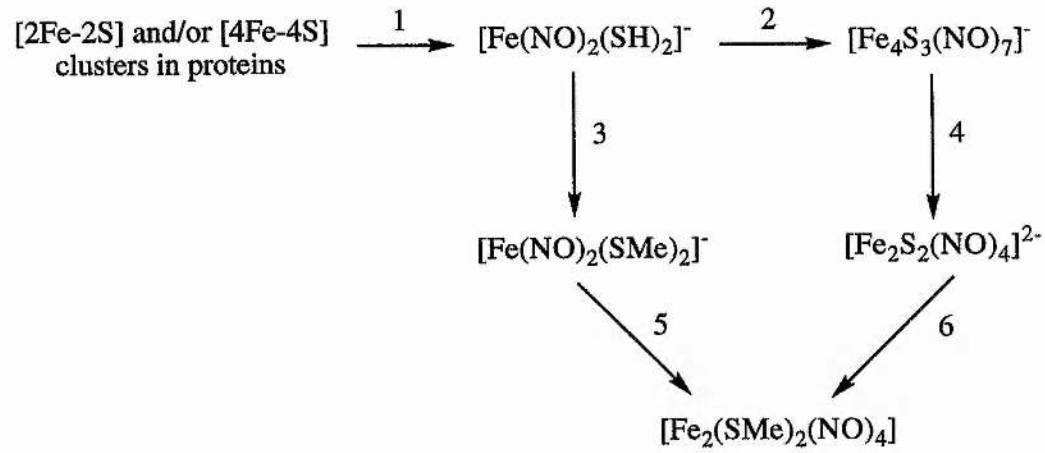


Figure 1.23; Possible biosynthetic pathways to $Fe_2(SMe)_2(NO)_4$

The formation of $[Fe(NO)_2(SH)_2]^-$ by reaction of nitrite with preformed iron-sulfur clusters in the first step has been shown to proceed readily[103,107]. In the second step, the paramagnetic species has been shown[35] to be a direct precursor of $[Fe_4S_3(NO)_7]^-$. Conversion to $[Fe_2S_2(NO)_4]^{2-}$, step 4, normally requires high pH[39,41,52,64], but enzyme-mediated sulfur transfer looks to be an attractive alternative. The methylation of $[Fe(NO)_2(SH)_2]^-$ to provide $[Fe(NO)_2(SMe)_2]^-$, step 3, has not yet been investigated. Similarly, the methylation of $[Fe_2S_2(NO)_4]^{2-}$, step 6, has been demonstrated by methylation by methyl halides[41,89], but not by biological methyl transfer, e.g., from methionine or S-adenosylmethionine. Recent work[37] has shown that MeS is transferred intact from methionine to $Fe_2(SMe)_2(NO)_4$. Conversion of $[Fe(NO)_2(SMe)_2]^-$ to $Fe_2(SMe)_2(NO)_4$, step 5, occurs spontaneously under the correct conditions of polarity/polarizability[35]. The most likely external source of NO for these reactions in biological systems is nitrite, introduced directly or as nitrate.

Vasodilatory Action

Muscle tissue can be divided into three types; smooth, striated and cardiac. Smooth muscle is present as sheets surrounding hollow organs and tubes, most significantly blood vessels, which have autonomous contractile properties. However, sustained contraction of vascular smooth muscle without compensation elsewhere in the body can lead to high blood pressure (hypertension). The relaxation of smooth muscle is a positive process and is brought about by the activation of the enzyme guanylate cyclase which converts guanosine triphosphate into cyclic guanosine monophosphate (see Figure 1.24).

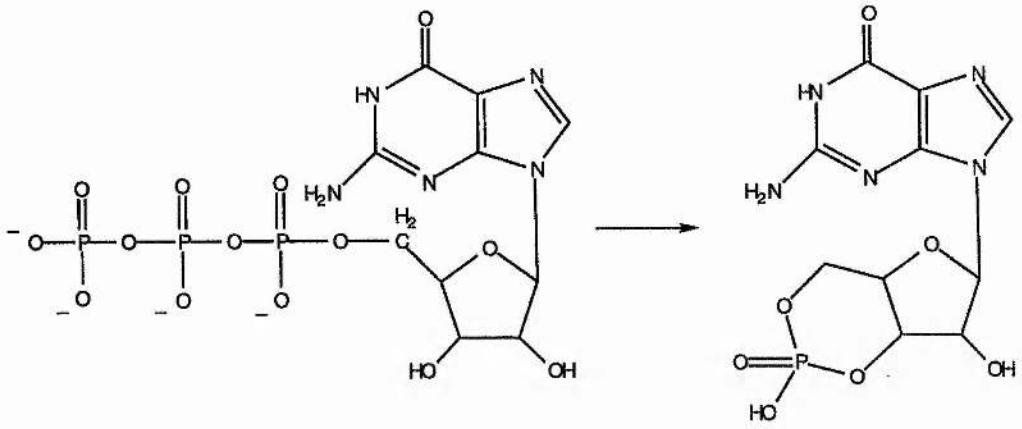


Figure 1.24; Conversion of guanosine triphosphate to cyclic guanosine monophosphate

A number of biological substances such as acetylcholine and bradykinin can trigger the process of relaxation and studies[128] of the effect of the former on isolated rings of rabbit aorta showed that the vasodilatory action was greatly diminished if the endothelial cells were removed or damaged. This led to the conclusion that it was not the action of acetylcholine on the muscle cells which caused relaxation, but rather the action upon the endothelial cells which in turn produced a 'second messenger' which diffused from the endothelial cells into the muscle and activated guanylate cyclase. The second messenger became known as endothelium-derived relaxing factor or EDRF. On the basis that a group of NO-containing compounds activate guanylate cyclase, proposals concerning the chemical identity of EDRF were made, which included S-nitrosocysteine[129] and proteins containing nitrosated thiol groups[130], but perhaps the most likely candidate is simply NO itself[131,132].

Synthetic NO-containing vasodilators include[133] S-nitroso-N-acetylpenicillamine (SNAP) and sodium nitroprusside (SNP). However the iron-sulfur cluster nitrosyls, Roussin's black anion, $[Fe_4S_3(NO)_7]^-$, and the cubane, $Fe_4S_4(NO)_4$, have also been shown[134] to have vasodilatory action. Both compounds are potentially able to transport large quantities of nitric oxide and have relaxation properties comprising an initial rapid drop of pressure, followed by incomplete recovery resulting in a plateau of reduced tone which can persist for several hours, in contrast to SNAP and SNP which display transient and fully reversible responses. The mechanism by which the iron-sulfur clusters release nitric oxide is not known, but extended Hückel molecular orbital calculations[135] show that the complexes are electron precise. Consequently, electron addition to $[Fe_4S_3(NO)_7]^-$ and either addition or removal of an electron in $Fe_4S_4(NO)_4$ will weaken the cage bonding, possibly causing release of NO.

1.5 Iron Complexes of Sulfur-Containing Amino Acids

Harris[136] observed that the violet colouration obtained from basic solutions of cysteine was due also to the presence of Fe(III) salts. A similar observation was made by Schubert[137] in the presence of Fe(II) salts, who attributed the violet colouration to the oxidation of the orange biscysteinatoiron(II) complex to a violet Fe(III) complex on addition of alkali. That the violet colour fades when the dissolved oxygen is used up was postulated to be due to the reduction back to the Fe(II) complex with the formation of cystine and iron(II) hydroxide (see Figure 1.25).

$$[Fe(cys)_2]^{2-} \xrightarrow{\text{Oxidation}} [Fe(OH)(cys)_2]_2^{4-} \longrightarrow [Fe(cys)_2]^{2-} + \text{cystine} + Fe(OH)_2$$

Figure 1.25; Catalytic oxidation of biscysteinatoiron(II)

Further investigation of the iron(II) and iron(III) cysteinate complexes by Tanaka et al[138] led to the suggestion that for Fe(III) in alkaline media, the principal species are $[Fe(OH)(cys)_2]^{2-}$ and $[Fe(cys)_3]^{3-}$. Leussing[139], however claimed that the former is the only principal complex. The equilibrium constants[140] for the oxidation of $[Fe(cys)_2]^{2-}$ by cystine to give $[Fe(cys)_3]^{3-}$ and for the oxidation of Fe(II) by cystine to give Fe(III) and cysteine were calculated to be equal to 2.5×10^{-3} and 5.3×10^{-24} respectively (see Figure 1.26). From the latter value, the oxidation potential of the cystine-cysteine system was calculated to be +0.08 volt vs N.H.E. at 25°C.

$$2[Fe(cys)_2]^{2-} + \text{cystine}^{2-} \rightleftharpoons 2[Fe(cys)_3]^{3-}$$

$$2Fe^{2+} + \text{cystine} + 2H^{+} \rightleftharpoons 2Fe^{3+} + 2\text{cysteine}$$

Figure 1.26; Oxidation of Fe(II) by cystine

The presence of a blue complex in acid solution and a red complex in neutral solution containing Fe(III) and cysteine were also reported[141] and were attributed[142] to complex ions of the type $[Fe(H_2cys)]^{3+}$. Page[142] determined the absorption spectra of the two complexes and found that the blue complex is strongly dependent upon the hydrogen concentration and has a low extinction coefficient ($\varepsilon_{max} = 150$), while the red complex has a much greater extinction coefficient ($\varepsilon = 3000$). He thus proposed that the red complex contained high spin Fe(III), whilst the blue complex is more typical of low spin Fe(III).

An investigation of the iron(III) catalysed oxidation of cysteine by Taylor et al[143] demonstrated that the reaction is zero order with respect to both cysteine and oxygen but two-thirds order with iron(III). Quantitative interpretation of these results led to the proposal that iron(III) forms a triligated complex with cysteine. An iron-cysteine $[Fe(Hcys)_3]^*$ active intermediate was postulated and the maximum catalytic rate of the scheme of oxidation (see Figure 1.27) was observed at pH 8.1. The Hcys* represents an intermediate which combines with itself to form cystine. Sufficient information was not available however to define the nature of the Hcys* intermediate, which may be a radical, an ionic species, a combination of activated units, or a molecular species of unusual reactivity.

$$Fe(Hcys)_3^* \rightleftharpoons Fe(Hcys) + 2Hcys^*$$

$$2Hcys^* \longrightarrow \text{cystine}$$

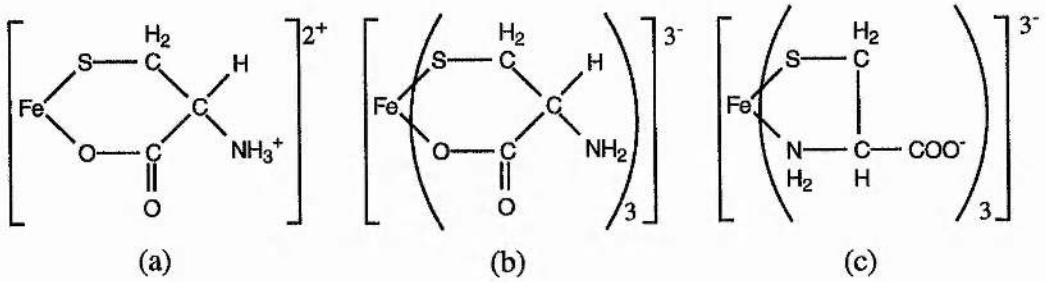
$$Fe(Hcys) + 2\text{cysteine} + 0.5O_2 \longrightarrow Fe(Hcys)_3 + H_2O$$

Figure 1.27; Mechanism for the iron(III) catalysed oxidation of cysteine by molecular oxygen

The kinetics of the reaction of molecular oxygen with iron(II) cysteinate complexes were studied by Gilmour & McAuley[144], who demonstrated that at pH = 6, the 1:1 complex Fe(cys) was the predominant species with the rate defined as $k'_1[Fe(cys)][O_2]$. The 1:2 complex, $[Fe(cys)_2]^{2-}$, took over as the predominant species in the pH range 9 to 10, with the observed rate constant expressed as $k'_2[Fe(cys)_2][O_2]$ where k'_2 was found to be four times the value of k'_1. A three centred π-system involving the sulfur, metal and oxygen atoms was proposed for the adduct formed between the metal complexes and the oxygen molecule, allowing greater facilitation of electron transfer.

Tomita et al[145] observed the reactions between iron(III) and cysteine in ethanolic solution which gave labile blue, red and violet Fe(III) complexes, which became inert when chilled to -78°C. Spectral data of the blue complex indicated that it is the same one obtained by Page in aqueous solution[142]. The similarity of the absorption spectra to that of the blue ferric thioglycolate complex led to the suggestion of a 1:1 complex (see Figure 1.28). The violet complex was isolated at higher pH and characterised using elemental analysis as the 1:3 complex, $[Fe(cys)_3]^{3-}$. Infra red data indicated that the complex is a S,O coordinated tris complex (see Figure 1.28). The red complex was assumed to be $[Fe(OH)(cys)_2]^{2-}$ as reported by Tanaka et al[138]. A green complex was also isolated, but only at very low temperature. That the violet tris complex was

obtained after complete washing of the green precipitate at -78°C suggested that it must also be a tris complex, which was proposed to be a S,N coordinated tris complex (see Figure 1.28).



(a) (b) (c)

Figure 1.28; The proposed structures of the (a) blue, (b) violet and (c) green complexes formed from ethanolic solutions of iron(III) and cysteine at low temperature

Jameson et al[146] followed the anaerobic oxidation of cysteine to cystine by iron(III) in basic media by use of stopped-flow high-speed spectrophotometry. By assuming that the bis-cysteine complex, $[Fe(OH)(cys)_2]^{2-}$, reacts with the mono-cysteine complex, [Fe(OH)(cys)], to yield two iron(III) ions, one cystine and an unoxidised cysteine, the second-order rate constant of 8.36×10^3 $dm^3mol^{-1}s^{-1}$ was calculated. Both complexes were violet in colour, but the predominant species was the bidentate $[Fe(OH)(cys)_2]^{2-}$. Another species did make its appearance during a run which could have been the tri-cysteine complex[138] $[Fe(cys)_3]^{3-}$ or a polymeric species but was not investigated further. Similarly, the anaerobic oxidation of cysteine by iron(III) was followed in acid media[147], from which it was suggested that the species responsible for the transient blue colour on the addition of iron(III) to acidified cysteine solution is $[Fe(cys)]^+$ (see Figure 1.29).

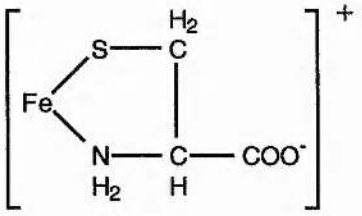


Figure 1.29; The proposed structure of the blue $[Fe(cys)]^+$ complex

The complex was assumed to be formed by the reaction of $[Fe(OH)]^{2+}$ with the cysteine ligand, followed by rapid deprotonation at the sulfur site, and the second-order rate constant for its formation was found to be 1.14×10^4 $dm^3mol^{-1}s^{-1}$. Since the final products of the reaction were cystine and iron(II) it was assumed that cystine is formed from the interaction of two free radicals (see Figure 1.30).

$$[Fe(cys)]^{+} + H^{+} \longrightarrow Fe^{2+} + Hcys\cdot$$

$$Hcys\cdot + Hcys\cdot \longrightarrow cystine$$

Figure 1.30; Formation of cystine from cysteine radicals

Murray & Newman[148] observed that the preparation of iron(II) complexes by reaction of cysteine with iron(II) salts in the presence of sodium hydroxide yielded initially a yellow 1:1 complex which on further addition of alkali dissolved to form a dark yellow 1:2 complex. Elemental analysis and infra-red spectroscopy measurements led to the assignment of structures $Fe(cys)(H_2O)_{1.5}$ and $Na_2[Fe(cys)_2](H_2O)$ respectively. Diffuse reflectance visible spectra were generally in accord with distorted octahedral symmetry around the iron(II) centres in the complexes. X-ray structure determination[149] of $[Fe(tga)H_2O]_n$ (H_2tga = thioglycollic acid) demonstrated a polymeric ligand bridged structure; thus it was proposed[148] that for the 1:1 cysteine complex to achieve pseudo-octahedral symmetry around the iron and to be compatible with the magnetic results obtained that it must also possess a polymeric ligand-bridged structure (see Figure 1.31).

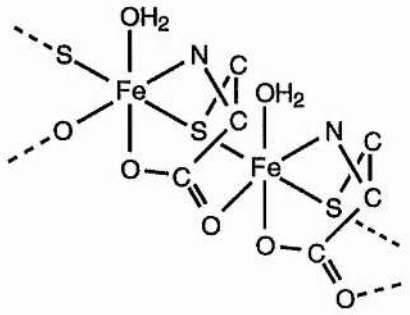


Figure 1.31; The proposed structure of the yellow $[Fe(cys)(H_2O)_{1.5}]_n$ complex

The 1:2 cysteine derivative was shown[148] to have bidentate S,N coordination similar to that observed in the crystal structure[150] of $Zn(cys)_2^{2-}$. The magnetic behaviour[148] suggested however that the iron(II) complex again has a polymeric structure, bridged most likely by cysteinyl sulfur atoms (see Figure 1.32).

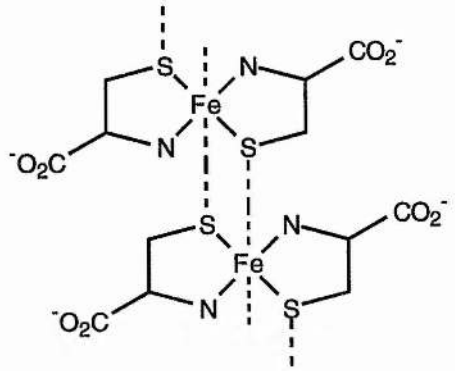


Figure 1.32; The proposed structure of the dark yellow $[Fe(cys)_2^{2-}]_n$ complex

Cremer[151] was the first to observe that solutions of bis(cysteinato)iron(II) absorbed carbon monoxide reversibly to form the dicarbonyl complex, $[Fe(cys)_2(CO)_2]^{2-}$, which was later isolated by Schubert[152]. ORD studies[153] confirmed Cremer's[151] claim that the bis(cysteinato)(dicarbonyl)iron(II) complex released carbon monoxide on illumination with light and picked up carbon monoxide in the dark reversibly. The magnetic moment of $[Fe(cys)_2]^{2-}$ was found by Wang et al[154] to be 4.74 BM, corresponding to four unpaired electrons per iron(II), which, on dicarbonylation showed diamagnetism due to six paired electrons. Studies using infra-red spectroscopy[155] of the dicarbonyl complex indicated that the carbon monoxide molecules occupy cis sites, indicating the presence of two configurational isomers. ORD studies[155] confirmed the presence of two diastereoisomers. Upon addition of an orange solution of silver ion in NH_4OH-NH_4NO_3 buffer to the dicarbonyl complex, an immediate colour change to give an intense crimson colloidal solution was observed, which was attributed to the change in coordination of the cysteinate to the iron from N,S to N,O, with the silver ions then being coordinated to S.

Tomita et al[153] observed that the reaction between bis(cysteinato)iron(II) and nitric oxide yielded a cystinato(dinitrosyl)iron(II) complex and suggested that the nitric oxide was responsible for the oxidation of cysteine to cystine. In contrast to the carbonyl complex, the reaction was irreversible; release of nitric oxide caused the complex to decompose giving free ferrous ion and cystine. McDonald et al[87] prepared the paramagnetic bis(cysteinato)(dinitrosyl)iron(II) complex which has been shown to exhibit antibacterial[108] and vasodilatory[156] activity. The very different ESR spectrum[87] obtained when cystine was used as the added ligand in place of cysteine suggested that the complexes involving cysteine and cystine were quite different, which was attributed to the differing bond behaviour of the -SH group of cysteine compared to the -S-S- group of cystine. The structures of the dinitrosyliron(II) complexes containing either

cysteine ethyl ester or penicillamine were assigned as being analogous to that of the cysteine complex.

The possible role of iron(II) amino acid complexes in nitrite depletion and inhibitor formation in cured meats was investigated by Larkworthy et al[157]. Reactions with nitric oxide gave the diamagnetic Fe(cystine)$(NO)_2$ complex from aqueous solutions of cysteine and iron(II) sulfate. However Fe(III) products were obtained from reaction with iron complexes containing methylcysteine or methionine, which led to the proposal that these complexes were likely to be mixtures of oxo- or hydroxo-bridged species.

Reaction of bis(cysteinato)iron(II) with MeCHO gives the bis(acetaldehydo)-bis(cysteinato)iron(II) complex[158] which is used as a remedy for anaemia.

Bielig & Bayer[159] reported the isolation of a bis complex of iron(III) and methionine, $[Fe(met)_2(OH)_2]^+$ and suggested a square planar configuration where the methionine was coordinated only through the amino groups. The neutral triligated complex $Fe(met)_3$ was reported by McAuliffe et al[160] and a high spin octahedral stereochemistry was proposed with coordination via the N and O atoms. That the S atom of the $-SCH_3$ group was still available for coordination was demonstrated by formation of the mixed-metal complex $[Ag_3Fe_2(met)_6]^-$ from reaction with silver ion. However Halbert and Rogerson[161] claim to have isolated the 1:1 complexes of methionine and iron(III), $Fe(met)(OH)X.2CH_3OH$, (X = Cl, NO_3) using a similar method to that of McAuliffe et al[160] and also reported coordination of methionine through the carboxylate group.

The trinuclear oxobridged iron(III) methionine nitrate complex (see Figure 1.33) and the similar iron(III) benzyl-cysteine perchlorate complex were prepared by Puri et al[162,163]. Spectral and magnetic properties indicated that the physical properties of these complexes were largely independent of the oxygen-containing ligands coordinated to the trimeric unit, $[Fe_3O]^{7+}$, and the nature of the counterion present.

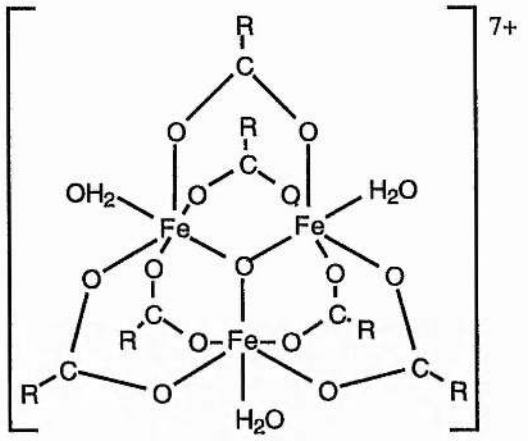


Figure 1.33; The proposed generalised molecular structure of the tetranuclear oxobridged iron(III)-methionine complex.

The bis(methioninato)iron(II) complex prepared by Murray and Newman[148] was proposed to contain methionine coordinated via the N,O,O groups. Spectral and magnetic data were indicative of a monomeric structure although a possible weakly associated structure involving the carboxylate ligands was suggested (see Figure 1.34). Larkworthy et al[164] made a similar suggestion and demonstrated the formation of the methylcysteine complex in likewise fashion. Again, spectral properties were indicative of a hexacoordinate iron(II) complex, likely involving carboxylate bridges to adjacent units. Similarly, the 1:1 cystine complex, Fe(cystine), was obtained and again its properties were suggestive of an extended structure to produce hexacoordinate iron(II).

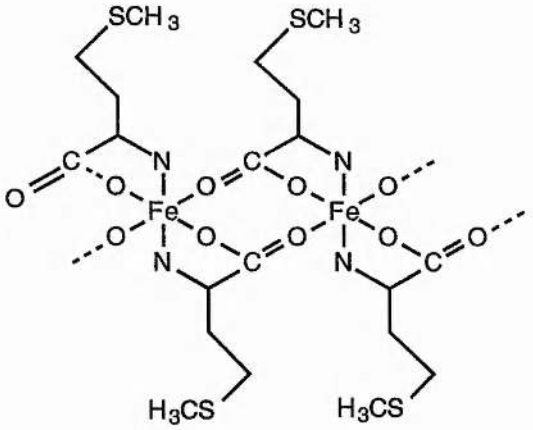


Figure 1.34; Proposed structure for $[Fe(met)_2]_n$

Glutathione complexation of iron(III) in the pH range 1 to 3 was reported by Khan et al[165]. Iron(III) chelation was also studied by Hamed et al[166] who observed rapid reduction of the metal at low pH. Subsequent[167] kinetic experiments under acidic conditions showed the presence of a blue intermediate which was proposed to be a 1:1

iron(II) glutathione complex. Further studies[168] demonstrated that when iron(III) salts react with glutathione, in all cases the final product contained iron(II). Kinetic measurements[169] on the iron catalysed oxidation of glutathione by molecular oxygen indicated the presence of a transient red species, the rate of production of which was oxygen dependent and required one oxygen per two iron atoms. Mössbauer data indicated that the red complex contained iron(III), whereas the initial complex, prior to oxygenation, contained only iron(II); and a scheme for the iron catalysed oxidation of glutathione by molecular oxygen was presented (see Figure 1.35). Two stoichiometries 1:1 and 1:2 have been established for iron(II)-glutathione materials[170]. Spectroscopic studies demonstrated that the 1:1 materials contained high spin iron(II) in distorted five and six coordinated environments, whereas the 1:2 materials contained only distorted six-coordinated high spin iron(II).

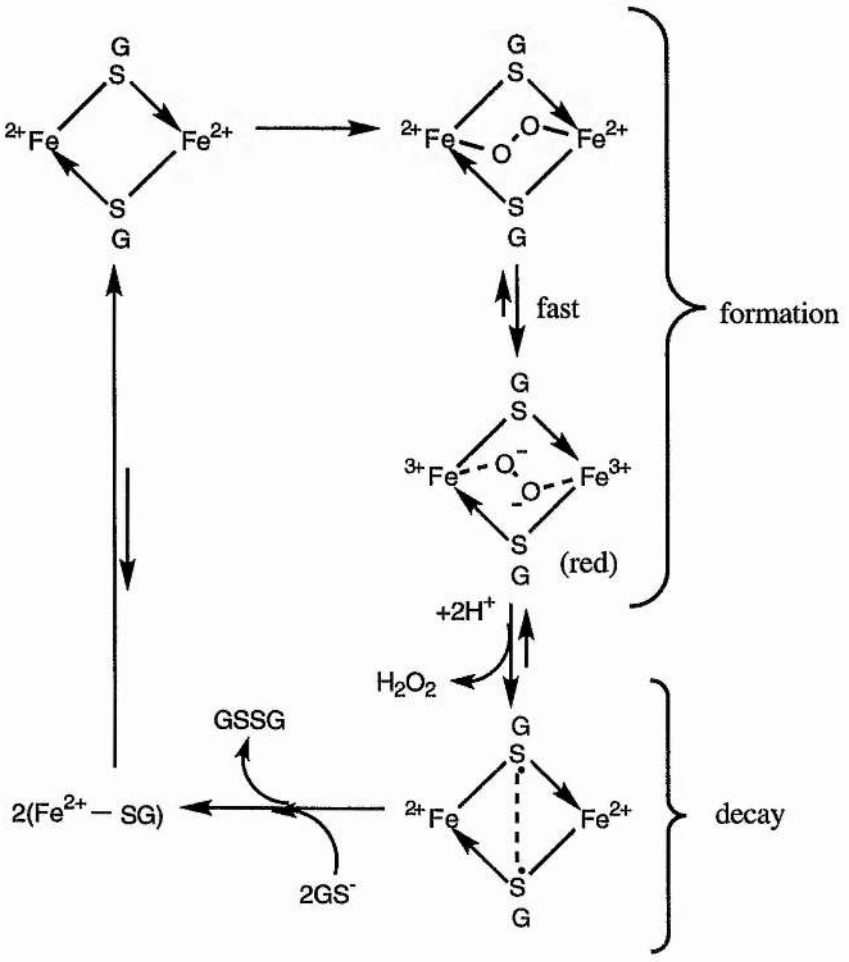


Figure 1.35; Scheme for the iron catalysed oxidation of glutathione by molecular oxygen

However it should be pointed out that much of the literature on iron-sulfur containing amino acid complexes is full of suggestions of possible structures and assumptions of mechanisms and very little definitive evidence is available. Thus very little can be taken as proven.

1.6 Direct Formation of Metal Nitrosyl Complexes from either Nitrite or Nitric Oxide

Vanadium

The violet coloured vanadium complex, $V(NO)(CO)_5$, can be prepared by the action of nitric oxide on vanadium carbonyl[171]. Fisher et al[172] prepared the cyclopentadienyl nitrosyl carbonyl of vanadium, $CpV(NO)_2(CO)$ by the interaction of nitric oxide with an acetone solution of $Na[CpV(CO)_3CN]$. Vanadium nitrosyl complexes in industrial usage are of the type $V(NO)(CO)_4L$, where L are phosphine ligands such as PPh_3, which are prepared from $V(CO)_4L_2$ and nitric oxide and used in a process for coating other metals with vanadium[173]. The reaction[174] between $V(Cp)_2$ and nitric oxide has been shown to have a 1:1 stoichiometry, but the initial product, presumed to be V(Cp)(NO) undergoes disproportionation to give a mixture of final products. However the reaction between $VI(Cp)_2$ and nitric oxide gives two products[174]; a brown insoluble polymer with ionic iodide, believed to contain bridging $N_2O_2^{2-}$ ligands and the green monomeric $VI(Cp)_2(NO)$.

Chromium

Chesneau[175] observed that aqueous solutions of chromium(II) chloride absorbed nitric oxide in the ratio 1:3, yielding a reddish-brown compound which he mistakenly proposed to be the complex $(CrCl_2)_3(NO)$. However, the oxidation of chromium(II) by nitric oxide produces not one, but three Cr(III) complexes, which were separated by Ardon et al[176] to give the dinuclear green ion Cr_2O^{4+}, the hexaquochromium(III) ion $[Cr(H_2O)_6]^{3+}$ and the red-brown $[Cr(H_2O)_5(NO)]^{2+}$ complex. The yellow pentamminenitrosylchromium(I) complex, $[Cr(NH_3)_5(NO)]^{2+}$ can be prepared in a similar manner[177] by treating a suspension of chromium(II) chloride in liquid ammonia with nitric oxide. With cyanide ion it yields the pentacyanonitrosylchromate(I) ion $[Cr(CN)_5(NO)]^{3-}$. The penta-methanolate and -ethanolate analogues of the type $[Cr(ROH)_5(NO)]^{2+}$, again could be prepared in a similar fashion by passing nitric oxide into alcoholic solutions of chromium(II) chloride. When the reaction between nitric oxide and chromium(II) chloride was carried out in acetone[178] in the presence of *o*-phenylenebis(dimethylarsine), (das), the chloride, $CrCl_2NO(das)_2$ could be isolated but proved difficult to purify. However addition of sodium perchlorate to acetone solutions of $CrCl_2NO(das)$ gave immediate precipitation of the perchlorate salt, $[CrClNO(das)_2]ClO_4$.

Bradley et al[179] found that trisdialkylamidochromium(III) compounds, CrL_3, (L = NPr^i_2, $N(SiMe_3)_2$, 2,6-dimethylpiperidide) react with nitric oxide forming coloured diamagnetic mononitrosyls of the type $Cr(NO)L_3$. The tris-*t*-butoxide, $Cr(NO)(OBut)_3$ can also be obtained by the action of nitric oxide on $LiCr(OBut)_4$. The red-black tetranitrosylchromium complex, $Cr(NO)_4$, was prepared[180] by photolysis of $Cr(CO)_6$ in pentane solution with an excess of nitric oxide. In the case[181] of $Cr(CN)_6$, reduction in the presence of nitrite gives the mononitrosyl product $[Cr(CN)_5(NO)]^{4-}$.

Piper et al[182] observed that greenish yellow crystals of the chromium complex, $CpCr(NO)_2Cl$ can be isolated from reaction of chromium(III) chloride with sodium cyclopentadienide and nitric oxide in tetrahydrofuran solution. The carbonylcyclopentadienide complex[183,184], $CpCr(CO)_2NO$ is prepared by reaction of nitric oxide with $[CpCr(CO)_3]_2$. The chromacycles $Cp^*(CO)_2Cr[R^1C{=}CR^2C(O)Me]$ ($Cp^* = \eta^5\text{-}C_5Me_5$; $R^1 = H$, $R^2 = Ph$; $R^1 = R^2 = Me$), when reacted with nitric oxide gave rise to the dinitrosyl nitro complex $Cp^*Cr(NO)_2(NO_2)$[185]. In the case where R^1 = H, R^2 = Ph; $Cp^*Cr(NO)_2(\eta^1\text{-}HC{=}CPhCOMe)$ is also formed (see Figure 1.36). Mixed nitrosyl nitro Schiff base complexes of the type $[(SB)Cr(NO)_2(NO_2)_2]$, where SB are Schiff base ligands, can be formed from reaction with $[(SB)Cr(CO)_4]$ with nitric oxide dissolved in benzene[186].

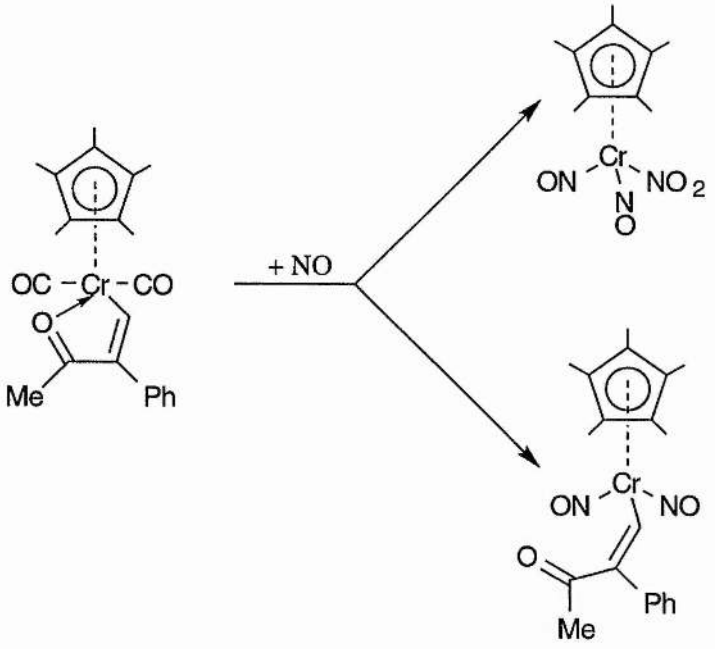


Figure 1.36; Formation of nitrosyl chromacycle complexes

Wayland et al[187] observed that addition of nitric oxide to tetraphenylporphyrin complexes of Cr(II)TPP and Cr(III)TPP(OMe) resulted in formation of 1:1 low spin complexes of CrTPP(NO) and CrTPP(NO)(OMe). The nitrosyl adducts[188],

$Cr(Me_2[14]tetraenatoN_4)(NO)$ and $[Cr(Me_6[14]4,11\text{-}dieneN_4)(NO_2)(NO)]PF_6$ were obtained by treatment of the Cr(III) and Cr(II) complexes of $Me_2[14]tetraenateN_4$ and $Me_6[14]4,11\text{-}dieneN_4$ respectively with alcoholic sodium nitrite. Spectral analysis indicated that both nitrosyl derivatives contained Cr(I) with a linear Cr-NO group.

The maroon chromium nitrosyldithiocarbamate complex, $Cr(NO)_2[S_2CN(C_2H_5)_2]_2$ was prepared[189] by bubbling nitric oxide through a cold saturated solution of sodium diethyldithiocarbamate in alcohol before addition to an alcoholic solution of chromium(II) acetate. It was observed[190] that the ß-modification of Cr-pc (pc = phthalocyanine) reacts with nitric oxide to form the purple-red mononitrosyl derivative Cr(NO)(pc). Wigley et al[191] observed that the reaction of nitric oxide with the homoleptic six-coordinate alkyl isocyanide complexes, $[Cr(CNR)_6](PF_6)_2$ led to the formation of the paramagnetic 17 electron species, $[Cr(NO)(CNR)_5](PF_6)_2$. These reactions, which may be viewed formally as leading to the one-electron reduction at the metal centre, proceeded smoothly in dichloromethane to give the complexes as bright yellow crystalline solids. Passage of nitric oxide through a solution of $Cr(CO)_4(Ph_2PCH_2CH_2AsPh_2)$ results in displacement of all the carbonyl groups leading to the isolation[192] of the yellow trinitrosyl complex, $Cr(NO)_3(Ph_2PCH_2CH_2AsPh_2)$.

Molybdenum

Complexes of the general type $Mo(NO)_2L_2X_2$, (L = monodentate ligand, X = Cl, Br) can be prepared by reaction of $MoCl_5$ and nitric oxide in acidified ethanol[193]. A further method of preparing such complexes was described by Anker et al[194] which involved reaction of nitric oxide with the isoelectronic tricarbonyl derivatives, $Mo(CO)_3L_2X_2$ in dichloromethane or benzene. The dinuclear molybdenum nitrosyl carbonyl, $[Mo(NO)(CO)_2(dipy)]_2$ can be prepared[195] by reaction of nitric oxide with the carbonyl bridged species $[Mo(CO)_3(dipy)]_2$. Passage of nitric oxide through an acetonitrile solution of $[Mo(CO)_2(bipy)(NCMe)][BF_4]_2$ leads to the isolation[196] of an emerald green powder which was shown to be *cis*-$[Mo(NO)_2(bipy)_2][BF_4]$ Reaction of nitric oxide with $[(OH)Mo(CO)_3H]_4.4OPPh_3$ in ethanol gives an orange compound[197,198] which was shown to be the cubic $[Mo(NO)(CO)_2(OH)]_4.4OPPh_3$ which possesses a structure containing molybdenum and oxygen tetrahedra interlocking in a 'cubane' arrangement. Attempts to produce the $[Mo(CO)_4(NO)]^-$ anion by refluxing $Mo(CO)_6$, sodium nitrite and potassium hydroxide in methanol led to the isolation of two unusual molybdenum carbonyl cluster ions[199]; $[(Ph_3P)_2N]^{3+}[Na[Mo_3(CO)_6(NO)_3(\mu_2\text{-}OCH_3)_3(\mu\text{-}O)]_2]^{3-}$ **1** and $[Me_4N]^+[Mo_3(CO)_6(NO)_3(\mu_2\text{-}OCH_3)]^-$ **2.** X-ray diffraction studies indicated that **1** differed from **2** in that for **1** the Mo triangle is

capped by a triply bridging oxygen atom, while **2** is capped by a triply bridging methoxy ligand. **1** exists as a triple ion in the solid state, with two Mo_3 units 'sandwiching' a sodium ion such that the Na^+ ion is octahedrally coordinated by the six oxygen atoms of the methoxy ligands. The yellow $[Mo(CO)_2(NO)(o\text{-phen or 2,2'-bipy})]_2$ complexes[192] can be formed by passage of a slow stream of nitric oxide gas through a benzene solution of $Mo(CO)_4(o\text{-phen or 2,2'-bipy})$. Under similar conditions $Mo(CO)_4(Ph_2PCH_2CH_2AsPh_2)$ gives $Mo(NO)_3(Ph_2PCH_2CH_2AsPh_2)$. Srivastava et al[186] demonstrated that the instantaneous reaction of nitric oxide in benzene with the complexes $[(SB)Mo(CO)_4]$ (SB = Schiff base) gave a mixture of $[(NO)_2(SB)Mo(NO_2)_2]$ and $[(NO)(SB)Mo(CO)_2(NO_2)]$ as yellow/brown diamagnetic solids. The yellow octahedral $[Mo(H_2CPz'_2)(CO)_2(NO)Br]$ (where Pz' is 3,5-dimethylpyrazol-1-yl) complex (see Figure 1.37) can be prepared[200] from reaction of $[Mo(H_2CPz'_2)(CO)_2Br_2]$ with $NaNO_2$, $n\text{-}Bu_4NNO_2$ or $AgNO_2$ in dichloromethane.

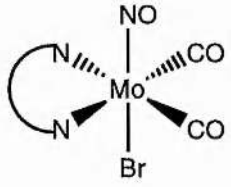


Figure 1.37; The yellow $[Mo(H_2CPz'_2)(CO)_2(NO)Br]$ complex

The reaction[201,202] of either $MoCl_5$ or $MoOCl_3$ with nitric oxide in C_6H_5Cl, $CHCl_3$ or CCl_4 produces the same dark green product, $[Mo(NO)_2Cl_2]_n$ which can readily react with PPh_3 or C_5H_5N to give the complexes[193,194], $Mo(NO)_2Cl_2L_2$. However, the reaction of $MoCl_5$ with nitric oxide in benzene produces a red nitrosyl from which a series of mononitrosyl complexes of molybdenum can be synthesised[203], including $[MoCl_4(NO)]^-$, $[MoCl_5(NO)]^{2-}$, $[MoCl_3(NO)L_2]$, $[MoCl_4(NO)Ll^-$, $[MoCl_3(NO)L_3]$, $[MoCl_3(NO)L_2]^-$, $[MoCl_2(NO)L_3]$ and $[MoCl(NO)L_4]$ (L = neutral ligand). Complexes of the type $MoCl_4L_2$ (L = tertiary phosphine) react with nitric oxide in benzene producing a mixture of three products, $[MoCl_3(NO)(L)_2]$, $[MoCl_2(NO)_2(L)_2]$ and $[MoCl_2O_2(L)_2]$, but by varying the reaction conditions, specific products could be isolated. The low temperature reaction[204] of the $MoCl_3(PMe_3)_3$ complex with nitric oxide in tetrahydrofuran solution gave the yellow, 18 electron species, $MoCl_3(NO)(PMe_3)_3$. Spectral analysis of the complex indicated a capped octahedral geometry (see Figure 1.38). The binuclear, nitrosyl-bridged complex $Cs[Mo_2Cl_5H(NO)_2]$ can be synthesised[205] from reaction of nitric oxide with the hydrido complex $Cs_3[Mo_2Cl_8H]$. Reduction by zinc amalgam in benzene of the dichloromolybdenum(IV) derivative $Mo(ttp)Cl_2$, where ttp is the dianion of meso-tetra-*p*-tolyporphyrin, under nitric oxide leads to the isolation[206] of green crystals of the diamagnetic $Mo(ttp)(NO)_2.C_6H_6$.

