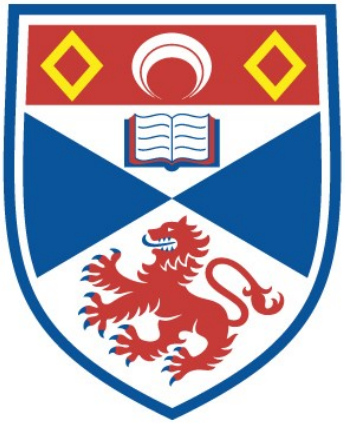
A STUDY OF THE NITROPRUSSIDE ANION AND SOME OF ITS ANALOGUES

John Reglinski

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



1981

A Study of the Nitroprusside Anion and some of its Analogues

A Thesis presented for the degree of

DOCTOR OF PHILOSOPHY

in the Faculty of Science of

the University of St. Andrews

by

John Reglinski, B.Sc.

October 1981

To My Parents

Their support has made this possible

Abstract

Sodium nitroprusside is a potent vasodilator and is widely used for lowering the blood pressure during major surgery. The physiological response is said to occur due to a nitrosation reaction at the smooth muscle membrane. The use of sodium nitroprusside has been restricted due to its ability to release toxic cyanide in-vivo and in-vitro, which can cause severe complications during surgery.

Chapter one is a study of the aqueous chemistry of sodium nitroprusside with amines and thiols. It is shown that steric factors play an important role and that thiols are more reactive than amines. The information is used to evaluate the chemical changes expected at the smooth muscle membrane and possible mechanisms for biochemical action. If the toxicity of nitroprusside anion cannot be aleviated the information can be used to evaluate the potential of other inorganic complexes as potential hypertensive agents.

Chapter two deals with the biochemical and medical problems associated with sodium nitroprusside therapy. The interaction of the complex with human erythrocytes is explored and the reasons for cyanide release are discussed. The toxicity associated with the complex is shown to be impossible to eradicate completely and short term measures to minimise the effects are given.

Chapter three explores the implications of the nitrosyl stretching frequency and how its value could be used to indicate whether a compound would be expected to exhibit nitroprusside-type chemistry. The reactivity of five nitrosylpentacyanometallates with the simple reagents previously discussed in chapter one are investigated to show that the nitrosyl group can be positive, neutral or negative and that

this information could be easily acquired from the stretching frequency of the nitrosyl group in the infra-red. A value for the nitrosyl stretching frequency is given, above which a compound would be expected to exhibit nitroprusside-type chemistry. These compounds would be expected to be vasodilators.

Declaration

I declare that this thesis is my own composition, that it is based on the results of experiments carried out by me, and it has not previously been presented for a higher degree.

This thesis describes the results of research carried out in the Department of Chemistry of the United College of St. Salvator and St. Leonard, University of St. Andrews, under the supervision of Dr. A.R. Butler and Dr. C. Glidewell between October 1978 and September 1981.

September 1981

John Reglinski

Certificate

I hereby certify that John Reglinski has spent twelve terms of research work under my supervision, has fulfilled the conditions of Ordinance General No. 12 and Resolution of the University Court, 1967, No. 1, and is qualified to submit the accompanying thesis in application for the degree of Doctor of Philosophy.

Dr. C. Glidewell
Director of Research

Certificate

I hereby certify that John Reglinski has spent twelve terms of research work under my supervision, has fulfilled the conditions of Ordinance General No. 12 and Resolution of the University Court, 1967, No. 1, and is qualified to submit the accompanying thesis in application for the degree of Doctor of Philosophy.

Dr. A. R. Butler
Director of Research

Acknowledgements

I wish to express my gratitude to Dr. A.R. Butler and Dr. C. Glidewell for their help and encouragement over the past three years and during the course of this work. There are many other people who have helped to make this study a success for me and I would like to mention them here; Dr. A.R. Butler, for his gift of blood; Ninewells Hospital Blood Transfusion Unit, for their help in removing the blood from Dr. Butler and myself; Dr. W.I.K. Eisset (Department of Anaesthesia, Ninewells Hospital) and Dr. M.G. Burdon (Department of Biochemistry and Microbiology, University of St. Andrews), for their helpful discussions on matters medical and biochemical; Ninewells Hospital, Department of Pharmacology and Therapeutics, for the animal testing and the technical staff of the Chemistry Department at St. Andrews, without whom all this would have been impossible.

There are many people who have given help and encouragement over the past three years in matters other than chemistry; these people I will never forget; they have helped to make my stay at St. Andrews happy and interesting, I owe them a debt. I would also like to thank Kristine Wieczorek for typing this work and my wife, Susan, for the endless cups of tea and kind words. It should now be possible to spend more time together.

I would like to thank the Scottish Hospitals Endownments Research Trust for the grant.

October 1981 John Reglinski

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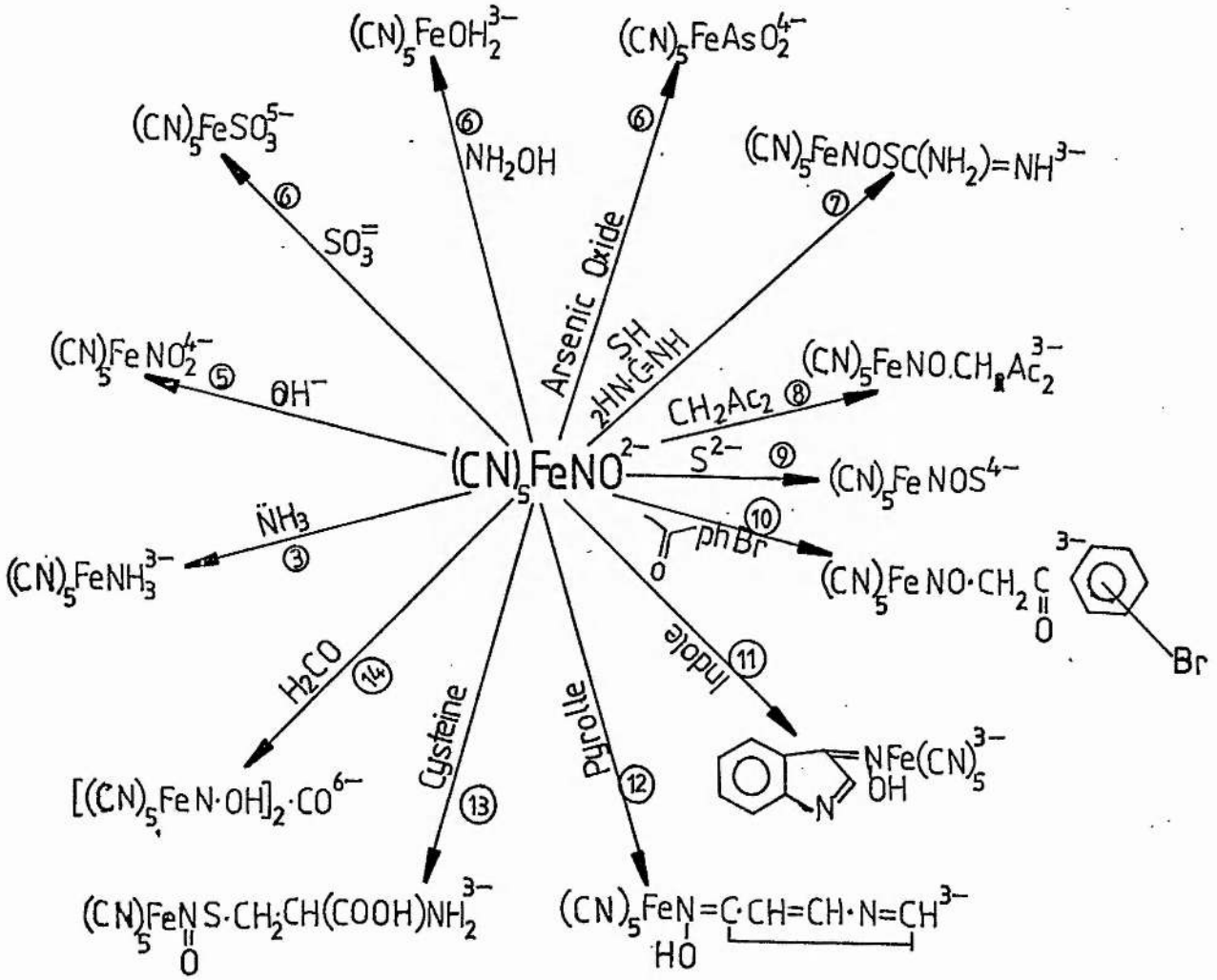
CHAPTER I

REACTIONS OF SODIUM NITROPRUSSIDE with Amines and Thiols

INTRODUCTION



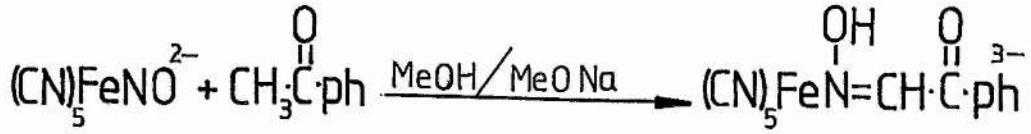
(Figure 1) Some Reactions of

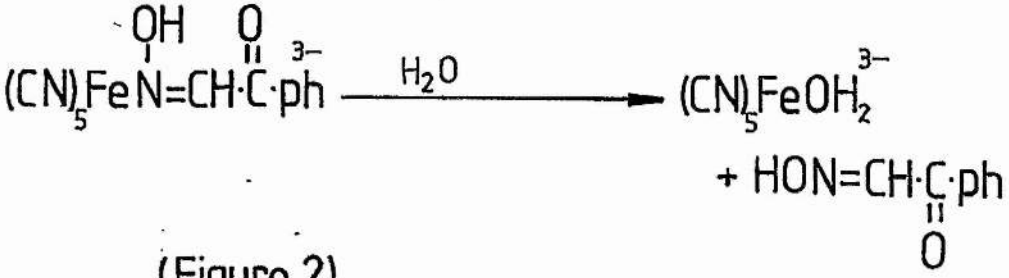
Sodium Nitroprusside

Sodium nitroprusside (sodium nitrosylpentacyanoferrate(II)) was first prepared by Lyon Playfair[1] in 1849. He claimed that his compound was a "New class of salt" even though he had failed to elucidate the correct formula. His analysis produced the empirical formula $Na_5Fe_5(CN)_{12}NO_3$. Playfair's study was limited to precipitation of the anion with various cations (copper(II), nickel(II), lead(II), zinc(II), mercury(II), potassium and sodium), but he did document reactions of the nitroprusside anion with three nucleophiles namely hydroxide, ammonia and sulphide (the mercury counter ion).

Städeler[2] gave the correct formula in 1868 as $Na_2[Fe(CN)_5NO]$ but it was Hofman[3-7] who began the real chemical study of the nitroprusside anion in the late 1890's. Realising that the complex could react with other anions rather than cations; he spent five years studying various reactions and the products (figure 1). Hofman[3-6] made no attempt to explain how the reactions came about or what had happened to the nitrosyl group, but was successful in preparing many new pentacyanoferrate complexes.

In 1914 Cambi[8, 11] discovered that sodium nitroprusside reacted with activated methylene groups. He managed to produce samples of the intermediates and products, both organic and inorganic (figure 2). This scheme was among the first to show that the reaction actually took place at the nitrosyl group and what subsequently happened at that site. This piece of work inspired Pavoline[10] to test various organic compounds. Although the paper does not study the reactions in great depth, he studied some fifty various compounds, some shown in





(Figure 2)

The Reaction of Sodium Nitroprusside and Acetophenone (Cambi 1914)

A Selection of Compounds Tested by Pavoline

Positive Response	Negative Response
Uracil	Glucose
Allentoin	Camphor
Thiourea	Barbituric Acid
Piperidine	Pyridine
Pyrocatachol	Furan
Resorcinol	Benzimidiazole

(table 1)

table 1. Reaction was said to occur if a colour change was observed; this change was red in most cases.

Since oxygen (as hydroxide)[5] reacted it was no surprise that sulphide[9], selenide[10] and telluride[10] also reacted (table 2).

Reactivity of the Group Six Elements

Species	Colour Change	Product
Hydroxide	Yellow	$(CN)_5FeNO_2^{4-}$
Sulphide	Red	$(CN)_5FeNOS^{4-}$
Selenide	Deep Blue	Not Given
Telluride	Black	Not Given

(table 2)

Selenium and tellurium are still curiosities today but sulphur has

attracted some attention. Sulphide[9,10], cysteine[13] and thiourea[7] are some of the reactions which appear in the literature (figure 1). The colour changes which occured with thiols were so marked that Scagliarini[13] in 1936 tried to develop a colorimetric test for the nitroprusside anion using cysteine.

All the chemistry reported so far was carried out without any intimate knowledge of the electronic structure of the compound and products they were investigating. They had relied quite successfully on empirical data, molecular ratios and comparative chemistry. Although Hofman[7] had formulated the iron asiron(III), he had no evidence to support his hypothesis. The question had to wait until the late 1950's early 1960's, when the techniques of infra-red, X-ray and ultra-violet spectroscopy had been developed and were used to study the nitroprusside anion.

Infra-red Data for Sodium Nitroprusside; KCl Disc[15]

Group	Frequency cm^{-1}
H_2O	3 580 (strong)
	3 440 (shoulder)
CN	2 152 (strong)
NO	1 938 (strong)
H_2O	1 618 (strong)
M-CN	663 (medium)

(table 3)

Although all groups agreed (within experimental error) about the infra-red data (table 3) there was some disagreement about the interpretation. Cotton[16] stated that the ion is iron(II), Bor[17] defined it as iron(III); Herington[15] said it was both. Cotton quotes Lewis[18] and his theory that if the nitrosyl frequency is between 1 575 cm^{-1} and 2 000 cm^{-1} the group can be formally written as NO^{+}, this is true for sodium nitroprusside leaving the iron in oxidation state two. Bor omitted to discuss the position of the nitrosyl frequency.

The X-ray structure was determined in 1963 by Manoharan[19] (table 4) and shown to have approximately C_{4v} symmetry. The iron was slightly displaced out of the plane in the direction of the nitrosyl group. The iron-nitrosyl grouping is linear suggesting strongly that the structure is iron(II) bound to a positive nitrosyl group. It is interesting to notice that the iron(III) nitrosyl grouping would be bent.

X-ray Data for Sodium Nitroprusside[19]

Grouping	Bond length $\pm$ 0.02Å	Grouping	Bond Angle
Fe—C	1.90	N—Fe—C_{eq}	96°
C≡N	1.16	Fe—N≡O	180°
Fe—N	1.63		
N≡O	1.13		

(table 4)

Following on from his X-ray study Manoharan did a molecular orbital calculation in conjunction with a study of the electronic spectrum. He again formally classed the anion as iron(II) with a positive nitrosyl group. His data are shown in table 5.

Ultra-violet Band Assignments for Sodium Nitroprusside[20]

Observed frequency cm^{-1}	Calculate frequency cm^{-1}	Assignment
50 000 (200 nm)	49 900	$d_{xy} \rightarrow \pi^*CN$
25 380 (394 nm)	25 090	$d_{xy}d_{yz} \rightarrow \pi^*NO$
20 080 (480 nm)	20 540	$d_{xy} \rightarrow \pi^*NO$

(table 5)

Manoharan[21] published the ^{13}CNMR data on pentacyanoferrates (table 6) in 1978. This produced no surprises, showing axial and equatorial cyanides (except in two cases) in the approximate ratio of one to four.

^{13}CNMR Data For Pentacyanoferrates

Ion	Equatorial ^{13}C ppm	Axial ^{13}C ppm
CN^-	135.2	-
$(CN)_5FeNO^{2-}$	104.2	102.2
$(CN)_5FeOH_2^{3-}$	147.1	-
$(CN)_5FeNO_2^{4-}$	146.1	145.8
$(CN)_5FeSO_3^{5-}$	147.3	145.7
$Fe(CN)_6^{4-}$	146.4	

All chemical shifts have been referred to t-butanol

(table 6)

$$(CN)_5FeNO^{2-} + 2\ddot{N}H_2R \longrightarrow (CN)_5FeNHR^{3-} + N_2 + H^+ + ROH$$

The Reaction of Sodium Nitroprusside and Primary Amines

(Figure 3)

The structural information compiled over the last two decades explains the early chemistry. The nitroprusside anion being formulated as iron(II) with a positive nitrosyl group[16,19,20] is d^6 substitution inert; this explains why attack by thiols[7,9,13] and activated methylene groups[8,10,12,14] is at the nitrosyl group and the integrity of the $Fe(CN)_5^{3-}$ species in the product.

Progress in the chemistry of sodium nitroprusside continued in 1960. Two groups of workers investigated the reactions of the anion with amines. Kenney[22] showed that simple primary amines reacted directly with the complex to produce aminopentacyanoferrates(II) in a molar ratio of two amines to one nitroprusside anion. Maltz[23] working on the same scheme showed that the organic products were the relevant alcohol and nitrogen (figure 3).

But the real innovation was the advent of mechanistic studies on the anion. Swinehart has made two such kinetic studies; the first being the hydroxide reaction[24,26] and the second the hydrogen-sulphide[25,26] reaction using spectrophotometric techniques. Swinehart's kinetic data and reaction schemes are in figure 4 and table 7 for the sodium nitroprusside-hydroxide system. His data for the sodium nitroprusside-hydrogen sulphide system are shown in figure 5 and table 8.

The Reaction of Sodium Nitroprusside with Hydroxide

$$(CN)_5FeNO^{2-} + OH^- \xrightarrow{k_{12}} (CN)_5FeNO_2H^{3-} \quad (1)$$

$$(CN)_5FeNO_2H^{3-} + OH^- \xrightarrow{\text{fast}} (CN)_5FeNO_2^{4-} + H_2O \quad (2)$$

$$(CN)_5FeNO_2^{4-} + H_2O \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} (CN)_5FeOH_2^{3-} + NO_2^- \quad (3)$$

(figure 4)

Reaction	k M^{-1} s^{-1}	K
$(CN)_5FeNO^{2-} + 2OH^- \rightleftharpoons (CN)_5FeNO_2^{4-} + H_2O$	0.55	$1.5\pm0.3\times10^{}$
$(CN)_5FeNO_2^{4-} + H_2O \rightleftharpoons (CN)_5FeOH_2^{3-} + NO_2^-$	1.4×10^{-4} (*)	$3.1\pm1\times10^{-4}$
	0.46 (**)	

* Rate constant for forward reaction

** Rate constant for reverse reaction

400 nm; 298 K; 1.0 M Ionic Strength (NaCl)

(table 6)

The Reaction of Sodium Nitroprusside with Hydrogen Sulphide

$$(CN)_5FeNO^{2-} + SH^- \xrightarrow{k_{12}} (CN)_5FeNOSH^{3-} \quad (4)$$

$$(CN)_5FeNOSH^{3-} + OH^- \xrightarrow{k_{23}} (CN)_5FeNOS^{4-} \quad (5)$$

This reaction is irreversible

(figure 5)

Reaction	k
$(CN)_5FeNO^{2-} + SH^- \longrightarrow (CN)_5FeNOSH^{3-}$	$170M^{-1}s^{-1}$
$(CN)_5FeNOSH^{3-} + OH^- \longrightarrow (CN)_5FeNOS^{4-} + H_2O$	$1.3x10^{-2}s^{-1}$

540 nm; 298 K; 1.0 M Ionic Strength (NaCl)

(table 8)

These kinetic studies are explained by attack of bases at the nitrosyl group. It is this type of reaction which will be the subject of the study to be reported. Apart from the interest in this particular metal nitrosyl for chemical reasons, there is another reason for the study. Sodium nitroprusside has become a common drug in surgery to lower blood pressure. A more detailed description of the biochemistry will be given in chapter 2, but what is important in this chapter is that the hypotensive properties are believed to be a nitrosation reaction of a thiol group[28,29] at the smooth muscle receptors. Notice that amines are also reactive and should not be dismissed. Listed in table 9 are the six common amino acid residues which could be reactive to sodium nitroprusside in their form at pH 7.0. Notice that only one thiol is present and chemically speaking it should be unreactive.

This study is a kinetic investigation of the reactions of amines and thiols with sodium nitroprusside. This will not only add to the wealth of information about the complex but enable the construction of a model for biochemical activity. This model will be used in the development of a non-cyanide based hypotensive drug; the reasons why an alternative drug to sodium nitroprusside is being sought will

become clear in chapter 2.