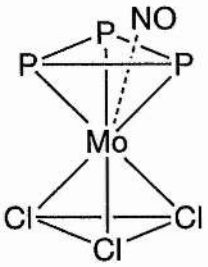


Figure 1.38; $MoCl_3(NO)(PMe_3)_3$

Tungsten

The carbonyl cyclopentadienyl complex, $CpW(NO)(CO)_2$ can be prepared[183] by the action of nitric oxide on the $[CpW(CO)_3]^-$ anion. Anker et al[194] observed that when nitric oxide was passed into dichloromethane or benzene solutions of the tungsten(II) tricarbonyl compounds, $M(CO)_3L_2X_2$ (L = PPh_3, $AsPh_3$, $SbPh_3$), that green complexes of the type $W(NO)_2L_2X_2$ could be isolated. The complexes, $[W(CO)_2(NO)(o\text{-phen or 2,2'-bipy})]_2$ are formed[192] when a stream of nitric oxide is passed through a benzene solution of $W(CO)_4$(*o*-phen or 2.2'-bipy). Under similar conditions, $W(CO)_4(Ph_2PCH_2CH_2AsPh_2)$ gives the nitrosyl carbonyl complex $W(CO)(NO)_2(Ph_2PCH_2CH_2AsPh)_2$. Reaction of nitric oxide with $[(OH)W(CO)_3H]_4.4OPPh_3$ in ethanol led to the isolation[198] of the nitrosyl derivative, $[(OH)W(CO)_2(NO)]_4.4OPPh_3$. The nitrosyl hydride, $HW_2(CO)_9(NO)$ can be prepared[207] by the reaction of $[HW_2(CO)_{10}]^-$ with sodium nitrite and acetic acid. Bencze[202] observed that reaction of WCl_6 with nitric oxide in dichloromethane gave the green $[W(NO)_2Cl_2]_n$ species which reacted with triphenylphosphine to give $W(NO)_2Cl_2(PPh_3)_2$. However Seyferth et al[208] stated that the nitrosylating reduction of WCl_6 with nitric oxide led to the formation of $WCl_3(NO)_4$, which on addition of different donor ligands, L, yielded complexes of the types $WCl_3(NO)L_2$ (L = $OPPh_3$, HMPT, pyridine) and $WX_2(NO)_2L_2$ (L = PPh_3, X = Cl; XL = acac) or mixtures of products (L = dipy, RCN, Et_4NCl) respectively. The yellow $WCl_3(NO)(PMe_3)_3$ complex can be prepared[204] by reaction of $WCl_3(PMe_3)_3$ with nitric oxide at low temperature (for general structure, see Figure 1.38).

Manganese

Piper and Wilkinson[182] observed that reaction of nitric oxide with $Mn(Cp)_2$ in tetrahydrofuran solution led to a compound of the stoichiometry $(Cp)_3Mn_2(NO)_3$. Infra-red data indicated bands typical of bridging and non-bridging nitric oxide groups and thus the structure shown in Figure 1.39 was proposed. The carbonyl cation,

$[CpMn(CO)_2NO]^+$, was isolated[209] as the chloroplatinate from reaction of $CpMn(CO)_3$ with a hot aqueous solution of sodium nitrite in concentrated sulfuric acid.

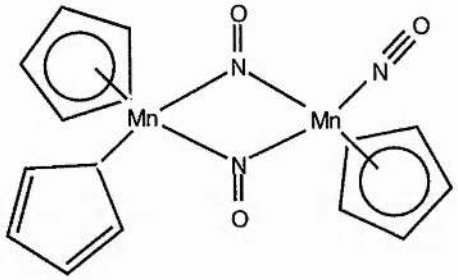


Figure 1.39; Proposed structure of $(Cp)_3Mn_2(NO)_3$

Reaction of nitric oxide with $Mn(CO)_5I$ or $[Mn(CO)_4I]_2$ yielded the green $Mn(NO)_3CO$ complex which was postulated to have essentially a tetrahedral structure[210]. Further reaction of the trinitrosyl complex with triphenylphosphine in benzene gave the dark green $Mn(NO)_3PPh_3$ which could also be prepared by reaction of nitric oxide with $Mn(CO)_4PPh_3$ in *p*-xylene at 100°C. Wawersik and Basolo[211] subsequently observed that a *p*-xylene solution of $Mn_2(CO)_8[P(C_6H_5)_3]_2$ reacts with nitric oxide within seconds, almost quantitatively, to produce equimolar amounts of $MnNO(CO)_4$ and $MnNO(CO)_3(PPh_3)$. $[N(PPh_3)_2](NO_2)$ is also a very effective nitrosylating agent, reacting with a solution of $[Mn(CO)_6]^+$ in acetonitrile almost instantaneously to form $MnNO(CO)_4$[212]. The related cation, $[Mn(CO)_5(CH_3CN)]^+$ yields the same nitrosyl product. In the presence of excess $[N(PPh_3)_2](NO_2)$ however, $MnNO(CO)_4$ is converted to the $[Mn(NO)_2(CO)_2]^-$ anion which X-ray crystallography demonstrated contained a tetrahedral geometry with disorded NO and CO ligands.

Treatment of $[TdpMn(CO)_4]_2$ (Tdp = tris(dimethylamino)phosphine) with excess nitric oxide gives the green volatile unstable nitrosyl[213] $[TdpMn(NO)_3]$. The complex $[Mn(CO)_3L_2Br]$ (L = $P(OPh)_3$) gives the dinitrosyl complex[214], $[Mn(NO)_2L_2Br]$ on reaction with nitric oxide. Similarly[215] the *mer-cis*-$[Mn(CO)_2L_3Br]$ (L = $P(OMe)_2Ph$) also reacts with nitric oxide to yield the equivalent dinitrosyl species $[Mn(NO)_2L_2Br]$. Upon contact with nitric oxide, both in the solid state and in tetrahydrofuran solution, the off-white complexes $[MnX_2(PR_3)]$ (X = Cl, Br or I; $PR_3 = PPr^n_3$, PBu^n_3, $PPhMe_2$ or $PPhEt_2$) become vividly coloured (X = Cl, deep red-purple, X = Br, deep blue, X = I, brown) due to formation of $[MnX_2(PR_3)(NO)]$ species[216,217]. For X = Cl or Br, the reaction was shown to be reversible but for X = I, the reaction is irreversible.

Nitric oxide forms 1:1 low spin complexes[187] with Mn(II)TPP and Mn(III)TPP(X) species (X = Cl, CH_3CO_2 or CN). The nitrosylmetalloporphyrin Mn(NO)TPP(MPip) (MPip = 4-methylpiperidine) can be readily prepared by treatment of a chloroform

solution of MnTPP(Cl) with nitric oxide in the presence of an excess of 4-methylpiperidine[218]. Nitrosylmanganese(II) complexes of the quinquedentate Schiff's base ligand (see Figure 1.40) can be prepared by reaction of nitric oxide with the manganese(II) complexes of the ligand[219]. Crystallographic studies demonstrated that the manganese displays approximately octahedral coordination by the five nitrogen atoms of the quinquedentate ligand and nitric oxide.

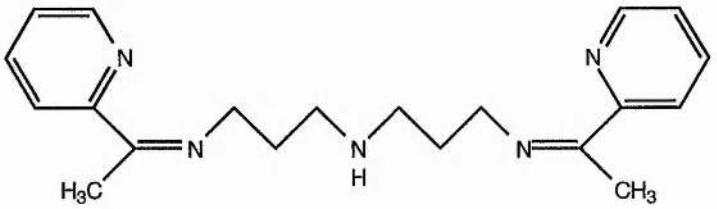


Figure 1.40; The quinquedentate Schiff's base ligand

The $K_3[Mn(NO)(CN)_5]$ salt can be prepared[220,221] by shaking a water-alcohol solution of $Mn(C_2H_3O_2)_2$ and KCN in the ratio 1:5 in an atmosphere of nitric oxide. Ercolani and Neri[190] found that the ß modification of Mn(pc) (pc = phthalocyanine) reacted with nitric oxide to form the Mn(pc)(NO) derivative. The benzyl manganese complex $(C_6H_5CH_2)_2Mn$ reacts with nitric oxide with formation[222] of dibenzyl and $(C_6H_5CH_2)Mn(NO)$ which contains the manganese in the valence state 0.

Iron

The first iron-sulfur-nitrosyl cluster was made by reaction of iron(II) sulfate, sodium nitrite and ammonium sulfide to form Roussin's black anion[40], $[Fe_4S_3(NO)_7]^-$ which was isolated as the sodium salt, but can also be isolated as the caesium[44], tetraphenylarsonium[45] and trimethylsulphonium[46] salt. A more convenient synthesis[52] of Roussin's black salt perhaps, is by reaction of iron(II) sulfate, sodium nitrite and hydrogen sulfide. Also[65], nitrosylations using sodium nitrite in aqueous ethanol of $[Fe_2S_2(CO)_6]$, $[Fe_2S_2(CO)_6]^{2-}$ and $[Fe_3S_2(CO)_9]$ all give $[Fe_4S_3(NO)_7]^-$; in addition, the reaction with $[Fe_2S_2(CO)_6]^{2-}$ also yields Roussin's red salt, $[Fe_2S_2(NO)_4]^{2-}$. The reaction of aqueous iron(II) salts and nitrite with a range of biological sulfur sources[36] including cysteine, homocysteine, sodium thioglycollate, penicillamine and potassium benzylpenicillamine all give $[Fe_4S_3(NO)_7]^-$; glutathione and cystine both give the same product in the presence of sodium ascorbate. The anion $[Fe_4S_3(NO)_7]^-$ can also be formed from reaction of either of the models for 2Fe-2S and 4Fe-4S redox proteins (see Figure 1.21) with aqueous nitrite[103]. Reaction of iron(II) sulfate with sodium nitrite and sodium hydrogen selenide[65] provides $Na[Fe_4Se_3(NO)_7]$ in a reaction entirely analogous to Roussin's original synthesis of the anion $[Fe_4S_3(NO)_7]^-$.

The neutral complexes $Fe_2(SR)_2(NO)_4$ are synthesised by reaction of iron(II) sulfate and nitric oxide in the presence of a thiol, RSH, to give the corresponding complex.[39,48,52,94] Similarly, reaction of an iron(II) salt with potassium thiosulfate and nitric oxide yields the $[Fe_2(S_2O_3)_2(NO)_4]^{2-}$ anion[39]. In the presence of iron(II) sulfate and sodium nitrite, methionine yields $Fe_2(SMe)_2(NO)_4$[37]. A similar reaction with ethionine yields only the ethionine ester, $Fe_2(SEt)_2(NO)_4$ confirming the transfer of intact SR fragments into $Fe_2(SMe)_2(NO)_4$. Reaction of an iron(II) salt with dialkyldithiocarbamate $[R_2NCS_2]Na$ under nitric oxide provides the iron(I) complexes[223], $[Fe(NO)(S_2CNR_2)_2]$ (see Figure 1.41). When the heterotrimetallic anion, $Fe(S_2MoS_2)_2^{3-}$ is treated with nitric oxide, the reaction product[224] is a salt of the heterometallic iron-dinitrosyl $[S_2MoS_2Fe(NO)_2]^{2-}$; the tungsten analogue is also synthesised in a similar manner.

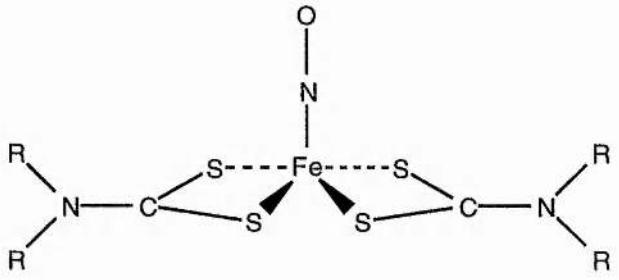


Figure 1.41; The $[Fe(NO)(S_2CNR_2)_2]$ complex

Sellman et al[225] synthesised a range of mercapto-dinitrosyl complexes from reaction of the carbonyl complexes with nitric oxide. Substitution of the carbonyl ligand by nitric oxide in the monocarbonyl complexes, $[Fe(CO)('S_5')]$ or $[Fe(CO)('OS_4')]$ led to the formation of dinitrosyl complexes of the general type $[Fe(NO)_2('XS_4')]$ (where X = S or O, see Figure 1.42). Spectral analysis suggested a *cis* dinitrosyl complex containing linear NO bonds with the ligands acting in a tridentate manner.

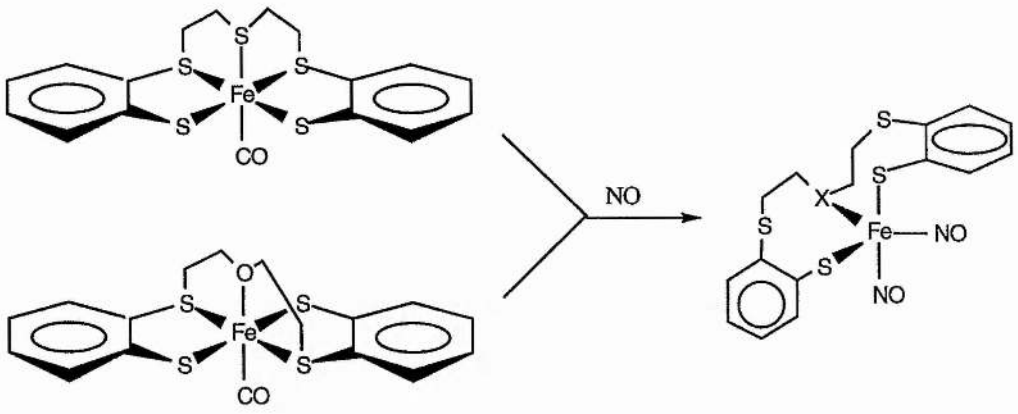


Figure 1.42; Proposed structure of $[Fe(NO)_2('XS_4')]$ (X = S,O) from reaction of $[Fe(CO)(('XS_4')\}$ with nitric oxide

Similarly the reaction of nitric oxide with the dicarbonyl complex $[Fe(CO)_2('S_4')]$ led to the isolation of the tridentate dinitrosyl complex $[Fe(NO)_2('S_4')]$ (see Figure 1.43); no mixed nitrosyl carbonyl species were observed.

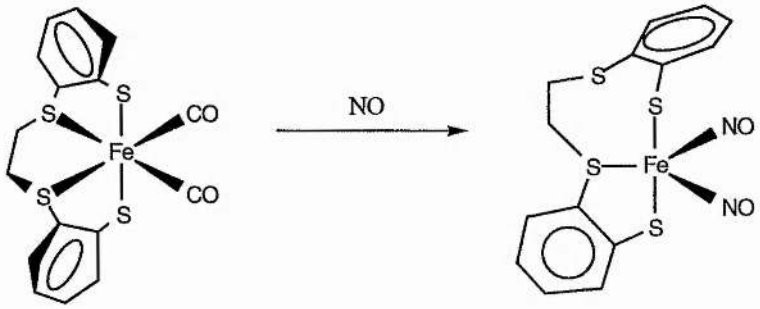


Figure 1.43: Proposed structure of $[Fe(NO)_2('S_4')]$ from reaction of $[Fe(CO)_2('S_4')]$ with nitric oxide

Aqueous solutions of iron(II) salts absorb nitric oxide to give the familiar colour of the 'brown ring test' which is due to the octahedral $[Fe(H_2O)_5NO]^{2+}$ cation[226]. The pentaethanol nitrosyliron(III) ion $[Fe(C_2H_5OH)_5NO]^{3+}$ can be prepared in a similar manner from reaction of nitric oxide with ethanolic solutions of iron(II) chloride. Aqueous solutions of $Na_3[Fe(CN)_5NH_3]$ absorb nitric oxide in the presence of acetic acid to form the analogous $[Fe(CN)_5NO]^{3-}$ ion.

In the reaction of $[CpFe(CO)_2]_2$ with nitric oxide in alkanes, $[CpFe(NO)_2]_2$ is formed[227] which possesses a dimeric structure containing an iron-iron double bond. Similarly, the dimeric $Fe_2(\eta 5\text{-}C_5H_4Me)_2(\mu\text{-}NO)_2$ complex[228] can be formed from reaction of $Fe_2(\eta^5\text{-}C_5H_4Me)_2(CO)_2(\mu\text{-}CO)_2$ with nitric oxide in octane. X-Ray crystallographic studies demonstrated a planar $Fe(NO)_2$ core. Treatment of the halo-π-

allyl-tricarbonyliron complexes (π-RCHCH=CH_2)$Fe(CO)_3X$, where R = H, Me or MeCO, with nitric oxide[229] gives the isoelectronic nitrosyls (π-RCHCH=CH_2)$Fe(NO)(CO)_2$. Broitman et al[230] observed that when nitric oxide reacts with solutions of $Fe(C_5H_7S_2)_2(CO)_2$ in $CHCl_3$, both carbonyl groups are replaced by NO to give the corresponding *cis*-dinitrosyl complex $Fe(C_5H_7S_2)_2(NO)_2$. This complex can also be obtained directly from reaction of $Fe(C_5H_7S_2)_2$ with nitric oxide in $CHCl_3$. Nitric oxide[231] also effects complete substitution of CO in $[Fe(CO)_3SCF_3]_2$ to give $[Fe(NO)_2SCF_3]_2$. A similar reaction has been reported[232] for $[Fe(CO)_3NH_2]_2$. The nitrosyl carbonyl $Na[Fe(CO)_3(NO)]$ can be prepared[233] from reaction of $Fe(CO)_5$ with sodium nitrite in $NaOCH_3$. Stevens et al[212] extended this work using $[N(PPh_3)_2](NO_2)$ to give the $[Fe(CO)_3(NO)]^-$ anion as the $[N(PPh_3)_2]$ salt from reaction with both $Fe(CO)_5$ and $Fe_3(CO)_{12}$ in tetrahydrofuran. The thallium(I) derivative, $TlFe(CO)_3NO$ is prepared in a similar fashion[234] from reaction of $Fe(CO)_5$ and potassium nitrite in methanol followed by addition of an aqueous solution of $TlNO_3$. Reaction of $Fe(CO)_3(PPh_3)_2$ with isopentyl nitrite and HPF_6 in benzene gives the yellow $[Fe(CO)_2(NO)(PPh_3)_2]PF_6$ complex[235].

Both methaemoglobin (Fe^{3+}) and haemoglobin (Fe^{2+}) react with nitric oxide to yield the same nitrosylhaemoglobin[236]. The Fe(III)(TPP)Cl complex reacts reversibly with nitric oxide in either toluene or chloroform to form Fe(TPP)(NO)Cl; however in the presence of methanol, Fe(III)(TPP)Cl reacts with excess nitric oxide to produce Fe(II)(TPP)(NO), which was shown to contain a square-pyramidal coordination group[237,238]. Iron(III) complexes of the type $[Fe(TPP)(NO)(H_2O)]ClO_4$ and $[Fe(OEP)(NO)]ClO_4$ can be prepared by reaction of nitric oxide with the corresponding (perchlorato)(porphyrinato)iron(III) complex[239]. Similarly the σ-bonded alkyl- and aryliron porphyrins, Fe(TPP)(R) and Fe(OEP)(R) (where R = CH_3, *n*-C_4H_9, C_6H_5, C_6H_4Me-*p*, C_6H_5OMe-*p* and C_6F_4H) react with nitric oxide to form the corresponding Fe(TPP)(R)(NO) and Fe(OEP)(R)(NO) derivatives[240]. The reaction of nitric oxide with [FeL].thf (H_2L = 5H,14H-6,8,15,17-tetramethyldibenzo[b,i][1,4,8,11]tetra-azacyclotetradecine) in tetrahydrofuran leads to the isolation of the deep green crystalline solid [FeL(NO)].thf which X-ray crystallography demonstrated contained a square-pyramidal geometry[241]. The β form of iron(II) phthalocyanine binds nitric oxide[190] to form Fe(pc)(NO); this complex when heated at low pressure or sublimed loses nitric oxide to regenerate Fe(β-pc).

Nitric oxide in the presence of base and alcohol was shown by Gwost & Caulton[242] to function as a reducing agent and nitrosylating agent towards $FeCl_2$ to give alkyl nitrite and an equilibrium mixture of $[Fe(NO)_2Cl]_2$ and $[Fe(NO)_2Cl_2]^-$. The $[FeL_4(NO)]BPh_4$ (L = $P(OMe)_3$, $P(OEt)_3$) complexes are prepared[243] by reaction of

alcoholic solutions of $FeBr_2$ with the appropriate phosphite in the presence of nitric oxide and $NaBPh_4$. NMR spectroscopy suggested a trigonal-bipyramidal structure for the cation, with the NO ligand in the equitorial position. Similarly ethanolic solutions of $Fe(BF_4)_2$ and $NaBPh_4$ in the presence of the tripodlike ligand tris(2-diphenylphosphinoethyl)amine, np_3, react with nitric oxide to form the trigonal-bipyramidal $[Fe(NO)(np_3)]BPh_4$ complex, which X-ray crystallography demonstrated contained the NO ligand in the axial position[244]. Nitric oxide also reacts with iron(II) halides to give complexes of the type $[Fe(NO)(das)_2X]X$ in the presence of *o*-phenylenebis(dimethylarsine)[245].

Hishinuma et al[246] demonstrated the aqueous solutions of Fe(II)EDTA bind nitric oxide to give Fe(NO)EDTA in a completely reversible reaction. The formation of some apparently pentacoordinate mononitrosyls[247] from reaction of nitric oxide with the high spin compounds, N,N'-ethylenebis(salicylideneiminato)iron(II) and its 5-Me, 3-NO_2, 5-Cl, 4-Cl and 5-NO_2 substituted derivatives was reported, which each contain three unpaired electrons at room temperature. However the reaction of nitric oxide with Fe(acacen) is very complex, occurring both at the metal and at the ligand resulting in a polymetallic aggregate[248] (see Figure 1.44). Iron complexes of the type Fe(J-R) (R = ph, mph) and Fe(bza-ph), where H_2(J-R) are Jäger-type ligands with the bridging group R (ph = *o*-phenylene, mph = 4-methyl-*o*-phenylene) combining two imino-nitrogens and H_2(bza-ph) is N,N'-bis(2-benzoylethylidene)-*o*-phenylenediamine, react with nitric oxide in dichloromethane solution to yield the mononitrosyl complexes[249] Fe(J-R)NO and Fe(bza-ph)NO.

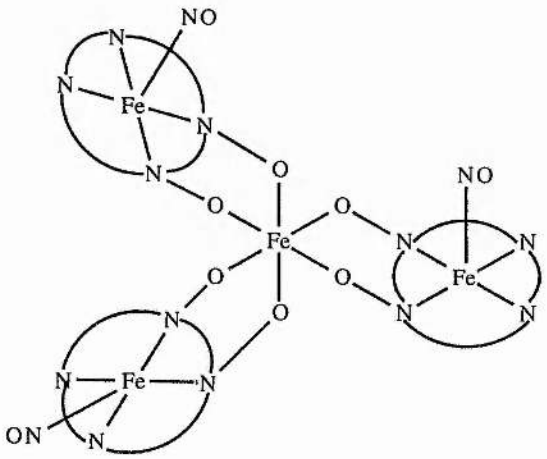


Figure 1.44; The tetranuclear [[ON-Fe{acacen$(NO)_2$]$_3$]Fe} complex

Ruthenium

Martin et al[250] observed that ruthenium tetroxide reacts with nitric oxide in tetrachloromethane forming the nitratonitrosylruthenium complex $RuN_3O_{7.5}$ and proposed a complex containing two bisnitrosyl ruthenium groups linked by an oxo bridge. The trichloronitrosyl complex, $Ru(NO)Cl_3$ is obtained from reaction of a NO-NO_2 mixture with ruthenium chloride in excess acid[251]. However reaction of ruthenium chloride with nitric oxide[252] or potassium nitrite[253] in excess acid yields the pentachloronitrosyl complex, $K_2Ru(NO)Cl_5$. The related cyanide complex, $K_2Ru(NO)(CN)_5$ is prepared by bubbling nitric oxide through a solution containing ruthenium chloride and potassium cyanide. The pentaaquonitrosyl, $[Ru(H_2O)_5(NO)]^{3+}$ and the pentaamminenitrosyl $[Ru(NH_3)_5(NO)]^{3+}$ complexes can similarly be prepared by passing nitric oxide through solutions containing $[Ru(H_2O)_6]^{3+}$ and $[Ru(NH_3)_6]^{3+}$ respectively[254,255]. Sodium nitrite however does not reacts with aqueous $[Ru(NH_3)_6]^{3+}$, but addition of hydrochloric acid leads to formation of the pentaamminenitrosyl complex[256]. Synthesis of the series of *cis* nitrosyl complexes[257] of the form *cis*-$[Ru(NH_3)_4(NO)X]^{n+}$ (X = OH_2, OH, Cl, Br or I) can be achieved by the direct stereospecific attack of nitric oxide upon dilute HX solutions of the corresponding *cis*-$[Ru(NH_3)_4X_2]^{m+}$ complexes.

The trinuclear $Ru_3(CO)_{12}$ readily forms $Ru_3(CO)_{10}(NO)_2$ on reaction with nitric oxide in benzene[258]. X-Ray studies demonstrated a structure whereby two ruthenium atoms were linked via a double nitrosyl bridge. However reaction of $Ru_3(CO)_{12}$ with $[(PPh_3)_2N](NO_2)$ yields the mononitrosyl bridged complex[212], $[Ru_3(CO)_{10}NO]^-$. Reaction of $[(PPh_3)_2N](NO_2)$ with the carbido cluster[259], $Ru_5C(CO)_{15}$ in tetrahydrofuran yields $[Ru_5C(CO)_{13}NO]^-$; similarly[212] $Ru_6C(CO)_{17}$ yields $[Ru_6C(CO)_{15}NO]^-$, both of which were believed to contain terminal nitrosyl ligands.

The mononitrosyl complex[260], $Ru(PPh_3)_2(NO)Cl_3$, can be prepared by bubbling nitric oxide through an ethanolic solution containing $RuCl_3.3H_2O$ and excess triphenylphosphine. The reaction of $Ru(PPh_3)_3Cl_2$ with nitric oxide was found by Cenini et al[261] to be solvent dependent. In the presence of chlorinated solvents, $Ru(PPh_3)_2(NO)Cl_3$ is obtained in quantitative yields. However in the absence of sources of halogens, $Ru(PPh_3)_2(NO)Cl_3$ is still formed but $Ru(PPh_3)_2(NO)_2$ is also observed suggesting that the reaction with nitric oxide is a disproportionation reaction. The solid tetrahydride $RuH_4(PPh_3)_3$ also reacts with nitric oxide yielding the same $Ru(PPh_3)_2(NO)_2$ complex[262,263]. Addition of aqueous sodium nitrite to an ethanolic solution of $CpRu(PPh_3)_2Cl$ and HPF_6 yields the red crystalline solid $[CpRu(PPh_3)(NO)Cl]PF_6$ which was shown to contain a distorted pseudo-octahedral structure[264]. Similarly[265], the $CpRu(PPh_3)_2CN$ complex yields

$[CpRu(PPh_3)(NO)CN]PF_6$. Complexes of the type $[Ru(bipy)_2(NO)X]^{2+}$ or $[Ru(phen)_2(NO)X]^{2+}$ (X = Cl, Br, NO_2 or py) can be prepared[266] by the addition of nitrite ion to acidic solutions of the corresponding aquo complexes $[Ru(bipy)_2(H_2O)X]^+$ or $[Ru(phen)_2(H_2O)X]^+$.

Osmium

Aràneo et al[267] synthesised the halogenonitrosyl complexes $[OsX_3(NO)(SbPh_3)_2]$ (X = Cl, Br, I) from reaction of $Os(SbPh_3)_3X_3$ and nitric oxide. The related complexes[268] $[OsX_3(NO)L_2]$ (where L = PPh_3 or $AsPh_3$) are prepared directly from reaction of the corresponding halogenosmate $[OsX_6]^-$ with nitric oxide and then with the ligand. However, when $K_2[OsX_6]$ is reacted with potassium nitrite, yellow crystals of $K_2[Os(NO)(OH)(NO_2)_4]$ are deposited, but if the reaction is carried out in the presence of caesium chloride, the caesium salts $Cs[Os(NO)X_5]$ are formed[269]. $[Os(NH_3)_5I]I_2$ reacts with nitric oxide to give a mixture of $[Os(NH_3)_3NO]I_3$ and $[OsI(NH_3)_4NO]I$, which is converted to $[Os(NH_3)_5NO]I_3$ in liquid ammonia[270]. Nitric oxide reacts with $Os(bipy)_2Cl_2$ and HPF_6 in aqueous ethylene glycol to give the bipyridyl nitrosyl complex[271], $[Os(bipy)_2Cl(NO)](PF_6)_2$. Zinc reduction[272] of *mer*-$[OsCl_3(PMe_2Ph)_3]$ in tetrahydrofuran with nitric oxide gives the dichloronitrosyl complex, $[OsI_2(NO_2)(NO)(PMe_2Ph)_2]$ which can also be isolated as the monochloronitrosyl product, $[OsCl(NO_2)(NO)(PMe_2Ph)_3]BPh_4$ when carried out in the presence of $NaBH_4$. Infra-red spectroscopy indicated that the NO_2 is bound as a nitro group which probably results from disproportionation of nitric oxide during the course of the reaction. The cyclopentadienylnitrosyl complex[265], $[CpOs(PPh_3)(NO)CN]PF_6$ can be prepared from reaction of sodium nitrite with an acidified ethanolic solution of $[CpOs(PPh_3)_2CN]$ and HPF_6.

The carbonyl complexes, $Os(CO)_{10}(NO)_2$ have been reported[258] from reaction of the tricarbonyl $Os_3(CO)_{12}$ with nitric oxide in benzene. However reaction of $[(PPh_3)_2N](NO_2)$ with $Os_3(CO)_{12}$ in tetrahydrofuran yields the mononitrosyl[212] $[(PPh_3)_2N][Os_3(CO)_{10}NO]$.

Cobalt

The reaction[273] of nitric oxide with ammoniacal solutions of $[Co(H_2O)_6]^{2+}$ yields two isomeric forms of $[Co(NO)(NH_3)_5]X_2$; a black series (X = Cl, NO_3, IO_3) and a red series (X = Br, NO_3, SO_4). The former decomposes readily in aqueous solution liberating NO but the latter are far more stable especially in dilute acid. X-Ray crystallography studies[274,275] demonstrated that the black series were monomeric but that the red series were dimeric containing a bent hyponitrite ligand. Nitric oxide reacts

directly[276] with the appropriate anhydrous CoX_2 in the presence of cobalt powder to yield complexes of the stoichiometry $Co(NO)_2X$ (X = Cl, Br, I). In the solid state, $Co(NO)_2I$ was shown[277] to consist of infinite chains formed by equally orientated $Co(NO)_2I_2$ tetrahedra sharing the corners of both iodine atoms (see Figure 1.45).

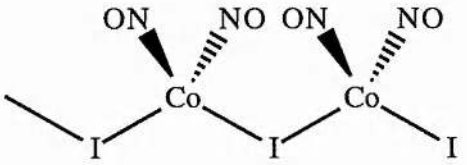


Figure 1.45; A section of the infinite chain of $[Co(NO)_2I]_n$

Slowly at room temperature, but almost instantaneously at 40°C, nitric oxide gas reacts with $Co_2(CO)_8$ to form the cherry red liquid[278] $Co(CO)_3NO$, which can also be prepared[212] from reaction of $Co_2(CO)_8$ with $[N(PPh_3)_2](NO_2)$. Alternatively, $[Co(CO)_4]^-$ in solution reacts with nitric oxide[279] or sodium nitrite[280] to yield the same nitrosyl tricarbonyl complex. Further addition of nitric oxide[281] at low pressure produces $Co(NO)_3$. The reaction is rapid and direct at room temperature but requires several days in the dark at 60°C. The same sequence of events, ie initial formation of $Co(CO)_3NO$ followed by complete conversion to $Co(NO)_3$ was observed[282] when nitric oxide was reacted with the cobalt carbonyls $Co_2(CO)_8$ and $Co_4(CO)_{12}$ supported on silica, γ-alumina, Na-Y and Na-X zeolites, and magnesia. However treatment of $Co_2(CO)_8$ in hexane solution with an excess of nitric oxide results in the formation[283] of $[Co(NO)_2(NO_2)]_n$ which has been shown to exist in two different forms in the solid state and in solution (see Figure 1.46).

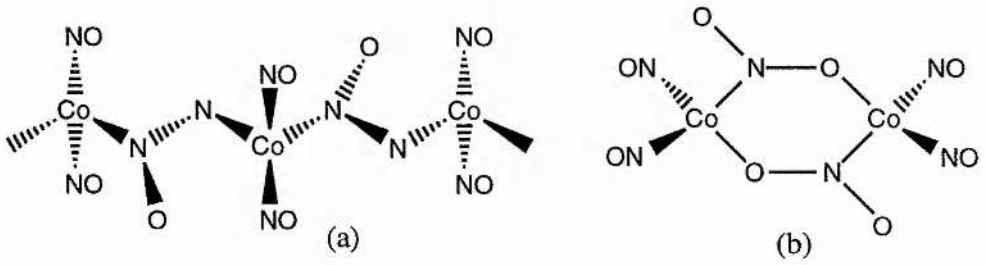


Figure 1.46; Structure of $[Co(NO)(NO_2)]_n$ in (a) solid state and (b) solution

In the reaction of the cyclopentadienyl complex, $CpCo(CO)_2$, with nitric oxide in hexane, the $[CpCo(NO)]_2$ complex is obtained which contains a double nitrosyl bridge linking the two Co atoms[284]. However the same reaction in the presence of norbornene[285] yields a cyclopentadienylnorbornenecobalt complex whereby norbornene is linked via two nitrosyl ligands across the norbornene double bond (see Figure 1.47).

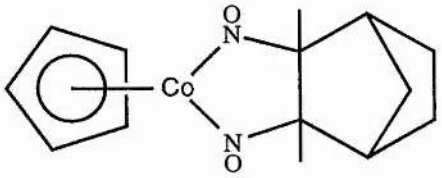


Figure 1.47; The $[CpCo(NO)_2(norbornene)]$ complex

The perfluoroalkyl complexes[286,287], $Co(NO)R_F(P(OPh)_3)$, are prepared by the action of nitric oxide on the isoelectronic carbonyls, $Co(CO)_3R_F(P(OPh)_3)$ and $Co(CO)_3(C_2F_5)(PPh_3)$ respectively. Nitric oxide reacts very rapidly with $Co(S_2PF_2)$ leading to formation of $Co(NO)_2(S_2PF_2)$[288]. The same product can also be obtained from the much slower reaction of nitric oxide with $Co(S_2PF_2)_3$.

The red diamagnetic mononitrosyl complex[289] $Co(NO)(P(CH_3)_3)$ can be formed from the addition of nitric oxide to solutions of $Co(PPh_3)_4$. The halogenophosphine derivatives, CoX_2L_2 (L = PMe_3, X = Cl, Br, I, NO_2, NCS; L = PEt_3, X = Cl, Br) similarly absorb nitric oxide to form the $CoX_2(NO)L_2$ complexes[290,291]. Ibers et al[292] synthesised the further series of nitrosylphosphine derivatives $CoCl_2(NO)L_2$ (L = $P(C_2H_5)_3$, $P(\eta\text{-}C_4H_9)_3$, $PPh(CH_3)_2$, PPh_2CH_3, PPh_3 and $P(p\text{-}CH_3C_6H_4)_3$) in an analogous fashion by direct reaction of nitric oxide with solutions of $CoCl_2L_2$. Solutions of the phosphite complexes, $[CoXL_4]^+$, (X = Cl, Br, I if L = $PhP(OEt)_2$ or $PhP(OMe)_2$; X = I if L = $P(OEt)_3$ or $P(OMe)_3$) and CoX_2L_2 (L = $P(OEt)_3$, $P(OMe)_3$; X = Cl, Br) take up nitric oxide with the formation of five-coordinate mononitrosyl cations of the type $[CoXL_3(NO)]^+$, which further react with nitric oxide to produce the dinitrosyl complexes[293], $[CoL_2(NO)_2]^+$ and $CoXL(NO)_2$. Bubbling nitric oxide through cobalt nitrite solution containing an excess of phosphite yields another series of five-coordinate mononitrosyl complexes[294] of the type $[CoL_4(NO)]^{2+}$ (L = $P(OMe)_3$, $P(OEt)_3$ or $PPh(OEt)_2$), but further reaction again leads to the dinitrosyl complex, $[CoL_2(NO)_2]^+$. Substitution by nitric oxide into the $CoH(np_3)$ complexes (where np_3 is the tripodlike ligand tris(2-diphenylphosphinoethyl)amine) yields the neutral complex[244] $Co(NO)(np_3)$. Attempts to prepare the $[Co(2{=}phos)_2(NO_2)_2]^+$ complex (where 2=phos is *cis*-1,2-bis-(diphenylphosphino)ethylene by Gray et al[295] from $[Co(2{=}phos)_2Cl_2]^+$ and nitrite gave instead the dinitrosyl product, $[Co(2{=}phos)(NO)_2]^+$. The $[Co(2{=}phos)_2(NO_2)_2]^+$ complex was thus inferred to be unstable due to highly unfavourable steric interactions involving NO_2^- and PPh_2 groups and undergoes decomposition to give the dinitrosyl species. Nitric oxide reacts with $[Co(P\text{-}SR)_2]^{2+}$ (P-SR = 1-(thioalkyl)-2-diphenylphosphino)ethane) to form the five coordinate mononitrosyl $[Co(NO)(P\text{-}SR)_2]^{2+}$ complexes[296] for which IR and NMR data suggested contained a trigonal-bipyramidal geometry. However, while these

complexes are air-stable in the solid state at room temperature, their solutions are relatively unstable, decomposing to yield the dinitrosyl $[Co(NO)_2(P\text{-}SR)_2]^+$ derivatives.

Dithiocarbamato complexes of the type, $Co(NO)(S_2CN(C_2H_5)_2)_2$ can be prepared by the reaction of $Na[S_2CN(C_2H_5)_2]$ with cobalt(II) acetate in the presence of nitric oxide[189]. Related complexes with the formulation $[Co(NO)(S_2C_2(CN)_2)_2]^{2-,1-}$ were prepared by McCleverty et al[297] from the action of nitric oxide on solutions containing the $[Co(S_2C_2(CN)_2)_2]^{2-}$ salts. The reaction at 0°C of nitric oxide with $Co(S_2C_5H_7)_2$ in dichloromethane yields the mononitrosyl complex[298] $Co(NO)(S_2C_5H_7)_2$. However the same reaction at room temperature yields the dinitrosyl complex $Co(NO)_2(S_2C_5H_7)_2$ which can also be formed from the spontaneous decomposition of the mononitrosyl complex in solution at room temperature. The series of six-coordinate mononitrosyl complexes[299] of the type $[CoX(NO)(das)_2]^+$ and $[CoX(NO)(en)_2]^+$ (das = *o*-phenylenebis(dimethylarsine), X = Cl, Br, NCS; en = ethylenediamine, X = Cl, Br, I, NO_3) can be prepared by the reaction of $CoX_2(das)_2$ or CoX_2 and ethylenediamine with nitric oxide.

A large number of cobalt(II) Schiff base complexes have been shown[300] to absorb nitric oxide giving the five-coordinate Co(NO)(Schiff base) complexes. Similarly, the cobalt complexes Co(J-R), where H_2(J-R) are Jäger-type ligands with bridging group R (R = ethylene, 1,2-propylene, *o*-phenylene) combining two imino nitrogens, absorb nitric oxide to yield the corresponding Co(NO)(J-R) complexes[249]. Solutions of the cobalt(II) porphyrins, Co(II)OEP and Co(II)TPP, absorb nitric oxide[301,302] to yield the Co(NO)OEP and Co(NO)TPP complexes respectively. Similarly the ß crystalline form of Co(pc) (pc = phthalocyanine) reacts with nitric oxide to form the corresponding mononitrosyl derivative[200].

Silvestroni et al[303] observed that colourless solutions containing the cobalt(II) dihistidine complex absorb nitric oxide reversibly to yield the violet $Co(His)_2(NO)$ species. Subsequently, the binding process of nitric oxide by the range of cobalt(II) complexes with amino acids (Gly, Ala, Sar, Orn, Thr, Arg, Lys) and also the cobalt(II) complexes with amino-acids and imidazole ($Co(a\text{-}a)_2(imid)$) was also shown to be reversible[304]. Electron spectroscopy indicated that as a result of the NO molecule being bound by the 'active' form, a cobalt(III) complex is formed, however even when the solution is fully saturated with nitric oxide, there is still a cobalt(II) complex in solution in equilibrium with the nitrosyl cobalt(III) complex.

Rhodium

The dinitrosyl chloride, $[Rh(NO)_2Cl]_n$, can be prepared[305] by passing nitric oxide over $Rh(CO)_2Cl$. A polymeric structure was proposed for the nitrosyl complex containing an essentially dimeric structure with polymerisation occurring through the rhodium-rhodium bonds[306]. Ethanolic solutions containing $[Rh(CO)_2Cl_2]^-$ or $RhCl_3.3H_2O$, when exposed to nitric oxide in the presence of triphenylphosphine gives the $RhCl_2(NO)(PPh_3)_2$ complex[260,307]. However bubbling nitric oxide through a boiling tetrahydrofuran solution containing $RhCl_3.3H_2O$ in the presence of excess tetrahydrofuran and granulated zinc affords the $Rh(NO)(PPh_3)_3$ species[308]. The fluorine analogue[309], $Rh(NO)(PF_3)_3$, can be obtained by passing carbon dioxide into an aqueous solution of $K[Rh(PF_3)_4]$ containing nitrite. The same nitrosyl compound can also be obtained by shaking $[RhCl(PF_3)]_2$ with copper, trifluorophosphine and nitric oxide at room temperature or more efficiently by displacement of the π-ally group in π-allyl$[Rh(PF_3)_3]$ with nitric oxide. Nitric oxide reacts with the square-planar complexes $RhCl(CO)(PPh_3)_2$ and $RhCl(PPh_3)_3$ to yield the same five-coordinate nitrosyl-nitro-derivative[310], $RhCl(NO_2)(NO)(PPh_3)_2$. Analogous behaviour is also exhibited by the $RhCl(CO)(AsPh_3)_2$ and $RhCl(AsPh_3)_3$ complexes.

Stable adducts of the type $[Rh(OOCCH_3)_2]_2(NO)_2$ are formed when nitric oxide is passed over the solid $Rh(OOCCH_3)]_2$ dimer[311]. The rhodium(III) porphyrin complexes $(RhOEP)_2$, RhOEP(H) and RhOEP(Cl) all react with nitric oxide to ultimately produce the same product[312], RhOEP(NO). Similarly, the reaction of RhTPP(Cl) with nitric oxide ultimately produces RhTPP(NO).

Iridium

Stevens et al[313] reported that the nucleophilic addition of $[N(PPh_3)_2]NO_2$ to $[Ir_6(CO)_{16}]$ leads to $[Ir_6(CO)_{15}NO]^-$, however an excess of nitrite causes further reduction to $[Ir_6(CO)_{15}]^2$. A much cleaner synthesis is via one-electron oxidation of $[Ir_6(CO)_{15}]^{2-}$, followed by addition of nitric oxide at low temperature[314]. X-Ray structure determination showed that the complex displays idealised C_S point group symmetry consisting of an octahedral framework of iridium atoms, a linear NO group, eleven terminal and three edge-bridging carbonyl ligands.

The $Ir(NO)(PPh_3)_3$ complex can be prepared either by bubbling nitric oxide through a boiling tetrahydrofuran solution containing $IrCl_3.3H_2O$ in the presence of excess triphenylphosphine and granulated zinc[308], or by passing nitric oxide through a benzene solution of $Ir(N_2)Cl(PPh_3)_2$ followed by reduction using a Na/Hg amalgam in the presence of triphenylphosphine[315]. Reaction between *trans*-$Ir(CO)Cl(PPh_3)_2$ and

sodium nitrite in benzene and aqueous alcohol affords the $[Ir(NO)(PPh_3)_2]O$ complex[316] (see Figure 1.48). Treatment of $[Ir(CO)_3(PPh_3)_2]^+$ with nitric oxide yields the $[Ir(NO)(CO)(PPh_3)_2]^+$ cation[317]. The neutral $Ir(NO)(CO)(PPh_3)_2$ complex[318] can be prepared by the action of nitric oxide on the equivalent hydride, $Ir(H)(CO)(PPh_3)_3$.

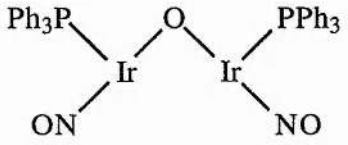


Figure 1.48; Structure of the $[Ir(NO)(PPh_3)_2]O$ complex

Nickel

The cyclopentadienylnickelnitrosyl CpNi(NO) can be obtained[209,319] either by treatment of nickelocene, or $Ni(CO)_4$ in the presence of cyclopentadiene, with nitric oxide. The carborane analogue[320], $Ni(NO)[1,7\text{-}B_9H_9CHPMe]$, can be isolated from the reaction of the $[7,9\text{-}B_9H_9CPMe]^-$ anion with nickel(II) chloride and nitric oxide in tetrahydrofuran.

Reaction of nickel(II) halides with nitrite and the triphosphines, tpp and tep (where tpp and tep are the 'tripod-like triphosphines, $CH_3C(CH_2PPh_2)_2$ and $CH_3C(CH_2PEt_2)_3$, respectively) yields the deep red, diamagnetic complexes[321] $[Ni(NO)(tpp)]X$ and $[Ni(NO)(tep)]X$ (where X = Cl, Br, I, BF_4, BPh_4). An X-ray structure determination has shown that the nickel atom is surrounded in a pseudo-tetrahedral fashion by three phosphorus atoms and a linearly bonded nitrosyl group. The tetrahedral $[Ni(NO)(np_3)]X$ and $[Ni(NO)(nas_3)]X$ complexes[244] (np_3 = $N(Ph_2PEt)_3$, X = I, NO_3, BF_4; nas_3 = $N(Ph_2ArEt)_3$, X = I) are formed from the action of nitric oxide on solutions of $[NiX(np_3)]$ and $[NiX(nas_3)]$. Nickel nitrosyl cations of the type $[Ni(NO(PR_3)_3]^+$ (PR_3 = PMe_3, PEt_3, PMe_2Ph) can be prepared[322] by the action of nitrite on the corresponding $NiCl_2(PR_3)_2$ complexes in the presence of carbon monoxide. The crystal structure[323] of the trimethylphosphine complex again showed a slightly distorted tetrahedron but with a slightly bent nitrosyl group. Tetracoordination is also supported in the case of the $[Ni(NO)(P\text{-}L)_2]^+$ complexes (P-L = 1-(2'-pyridyl)-2-(diphenylphosphino)ethane and 1-(thioethyl)-2-(diphenylphosphino)ethane), which can be formed from the action of nitrite on solutions containing $[Ni(P\text{-}L)_2]_2^+$ under carbon monoxide, where one P-L molecule acts as a monodentate ligand[324].

Sodium nitrite reacts with the $NiX_2(PPh_3)_2$ complexes (X = Cl, Br, I) in the presence of triphenylphosphine to give the halogenonitrosyl complexes $NiX(NO)(PPh_3)_2$ which again are believed to contain a tetrahedral structure[325]. Similarly NiX_2L_2 reacts with nitrite in the presence of carbon monoxide to yield the

$NiX(NO)L_2$ complexes[326] (X = Cl, Br, NO_2; L = $1/2Ph_2PCH_2CH_2PPh_2$, PPh_3, PPr^n_3, $OPPh_3$, NC_5H_5). Binuclear complexes of the type $[Ni_2(NO)_2(L\text{-}L)_3]^{2+}$ (L-L = $Ph_2P(CH_2)_nPPh_2$; n = 2(dppe) or 3(dppp) and $Me_2PCH_3PMe_2$ (dmpp)) can be prepared either by the action of nitrite on $[Ni(dppe)_2]^{2+}$ or $[Ni(H_2O)_6]^{2+}$ in the presence of the dppp or dmpp ligands[327]. The bidentate complexes possess a pseudo-tetrahedral coordination for the nickel atoms which are linked via a diphosphine bridge and contain linear Ni-NO groups (see Figure 1.49). Nitrosyl complexes containing chelating sulfur ligands have been generated by the passage of nitric oxide through toluene solutions of $Ni(S_2CNEt_2)_2$ or $Ni(S_2COEt)_2$[328]. Spectroscopic analysis suggested the formation of neutral square pyramidal complexes of the type $Ni(NO)(S_4)$, where $S_4 = S_2CNEt_2$ and S_2COEt.

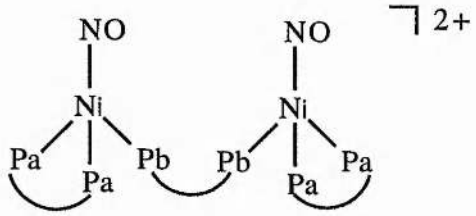


Figure 1.49; Structure of the $[Ni_2(NO)_2(L\text{-}L)_3]^{2+}$ cation

Nitric oxide reacts with the carbonyl complex, $Ni(CO)_2(PPh_3)_2$ to give the nitrosyl oxide, $[Ni(NO)(PPh_3)_2]_2O$ which rapidly reacts with water giving $Ni(OH)(NO)(PPh_3)_2$[329]. However in the presence of nitrogen dioxide, the nitrite product, $Ni(NO_2)(NO)(PPh_3)_2$ is obtained. Passage of nitric oxide into inert solutions of $Ni(CO)_4$ containing traces of water yields the blue paramagnetic compound[305], $Ni(NO)(OH)_3$. In methanol solution, the methoxy complex, $Ni(NO)(OH)(OMe)_2$ is formed. Similarly, $Ni(CO)_4$ reacts with ammonia and nitric oxide in the presence of water to give the ammine complex[330], $[Ni(NO)(NH_3)OH](OH)_2$. Again, in methanol solution the methoxy complex, $[Ni(NO)(NH_3)(OMe)(OH)](OH)$ is formed.

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CHAPTER TWO

TABLE OF CONTENTS

Formation of Iron-Sulfur Nitrosyl Complexes from Dietary Components and the Effect of pH

2.1 Introduction

Glidewell et al[1] demonstrated that in the presence of iron(II) salts, nitrite reacts with cysteine under a range of experimental conditions relevant to food processing to yield the anti-microbial iron-sulfur nitrosyl salt, $Na[Fe_4S_3(NO)_7]$; the yield is increased when the reducing agent sodium ascorbate is also present. Sulfur is similarly captured, to form $Na[Fe_4S_3(NO)_7]$, from a range of other sources including several derivatives of cysteine; homocysteine, sodium thioglycolate, penicillamine and potassium benzylpenicillin, as well as from sodium thiosulfate. Glutathione and cystine also provided $Na[Fe_4S_3(NO)_7]$ although both required the presence of sodium ascorbate. Both acid-hydrolysed casein and enzyme-hydrolysed casein, which contain low levels of both cystine and iron, react with sodium nitrite in the presence of sodium ascorbate to form $Na[Fe_4S_3(NO)_7]$. Slurries of de-fatted pork leg muscle were also shown[2] to form $Na[Fe_4S_3(NO)_7]$ when reacted with sodium nitrite and sodium ascorbate in the presence of added iron(II). Under autoclave conditions the tetranuclear complex was detected at added iron levels down to 0.004% by weight iron(II), similar in fact to levels of iron typically encountered in beef products[3].

The dinuclear iron-sulfur nitrosyl complex, $Fe_2(SMe)_2(NO)_4$ can be isolated from reactions between methionine and iron(II) salts in the presence of sodium nitrite and sodium ascorbate[4]. Methionine ethyl ester similarly gave $Fe_2(SMe)_2(NO)_4$ while S-methyl cysteine gave a mixture of dinuclear $Fe_2(SMe)_2(NO)_4$ and tetranuclear $Na[Fe_4S_3(NO)_7]$. Incubation of parsley, *Petroleselinum crispum*, with sodium nitrite also provided[5] $Fe_2(SMe)_2(NO)_4$.

Thus the dinuclear $Fe_2(SMe)_2(NO)_4$ and the tetranuclear $Na[Fe_4S_3(NO)_7]$ iron-sulfur nitrosyl complexes can be formed from simple precursors, many of which can be found in food products (see Section 1.1; Sources of Nitrite and Nitrate in the Diet). In fact the conditions under which these reactions occur are very similar to those employed in the meat curing industry where nitrite and sodium ascorbate are added to the meat prior to heating to high temperature. The tetranuclear complex, $[Fe_4S_3(NO)_7]^-$, has been shown[6,7] to exhibit wide-spectrum antibacterial action but the dinuclear complex, $Fe_2(SMe)_2(NO)_4$, although not itself carcinogenic and only weakly mutagenic, potentiates the action of other carcinogens, including N-nitrosamines and polycyclic aromatic hydrocarbons[8-10] such as those found in cigarette smoke and therefore is a cause for concern.

However a number of problems still remain to be addressed for the formation of $Na[Fe_4S_3(NO)_7]$ and $Fe_2(SMe)_2(NO)_4$ from the reaction of cysteine or methionine with iron(II) salts and nitrite. As both iron(III) and nitrate can also be found in food products and nitrate can additionally be present in drinking water, could iron(III) salts replace iron(II) salts or nitrate replace nitrite? Also, since not all foods have neutral pH and in fact most foods are acidic (see Table 2.1) and conditions in the stomach are extremely acidic with a pH of around 2, what would be the effect of lowering the pH?

Food	pH
Apples	2.9 - 3.3
Beans	5.0 - 6.0
Bread, white	5.0 - 6.0
Butter	6.1 - 6.4
Carrots	4.9 - 5.3
Cheese	4.8 - 6.4
Limes	1.8 - 2.0
Milk, cows	6.3 - 6.6
Peas	5.8 - 6.4
Potatoes	5.6 - 6.0
Tuna	5.9 - 6.1

Table 2.1; Approximate pH values of selected foods

2.2 Results and Discussion

2.2.1 Reactions of Cysteine with Iron(II)/(III) Salts and Sodium Nitrite/Nitrate with and without Sodium Ascorbate

Control reactions

When cysteine (16.5 mmol) was autoclaved at 118°C in aqueous solution with iron(II) sulfate and sodium nitrite (1.80 mmol and 1.45 mmol respectively), the sodium salt of Roussin's black anion, $Na[Fe_4S_3(NO)_7]$ was formed in ca. 40% yield. The yield of $Na[Fe_4S_3(NO)_7]$ obtained was increased to 72% when sodium ascorbate (10.1 mmol) was also present.

The formation of $Na[Fe_4S_3(NO)_7]$ requires cleavage of the carbon-sulfur bonds in cysteine to liberate sulfide and reduction of iron(II) to a mean oxidation state of -1/2, followed by spontaneous self-assembly[11]. Thus it has been suggested[1] that the increase in yield of $Na[Fe_4S_3(NO)_7]$ obtained when sodium ascorbate is also present is due to the reducing action of sodium ascorbate in keeping the cysteine in the reduced thiol form, $HSCH_2CH(NH_2)COOH$. It is known[12] that the very slow oxidation by molecular oxygen of thiols, such as cysteine and glutathione, to the disulfide forms, cystine and diglutathione, is accelerated by iron salts (see Figures 1.25 and 1.33 for the iron catalysed oxidation by molecular oxygen reaction schemes for cysteine and glutathione). Further supporting[1] this interpretation, when cystine was employed as the sulfur source, in the absence of sodium ascorbate no $Na[Fe_4S_3(NO)_7]$ was formed; only when the reducing agent sodium ascorbate was also present was $Na[Fe_4S_3(NO)_7]$ formed. This led to the suggestion that a thiol group might be essential for sulfur capture from organosulfur compounds into $[Fe_4S_3(NO)_7]^-$. However subsequent investigation of a whole range of thiols and thiones showed that this was an over-simple hypothesis.

Although a number of other thiols including N-acetylcysteine, cysteine methyl ester hydrochloride, cysteine ethyl ester hydrochloride, homocysteine, sodium thioglycolate and penicillamine all gave $Na[Fe_4S_3(NO)_7]$ in the absence of sodium ascorbate, several thiols including glutathione, N-acetyl penicillamine, 2-mercaptopyridine and 2-mercaptobenzothiazole required the presence of sodium ascorbate for formation of $Na[Fe_4S_3(NO)_7]$, while the apparently related heterocyclic thiols (2-mercaptopyrimidine, 2-mercaptothiozoline, 2-mercapto-1-methylimidazole and 2-mercaptobenzimidazole) gave no $Na[Fe_4S_3(NO)_7]$ even in the presence of sodium ascorbate[1]. In contrast, it was found that several thiones, including N-methylthiourea,

N,N'-dimethylthiourea, N,N'-diphenylthiourea, thioacetanilide and thiocarbonyldi-imidazole all provided $Na[Fe_4S_3(NO)_7]$ without added ascorbate.

Another factor in the increased yield of $Na[Fe_4S_3(NO)_7]$ observed when sodium ascorbate is also present could be that sodium ascorbate, being a reducing agent could also prevent, or at least partially prevent, nitrite from oxidising iron(II) to iron(III).

Iron(III) reactions

When iron(III) chloride was substituted for iron(II) sulfate (see Section 2.2.3) in the reaction between cysteine and sodium nitrite in aqueous solution, $Na[Fe_4S_3(NO)_7]$ was isolated in 42% yield. However, unlike the control reactions, the yield of $Na[Fe_4S_3(NO)_7]$ obtained did not significantly increase when sodium ascorbate was also present.

In the absence of sodium ascorbate, the yield of $Na[Fe_4S_3(NO)_7]$ obtained during the reaction of cysteine with nitrite in the presence of iron(III) salts is very similar to that obtained from the control reaction with iron(II) salts in the absence of sodium ascorbate. However, in the absence of sodium ascorbate it is likely that nitrite will oxidise iron(II) to iron(III). The iron atoms present in $Na[Fe_4S_3(NO)_7]$ exist in the mean oxidation state of -1/2 (each of the three basal iron atoms are Fe(-1), d^9 and the unique apical iron atom has been assigned as Fe(I), d^7). Hence in order to form $Na[Fe_4S_3(NO)_7]$, iron(III) would be required to be reduced further than its iron(II) counterpart. However it should also be noted that during the oxidation of iron(II) to iron(III) by nitrite, that nitrite is consumed which may also acount for the decrease in yield observed in the control reactions in the absence of ascorbate. This does however show that the decrease in yield of $Na[Fe_4S_3(NO)_7]$ obtained in the control reaction when carried out in the absence of sodium ascorbate, compared to that obtained in the control reaction carried out in the presence of sodium ascorbate, is not due to a partial oxidation of iron(II) to iron(III) by nitrite whereby the formation of $Na[Fe_4S_3(NO)_7]$ could be hindered due to the inactivity of iron(III).

Taylor et al[13] observed that addition of iron(III) to an aqueous solution of cysteine resulted in the formation of a reddish blue to blue coloured complex, followed by conversion to cystine. A more detailed study demonstrated that the reaction is two-thirds order with respect to both cysteine and oxygen. It was thus proposed that the iron(III) ion forms a complex with three cysteine molecules which may undergo ionisation due to the carboxyl and amino groups on the cysteine ligands. One of these forms was proposed to be specially reactive and was designated $Fe(Hcys)_3$*, which then decomposes to form an iron-monocysteine complex and two cysteinyl species

which may combine together to form cystine. Reformation of the iron-tricysteine complex is achieved by the oxidation of the iron-monocysteine complex in the presence of cysteine (see Figure 2.1).

$$Fe^{III}(Hcys)_3^* \rightleftharpoons Fe^{I}(Hcys) + 2Hcys^*$$

$$2Hcys^* \longrightarrow \text{cystine}$$

$$Fe^{I}(Hcys) + 2\text{cysteine} + 0.5O_2 \longrightarrow Fe^{III}(Hcys)_3 + H_2O$$

Figure 2.1; Scheme for the iron(III) catalysed oxidation of cysteine by molecular oxygen

Subsequent work has shown that the formation of $Na[Fe_4S_3(NO)_7]$ from cystine requires the presence of a reducing agent[1], and so in the absence of sodium ascorbate, the decrease in yield of $Na[Fe_4S_3(NO)_7]$ obtained from the reaction between cysteine, iron(III) chloride and sodium nitrite may be explained due to the formation of cystine from the iron(III) catalysed oxidation of cysteine.

However, the presence of sodium ascorbate during the reaction of iron(III) chloride with cysteine and nitrite does not lead to a significant increase in yield of $Na[Fe_4S_3(NO)_7]$. This is in contrast to that observed with the control reactions using iron(II) salts. Glidewell et al[1] had previously demonstrated that the reaction of cystine with iron(II) salts and nitrite required the presence of sodium ascorbate, and proposed that sodium ascorbate reduced the disulfide cystine to its thiol form cysteine. However, in the presence of iron(III) salts, the reduction of cystine to cysteine by ascorbate may continually be reversed by the stronger tendency for iron(III) to oxidise cysteine back to cystine. Additionally, the self-assembly of $Na[Fe_4S_3(NO)_7]$ again may be less favoured by the higher oxidation state present in iron(III).

Nitrate reactions

In a similar manner, when cysteine and iron(II) sulfate were autoclaved at 118°C in aqueous solution with sodium nitrate (in place of sodium nitrite) no nitrosyl products were formed with or without sodium ascorbate present.

The nitrosating action of nitrite is believed to proceed via a series of steps. In aqueous solution, but favoured by acidic conditions, nitrite is converted to nitrous anhydride via nitrous acid.

$$2NO_2^- + 2H^+ \rightleftharpoons 2HNO_2$$

$$2HNO_2 \rightleftharpoons N_2O_3 + H_2O$$

Nitrous anhydride is a very reactive nitrosating species in water, reacting for example, with amines at $pK_a > 5$ with a rate constant close to the diffusion limit. However, in the case of nitrate, the first step in the nitrosation process would form nitric acid which would be unfavourable since nitric acid is a very strong acid.

$$NO_3^- + H^+ \rightleftharpoons HNO_3$$

Thus nitrate does not react with cysteine and iron(II) salts with or without sodium ascorbate present to form nitrosyl complexes since nitrate is not as effective a nitrosating agent as nitrite. Nor is there any reduction of nitrate to nitrite by either iron(II) or cysteine. This is perhaps surprising in view of the great ease of oxidation of both components (see Appendix I).

2.2.2 Reactions of Cysteine with Iron(II) Sulfate and Sodium Nitrite with and without Sodium Ascorbate versus pH

Results

When sodium nitrite and iron(II) sulfate (1.45 mmol and 1.80 mmol respectively) were autoclaved at 118°C at pH 7 in a buffered solution containing potassium dihydrogen orthophosphate and sodium hydroxide with cysteine (16.5 mmol) in the presence of sodium ascorbate, the sodium salt of Roussin's black anion, $Na[Fe_4S_3(NO)_7]$ was isolated in 87% yield and identified by FTIR (see Figure 2.2). In a like maner, when using aqueous solutions in the pH range of 7 to 1, $Na[Fe_4S_3(NO)_7]$ was similarly formed, but the yield isolated fell as the pH was decreased. However, at pH 2, more than one product was observed on a tlc plate and at pH 1, no $Na[Fe_4S_3(NO)_7]$ was isolated.

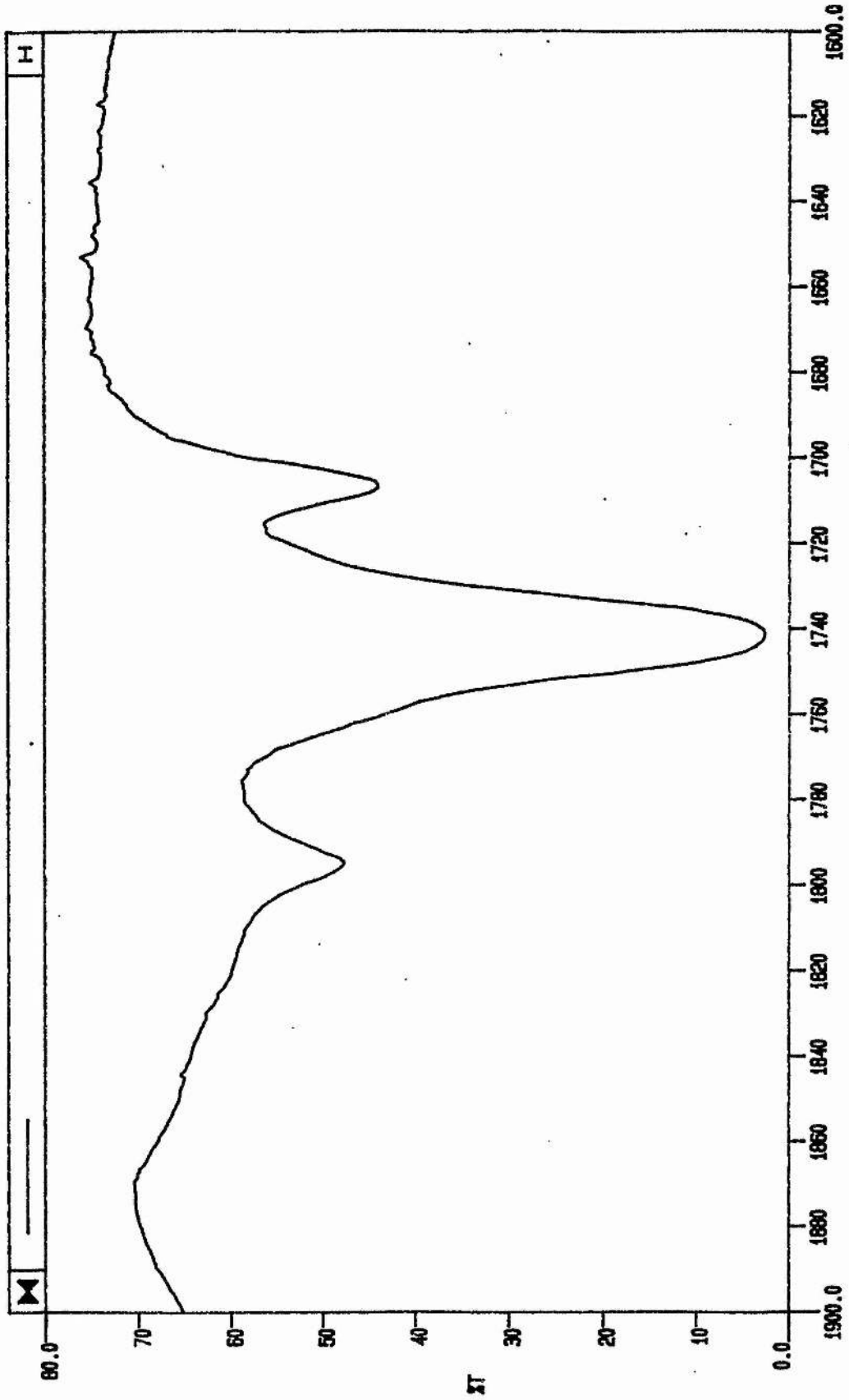


Figure 2.2; FTIR spectrum of $Na[Fe_4S_3(NO)_7]$ in tetrahydrofuran

pH	% yield $Na[Fe_4S_3(NO)_7]$
7	87
6	67
5	'68' (sticky)
4	51
3	64
2	<'52' (sticky)
1	-

Table 2.2; Percentage yield of $Na[Fe_4S_3(NO)_7]$ obtained at different levels of pH from the reaction of cysteine, sodium nitrite, iron(II) sulfate and sodium ascorbate

However a problem was encountered when using buffered solutions below pH 6. When the buffer was changed to potassium hydrogen phthalate and sodium hydroxide to control the pH at pH 5, the yield of $Na[Fe_4S_3(NO)_7]$ obtained dropped to about half that isolated at pH 6, and at pH 4 using a potassium hydrogen phthalate and hydrochloric acid buffer no $Na[Fe_4S_3(NO)_7]$ was observed. When $Na[Fe_4S_3(NO)_7]$ itself was dissolved in the buffered solutions at pH 5 and 4 it was seen that at pH 5, when evaporating the ether extract to dryness, transfer of the extract to a smaller flask resulted in some solid remaining in the larger flask which could not be redissolved in ether. FTIR analysis of the ether extract in tetrahydrofuran demonstrated the presence of $Na[Fe_4S_3(NO)_7]$. However at pH 4, a yellow/brown solid was obtained which was insoluble in tetrahydrofuran thus indicating that the potassium hydrogen phthalate buffer was reacting with $Na[Fe_4S_3(NO)_7]$ itself leading to its decomposition. That it is the potassium hydrogen phthalate and not the hydrochloric acid which is reacting with $Na[Fe_4S_3(NO)_7]$ was demonstrated by dissolving $Na[Fe_4S_3(NO)_7]$ in an aqueous hydrochloric acid solution and working up to give quantitative recovery of $Na[Fe_4S_3(NO)_7]$. However it was shown that refluxing aqueous hydrochloric acid for one hour resulted in the evolution of hydrogen chloride gas, thus neutralising the solution. Sulfuric acid was shown to remain in aqueous solution thus allowing the pH to remain at a constant level. Subsequently, reaction of cysteine, sodium nitrite, iron(II) sulfate and sodium ascorbate in a sulfuric acid solution at pH 6 gave $Na[Fe_4S_3(NO)_7]$ in 67% yield which was the same as that obtained using the potassium dihydrogen

orthophosphate and sodium hydroxide buffer. Therefore sulfuric acid solutions were used to control the pH below pH 6.

Another problem encountered was that it was very difficult to isolate $Na[Fe_4S_3(NO)_7]$ as a dry solid. Rather the product tended to be obtained as a black sticky solid, resulting in most of the experiments having to be repeated as many as five times in order to obtain a suitable dry sample.

When cysteine was similarly autoclaved with sodium nitrite and iron(II) sulfate in a buffered aqueous solution at pH 7 in the absence of sodium ascorbate, $Na[Fe_4S_3(NO)_7]$ was isolated in 45% yield. Investigation of the series of reactions with no sodium ascorbate present in the pH range 7 to 1, using sulfuric acid solutions to control the pH below pH 6, demonstrated that as pH is decreased, the yield of $Na[Fe_4S_3(NO)_7]$ obtained remained largely unaffected. However, at the very low value of pH 2, several spots were observed on a tlc plate and on further reduction of the pH to pH 1 resulted in no formation of $Na[Fe_4S_3(NO)_7]$.

pH	% yield $Na[Fe_4S_3(NO)_7]$
7	45
6	51
5	44
4	'49' (sticky)
3	'52' (sticky)
2	<40
1	-

Table 2.3; Percentage yield of $Na[Fe_4S_3(NO)_7]$ obtained at different levels of pH from the reaction of cysteine, sodium nitrite and iron(II) sulfate

Again, problems were encountered when trying to isolate $Na[Fe_4S_3(NO)_7]$ as a dry solid, resulting in many attempts to achieve a suitable sample. Although the results obtained in both Tables 2.2 and 2.3 contain some 'sticky' samples, the overall trend can clearly be seen.

The control reactions whereby $Na[Fe_4S_3(NO)_7]$ was dissolved in aqueous solution at pH values ranging from 7 to 1 and either autoclaved at 118°C or refluxed under nitrogen gas demonstrated quantitative recovery of $Na[Fe_4S_3(NO)_7]$ between pH 7 and 3. However below pH 3, the yield of $Na[Fe_4S_3(NO)_7]$ obtained decreased dramatically and thin layer chromatography demonstrated the presence of other products.

pH	% recovery $Na[Fe_4S_3(NO)_7]$
7	100
6	100
5	'122' (very sticky)
4	100
3	99
2	<45
1	<45 (sticky)

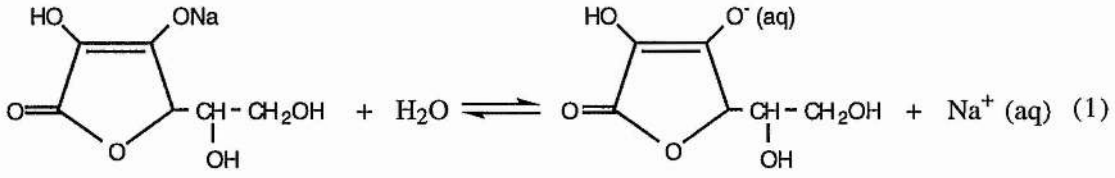
Table 2.4; Percentage recovery of $Na[Fe_4S_3(NO)_7]$ when autoclaved at 118°C at different levels of pH

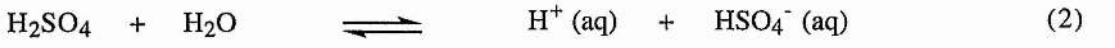
Discussion

When cysteine is reacted with sodium nitrite and iron(II) sulfate in the presence of sodium ascorbate, decreasing the pH hinders the formation of $Na[Fe_4S_3(NO)_7]$. However when sodium ascorbate is absent, initially decreasing the pH has little effect; control reactions demonstrate that during this pH range (pH 7 to 3), $Na[Fe_4S_3(NO)_7]$ itself is not affected by pH, suggesting that between pH 7 and 3 the sulfuric acid used to control the pH may protonate the sodium ascorbate.

When sodium ascorbate is added to an aqueous solution, an equilibrium is established (Figure 2.3; equation 1) producing ascorbate and sodium ions. However addition of sulfuric acid establishes a further equilibrium (Figure 2.3; equation 2) whereby protons are also donated to the system. However, Le Chatelier's principle states that; "A system at equilibrium, when subjected to a perturbation, responds in a way that tends to minimise its effect." Therefore a third equilibrium (Figure 2.3;

equation 3) could be set up whereby the protons are donated to the ascorbate anion to form ascorbic acid. However ascorbic acid is not as powerful a reducing agent as sodium ascorbate. Although addition of a strong acid to the sodium salt of an organic acid will precipitate out the organic acid, which is always less soluble than the sodium salt, solubility is unlikely to have an effect at the concentrations employed here. The diminished effect of ascorbic acid as a reducing agent, combined with the action of nitrite as an oxidising agent, will lead to a greater degree of iron(II) to iron(IIII) oxidation on decreasing pH. As discussed in Section 2.2.1, the presence of iron(III) will lead to a decrease in yield of $Na[Fe_4S_3(NO)_7]$ formed.





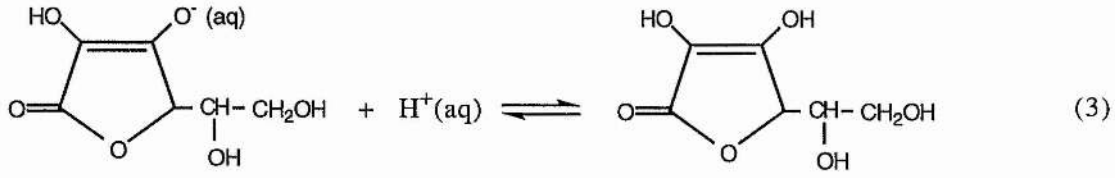


Figure 2.3; Scheme whereby sodium ascorbate is converted to ascorbic acid

In the series of reactions with no sodium ascorbate present, the yield of $Na[Fe_4S_3(NO)_7]$ isolated does not initially decrease with pH as nitrite will oxidise iron(II) to iron(III) in the absence of a reducing agent.

However at pH 2, both the reactions with and without sodium ascorbate present show a drop in yield of $Na[Fe_4S_3(NO)_7]$ with the observation of more than one product on a tlc plate; with no $Na[Fe_4S_3(NO)_7]$ isolable at pH 1. The reaction of nitrite with iron(II) sulfate and sulfuric acid is an established method of producing nitric oxide gas. The levels of $Na[Fe_4S_3(NO)_7]$ produced between pH 7 and 3 are not significantly altered by pH when nitrite is reacted with iron(II) sulfate and cysteine in the absence of

sodium ascorbate; this indicates that nitric oxide production does not play a significant role in this pH range. However at very low pH it is likely that nitric oxide production is responsible for the absence of any $Na[Fe_4S_3(NO)_7]$ as nitric oxide would quickly oxidise in the presence of air to form nitrogen dioxide which presumably cannot react to form $[Fe_4S_3(NO)_7]^-$. The control reactions show a large drop in yield of $Na[Fe_4S_3(NO)_7]$ at pH 2 and 1 with the observation of more than one product using thin layer chromatography suggesting that at very low levels of pH $Na[Fe_4S_3(NO)_7]$ itself is unstable and decomposes to form iron oxides.

2.2.3 Reactions of Methionine with Iron(II)/(III) Salts, Sodium Nitrite/Nitrate and Sodium Ascorbate

Control reaction

When an aqueous mixture of methionine, iron(II) sulfate and sodium nitrite (6.7 mmol, 6.1 mmol and 14.5 mmol respectively) was refluxed under anaerobic conditions in the presence of sodium ascorbate (13.6 mmol), the neutral dinuclear iron complex, $Fe_2(SMe)_2(NO)_4$ was isolated in 13% yield (see Figure 2.4).

Iron(III) reaction

When iron(III) chloride was substituted for iron(II) sulfate in the reaction between methionine with sodium nitrite and sodium ascorbate under anaerobic conditions, $Fe_2(SMe)_2(NO)_4$ was isolated in less than 3% yield. (Glidewell et al[4] showed that the reaction between methionine with iron(II) salts and nitrite is anion independent with respect to the iron salt whilst demonstrating that methionine is the source of sulfur in $Fe_2(SMe)_2(NO)_4$. Thus iron(III) chloride was chosen to replace iron(II) sulfate rather than perhaps the more obvious choice, iron(III) sulfate, due to its increased solubility in aqueous solvents).

It has previously been shown[2] that when the reaction between methionine, iron(II) sulfate and nitrite was conducted at 100°C, under anaerobic conditions at ambient pressure, rather than at 118°C in an autoclave, that the yield of $Fe_2(SMe)_2(NO)_4$ isolated rose reproducibly from ca. 4% to 10% and so it was suggested that regardless of the temperature, the establishment of a reducing environment is more critical for the formation of $Fe_2(SMe)_2(NO)_4$ from methionine than for the formation of $Na[Fe_4S_3(NO)_7]$ from cysteine where the yield of $Na[Fe_4S_3(NO)_7]$ obtained actually rose when the reaction was carried out in the autoclave rather than under nitrogen. That the yield of $Fe_2(SMe)_2(NO)_4$ obtained from reaction with iron(III) chloride is less than that obtained from iron(II) sulfate would further indicate the importance of a reducing environment. For the assignment of formal oxidation states in iron sulfur nitrosyl

complexes, it is accepted that sulfur is present as S^{2-} and that the nitrosyl ligands are linear NO^+. Therefore $Fe_2(SMe)_2(NO)_4$ contains iron as Fe(-1), d^9 and thus its formation would require reduction of both iron(II) sulfate and iron(III) chloride. However iron(III) chloride would require to be reduced further than its iron(II) sulfate counterpart and this may explain why the reaction with iron(III) chloride does not proceed as effectively.

Nitrate reaction

When methionine is refluxed with iron(II) sulfate and sodium nitrate under nitrogen in the presence of sodium ascorbate no nitrosyl products are formed. See Section 2.2.1(c) for a detailed explanation.

2.2.4 Reactions of Methionine and/or Iron(II) Sulfate and/or Sodium Nitrite with Sodium Ascorbate

When methionine (6.7 mmol) was refluxed in aqueous solution with iron(II) sulfate and sodium nitrite (6.1 mmol and 14.5 mmol respectively) under nitrogen for 2 hours in the presence of sodium ascorbate (13.6 mmol), the neutral dinuclear iron complex, $Fe_2(SMe)_2(NO)_4$ was isolated in 13% yield. However, if any of the reactants (other than sodium ascorbate) were omitted, no nitrosyl products were formed thus demonstrating that all three reactants are necessary for the formation of $Fe_2(SMe)_2(NO)_4$.