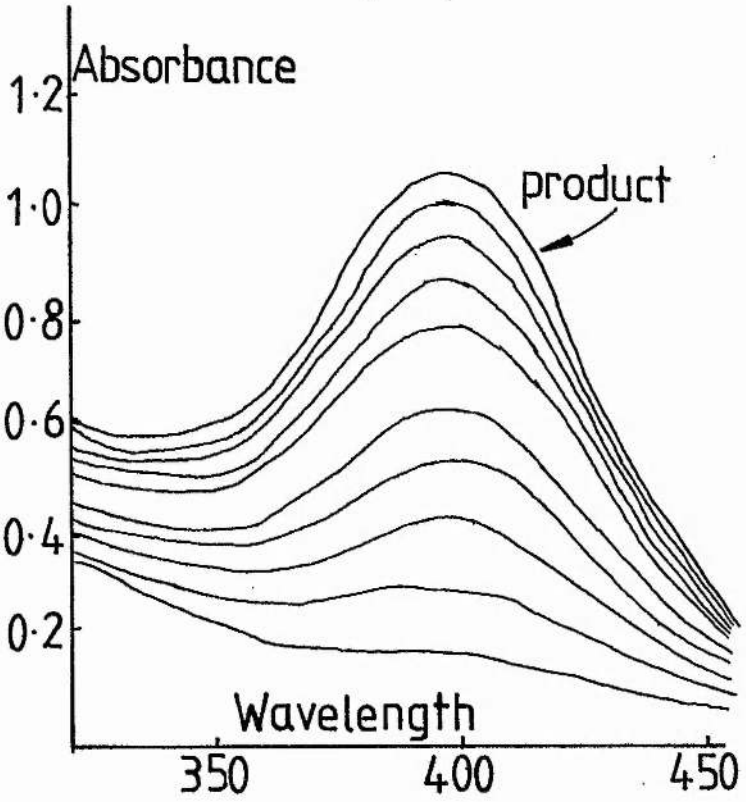
The Amino Acid Residues which may be reactive with Sodium Nitroprusside

Amino Acid	Formula	pK_a (residue)
Cysteine	$HS-CH_2-$	8.33
Arginine	$NH_2-\underset{\underset{+NH_2}{\|}}{C}-NH-(CH_2)_3-$	12.48
Asparagine	$NH_2-\underset{\underset{O}{\|}}{C}-CH_2-$	-
Glutamine	$NH_2-\underset{\underset{O}{\|}}{C}-(CH_2)_2-$	-
Histidine	$CH_2-\overset{+}{N}H=CH-NH-CH-CH_2-$	6.0
Lysine	$\overset{+}{N}H_3-(CH_2)_4-$	10.53

(table 9)

RESULTS AND DISCUSSION

(Figure 6)



$[(CN)_5FeNO^{2-}] = 5{\cdot}0\times10^{-3}$ M

Reading every two minutes

Reaction of Sodium Nitroprusside and Ethylamine

1. Primary Amines

Reactions of sodium nitroprusside with primary amines are well known. Hofman[3] studied the reaction of ammonia and successfully identified the aminopentacyanoferrate(II) anion as the product. Kenny[22] investigated the inorganic products of the reactions with methylamine, ethylamine, propylamine and butylamine. He states that the relevant aminopentacyanoferrate(II) is produced. Maltz[23] investigated the organic products of sodium nitroprusside and benzylamine, butylamine, allylamine, cyclohexamine and octylamine. In the absence of oxygen the first three produce only the respective alcohol, the others produced predominantly the alcohol but with some elimination product (cyclohexene, 1-octene and 2-octene). Their proposed reaction is shown in figure 3.

1.1 Ethylamine

The proposed reaction of sodium nitroprusside and ethylamine, which produces ethylaminopentacyanoferrate(II) is shown in scheme 1. The unprotonated, rather than the protonated form of the amine is viewed as the reactive species. The product of reaction is highly coloured and its formation is shown in figure 6. In every instance the reaction was first order in the appearance of the product.

The reaction is between sodium nitroprusside and the unprotonated form of the amine if the mathematical expression given below is a constant.

$$k_{obs} / [EtNH_2] = \text{Constant } M^{-1}s^{-1} \qquad (6)$$

where

$[EtNH_2]_t$ = Total Amine Concentration

$[EtNH_2]$ = Concentration of the Unprotonated Amine

$[EtNH_3^+]$ = Concentration of the Protonated Amine

By varying the degree of neutralisation and monitoring the observed rate constant we get the results shown in table 10; column five is a constant (within experimental error), proving the above hypothesis to be correct.

The Effect of the Degree of Neutralisation on k_{obs}

$[EtNH_2]_t/M$	$[EtNH_2]/M$	Degree of Neutralisation	$10^{+3}k_{obs}/s^{-1}$	Ratio $10^{+2}M^{-1}s^{-1}$
0.20	0.16	1/5	9.19	5.7
0.20	0.12	2/5	6.11	5.1
0.20	0.10	1/2	5.70	5.7
0.20	0.08	3/5	5.20	6.5

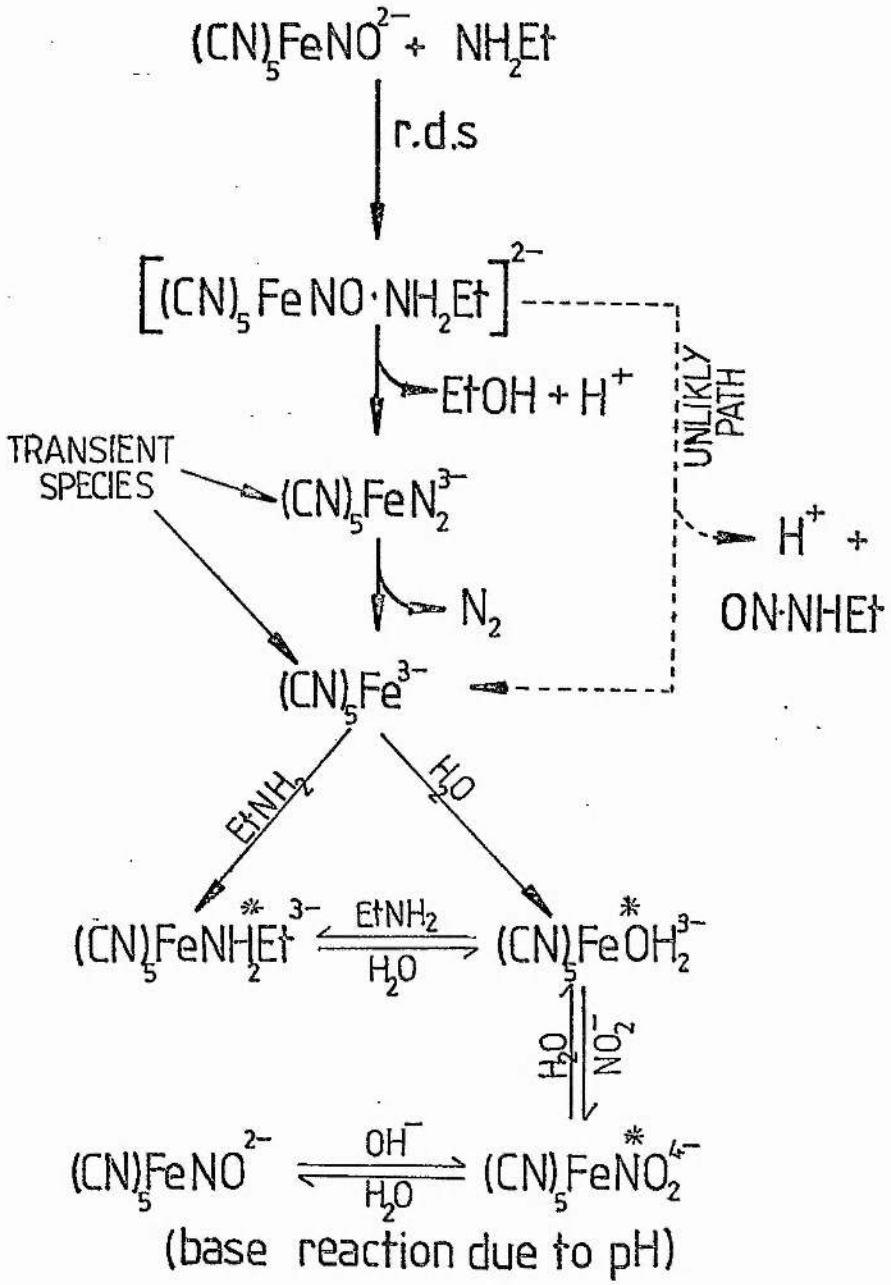
400 nm; 303 K; 1.0 M Ionic Strength (KCl)

Sodium Nitroprusside concentration = 2.5×10^{-3}M

(table 10)

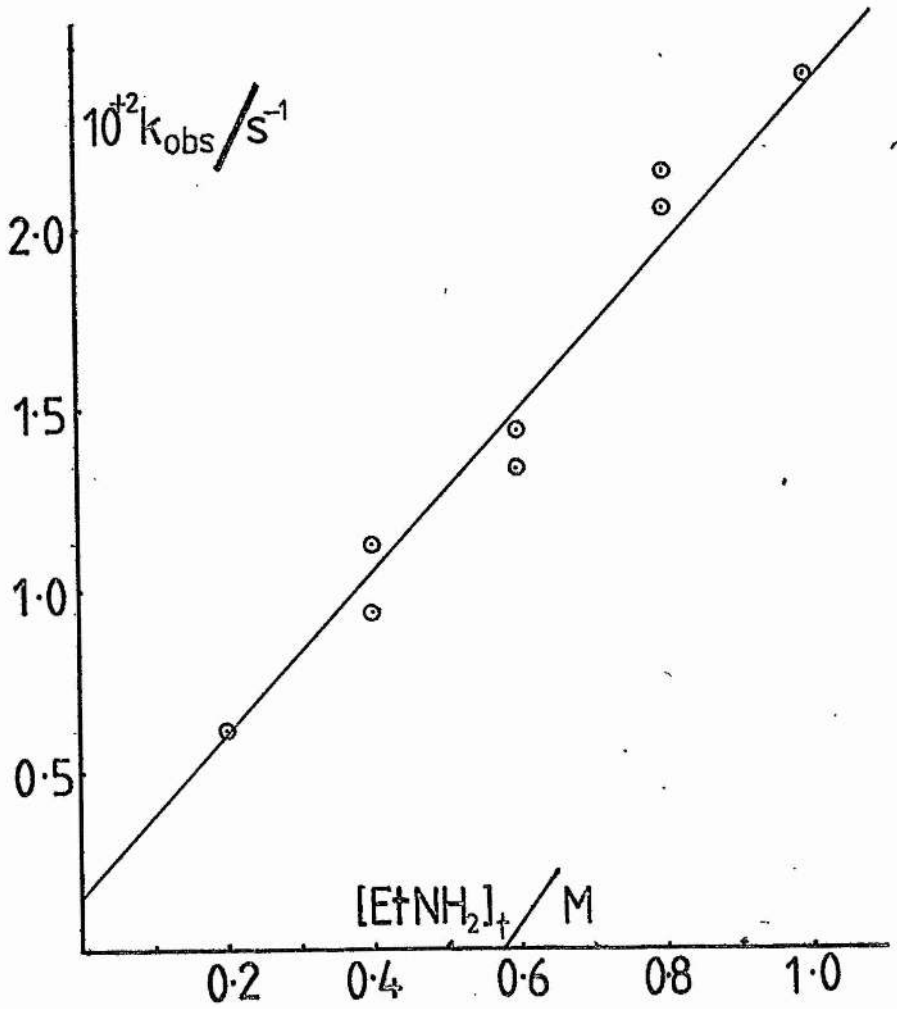
Evidence that the kinetic scheme proposed is correct is shown by the amine dependant kinetics displayed in table 11 and figure 7.

Scheme 1



* Mixed product identified using. I.R.

The Reaction of Sodium Nitroprusside and Ethylamine



Rate Constant = 0·0237 $M^{-1}s^{-1}$

Intercept = $8{\cdot}4\times10^{-4}s^{-1}$

Plot of the Amine Dependent Kinetic Data for the Sodium Nitroprusside-Ethylamine Reaction (Figure 7)

Kinetic Data for the Ethylamine Kinetics of Sodium Nitroprusside

$[EtNH_2]_t/M$	$10^{+2}k_{obs}/s^{-1}$
0.99	2.42 ; 2.42
0.79	2.05 ; 2.16
0.60	1.35 ; 1.45
0.40	1.14 ; 0.95
0.20	0.61 ;

400 nm; 303 K; 1.0 M Ionic Strength (KCl)

Sodium Nitroprusside concentration = 2.54×10^{-3}M

(table 11)

The reaction is first order in both sodium nitroprusside and total amine concentration. This is consistent with the belief that the rate determining step is the formation of the anion-amine adduct shown in scheme 1. The total amine concentration is directly related to the concentration of the unprotonated form of the amine. The intercept in figure 7 can be explained as the hydroxide reaction of sodium nitroprusside[24,25].

Calculation of the Value of k_{obs} due to the Hydroxide Reaction present in the Sodium Nitroprusside-Ethylamine reaction

$$NH_2Et + H_3O^+ \rightleftharpoons {}^{+}NH_3Et + H_2O$$

$$K_a = [NH_2Et][H_3O^+] \Big/ [{}^{+}NH_3Et][H_2O] \qquad (7)$$

$$= 1.56\times10^{-11}$$

The amine was half neutralised for internal buffering purposes.

therefore

$$[NH_2Et] = [\overset{+}{N}H_3Et]$$

$$\Rightarrow [NH_2Et]_t = 2[NH_2Et]$$

also

$$pH = pK_a = 10.81$$

$$= [OH^-] \simeq 8.4\times10^{-4}M$$

Rate Constant k_{12} for the hydroxide reaction (figure 4) $= 0.55M^{-1}s^{-1}$

$=$ Expected k_{obs} for hydroxide reaction in the amine kinetics $= 0.55\times8.4\times10^{-4}$

$= 5\times10^{-4}s^{-1}$

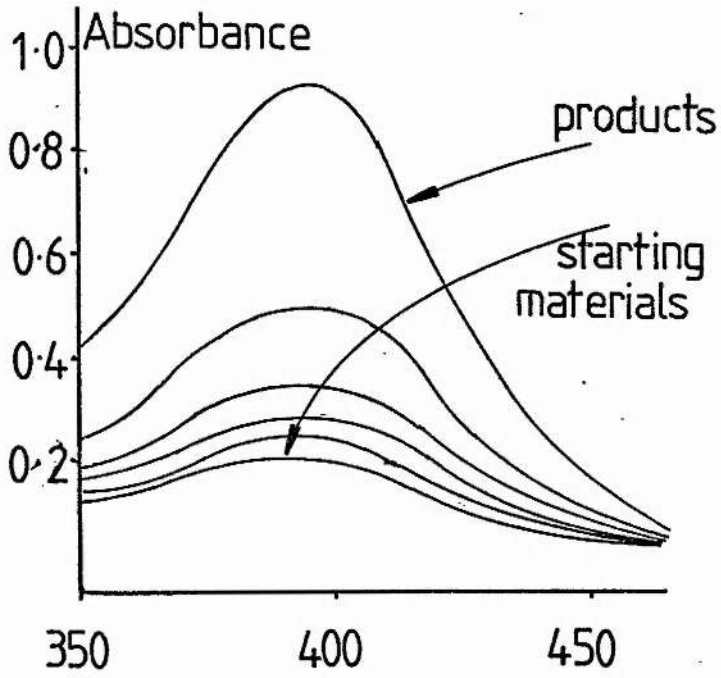
Observed Intercept $\div 8\times10^{-4}s^{-1}$

These two figures are the same within experimental error

(figure 8)

Swinehart's[24,25] value for the rate constant of the hydroxide reaction is $0.55M^{-1}s^{-1}$. The pK_a of ethylamine is documented[30] as 10.807, since the amine is half neutralised this is also the approximate pH of solution (figure 8). The approximate hydroxide concentration is $8.4\times10^{-4}M$. The observed rate constant at this concentration for the hydroxide-sodium nitroprusside reaction is $5\times10^{-4}s^{-1}$. This value compares favourably with that of the intercept.

The mechanism is believed to go via the production of the inorganic diazonium salt rather than the ejection of N-ethylnitrosamine. The elimination of the nitrosamine is ruled out, as this is the

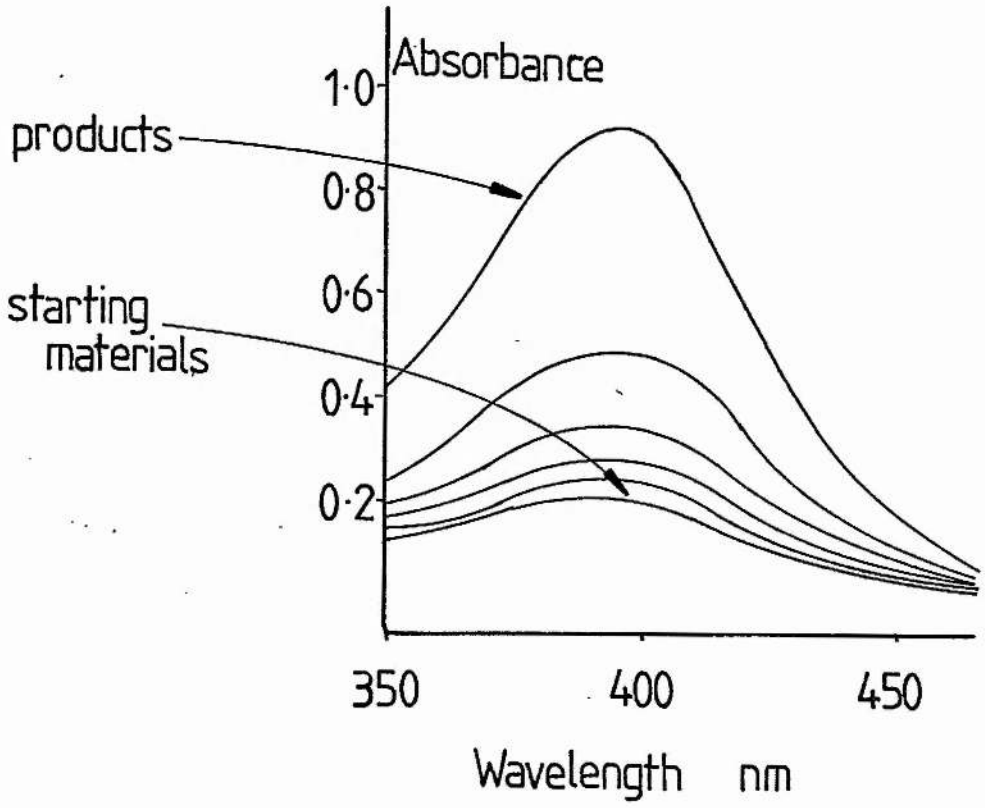


$[(CN)_5FeNO^{2-}] = 2{\cdot}0 \times 10^{-3}$ M

Reading every two minutes.

The Reaction of Sodium Nitroprusside in Isopropylamine Solution

(Figure 9)



$[(CN)_5FeNO^{2-}] = 2{\cdot}0 \times 10^{-3}M$

Reading every two minutes

The Reaction of Sodium Nitroprusside in Basic Solution

(Figure 10)

ejection of a poor leaving group from a d^6 substitution inert species. This, if it occurred, would be an extremely slow, rate determining process with approximately amine independant kinetics. The mechanism producing nitrogen which is an excellent leaving group is favoured. The steps involved in this process are hydrogen transfer and nitrogen elimination both of which could be expected to be rapid.

No deviation from first order kinetics is observed when the amine concentration is reduced and therefore no change of mechanism is thought to occur.

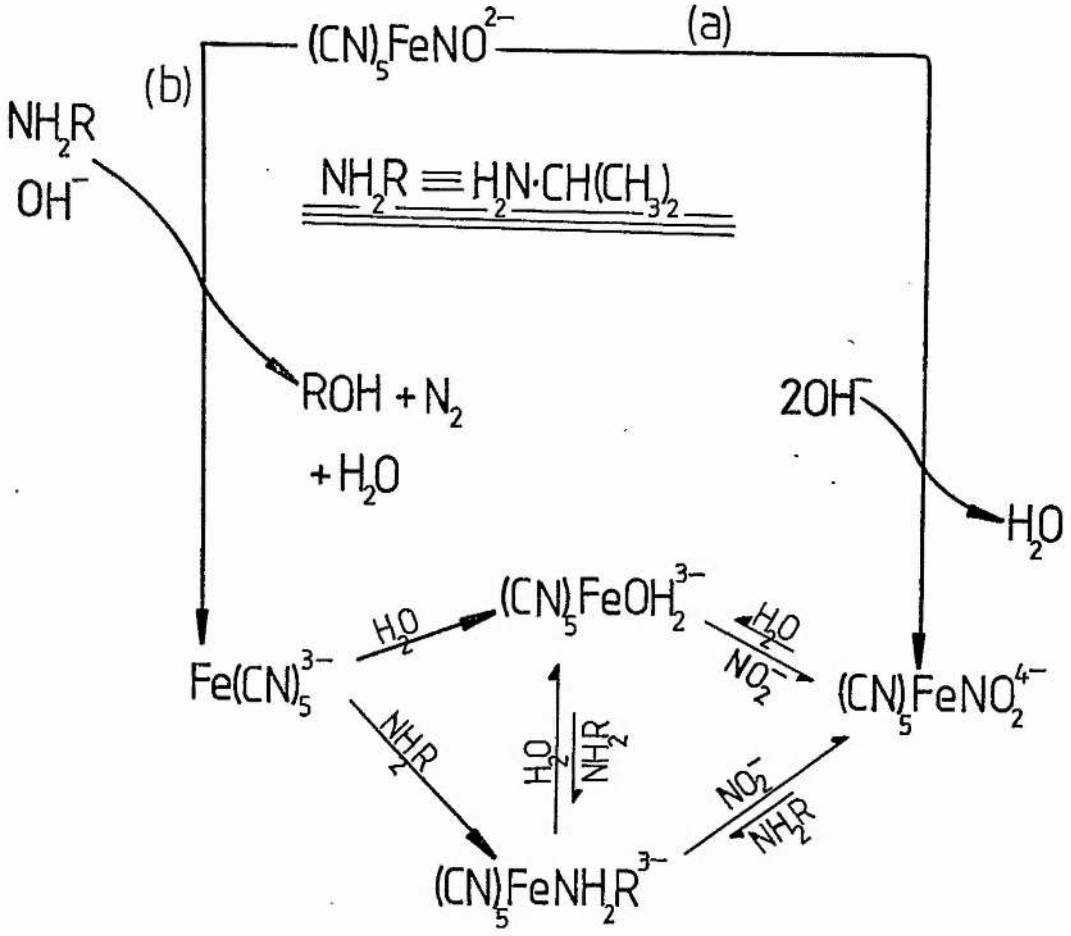
1.2 Isopropylamine

The proposed reaction for sodium nitroprusside with isopropylamine producing a mixture of nitropentacyanoferrate(II), aquopentacyanoferrate(II) and isopropylaminopentacyanoferrate(II) (Scheme 2). The u.v.-visible spectral change between 350 nm and 450 nm is shown in figure 9. This is identical to the u.v.-visible spectral change in the same region for the hydroxide reaction at the same sodium nitroprusside concentration (figure 10).

Evidence that it is the hydroxide reaction which is being observed is indicated by the wide range of amine independant kinetics shown in table 12. The reactions were all first order in appearance of product.