2.2.5 Reactions of Methionine with Iron(II) Sulfate and Sodium Nitrite with and without Sodium Ascorbate versus pH

Results

When sodium nitrite and iron(II) sulfate (14.5 mmol and 6.1 mmol respectively) were refluxed under nitrogen gas in a buffered solution containing potassium dihydrogen orthophosphate and sodium hydroxide at pH 7 with methionine (6.7 mmol) and sodium ascorbate (13.6 mmol), the neutral dinuclear iron complex $Fe_2(SMe)_2(NO)_4$ was isolated in 13% yield and identified by FTIR (see Figure 2.4). In a similar manner using aqueous solutions in the pH range of 7 to 1 (aqueous sulfuric acid solutions were used to control the pH below pH 6), $Fe_2(SMe)_2(NO)_4$ was still formed, but the yield isolated fell slightly with decreasing pH then dropped drastically at pH 1 to less than 1%. Infra-red spectroscopy and thin layer chromatography demonstrated that no other nitrosyl products were formed.

pH	% yield $Fe_2(SMe)_2(NO)_4$
7	13
6	10
5	7
4	7
3	8
2	9
1	<1

Table 2.5; Percentage yield of $Fe_2(SMe)_2(NO)_4$ isolated at different levels of pH from reaction of methionine, sodium nitrite, iron(II) sulfate and sodium ascorbate

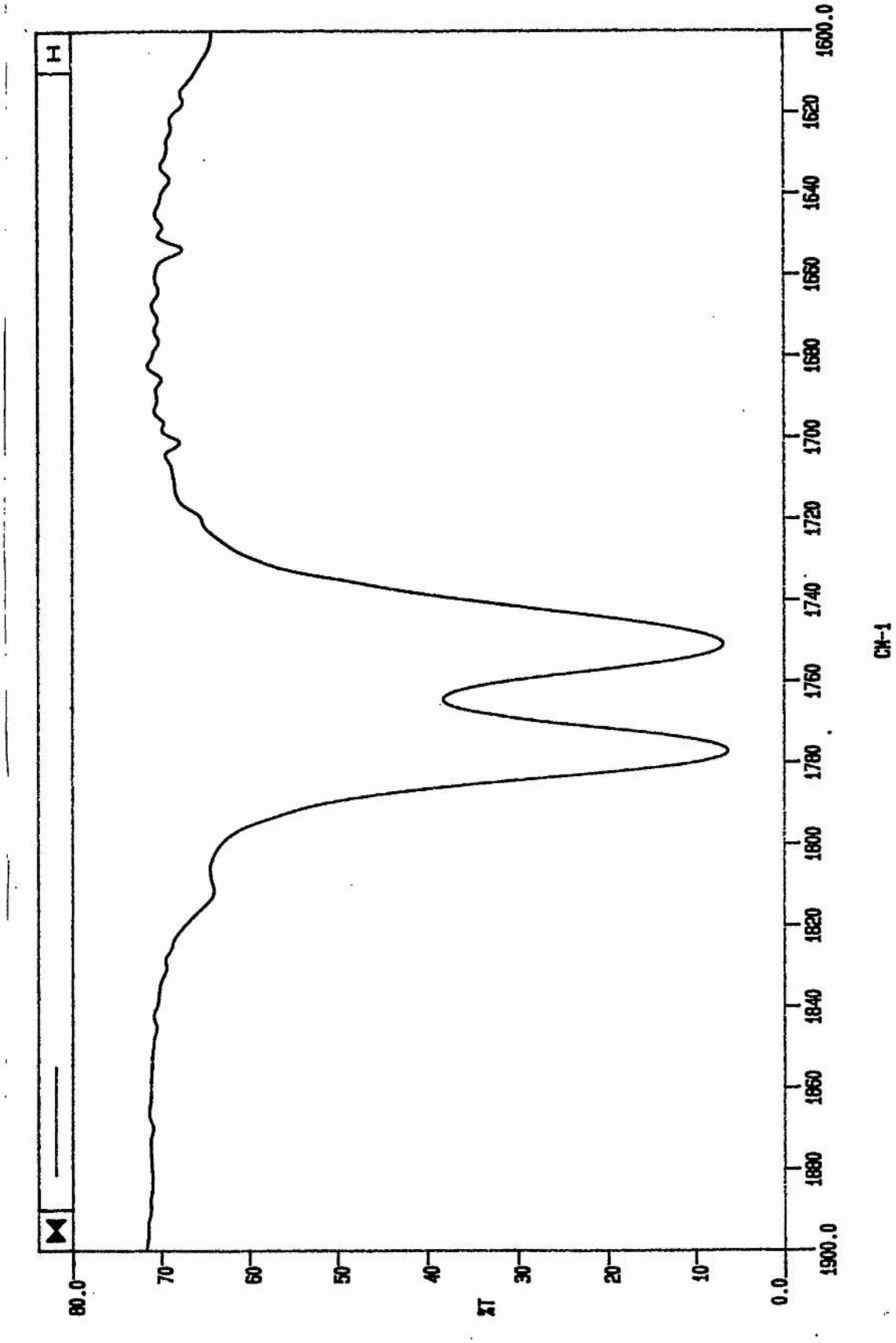


Figure 2.4; FTIR spectrum of $Fe_2(SMe)_2(NO)_4$ in dichloromethane

The control reactions whereby $Fe_2(SMe)_2(NO)_4$ was similarly dissolved in a range of aqueous solutions between the pH values 7 to 1 and refluxed under nitrogen gas showed that $Fe_2(SMe)_2(NO)_4$ was initially recovered in quantitative yield between pH 7 to 3 then fell slightly to give ca. 90% recovery at pH 2 and 1.

pH	% recovery $Fe_2(SMe)_2(NO)_4$
7	100
6	94
5	100
4	100
3	100
2	91
1	87

Table 2.6; Percentage recovery of $Fe_2(SMe)_2(NO)_4$ at different levels of pH

Discussion

The results indicate that pH really only plays a significant role at very low pH during the formation of the $Fe_2(SMe)_2(NO)_4$ complex from the reaction of methionine with sodium nitrite and iron(II) sulfate in the presence of sodium ascorbate. The control reactions however are not affected to any great degree by a decrease in pH suggesting again that nitric oxide production may be the limiting factor at very low values of pH.

2.3 Experimental

FTIR spectra were recorded in tetrahydrofuran (THF) or dichloromethane (DCM) solutions using 0.2 mm pathlength cells and KBr plates with a Perkin-Elmer Model 1710 FTIR spectrophotometer. ^{14}N NMR spectra were recorded at 21.638 MHz in acetone solution, at ambient temperature, relative to 50% CH_3NO_2 in acetone, using a Brucker AM-300 spectrophotometer. pH measurements were recorded using a Piccolo ATC pH-meter on buffered solutions of KH_2PO_4 & NaOH at pH 6 & 7 and H_2SO_4 solutions at pH 6 and below. The autoclave employed had a capacity of 14.3 dm^3.

2.3.1 Preparation of Sodium Heptanitrosyl-tri-(μ_3-thio)-tetraferrate(1-), $Na[Fe_4S_3(NO)_7]$

Sodium nitrite (8.0 g, 116 mmol) and sodium sulfide nonahydrate (11.3 g, 47 mmol) were dissolved in distilled deoxygenated water (160 cm^3) and heated to boiling under nitrogen. A solution of iron(II) sulfate heptahydrate (20.0 g, 72 mmol) in deoxygenated water (80 cm^3) was added followed immediately by 20% aqueous ammonia (20 cm^3). The resulting thick black mixture was filtered through preheated hyflo before cooling in ice/water. The crude product was filtered off and recrystallised from distilled water to give a shiny black crystalline solid (2.0 g, 20%).

IR (THF)	υ(NO)	1795 cm^{-1} (w)	1742 cm^{-1} (vs)	1707 cm^{-1} (m)	
^{14}N NMR (Acetone)	δ_N	77 ppm	38.5 ppm	11 ppm	(broad)

TLC Gave a single brown spot moving with the solvent front in methanol.

2.3.2 Reactions of Cysteine with Iron(II)/(III) Salts and Sodium Nitrite/Nitrate with and without Sodium Ascorbate

Control Reactions

L-Cysteine (2.0 g, 16.5 mmol), iron(II) sulfate heptahydrate (0.50 g, 1.80 mmol), sodium nitrite (0.100 g, 1.45 mmol) and sodium ascorbate (2.0 g, 10.1 mmol) were dissolved in distilled water (1 dm^3) and the mixture autoclaved at 118°C for 20 min. The mixture was cooled and filtered, and the filtrate exhaustively extracted with diethyl ether, until the extracts were colourless. The combined ether extracts were washed with water, dried over magnesium sulfate and evaporated to provide $Na[Fe_4S_3(NO)_7]$ (0.082 g, 72%) as a black solid.

IR (THF)	υ(NO)	1795 cm^{-1} (w)	1742 cm^{-1} (vs)	1707 cm^{-1} (m)

A similar reaction was carried out (L-cysteine 2.0 g, 16.5 mmol; iron(II) sulfate heptahydrate 0.5 g, 1.80 mmol; sodium nitrite 0.100 g, 1.45 mmol) but without sodium ascorbate present to provide $Na[Fe_4S_3(NO)_7]$ (0.045 g, 40%).

IR (THF) υ(NO) 1795 cm^{-1} (w) 1742 cm^{-1} (vs) 1707 cm^{-1} (m)

Iron(III) reactions

L-Cysteine (2.0 g, 16.5 mmol), iron(III) chloride (0.311 g, 1.92 mmol), sodium nitrite (0.100 g, 1.45 mmol) and sodium ascorbate (2.0 g, 10.1 mmol) were dissolved in distilled water (1 dm^3) and the mixture autoclaved at 118°C for 20 min. After cooling, the mixture was filtered, and the filtrate exhaustively extracted with diethyl ether until the extracts were colourless. The combined ether extracts were washed with water, dried over magnesium sulfate and evaporated to provide $Na[Fe_4S_3(NO)_7]$ (0.054 g, 47%) as a black solid.

IR (THF) υ(NO) 1795 cm^{-1} (w) 1742 cm^{-1} (vs) 1707 cm^{-1} (m)

The analogous reaction was carried out (L-cysteine 2.0 g, 16.5 mmol; iron(III) chloride 0.285 g, 1.76 mmol; sodium nitrite 0.120 g, 1.73 mmol) without sodium ascorbate present to provide $Na[Fe_4S_3(NO)_7]$ (0.057 g, 42%).

IR (THF) υ(NO) 1795 cm-1 (w) 1742 cm-1 (vs) 1707 cm-1 (m)

Nitrate reactions

L-Cysteine (2.0 g, 16.5 mmol), iron(II) sulfate heptahydrate 0.50 g, 1.80 mmol), sodium nitrate (0.134 g, 1.57 mmol) and sodium ascorbate (2.0 g, 10.1 mmol) were dissolved in distilled water (1 dm^3) and the mixture autoclaved at 118°C for 20 min. The mixture was cooled and filtered, and the filtrate exhaustively extracted with diethyl ether until the extracts were colourless. The combined ether extracts were washed with water, dried over magnesium sulfate and evaporated to dryness. No nitrosyl products were observed.

The analogous reaction was carried out (L-cysteine 2.0 g, 16.5 mmol; iron(II) sulfate heptahydrate 0.50 g, 1.80 mmol; sodium nitrate 0.154 g, 1.82 mmol) and again no nitrosyl products were observed.

2.3.3 Reactions of Cysteine with Iron(II) Sulfate and Sodium Nitrite with and without Sodium Ascorbate versus pH

L-Cysteine (2.0 g, 16.5 mmol), iron(II) sulfate heptahydate (0.50 g, 1.80 mmol) and sodium nitrite (0.10 g, 1.45 mmol) were dissolved with sodium ascorbate (2.0 g, 10.1 mmol) in a aqueous solution of known pH (500 cm^3) and the mixture was autoclaved at 118°C for 30 min. After cooling, the reaction mixture was filtered and exhaustively extracted with diethyl ether until the extracts were colourless. The combined ether extracts were washed with water and dried over magnesium sulfate before removal of the solvent. Duplicate runs were made omitting the sodium ascorbate.

2.3.4 Reactions of $Na[Fe_4S_3(NO)_7]$ versus pH

Autoclave reactions

$Na[Fe_4S_3(NO)_7]$ (0.100 g, 0.181 mmol) was dissolved in an aqueous solution of known pH (200 cm^3) and the mixture was autoclaved at 118°C for 30 min. After cooling, the reaction mixture was filtered and exhaustively extracted with diethyl ether until the extracts were colourless. The combined ether extracts were washed with water and dried over magnesium sulfate before removal of the solvent.

Anaerobic reactions

$Na[Fe_4S_3(NO)_7]$ (0.100 g, 0.181 mmol) was dissolved in a deoxygenated aqueous solution of known pH (100 cm^3) and the mixture refluxed under nitrogen for 1 hour. After cooling, the reaction mixture was filtered and exhaustively extracted with diethyl ether until the extracts were colourless. The combined ether extracts were washed with water and dried over magnesium sulfate before removal of the solvent.

2.3.5 Preparation of Bis(μ-methylthiolato)bis(dinitrosyliron), $Fe_2(SMe)_2(NO)_4$

The dinuclear complex, $Fe_2(SMe)_2(NO)_4$ was prepared from sodium bis(μ-thiosulphato-S)-bis(dinitrosylferrate), $Na_2[Fe_2(S_2O_3)_2(NO)_4]$, which was characterised by conversion to the bis(triphenylphosphoranylidene)ammonium salt, $[N(PPh_3)_2]_2[Fe_2(S_2O_3)_2(NO)_4]$.

Preparation of $Na_2[Fe_2(S_2O_3)_2(NO)_4]$

To a well stirred solution of sodium nitrite (5.68 g, 82 mmol) and sodium thiosulfate pentahydrate (19.8 g, 80 mmol) in nitrogen flushed distilled water (80 cm^3)

was added, under nitrogen, a solution of iron(II) sulfate heptahydrate (11.04 g, 39.6 mmol) in deoxygenated distilled water (60 cm^3). The solution turned yellow and darkened as the reaction proceeded and iron oxides were deposited on the sides of the flask. The mixture was stirred for 2 hours and then filtered. The solid residue was extracted with analaR acetone (1 dm^3) to leave a green residue. The extracts were combined and dried over magnesium sulfate, then filtered and reduced to small volume. Addition of a dichloromethane/40-60 petroleum ether solution (1:5 v/v) precipitated $Na_2[Fe_2(S_2O_3)_2(NO)_4]$ as a brown solid which was filtered and sucked dry.

Conversion to $[N(PPh_3)_2]_2[Fe_2S_2O_3)_2(NO)_4]$

To a solution of $Na_2[Fe_2(S_2O_3)_2(NO)_4]$ (0.25 g, 0.5 mmol) in deoxygenated distilled water (20 cm^3) at 60°C was added, under nitrogen , $[N(PPh_3)_2]Cl$ (0.58 g, 1 mmol). The mixture was stirred for 20 minutes, filtered and the solid product was washed with a little warm distilled water. The solid was dissolved in dichloromethane (50 cm^3) and the solution dried over magnesium sulfate. The solution was reduced to small volume, flushed with nitrogen and cooled in an ice bath to give the product as brown crystals which could be identified using FTIR spectroscopy.

IR (DCM) 1787 cm^{-1} 1757 cm^{-1}

Conversion to $Fe_2(SMe)_2(NO)_4$

To a mixture of $Na_2[Fe_2(S_2O_3)_2(NO)_4]$ (1.4 g, 2.8 mmol) and sodium thiosulfate pentahydrate (1.0 g, 5.6 mmol) in deoxygenated distilled water (45 cm^3) was added, under nitrogen, a solution of sodium sulfide nonahydrate (8.2 g, 33.6 mmol) and sodium hydroxide (1.4 g) in deoxygenated water (45 cm^3). After brief stirring, tetrahydrofuran (90 cm^3) was added and the mixture filtered. To the filtrate was added, under nitrogen, methyl iodide (3.0 g, 21 mmol) and the solution was stirred for 30 minutes whence it gradually turned green. It was appreciated at this stage that methyl iodide is a highly toxic compound and it was handled accordingly with gloves in a well-ventilated fume cupboard. The solution was extracted with dichloromethane and dried over magnesium sulfate. The extract was reduced to small volume and purified by elution through a silica chromatography column. Subsequent reduction to dryness gave $Fe_2(SMe)_2(NO)_4$ as a red solid.

IR (DCM) 1776 cm^{-1} 1750 cm^{-1}

TLC Gave a single red spot moving with the solvent front in dichloromethane.

2.3.6 Reactions of Methionine with Iron(II)/(III) Salts and Sodium Nitrite/Nitrate and Sodium Ascorbate

Control reaction

DL-Methionine (1.0 g, 6.7 mmol), iron(II) sulfate heptahydrate (1.701 g, 6.1 mmol) and sodium nitrite (1.0 g, 14.5 mmol) were dissolved with sodium ascorbate (2.7 g, 13.6 mmol) in deoxygenated distilled water (100 cm^3) and the mixture refluxed under nitrogen for 2 hours. After cooling, the reaction mixture was filtered and the filtrate exhaustively extracted with diethyl ether. The combined ether extracts were washed with water and dried over magnesium sulfate. The solid obtained on the condenser surface, on the sides of the reaction vessel and on the filter paper was dissolved in dichloromethane, washed well with water and dried over magnesium sulfate. Both extracts were evaporated to dryness, redissolved in the minimum volume of dichloromethane and eluted through a silica chromatography column. The eluant was evaporated to dryness to provide $Fe_2(SMe)_2(NO)_4$ (0.131 g, 13%) as a red solid.

IR (DCM) 1776 cm^{-1} 1751 cm^{-1}

TLC Gave a single red spot moving with the solvent front in dichloromethane

Iron(III) reaction

DL-Methionine (1.0 g, 6.7 mmol), iron(III) chloride hexahydrate (1.653 g, 6.1 mmol) and sodium nitrite (1.0 g, 14.5 mmol) were dissolved with sodium ascorbate (2.7 g, 13.6 mmol) in deoxygenated distilled water (100 cm^3) and the mixture refluxed under nitrogen for 2 hours. After cooling, the reaction mixture was filtered and exhaustively extracted with diethyl ether. The combined ether extracts were washed with water and dried over magnesium sulfate. The solid obtained on the condenser surface, on the sides of the flask and on the filter paper was dissolved in dichloromethane, washed well with water and dried over magnesium sulfate. Both extracts were combined and evaporated to dryness to yield $Fe_2(SMe)_2(NO)_4$ (0.034 g, 3%) as an impure solid.

IR (DCM) 1776 cm^{-1} 1751 cm^{-1}

TLC Two spots moving with the solvent front in dichloromethane

Nitrate reaction

DL-Methionine (1.0 g, 6.7 mmol), iron(II) sulfate heptahydrate (1.70 g, 6.1 mmol), and sodium nitrate (1.22 g, 14.4 mmol) were dissolved with sodium

ascorbate (2.7 g, 13.6 mmol) in deoxygenated distilled water (100 cm^3) and the mixture refluxed under nitrogen gas for 2 hours. After cooling, the reaction mixture was filtered and exhaustively extracted with diethyl ether. The combined extracts were washed with water and dried over magnesium sulfate before evaporating to dryness. FTIR analysis demonstrated that no nitrosyl products were formed.

2.3.7 Reactions of Methionine and/or Iron(II) Sulfate and/or Sodium Nitrite

Control reaction

See 2.3.6 (a)

Absence of iron(II)

DL-Methionine (1.0 g, 6.7 mmol) and sodium nitrite (1.0 g, 14.5 mmol) were dissolved with sodium ascorbate (2.7 g, 13.6 mmol) in deoxygenated distilled water (100 cm^3) and the mixture refluxed under nitrogen for 2 hours. After cooling, the reaction mixture was filtered and the filtrate exhaustively extracted with diethyl ether. The combined ether extracts were washed with water, dried over magnesium sulfate and evaporated to dryness. FTIR analysis demonstrated that no nitrosyl complexes were formed.

Absence of methionine

Iron(II) sulfate heptahydrate (1.70 g, 6.1 mmol) and sodium nitrite (1.0 g, 14.5 mmol) were dissolved with sodium ascorbate (2.7 g, 13.6 mmol) in deoxygenated distilled water (100 cm^3) and the mixture refluxed under nitrogen for 2 hours. After cooling, the reaction mixture was filtered and the filtrate exhaustively extracted with diethyl ether. The combined ether extracts were washed with water, dried over magnesium sulfate and evaporated to dryness. FTIR analysis demonstrated that no nitrosyl complexes were formed.

Absence of nitrite

DL-Methionine (1.0 g, 6.7 mmol) and iron(II) sulfate heptahydrate (1.70 g, 6.1 mmol) were dissolved with sodium ascorbate (2.7 g, 13.6 mmol) in deoxygenated distilled water (100 cm^3) and the mixture refluxed under nitrogen for 2 hours. After cooling, the reaction mixture was filtered and the filtrate exhaustively extracted with diethyl ether. The combined ether extracts were washed with water, dried over

magnesium sulfate and evaporated to dryness. FTIR analysis demonstrated that no nitrosyl complexes were formed.

2.3.8 Reactions of Methionine with Sodium Nitrite, Iron(II) Sulfate and Sodium Ascorbate versus pH

DL-Methionine (1.0 g, 6.7 mmol), iron(II) sulfate heptahydrate (1.700 g, 6.1 mmol) and sodium nitrite (1.0 g, 14.5 mmol) were dissolved with sodium ascorbate (2.7 g, 13.6 mmol) in an aqueous solution of known pH (100 cm^3) and the mixture refluxed under nitrogen for 2 hours. After cooling, the reaction mixture was filtered and exhaustively extracted with diethyl ether. The combined ether extracts were washed with water and dried over magnesium sulfate. The solid obtained on the condenser surface, on the sides of the flask and on the filter paper was dissolved in dichloromethane, washed well with water and dried over magnesium sulfate. Both extracts were evaporated to dryness, redissolved in the minimum volume of dichloromethane and eluted through a silica chromatography column.

2.3.9 Reactions of $Fe_2(SMe)_2(NO)_4$ versus pH

$Fe_2(SMe)_2(NO)_4$ (0.100 g, 0.31 mmol) was dissolved in an aqueous solution of known pH (150 cm^3) and the mixture refluxed under nitrogen for 2 hours. After cooling, the reaction mixture was filtered and exhaustively extracted with diethyl ether. The combined ether extracts were washed with water and dried over magnesium sulfate The solid obtained on the condenser surface, on the sides of the flask and on the filter paper was dissolved in dichloromethane, washed well with water and dried over magnesium sulfate. Both extracts were evaporated to dryness, redissolved in the minimum volume of dichloromethane and eluted through a silica chromatography column.

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CHAPTER THREE

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Iron Complexes with Sulfur Containing Amino Acids and their Reactions with Nitrite

3.1 Introduction

Recent work[1] has shown that the reaction of cysteine with iron(II) salts and sodium nitrite in aqueous solution yields the tetranuclear iron-sulfur-nitrosyl cluster, $Na[Fe_4S_3(NO)_7]$. Similar reactions[2] carried out in the presence of methionine yield the dinuclear iron-sulfur-nitrosyl complex, $Fe_2(SMe)_2(NO)_4$. However, as yet the mechanism by which these reactions proceed remain unknown. A possible explanation could be via an iron-amino acid intermediate

An extensive literature survey on iron-sulfur containing amino acids was conducted (see Chapter 1.5) from which several of the more promising synthetic procedures were selected. These included the preparation of some iron(III)-amino acid nitrates[3] and chlorides of the general formulae $[Fe_3O(\text{amino acid})_6(H_2O)_3](X)_7$; the triligated iron-methionine complex[4], $Fe(\text{methionine})_3$; and some 1:1 iron-methionine complexes[5] of the type $Fe(\text{methionine})(OH)Cl,2CH_3OH$ and $Fe(\text{methionine})(OH)NO_3,2CH_3OH$.

The trinuclear oxobridged iron(III) amino acid complexes, $[Fe_3O(\text{amino acid})_6(H_2O)_3](X)_7$ were first prepared by Puri et al[3,6] in an attempt to model the structure of the ferritin iron core. The overall structure (see Figure 3.1) of the known iron(III) amino acid complexes consists of a central oxygen bonded to three iron atoms in approximately the same plane. Pairs of iron atoms are bridged by two amino acids through the oxygens of the carboxyl groups; the sixth coordination site is occupied by the oxygen of a water molecule. The triligated iron-methionine complex, $Fe(met)_3$ was prepared by McAuliffe et al[4] during a general study of metal complexes with methionine. An octahedral stereochemistry was proposed and infra red spectroscopy indicated coordination via the carboxyl and amino groups. Halbert and Rogerson[5] reported the formation of two 1:1 complexes of iron(III) and methionine, $Fe(met)(OH)X,2CH_3OH$, with X = Cl, NO_3 and suggested coordination via the carboxylate group. Again however, as with the findings of McAuliffe et al[4], no crystal structures were determined and characterisation was based mainly on infra red and magnetic studies.

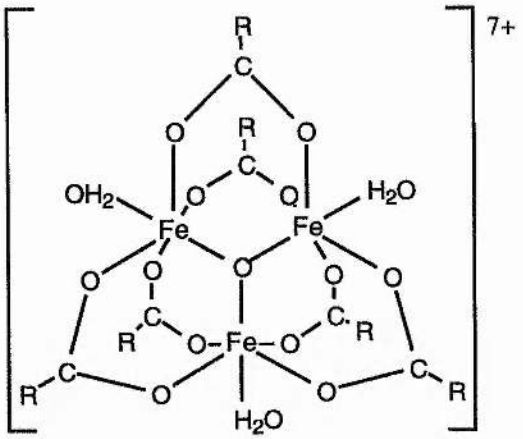


Figure 3.1; Proposed structure of the $[Fe_3O(\text{amino acid})_6(H_2O)_3]^{7+}$ cation

It was thus proposed to synthesise and further characterise these iron-amino acid complexes and to study their reactions with nitrite, both in the autoclave and under nitrogen.

3.2 Discussion

3.2.1 Formation of $[Fe_3O(amino\ acid)_6(H_2O)_3](X)_7$ Complexes

The preparation of models of the ferritin core has been a challenge to synthetic inorganic chemists. Thus Puri and Asplund[3] prepared a range of trinuclear oxobridged iron(III)-L-amino acid nitrates of the general type $[Fe_3O(amino\ acid)_6(H_2O)_3](NO_3)_7$ in order to compare their spectral and magnetic properties with those of the ferritin iron core. Ferritin[7] acts as a depot in which excess iron can be stored within cells in a nontoxic form and from which it can be released in usable form, as required. It is widely distributed throughout the various organs of all mammals with high concentrations found in the liver, spleen and bone marrow. It is also found in plants and bacteria. Ferritin consists of a shell of protein surrounding a core which contains iron(III) as a polymeric oxy-hydroxo complex. Approximately 1200 iron atoms are usually stored in the cavity, although it can accomodate up to 4500. The composition of the horse spleen ferritin core is approximately $\{[FeO(OH)]_8[FeO(H_2PO_4)]\}_n$ and it has a diffraction pattern similar to that of the mineral ferrihydrite, $5Fe_2O_3.9H_2O$. The structure is based on a close-packed array of oxide ions and OH^- ions, with iron atoms in octahedral interstices.

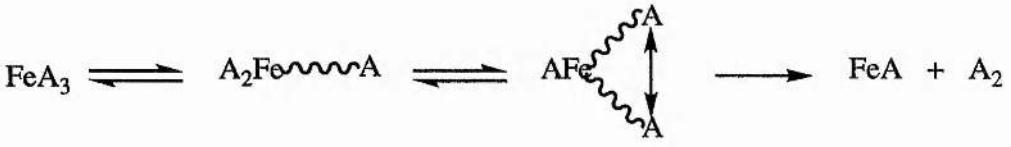
The study of the spectral and magnetic properties of the trinuclear oxobridged iron(III)-L-amino acid nitrates and the close similarity of these properties with those of the already known[6,8,9] trinuclear oxobridged iron(III)-L-amino acid perchlorates and trinuclear oxobridged iron(III)-carboxylates suggested that the above physical properties of all the complexes were largely independent of the oxygen containing ligands coordinated to the trimeric unit $[Fe_3O]^{7+}$, and the nature of the counter-ion present in these complexes, and further supported the view that iron(III) complexes containing the structural unit $[Fe_3O]^{7+}$ remain strong contenders as models for the ferritin iron core.

The preparation[3] of the trinuclear oxobridged iron(III)-amino acid complex, $[Fe_3O(met)_6(H_2O)_3](NO_3)_7$ consisted of mixing iron(III) nitrate and methionine in a 1:2 molar ratio. Evaporation to dryness yielded the product as a powder after maceration with acetonitrile. It was proposed to repeat this synthesis and to attempt to prepare the anolagous complexes containing the amino acids cysteine, methylcysteine and benzylcysteine in a similar manner.

Preparation of the methionine and the analogous methylcysteine complexes proved to be relatively simple, but problems were encountered when attempting to synthesise

the benzylcysteine complex due to the limited solubility of benzylcysteine in aqueous solution. CHN analysis results for the benzylcysteine complex proved to be disappointing so it was decided to concentrate primarily on the methionine and methylcysteine analogues.

In the case of cysteine, it was observed that when added to the iron(III) nitrate solution, an intense blue colour developed, then quickly faded to give a white solid which was identified by microanalysis to be cystine. The filtrate when reduced to dryness yielded a black powder which was shown to be Fe_3O_4 (magnetite) using powder diffraction analysis. The oxidation of cysteine to cystine is well documented in the literature. An investigation into the effect of iron(III) on the oxidation of cysteine by Taylor et al[10] demonstrated that the reaction is zero order with respect to both cysteine and oxygen but two-thirds order with respect to iron. The reaction was shown to be extremely slow in the absence of iron. The two-thirds order with respect to iron was explained by a proposal that the iron(III) ion forms a complex with three cysteine molecules. This proposal was favoured over the formation of an iron-dicysteine complex because of the more reasonable octahedral structure. The tris complex although quantitative in its formation is subject to decomposition to the iron-monocysteine complex and two unidentified cysteinyl species which may combine together to form cystine. The final reaction is the reformation of the iron-tricysteine complex (for mechanism see Figure 1.27). Such a proposal may be represented as



As it was intended to study the reaction of these complexes with nitrite, preparation of the methionine, methylcysteine complexes and benzylcysteine complexes as their sulfate salts were also attemped in order to eliminate the possibility of reaction with the nitrate counter-ion. However CHN analysis of the prepared materials did not show promising results, perhaps due to the poor solubility of iron(III) sulfate in aqueous solution. Successful preparation of the chloride salts of the methionine and methylcysteine complexes was achieved.

In the original synthesis[3] of the $[Fe_3O(met)_6(H_2O)_3](NO_3)_7$ complex, the iron(III) salt is added to an aqueous solution of the amino acid, stirred, filtered and left to evaporate at room temperature. Maceration of the sticky solid produced using acetonitrile yielded the product as a powder. This process however proved to be extremely time consuming so it was attempted speed up the process by removal of the aqueous solvent at 40°C using a Büchi. However this caused the solid which formed to

decompose rather dramatically. Another method was tried whereby a stream of air was passed over the liquid surface via a water pump. Again this proved to be very time consuming. Success was eventually achieved using freeze-drying techniques ie. the solution was frozen using liquid nitrogen and the solvent sublimed off using a vacuum pump. This cut down the preparation time of each sample to about two weeks.

Another problem encountered during synthesis of the chloride salts was that after maceration with acetonitrile, in order to remove any acetonitrile adduct present, the solid was washed with cold absolute ethanol. However the chloride salts proved to be soluble in ethanol. Super-dry ethanol was prepared in attempt to solve the problem but proved to be unsuccessful. Eventually it was found that washing with cold sodium-dried ether was the answer as this gave more promising CHN analysis results. However it is likely that the product is still contaminated with some acetonitrile adduct as the results obtained from CHN analysis remain rather high.

Thus preparation of complexes of the type $[Fe_3O(\text{amino acid})_6(H_2O)_3](X)_7$ can be achieved provided both the amino acid and the iron(III) salt have a reasonable degree of solubility in aqueous media. Spectral analysis of the methionine and methylcysteine complexes (see Figures 3.2 - 3.5 for FTIR spectra) as their nitrate or chloride salts correlate well with the observations of Puri et al[5,6]. Unfortunately as the compounds could not be crystallised, due to the insolubility of the complexes in organic solvents, no structural characterisations could be made.

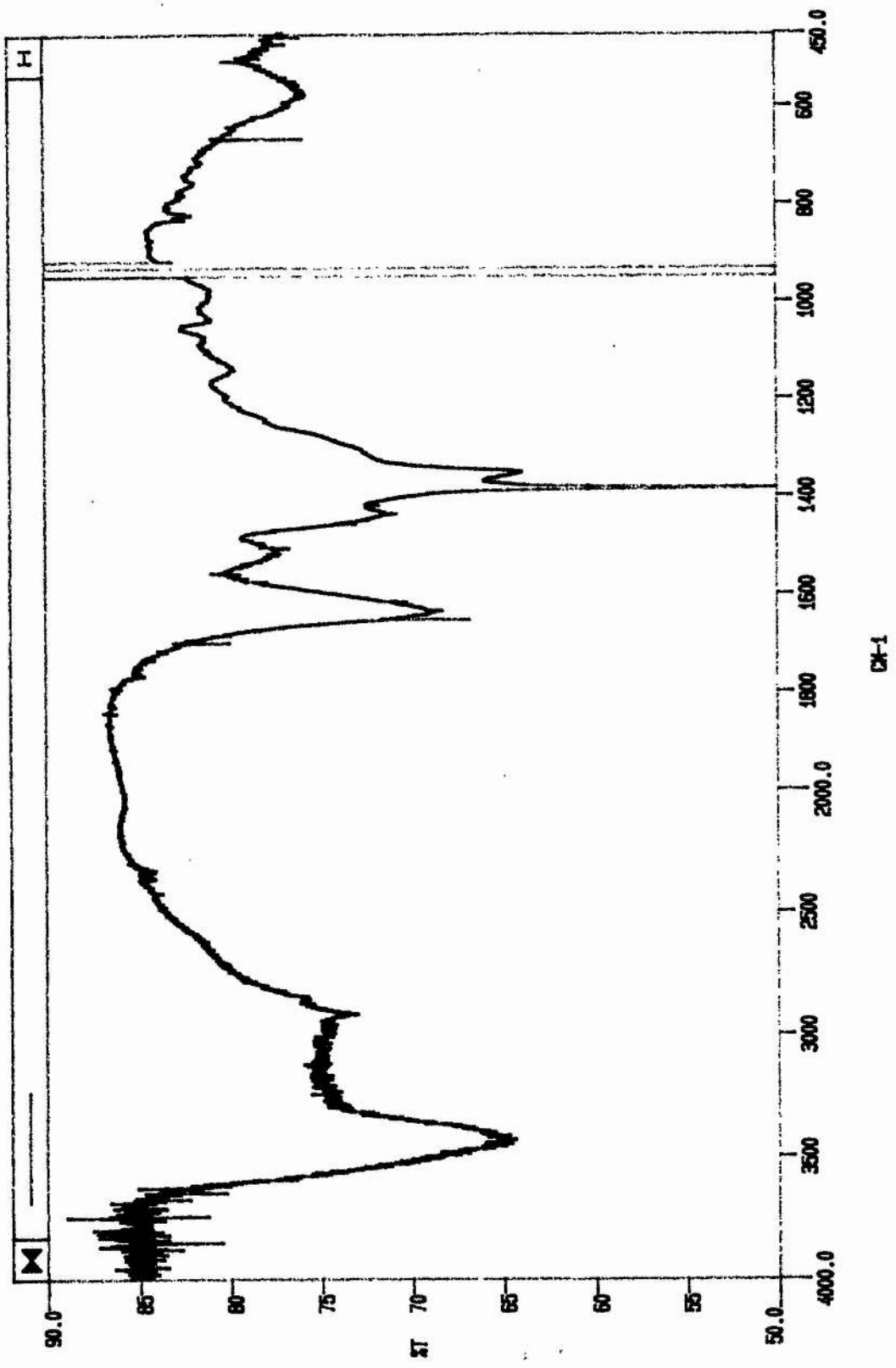


Figure 3.2; FTIR spectrum of $[Fe_3O(met)_6(H_2O)_3](NO_3)_7$, KBr disc

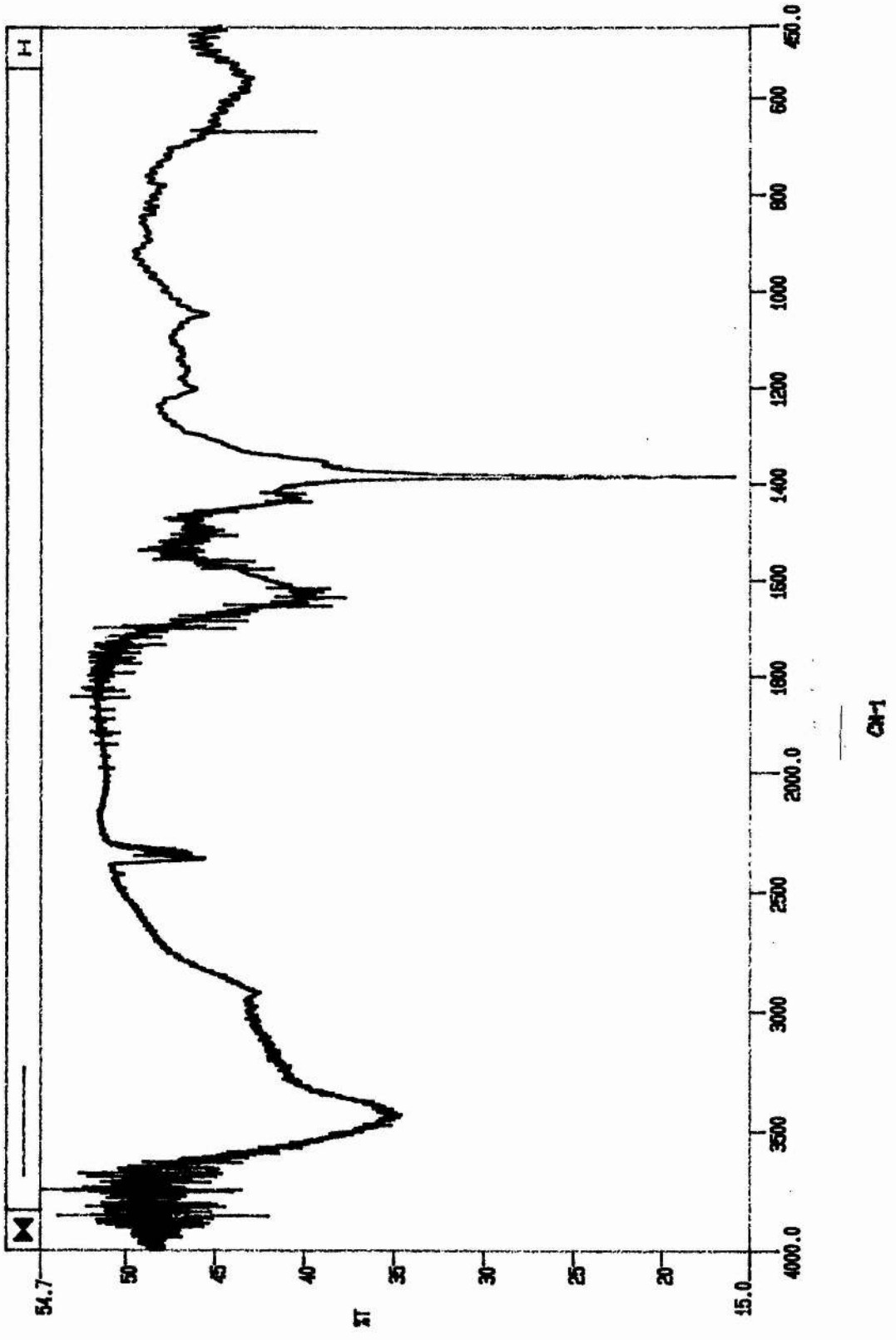


Figure 3.3; FTIR spectrum of $[Fe_3O(mcys)_6(H_2O)_3](NO_3)_7$, KBr disc

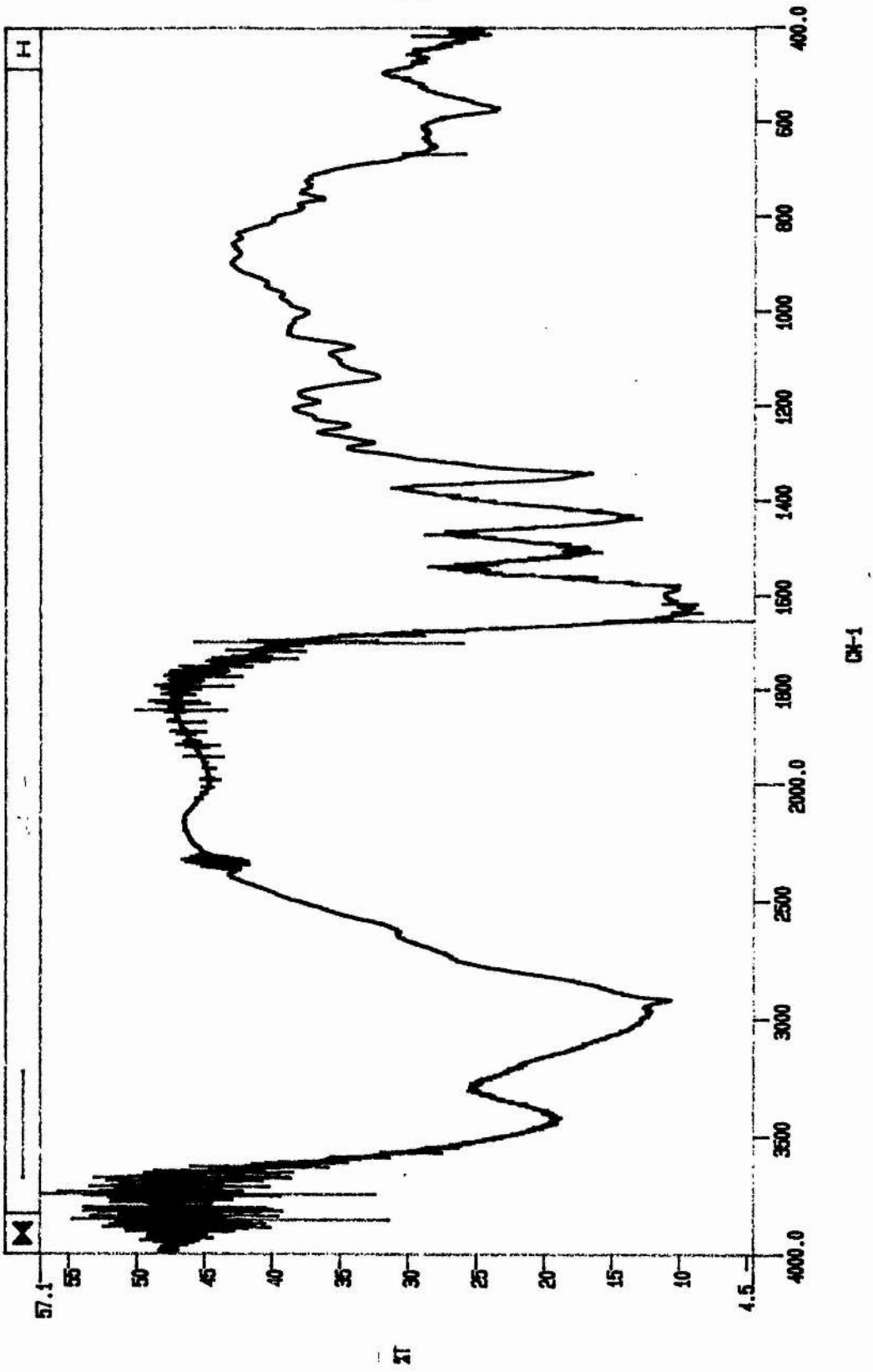


Figure 3.4; FTIR spectrum of $[Fe_3O(met)_6(H_2O)_3]Cl_7$, KBr disc

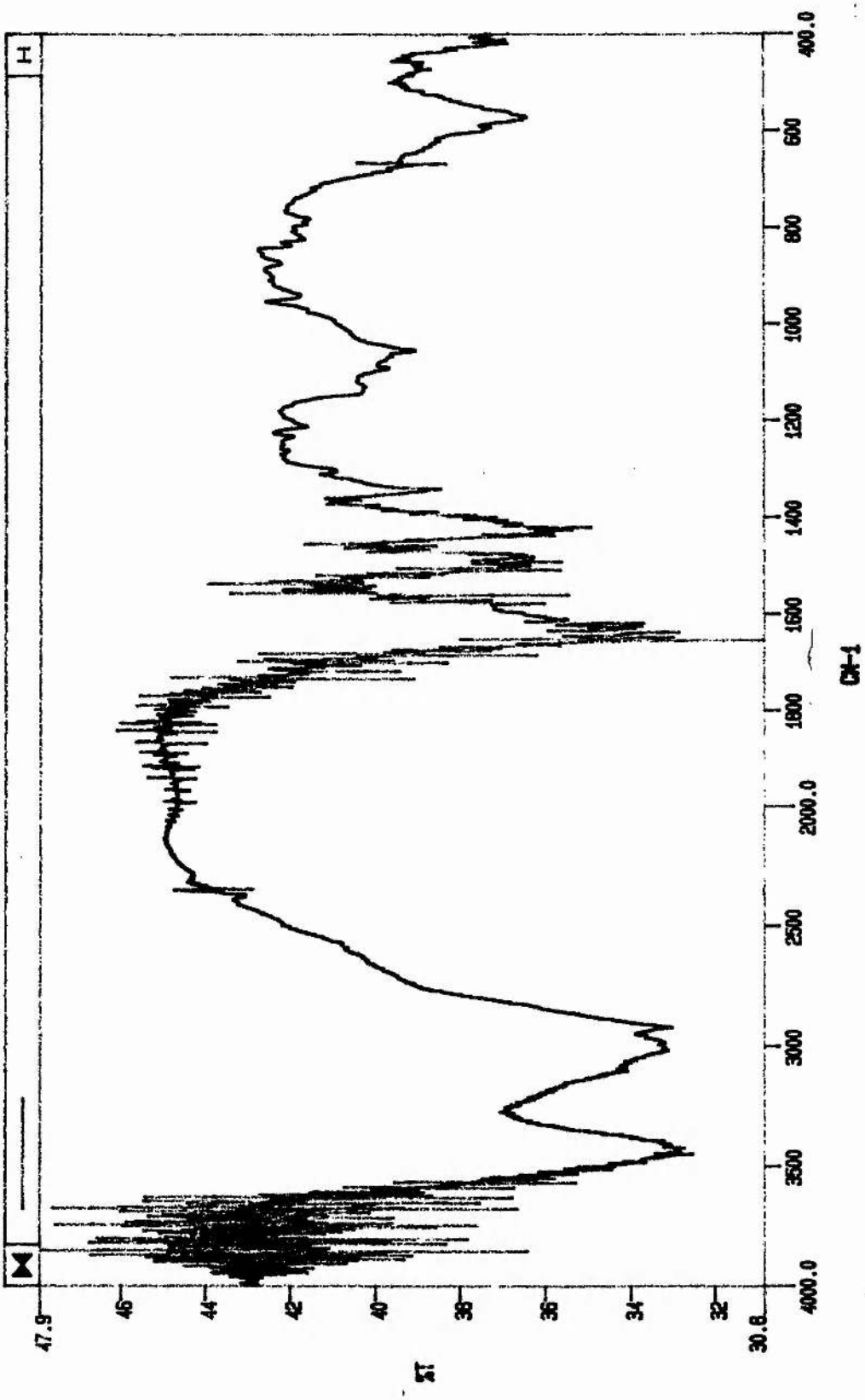


Figure 3.5; FTIR spectrum of $[Fe_3O(mcys)_6(H_2O)_3]Cl_7$, KBr disc

3.2.2 Attempted Preparation of Other Iron Complexes with Methionine

Iron(III) complexes of methionine with the general formula $Fe(met)(OH)X,2CH_3OH$, where X = Cl or NO_3 were reported by Halbert and Rogerson[5]. The complexes were prepared by the addition of iron(III) chloride (or nitrate) to a solution of lithium methioninate in methanol. The product could then be precipitated out by the gradual addition of sodium-dried ether. Comparison of the infra-red data from the iron-amino acid complexes with the lithium salt suggested that methionine is still coordinated through the carboxylate group. That methionine does not coordinate to iron(III) through the thioether group was ascertained by NMR spectroscopy. Since the signals due to protons of the thioether group remain least influenced by increasing the concentration of iron(III), while the signals due to the protons of the α-carbon atom were affected most, the thioether group was concluded to have little interaction with iron(III).

The synthetic procedure was repeated for both the chloride and the nitrate complexes. However, in the case of the nitrate complex, addition of sodium-dried ether to a solution of iron(III) nitrate and lithium methioninate in methanol only resulted in the deposition of a red oil. Preparation of the chloride complex met with a greater degree of success. A yellow solid precipitated out of the methanolic solution containing iron(III) chloride and lithium methioninate on addition of sodium-dried ether. Purification of the solid was attempted by redissolving in methanol and precipitating back out again with diethyl ether. However the solid did not contain a uniform colouration, suggesting that it still retained a degree of impurity. It was unsuccessfully attempted to recrystallise the complex from hot ethanol.

Comparison of the carboxylate stretching region at ~3000 cm^{-1} in the infra-red spectrum of the compound compared to that of free methionine showed the disappearance of the -OH stretching frequency due to the carboxylic acid group in methionine which is consistant with coordination via a $-COO^-$ group (see Figure 3.6). However CHN analysis results were poor. On the basis of infra-red analysis it is likely that an iron-methionine complex was formed but that it contains a degree of impurity.

McAuliffe et al[4] reported synthesis of the octahedral $Fe(met)_3$ complex during a study of the donor proporties of the amino acid methionine with a range of transition and nontransition metal ions. Methionine can be regarded as a potentially tridentate chelating ligand, with coordination through the $-SCH_3$, $-NH_2$ and $-COO^-$ groups. However reaction with iron salts demonstrated a triligated complex with coordination only via $-NH_2$ and $-COO^-$ groups. The group reported preparation of the complex by

addition of methionine to a suspension of lithium hydroxide in ethanol which was stirred for 20 minutes at 60°C. After filtration of the unreacted lithium hydroxide, a solution of iron(III) perchlorate was added slowly and the reaction mixture heated on a steam bath until crystallisation began. The complex was isolated as a red-brown solid which was soluble in a wide range of organic solvents.

Attempts to repeat the synthesis only resulted in the formation of an insoluble pale-orange powder. Due to the explosive nature of iron(III) perchlorate, the reaction was also attempted using iron(III) chloride. Instead of red-brown crystals as reported by McAuliffe et al[4], a pale red-brown powder was deposited on heating of the reaction mixture. Infra-red analysis of the complex indicated coordination of methionine via the carboxylate group. That an iron-methionine complex had been formed was further supported by the fact that the solid melted between 192 - 209°C (reported mp = 203°C) indicating that the complex formed was impure. There was also some evidence of melting between 40 and 60°C which was probably due to some residual iron(III) nitrate. Consequently CHN analysis results were poor. However, in contrast to the published results, the complex was found to be insoluble in all of the organic solvents reported to afford solubility. It is feasible that an impure iron-methionine complex was formed, but it is interesting to note that Halbert and Rogerson[5] also failed to repeat this synthesis. It may also be significant that no structural characterisations, by X-ray analysis, have been reported for any of the many iron-methionine and related complexes discussed in the literature.

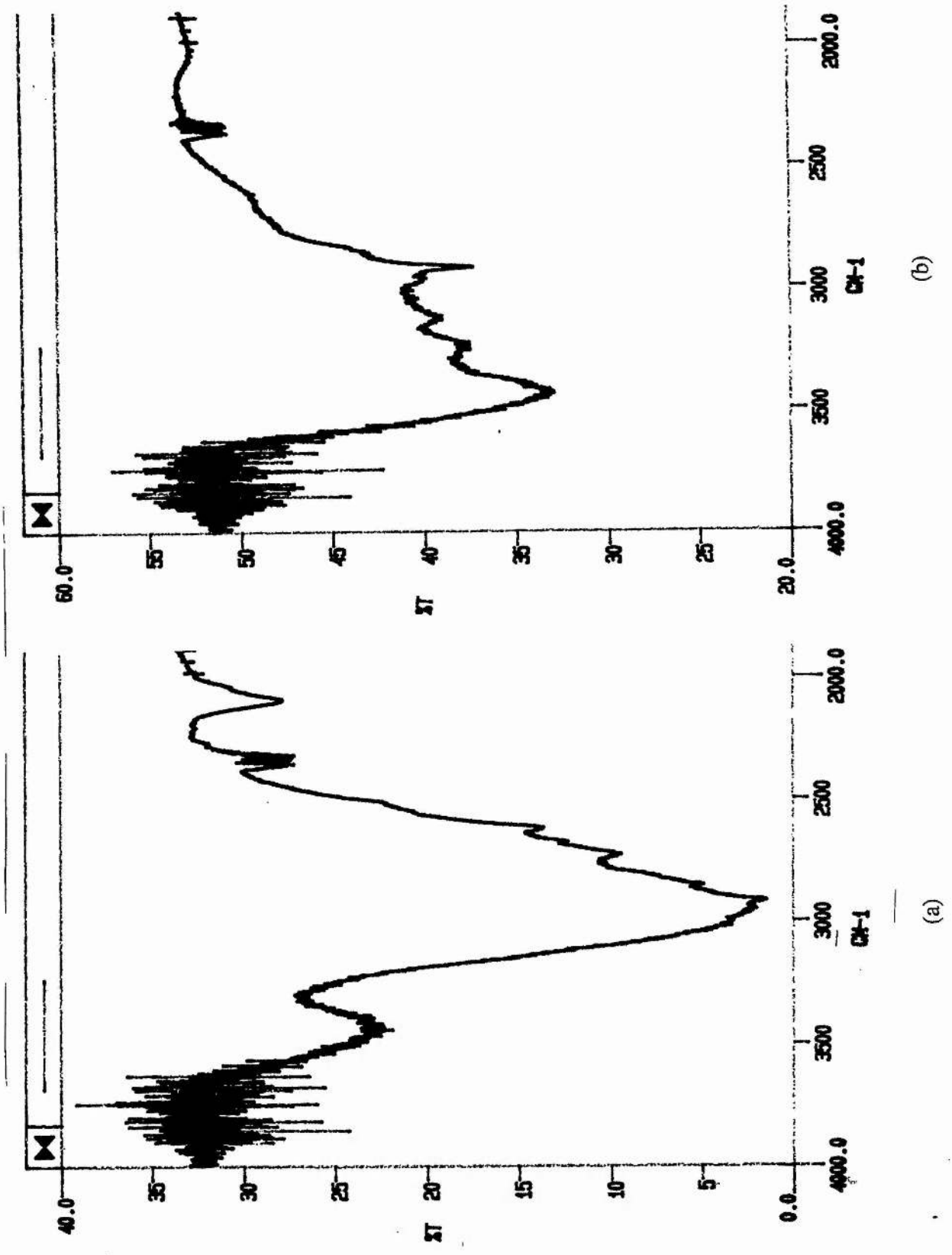


Figure 3.6; Comparison of the carboxylic acid/carboxylate stretching frequency in (a) free methionine and (b) complexed methionine

3.2.3 Reaction of the $[Fe_3O(amino\ acid)_6(H_2O)_3](X)_7$ Complexes with Nitrite

The sulfur containing amino acid methylcysteine has been shown[11] to react with sodium nitrite and iron(II) sulfate in the presence of sodium ascorbate in aqueous solution to give a mixture of Roussin's black salt $Na[Fe_4S_3(NO)_7]$ and the neutral dinuclear iron complex $Fe_2(SMe)_2(NO)_4$ under autoclave conditions. When an aqueous solution containing methionine, sodium nitrite and iron(II) sulfate was autoclaved in the presence of sodium ascorbate only $Fe_2(SMe)_2(NO)_4$ was isolated. However the yield of $Fe_2(SMe)_2(NO)_4$ obtained was increased when the reaction was conducted at 100°C under anaerobic conditions at ambient pressure, rather than at 118°C in an autoclave with air present. Thus the reactions of the preformed iron-methylcysteine and iron-methionine complexes were conducted in the autoclave and under nitrogen respectively, as optimum yields of the corresponding products were isolated when using the free amino acids under these conditions.

When $[Fe_3O(met)_6(H_2O)_3]Cl_7$ was reacted under nitrogen with sodium nitrite in the presence of sodium ascorbate, no identifiable iron-sulfur-nitrosyl complexes could be detected using infra-red spectroscopy. However the methylcysteine complex $[Fe_3O(mcys)_6(H_2O)_3]Cl_7$, when reacted with sodium nitrite and sodium ascorbate in the autoclave gave both the tetranuclear $[Fe_4S_3(NO)_7]^-$ (I) and the dinuclear $Fe_2(SMe)_2(NO)_4$ (II) complexes which were identified by infra-red spectroscopy.

Complex $[Fe_3O(A)_6(H_2O)_3]Cl_7$	Reaction Conditions	υ(NO) (cm^{-1}) THF	DCM
A = methionine	Nitrogen	1716 (w)	1713 (w)
A = methylcysteine	Autoclave	1795 (I),1775 (II), 1742 (I), 1708 (I)	1797 (I), 1777 (II), 1747 (I), 1708 (I)

Table 3.1; υ(NO) observed after reaction of $[Fe_3O(A)_6(H_2O)_3]Cl_7$ with sodium nitrite in the presence of sodium ascorbate

The isolation of iron-sulfur-nitrosyl complexes from reaction of the preformed methylcysteine complex with sodium nitrite in the presence of sodium ascorbate suggests the possibility of an iron-amino acid intermediate during the formation of iron-sulfur nitrosyl complexes from free methylcysteine, iron salts and nitrite.

Fe^{x+} + amino acid ⟶ Fe/amino acid complex $\xrightarrow{NO_2^-}$ iron-sulfur nitrosyl complex

That peaks due to $[Fe_4S_3(NO)_7]^-$, from reaction of the methylcysteine complex, were observed in dichloromethane indicates that the counter-ion is not Na^+ from sodium nitrite ($Na[Fe_4S_3(NO)_7]$ is insoluble in dichloromethane) but that of a large organic cation. Subtraction of a spectrum of $Fe_2(SMe)_2(NO)_4$ in dichloromethane from the spectrum obtained from reaction of the methylcysteine complex with nitrite in the presence of ascorbate demonstrated not only peaks due to $[Fe_4S_3(NO)_7]^-$, but also peaks at 2931 cm^{-1} and at 1113 cm^{-1}, which may correspond to CH and S=O stretches respectively.

That $Fe_2(SMe)_2(NO)_4$ could not be observed from reaction of the preformed methionine complex may simply be that the yield obtained was simply too small to be detected by FTIR spectroscopy.

The reactions were duplicated both under nitrogen and in the autoclave in the absence of sodium ascorbate, which led to the formation of a different set of reaction products which FTIR spectroscopy demonstrated absorbed strongly in the υ(NO) stretching region.

Complex $[Fe_3O(A)_6(H_2O)_3]Cl_7$	Reaction Conditions	υ(NO) (cm^{-1}) THF	DCM
A = methionine	Nitrogen	1780, 1729	1774, 1704
A= methionine	Autoclave	1781, 1728	1774, 1704
A = methylcysteine	Nitrogen	1734	1709
A = methylcysteine	Autoclave	1734	1708

Table 3.2; υ(NO) observed after reaction of $[Fe_3O(A)_6(H_2O)_3]Cl_7$ with sodium nitrite in the absence of sodium ascorbate

That the peaks observed using FTIR are consistent regardless of whether the reaction is carried out under nitrogen or in the autoclave demonstrates that the reaction products are independent of the reaction conditions. An analogous set of reactions were carried out using the nitrate salts to find out the effect, if any, of the counter-ion.

Complex $[Fe_3O(A)_6(H_2O)_3](NO_3)_7$	Reaction Conditions	υ(NO) (cm^{-1}) THF	DCM
A = methionine	Nitrogen	1781, 1725	1777
A= methionine	Autoclave	1785, 1729	1782
A = methylcysteine	Nitrogen	1726, 1711	1712
A = methylcysteine	Autoclave	1735, 1711	1708

Table 3.3; υ(NO) observed after reaction of $[Fe_3O(A)_6(H_2O)_3](NO_3)_7$ with sodium nitrite in the absence of sodium ascorbate

The species formed from the nitrate salts compare well with those formed from the chloride salts, especially in THF, with the exception of the products obtained from reaction of the methylcysteine complex under nitrogen. However, overall the reaction products appear independent of the nature of the counter-ion.

However comparison of the reactions of the chloride salts with and without sodium ascorbate present demonstrate the dramatic effect of sodium ascorbate on the reaction products formed. It is known that ascorbate sequesters iron(III) from the ferritin core[14] and keeps it in the reduced state. If this is the case then it may be that ascorbate is removing iron(III) from the core of the $[Fe_3O(\text{amino acid})_6(H_2O)]Cl_7$ complex causing breakdown of the molecule into the free amino acid and iron(II) which could then react with nitrite in the manner shown in Chapter 2 (see Section 2.2.1). The identity of the reaction products from reaction of the preformed methionine and methylcysteine complexes with nitrite in the absence of sodium ascorbate remain unidentified. Perhaps the most 'obvious' deduction would be the formation of an N-nitrosamine. This could be supported by the fact that N-nitrosamines are relatively stable compounds and that compounds such as ascorbic acid inhibit nitrosation which would explain why these species are absent in the presence of sodium ascorbate. However nitrite cannot react directly with amines, but must first be converted to nitrous anhydride (N_2O_3) which is dependant on acidic conditions. All the reactions studied were carried out in neutral solution. Another factor is the absence of organic peaks, other than those of the solvent, in the FTIR spectrum suggesting perhaps, the formation of an inorganic nitrosyl complex.

3.3 Experimental

FTIR spectra were recorded in tetrahydrofuran (THF) or dichloromethane (DCM) solutions using 0.2 mm pathlength cells or from KBr plates with a Perkin-Elmer Model 1710 FTIR spectrophotometer. All solution electronic spectra were run in H_2O solutions using a SP8-150 UV/VIS spectrophotometer. Magnetic susceptibility measurements on solid samples were run on a Johnson-Matthey Guoy balance. The autoclave employed had a capacity of 14.3 dm^3. Powder diffraction spectra were recorded using Cu Kα radiation where λ = 1.5148Å on a Philips PW1050 spectrometer by Jamie Thomson.

3.3.1 Preparation of $[Fe_3O(methionine)_6(H_2O)_3](NO_3)_7$

To a an aqueous solution of DL-methionine (2.98g, 20 mmol) in distilled water (50 cm^3) was added a solution of iron(III) nitrate nonahydrate (4.04g, 10 mmol) in distilled water (30 cm^3) and the resulting red/orange mixture stirred for 2 hours. The mixture was filtered through hyflo and the filtrate freeze-dried to yield a gummy solid, which on maceration with acetonitrile yielded a powder. The product was washed with cold absolute ethanol to remove any acetonitrile adduct present before drying in vacu. to give the product as an orange powder (3.21g, 62%).

IR (KBr)	$\upsilon(H_2O)$	3400 - 3500 cm^{-1}	
	$\upsilon(NH_3^+)$	3200 - 2800 cm^{-1}	
	$\upsilon(COO^-)$	1655 cm^{-1}	1435 cm^{-1}
	$\upsilon(NO_3^-)$	1385 cm^{-1}	1350 cm^{-1}
	$\upsilon(FeO)$	590 cm^{-1}	

UV/VIS 320 nm (H_2O)

Magnetic Susceptibility

	χ_M	μ_{eff}
Lit	5.465x10-3 cgsu	3.60 B.M.
Found	4.288x10-3 cgsu	3.16 B.M.

CHN analysis

	%C	%H	%N
Calcd	23.09	4.26	11.67
Found	22.75	4.55	10.28

3.3.2 Preparation of $Fe_3O(methylcysteine)_6(H_2O)_3](NO_3)_7$

To a solution of S-methyl-L-cysteine (2.70 g, 20 mmol) in distilled water (50 cm^3) was added a solution of iron(III) nitrate nonahydrate (4.04 g, 10 mmol) in distilled water (30 cm^3) and the resulting deep red mixture stirred for 2 hours. The mixture was filtered through hyflo and the filtrate freeze-dried to yield a gummy solid, which on maceration with acetonitrile yielded a powder. The product was washed with cold absolute ethanol to remove any acetonitrile adduct present before drying in vacu. to give the product as a brown powder (3.27 g, 60%).

IR (KBr)	$\upsilon(H_2O)$	3450 - 3350 cm^{-1}	
	$\upsilon(NH_3^+)$	3200 - 2900 cm^{-1}	
	$\upsilon(COO^-)$	1640 cm^{-1}	1440 cm^{-1}
	$\upsilon(NO_3^-)$	1385 cm^{-1}	1350 cm^{-1}
	$\upsilon(FeO)$	570 cm^{-1}	

UV/VIS 272 nm (H_2O)

Magnetic Susceptibility

χ_M	1.138 x 10^{-2} cgsu
μ_{eff}	5.14 B.M.

CHN analysis

	%C	%H	%N
Calcd	19.53	3.69	12.34
Found	19.37	3.60	8.42

3.3.3 Attempted Preparation of $[Fe_3O(benzylcysteine)_6(H_2O)_3](NO_3)_7$

To a suspension of S-benzyl-L-cysteine (4.23 g, 20 mmol) in distilled water (300 cm^3) was added a solution of iron(III) nitrate nonahydrate (4.04g, 10 mmol) in distilled water (50 cm^3) and the resulting green mixture which rapidly turned brown was stirred for 5 hours. The mixture was filtered through hyflo and the filtrate freeze-dried to yield a gummy solid, which on maceration with acetonitrile yielded a powder. The product was washed with cold absolute ethanol to remove any acetonitrile adduct present before drying in vacu. to yield an orange/brown powder (2.28 g).

IR (KBr)	$\upsilon(H_2O)$	3450 - 3350 cm^{-1}	
	$\upsilon(NH_3^+)$	3300 - 2800 cm^{-1}	
	$\upsilon(COO^-)$	1655 cm^{-1}	1560 cm^{-1}

$\upsilon(NO_3^-)$	1385 cm^{-1}
υ(FeO)	590 - 610 cm^{-1}

UV/VIS 318 nm (H_2O)

Magnetic Susceptibility

χ_M	9.938 x 10^{-3} cgsu
μ_{eff}	4.81 B.M.

CHN analysis

	%C	%H	%N
Calcd	37.27	4.07	9.42
Found	11.86	2.57	2.98

3.3.4 Attempted Preparation of $[Fe_3O(cysteine)_6(H_2O)_3](NO_3)_7$

To a solution of L-cysteine (2.42g, 20 mmol) in distilled water (100 cm^3) was added a solution of iron(III) nitrate nonahydrate (4.04g, 10 mmol) in distilled water (50 cm^3). The mixture immediately turned an intense blue colour (due to formation of Fe(cysteine)[10]), which quickly faded to yield cystine as a white solid. The white solid was filtered off and dissolved in 1.5M hydrochloric acid and the pH adjusted to neutral using aqueous ammonia. The creamy coloured solid which precipitated out was washed with copious amounts of distilled water before drying in vacu.. The solid was then recrystallised from distilled water to give cystine as shiny white crystals which were washed with cold water and dried in vacu..

CHN analysis

	%C	%H	%N
Calcd.	30.00	5.03	11.66
Found	29.71	4.72	11.31

The filtrate was reduced to dryness to yield a black polycrystalline solid which powder diffraction analysis showed to be Fe_3O_4.

Powder diffraction analysis

Found		Literature values	
2θ	Intensity	2θ	Intensity
18.8	10.0	18.269	8.0
30.2	34.0	30.095	30.0
35.6	100.0	35.422	100
37.0	6.0	37.052	8.0
43.2	23.0	43.052	20.0
53.6	15.0	53.391	10.0
57.1	27.0	56.942	30.0
62.7	39.0	62.515	40.0
74.1	8.0	73.948	10.0
89.8	12.0	89.617	12.0

3.3.5 Attempted Preparation of $Fe_3O(methionine)_6(H_2O)_3]_2(SO_4)_7$

To a solution of DL-methionine (2.97 g, 20 mmol) in distilled water (100 cm^3) was added a solution of iron(III) sulfate (4.00 g, 10 mmol) in distilled water (50 cm^3) and the red mixture stirred for 48 hours. The mixture was filtered and the filtrate left to evaporate to dryness at room temperature to yield a gummy solid, which on maceration with acetonitrile yielded a powder. The product was washed with cold absolute ethanol to remove any acetonitrile adduct present before drying in vacu. to give the product as a brown powder.

IR (KBr)	$\upsilon(H_2O)$	3450 - 3400 cm^{-1}			
	$\upsilon(NH_3^+)$	3200 - 2700 cm^{-1}			
	$\upsilon(COO^-)$	1630 cm^{-1}	1445 cm^{-1}		
	$\upsilon(SO_4^{2-})$	1220 cm^{-1}	1130 cm^{-1}	1070 cm^{-1}	1000 cm^{-1}
		600 cm^{-1}			
	$\upsilon(FeO)$	480 cm^{-1}			

UV/VIS 312 nm (H_2O)

CHN analysis

	%C	%H	%N
Calcd	24.63	4.54	5.74
Found	19.30	4.14	4.45

3.3.6 Attempted Preparation of $Fe_3O(methylcysteine)_6(H_2O)_3]_2(SO_4)_7$

To a solution of S-methyl-L-cysteine (2.70 g, 20 mmol) in distilled water (100 cm^3) was added a solution of iron(III) sulfate (4.00 g, 10 mmol) in distilled water (50 cm^3) and the mixture was stirred for 5 hours. The mixture was filtered and the filtrate evaporated to dryness at room temperature to yield a gummy solid, which on maceration with acetonitrile yielded a powder. The product was washed with cold absolute ethanol to remove any acetonitrile adduct present before drying in vacu. to give the product as a brown powder.

IR (KBr)	$\upsilon(H_2O)$	3450 - 3400 cm^{-1}			
	$\upsilon(NH_3^+)$	3200 - 2800 cm^{-1}			
	$\upsilon(COO^-)$	1635 cm^{-1}	1440 cm^{-1}		
	$\upsilon(SO_4^{2-})$	1210 cm^{-1}	1130 cm^{-1}	1040 cm^{-1}	1000 cm^{-1}
		600 cm^{-1}			
	υ(FeO)	475 cm^{-1}			

UV/VIS 320 nm (H_2O)

CHN analysis

	%C	%H	%N
Calcd	20.92	3.95	6.10
Found	15.19	3.68	4.33

3.3.7 Attempted Preparation of $[Fe_3O(benzylcysteine)_6(H_2O)_6]_2(SO_4)_7$

To a suspension of S-benzyl-L-cysteine (4.23 g, 20 mmol) in distilled water (200 cm^3) was added a solution of iron(III) sulfate (4.00 g, 10 mmol) in distilled water (50 cm^3) and the mixture was stirred for 24 hours. The mixture was filtered and the filtrate evaporated to dryness to yield a gummy solid, which on maceration with acetonitrile yielded a powder. The product was washed with cold absolute ethanol to remove any acetonitrile adduct present before drying in vacu. to give the product as a brown powder (1.82 g).

IR (KBr)	$\upsilon(H_2O)$	3450 - 3350 cm^{-1}			
	$\upsilon(NH_3^+)$	3300 - 2900 cm^{-1}			
	$\upsilon(COO^-)$	1635 cm^{-1}	1440 cm^{-1}		
	$\upsilon(SO_4^{2-})$	1230 cm^{-1}	1130 cm^{-1}	1080 cm^{-1}	1000 cm^{-1}
		600 cm^{-1}			
	υ(FeO)	490 cm^{-1}			

UV/VIS 320 nm (H_2O)

3.3.8 Preparation of $[Fe_3O(methionine)_6(H_2O)_3]Cl_7$

To a solution of DL-methionine (2.98 g, 20 mmol) in distilled water (50 cm^3) was added a solution of iron(III) chloride heptahydrate (2.70 g, 10 mmol) in distilled water (30 cm^3) and the resulting red/orange mixture stirred for 2 hours. The mixture was filtered through hyflo and the filtrate freeze-dried to yield a gummy solid, which on maceration with acetonitrile yielded a powder. The product was washed with cold sodium-dried ether to remove any acetonitrile adduct present before drying in vacu. to give the solid as an orange powder (3.16 g, 69%).

IR (KBr)	$\upsilon(H_2O)$	3450 - 3400 cm^{-1}	
	$\upsilon(NH_3^+)$	2950 - 2900 cm^{-1}	
	$\upsilon(COO^-)$	1635 cm^{-1}	1435 cm^{-1}
	$\upsilon(FeO)$	650 - 570 cm^{-1}	

Magnetic Susceptibility

χ_M	1.15 x 10^{-2} cgsu
μeff	5.21 B.M.

CHN analysis

	%C	%H	%N
Calcd	26.21	4.84	6.11
Found	27.87	5.56	6.43

3.3.9 Preparation of $[Fe_3O(methylcysteine)_6(H_2O)_3]Cl_7$

To a solution of S-methyl-L-cysteine (2.70 g, 20 mmol) in distilled water (50 cm^3) was added a solution of iron(III) chloride heptahydrate (2.70 g, 10 mmol) in distilled water (30 cm^3) and the resulting red/purple mixture stirred for 2 hours. The mixture was filtered through hyflo and the filtrate freeze-dried to yield a gummy solid, which on maceration with acetonitrile yielded a powder. The product was washed with cold sodium-dried ether to remove any acetonitrile adduct present before drying in vacu. to give the product as a brown powder (2.64 g, 61%).

IR (KBr)	$\upsilon(H_2O)$	3400 - 3500 cm^{-1}	
	$\upsilon(NH_3^+)$	3015 cm^{-1}	2920 cm^{-1}
	$\upsilon(COO^-)$	1650 cm^{-1}	1430 cm^{-1}
	$\upsilon(FeO)$	575 cm^{-1}	

Magnetic Susceptibility

χ_M 1.67×10^{-2} cgsu
μeff 6.25 B.M.

CHN analysis

	%C	%H	%N
Calcd	22.35	4.22	6.51
Found	24.13	5.29	6.99

3.3.10 Preparation of Lithium Methioninate

Ethanol (40 cm^3) was added to lithium hydroxide monohydrate (0.4 g, 19 mmol) and DL-methionine (3.0 g, 20 mmol) and the resulting mixture stirred for 30 mins at 60°C. The mixture was filtered and allowed to cool slowly to yield lithium methioninate as a white crystalline solid (1.1 g, 38%).

CHN analysis

	%C	%H	%N
Calcd	38.71	6.50	9.03
Found	38.78	6.34	8.97

3.3.11 Attempted Preparation of Fe(methionine)(OH)Cl,2CH_3OH

To lithium methionate (1.1 g, 7.10 mmol) in analaR methanol (20 cm^3) was added anhydrous iron(III) chloride (0.33 g, 2.03 mmol) and the yellow/brown mixture stirred for 16 hours. The mixture was filtered and addition of sodium dried ether resulted in the precipitation of a sticky dark yellow solid. The solid was purified by redissolving in the minimum volume of analaR methanol and precipitating out again with sodium-dried diethyl ether (25 cm^3) which was filtered and washed well with diethyl ether to give the product as a yellow powder (0.36 g).

IR (KBr)	$\upsilon(H_2O)$	3500 cm^{-1} (b)	1340 cm^{-1}	
	$\upsilon(NH_3^+)$	3240 cm^{-1}	3130 cm^{-1}	2335 cm^{-1} (w)
		1635 cm^{-1} (b)		
	$\upsilon(COO^-)$	1635 cm^{-1} (b)	1440 cm^{-1}	
	υ(C-O)	1120 cm^{-1}		

CHN analysis

	%C	%H	%N
Calcd	26.23	5.97	4.37
Found	29.92	5.64	6.86

3.3.12 Attempted Preparation of Fe(methionine)(OH)NO_3,2CH_3OH

To lithium methionate (1.06 g 6.83 mmol) in analaR methanol (20 cm^3) was added iron(III) nitrate nonahydrate (2.60 g, 6.43 mmol) and the deep orange/red solution stirred for 2 hours. After filtration of the solution, addition of sodium-dried diethyl ether resulted only in the formation of a red oil.

3.3.13 Attempted Preparation of Fe(methionine)$_3$

DL-methionine (3.0 g, 20.1 mmol) was added to a suspension of lithium hydroxide monohydrate (0.8 g, 19 mmol) in ethanol (40 cm^3) and stirred at 60°C for 20 mins. The mixture was filtered and a solution of iron(III) chloride hexahydrate (1.80 g, 6.6 mmol) in ethanol (20 cm^3) was added to the filtrate. The resulting mixture was then refluxed for 30 mins, filtered and left to cool slowly. The mixture was filtered to yield a red-brown solid (1.12 g).

IR(KBr)	$\upsilon(NH_3^+)$	2955 cm^{-1}	1580 cm^{-1}
	$\upsilon(COO^-)$	1625 cm^{-1}	1415 cm^{-1}

CHN analysis

	%C	%H	%N
Calcd	36.00	6.04	8.40
Found	33.89	6.32	7.73

M.P. 192-209°C

3.3.14 Reaction of [Fe_3O(methionine)$_6$(H_2O)$_3$]Cl_7 with Sodium Nitrite and Sodium Ascorbate under Nitrogen

To a solution of [Fe_3O(methionine)$_6$(H_2O)$_3$]Cl_7 (0.88 g, 0.64 mmol) and sodium ascorbate (2.0 g, 10.1 mmol) in deoxygenated distilled water (70 cm^3) was added a solution of sodium nitrite (0.10 g, 1.45 mmol) in deoxygenated distilled water (30 cm^3) and the mixture refluxed under nitrogen gas for 5 hours. After cooling, the mixture was filtered and the filtrate exhaustively extracted with diethyl ether. the combined ether

extracts were washed with water and dried over magnesium sulfate before evaporating to dryness.

IR (THF) 1716 cm^{-1} (w)
IR (DCM) 1713 cm^{-1} (w)

3.3.15 Reaction of $[Fe_3O(methylcysteine)_6(H_2O)_3]Cl_7$ with Sodium Nitrite and Sodium Ascorbate in the Autoclave

$[Fe_3O(methionine)_6(H_2O)_3]Cl_7$ (0.83 g, 0.64 mmol) and sodium nitrite (0.10 g, 1.45 mmol) were dissolved in distilled water (100 cm^3) and autoclaved at 118°C for 30 mins. After cooling, the mixture was filtered and the filtrate exhaustively extracted with diethyl ether. The combined ether extracts were washed with water and dried over magnesium sulfate before evaporating to dryness.

IR (THF)	1795 cm^{-1}	1775 cm^{-1}	1742 cm^{-1}	1708 cm^{-1}
IR (DCM)	1797 cm^{-1}	1777 cm^{-1}	1747 cm^{-1}	1708 cm^{-1}
	2931 cm^{-1}	1113 cm^{-1}		

3.3.16 Reaction of $[Fe_3O(methionine)_6(H_2O)_3]Cl_7$ with Sodium Nitrite under Nitrogen

To a solution of $[Fe_3O(methionine)_6(H_2O)_3]Cl_7$ (0.88 g, 0.64 mmol) in deoxygenated distilled water (70 cm^3) was added a solution of sodium nitrite (0.10 g, 1.5 mmol) in deoxygenated distilled water (30 cm^3) under nitrogen gas and the mixture refluxed for 5 hours. After cooling, the mixture was filtered and exhaustively extracted with diethyl ether. The combined ether extracts were washed with water and dried over magnesium sulfate before evaporating to dryness.

IR (THF)	1780 cm^{-1}	1729 cm^{-1}
IR (DCM)	1774 cm^{-1}	1704 cm^{-1}

3.3.17 Reaction of $[Fe_3O(methionine)_6(H_2O)_3]Cl_7$ with Sodium Nitrite in the Autoclave

$[Fe_3O(methionine)_6(H_2O)_3]Cl_7$ (0.88 g, 0.64 mmol) and sodium nitrite (0.10 g, 1.45 mmol) were dissolved in distilled water (100 cm^3) and autoclaved at 118°C for 30 mins. After cooling, the mixture was filtered and the filtrate exhaustively extracted with diethyl ether. The combined ether extracts were washed with water and dried over magnesium sulfate before evaporating to dryness.

IR (THF)	1781 cm^{-1}	1728 cm^{-1}

IR (DCM) 1774 cm^{-1} 1704 cm^{-1}

3.3.18 Reaction of $[Fe_3O(methylcysteine)_6(H_2O)_3]Cl_7$ with Sodium Nitrite under Nitrogen

To a solution of $[Fe_3O(methylcysteine)_6(H_2O)_3]Cl_7$ (0.83 g, 0.64 mmol) in deoxygenated distilled water (70 cm^3) was added a solution of sodium nitrite (0.10 g, 1.5 mmol) in deoxygenated distilled water (30 cm^3) under nitrogen gas and the mixture refluxed for 5 hours. After cooling, the mixture was filtered and exhaustively extracted with diethyl ether. The combined ether extracts were washed with water and dried over magnesium sulfate before evaporating to dryness.

IR (THF) 1734 cm^{-1}
IR (DCM) 1709 cm^{-1}

3.3.19 Reaction of $[Fe_3O(methylcysteine)_6(H_2O)_3]Cl_7$ with Sodium Nitrite in the Autoclave

$[Fe_3O(methylcysteine)_6(H_2O)_3]Cl_7$ (0.83 g, 0.64 mmol) and sodium nitrite (0.10 g, 1.45 mmol) were dissolved in distilled water (100 cm^3) and autoclaved at 118°C for 30 mins. After cooling, the mixture was filtered and the filtrate exhaustively extracted with diethyl ether. The combined ether extracts were washed with water and dried over magnesium sulfate before evaporating to dryness.

IR (THF) 1734 cm^{-1}
IR (DCM) 1708 cm^{-1}

3.3.20 Reaction of $[Fe_3O(methionine)_6(H_2O)_3](NO_3)_7$ with Sodium Nitrite under Nitrogen

To a solution of $[Fe_3O(methionine)_6(H_2O)_3](NO_3)_7$ (1.0 g, 0.64 mmol) in deoxygenated distilled water (70 cm^3) was added a solution of sodium nitrite (0.10 g, 1.5 mmol) in deoxygenated distilled water (30 cm^3) under nitrogen gas and the mixture refluxed for 5 hours. After cooling, the mixture was filtered and exhaustively extracted with both diethyl ether and dichloromethane. Both extracts were washed with water and dried over magnesium sulfate before evaporating to dryness.

IR (THF) of ether extract 1781 cm^{-1} 1725 cm^{-1} (b)
IR (DCM) 1777 cm^{-1}

3.3.21 Reaction of $[Fe_3O(methionine)_6(H_2O)_3](NO_3)_7$ with Sodium Nitrite in the Autoclave

$[Fe_3O(methionine)_6(H_2O)_3](NO_3)_7$ (1.0 g, 0.64 mmol) and sodium nitrite (0.10 g, 1.45 mmol) were dissolved in distilled water (100 cm^3) and autoclaved at 118°C for 30 mins. After cooling, the mixture was filtered and the filtrate exhaustively extracted with both diethyl ether and dichloromethane. Both extracts were washed with water and dried over magnesium sulfate before evaporating to dryness.

IR (THF) of ether extract	1785 cm^{-1}	1725 cm^{-1}
IR (DCM)	1782 cm^{-1}	

3.3.22 Reaction of $[Fe_3O(methylcysteine)_6(H_2O)_3](NO_3)_7$ with Sodium Nitrite under Nitrogen

To a solution of $[Fe_3O(methylcysteine)_6(H_2O)_3](NO_3)_7$ (0.94 g, 0.64 mmol) in deoxygenated distilled water (100 cm^3) was added a solution of sodium nitrite (0.10 g, 1.5 mmol) in deoxygenated distilled water (30 cm^3) under nitrogen gas and the mixture refluxed for 5 hours. After cooling, the mixture was filtered and exhaustively extracted with both diethyl ether and dichloromethane. Both extracts were washed with water and dried over magnesium sulfate before evaporating to dryness.