The observed rate constants are of a magnitude which would be expected for the base reaction at the amine buffering pH ($\sim$11).

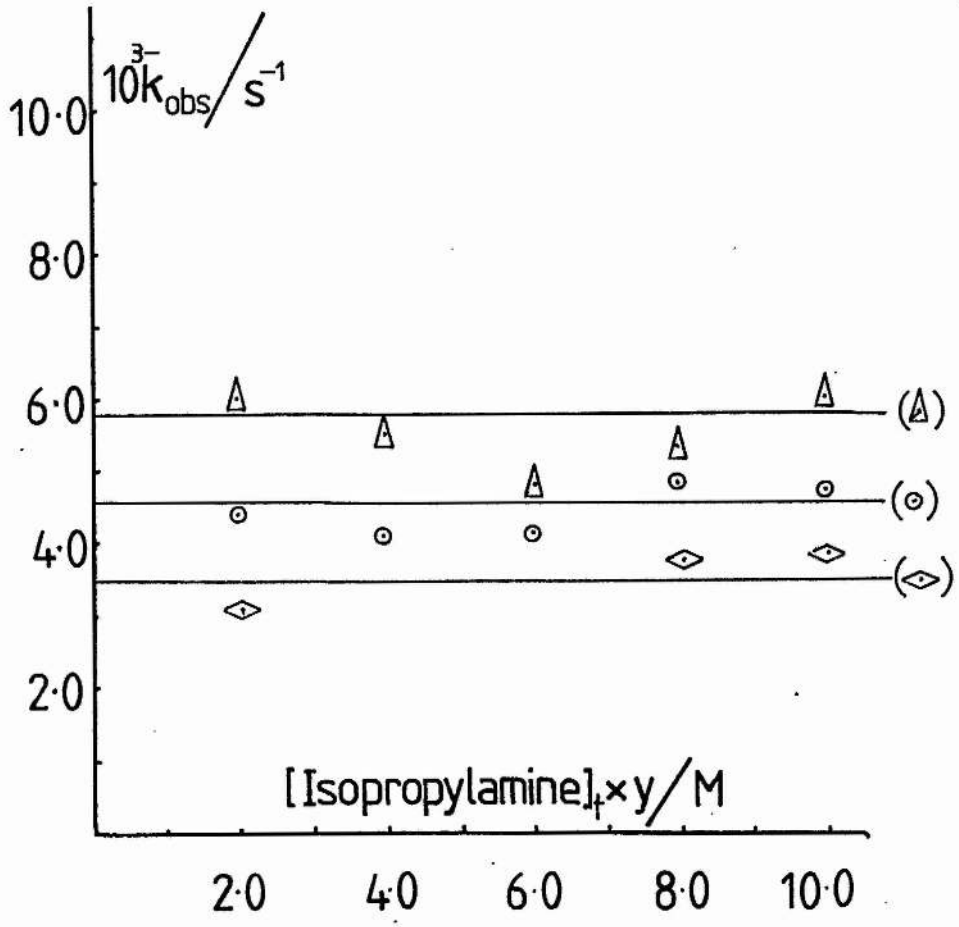
Scheme 2



(a) The main reaction: Production of Nitropentacyanoferrate II

(b) A minor reaction in aqueous solution

The Reaction of Sodium Nitroprusside in Isopropylamine Solution



⊙ $y = 10^{-1}$

Δ $y = 10^{-2}$

◇ $y = 5 \times 10^{-4}$

Plot of the Kinetic Data for the Amine Independant Kinetics of Sodium Nitroprusside in Isopropylamine Solution

(Figure 11)

Kinetic Data for the Isopropylamine Independant Kinetics of Sodium Nitroprusside

$[Isopropylamine]_t/M$	1.0	0.8	0.6	0.4	0.2
$10^{+3}k_{obs}/s^{-1}$	4.7	4.8	4.1	4.1	4.4

Sodium Nitroprusside concentration = 2.61×10^{-3}M

temperature = 301 K

$[Isopropylamine]_t/M$	0.10	0.08	0.06	0.04	0.02
$10^{+3}k_{obs}/s^{-1}$	6.0	5.3	4.8	5.5	6.0

Sodium Nitroprusside concentration = 2.47×10^{-3}M

temperature = 303 K

$[Isopropylamine]_t/M$	0.020	0.016	0.004
$10^{+3}k_{obs}/s^{-1}$	3.9	3.8	3.1

Sodium Nitroprusside concentration = 2.97×10^{-3}M

temperature = 301 K

All reactions carried out at 400 nm and 1.0 M Ionic Strength (KCl)

(table 12)

Some aminopentacyanoferrate(II) is present in low concentration in the product isolated from aqueous solution. Its presence is indicated by the comparison of the infra-red spectra of the aqueous product and the methanolic product ("pure" isopropylaminopentacyanoferrate(II)). A weak CH stretching frequency at 2980cm^{-1} in the aqueous product corresponds exactly with a strong absorption at the same frequency in

the methanolic product.

The aminopentacyanoferrate(II) could be produced in two ways. Since the aminopentacyanoferrate(II) can be made in methanol, possibly through a similar mechanism to ethylamine, it is reasonable to suggest that the same reaction occurs in aqueous solution but that it is much slower than the hydroxide reaction. The hydroxide reaction is believed to be dominant at the buffering pH. Another method of producing aminopentacyanoferrates is by an extension of a proven reaction. Swinehart[24,25] has shown that the product of hydroxide action on sodium nitroprusside, the nitropentacyanoferrate(II), equilibrates with the solvent, as shown below.

$$(CN)_5FeNO_2^{4-} + H_2O \rightleftharpoons (CN)_5FeOH_2^{3-} + NO_2^- \qquad (3)$$

$$K = \frac{[(CN)_5FeNO_2^{4-}][H_2O]}{[(CN)_5FeOH_2^{3-}][NO_2^-]}$$

$$= 3.1\times10^{-4}$$

The final spectrum in figure 10 is therefore the mixed product. It is not impossible that the small amounts of aminopentacyanoferrate(II) is produced by a similar equilibration as the solvent in this study could be viewed as being mixed. Manoharan[21] gives a series of ligands and their relative binding strength to the $Fe(CN)_5^{3-}$ species (figure 11). Using his information we would subsequently expect the relative concentrations of the three products to form a similar sequence with respect to their concentrations (figure 11). The isopropylamino-pentacyanoferrate(II) would at low concentrations have no impact on the

u.v.-visible spectrum.

Relative Preference of Binding of Ligands to $Fe(CN)_5^{3-}$

$$NO^+ > CO > CN^- > SO_3^{2-} \geq NO_2^- > H_2O > NH_3$$

$$[(CN)_5FeNO_2]^{4-} >>> [(CN)_5FeOH_2]^{3-} >>> [(CN)_2FeNH_2R]^{3-}$$

where

NH_2R = Isopropylamine

(figure 11)

It is important to realise that isopropylamine is capable of reacting with sodium nitroprusside to form isopropylaminopentacyanoferrate(II) even although the reaction is slow.

1.3 t-Butylamine

There is no direct action between sodium nitroprusside and t-butylamine. This is substantiated by the products of reaction in aqueous solution being the nitropentacyanoferrate(II) and the aquo-pentacyanoferrate(II). The aqueous reaction which occurred had exactly the same u.v.-visible spectral change as that displayed in figure 9. Further evidence is the amine independant kinetics observed in table 12. All the kinetics were first order in the appearance of the product. The observed rate constants are of comparable magnitude to that expected for the hydroxide reaction at the buffering pH of the amine.

Kinetic Data for the t-Butylamine Independant Kinetics of Sodium Nitroprusside

$[\text{t-butylamine}]_t/M$	1.0	0.8	0.6	0.4	0.2
$10^{+3}k_{obs}/s^{-1}$	6.0	6.1	6.1	5.1	5.7

Sodium Nitroprusside concentration = 2.48×10^{-3}M

temperature = 303 K

$[\text{t-butylamine}]_t/M$	0.10	0.08	0.06	0.04
$10^{+3}k_{obs}/s^{-1}$	10.9	10.7	11.2	12.1

Sodium Nitroprusside concentration = 2.21×10^{-3}M

temperature = 302.5

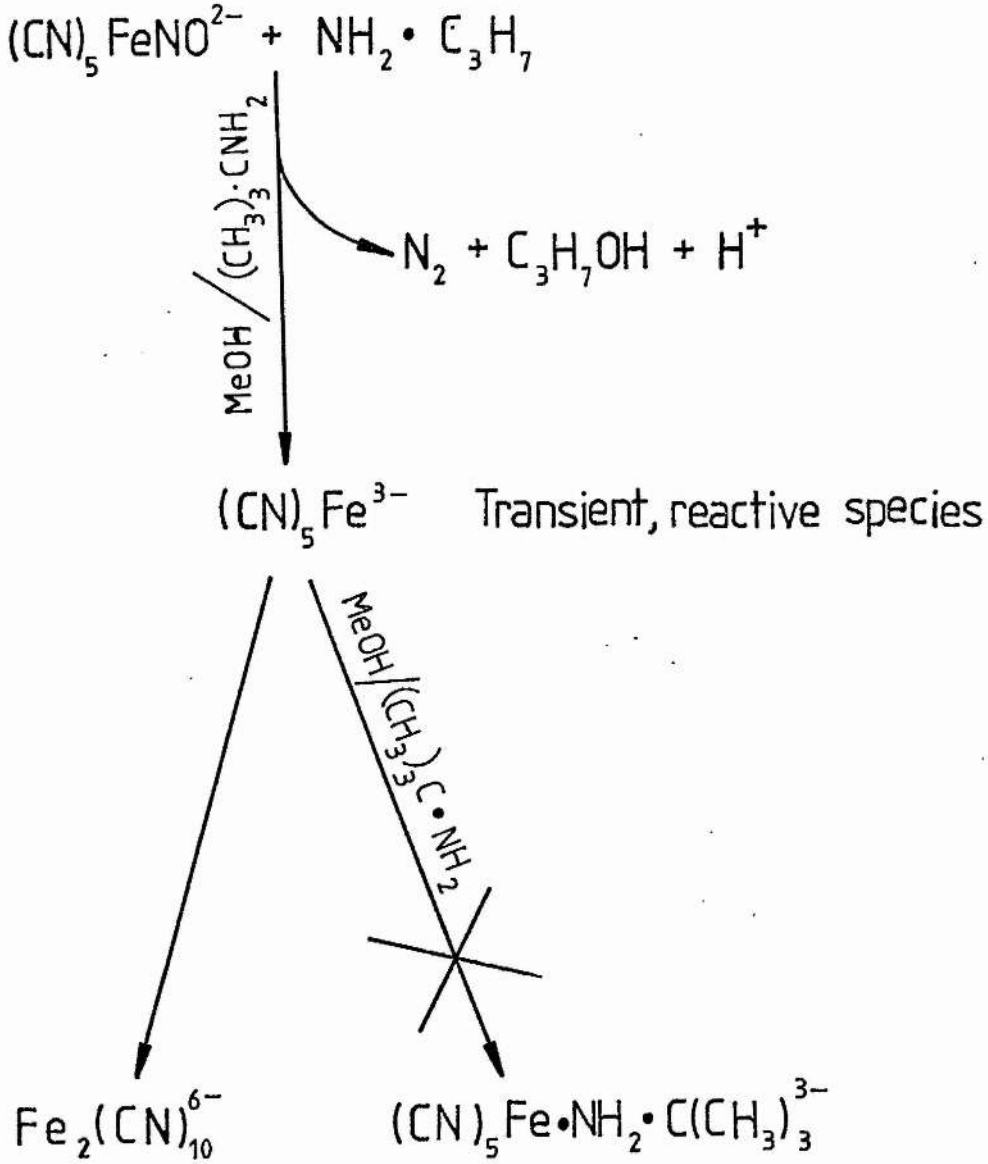
All reactions were carried out at 400 nm and 1.0 M Ionic Strength (KCl)

(table 13)

The results show that the lower amine concentration range is causing a faster reaction even though it has a lower temperature and sodium nitroprusside concentration. This could only be explained as a medium effect or an error in half neutralisation of the solution.

Unlike isopropylamine; t-butylamine does not react in methanol. Steric factors must be playing an important role, preventing attack at the nitrosyl group. An experiment was designed to try and make t-butylaminopentacyanoferrate(II) (Scheme 3). Sodium nitroprusside is allowed to react with n-propylamine (extremely dry) in the presence of excess t-butylamine in dry methanol. (The sodium nitroprusside concentration being approximately equal to the n-propylamine

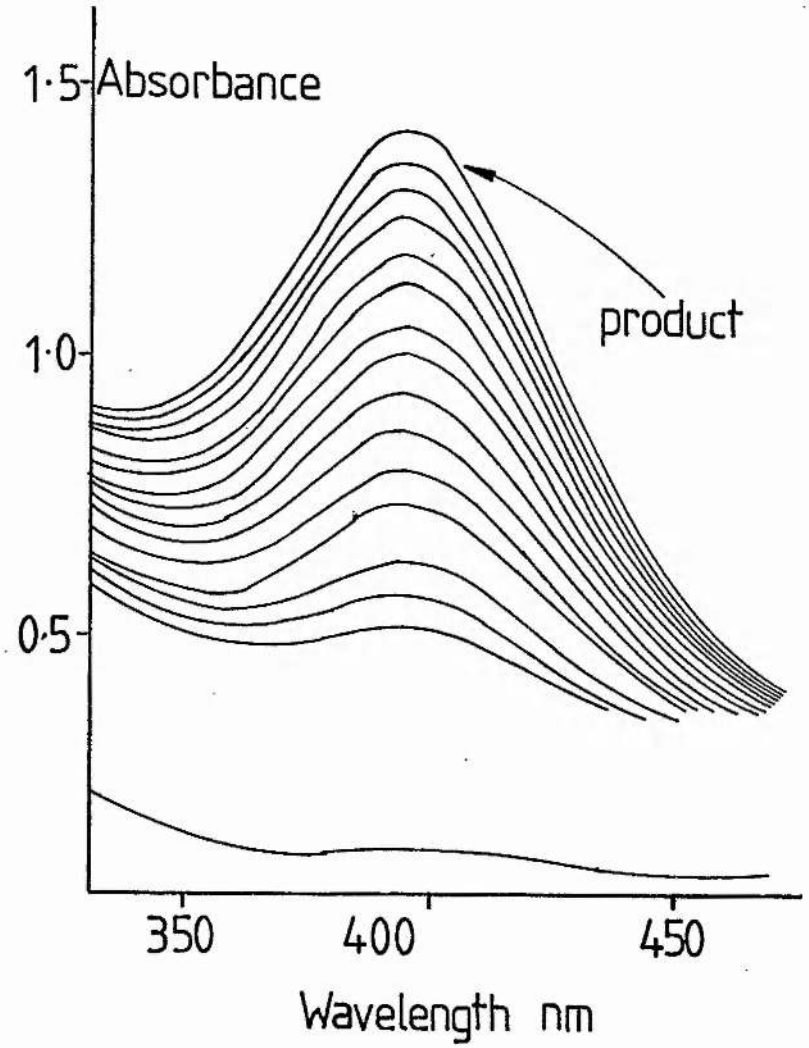
Scheme 3



Product isolated from methanolic solution

Not observed in product

Attempt to produce t-butylaminopentacyanoferrate II



$[(CN)_5FeNO^{2-}] = 5 \times 10^{-3} M$

Reading every two minutes

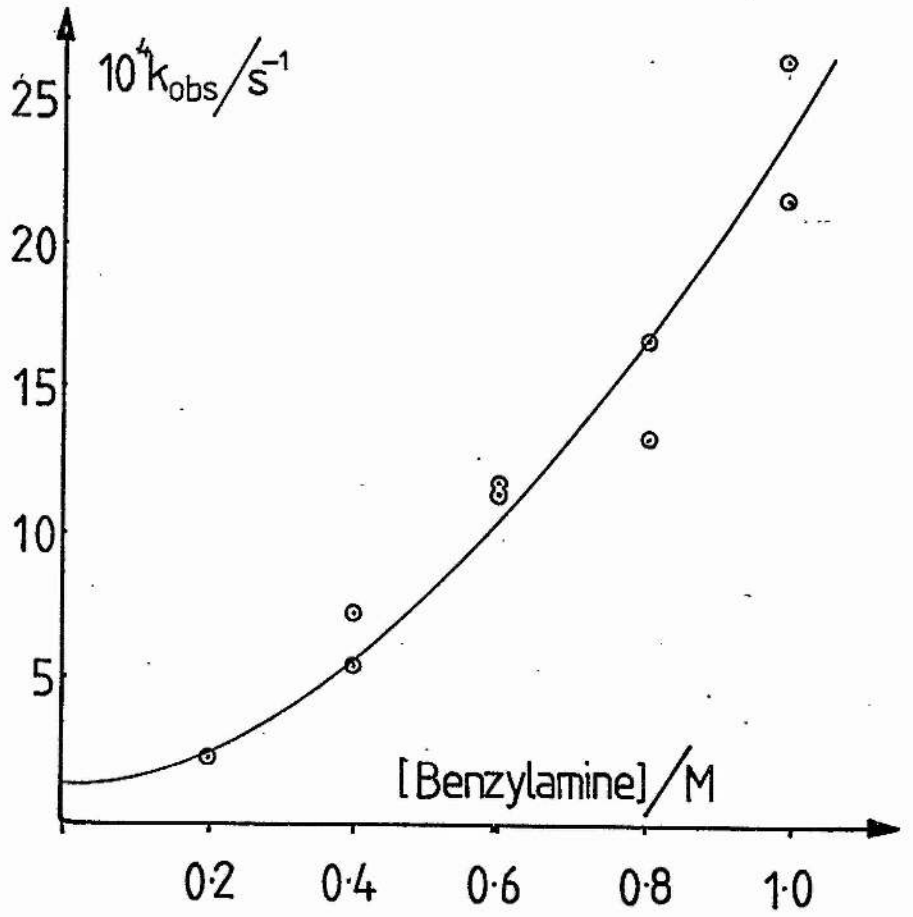
Reaction of Sodium Nitroprusside and Benzylamine

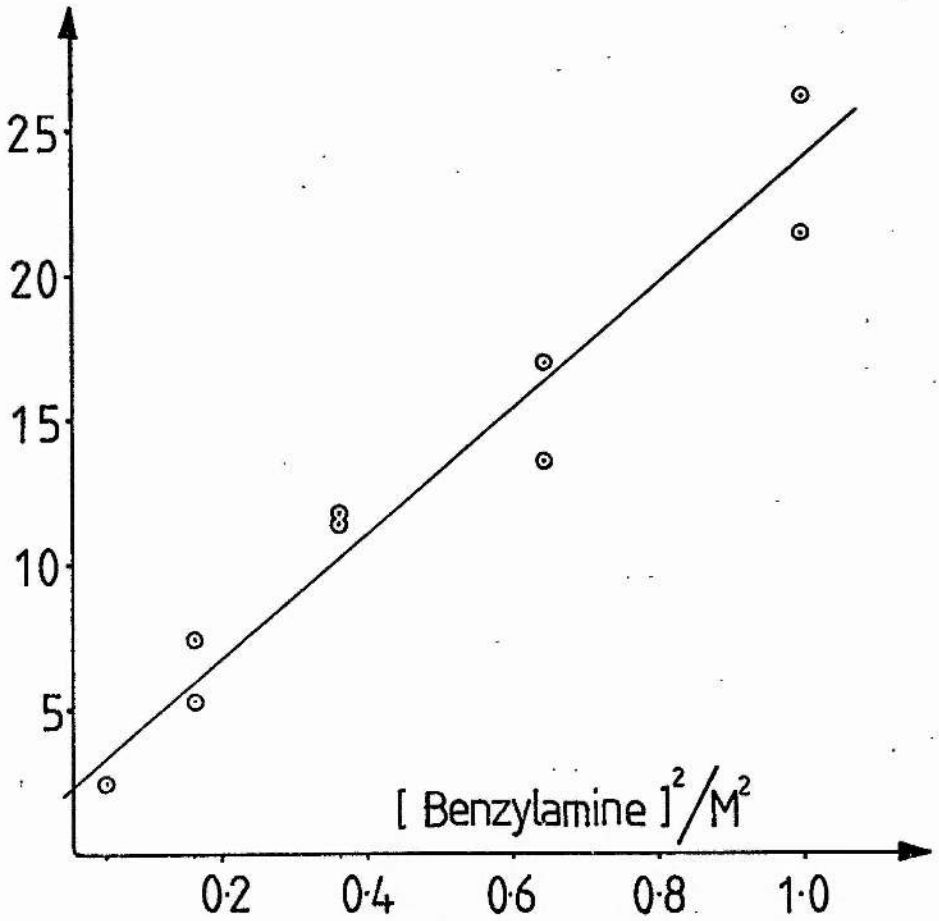
(Figure 12)

concentration.) Diazotisation of the simple amine would be expected with the subsequent production of $Fe(CN)_5^{3-}$ in solution. This should be highly reactive and attack t-butylamine, since there should be no propylamine or any other reactive species present. This should produce t-butylaminopentacyanoferrate(II) but the reaction does not occur and the methanolic solution turns red. Aminopentacyanoferrates(II) are yellow and insoluble in methanol as has been shown by the other members of this class of complex, it is therefore proposed that t-butylamine cannot, for steric reasons, complex to the $Fe(CN)_5^{3-}$ species.

1.4 Benzylamine

The reaction of sodium nitroprusside and benzylamine produces benzylaminopentacyanoferrate(II) and aquopentacyanoferrate(II) and the proposed reaction is set out in scheme 4. The products of reaction is highly coloured and their formation is shown in figure 12, in every instance the reaction was first order in appearance of product. Both aquopentacyanoferrate(II) and benzylaminopentacyanoferrate(II) as "pure" products absorb strongly at 400 nm. Evidence that the scheme is correct is supported by the amine dependant kinetics displayed in figure 13 and table 14. The data are explained by allowing the rapid formation of a nitroprusside-amine equilibrium. A nitroprusside-amine adduct then reacts further with another amine in the slower rate determining step. This rate determining step is the abstraction of a proton by benzylamine from the nitroprusside-amine adduct.