IR (THF) of ether extract	1726 cm^{-1}	1711 cm^{-1}
IR (DCM)	1712 cm^{-1}	

3.3.23 Reaction of $[Fe_3O(methylcysteine)_6(H_2O)_3](NO_3)_7$ with Sodium Nitrite in the Autoclave

$[Fe_3O(methylcysteine)_6(H_2O)_3](NO_3)_7$ (0.94 g, 0.64 mmol) and sodium nitrite (0.10 g, 1.45 mmol) were dissolved in distilled water (100 cm^3) and autoclaved at 118°C for 30 mins. After cooling, the mixture was filtered and the filtrate exhaustively extracted with diethyl ether. The combined ether extracts were washed with water and dried over magnesium sulfate before evaporating to dryness.

IR (THF)	1735 cm^{-1}	1711 cm^{-1}
IR (DCM)	1708cm^{-1}	

3.4 Chapter Three Bibliography

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CHAPTER FOUR

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Conversion of $Fe_4S_4(NO)_4$ to $[Fe_4S_3(NO)_7]^-$; an FTIR and ESR Study

4.1 Introduction

The neutral cubane cluster $Fe_4S_4(NO)_4$ has been shown to convert to Roussin's black anion, $[Fe_4S_3(NO)_7]^-$ when dissolved in DMSO (see Figure 4.1).

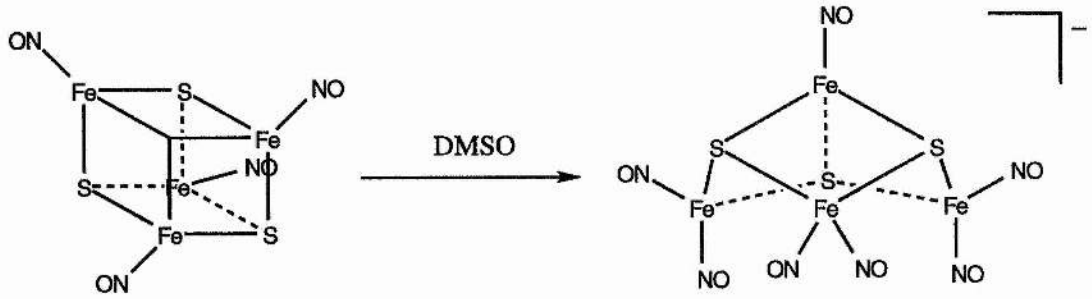


Figure 4.1; Conversion of $Fe_4S_4(NO)_4$ to $[Fe_4S_3(NO)_7]^-$

The aim of this work was to elucidate the intermediate product(s) and the cation of Roussin's black salt obtained during the conversion of $Fe_4S_4(NO)_4$ to $[Fe_4S_3(NO)_7]^-$ using FTIR and ESR studies. A large volume of work suggested[1-8] that the conversion occurred via a mechanism of extensive fragmentation to form mononuclear, paramagnetic iron species followed by reassembly.

The cubane-type cluster was first prepared[1] in 1974 by refluxing the mononuclear mercurial salt $[Fe(CO)_3NO]_2Hg$ with elemental sulfur in toluene according to the following equation.

$$2Hg[Fe(CO)_3NO]_2 + 3/4S_8 \longrightarrow Fe_4S_4(NO)_4 + 2HgS + 12CO$$

The cubane complex can similarly be prepared[2] from polynuclear complexes by refluxing the sodium salt of Roussin's black anion, $Na[Fe_4S_3(NO)_7]$ with elemental sulfur in toluene in the following reaction.

$$Na[Fe_4S_3(NO)_7] \xrightarrow{S_8,\ \text{toluene}} Fe_4S_4(NO)_4$$

However, Chu and Dahl accidentally converted the cubane $Fe_4S_4(NO)_4$ back to the black salt anion $[Fe_4S_3(NO)_7]^-$ whilst attempting to isolate the cubane monoanion $[Fe_4S_4(NO)_4]^-$ by Na/Hg amalgam for cyclic voltammetric studies[3].

$$Fe_4S_4(NO)_4 \xrightarrow{Na/Hg} Na[Fe_4S_3(NO)_7]$$

The formation of $Na[Fe_4S_3(NO)_7]$ was explained as being a decomposition product from the $[Fe_4S_4(NO)_4]^-$ monoanion during the acetone extraction and/or the methylene chloride/heptane recrystallisation stage. However the reduction was carried out in THF solution and an alternative explanation could be that the cluster formed mononuclear solvocomplexes on reduction in THF, which reassembled in acetone to give $[Fe_4S_3(NO)_7]^-$. That both $Fe_4S_4(NO)_4$ and $[Fe_4S_3(NO)_7]^-$ can be synthesised by spontaneous self-assembly from mononuclear precursors leads plausibly to this suggestion.

Butler and Glidewell et al[4] demonstrated that in DMF solution, $Fe_4S_4(NO)_4$ exhibited a very weak ESR spectrum indicating the presence of at least three paramagnetic centres. However addition of RS^- caused replacement of this spectrum with a new spectrum which was assigned as due to $[Fe(SR)_2(NO)_2]^-$ together with $[Fe_4(SR)_3(NO)]^-$ and their formulation was postulated as follows.

$$Fe_4S_4(NO)_4 \xrightarrow{12RS^-} 4[Fe(SR)_3NO]^- + 4S^{2-}$$

$$2[Fe(SR)_3NO]^- \rightleftharpoons [Fe(SR)_2(NO)_2]^- + [Fe(SR)_4]^-$$

$$2Fe(I) \rightleftharpoons Fe(-I) + Fe(III)$$

The iron-sulfur-nitrosyl clusters, $Fe_4S_4(NO)_4$, $[Fe_4S_3(NO)_7]^-$ and $[Fe_2S_2(NO)_4]^{2-}$ are closely related to each other chemically, as shown by the transformations[2,3,5,6].

$$[Fe_2S_2(NO)_4]^{2-} \underset{OH^-}{\overset{H^+}{\rightleftharpoons}} [Fe_4S_3(NO)_7]^- \underset{Na/Hg}{\overset{S_8}{\rightleftharpoons}} Fe_4S_4(NO)_4$$

The complexes are all diamagnetic in the solid state and in weak donor solvents[7]. However in powerful donor solvents such as DMF or DMSO, each one is converted[4] to paramagnetic, mononuclear complexes of the type $[Fe(SR)_2(NO)_2]^-$ further supporting the idea of fragmentation followed by reassembly.

Isotopic exchange reactions carried out by Butler and Glidewell et al[8] between iron-sulfur-nitrosyl clusters and nitrite suggested fragmentation followed by reassembly. It was shown using ^{15}N NMR that the anion $[Fe_4S_3(NO)_7]^-$ underwent slow exchange with labelled nitrite $[^{15}NO_2]^-$ to yield $[Fe_4S_3(^{14}NO)(^{15}NO)_6]^-$ in which complete isotopic exchange had occurred at the basal $Fe(NO)_2$ groups, but with no exchange at the apical Fe(NO) group. The neutral $Fe_4S_4(NO)_4$ complex however reacted rapidly with $[^{15}NO_2]^-$ to give the fully exchanged $[Fe_4S_3(^{15}NO)_7]^-$ product. It was proposed that the transformation of $Fe_4S_4(NO)_4$ to $[Fe_4S_3(NO)_7]^-$ proceeded via electron transfer leading to cluster fragmentation to monoiron complexes, followed by complete isotopic exchange and rapid reassembly.

The electronic structure of the binuclear and tetranuclear iron-sulfur-nitrosyl clusters, $[Fe_4S_3(NO)_7]^-$, $Fe_4S_4(NO)_4$ and $[Fe_2S_2(NO)_4]^{2-}$ was investigated[7] and it was found that the Lowest Unoccupied Molecular Orbital (LUMO) was antibonding in nature and so it is hardly surprising that powerful donor solvents should destabilise the structure yielding monoiron species. It also reinforces the possibility that interconversion of $Fe_4S_4(NO)_4$ and $[Fe_4S_3(NO)_7]^-$ may occur via a mechanism of extensive fragmentation and reassembly.

4.2 Results and Discussion

4.2.1 FTIR Study of $Fe_4S_4(NO)_4$ in a Range of Solvents

This study began with the observation that the cubane-like cluster, $Fe_4S_4(NO)_4$ when dissolved in DMSO, disproportionates to give Roussin's black anion $[Fe_4S_3(NO)_7]^-$ as shown by FTIR spectroscopy. The study was repeated in an attempt to identify the intermediate(s) present during the conversion. $Fe_4S_4(NO)_4$ was dissolved in DMSO and at regular time intervals, measured aliquots were removed and quenched in distilled water. Work up via diethyl ether extraction yielded samples suitable for FTIR analysis. Petroleum ether 40/60 (PE40-60) was used as the solvent for FTIR analysis for the first two days of the study. However by the third day it was noted that not all of the solid isolated dissolved in PE40-60. The remaining solid however proved to be readily soluble in THF and so subsequent spectral analyses were made using both PE40-60 and THF. The results are displayed in Table 4.1.

Time	Solvent	FTIR Peaks	Assignment
0 hrs	PE40-60	1790 cm^{-1}	$Fe_4S_4(NO)_4$
24 hrs	PE40-60	1790 cm^{-1}	$Fe_4S_4(NO)_4$
48 hrs	PE40-60	1790 cm^{-1}	$Fe_4S_4(NO)_4$
	THF	1795 cm^{-1}	$[Fe_4S_3(NO)_7]^-$
		1741 cm^{-1}	$[Fe_4S_3(NO)_7]^-$
		1724 cm^{-1}	Intermediate
		1707 cm^{-1}	$[Fe_4S_3(NO)_7]^-$
72 hrs	PE40-60	1790 cm^{-1}	$Fe_4S_4(NO)_4$
	THF	1795 cm^{-1}	$[Fe_4S_3(NO)_7]^-$
		1741 cm^{-1}	$[Fe_4S_3(NO)_7]^-$
		1724 cm^{-1}	Intermediate
		1707 cm^{-1}	$[Fe_4S_3(NO)_7]^-$

96 hrs	PE40-60	1790 cm^{-1}	$Fe_4S_4(NO)_4$
	THF	1795 cm^{-1}	$[Fe_4S_3(NO)_7]^-$
		1741 cm^{-1}	$[Fe_4S_3(NO)_7]^-$
		1724 cm^{-1}	Intermediate
		1707 cm^{-1}	$[Fe_4S_3(NO)_7]^-$
120 hrs	PE40-60	1790 cm^{-1}	$Fe_4S_4(NO)_4$
	THF	1795 cm^{-1}	$[Fe_4S_3(NO)_7]^-$
		1759 cm-1	Side-product
		1741 cm^{-1}	$[Fe_4S_3(NO)_7]^-$
		1707 cm^{-1}	$[Fe_4S_3(NO)_7]^-$
144 hrs	PE40-60	1790 cm^{-1}	$Fe_4S_4(NO)_4$
	THF	1795 cm^{-1}	$[Fe_4S_3(NO)_7]^-$
		1759 cm^{-1}	Side-product
		1741 cm^{-1}	$[Fe_4S_3(NO)_7]^-$
		1707 cm^{-1}	$[Fe_4S_3(NO)_7]^-$

Table 4.1; Assignment of FTIR spectrum of $Fe_4S_4(NO)_4$ in DMSO versus time

As measured volumes of solvent were not used to record the infra-red spectra then the assignment of the peaks observed can only be reported qualitatively. However, it could generally be seen that as time progressed, the peaks due to $Fe_4S_4(NO)_4$ became weaker in intensity as the $[Fe_4S_3(NO)_7]^-$ peaks grew in intensity, demonstrating that the $Fe_4S_4(NO)_4$ cluster is indeed being converted to give Roussin's black anion, $[Fe_4S_3(NO)_7]^-$. However between 48 and 96 hours (days 2 and 4), an additional peak is observed at 1724 cm^{-1} and this could perhaps be due to an intermediate species. This intermediate peak was replaced in the later stages of the conversion (120 and 144 hours; days 5 and 6) by another peak at 1759 cm^{-1} which may be due to a side-product during the formation of $[Fe_4S_3(NO)_7]^-$ from the intermediate.

It was decided to repeat the experiment, but recording the spectra in THF from the start. However the control spectrum of $Fe_4S_4(NO)_4$ in THF showed two peaks, one at 1785 cm^{-1} which can be assigned to $Fe_4S_4(NO)_4$ and another at 1724 cm^{-1}. This proved to be very interesting as it suggested that $Fe_4S_4(NO)_4$ was perhaps being converted to $[Fe_4S_3(NO)_7]^-$ in THF as well. The FTIR spectrum of $Fe_4S_4(NO)_4$ in dichloromethane showed only a single peak at 1790 cm^{-1} attributable to $Fe_4S_4(NO)_4$ and thin layer chromatography of $Fe_4S_4(NO)_4$ in dichloromethane demonstrated only a single spot moving with the solvent front. To establish whether $Fe_4S_4(NO)_4$ was indeed being converted to $[Fe_4S_3(NO)_7]^-$ in THF, an FTIR study of $Fe_4S_4(NO)_4$ in THF was made over time.

The initial study involved dissolving $Fe_4S_4(NO)_4$ in THF, bubbling the solution through with nitrogen and sealing up. When samples were removed for FTIR analysis, they were returned to the flask which was then bubbled through with nitrogen again and sealed. The results (shown in Table 4.2) proved to be very promising.

Time	Observation
5 mins	Intense peak at 1785 cm^{-1}. Smaller peak at 1724 cm^{-1}
2 hrs	Intense peak at 1785 cm^{-1}. Smaller peak at 1724 cm^{-1}
19 hrs	Intense peak at 1785 cm^{-1}. Peak at 1724 cm^{-1} increased in intensity. Broad shoulder at 1735 cm^{-1}
22 hrs	Peaks at 1785 and 1724 cm^{-1} unchanged. Shoulder sharpened to give peak at 1740 cm^{-1}
27 hrs	Peak at 1785 cm^{-1} decreased greatly in intensity. Peak at 1724 cm^{-1} increased in intensity. Peak at 1740 cm^{-1}. Shoulder at 1757 cm^{-1}
42 hrs	Peak at 1785 cm^{-1} sharpened up and is now closer to 1793 cm^{-1}. Shoulder at 1757 cm^{-1}
50 hrs	Peak at 1740 cm^{-1} increased slightly with respect to peak at 1724 cm^{-1}. Shoulder at 1757 cm^{-1} not so prominent
118 hrs	Peak at 1740 cm^{-1} now more intense than peak at 1724 cm^{-1}. Small shoulder at 1757 cm^{-1}

147 hrs	Peak at 1740 cm^{-1} grown at expense of peak at 1724 cm^{-1}. Small shoulder at 1757 cm^{-1}
156 hrs	Three peaks at 1793, 1741 and 1707 cm^{-1}. See shoulder at 1724 cm^{-1} on intense peak at 1741 cm^{-1} and small shoulder at 1757 cm^{-1}
196 hrs	Only peaks at 1793, 1741 and 1707 cm^{-1}

Table 4.2; FTIR spectrum observed when $Fe_4S_4(NO)_4$ is dissolved in THF versus time

The results clearly show the disappearance of the $Fe_4S_4(NO)_4$ peak at 1785 cm^{-1} being replaced by an intermediate species at 1724 cm^{-1} and later at 1757 cm^{-1} which are then replaced by Roussin's black anion with peaks at 1793, 1741 and 1707 cm^{-1}. The conversion of $Fe_4S_4(NO)_4$ to $[Fe_4S_3(NO)_7]^-$ is very clearly shown by FTIR analysis in THF solution (Figures 4.2-4.12).

The FTIR spectrum of $Fe_4S_4(NO)_4$ in THF in the presence of air was then studied. This resulted in much the same sequence of events, but occurred at a much faster rate. A shoulder on the main peak due to $[Fe_4S_3(NO)_7]^-$ at 1741 cm^{-1} may have been observed at 1757cm^{-1}. However the resolution of these spectra mean that this observation is uncertain. The peak at 1724 cm^{-1} did not grow to the same extent as that in the system where the solution was bubbled through with nitrogen and sealed after each sample was removed for analysis.

Another FTIR study of $Fe_4S_4(NO)_4$ in THF was undertaken, this time the solution was kept continuously under a slow stream of nitrogen. Samples were removed for direct FTIR analysis via a suba seal using a syringe. A very slow conversion of $Fe_4S_4(NO)_4$ to $[Fe_4S_3(NO)_7]^-$ was observed over a period of 40 days in comparison to a conversion of just 8 days when the solution was exposed to air during analysis. Again a peak at 1724 cm^{-1} was seen to grow in intensity then to gradually disappear during this period, indicating the presence of an intermediate species. However only one of the 34 spectra recorded over the 40 day period displayed evidence of a peak at 1757 cm^{-1} and even then it was only just observed as a very small shoulder on the $Na[Fe_4S_3(NO)_7]$ peak at 1741 cm^{-1}.