$10^4 k_{obs}/s^{-1}$
25
20
15
10
5
[Benzylamine]/M
0·2
0·4
0·6
0·8
1·0


25
20
15
10
5
[Benzylamine]^2/M^2
0·2
0·4
0·6
0·8
1·0

Third Order Rate Constant $= 2{\cdot}39 \times 10^{-3} M^{-2} s^{-1}$

Intercept $= 1{\cdot}8 \times 10^{-4} s^{-1}$

Plots of the Amine Dependant Kinetic Data for the Sodium Nitroprusside–Benzylamine Reaction

(Figure 13)

Amine dependant Kinetics for Benzylamine -
Sodium Nitroprusside Reaction

$[\text{Benzylamine}]_t/\text{M}$	$[\text{Benzylamine}]_t^2/\text{M}^2$	$10^{+4}k_{obs}/\text{s}^{-1}$
1.0	1.00	21.59 ; 26.24
0.8	0.64	16.90 ; 13.35
0.6	0.36	11.53 ; 11.76
0.4	0.16	5.35 ; 7.28
0.2	0.04	2.40 ;

400 nm; 301.5 K; 1.0 M Ionic Strength (KCl)

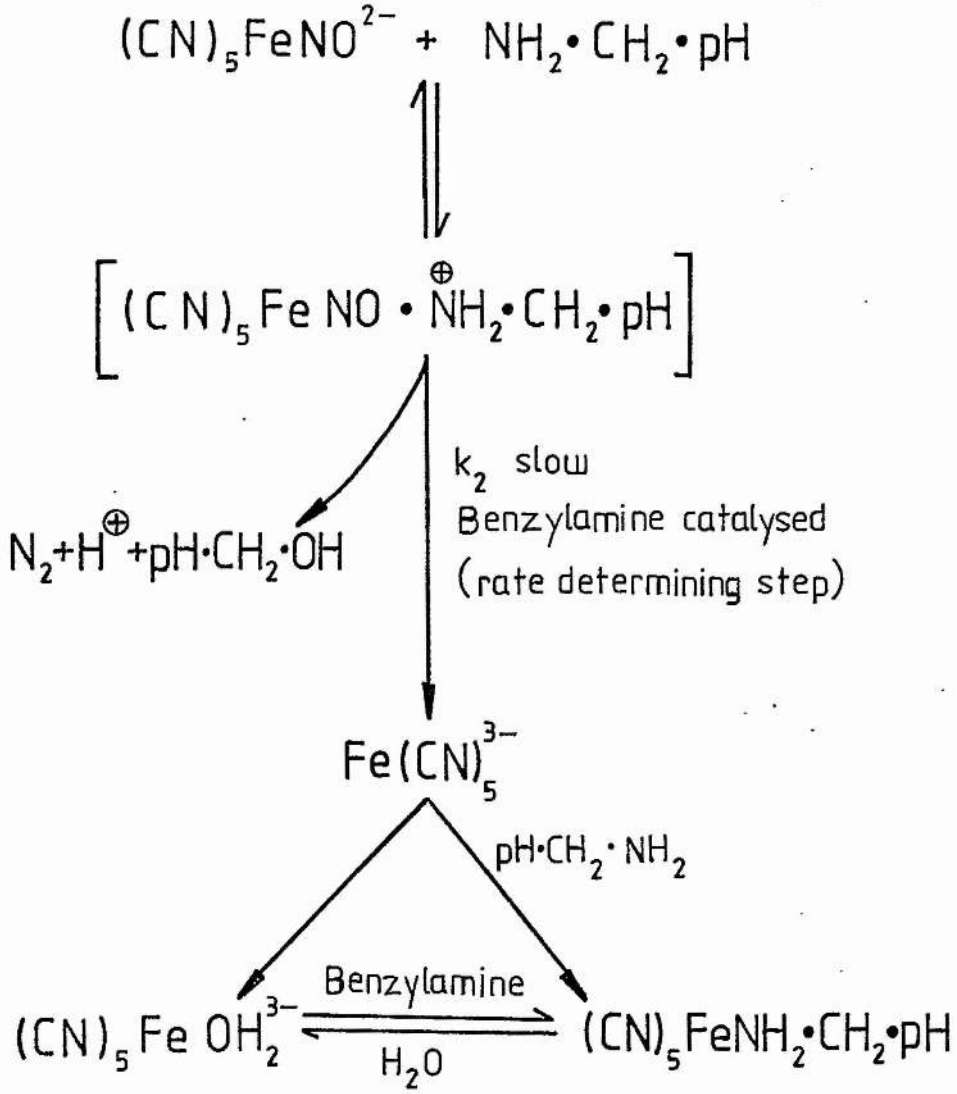
Sodium Nitroprusside concentration = $2.42\text{x}10^{-3}$M

(table 14)

The intercept in figure 13 can again be explained as the hydroxide reaction, but at the buffering pH of the amine ($\sim$9.5) it does not make a great impact on the overall reaction. No nitropentacyanoferrate(II) is observed in the infra-red of the aqueous product. (The stretching frequencies due to NO_2^- are quite distinctive.) The low pH (considering other amine studies) at which this reaction is studied may have an important effect on the rate determining step. At higher pH's hydrogen abstraction could not be viewed as a slow, rate determining, process. At higher pH's the hydroxide reaction would become more prominent also, possibly taking precident over the benzylamine reaction, this is observed with isopropylamine.

The mixed product of the aquopentacyanoferrate(II) and benzylaminopentacyanoferrate(II) can be produced by either competition for the

Scheme 4



Reaction of Sodium Nitroprusside and Benzylamine

$Fe(CN)_5^{3-}$ species which must exist or equilibration similar to that described previously and shown below for this particular amine.

$$H_2O + (CN)_5FeNH_2CH_2pH^{3-} \rightleftharpoons (CN)_5FeOH_2^{3-} + NH_2CH_2pH$$

2. Secondary and Tertiary Amines

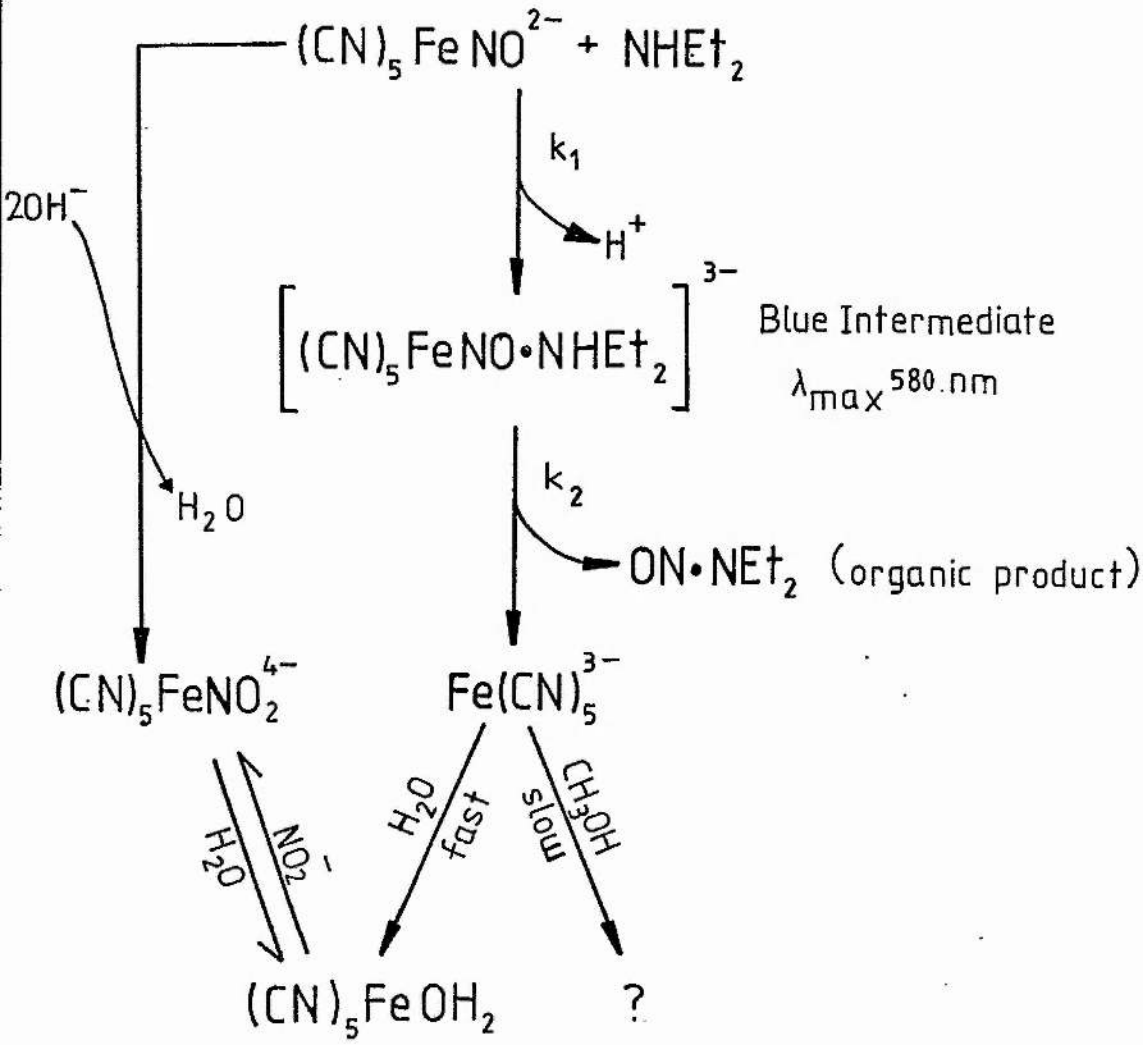
Reactions of sodium nitroprusside with secondary and tertiary amines are not so well documented. Maltz[23] briefly studied a reaction between the nitroprusside anion and diethylamine and stated that the product of reaction was N,N,-diethylnitrosamine, but no mention was made of the inorganic product. Scagliarini[12] studied pyrrole and Cambi[11] indole, 2-methylindole and 3-methylindole. The first three react but not at the amine group, 3-methylindole does not even react. The organic products are said to be oximes, but again the inorganic products escape identification.

Diethylamine and Triethylamine have been chosen for study as they complete a series of simple amines, the first member of which was ethylamine.

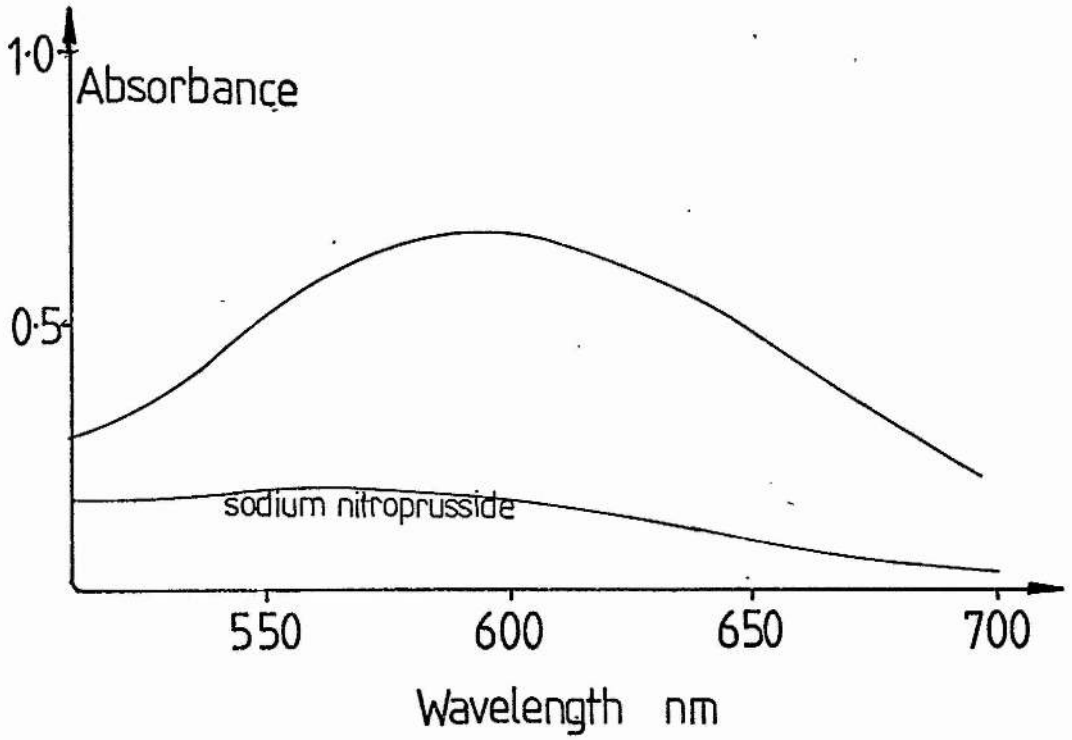
2.1 Diethylamine

The proposed reaction of sodium nitroprusside and diethylamine is shown in scheme 5. Unlike the previous amine investigated a deeply coloured intermediate is formed (figure 14) in solution (λ max 580 nm), this subsequently reacts further to produce a highly coloured product (figure 15) at 400 nm. The reactions were all good first order in the appearance of the intermediate and the product. There is also an

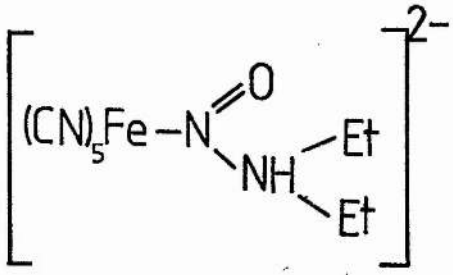
Scheme 5



Reaction of Sodium Nitroprusside and Diethylamine

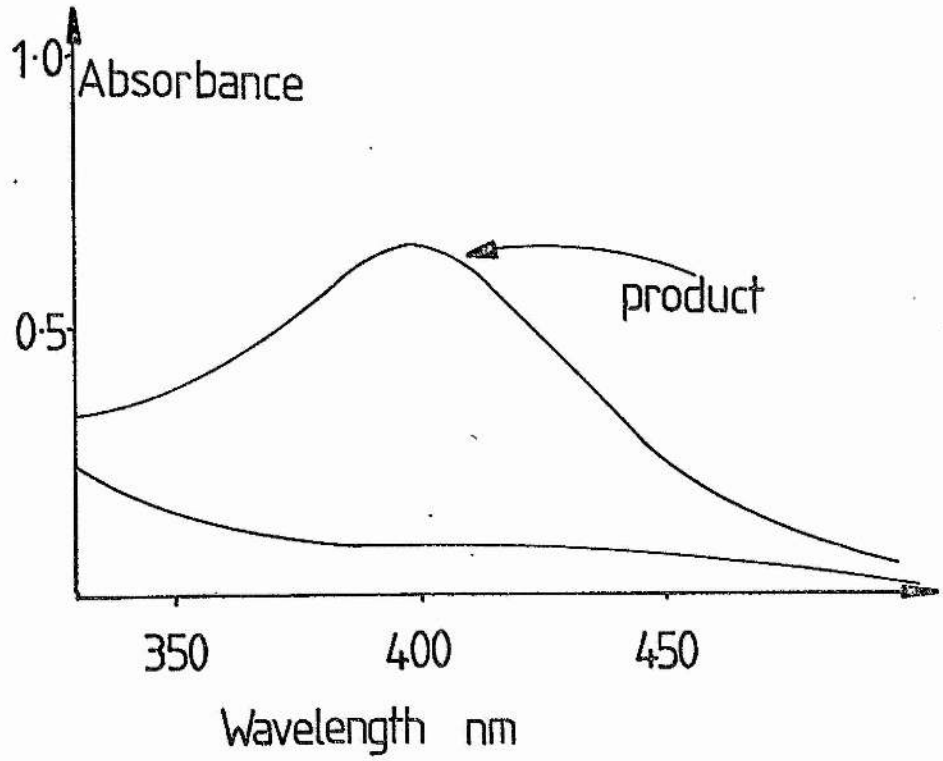


$[(CN)_5FeNO^{2-}] = 0{\cdot}05M$

proposed structure 

Visible Spectrum of the Blue Intermediate (λ_{max} 580nm) in the Reaction of Sodium Nitroprusside and Diethylamine

(Figure 14)



$[(CN)_5FeNO^{2-}] = 2{\cdot}5 \times 10^{-3}$ M

spectrum recorded after the reaction had gone to completion.

The Product of the Reaction of Sodium Nitroprusside and Diethylamine

(Figure 15)

isosbestic point at 460 nm, proving that this is indeed a sequence of reactions with very different rates. Attempts to isolate the intermediate failed and its structure has had to be inferred. The product of aqueous reaction is not the diethylaminopentacyanoferrate(II) as would be expected but the aquopentacyanoferrate(II) with no evidence for any amino product being present. In methanol there is strong evidence that the bispentacyanoferrate(II) anion is the product and again not the amino product. This is an intermediate situation between ethylamine and t-butlylamine, the amine is capable of reacting with the nitrosyl group but unable to occupy the vacant site of the nitrosamine ejected. Maltz has previously stated that the organic product was N,N,-diethylnitrosamine.

The reaction scheme proposed is supported by two sets of amine dependant kinetics. Table 15 and figure 16 show the kinetic data for the rise in absorbance associated with the production of the blue intermediate at 580 nm.

This result is explained by allowing the reaction of one nitroprusside anion with one diethylamine molecule to be the rate limiting step. This is the basis for the belief that the blue complex being observed has the structure shown in scheme 5.

Table 16 and figure 16 show the kinetic data associated with the fall in absorbance at 580 nm the decomposition of the blue intermediate.

The kinetics for this process can be described simply as the decomposition of the intermediate to products by an internal reaction.

Kinetics of Formation of the Blue Intermediate (580 nm)

$[Et_2NH]_t/M$	$10^{+2}k_{obs}/s^{-1}$
0.27	2.33
0.50	4.56
0.53	4.22
0.60	4.35
0.80	6.34
1.00	7.63

580 nm; 303 K; 1.0 M Ionic Strength (KCl)

Sodium Nitroprusside concentration = 2.0×10^{-3} M

(table 15)

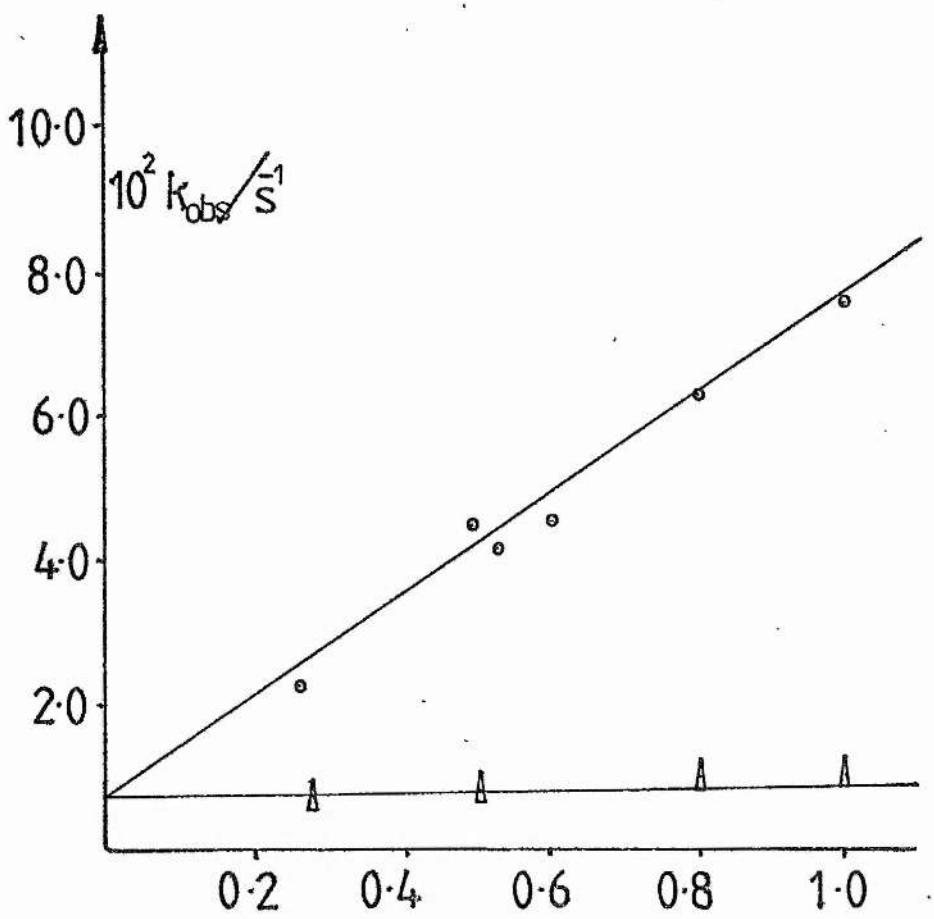
Kinetic Data for the Decomposition of the Blue Intermediate (580 nm)

$[Et_2NH]_t/M$	$10^{+3}k_{obs}/s^{-1}$
1.00	10.6
0.80	9.6
0.50	7.7
0.27	6.7

580 nm; 303 K; 1.0 M Ionic Strength (KCl)

Sodium Nitroprusside concentration = 2.0×10^{-3} M

(table 16)



∘ Data for the formation of the intermediate

Rate Const = 7.1×10^{-2} $M^{-1}s^{-1}$

Δ Data for the decomposition of the intermediate

Plot of the Kinetic Data for the Sodium Nitroprusside – Diethylamine Reaction

(Figure 16)

The kinetic data (figure 16) shows that the first reaction, the formation of the blue intermediate, is amine dependent while the second, the decomposition of the intermediate, is amine independent. This is to be expected in a system whose one reactant is rapidly converted to an intermediate which slowly breaks down to give products.

A study of the amine dependant kinetics at 400 nm (figure 15) was unsuccessful giving results which were inconsistent and in poor agreement. Diethylamine would seem to undergo a reaction in aqueous solution when half neutralised. A definite yellowing of stock solutions was observed over a short period of time. This reaction would seem to interfere with the observations at 400 nm. The reaction observed at this wavelength is believed to be the slow process associated with the decomposition of the blue intermediate. This is reasonable as this reaction is believed to be the reaction of the nitrosamine from the intermediate (k_2). The iron is d^6 substitution inert and any process involving ligand exchange (eg. water for nitrosamine) would be expected to be very slow and a likely rate determining step for the overall reaction.

The fact that there are two reactions present could partially explain why Maltz[23] only achieved a 44% yield of nitrosamine. Another reason for such a poor yield could be that sodium nitroprusside reacts with N,N -diethylamine.

2.2 N,N,-Diethylnitrosamine

It has already been mentioned that Maltz[23] in his early study on

diethylamine proved that N,N -diethylnitrosamine was the organic product of reaction. He achieved a maximum yield of 44%. It is of some interest to discover if this compound reacts further with more sodium nitroprusside (explaining such a low yield).

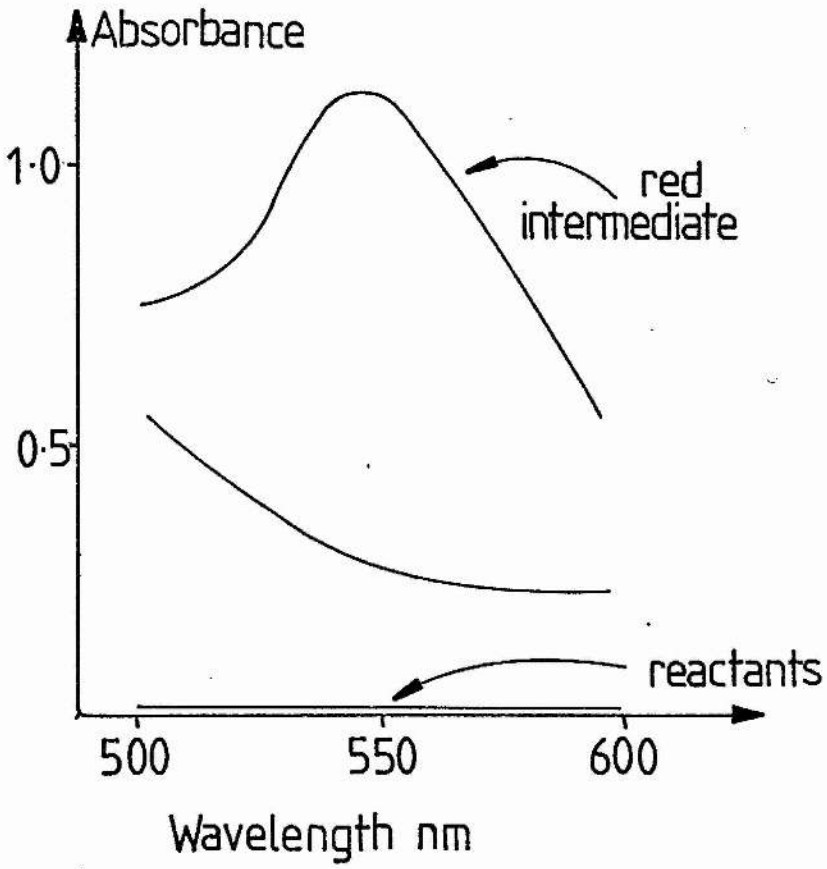
There is no spectral evidence for a reaction between sodium nitroprusside and N,N -diethylnitrosamine. The only reason for Maltz achieving such a low yield is the argument put forward in the previous section about competitive reactions. Considering both of the reactions present in diethylamine solution produce the same inorganic product (aquopentacyanoferrate(II)) Maltz would not have suspected that there was anything wrong with his data.

2.3 Tri-ethylamine

There is no spectral evidence for a reaction between sodium nitroprusside and tri-ethylamine. There is still, however, the familiar hydroxide reaction caused by the buffering action of the amine. Reaction at the nitrosyl group (with amines) requires the amine to have a labile proton; this is not the case with tertiary amines and it is subsequently no surprise that they do not react.

3. Thiols

Apart from Swinehart's[25,26] work on hydrogen sulphide there has been no recent reports or studies on sodium nitroprusside and thiols.



$[(CN)_5FeNO^{2-}] = 2{\cdot}0 \times 10^{-3}M$

Readings thirty seconds apart

The intermediate starts to decompose before the spectrum can be properly recorded

Reaction of Sodium Nitroprusside and Cysteine at 540nm

(Figure 17)

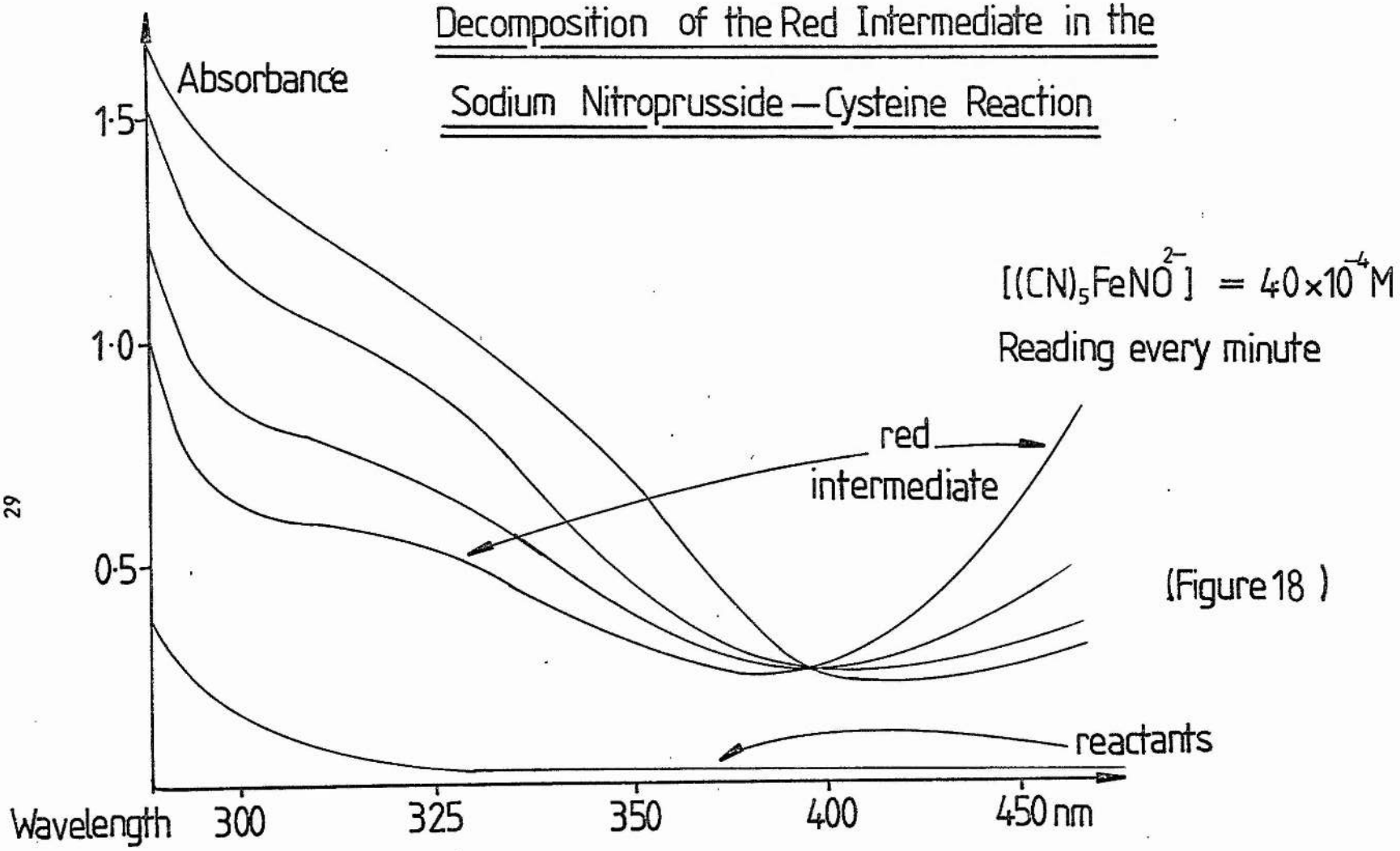
3.1 Cysteine

Cysteine was chosen as an example of the thiols as it is an easily accessible and safe member of the group, it is also important biologically. Cysteine has been briefly looked at by Scagliarini[13]; he isolated a red product from methanolic solution and formulated it as $[(CN)_5FeNOSCH_2(COOH)NH_2]^{3-}$.

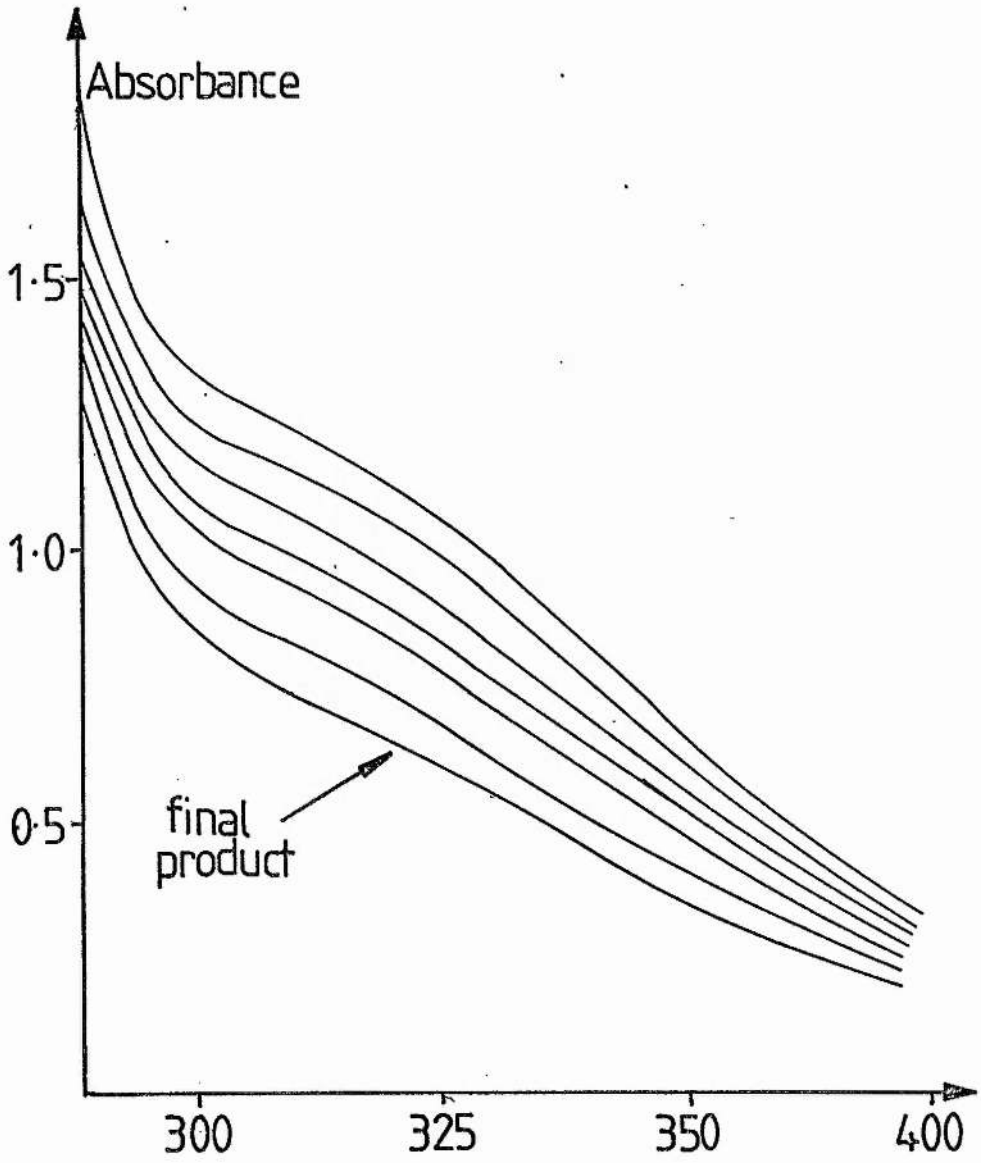
The reaction between cysteine and sodium nitroprusside is a three stage reaction (figures 17, 18, 19). The first reaction (figure 17) is the rapid rise in absorbance at 540 nm associated with the formation of Scagliarini's red product. The second reaction is the fall in absorbance at 540 nm and the subsequent rise in absorbance at 300 nm (figure 18). There is an isosbestic point at 400 nm indicating that these two processes are the same chemical reaction, the product of which was not isolated and has been inferred. The third and final reaction (figure 19) is the slow fall in absorbance at 300 nm which produces the eventual product of reaction: S-mercaptoalanyl-pentacyanoferrate(II) (hereafter thiopentacyanoferrate(II)). Due to the pH (8 to 9) of solution little or no nitropentacyanoferrate(II) is produced via a hydroxide reaction. We would expect sulphur complexes to bind more strongly than water to the iron (figure 11), which explains the high concentration of the thiopentacyanoferrate(II) in the product and the u.v.-visible spectral evidence that there is little or no aquopentacyanoferrate(II) (no absorption at 400 nm by product).

A great problem with this reaction is its sensitivity to oxygen. If, when the solution is a pale yellow, stage 2 to 3, the vessel is

Decomposition of the Red Intermediate in the Sodium Nitroprusside—Cysteine Reaction



(Figure 18)



$[(CN)_5FeNO^{2-}] = 4{\cdot}0 \times 10^{-4} M$

Reading every five minutes

Formation of the Final Product in the Sodium Nitroprusside —Cysteine Reaction

(Figure 19)

shaken, the solution will return to the red form. This reverse process is not complete and the system eventually exhausts itself. Solutions used for study were de-oxygenated using a nitrogen flush but traces of oxygen will always be present.

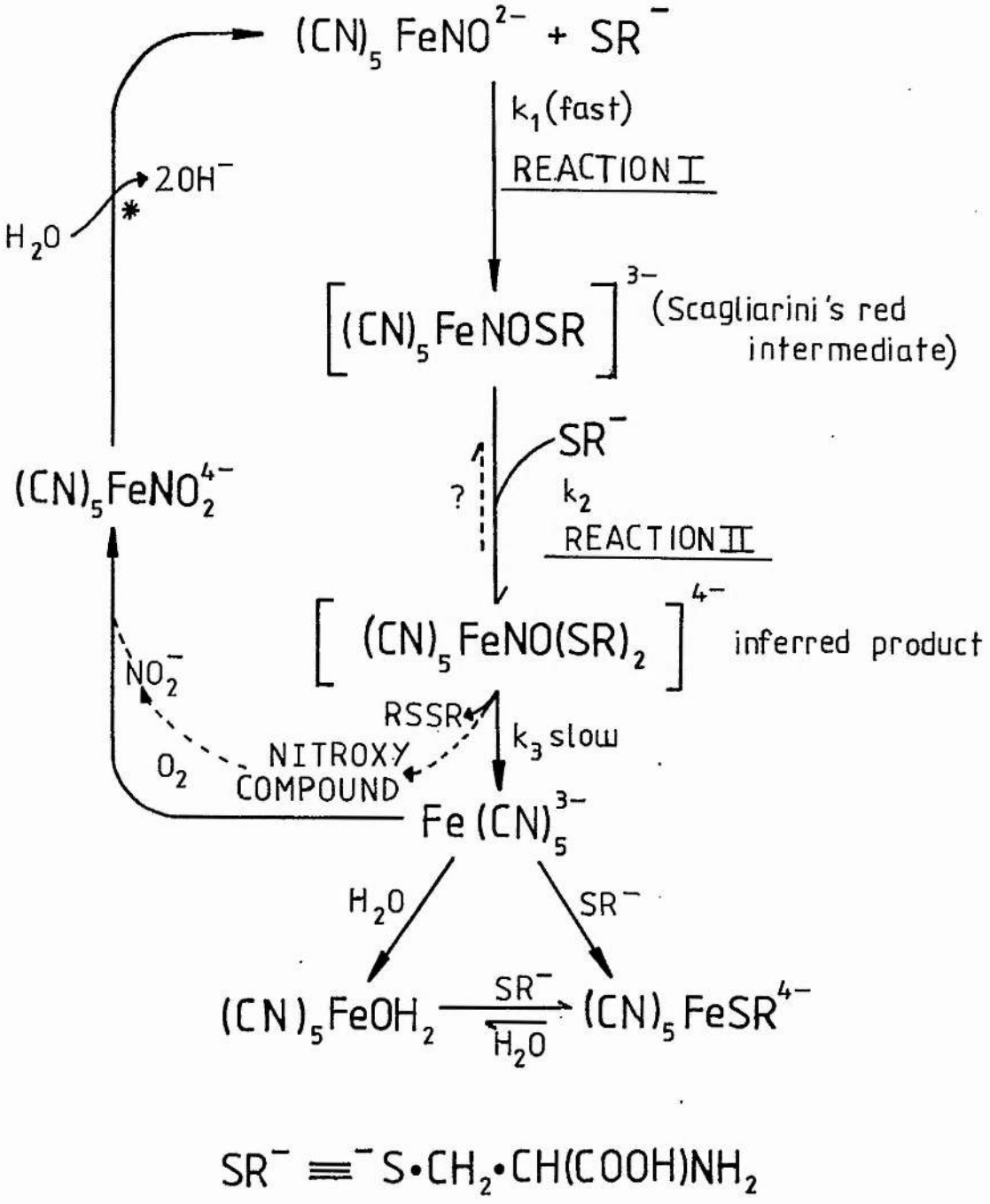
The organic product of reaction is cystine which precipitates in large amounts from the reacting solution, being highly insoluble. The proposed reaction of sodium nitroprusside and cysteine to produce thiopentacyanoferrate(II) and cysteine is shown in scheme 6. The hypothesis goes unsupported by any kinetic data, for reasons to be explained, but does not contradict any of the simple chemical evidence observed.

Reaction I. Formation of $(CN)_5FeNO.S.CH_2.CH(COOH)NH_2$. Reaction I shown in figure 17 is the formation of Scagliarini's red product. This reaction was extremely rapid, too rapid even for stopped-flow kinetic. The reaction was complete in five milliseconds or less. Although it cannot be proved at this moment in time, it is believed that this reaction is unidirectional in a similar fashion to the hydrogen sulphide system.

Reaction II. Decomposition of Scagliarini's Product.

An attempt was made to investigate the kinetics of this process using both stopped-flow (540 nm) and conventional (300 nm) kinetics. Unfortunately, there was wide scatter and poor agreement throughout the results and no mechanistic information could be determined. The

Scheme 6



$SR^- \equiv {}^-S{\cdot}CH_2{\cdot}CH(COOH)NH_2$

* Reverse hydroxide reaction taking place at low pH(9·0)

Reaction of Sodium Nitroprusside and Cysteine

cause of the scatter in the data can be attributed to oxygen in solution. Although precautions are taken oxygen will re-disolve quite rapidly during the transfer operations before the kinetic investigation starts. But for the conditions used (300 K; 1.0 M Iionic Strength; Sodium nitroprusside concentration ≈ 5×10^{-4}M; Cysteine concentration 0.10 - 0.02M) the reaction was rapid, being complete within two minutes. This reaction cannot therefore be associated with the dissociative process producing the transient species pentacyanoferrate(II), which should be its last spectral change and very slow (of the order of tens of minutes).

It is thought that another thiol species attacks the nitrosyl group producing a ternary product described in scheme 6. This species would be expected to be unstable in solid form due to its crowded nature and it is no surprise that it could not be isolated. It does, however, have some structural resemblance to a transient species observed by West[31] in the ESR spectrum ($(CN)_5FeNO(SO_3)_2^{4-}$).

Reaction III. Formation of Thiopentacyanoferrate(II).

The solutions used for studying this process were exactly the same as used in the previous section. The problem of oxygen in solution was similar and no kinetic results leading to a mechanistic argument were obtained. The reaction however was slow taking some thirty minutes to be complete, this was taken as an indication that what was being observed was a dissociative process, that is, the ejection of the hydroxylamine-N,N-dicysteinate anion. This rapidly

decomposes to produce cysteine and hyponitrous acid (or some other nitroxy compound). This dissociative process produces the now familiar $Fe(CN)_5^{3-}$ species which combines with more thiol to produce the essential inorganic product (thiopentacyanoferrate(II)).

An explanation of why oxygen should allow the reforming of one of the components in the reaction scheme is simple but difficult to prove. The hypothesis is that the hydroxylamine-N,N-dicysteinate anion is ejected from the complex (reaction III) to produce $Fe(CN)_5^{3-}$ (trapped as thiopentacyanoferrate(II)). This dicysteinate species undergoes various reactions to produce cysteine and nitrite. Scheme 6 shows the formation of cysteine and hyponitrous acid which further reacts with oxygen to give nitrite. There is no evidence for hyponitrous acid being present as an intermediate and other nitroxy compounds could be present in its place. Any nitrite produced would react with pentacyanoferrate(II) to produce nitropentacyanoferrate(II) and at the pH of these studies the nitropentacyanoferrate(II) would react further to produce the nitroprusside anion. This would rapidly complex with free thiol to produce the red intermediate. It is important to realise that the extinction coefficient of the red intermediate is very large (but impossible to measure). The only piece of this argument which can be proved is that at pH 8.5 nitropentacyanoferrate(II) reacts with cysteine to produce the red intermediate. Another point is that aquopentacyanoferrate(II) in the presence of nitrite will also react with cysteine to form the red complex. The sequence of reactions which are fact are shown below.