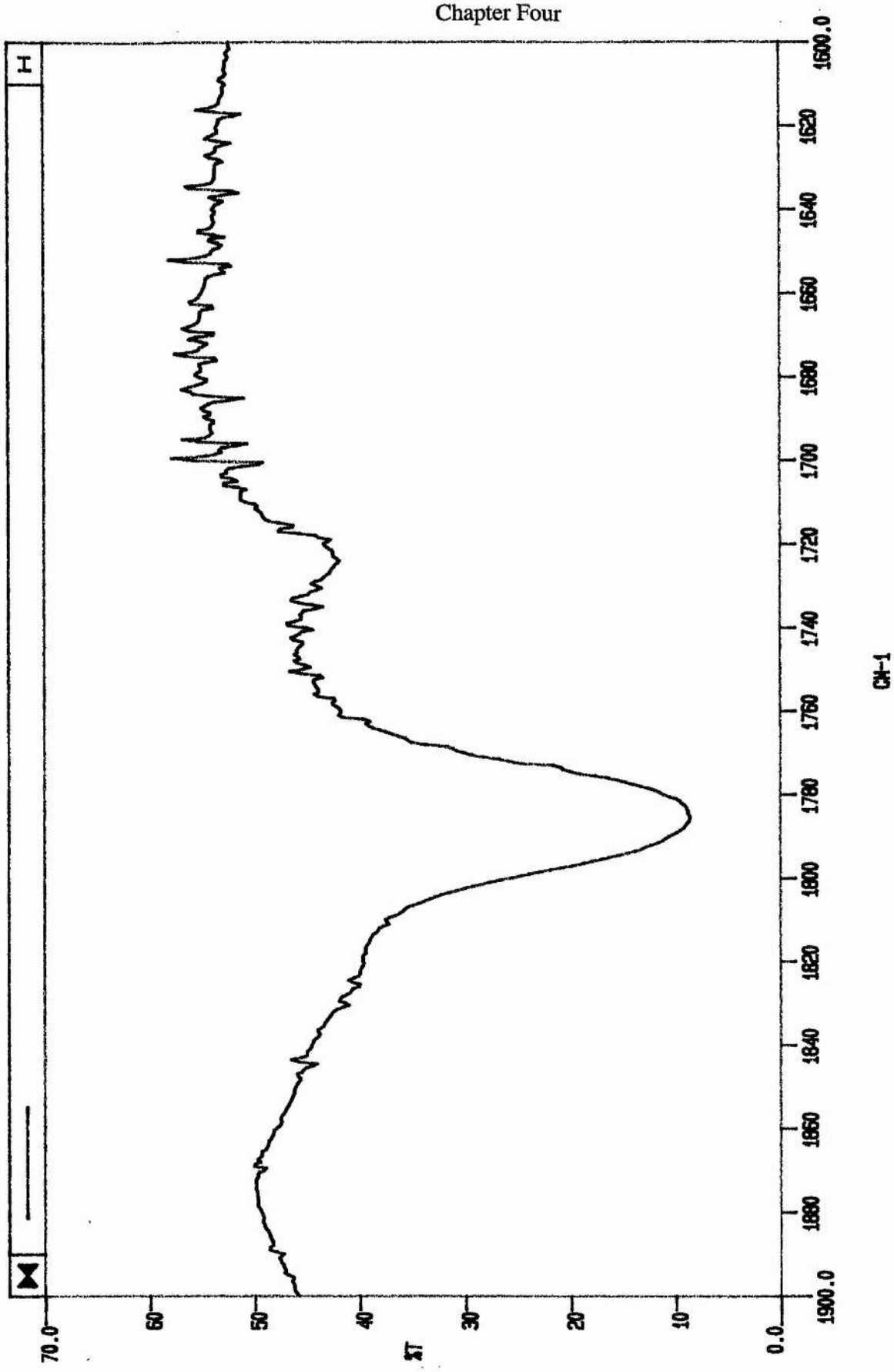


Figure 4.2; FTIR spectrum of $Fe_4S_4(NO)_4$ in THF after 5 minutes

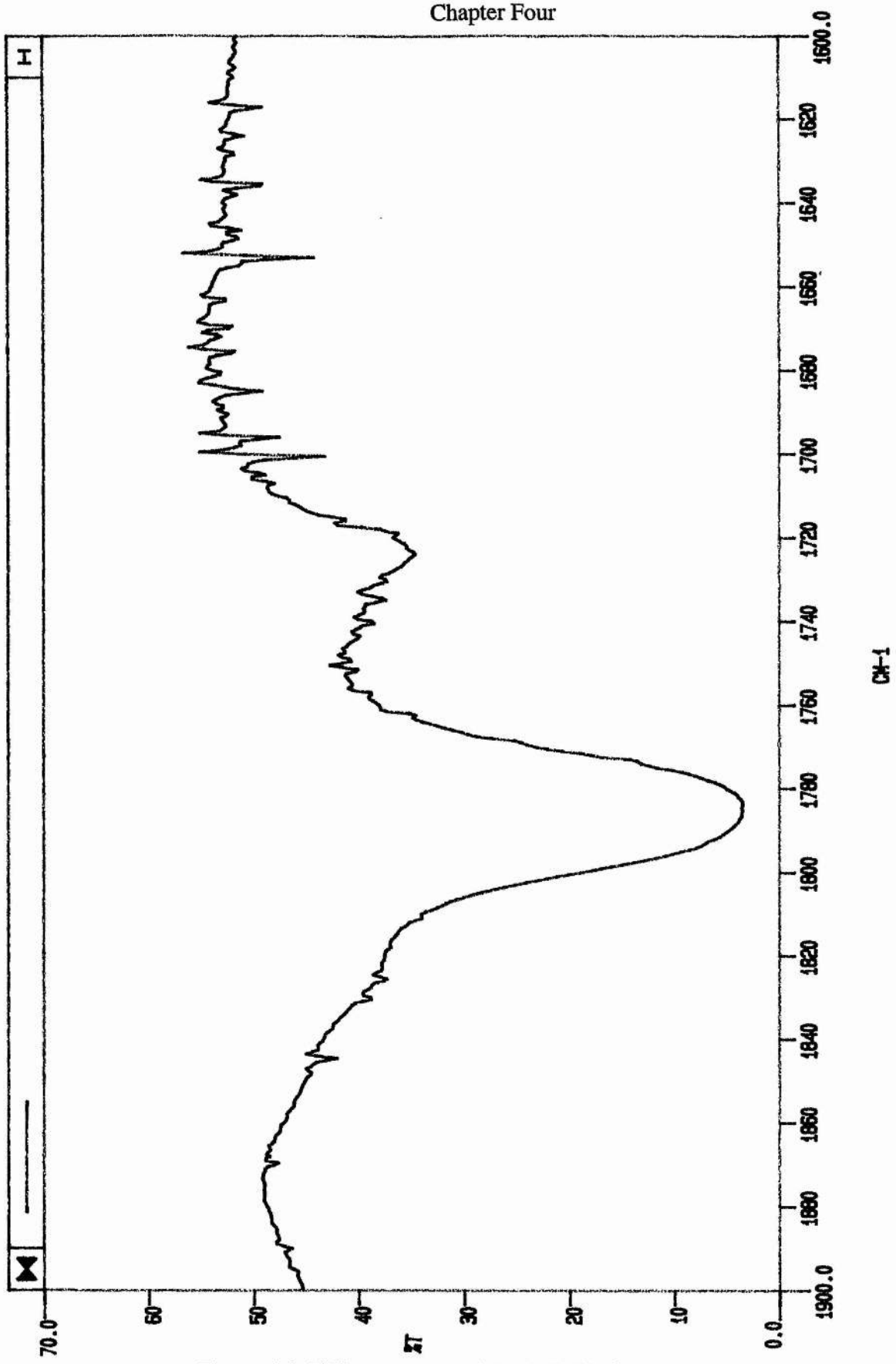


Figure 4.3; FTIR spectrum of $Fe_4S_4(NO)_4$ in THF after 2 hours

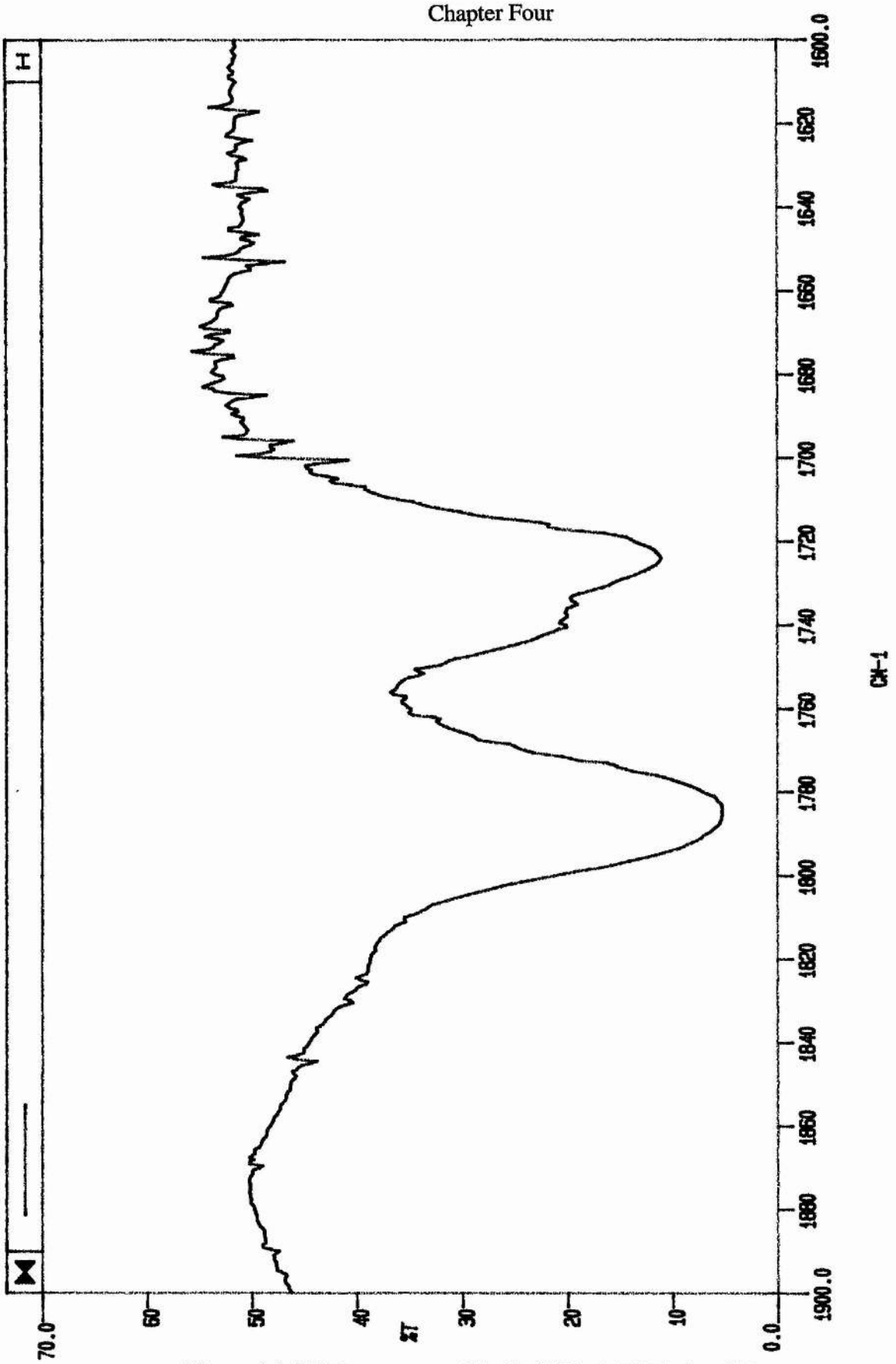


Figure 4.4; FTIR spectrum of $Fe_4S_4(NO)_4$ in THF after 19 hours

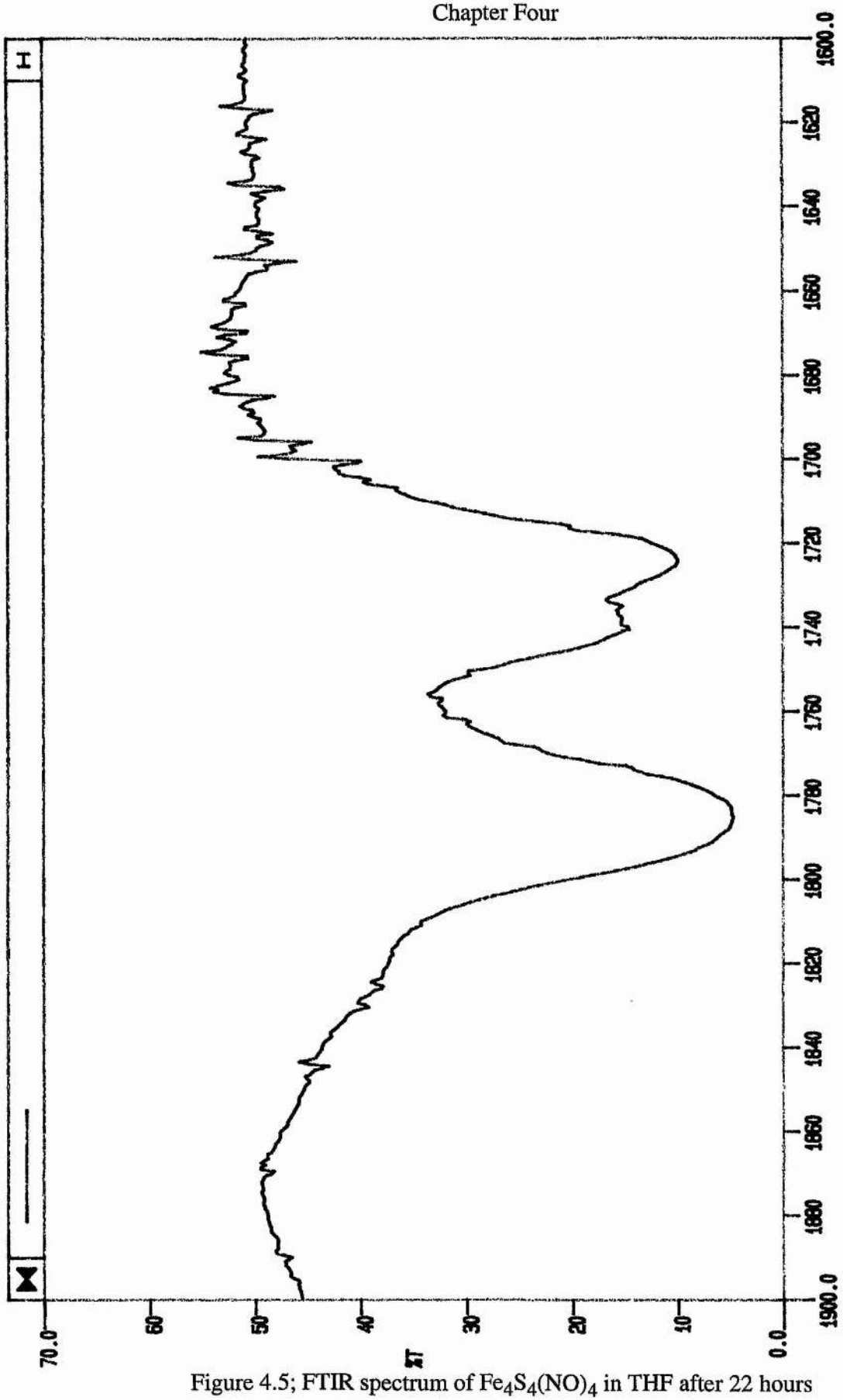


Figure 4.5; FTIR spectrum of $Fe_4S_4(NO)_4$ in THF after 22 hours

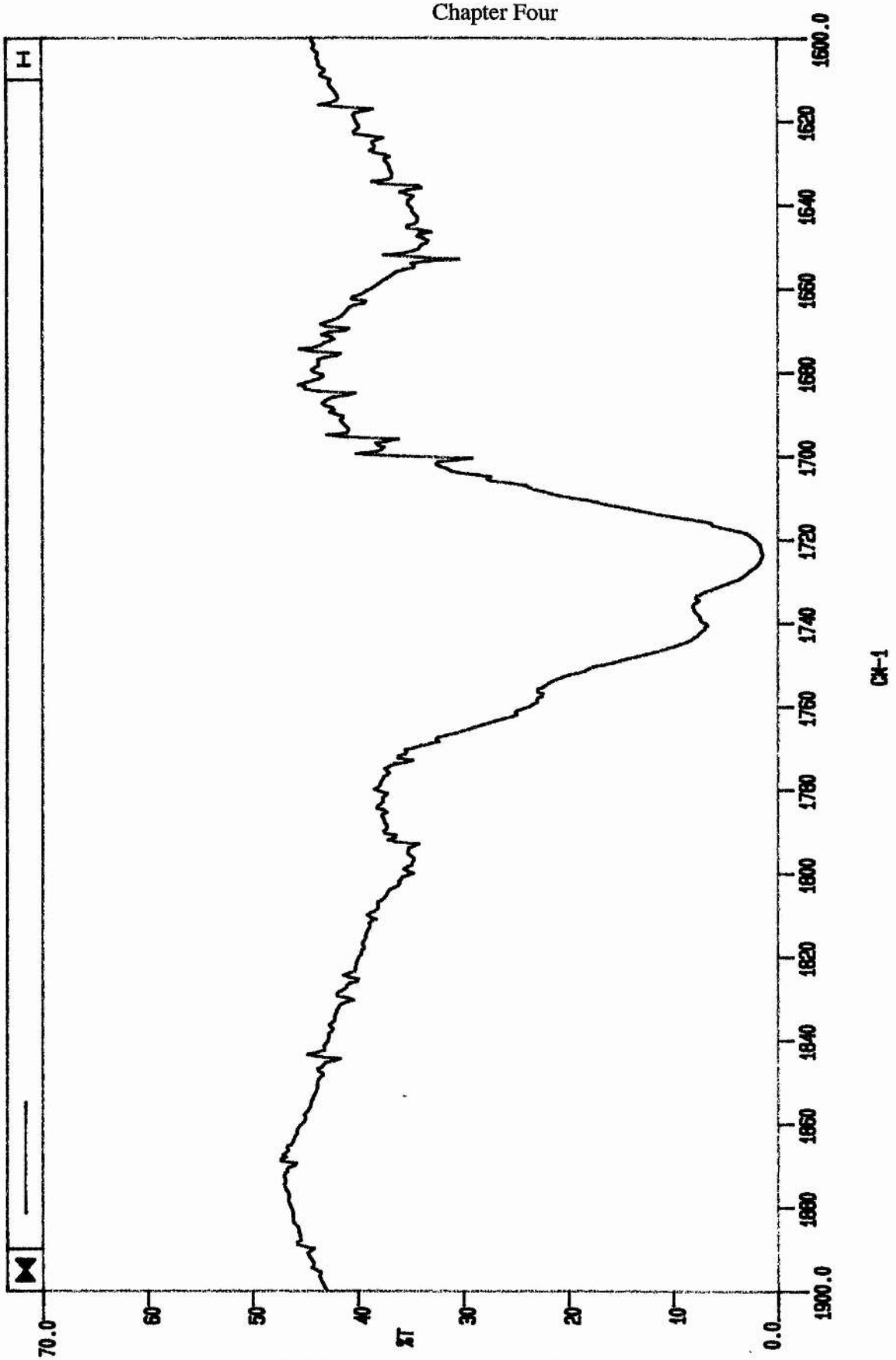


Figure 4.6; FTIR spectrum of $Fe_4S_4(NO)_4$ in THF after 27 hours

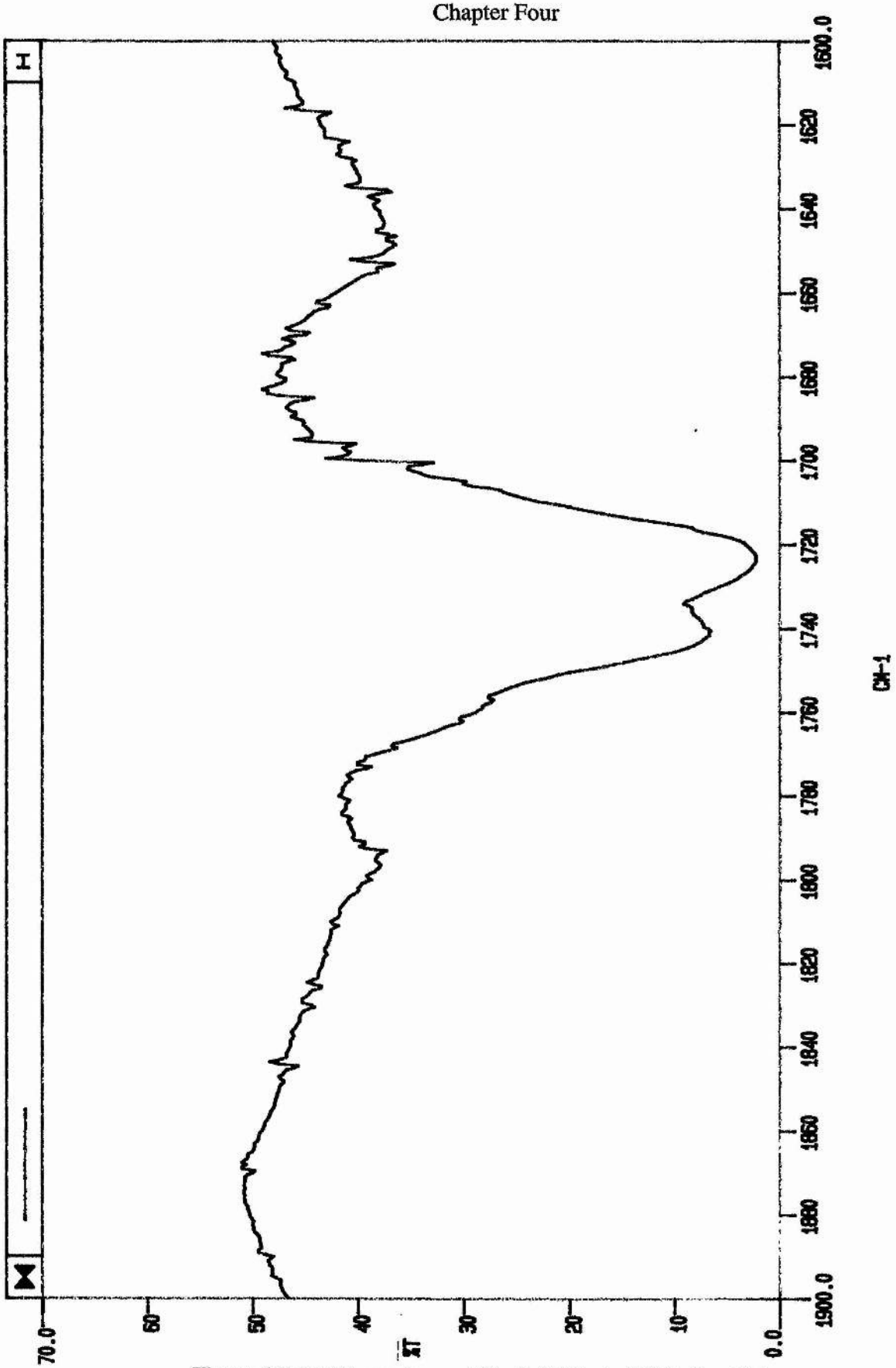


Figure 4.7; FTIR spectrum of $Fe_4S_4(NO)_4$ in THF after 42 hours

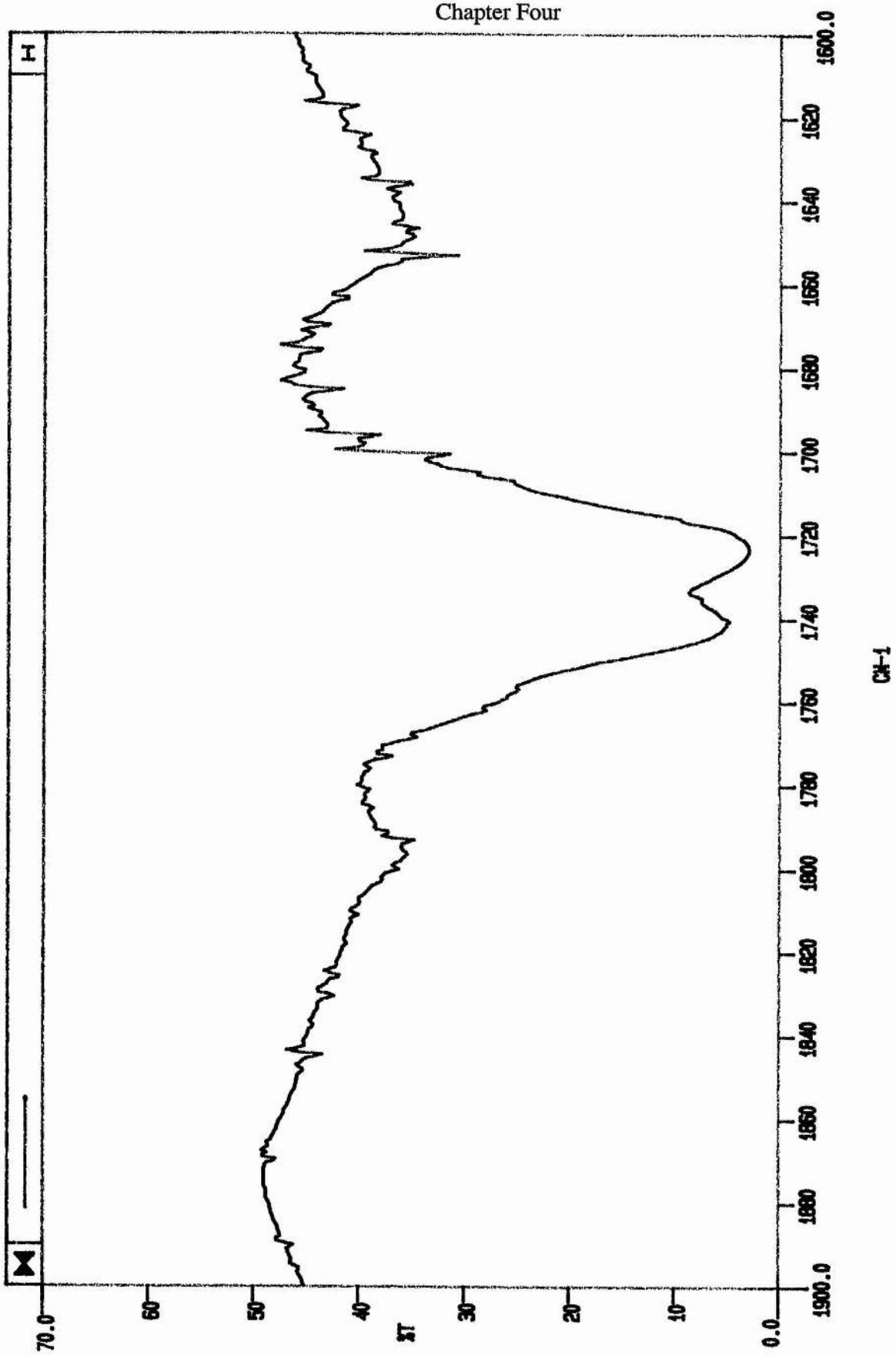


Figure 4.8; FTIR spectrum of $Fe_4S_4(NO)_4$ in THF after 50 hours

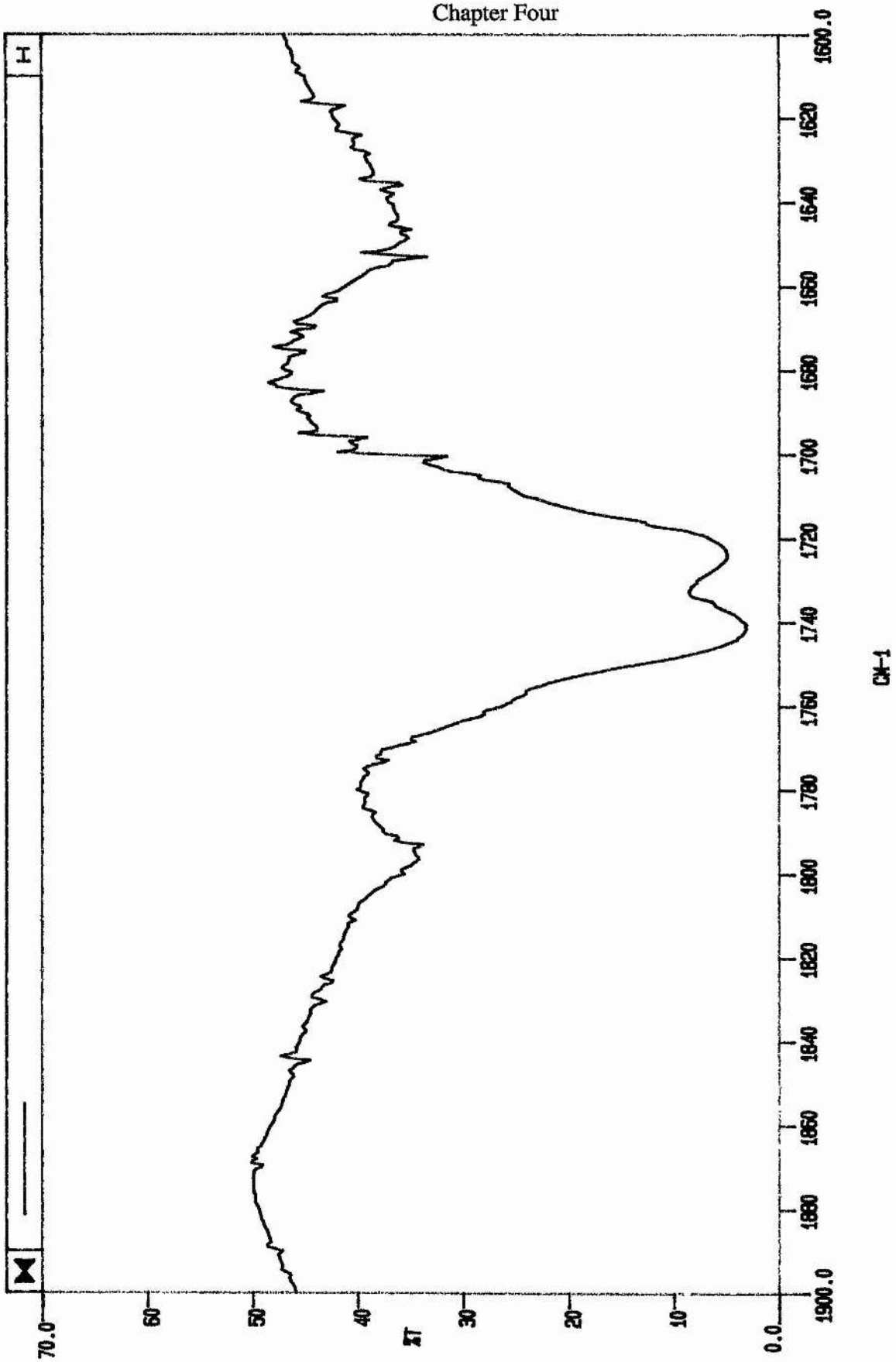


Figure 4.9; FTIR spectrum of $Fe_4S_4(NO)_4$ in THF after 118 hours

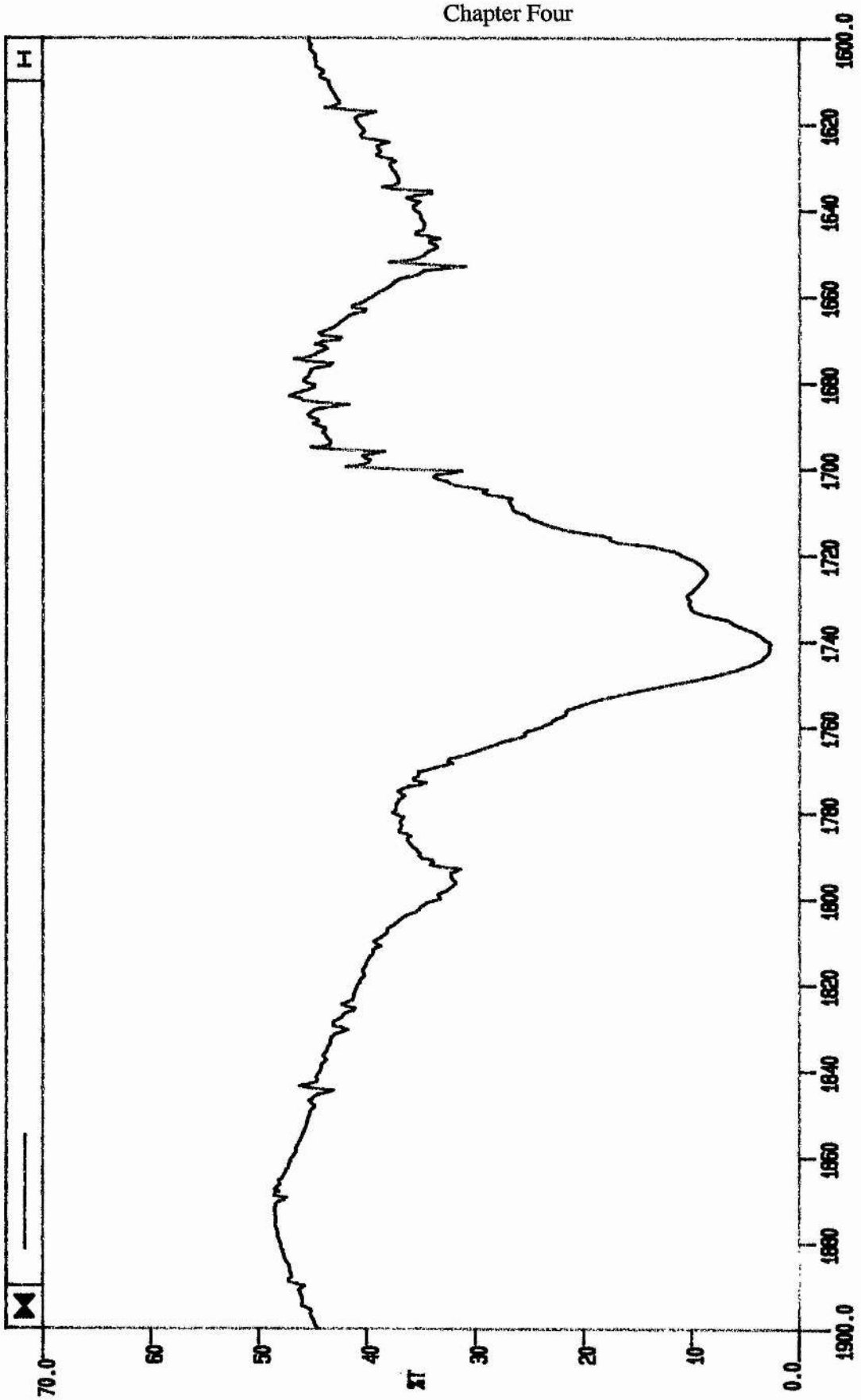


Figure 4.10; FTIR spectrum of $Fe_4S_4(NO)_4$ in THF after 147 hours

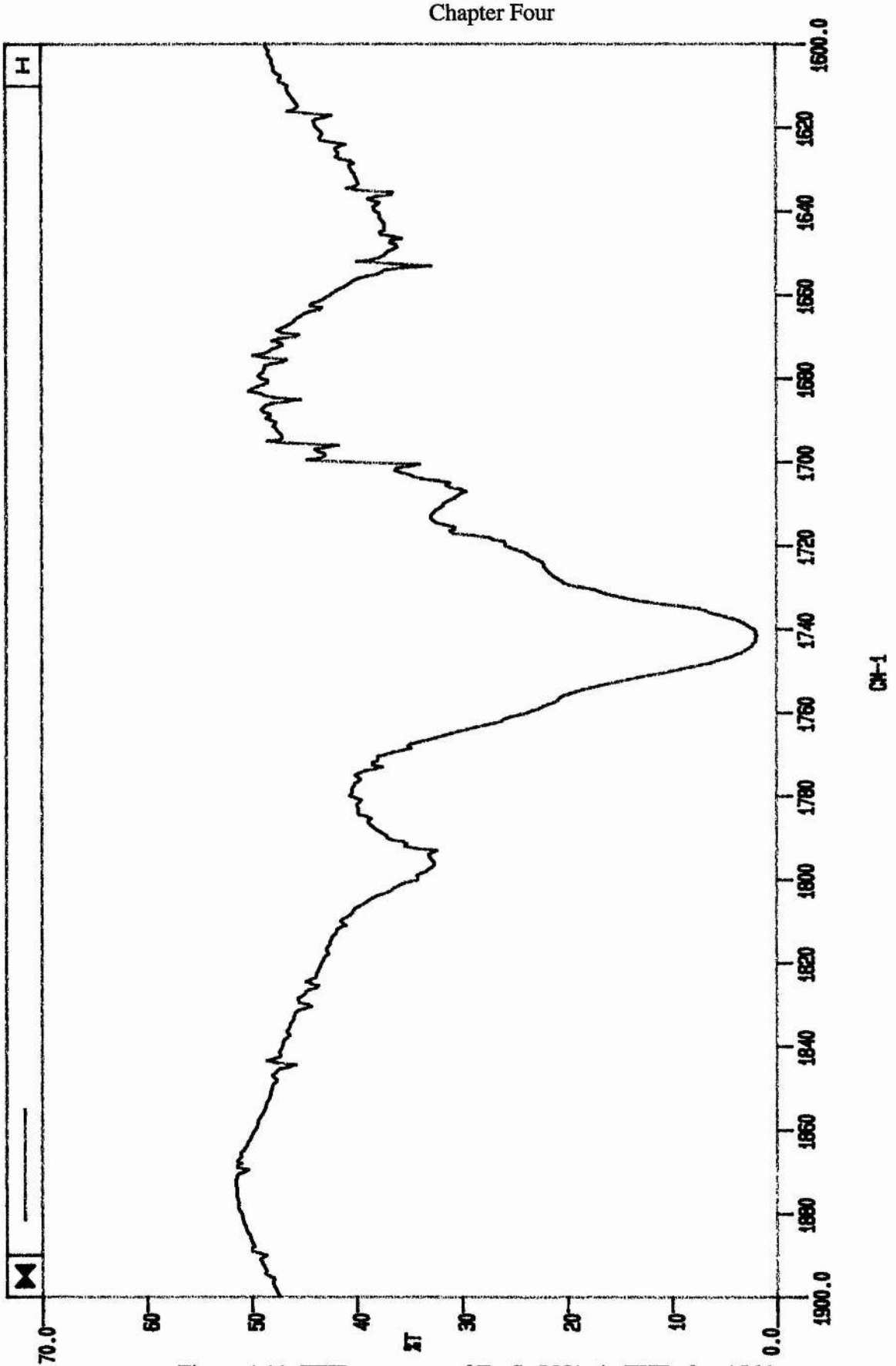


Figure 4.11; FTIR spectrum of $Fe_4S_4(NO)_4$ in THF after 156 hours

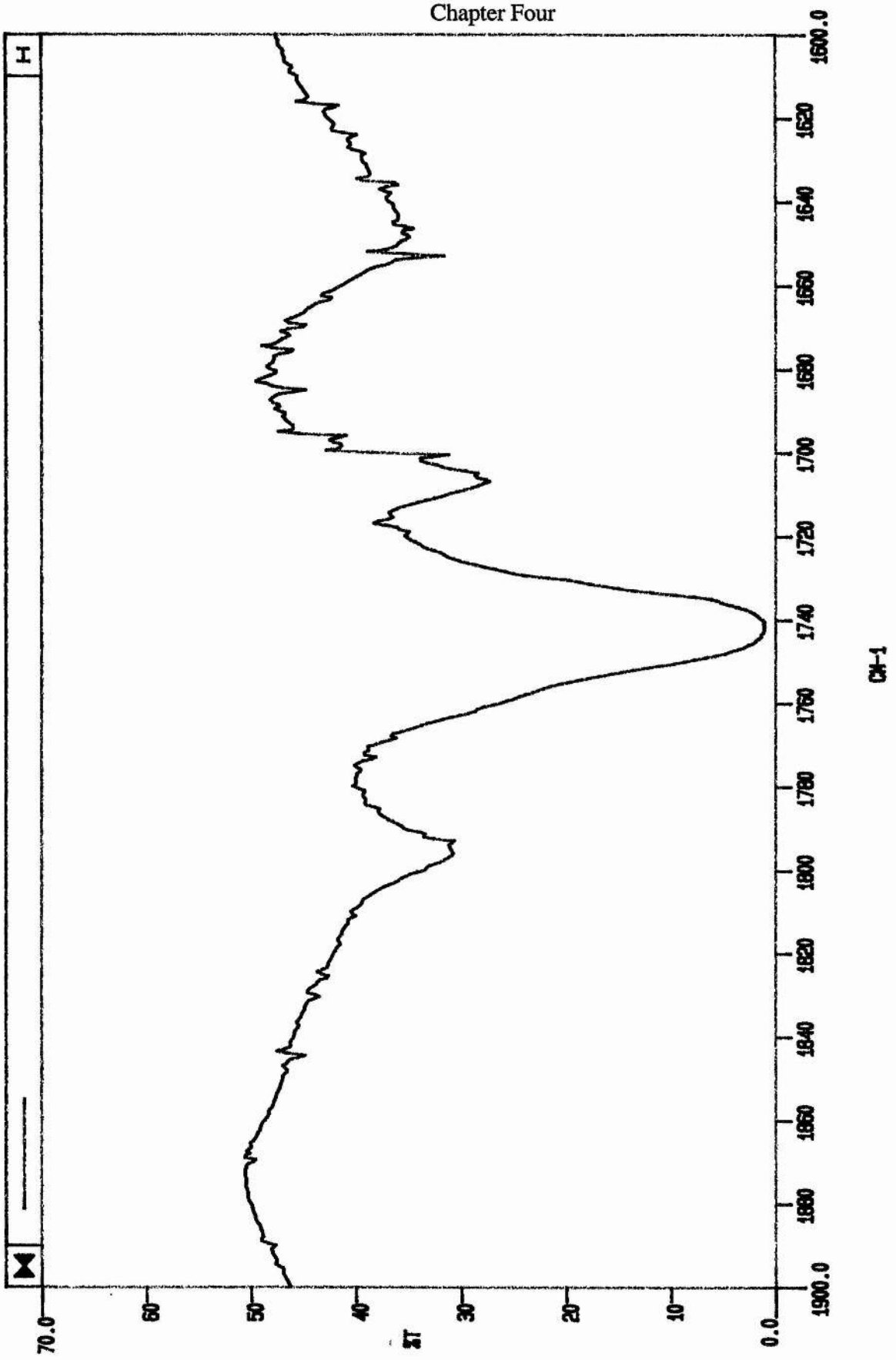


Figure 4.12; FTIR spectrum of $Fe_4S_4(NO)_4$ in THF after 196 hours

In a similar manner, the FTIR spectrum of $Fe_4S_4(NO)_4$ in both sodium-dried diethyl ether and dichloromethane (DCM) was studied over time. However even after a period of 21 days, no change was observed in the spectrum of either solvent, demonstrating that the conversion of $Fe_4S_4(NO)_4$ to $[Fe_4S_3(NO)_7]^-$ does not occur in diethyl ether or DCM. It may be argued that it would not be expected to observe peaks due to $[Fe_4S_3(NO)_7]^-$ from the infra red spectrum of $Fe_4S_4(NO)_4$ in DCM as $Na[Fe_4S_3(NO)_7]$ is insoluble in DCM and so would not display an FTIR spectrum! This argument can easily be refuted by the fact that no Na^+ is present in the system and so the cation must, at least partly, come from the solvent itself and so would be largely organic thus allowing solubility. If $Fe_4S_4(NO)_4$ were to convert to $[Fe_4S_3(NO)_7]^-$ in DCM then the infra-red peak at 1790 cm^{-1} due to the cubane species would decrease with time. This did not happen, further confirming that the $Fe_4S_4(NO)_4$ complex remains intact in DCM solution.

The powerful donor solvent dimethylformamide (DMF) was chosen as the next solvent in which an FTIR study of $Fe_4S_4(NO)_4$ was made versus time. However a problem was immediately encountered in that DMF absorbs strongly in the 1700-1800 cm^{-1} region of the infra-red spectrum. To overcome this, 3 cm^3 aliquots of the $Fe_4S_4(NO)_4$ in DMF solution, which was kept under a slow stream of nitrogen, were removed via a suba seal using a syringe and quenched in 100 cm^3 water for 15 minutes. The solution was then exhaustively extracted with diethyl ether, washed and dried over magnesium sulfate before evaporating to dryness. This procedure was repeated in an identical manner for each sample taken. FTIR analysis of the solid was made using diethyl ether and the results are as shown in Table 4.3. However due to a fault in the FTIR machine at the high resolution normally used to record the spectra the results for the conversion in DMF had to be recorded at a lower resolution.

Time	Observation
21 hrs	Intense peak at 1790 cm^{-1}. Small peak at 1747 cm^{-1}
42 hrs	No great change in spectrum
98 hrs	Peak at 1747 cm^{-1} grown slightly at expense of peak at 1790 cm^{-1}
141 hrs	No great change in spectrum

213 hrs	Peak at 1747 cm^{-1} grown slightly at expense of peak at 1790 cm^{-1}. Very weak peak at 1709 cm^{-1}
285 hrs	Peak at 1747 cm^{-1} grown slightly at expense of peak at 1790 cm^{-1}
381 hrs	No great change in spectrum
453 hrs	No great change in spectrum
789 hrs	Peak at 1747 cm^{-1} now more intense than peak at 1790 cm^{-1}
1197 hrs	Only peaks at 1782, 1747 and 1709 cm^{-1}

Table 4.3; FTIR spectrum observed when $Fe_4S_4(NO)_4$ is dissolved in DMF versus time

The results show that DMF effects the conversion of $Fe_4S_4(NO)_4$ to $[Fe_4S_3(NO)_7]^-$, however the conversion proceeds more slowly in DMF than in THF, i.e. 51 days compared to 40 days when THF was kept under N_2. However, no peaks were observed at 1724 cm^{-1} or at 1759 cm^{-1} in the FTIR spectrum, as was observed in THF and DMSO solution, but this may be due to the low resolution under which the spectra were recorded.

In summary, the solvents DMSO, THF and DMF all allow conversion of $Fe_4S_4(NO)_4$ to $[Fe_4S_3(NO)_7]^-$, while the solvents diethyl ether and DCM do not. The conversion of $Fe_4S_4(NO)_4$ occurs faster in THF than in either DMF or DMSO although no comparison can be made between the rates of conversion of $Fe_4S_4(NO)_4$ in DMF and DMSO as the former was carried out under nitrogen and the latter in the presence of air. That the conversion does not occur in DCM is probably due to the fact that DCM is a very poor coordinating solvent.

DMF, DMSO, THF and diethyl ether (see Figure 4.13) are all aprotic solvents and belong to the class of *nonionised but strongly solvating (generally polar) solvents*. They are Lewis bases and so will be attracted to a positive charge, thus enabling stabilisation of a positively charged counter-ion.

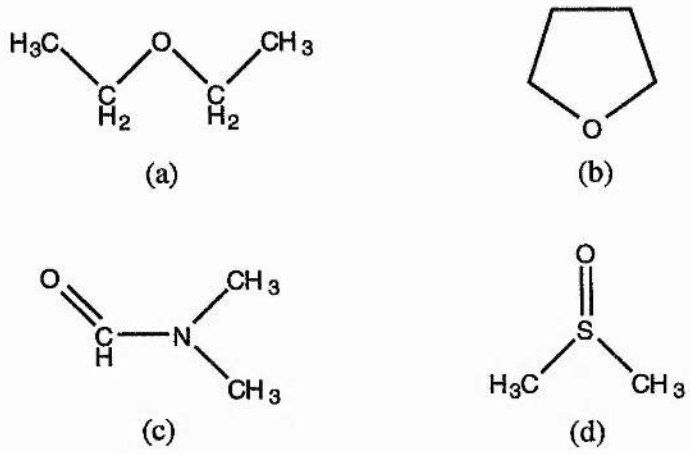


Figure 4.13; Chemical structure of (a) diethyl ether, (b) tetrahydrofuran (THF), (c) dimethylformamide (DMF), (d) dimethyl sulfoxide (DMSO)

One such property of these solvents is that they solvate cations very well; by orienting their negative ends around the cation and by donating unshared electron pairs to vacant orbitals of the cation (see Figure 4.14). This would allow stability of a cationic intermediate.

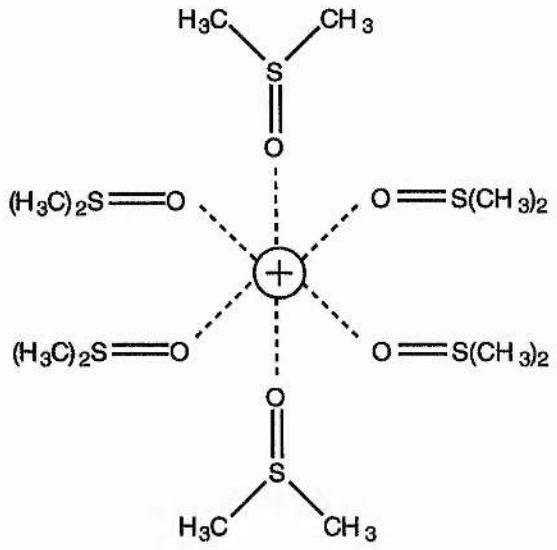


Figure 4.14; A cation solvated by molecules of the aprotic solvent dimethyl sulfoxide

Solvents intervene in chemical processes by producing species from solutes that are more reactive than if the solvent were not present. When a covalent solute is dissolved, two processes may occur; the solvation of molecules or the formation of ions. The formation of ions from covalent molecules is a measure of the ionising power of the solvent and this generally, although not always, correlates with the dielectric constant ε of the solvent (see Table 4.4). The π^* scale of solvent polarity[9] correlates

solvatochromatic effects on $p \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ electronic spectral transitions and is a measure of the dipolarity/polarizability of a given solvent (see Table 4.4). The extent to which a substance is ionised by neutral donor (solvent) molecules should increase with increasing stability of the cation resulting from nucleophilic attack compared with that of the unionised solute.

$$S: \quad + \quad X\text{-}Y \quad \rightarrow \quad S\text{-}X^+ \quad + \quad Y^-$$

The strength of the coordinate covalent bond formed is related to the donor ability of S, the acceptor ability in the species XY and the magnitude of solvent-solute interactions. The donor strengths expressed as donicity D*n* (see Table 4.4) of solvent molecules have been found relative to the reference acceptor $SbCl_5$; the enthalpy of the reaction between $SbCl_5$ and a series of donors in an inert medium is taken as a measure of donicity[10]. The donicity of a solvent has been interpreted as a measure of its donor strength, nucleophilicity, or Lewis base strength.

Solvent	D*n*	ε	π*
Diethyl ether	19.2	4.3	0.27
THF	20.0	7.6	0.58
DMF	26.6	36.1	0.88
DMSO	29.8	45.0	1.00

Table 4.4; Donicity (D*n*), dielectric constants (ε) and solvatochromatic parameters (π*) of selected solvents.

From the above table it can clearly be seen that diethyl ether has a lower value of donicity and a greatly reduced solvent dipolarity/polarizability than that of THF, DMF and DMSO which may explain why diethyl ether is unable to solvate an intermediate cationic species via the lone pair on the oxygen atom. Diethyl ether may simply not be polar enough to allow stabilisation of a potential cationic intermediate.

Diethyl ether may also experience more steric crowding than that of THF, DMF and DMSO due to the presence of straight-chain alkyl groups. DMF, DMSO and THF (containing a ring structure) may be able to arrange coordination around the cation easier than diethyl ether (see Figure 4.15). However this picture is rather oversimplified as it only shows the arrangement of solvent in one dimension but even in a three-

dimensional model, diethyl ether will experience more steric effects than THF, DMF and DMSO.

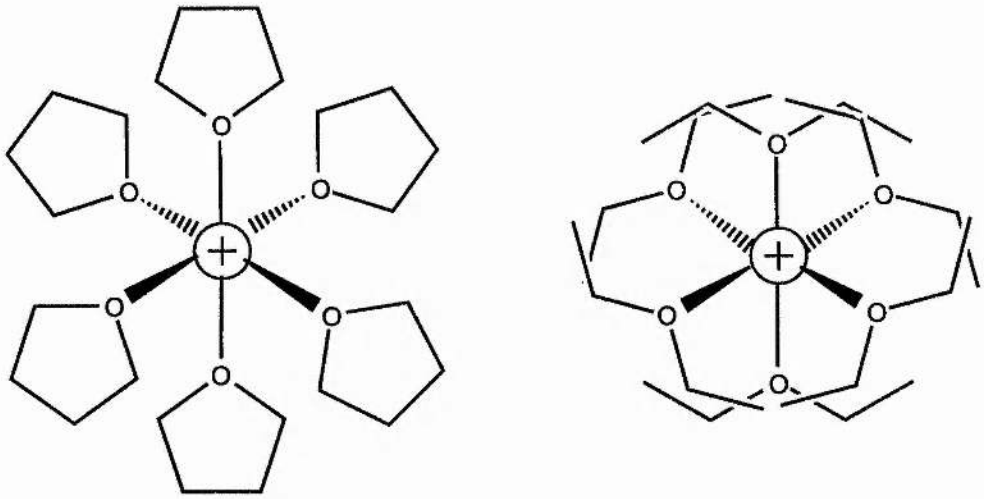
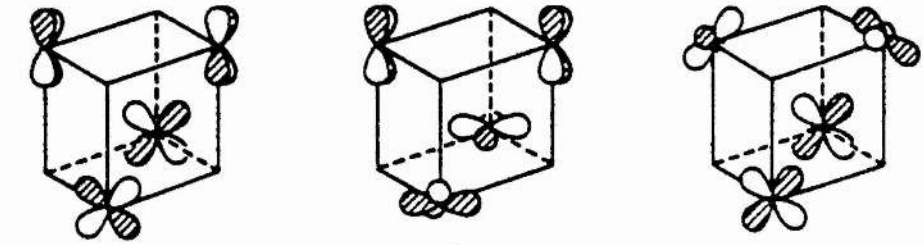


Figure 4.15; (a) Coordination of THF around the cation, (b) steric crowding following coordination of diethyl ether round cation

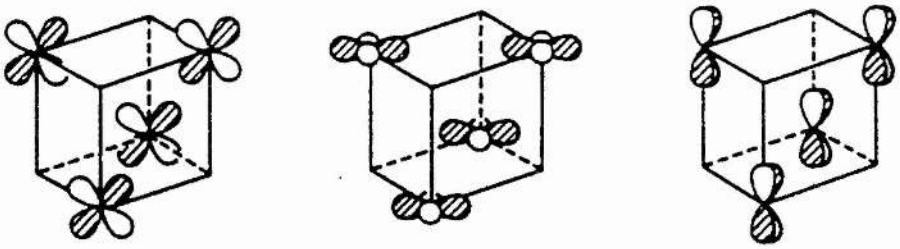
That the conversion of $Fe_4S_4(NO)_4$ to $[Fe_4S_3(NO)_7]^-$ occurred faster in THF than in DMF or DMSO may be that DMF and DMSO which contain a greater degree of dipolarity/polarizability form a more stable intermediate. The intermediate involving THF may be less stable and thus forms $[Fe_4S_3(NO)_7]^-$ more rapidly.

However evidence of an anionic iron-sulfur-nitrosyl intermediate is suggested following the work of Scott and Holm[11]. During a study of tetranuclear cuboidal and hexanuclear prismatic iron-sulfur-nitrosyl clusters, the solvated salt of $[Fe_4S_4(NO)_4]^-$ was isolated and identified by crystallography as $[Fe(DMF)_6][Fe_4S_4(NO)_4]_2$. The infra-red spectrum of the $[Fe_4S_4(NO)_4]^-$ anion exhibited υ(NO) peaks at 1787 and 1724 cm^{-1}. These υ(NO) values correlate well with those observed when $Fe_4S_4(NO)_4$ is dissolved in THF and DMSO at room temperature, suggesting formation of a $[Fe(solvent)_6][Fe_4S_4(NO)_4]_2$ complex. This strengthens the discussion about solvent effects as both THF and DMSO can coordinate to Fe(II) via the lone pair on their oxygen atoms. Diethyl ether, being less polar as well as containing larger side groups in its lower energy *trans* form would experience more steric interactions and thus would not form a stable $[Fe(solvent)_6]^{2+}$ cation. However no peaks were observed at 1724 cm^{-1} in DMF, despite the fact that it is a powerful donor solvent but this may be due to the fact that the FTIR spectra using DMF had to be recorded at a lower resolution due to a fault in the FTIR machine.

Qualitative calculations[7] on $Fe_4S_4(NO)_4$ using the extended Hückel molecular orbital (EHMO) method show that the highest occupied molecular orbital (HOMO) of t_2 type is nonbonding in the Fe_4 cage, while the LUMO of t_1 type is antibonding in Fe_4 (see Figure 4.16). Thus addition of electrons to $Fe_4S_4(NO)_4$, as in the case of the potential $[Fe(solvent)_6][Fe_4S_4(NO)_4]_2$ intermediate, should weaken the cluster bonding perhaps resulting in fragmentation to mononuclear paramagnetic species, which could then reassemble to give $[Fe_4S_3(NO)_7]^-$.



LUMO (T_1)



HOMO (T_2)

Figure 4.16; Principal contributing orbitals to the (a) LUMO (T_1) and (b) HOMO (T_2) of $Fe_4S_4(NO)_4$[7]

In DMSO and THF another peak was observed at 1757 cm^{-1} in the latter stages of the conversion. This may be due to a dinuclear species of the type $Fe_2(SR)_2(NO)_4$ which typically displays a characteristic pair of absorptions in the υ(NO) region[12]. That the peak is observed as a shoulder on the main $[Fe_4S_3(NO)_7]^-$ peak at 1741 cm^{-1} suggests that its sister peak may well be hidden beneath the main $[Fe_4S_3(NO)_7]^-$ peak. $Fe_2(SR)_2(NO)_4$ complexes can readily be formed[4] from a wide range of mononuclear

precursors of the type $[Fe(NO)_2(SR)_2]^-$ (R≠H) which further supports this observation. The peak at 1757 cm^{-1} is not easily observed in DMF solution but this may be due to the lower resolution which had to be used to record the FTIR spectra.

4.2.2 Attempted Isolation of Intermediate and $X[Fe_4S_3(NO)_7]$

Scott and Holm[11] reported formation of $[Fe(DMF)_6][Fe_4S_4(NO)_4]_2$ from $Fe_4S_4(NO)_4$ with PPh_3 in DMF solution with υ(NO) at 1787 and 1725 cm^{-1} and thus it follows that if a $[Fe(THF)_6][Fe_4S_4(NO)_4]_2$ intermediate species were formed from $Fe_4S_4(NO)_4$ in THF solution, then it could be isolated in a similar manner. However the paper published by Scott and Holm does not clearly state how the complex was isolated. The analogous cluster salt $[Fe(DMF)_6][Fe_6S_6(NO)_6]$ was precipitated by ether addition and so it was attempted to precipitate out the intermediate species formed from $Fe_4S_4(NO)_4$ in THF with diethyl ether. $Fe_4S_4(NO)_4$ was dissolved in THF solution and after five days FTIR analysis showed peaks due to $Fe_4S_4(NO)_4$ and $[Fe_4S_3(NO)_7]^-$ together with a band at 1724 cm^{-1}. However no indication of the complex was seen using FTIR spectroscopy after precipitation with diethyl ether. This is not surprising since the intermediate peak at 1724 cm^{-1} is also observed after ether extraction when $Fe_4S_4(NO)_4$ is dissolved in DMSO indicating that the intermediate species is soluble in diethyl ether.

Isolation of the intermediate was also attempted using chromatography. After five days a $Fe_4S_4(NO)_4$/THF mixture was evaporated to dryness. The solid was redissolved in the minimum volume of dichloromethane and eluted through a silica chromatography column. FTIR analysis demonstrated only the presence of cubane.

It was also attempted to duplicate the (rather vague) published method of Scott and Holm[11] in order to obtain an authentic sample of $[Fe(DMF)_6][Fe_4S_4(NO)_4]_2$ for comparative properties and for ESR analysis, but despite several attempts, the complex could not be isolated.

Attempts were also made to isolate the $X[Fe_4S_3(NO)_7]$ salt after conversion of $Fe_4S_4(NO)_4$ in THF either by immediate precipitation with petroleum ether or by crystallisation from aqueous solution, but met with no success.

4.2.3 ESR Study of $Fe_4S_4(NO)_4$ in a Range of Solvents

An ESR study of $Fe_4S_4(NO)_4$ in THF was made over time in an attempt to identify the intermediate species observed when using FTIR spectroscopy. Spectra were recorded at 2 hourly intervals over a time period of 4 days using capped tubes to contain the solution which had previously been exposed to air. A single peak was observed at g = 2.032 which is typical of a dinitrosyl $[Fe(NO)_2(X)_2]^{n+}$ species (where X could be the solvent THF). The peak integral was recorded at each time interval (see Table 4.5).

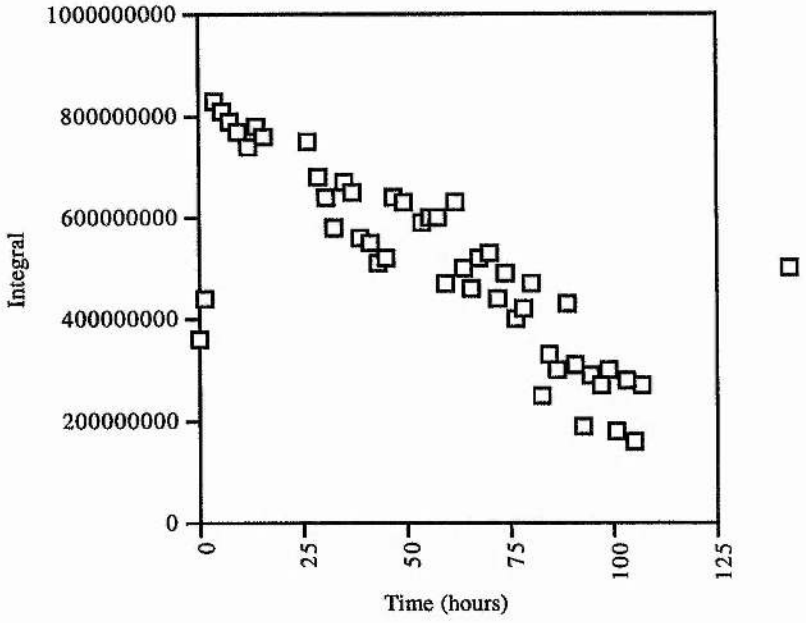
Time (hours)	Integral (x 10^8)
0	3.6
1.42	4.4
3.48	8.3
5.55	8.1
7.62	7.9
9.68	7.7
11.75	7.4
13.82	7.8
15.88	7.6
26.52	7.5
28.58	6.8
30.65	6.4
32.72	5.8
34.77	6.7
36.83	6.5

38.90	5.6
40.97	5.5
43.03	5.1
45.10	5.2
47.17	6.4
49.22	6.3
53.50	5.9
55.42	6.0
57.48	6.0
59.55	4.7
61.62	6.3
63.68	5.0
65.75	4.6
67.80	5.2
69.87	5.3
71.99	4.4
74.00	4.9
76.07	4.0
78.13	4.2
80.20	4.7
82.27	2.5
84.33	3.3

86.40	3.0
88.47	4.3
90.53	3.1
92.60	1.9
94.67	2.9
96.73	2.7
98.80	3.0
100.87	1.8
102.93	2.8
105.00	1.6
107.07	2.7

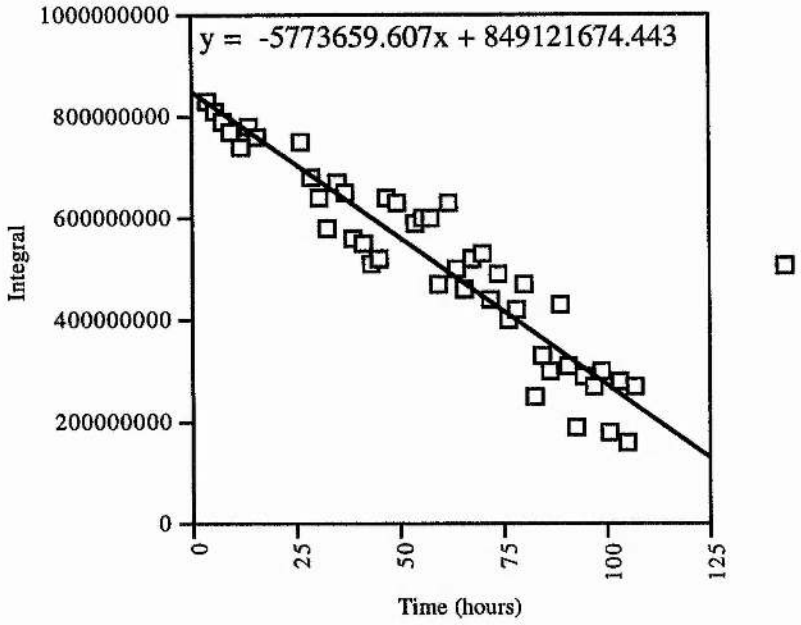
Table 4.5; Change in integral size of the ESR spectrum versus time when $Fe_4S_4(NO)_4$ is dissolved in THF

The peak integral was plotted against time (see Graph 4.1) to give a graph which demonstrated the initial rapid growth of the peak over a period of 3 hours followed by its gradual decay over the next 4 days.