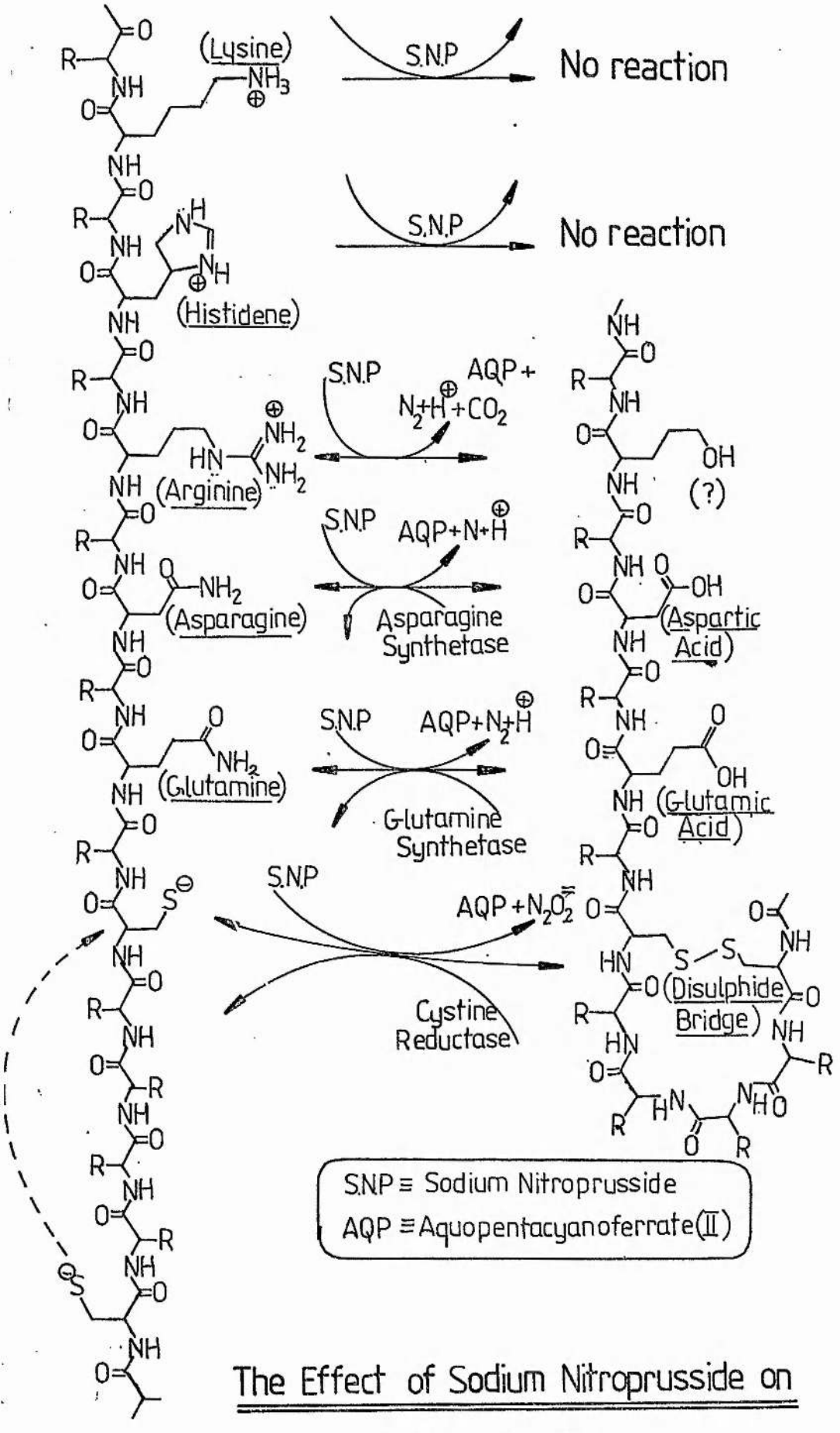
$$(CN)_5FeOH_2^{3-} \underset{H_2O}{\rightleftharpoons} [(CN)_5Fe^{3-}] \underset{\text{Intermediate}}{\overset{NO_2^-}{\rightleftharpoons}} (CN)_5FeNO_2^{4-} \overset{H_2O}{\rightleftharpoons} (CN)_5FeNO^{2-} \qquad (10)$$

If aquopentacyanoferrate(II) is mixed with cysteine, thiopentacyanoferrate(II) forms; on the addition of nitrite there is no reaction. Binding of thiols to $Fe(CN)_5^{3-}$ is stronger than nitrite.

3.2 Methionine

There are no chemical reports on reactions of this compound with sodium nitroprusside, but it would be expected to react via the amine function. Hills[32] when discussing the hypotensive properties of sodium nitroprusside suggested that the physiological response stemmed from a reaction with either cysteine or methionine via the sulphur. A reaction of sodium nitroprusside with cysteine has been documented[13] since 1933 but there has been no mention of a thiol-type reaction with this complex.

A simple spot test was employed using methionine solutions at an appropriately low pH (8-9); this minimises any amine and hydroxide reaction. Reactions of sodium nitroprusside and thiols are normally rapid producing deeply coloured intermediates (reds and blues). No reaction of this type was observed and Hills theory that nitroprusside reacts with this particular amino acid residue can be discounted.



The Effect of Sodium Nitroprusside on Amino-Acid Residues

(Figure 20)

Concluding Remarks

The reactions of sodium nitroprusside with amines has been shown to produce alcohols and nitrosamines[23] from primary and secondary amines respectively. The inorganic product of these reactions is a mixture of aquopentacyanoferrate(II) and aminopentacyanoferrate(II), the presence of amino pentacyanoferrate is dependant on steric factors. Steric factors are also important if any reaction is to take place; bulky alkyl groups prevent the approach of the amine to the nitrosyl group. A comparison between the reactions of diethylamine and ethylamine proves that the reactions of primary amines proceeds via hydrogen transfers and nitrogen production by the involvement of a diazonium salt. If this route was not used a build up of an amine-nitroprusside adduct would be expected similar to the diethylamine system and an intermediate would be observed in solution. The rate constant would be almost amine independant and the overall reaction would be slow. The reactions of sodium nitroprusside and cysteine produces cystine, thiopentacyanoferrate(II), and a nitroxy compound (the exact nature of which is still unknown).

In the introduction it was mentioned that biological implications are important. The nitroprusside anion has important therapeutic value in the treatment of hypertension. The biological response effected by this complex is believed to stem from a reaction at the smooth muscle receptors[28,29]. The six amino acids (potential muscle receptors) are now re-examined in view of the information reported here. Figure 27 shows the result of each amino acid which would be expected after interaction with sodium nitroprusside.

(a) Lysine is unreactive; as it is in the totally protonated form.

(b) Histidine is unreactive; the amine function would be expected to be too crowded similar to the indole's.

(c) Arginine would react, through various stages, to produce an unknown hydroxy amino acid.

(d) Asparagine would react to produce aspartic acid.

(e) Glutamine would react to produce glutamic acid.

(f) Cysteine requires a special biological environment to ensure that the thiol group is in the ionised form. If this is the case a disulphide bridge would be produced (assuming there is another cysteine residue in the vicinity).

Only the last three produce biologically common products. This is extremely important; if the induced condition is to pass, the amino acid residue must have a mechanism by which they can be reformed to their original structure. Figure 27 shows the enzymes which can effect the reverse reactions in (d), (e), and (f). Since (c) produces a biologically unknown product there is no simple reverse mechanism. If arginine was the receptor the condition would last a long time compared to a glutamine, asparagine, or cysteine induced mechanism.

The reaction of sodium nitroprusside with these amino acid residues would effect a conformational change in the membrane structure; this would result in a physiological response which would pass when the biological mechanism returns the membrane to its normal conformation.

The amino acids, glutamine and asparagine, would be expected to be

slightly deactivated by the ketonic group. Reactions with these two amines would be slow (compared to cysteine), but all three amino acids will produce aquopentacyanoferrate(II), in the absence of a better ligand, as the inorganic product. The amines will produce nitrogen whereas the thiol produces a nitroxy compound which will be reactive and possibly toxic. A nitroxyhaemoglobin species has been observed by Smith[33] in biological medium.

It is for these simple reasons: one, that the amine mechanism would be expected to give a slow response, and two, that a nitroxy species has been observed, that the biological action of sodium nitroprusside is believed to be a reaction with cysteine producing a disulphide bridge.

EXPERIMENTAL

Materials

Ethylamine, n-propylamine, isopropylamine, t-butylamine, benzylamine, diethylamine, tri-ethylamine, N,N,-diethylnitrosamine, cysteine hydrochloride, cystine and sodium nitroprusside were all bought commercially. Sodium nitropentacyanoferrate(II)[5] and sodium aquopentacyanoferrate(II)[6] were prepared by published methods. Amines, apart from N,N,-diethylnitrosamine, were redistilled and stored over molecular sieve.

Sodium nitropentacyanoferrate(II) was prepared by the method of Hofman[5] as follows. Sodium nitroprusside (0.3g) was dissolved in 100ml of aqueous methanolic solution (1:1) and treated with concentrated sodium hydroxide (25ml, 10 M). The solution was cooled to $2^{\circ}C$ in an ice bath where the yellow product precipitates out. The salt was filtered, redissolved in water, reprecipitated with ethanol, filtered, washed with ethanol and dried in a desiccator.

Sodium aquopentacyanoferrate(II) was prepared by the method of Hofman[6] as follows. Sodium nitroprusside (0.3g) was dissolved in 50ml of water. This was added to a solution of hydroxylamine (0.1g) in 50ml of sodium carbonate (0.1 M). After one hour, ethanol (500ml) was added and a yellow-green oil formed. The ethanol layer was decanted off and more ethanol added, this procedure was repeated until the oil became a green powder. The powder was redissolved in water and precipitated with ethanol and dried in a desiccator.

Kinetics

The kinetic studies on ethylamine, isopropylamine, t-butylamine

(higher concentration range), and benzylamine were carried out on a Pye-Unicam SP 700. The kinetic studies on t-butylamine (lower concentration range), diethylamine, and cysteine at 300 nm were carried out on a Pye-Unicam SP 8-100. The cysteine kinetics at 540 nm were carried out on a Canterbury stopped-flow apparatus and data-lab transient recorder. For all the amine kinetic studies the observed wavelength was 400 nm with additional studies on diethylamine at 580 nm. As stated previously the reactions of cysteine were observed at 540 nm and 300 nm. The u.v.-visible spectra of reactants and products were recorded on a Pye-Unicam SP 800. Rate constants were calculated by the method of Kedzy[34] and Swinbourne[35] (Appendix I).

General Kinetic Technique

Having ascertained that a reaction exists using very simple experiments on a Unicam SP 800, a concentration of sodium nitroprusside is found which gives a good spectral change.

Stock solutions of the species to be studied, in an appropriate range are prepared and the ionic strength is brought up to 1.0 M with potassium chloride. The stock sodium nitroprusside is prepared and again the ionic strength is brought up to 1.0 M with potassium chloride.

For studies involving conventional kinetics on the Unicam SP 8-100 and SP 700 the solutions were brought to the required temperature in a bath. Set equal volumes of both solutions are added together in a cuvette mixed and reaction observed. Buffering of these solutions is by an internal mechanism. The primary amines were half neutralised using analar hydrochloric acid and the cysteine was half neutralised

using sodium hydroxide (notice that the acid groups had to first be removed from the cysteine hydrochloride). Diethylamine was treated slightly different; the amine was half neutralised just before reaction commenced. There were, in this study, three solutions: an amine solution, an appropriate acid solution, and a sodium nitroprusside solution. Set equal volumes of these three solutions were added together to effect reaction. The reasons for this deviation in procedure is given in the results and discussion section.

For the stopped-flow study, solutions are decanted into reservoirs in the apparatus; these reservoirs are partially submerged in a bath to bring the solutions to the required temperature. The operation of the apparatus is by the addition of equal volumes of the two solutions rapidly in a mixing chamber, where the kinetic reaction is observed. Buffering is, as was described for conventional kinetics.

Products

1. Isopropylamine

Preparation of the products of reaction in aqueous solution for this amine, by the method of Kenney[22], gave products which were difficult to purify with no satisfactory analysis. However, spectroscopic evidence indicates that it is a mixture of nitropentacyanoferrate(II), aquopentacyanoferrate(II), and a small amount of isopropylamino-pentacyanoferrate(II).

Sodium nitroprusside (1.3g) was dissolved in 50ml of water. Sodium acetate (0.1g) and 10ml of the amine were added and mixed. After 30 minutes the mixture was added to 500ml of ethanol and cooled

in an ice bath at 2°C to precipitate a yellow powder. This was collected, redissolved, reprecipitated, washed with ethanol, and dried in a desiccator.

There is no ^{1}HNMR. Infra-red stretching frequencies (ν max) 3 550-3 400 cm^{-1} (H_2O) strong; 2 960 cm^{-1} (CH) weak (low concentration of amino product); 2 030 cm^{-1} (CN) strong, 1 620 cm^{-1} (H_2O) strong; 1 350 cm^{-1}-1 250 cm^{-1} (NO_2^-) strong.

Using the same preparation technique except with methanol as solvent gives a product which had to be treated as a crude product; no satisfactory analysis was obtained. Spectroscopic evidence indicates that it is isopropylaminopentacyanoferrate(II).

Sodium nitroprusside (0.3g) was dissolved in 50ml of methanol. Sodium acetate (0.1g) and 10ml of the amine were added and mixed. The methanolic solution precipitates a yellow product directly. This was collected, washed with ethanol, and dried in a desiccator.

^{1}HNMR (D_2O @ 25°C) 0.9 (3H), 1.2 (6H), and 2.4 (2H). Infra-red stretching frequency (ν max) 3 350 cm^{-1}-3 500 cm^{-1} (H_2O,NH) strong, 2 960 cm^{-1} (CH) strong, 2 040 cm^{-1} (CN) strong, 1 600 cm^{-1}-1 650 cm^{-1} (H_2O,CH) strong and other organic frequencies which are unassignable: 1 520 cm^{-1}, 1 370 cm^{-1}, 1 200 cm^{-1}, 1 160 cm^{-1}, 1 080 cm^{-1} - all medium.

2._ t-Butylamine

Preparation of the products of reaction in aqueous solution for this amine, by the method of Kenney[22], gave products which were difficult to purify with no satisfactory analysis. However, spectroscopic evidence indicates that it is a mixture of nitropentacyanoferrate(II) and

aquopentacyanoferrate(II).

Sodium nitroprusside (0.3g) was dissolved in 50ml of water. Sodium acetate (0.1g) and 10ml of the amine were added and mixed. After 30 minutes the mixture was added to ethanol (500ml) and cooled in an ice bath at 2°C to precipitate a yellow powder. This was collected, redissolved, reprecipitated, washed with ethanol and dried in a desiccator.

There is no ^{1}HNMR. Infra-red stretching frequencies correspond exactly to the product of aqueous hydroxide action (nitropentacyanoferrate(II)/aquopentacyanoferrate(II) mixture). 3 550 cm^{-1}-3 400 cm^{-1} (H_2O) strong, 2 030 cm^{-1} (CN) strong, 1 620 cm^{-1} (H_2O) strong, and 1 350 cm^{-1}-1 250 cm^{-1} (NO_2^-) strong.

There is no product from methanolic solution.

3._ Benzylamine

Preparation of the product of reaction in aqueous solution, by the method of Kenney[22], gave a mixed product. Spectroscopic evidence indicates that it is a mixture of aquopentacyanoferrate(II) and benzylaminopentacyanoferrate(II).

The preparation used the same amounts of sodium nitroprusside, sodium acetate and benzylamine as was used previously for the other primary amines.

The product from methanolic solution gave:

^{1}HNMR (D_2O @ 25°C) 3.6 (2H), 7.3 (5H). Infra-red stretching frequencies 3 650 cm^{-1} (NH) strong, 2 900 cm^{-1}-3 500 cm^{-1} (CH) strong, 2 050 cm^{-1} (CN) strong, 1 600 cm^{-1} (CH,NH) strong, 750 cm^{-1} and 700 cm^{-1}

(Aromatic) strong.

The product from aqueous solution gave no ^{1}HNMR. Infra-red stretching frequencies 3 650 cm^{-1} (NH) weak, 3 300 cm^{-1}-3 500 cm^{-1} (CH,H_2O) medium, 2 050 cm^{-1} (CN) strong, 1 600 cm^{-1} (H_2O) strong, 700 cm^{-1} and 750 cm^{-1} (Aromatic) weak.

4. Diethylamine

Preparation of the product of reaction in aqueous solution, by simple chemical techniques, gave products which were difficult to purify with no satisfactory analysis. However, spectroscopic evidence indicates that the major product is aquopentacyanoferrate(II).

Sodium nitroprusside (0.1g) were dissolved in 250ml of water. Sodium acetate (0.1g) and 10ml of diethylamine were added. After one hour, 75ml of the solution was filtered and lyophilised to give a crude powder. This was washed with ethanol, to remove excess sodium acetate, redissolved in a minimum amount of water and reprecipitated with ethanol. The product was collected on a filter and dried in a desiccator.

There was no ^{1}HNMR. Infra-red stretching frequencies (ν max) 3 550 cm^{-1}-3 400 cm^{-1} (H_2O) strong, 2 030 cm^{-1} (CN) strong, and 1 620 cm^{-1} (H_2O) strong.

Sodium nitroprusside (0.1g) was dissolved in 250ml of water. 10ml of diethylamine was added and after one hour excess silver nitrate was added. A yellow solid precipitated, collected, and washed with warm water @ 70°C, ethanol and ether, then dried in a desiccator.

There was no ^{1}HNMR. Infra-red stretching frequencies (νmax)

3 200 cm^{-1}-3 600 cm^{-1} (H_2O) strong, 2 020 cm^{-1} (CN) strong, 1 620 cm^{-1} (H_2O) strong.

Using methanol as solvent gives a different but related product. Ultra-violet spectroscopy indicates that the species is the same as that which persists in concentrated diethylamine-sodium nitroprusside solution (mixed solvent H_2O-$HHEt_2$) after long periods of time. The product was difficult to purify and poor analytical results were obtained; these in conjunction with the limited spectroscopic evidence were used to confirm a result consistent with bispentacyanoferrate(II).

Sodium nitroprusside (0.3g) was dissolved in 40ml of methanol and added to 10ml of diethylamine. The mixture was allowed to stand for two hours whereupon it was filtered.

10ml of the solution was taken and added to 50ml of aqueous silver nitrate solution. A purple complex precipitates which was washed with hot distilled water, ethanol and dried in a desiccator.

40ml of the solution was taken and added to 20ml of methanolic zinc acetate. An orange complex precipitates which was collected and washed with 0.1 M nitric acid to remove excess zinc acetate and other zinc salts which may be present. The complex turns green, is collected on a filter, and washed with water, ethanol and dried in a desiccator.

Analytical data for silver salt

Infra-red stretching frequencies (ν max) 3 320 cm^{-1}-3 480 cm^{-1} (H_2O) weak, 2 160 cm^{-1} (CN) weak, 2 140 cm^{-1} (CN) weak, 2 060 cm^{-1} (CN) strong, 1 650 cm^{-1} (H_2O) weak. Found: C,12.29; H,0.18; N,13.78%; $Ag_6Fe_2(CN)_{10}$ requires C,11.80; H,0.18; N,13.78%.

Analytical data for the zinc salt

No ^{1}HNMR. Infra-red stretching frequencies (νmax) 3 600 cm^{-1}-3 300 cm^{-1} (H_2O) strong, 2 080 cm^{-1} (CN) strong, 1 610 cm^{-1} (H_2O) medium. Found: C,19.41; H,1.12; N,19.08%. $Zn_3Fe_2(CN)_{10}$. $4H_2O$ requires: C,18.92; H,1.27; N,22.07%.

The zinc to iron ratio was calculated by atomic absorption Found 2.94:2. The atomic absorption result was obtained using a chemical iterative technique as the zinc and iron signals interfere with one another. A sample was analysed to give an approximate zinc and iron concentration. New standards were prepared with a constant zinc concentration for iron analysis and a constant iron concentration for zinc analysis. (The actual amounts depended on the first result.) This result can only give a zinc to iron ratio as both signals are affected by an unknown amount, making precise analytical measurements impossible.

5. Cysteine

Preparation of products was by simple precipitation techniques. Elemental analysis and spectroscopic techniques give a good correlation with sodium S-mercaptoalanylpentacyanoferrate(II) (sodium thiopentacyanoferrate(II)).

Sodium nitroprusside (0.3g) was dissolved in 50ml of water. Cystein hydrochloride (2.0g) and sodium hydroxide (3.0g) were dissolved in 25ml of water. These two solutions were added together and rigorously protected from the light. A white powder appeared in solution and was filtered off washed with warm water (@ 60°C), ethanol and dried in a desiccator.

The mother liquor was checked for further reaction by shaking (solution would turn red). Excess zinc nitrate was added to the mother liquor precipitating a green complex. This was collected on a filter, then washed with warm nitric acid (0.1 M) to remove impurities. This was filtered, washed with water, ethanol and dried in a desiccator.

Analytical data for the white powder

Insoluble - no ^{1}HNMR. Infra-red was exactly the same as a commercially bought sample of cystine. Found: C,29.78; H,4.65; N,11.28%; $[NH_2(COOH)CHCH_2S-]_2$ requires C,30.00; H,5.03; N,11.66%.

Analytical data for the zinc salt

Insoluble - no ^{1}HNMR. Infra-red stretching frequency (ν max) 3 400 cm^{-1}-3 680 cm^{-1} (NH,OH) strong, 3 120 cm^{-1}-3 240 cm^{-1} (CH) strong, 2 080 cm^{-1}-2 120 cm^{-1} (CN) strong, 1 740 cm^{-1} (C=O), 1 620 cm^{-1}-1 670 cm^{-1} (H_2O) strong, 1 500 cm^{-1} (NH) medium, 1 400 cm^{-1} (CH) medium. Found: C,20.15; H,2.37; N,17.19%; $Zn_3[Fe(CN)_5SCH_2CH(COOH)NH_2]$. $.8H_2O$ requires C,20.10; H,3.20; N,17.6%.

Atomic absorption gives a zinc to iron ratio of 2.8:2 (3:2). A similar situation to that described for the isolation of a product with diethylamine exist here. The zinc and iron ratio is the limit of the information which can be gained from atomic absorption.

CHAPTER II

REACTIONS OF SODIUM NITROPRUSSIDE in Biological Media

INTRODUCTION

Johnson[1] first described the hypotensive action of sodium nitroprusside in 1929, but it was the mid 1960's[2] before it became established as the potent rapid acting vasodilator used in anaesthesia as a treatment for hypertension to lower the blood pressure. More recently it has found wider use in the treatment of myocardial infarction (heart attacks)[3,4,5], chronic low cardiac output[6], and cardiac surgery[7,8,9] as it relaxes the arteries reducing stress on the weakened heart. On infusion of the nitroprusside anion into the vascular system an almost instantaneous reduction in blood pressure results. The compound is metabolised rapidly and consequently a safe, steady, low blood pressure can be established and maintained. Rapid metabolism is said to occur because immediately after infusion stops the blood pressure gently rises to normal and does not overshoot. The compound, being an inorganic complex is extremely water soluble and lipid insoluble, passes into the blood stream remaining there during action unlike organic hypotensive agents, which have a problem of becoming membrane bound. This association with the membrane causes problems of achieving rapid stabilisation of the blood pressure both before and after the operation as the organic compounds diffuse slowly into and out of the lipid phase.

Chemical comparisons between sodium nitroprusside and sodium nitrite have been made[10]. Both are good nitrosating agents reacting with amines[10,11], thiols[10,12], sulphite[13,14], and activated methylene[15] groups (table 1) except that sodium nitrite is effective in acidic media and sodium nitroprusside in a basic one. Although this does not affect the first three classes of compound mentioned it does limit the

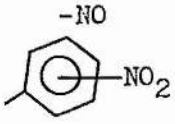
Reactions of Sodium Nitroprusside and Sodium Nitrite

Reagent	Formula	Product using Sodium Nitroprusside	Product using Sodium Nitrite	Product
Primary Amines	NH_2R	N_2 ; ROH	N_2 ; ROH	Alcohols
Secondary Amines	NHR_2	$ONNR_2$	$ONNR_2$	Nitrosamines
Thiols	RS^-	RSSR	RSSR	Dimers
Sulphite	SO_3^{2-}	$ON(SO_3)_2^{2-}$	$ON(SO_3)_2^{2-}$	Fremy's Salt
Activated Methylene Group	$R\overset{O}{\overset{\|}{C}}CH_2R$	$HON{=}CR\overset{O}{\overset{\|}{C}}R$	$HON{=}CR\overset{O}{\overset{\|}{C}}R$	Oximes

(table 1)

activating group on the methylene group to one which is media specific. Table 1 shows the carbonyl group which is one exception being active in both acidic and basic medium.

Media Specific Activating Groups

$-NO_2$	Acid media specific
$-C{\equiv}N$	Acid media specific
$-\overset{O}{\overset{\|}{C}}-R$	Both acid and basic active
$-NO$	Basic media specific
	Basic media specific

(figure 1)

The first four reagents (table 1) produce exactly the same organic product (Fremy's salt is classed as organic compound for this argument), but this is not generally true of activated methylene groups. Since sodium nitrite and sodium nitroprusside are similar in their reactions it comes as no surprise that they are both vasodilators[1], sodium nitrite being vastly inferior (possibly due to the pH of the blood stream) with the nitroprusside anion being reported as 50-1000 times more efficient. The action is associated with a nitrosation reaction at the smooth muscle receptors in particular thiol groups[16,17] (cysteine residues), but as was discussed in chapter I amines are also reactive and should not be totally dismissed. It was reported that sodium nitroprusside has toxic side effects releasing cyanide in the presence of erythrocytes (red blood cells) both in vivo[18,19,20] and more important in vitro[21,22]. Patients on long term treatment show signs of cyanide poisoning, the origin of which must be the nitroprusside ion. A number of fatalities have been reported[23,24,25] and the compound was said to be highly dangerous and extremely toxic. The liver is the site of detoxification converting cyanide to thiocyanate (figure 2), but although the liver is said to be an efficient processor, the compound is believed to be so

Method of Detoxification of Cyanide

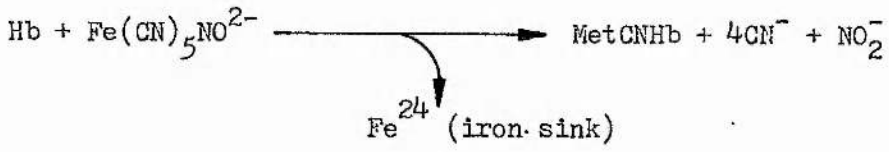
$$Na_2S_2O_3 + NaCN \xrightarrow[\text{liver}]{\text{Rhodanase}} NaSCN + NaHCO_3$$

(figure 2)

toxic that it is recommended that hydroxycobalamin[19] is used in

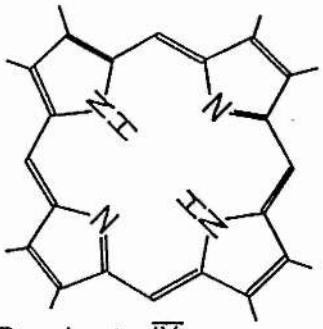
conjunction with sodium nitroprusside during therapy to "soak" up the cyanide ion produced in solution. Quantitative release of cyanide is claimed[21,22] with the cyanide concentration peaking some 30-45 minutes after infusion[20] stops (this time is dose dependant). This release is said to occur by an interaction with haemoglobin producing metcyanohaemoglobin (figure 3). This was shown by Smith[22] in his study of the u.v.-visible spectra of haemoglobin in the presence of sodium nitroprusside. The characteristic bands of haemoglobin being

Decomposition of Sodium Nitroprusside in Blood



(figure 3)

replaced, after reaction, with that of metcyanohaemoglobin. The weight of this information has led to the manufacturers[27] and users[18,20,26] giving conservative guidelines to the maximum dosage, which they stress should not be exceeded. The toxicity and hypertensive action are thought to be unrelated considering that the toxicity is an interaction with the red blood cells and the hypertensive ability an interaction with the smooth muscle receptors.

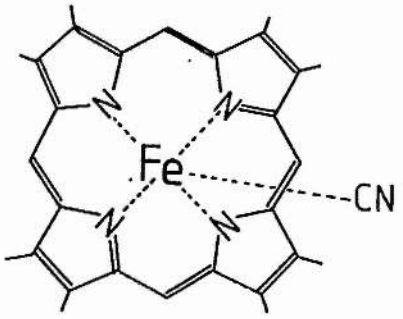


Porphyrin IX

+ $(CN)_5FeNO^{2-}$

$4(CN^-)$

+ NO_2^-



Metcyanohaemoglobin

Sequestering of Iron(II) from Sodium Nitroprusside by Porphyrin IX

(figure 4)

Formation Constants of Iron Complexes

Compound	$\log_{10}$ Formation Constant
$(CN)_5FeNO^{2-}$	30.0
$(CN)_5FeOH_2^{3-}$	22.8
$Fe(CN)_6^{4-}$	35.4
$Fe(CN)_6^{3-}$	43.6
$FeNH(CH_2CH_2NH_2)_2$	6.2
$FeN(CH_2CH_2NH_2)_3$	8.6
Fe[1,2Diamino-N-(1,2hydroxyethyl)-N-N-N-triacetic acid]	11.6
Fe[1,2Diamino,N,N,N,N-tetra-acetic acid]	14.1

(table 2)

The formation constants of sodium nitroprusside, possible products and some simple peptide complexes are given in table 2[28]. For the cyanide complexes to release their cyanide would require the formation of an iron complex with a larger formation constant than 10^{46}. Simple peptides and other ligands present in plasma do not have this capability. One candidate could be the porphyrin IX system (figure 4). This hypothesis would give the quantitative release of cyanide expected and said to occur[21,22]. But the number of porphyrin IX systems in the plasma is very low and they are not abundant enough to convert completely a normal dose as rapidly as is required. Curiously, treatment for cyanide poisoning is the administration of iron(II) sulphate; it would seem that the formation constant of hexacyanoferrate(II) is after all higher than the proposed porphyrin system. Two chemical points must be

considered. Firstly, no chemical reaction of sodium nitroprusside is known which releases cyanide and, secondly, the nitroprusside anion and its products of reaction (with hydroxide, amines and thiols) are known as d^6 substitution inert. This rules out any rapid exchange of cyanide ligands with the bulk solvent.

It would seem that we have a contradiction, medically sodium nitroprusside is toxic releasing cyanide, but chemically it is stable and should be safe. A re-investigation of the effect of erythrocytes on this normally stable inorganic complex is necessary (to discover why the complex is unstable in biological media and) to understand fully the properties required of a new inorganic based hypotensive drug. A first step will be the simple repitition of previous work using a cyanide sensitive electrode.

RESULTS AND DISCUSSION

As satated previously there are many claims in the literature[18-27] that cyanide is released by sodium nitroprusside in the presence of red blood cells (erythrocytes). This claim originated in 1929[1] and there is now no doubt in the medical sphere that sodium nitroprusside is capable of rapidly producing high, toxic levels of cyanide during therapy. The medical reports are mostly in-vivo studies except for Spiegel[21] and Smith[22], who both state that cyanide is released in-vitro when sodium nitroprusside is incubated with whole blood. For reasons outlined previously this lability is a chemically interesting occurrence, which is the only exception to a rule that low spin d^6 octahedral complexes are inert to substitution. It is not within the scope of this study to investigate the in-vivo work but it is possible to investigate Spiegel andSmith's claim that sodium nitroprusside reacts in-vitro.

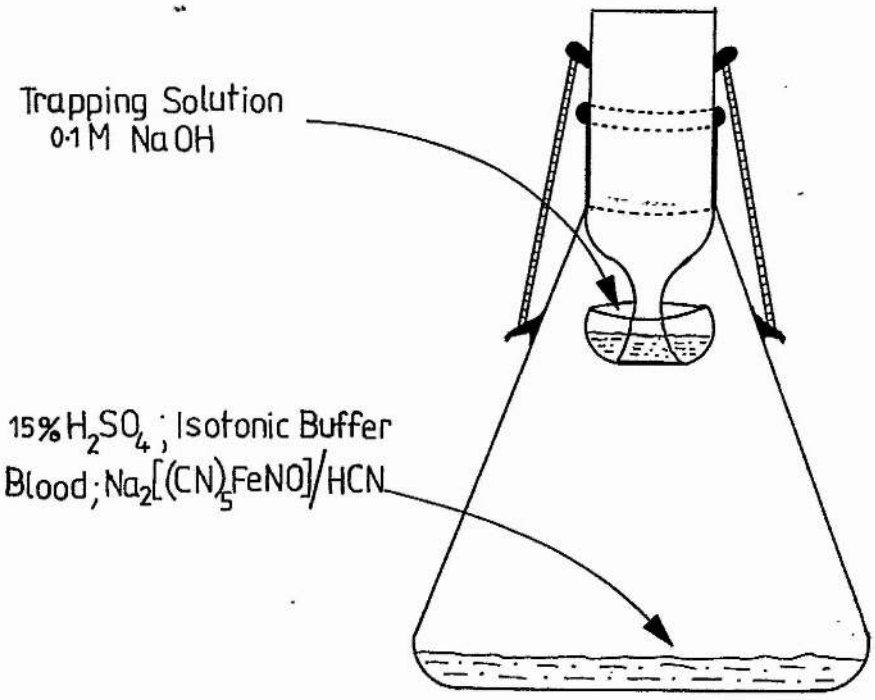
From communications with Ninewells Hospital certain important facts associated with sodium nitroprusside therapy have come to light.

#1 Patients on long term treatment, which can last several hours, do show signs of cyanide poisoning during surgery. Precautions are taken during and after the operation to prevent fatal cyanosis (cyanide poisoning). This is normally the infusion of hydroxycobalamin during the operation, with further doses in conjunction with oxygen if there is signs of cyanide poisoning afterwards.

#2 Patients would seem to build up an immunity to the drug. As the operation proceeds, the infusion rate (amount of complex being administered) is normally required to be increased to maintain a steady, low blood pressure.

3 There is no definite "type" of patient who are especially susceptible to the drug. Children and elderly patients would be expected to succumb to poisoning more rapidly than a "healthy" male adult; this is not observed. Certain patients would seem to resist nitroprusside therapy. Both Davies[23] and Jack[25] in their reports state that they could not get their respective patients blood pressure below 75mmHg to the required operating pressure of 60mmHg (normal systolic blood pressure is 120mmHg). Incidentally, both patients died in post-operative care due to cyanide poisoning.

Simple breakdown of the complex to form toxic by-products cannot totally account for this behaviour. Careful analysis and determination of the dose given should be possible with the result that cyanosis does not occur. It should be possible to predict exactly when the cyanide concentration will be at its most dangerous and prevent any complications. The simple remedy of administering high doses of hydroxycobalamin to the patient is not a satisfactory form of treatment. Hydroxycobalamin is itself toxic and its toxicity is relieved by reaction with cyanide; these two compounds must be balanced to prevent any adverse reactions. Certain "types" of patient should be at more risk; there should be more incidents of cyanide poisoning involving children. Of the four deaths reported[24,25,26,27] three were adults and one was a child. A clinical fact is that when infusion of the drug ceases, the blood pressure rises quickly to normal without overshooting the normal systolic blood pressure of 120mmHg (the blood pressure will not rise much above 120mmHg), even after many hours of therapy. This is not the behaviour expected of a drug, which is losing its effect.

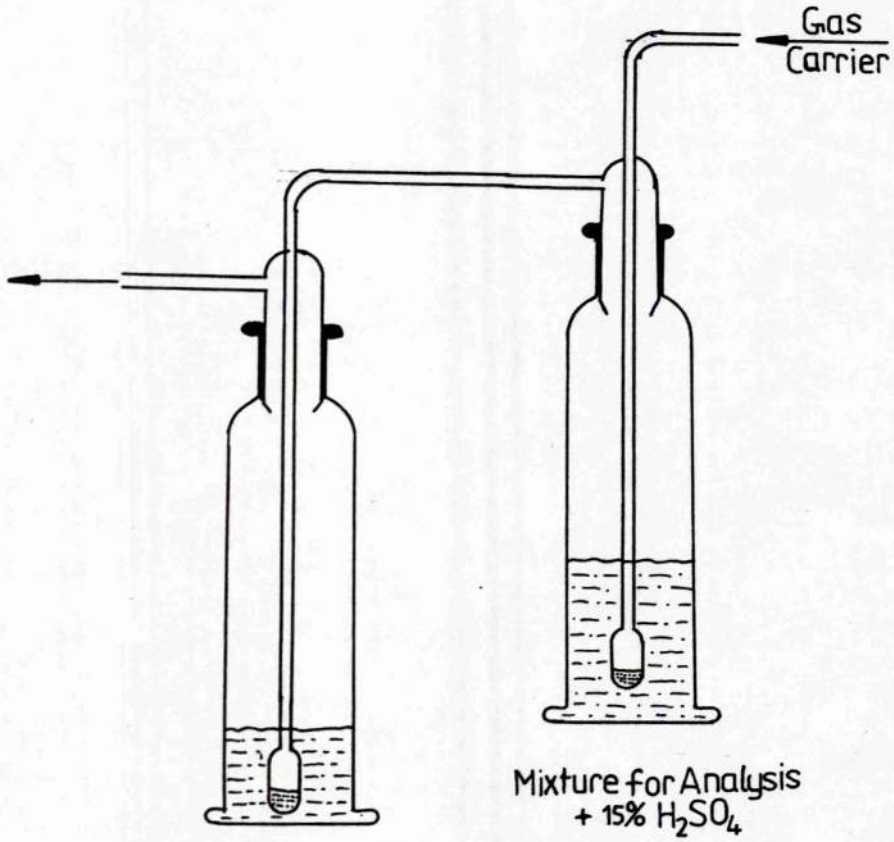


A Cavette Flask

(Figure 5)

For medical reasons this problem of cyanide production is important and for chemical reasons it is very interesting. There is no reaction of sodium nitroprusside which releases cyanide quantitatively in the manner described by Smith[22] and Spiegel[23]. A normally stable complex with a huge potential medically is in this one instance unstable in aqueous solution.

It is proposed to discuss the three methods of cyanide determination which will be used at various stages in the following experiments. The cavette flask or microdiffusion apparatus (figure 5) is the technique employed by Spiegel[21] and operates as its name suggests on a small scale. The solutions for analysis are placed on the base of the flask and treated with a small volume of sulphuric acid (15%) to volatalise the cyanide present in solution. If the solution for analysis is viscous, like erythrocyte suspensions, solvent is added (in the case of erythrocytes: isotonic buffer); this reduces the viscosity and helps diffusion. The ratio (in millilitres) of reagents for cyanide analysis in whole blood would normally be 1:4:8 H_2SO_4(15%): whole blood:isotonic buffer. A closed system is formed by fitting the stopper firmly; this stopper has a bowl attached, which holds a trapping solution of sodium hydroxide (0.3ml;0.1 M). Diffusion of cyanide is allowed to proceed for two hours, after which time all the cyanide present in the solution on the base of the flask is said to have diffused into the trapping solution in the bowl. The trapping solution is then analysed using Faulkof's technique[29] (Appendix II) for cyanide determination. This technique relies on a series of reactions initiated by the cyanide ion producing a deep blue colour (λmax 615 nm).



Modified method of Boxer and Rickards[30]

for Cyanide Analysis

(Figure 6)

The intensity of the blue colour is directly proportional to the initial cyanide concentration.

The second technique, a modification of the method of Boxer and Rickards[30], is the use of bubbler units and frittered discs (figure 6). Cyanide is flushed from solution (right) using a gas carrier and trapped in an alkali solution (left). Sulphuric acid (15%) is again used as a volatilising agent, although for reasons to be mentioned it may not be necessary. The alkali solution can be tested colorimetrically in a similar fashion to the microdiffusion experiments. Since the volume of solution is larger, the cyanide concentration can be measured directly using a cyanide sensitive electrode. This is a new piece of apparatus to these studies, all the previous work reported has relied on colorimetric determination of cyanide concentrations. The actual operation of the electrode will be described later. This is a better technique than microdiffusion as it allows for constant monitoring of the cyanide concentration and provides enough solution for the analytical results to be repeated. The cavette flask provides only enough solution for one spectrophotometric measurement.

It is generally believed that the pH has to be quite low ($\sim$4) before hydrogen cyanide will be produced. This is not true. The pK_a of hydrogen cyanide is 9.2; cyanide will diffuse from neutral and even mild alkali solution. It is the rate of diffusion which is affected by the pH. A more important effect of the acid is that it stops any further reaction of sodium nitroprusside (in-vitro) by destroying the biological material. Sodium nitroprusside is stable in acidic media; any cyanide detected cannot therefore be attributed to the acid lability

of the complex.

As mentioned briefly it is now possible to measure cyanide concentrations directly and immediately using a Corning cyanide sensitive electrode; this is in contrast to the long waiting period ($\sim$2 hours) required to isolate the cyanide from solution using the other two techniques. The electrode gives accurate results only at pH values in excess of 12; it is required that an equal volume of concentrated sodium hydroxide (2.0 M) is added to the biological mixture to quench the reaction and bring the pH up to a suitable value.

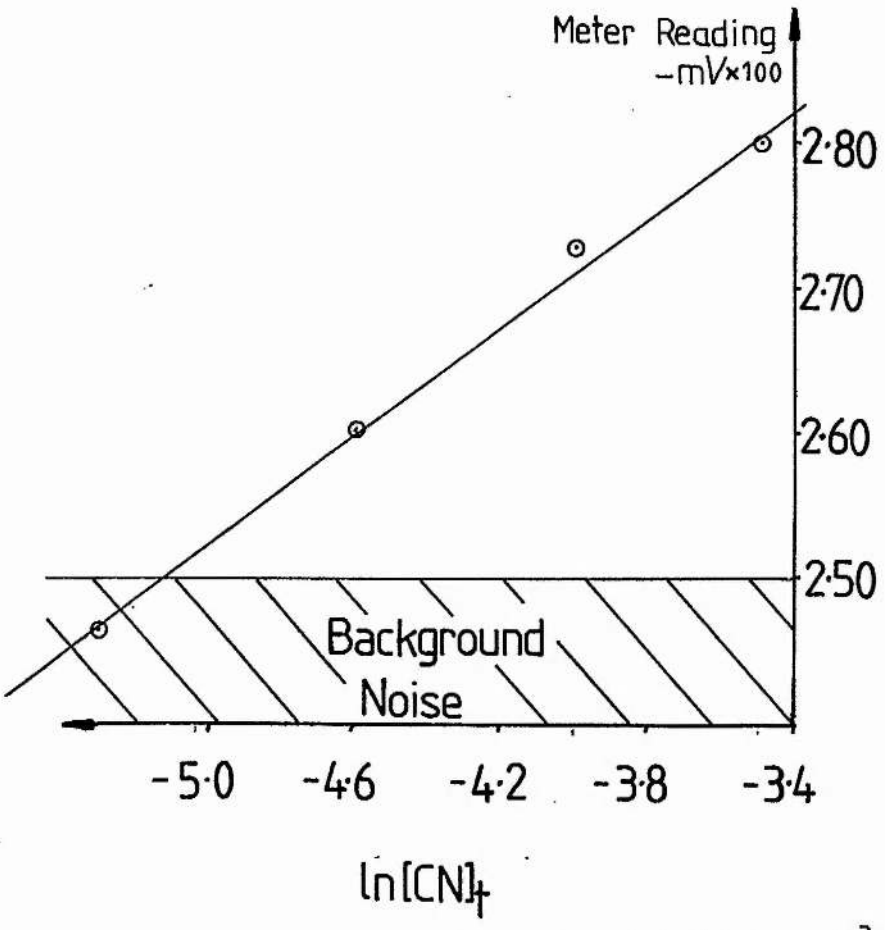
It is proposed to open this study of the lability of sodium nitroprusside in biological material by an electrode study of the behaviour of cyanide and sodium nitroprusside in biological material.