Graph 4.1; Graph of integral versus time observed when studying ESR spectrum of $Fe_4S_4(NO)_4$ in THF

The decay of the peak only (i.e. integral size vs time after the initial 3 hours) was plotted against time (see Graph 4.2) from which the straight line equation $y = -5.77x10^6x + 8.48x10^8$ could be calculated.



Graph 4.2; Graph of decay integral versus time observed when studying ESR spectrum of $Fe_4S_4(NO)_4$ in THF

The rate of a zero-order reaction is independent of the concentration of reactant and so the rate can be expressed as $k[A]^o$ and since $[A]^o = 1$, then rate = k. The differential form of the rate equation for a zero-order rate equation is

$$-d[A]/dt = k \qquad (1)$$

which can be converted into

$$[A] = -kt + [A]^o \qquad (2)$$

Comparison of equation (2) with the equation derived from Graph 4.2 for the straight line y = -mx + c, and assuming that the integral size is directly related to the concentration of the species responsible for the peak, shows that the plot of integral size against t corresponds to a zero-order reaction.

A more intense ESR spectrum was observed when $Fe_4S_4(NO)_4$ was dissolved in the more polar coordinating solvent DMSO. A single broad peak at g = 2.032, typical of a $[Fe(NO)_2X_2]^{n+}$ species, was similarly observed where X could be DMSO (see Figure 4.16). A very small peak was also observed at g = 4.583 which can be assigned to the iron(II)/(III) oxidation states.

$Fe_4S_4(NO)_4$ was then dissolved in toluene, a non-coordinating solvent, and irradiated with UV light. This resulted in an ESR spectrum displaying a singlet at g = 2.033 and a triplet at g = 2.039 (see Figure 4.17). The singlet again, is typical of a $[Fe(NO)_2X_2]^{n+}$ type species but the triplet remains at present unidentified. On prolonged irradiation the singlet was observed to grow at the expense of the triplet, so it would appear that the unknown triplet species, which may be an excited product due to UV irradiation, is then forming the $[Fe(NO)_2(X)_2]^{n+}$ species.

Potassium superoxide, which is an electron donor, was added to a solution of $Fe_4S_4(NO)_4$ in toluene. The ESR spectrum (see Figure 4.18) showed a singlet and a triplet at g = 2.035 and g = 2.021 respectively. However the singlet is rather broad and somewhat displaced from previous spectra displaying $[Fe(NO)_2X_2]^{n+}$ species, but this may be due to the presence of potassium superoxide and $[Fe(NO)_2X_2]^{n+}$ may well be present. However potassium superoxide, being a one-electron donator may donate an electron to $Fe_4S_4(NO)_4$ forming the anion $[Fe_4S_4(NO)_4]^-$. The symmetry of $[Fe_4S_4(NO)_4]^-$ in the $[K(2,2,2\text{-crypt})]^+$ salt[13] is D_{2d} in which all the nitrosyl environments are identical. This in theory would give rise to a nine line spectrum. However the A values are likely to be very small as the single unpaired electron is contained in a relatively large cage structure and so a broad singlet is more likely to be observed. The broad singlet actually observed at g = 2.035 may be due to both the $[Fe_4S_4(NO)_4]^-$ and the $[Fe(NO)_2(X)_2]^{n+}$ species. The triplet is also not very well resolved so there may be other peaks present in addition to the unidentified triplet, perhaps a pair of triplets.

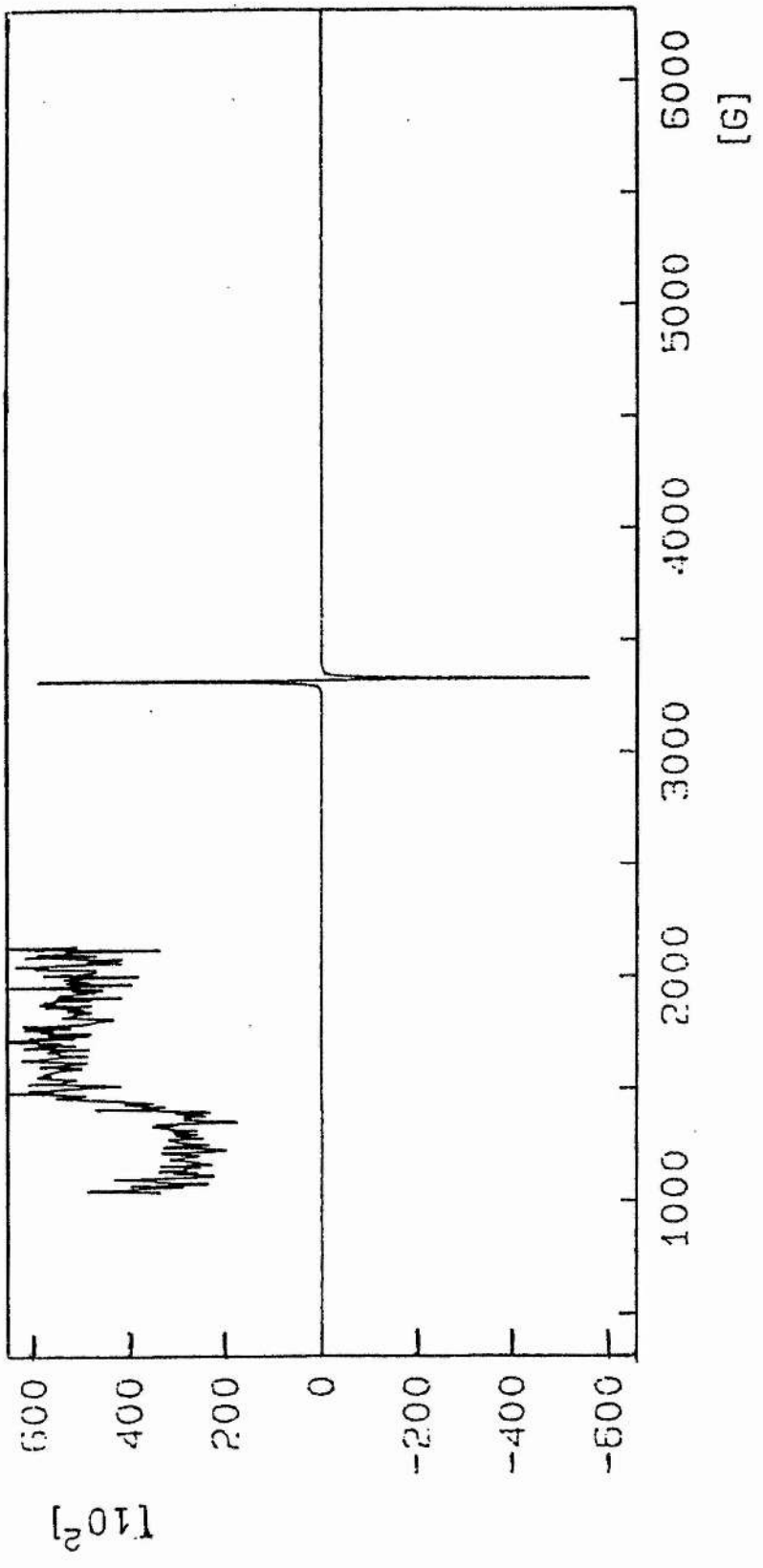


Figure 4.16; ESR spectrum of $Fe_4S_4(NO)_4$ in DMSO

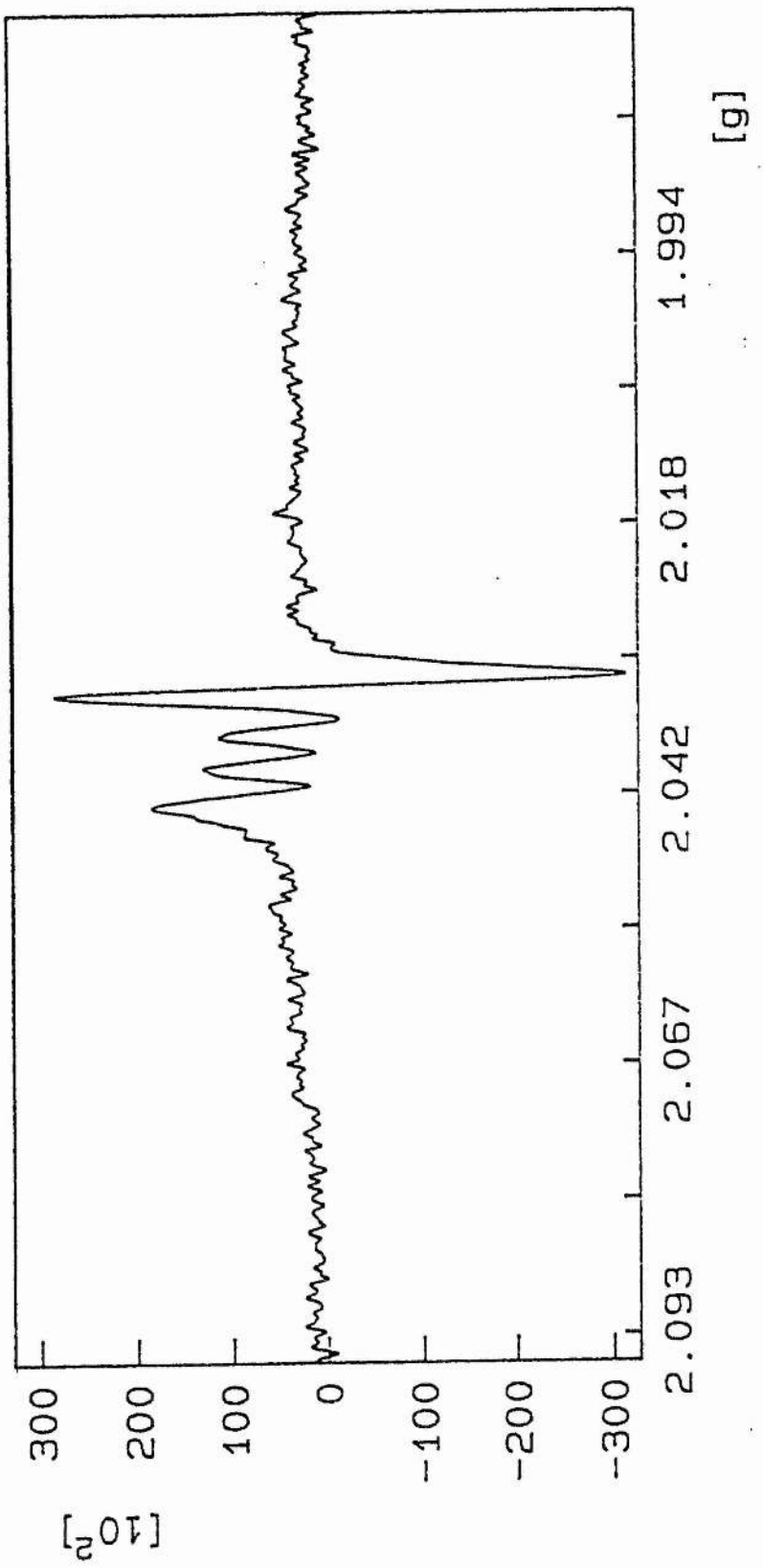


Figure 4.17; ESR spectrum of $Fe_4S_4(NO)_4$ in toluene irradiated with UV light

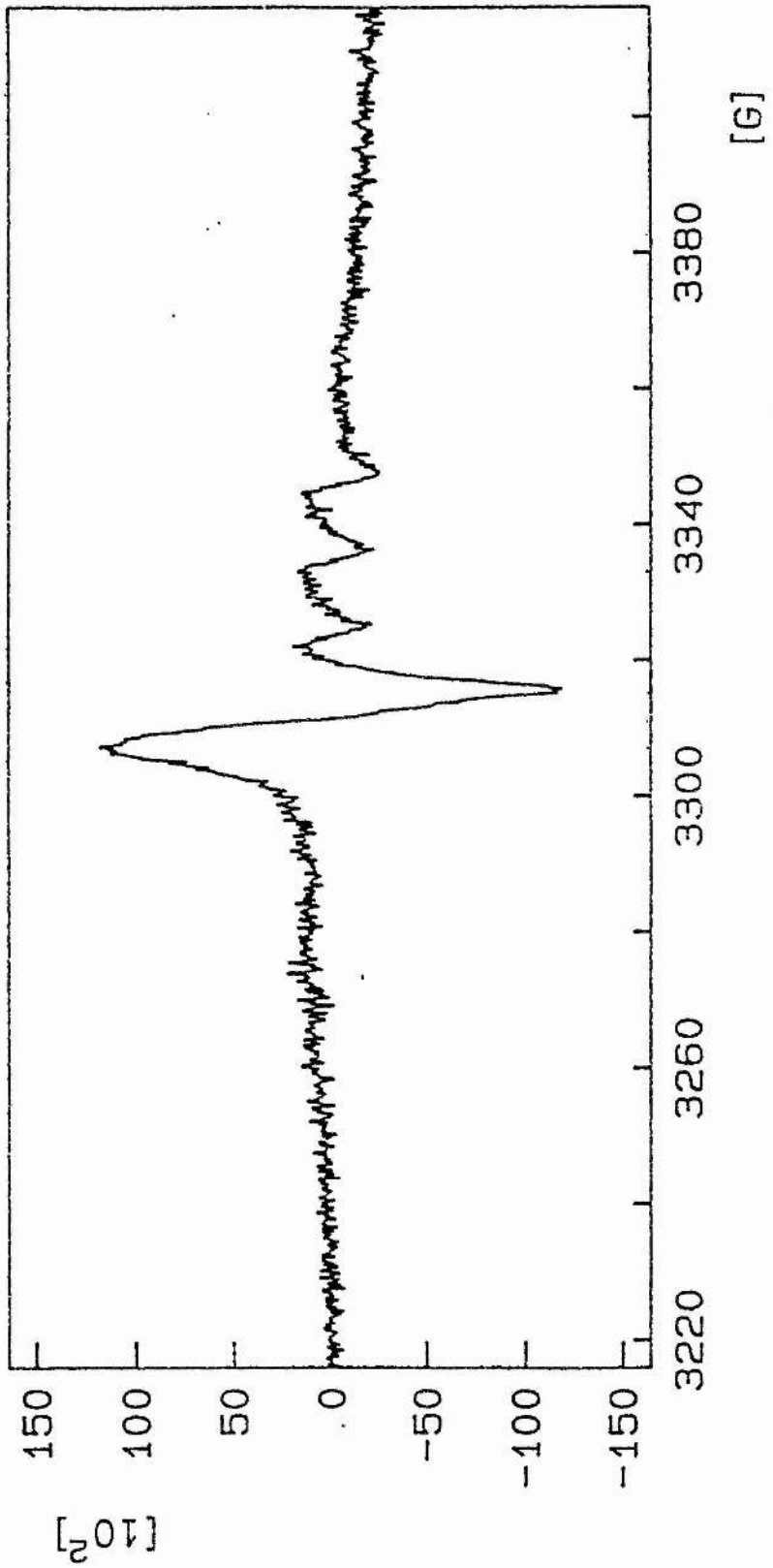


Figure 4.18; ESR spectrum of $Fe_4S_4(NO)_4$ and potassium superoxide in toluene

4.2.4 Discussion

When $Fe_4S_4(NO)_4$ is dissolved in the polar coordinating solvents THF, DMSO and DMF; FTIR spectroscopy demonstrates conversion to $[Fe_4S_3(NO)_7]^-$. An intermediate peak is observed during this conversion pathway which is postulated to be $[Fe(solvent)_6][Fe_4S_4(NO)_4]_2$. However ESR analysis of $Fe_4S_4(NO)_4$ in both THF and DMSO shows formation of a complex of type $[Fe(NO)_2(X)_2]^{n+}$ as the predominant species observed. Thus mononuclear species appear to be favoured in the dilute solutions required for ESR analysis and polynuclear species in the more concentrated solutions needed for FTIR analysis.

mononuclear complexes $\underset{\text{dilute solution}}{\overset{\text{concentrated solution}}{\rightleftharpoons}}$ polynuclear complexes

Therefore both mononuclear and polynuclear intermediate species are formed in the conversion of $Fe_4S_4(NO)_4$ to $[Fe_4S_3(NO)_7]^-$, but the predominant species observed is dependent on the concentration of the solution. As the solution becomes more concentrated, the equilibrium will shift according to Le Chatelier's principle to minimise the effect of increasing concentration by forming polynuclear complexes and vice versa.

FTIR analysis also suggested formation of a dinuclear complex of type $Fe_2(SR)_2(NO)_4$ in the later stages of the conversion which would be consistent with the already known synthesis[4] of $Fe_2(SR)_2(NO)_4$ from mononuclear precursors of the type $[Fe_2(NO)_2(SR)_2]^-$. That the peak responsible for $Fe_2(SR)_2(NO)_4$ in THF does not persist throughout the entire time period studied is because $Fe_2(SR)_2(NO)_4$ is unstable in solution.

4.3 Experimental

FTIR spectra were recorded in a variety of solvents using 0.2 mm pathlength cells with a Perkin-Elmer Model 1710 FTIR spectrophotometer. ^{14}N NMR spectra were recorded at 21.638 MHz in acetone solution, at ambient temperature, relative to 50% CH_2NO_2 in acetone, using a Brucker AM-300 spectrophotometer. ESR spectra were recorded by Sheila Glidewell using a Brucker ESP300 spectrophotometer in a variety of solvents using capped tubes with an inside diameter of 2.8 mm.

4.3.1 Preparation of Sodium Heptanitrosyl-tri-(μ_3-thio)-tetrairon(1-), $Na[Fe_4S_3(NO)_7]$

Sodium nitrite (8.0 g, 116 mmol) and sodium sulfide nonahydrate (11.3 g, 47 mmol) were dissolved in distilled deoxygenated water (160 cm^3) and heated to boiling under nitrogen. A solution of iron(II) sulfate heptahydrate (20.0 g, 72 mmol) in deoxygenated water (80 cm^3) was added followed immediately by 20% aqueous ammonia (20 cm^3). The resulting thick black mixture was filtered through preheated Hyflo before cooling in ice/water. The crude product was filtered off and recrystallised from distilled water to give a shiny black crystalline solid.

IR (THF)	υ(NO)	1795 cm^{-1} (w)	1742 cm^{-1} (vs)	1707 cm^{-1} (m)	
^{14}N NMR (Acetone)	∂_N	77 ppm	38.5 ppm	11 ppm	(broad)

TLC Gave a single brown spot moving with the solvent front in methanol.

4.3.2 Preparation of Tetranitrosyl-tetra-(μ3-thio)-tetrahedro-tetrairon, $Fe_4S_4(NO)_4$

$Na[Fe_4S_3(NO)_7]$ (0.30 g, 0.64 mmol) and elemental sulfur (0.50 g, 1.95 mmol) were heated under reflux in dry toluene (50 cm^3) for 16 hours under nitrogen. The solvent was evaporated off and the product purified by chromatography on a 30 cm x 1 cm (dia) silica column, eluting with dichloromethane. Evaporation of the solvent yielded shiny black crystals (0.31 g, 17%).

IR (DCM)	υ(NO)	1790 cm^{-1}

TLC Gave a single black spot moving with the solvent front in dichloromethane

4.3.3 FTIR Study of $Fe_4S_4(NO)_4$ in DMSO

$Fe_4S_4(NO)_4$ (0.11 g, 0.23 mmol) was dissolved in DMSO (20 cm^3). At 24 hour intervals 3 cm^3 aliquots of the solution were quenched in H_2O (100 cm^3) and left for 15 minutes. The solution was exhaustively extracted with diethyl ether. The combined ether extracts were washed with water and dried over magnesium sulfate before evaporating to dryness.

4.3.4 FTIR Study of $Fe_4S_4(NO)_4$ in THF

$Fe_4S_4(NO)_4$ (0.2 g, 0.42 mmol) was dissolved in THF (25 cm^3) which had been dried over molecular sieves and sealed under nitrogen. The solution was exposed to air during the removal of samples for FTIR analysis before being resealed under nitrogen.

$Fe_4S_4(NO)_4$ (0.4 g, 0.84 mmol) was dissolved in sodium-dried THF (50 cm^3) under nitrogen. Samples were removed for direct FTIR analysis via a suba seal at regular intervals.

A similar study was made using a solution of $Fe_4S_4(NO)_4$ (0.2 g, 0.42 mmol) in sodium-dried THF (25 cm^3) which was exposed to air.

4.3.5 FTIR Study of $Fe_4S_4(NO)_4$ in Diethyl Ether

$Fe_4S_4(NO)_4$ (0.2 g, 0.42 mmol) was dissolved in sodium-dried ether (25 cm^3) under nitrogen. Samples were removed for direct FTIR analysis via a suba seal at regular intervals.

4.3.6 FTIR Study of $Fe_4S_4(NO)_4$ in DCM

$Fe_4S_4(NO)_4$ (0.2 g, 0.42 mmol) was dissolved in DCM (25 cm^3), which had previously been dried over molecular sieves, under nitrogen. Samples were removed for direct FTIR analysis via a suba seal at regular intervals.

4.3.7 FTIR Study of $Fe_4S_4(NO)_4$ in DMF

$Fe_4S_4(NO)_4$ (0.40 g, 0.84 mmol) was dissolved in dry, distilled DMF (50 cm^3) under nitrogen. At regular intervals 3 cm^3 aliquots were removed via a suba seal and quenched in H_2O (100 cm^3) for 15 minutes. The solution was exhaustively extracted with diethyl ether. The combined ether extracts were washed with water and dried over magnesium sulfate before evaporating to dryness.

4.3.8 Attempted Isolation of Intermediate in the Conversion of $Fe_4S_4(NO)_4$ to $[Fe_4S_3(NO)_7]^-$ in THF

$Fe_4S_4(NO)_4$ (0.1 g, 0.21 mmol) was dissolved in sodium-dried THF (25 cm^3) under nitrogen. After 5 days, FTIR analysis showed peaks at 1786, 1741 and 1725 cm^{-1} which can be assigned to $Fe_4S_4(NO)_4$, $[Fe_4S_3(NO)_7]^-$ and the intermediate respectively. The solution was filtered and evaporated to dryness. Routes (a) and (b) were then followed.

(a) Attempted Precipitation using Diethyl Ether

The solid was redissolved in the minimum volume of sodium-dried THF to which sodium-dried diethyl ether (100 cm^3) was added dropwise and the mixture refrigerated for 16 hours. The mixture was filtered and washed with ether. However any solid which may have formed could not be scraped back off the sintered funnel. Washing the sinter with dichloromethane produced a mildly yellow coloured extract which was evaporated to small volume. No IR peaks were detected in the nitrosyl stretching region.

(b) Attempted Separation from $[Fe_4S_3(NO)_7]^-$

The solid was redissolved in the minimum volume of dichloromethane and eluted through a silica chromatography column. Evaporation to dryness yielded a silvery black coloured solid. However FTIR analysis in dichloromethane showed only a single peak at 1790 cm^{-1} due to $Fe_4S_4(NO)_4$.

4.3.9 Attempted Preparation of $[Fe(DMF)_6][Fe_4S_4(NO)_4]_2$

$Fe_4S_4(NO)_4$ (0.36 g, 0.76 mmol) and triphenylphosphine (0.40 g, 1.52 mmol) were dissolved in dry DMF (20 cm^3) and stirred at 80°C under nitrogen for 12 hours. After cooling, the mixture was filtered and sodium-dried ether (300 cm^3) was added dropwise. However, no precipitate formed even after placing in the refrigerator for 24 hours.

4.3.10 Attempted Isolation of $X[Fe_4S_4(NO)_7]$ from $Fe_4S_4(NO)_4$ in THF

$Fe_4S_4(NO)_4$ (0.2 g, 0.42 mmol) was dissolved in sodium-dried THF (50 cm^3) under nitrogen and left until all of the $Fe_4S_4(NO)_4$ had converted to $[Fe_4S_3(NO)_7]^-$. Routes (a) and (b) were then followed.

(a) Attempted Precipitation of $X[Fe_4S_3(NO)_7]$ using Petroleum Ether

The solution was filtered and the volume reduced using a rotary evaporator. A large excess of petroleum ether (40/60) was added in an attempt to precipitate out the salt. However this resulted in the immediate precipitation of an iron oxide decomposition product.

(b) Attempted Recrystallisation of $X[Fe_4S_3(NO)_7]$ from Aqueous Solution

The solution was filtered through hyflo and evaporated to dryness to yield a black solid which slowly turned brown when left overnight on a vacuum line. The solid was sparingly dissolved in the minimum volume of boiling distilled water, filtered off and left to crystallise. No crystals formed.

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CHAPTER FIVE

TABLE OF CONTENTS

SUMMARY

5.1 Formation of Iron-Sulfur-Nitrosyl Complexes from Dietary Components and the Effect of pH

Sodium and potassium nitrites are employed as preservatives in meat to prevent the growth of the bacterium, *Clostridium botulinum*, as well as to provide an attractive colour. As yet, studies[1,2] of the effect of nitrite on the culture of *Clostridial* species in growth media and in meat have failed to show the mechanism of inhibition. A clue as to this mode of action comes from the Perigo effect[3] - the greater, but almost pH independent inhibition of growth found after heating in a laboratory medium, even with no residual nitrite present. Recent work[4] based on the suggestion that iron salts and a sulfur source are required for Perigo-type inhibition has shown that the reaction of cysteine with iron(II) sulfate heptahydrate and sodium nitrite in aqueous solution yields $Na[Fe_4S_3(NO)_7]$, known to be a wide-spectrum anti-bacterial agent. The yield of $Na[Fe_4S_3(NO)_7]$ isolated is increased when sodium ascorbate is also present. However it has also been shown[5] that similar reactions carried out in the presence of methionine yields the dinuclear iron-nitrosyl complex, $Fe_2(SMe)_2(NO)_4$, known to promote the tumorigenic activity of environmental carcinogens such as nitrosamines and the polyaromatic hydrocarbons found, for example, in cigarette smoke.

All of the components in the above reactions are present in the normal human diet, and the problems addressed as part of my PhD research were: can iron(III) which is also present in the human diet mimic iron(II) in these reactions; can nitrate (present in drinking water and in the human diet) mimic the action of nitrite; or are all the above components necessary for formation of bioactive iron-nitrosyl complexes $Na[Fe_4S_3(NO)_7]$ and $Fe_2(SMe)_2(NO)_4$?

It was found that substitution of iron(III) for iron(II) in the absence of sodium ascorbate did not significantly alter the yield of $Na[Fe_4S_3(NO)_7]$ isolated. However substitution of iron(III) for iron(II) in the presence of sodium ascorbate resulted in a large decrease in yield of $Na[Fe_4S_3(NO)_7]$, similar in fact to that obtained for both the iron(II) and iron(III) reactions in the absence of abscorbate. Thus the absence of a reducing agent may allow oxidation of iron(II) to iron(III) by nitrite. The reduced yield of $Na[Fe_4S_3(NO)_7]$ observed in the presence of iron(III) salts may be attributed to the iron(III) catalysed oxidation of cysteine to the disulfide form, cystine and/or to the greater reduction required to reduce iron(III) to the very low iron oxidation states present in $Na[Fe_4S_3(NO)_7]$. Similarly substitution of iron(III) for iron(II) in the reaction of methionine with iron(II) salts and nitrite in the presence of sodium ascorbate

resulted in reduced yield of $Fe_2(SMe)_2(NO)_4$. No iron-sulfur-nitrosyl complexes were detected if nitrate was substitued for nitrite in either of the cysteine or methionine reactions. Similarly no iron-sulfur-nitrosyl complexes were formed if either sodium nitrite or iron(II) were omitted in the reaction of methionine with iron(II) salts and nitrite in the presence of sodium ascorbate.

The effect of pH, an important variable both in foodstuffs and in the human digestive system, on the formation of iron-nitrosyl complexes $Na[Fe_4S_3(NO)_7]$ and $Fe_2(SMe)_2(NO)_4$ was so far unknown, and hence a series of reactions of sodium nitrite with cysteine and methionine in the presence of iron(II) salts at various pH was investigated, under heating conditions relevant to food processing.

In the presence of sodium ascorbate the yield of $Na[Fe_4S_3(NO)_7]$ isolated from the cysteine reactions fell slightly with decreasing pH. Protonation of sodium ascorbate to form the weaker reducing agent ascorbic acid would account for this observation. However at very low pH, both cysteine reactions with and without sodium ascorbate present did not yield $Na[Fe_4S_3(NO)_7]$ which may be due to the formation of nitric oxide from the action of sulfuric acid with nitrite in the presence of an iron(II) salt, and also to the instability of $Na[Fe_4S_3(NO)_7]$ itself which was demonstrated at acidic conditions. The dinuclear complex $Fe_2(SMe)_2(NO)_4$ was shown to be very stable to conditions of low pH, but its formation at very acidic conditions was severely hindered, again due to the formation of nitric oxide from nitrite. Perhaps instead of using sulfuric acid solutions to control the pH in these reactions, an attempt to find a suitable buffer medium which would not cause chelation of iron from either the reaction materials or the product should be made.

In conclusion, Chapter Two has demonstrated the formation of the iron-sulfur-nitrosyl clusters, $Na[Fe_4S_3(NO)_7]$ and $Fe_2(SMe)_2(NO)_4$ from a very wide range of precursors including iron(III) salts and nitrate, which both can commonly be found in food products. The effect of pH, an important variable in foodstuffs, has shown that at the acidity levels typically found in foodstuffs, the yields of the tetranuclear cluster, $Na[Fe_4S_3(NO)_7]$ and the neutral dinuclear complex, $Fe_2(SMe)_2(NO)_4$ from the reactions of cysteine or methionine respectively with iron(II) salts and nitrite with sodium ascorbate can approach quantitative capture of the nitrite.

5.2 Iron Complexes with Sulfur Containing Amino Acids and their Reactions with Nitrite

Recent work[4,5] has demonstrated the formation of $Na[Fe_4S_3(NO)_7]$ and $Fe_2(SMe)_2(NO)_4$ from reaction of the sulfur containing amino acids, cysteine and methionine respectively, with iron(II) salts and nitrite. However the mechanism by which these reactions proceed is as yet unknown and a possible explanation may be via an iron-amino acid intermediate. Following an extensive literature survey on iron complexes with sulfur containing amino acids, several of the more promising synthetic procedures were selected[6-9]. These included complexes of the type $Fe(met)_3$, $Fe(met)(OH)X,2CH_3OH$ and $[Fe_3O(\text{amino acid})_6(H_2O)_3]X_7$. That the complexes chosen all contained iron(III) was due to their increased stability in air, in contrast to the iron(II) complexes reported which were largely air sensitive.

However attempts to synthesise the iron-amino acid complexes of the type $Fe(met)(OH)X,2CH_3OH$ and $Fe(met)_3$ using the published methods[8,9] were unsuccessful, shedding some doubt on the credibility of some of the rather dated literature on iron-sulfur containing amino acid complexes.

Greater success was achieved during the synthesis of a range of iron-sulfur containing amino acid complexes of the type $[Fe_3O(\text{amino acid})_6(H_2O)_3]X_7$, using the amino acids methionine and methylcysteine with iron(III) nitrate or chloride salts. The preformed complexes were studied in the presence of nitrite under different heating conditions. Thus in the presence of sodium ascorbate, when the amino acid is methylcysteine, both $[Fe_4S_3(NO)_7]^-$ and $Fe_2(SMe)_2(NO)_4$ were isolated after reaction of the preformed complex with nitrite. That $[Fe_4S_3(NO)_7]^-$ was observed using FTIR spectroscopy in dichloromethane demonstrated that the cation is not Na^+, but must be largely organic. However no identifiable iron-sulfur-nitrosyl complexes could be detected when the amino acid was methionine but this may be due to the insensitivity of the FTIR instrument. In the absence of sodium ascorbate, both the preformed methylcysteine and methionine complexes when reacted with nitrite yielded a range of complexes which infra-red spectroscopy demonstrated absorbed strongly in the $\upsilon(NO)$ stretching region. The reaction products were shown to be independent of the heating conditons and of the nature of X (where X = Cl or NO_3) in the starting complex. Due to the absence of any organic peaks, these complexes are believed to be inorganic nitrosyl complexes. To establish whether the peaks observed are indeed those due to an inorganic nitrosyl complex, the reactions of the preformed iron-amino acid complexes could be repeated using ^{15}N labelled $NaNO_2$ as FTIR spectroscopy would demonstrate a shift of the $\upsilon(^{15}NO)$ peaks to a region of lower frequency.

Thus Chapter Three has demonstrated that iron-sulfur containing amino acid complexes of the type $[Fe_3O(\text{amino acid})_6(H_2O)_3]X_7$ may be intermediates during the formation of iron-sulfur-nitrosyl complexes from the reaction of amino acids with iron(II)/(III) salts and nitrite in the presence of sodium ascorbate. That complexes of the type $[Fe_3O(\text{amino acid})_6(H_2O)_3]X_7$ are readily formed simply by mixing the appropriate amino acid with an iron(III) salt in aqueous solution would support this observation. However the trinuclear complexes are unstable at high temperatures and may simply decompose to give the free amino acid on heating.

5.3 Conversion of $Fe_4S_4(NO)_4$ to $[Fe_4S_3(NO)_7]^-$; an FTIR and ESR Study

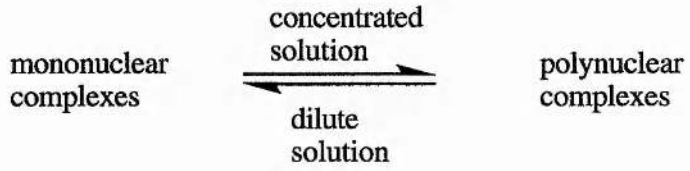
FTIR spectroscopy has demonstrated the conversion of the cubane type iron-sulfur-nitrosyl cluster, $Fe_4S_4(NO)_4$ to $[Fe_4S_3(NO)_7]^-$ in DMSO. FTIR and ESR studies of the conversion were carried out in a range of organic solvents in an attempt to elucidate the conversion pathway. That both $Fe_4S_4(NO)_4$ and $[Fe_4S_3(NO)_7]^-$ can readily be formed from a wide range of mononuclear precursors[10-14] suggests that the interconversion may occur via a mechanism of extensive fragmentation and reassembly.

When $Fe_4S_4(NO)_4$ was dissolved in the polar coordinating solvents THF, DMSO and DMF; FTIR spectroscopy demonstrated conversion to $[Fe_4S_3(NO)_7]^-$. No such conversion was observed in diethyl ether or dichloromethane. When the conversion was followed in THF and DMSO, an intermediate peak was clearly obseved at 1724 cm^{-1} in addition to those peaks responsible for $Fe_4S_4(NO)_4$ (1785 cm^{-1}) and $[Fe_4S_3(NO)_7]^-$ (1793, 1741 and 1707 cm^{-1}). Scott and Holm[15] had reported υ(NO) for the complex $[Fe(DMF)_6][Fe_4S_4(NO)_4]_2$, at 1787 and 1724 cm^{-1}. This suggested that a similar solvated complex of the type $[Fe(solvent)_6][Fe_4S_4(NO)_4]_2$ was responsible for the intermediate peak observed at 1724 cm^{-1} during the conversion of $Fe_4S_4(NO)_4$ to $[Fe_4S_3(NO)_7]^-$. The conversion occured at a faster rate in THF than in DMF and DMSO and this was postulated to be due to the formation of a less stable intermediate species with THF, which does not contain as strong coordinating properties as DMF and DMSO. Another peak at 1757 cm^{-1} was observed during the latter stages of the conversion which may be due to the formation of a dinuclear complex of the type $Fe_2(SR)_2(NO)_4$. However, this was not believed to be an intermediate species.

ESR spectroscopy of $Fe_4S_4(NO)_4$ in both THF and DMSO demonstrated a single peak at g = 2.032, typical of a mononuclear $[Fe(NO)_2(X)_2]^{n+}$ species where X could be the solvent. Measurement of the peak integral size indicated that after the rapid initial growth period, a zero order of decay was followed over the remaining 4 days.

Evidence of the mononuclear complex, $[Fe(NO)_2(X)_2]^{n+}$ (g = 2.033) was also obtained using ESR spectroscopy when a solution of $Fe_4S_4(NO)_4$ in the non-coordinating solvent toluene was irradiated with UV light. However a triplet at g = 2.039 was also observed which may be due to an excited species, but on prolonged irradiation the mononuclear complex was seen to grow at the expense of the triplet suggesting that the $[Fe_2(NO)_2(X)_2]^{n+}$ complex was formed from the excited triplet species. Addition of potassium superoxide to a solution of $Fe_4S_4(NO)_4$ in toluene led to a very poorly resolved singlet and a triplet at g = 2.035 and 2.021 respectively. The singlet is believed to be due to a mixture of $[Fe_4S_4(NO)_4]^-$ and $[Fe(NO)_2(X)_2]^{n+}$. The triplet remains at present unidentified but due to the very poor resolution imposed by the presence of potassium superoxide may contain more than one triplet species.

Thus mononuclear species appear to be favoured in the dilute solutions required for ESR analysis and polynuclear species in the more concentrated solutions needed for FTIR analysis.



Attempts to isolate the intermediate species during FTIR analysis via precipitation or chromatography techniques proved unsuccessful. Also, preparation of an authentic sample of $[Fe(DMF)_6][Fe_4S_4(NO)_4]_2$ for comparative properties according to the method of Scott and Holm[15] could not be duplicated. Isolation of $X[Fe_4S_3(NO)_7]$, in order to determine the cation present, by precipitation from petroleum ether or by recrystallisation from water again met with no success.

Chapter Four has therefore provided a step forward in the elucidation of the reaction pathway during the spontaneous conversion of $[Fe_4S_4(NO)_4]$ to $[Fe_4S_3(NO)_7]^-$ in polar coordinating solvents.

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APPENDIX I

Electrode Reaction	E^o/V
$NO_3^- + H^+ + 2e^- \rightarrow NO_2^- + H_2O$	+0.835
$Fe^{3+} + e^- \rightarrow Fe^{2+}$	+0.771
cystine + $2H^+ + 2e^- \rightarrow$ 2cysteine	-0.22*

* $E^{o'}$ (at pH 7 and 30°C)

Reduction of Nitrate by Cysteine

(a) $NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$ $\quad Eo = +0.835$ V

$\Delta G^o = -nFE^o$

$= -$ (2 mol) x (96485 C mol^{-1}) x (0.835 V)

$= (-1.6113 \times 10^5)$ J mol^{-1}

$Q = 1/[H^+]^2$

At pH 7 and 30°C, $[H^+] = 10^{-7}$ M

$\Delta G = \Delta G^o + RT\ln Q$

$= (-1.6113 \times 10^5) + [(8.314 \text{ J } K^{-1} \text{ mol}^{-1}) \times (303 \text{ K}) \times (2.\ln 10^7)$

$= (-6.832 \times 10^4)$ J mol^{-1}

(b) Cystine + $2H^+ + 2e^- \rightarrow$ 2cysteine $\quad E^{o'} = -0.22$ V

where $E^{o'}$ is value obtained at pH 7 and 30°C

$\Delta G^{o'} = -nFE^{o'}$

$= -$ (2 mol) x (96485 C mol^{-1}) x (-0.22 V)

$= (4.245 \times 10^4)$ J mol^{-1}

Required reaction is

(a) $NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$

(-b) $2\text{cysteine} \rightarrow \text{cystine} + 2H^+ + 2e^-$

(c) $2\text{cysteine} + NO_3^- \rightarrow \text{cystine} + NO_3^- \; H_2O$

$\Delta G(c) = \Delta G(a) - \Delta G(b)$

$= (-6.832 \times 10^4 \text{ J mol}^{-1}) - (4.245 \times 10^4 \text{ J mol}^{-1})$

$= -1.108 \times 10^5 \text{ J mol}^{-1}$

$= -110.8 \text{ kJ mol}^{-1}$

Reduction of Nitrate by Iron(II)

(a) $NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$ $\quad E^o = +0.835$ V

$\Delta G = (-6.832 \times 10^4) \text{ Jmol}^{-1}$

(b) $Fe^{3+} + e^- \rightarrow Fe^{2+}$ $\quad E^o = +0.771$ V

$\Delta G^o = -nFE^o = \Delta G$

$= -(1 \text{ mol}) \times (96485 \text{ C mol}^{-1}) \times (0.771 \text{ V})$

$= (-7.439 \times 10^4) \text{ J mol}^{-1}$

Required reaction is

(a) $NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$

(-2b) $2Fe^{2+} \rightarrow 2Fe^{3+} + 2e^-$

(c) $2Fe^{2+} + NO_3^- + 2H^+ \rightarrow 2Fe^{3+} + NO_2^- + H_2O$

$\Delta G(c) = \Delta G(a) - 2\Delta G(b)$

$= (-6.832 \times 10^4 \text{ J mol}^{-1}) - (2 \times -7.439 \times 10^4 \text{ J mol}^{-1})$

$= 1.488 \times 10^5 \text{ J mol}^{-1}$

$= 148.8 \text{ kJ mol}^{-1}$

APPENDIX II

The postgraduate lecture courses passed during the first and second year of my PhD were;

Year One

Clusters - C. Glidewell

Ligand Design - R. W. Hay

Organic Synthesis - D. Gani

Year Two

Advanced N.M.R. - F. Riddell

Advanced Spectroscopic Problems in Inorganic Chemistry - J. A. Crayston

Recent Developments in Inorganic Chemistry - D. T. Richens