1. Electrode Studies on the Interaction of Blood Fractions with Cyanide and Sodium Nitroprusside

Before quantitative experiments can be carried out, the electrode must be calibrated in the media to be studied, namely plasma, isotonic buffer and erythrocytes (red blood cells). The calibration charts for cyanide in these various media are given in figures 7, 8, and 9, and tables 3 and 4.

The results for plasma (table 3, figure 7) were produced by simply doping plasma (10ml) with potassium cyanide and waiting a required time (10 minutes) before the solution was analysed in the usual manner.

The red blood cells and isotonic buffer as media for cyanide analysis are related. In experiments to come it is proposed to dissolve sodium nitroprusside in isotonic buffer; this will allow the introduction of sodium nitroprusside to the red blood cells without haemolysis



Minimum Cyanide Concentration Detectable = 5×10^{-3} M

Cyanide Calibration Chart for Plasma

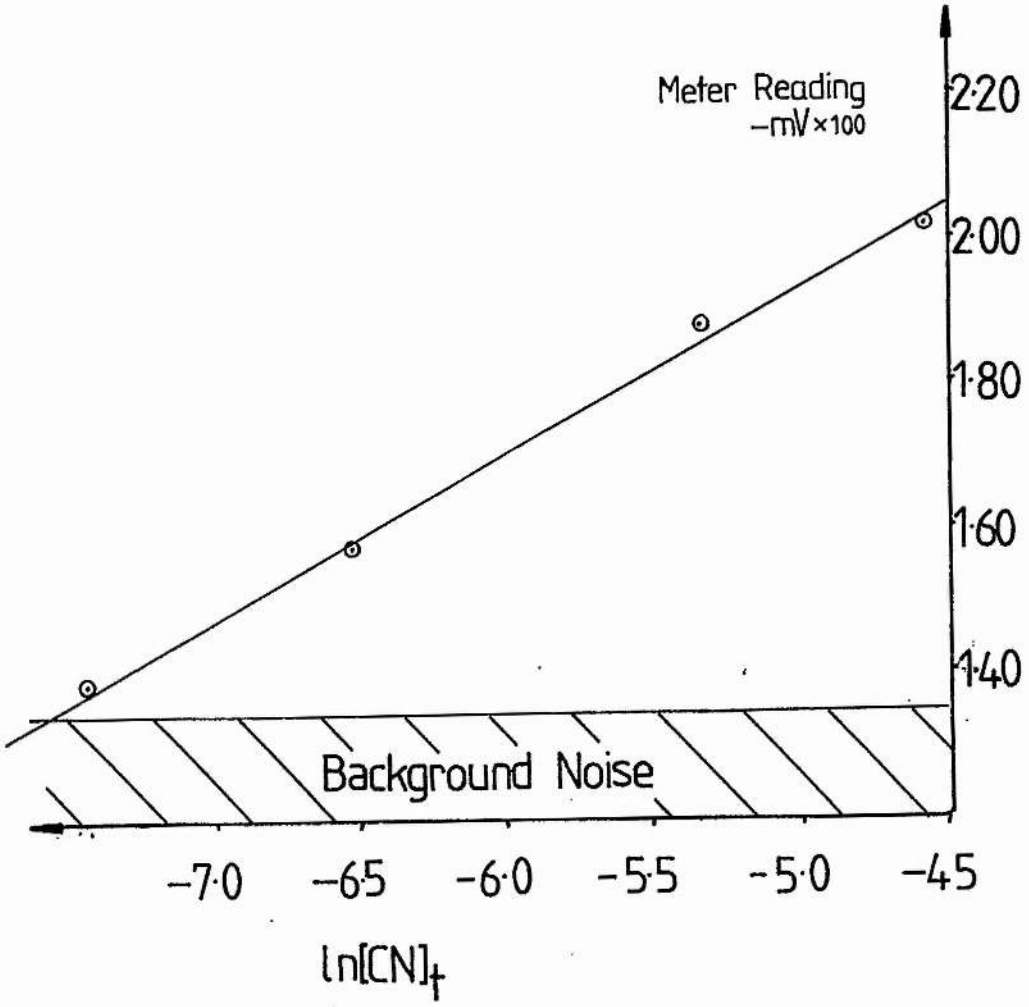
(Figure 7)

Calibration of the Cyanide Concentration in Plasma

Number of grammes of Potassium Cyanide	$[CN^-]_t$ / $10^{+2}M$	$-In[CN]_t$	Meter Reading (-mVx100)/Volts
0.000	-	-	2.514
0.000	-	-	2.542
0.000	-	-	2.485
0.0066	0.50	5.30	2.475
0.0133	1.00	4.60	2.605
0.0240	1.85	3.99	2.729
0.0396	3.05	3.49	2.803

(table 3)

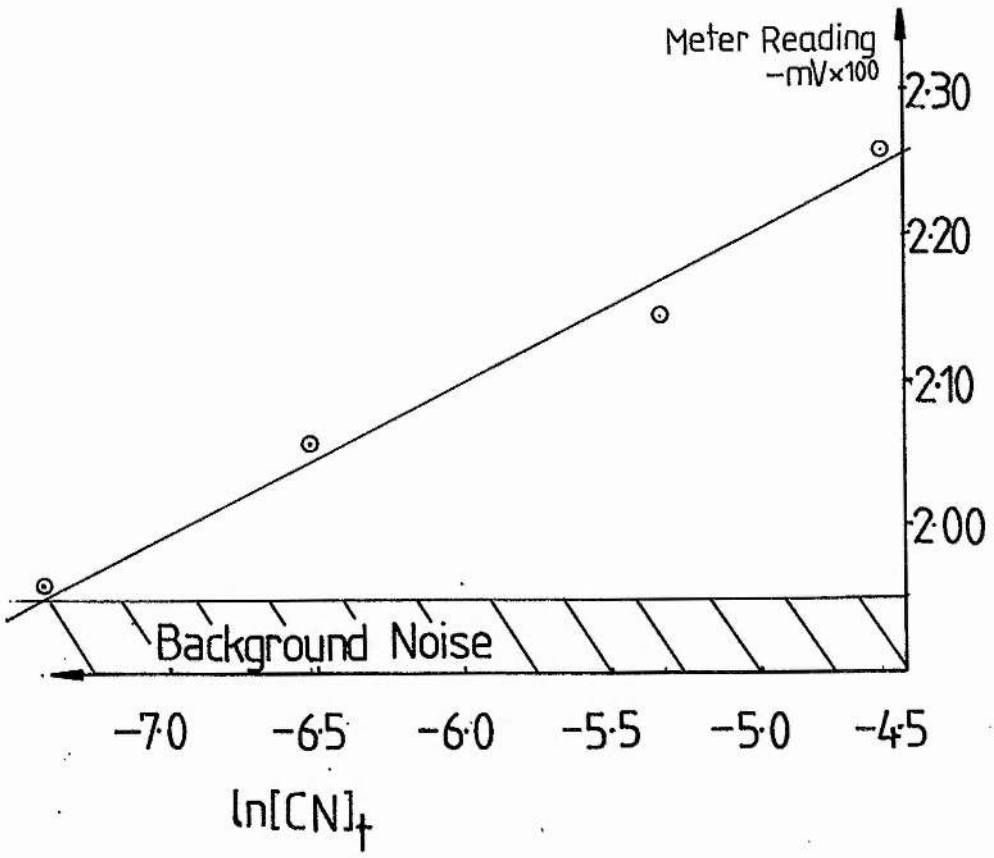
(disruption of the red blood cells). Equal volumes of blood cells and buffer will be used and any cyanide produced would be expected to partition itself between the phases. It is appropriate that the calibration procedure should be the same ensuring that any partition of the cyanide produced be monitored properly. Potassium cyanide is dissolved in isotonic buffer (20ml) producing solutions with concentrations shown in table 4. These solutions were added to erythrocytes (20ml) and allowed to stand for 15-20 minutes. There is a possibility of a reaction of cyanide and haemoglobin, which must be allowed to equilibrate. The mixture was separated in the normal fashion. Figures 8 and 9 show that partition actually takes place and is directly related to the initial cyanide concentration. In subsequent experiments any result which indicates that cyanide is present in the red



Minimum Cyanide Concentration Detectable = 5.5×10^{-4}

Cyanide Calibration Chart for Isotonic Buffer

(Figure 8)



Minimum Cyanide Concentration Detectable = $1{\cdot}2\times10^{-3}$

Cyanide Calibration Chart for Erythrocytes

(Figure 9)

Calibration of Cyanide Concentration in Isotonic Buffer and Erythrocytes

$10^{-3}[CN^-]_t/M$ (in isotonic buffer)	$-In[CN]_t$	Isotonic Buffer Reading (-mVx100)/Volts	Erythrocytes Reading (-mVx100)/Volts
0.00	-	1.347	1.968
0.00	-	1.340	1.970
0.58	7.44	1.390	1.970
1.46	6.53	1.578	2.056
4.79	5.34	1.888	2.146
10.26	4.58	2.060	2.260

(table 4)

blood cells must also produce a positive result in the isotonic buffer.

Figures 7, 8, and 9 show that it is possible to measure cyanide concentrations only in excess of a certain value in each media. This difficulty arises from what the manufacturers call interfering ions, one of the most problematic of these ions being the sulphide ion. These ions cause "psuedo" cyanide concentrations; unfortunately, thiols and sulphurous compounds are present in biological fluids and these are responsible for the erroneous cyanide concentrations. The importance of controls cannot be emphasised too strongly. The calculation of real cyanide concentrations is done by simple subtraction of the cyanide concentration caused by the control (a ghost) from the cyanide concentration recorded in the media for the corresponding experiment.

Concentrated sodium nitroprusside solutions were used for the following experiments. If a small faction of the complex is converted

to cyanide (the system is bound to exhaust itself at some point) it should be detectable using the electrode in the respective media. 100% conversions are not expected, only an indication that cyanide is being released.

1.1 Reaction of Sodium Nitroprusside in Plasma

To plasma (10ml) was added a strong sodium nitroprusside solution (1ml, 1.0 M). Three such samples were prepared and left for varying times and then treated in the normal fashion. The results are shown in table 5 and indicate that no reaction is occuring, which releases cyanide.

Reaction of Sodium Nitroprusside in Plasma

Sample	Time	Meter Reading (-mVx100)/Volts	$[CN^-]_p$/M
Control	10 minutes	2.475	0.00 (by def)
Control	10 minutes	2.415	0.00 (by def)
# 1	5 minutes	2.397	Below Zero
# 2	30 minutes	2.286	Below Zero
# 3	3 days	2.284	Below Zero

$[CN^-]_p$ = concentration of cyanide in plasma

(table 5)

The reduced meter readings can be explained. In the previous chapter the reactions of sodium nitroprusside with thiols and amines were described. If thiols (main cause of interfering ions) were to react with sodium nitroprusside, their concentration would be reduced and so

also would the background noise.

1.2 Reaction of Sodium Nitroprusside with Erythrocytes

Equal volumes of red blood cells and sodium nitroprusside (0.416 M) in isotonic buffer are mixed, allowed to stand for an allotted time, and treated in the usual manner.

Reaction of Sodium Nitroprusside in Erythrocytes

Sample	Time	Meter Reading Isotonic Buffer (-mVx100)/Volts	$[CN^-]_{IB}/M$	Meter Reading Erythrocytes (-mVx100)/Volts	$[CN^-]_E/M$
Control	15 minutes	1.104	0.000	2.019	0.000
Control	15 minutes	1.062	0.000	2.019	0.000
# 1	15 minutes	0.924	-	2.044	3.5×10^{-4}
# 2	15 minutes	0.927	-	2.046	3.5×10^{-4}
# 3	1 day	1.037	-	2.028	8.5×10^{-5}

$[CN^-]_{IB}$ = concentration of cyanide in Isotonic Buffer

$[CN^-]_E$ = concentration of cyanide in Erythrocytes

(table 6)

The results fail to support the theory that cyanide is released by sodium nitroprusside in-vitro. Although low cyanide concentrations are detected in one faction, the erythrocytes, this has no support from the more sensitive buffer fraction, which as stated previously would be expected. The supposed low conversion by one faction can therefore be attributed to experimental error.

These experiments were repeated with exactly the same result; no evidence for substantial cyanide release. The electrode operates efficiently in the media under observation, except that in each there is a minimum detectable concentration of cyanide which must be present. Taking this fact into account the electrode studies do not support the theory that sodium nitroprusside is highly unstable in the presence of blood. The results indicate that the complex is stable or at worst will only decompose very slowly producing levels of cyanide which should easily be disposed of by the liver. There should, from the evidence, be no medical hazard due to sodium nitroprusside.

2. Spot Tests on Sodium Nitroprusside - Erythrocyte Mixtures

It is proposed to use simple spot tests to produce conclusive evidence of the decomposition of sodium nitroprusside to cyanide. The experiment was conducted at very low sodium nitroprusside concentrations and the amount of cyanide produced would in some cases be impossible to detect using the electrode. There is a degree of overlap with the electrode studies as an added control. It should be possible to show that sodium nitroprusside reacts with blood components by the failure of the sodium nitroprusside spot test (Legal[31] reaction). The Legal reaction is the common name for the reaction of sodium nitroprusside and malonitrile (figure 10).

The Legal Reaction; A Spot Test for Sodium Nitroprusside

$$(CN)_5FeNO^{2-} + NC\bar{C}HCOO^- \xrightarrow{OH^-} \underset{\text{(Deep Red)}}{[(CN)_5FeNOCH(CN)COO]^{4-}} \longrightarrow (CN)_5FeOH_2^{3-} + \text{Oxime}$$

(figure 10)

The colour produced is only transient and quantitative results are impossible (the reaction is of stopped-flow scale). The detection limit is $5x10^{-5}M$. By comparison using Faulkofs[30] technique as a spot test for the cyanide anion (Appendix II), the production of cyanide should be detected in the various media by the characteristic formation of a deep blue colour.

Spot Test for Sodium Nitroprusside in Plasma

$[(CN)_5FeNO^{2-}]_t/M$	Spot Test Result	Expected Electrode Response
$1.0x10^{-3}$	Positive	Yes
$5.0x10^{-4}$	Positive	Possibly
$1.0x10^{-4}$	Positive	No

The expected electrode response; or would the electrode detect cyanide in the solution if there was a 100% conversion.

(table 7)

Solutions of sodium nitroprusside in plasma were prepared (to the values shown). These solutions were allowed to incubate at $4^{o}C$ in a fridge for 20 minutes, they were then allowed to come to room temperature before they were tested with strong malonitrile solution. Table 7 shows that there is no substantial decomposition of sodium nitroprusside in plasma.

Solutions of sodium nitroprusside in isotonic buffer were prepared. These were added to an equal volume of erythrocytes, mixed and allowed to incubate at $4^{o}C$ in a fridge for 20 minutes. The mixture was then

Spot Test for Sodium Nitroprusside in Erythrocytes

$[Fe(CN)_5NO^{2-}]_t/M$	Spot Test Result	Expected Electrode Response
$1.0x10^{-3}$	Positive	Yes
$5.0x10^{-4}$	Positive	Yes
$2.5x10^{-4}$	Positive	Possibly
$1.0x10^{-4}$	Positive	No
$5.0x10^{-5}$	Positive	No

$[Fe(CN)_5NO^{2-}]_t$ is the concentration of sodium nitroprusside in the erythrocytes/buffer mix.

The expected electrode response; or would the electrode detect cyanide in the solution if there was a 100% conversion.

(table 8)

separated in the usual manner. Only the isotonic buffer phase could be tested for sodium nitroprusside as the colour of the erythrocytes (also deep red) mask completely any reaction which should be observed. Table 8 shows that there is no substantial decomposition of sodium nitroprusside in the erythrocyte faction.

Using solutions which were identical to those used in the initial electrode studies the simple spot test for cyanide was used. The initial amount of sodium nitroprusside present is again of no consequence; what is desired is an indication that some small faction has decomposed. A control using potassium cyanide in both erythrocytes and plasma was also tested. Apart from the control all the other samples were negative. There is again no evidence for cyanide release by sodium nitroprusside in

the presence of blood factions. These simple experiments were repeated; giving exactly the same result.

The experiments reported so far indicate that cyanide is not released by sodium nitroprusside in a rapid reaction with blood, contrary to previous reports. The major difference between the experiments reported here and those previously reported is not the basic experiments but the method of analysis.

3. The Effect of Mild Acid on Sodium Nitroprusside

There is a possibility that cyanide said to be the product of biochemical action on sodium nitroprusside is being produced during analysis. As stated previously the electrode is a new instantaneous technique to studies such as these, previous studies employed cavette flasks and other diffusion apparatus which required a lengthy waiting period. Electrode experiments using long time periods failed to give an improved decomposition. Another major difference is the use of sulphuric acid to quench reaction and volatilise the cyanide. In the electrode studies sodium hydroxide is used for this purpose.

Although Hills[32] states that sulphuric acid has no effect on sodium nitroprusside it was decided to check this as a first step in a deeper investigation of the analytical techniques which depend on diffusion. A simple experiment was devised using the apparatus shown in figure 6, where a solution of sodium nitroprusside (0.1g) in sulphuric acid (100ml 15%) was purged with nitrogen using a frittered disc. The gas stream was then passed through a trapping solution of sodium carbonate (0.1 M, 100ml) again using a frittered disc. A simple control, using potassium cyanide

(0.1g) instead of sodium nitroprusside, was employed. At this point in the study fume cupboard space was being shared. My colleague was working on a photolytic oxidation of a long chain unsaturated lipid. The exact details of his experiment is the subject of another Ph.D thesis, but what is important to this study is that he was working with high light intensities (4x150W bulbs). The strong lights in such close proximity created abnormal conditions for the study being reported here; all the previous work had been carried out in normal diffuse light. It was important that this particular experiment was properly monitored and controlled. It was decided to employ one further control and cover an extra vessel containing sodium nitroprusside in foil while leaving one vessel in the light. The test for cyanide was by the colorimetric

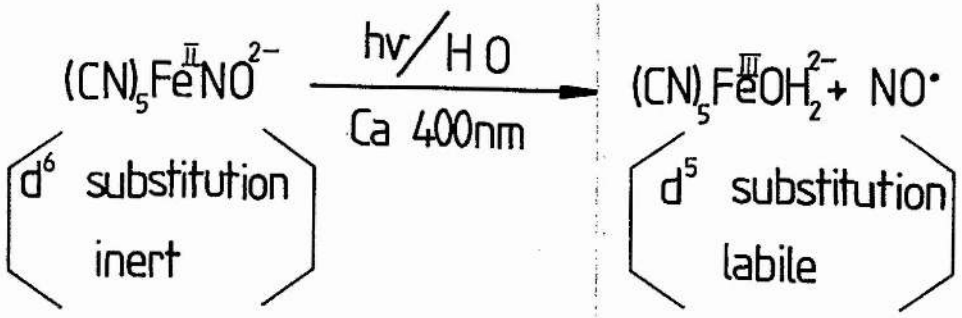
The Photolability of Sodium Nitroprusside

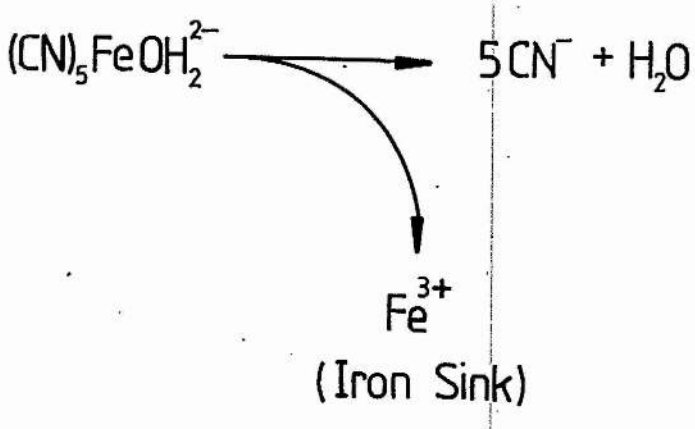
Contents of Flask	In Light or Dark	Response to Test
Sodium Nitroprusside (0.1g)	Dark	Negative
Sodium Nitroprusside (0.1g)	Light	Positive
Potassium Cyanide	Light	Positive

(table 9)

analysis of Falkof.

The result shown in table 9 mean that cyanide is released from sodium nitroprusside in the light but not in the dark. The photochemistry of sodium nitroprusside has been reported by Swinehart[33] but he used a 550 watt medium pressure mercury vapour lamp. His study shows





The Photochemistry of Sodium Nitroprusside

(Figure 11)

that the primary photochemical step to be the ejection of a nitric oxide moeity (figure 11). This is a direct result of the excitation of the iron $d_{xy}d_{yz} \longrightarrow \pi^{*}NC$ absorbance band ca 400 nm (visible region) by the absorption of a photon. The aquopentacyanoferrate(III) is d^5 substitution labile. It is not surprising that cyanide is released into solution as a result of a photochemically initiated reaction.

There has been no mention of precautions to prevent the photolysis of sodium nitroprusside in any of the work previously reported. It can only be assumed no precautions were taken and that substantial amounts of the cyanide detected are due to a photolytic reaction rather than a biochemical one. Photolysis is not a problem with the electrode studies. Mixtures were either treated within minutes of preparation or stored in the fridge in complete darkness. There was no opportunity for substantial photolytic decomposition.

It is not proposed to undertake an in-depth photochemical study of sodium nitroprusside. The elucidation of the actual mechanism and nature of the excited states involved in the mechanism are not the purpose of this study; what is important here is the photochemical production of a d^5 substitution labile species (figure 11), which is capable of cyanide release.

Are substantial amounts of the cyanide detected produced during analysis or in solution before an operation or experiment commences? If the latter is true, then much of the cyanide released, previously associated with the nitroprusside anion in biological media can be prevented by proper preparative and handling techniques. It is proposed to use the two analytical techniques described at the beginning of this

section to prove beyond doubt that sodium nitroprusside releases cyanide in the light but that it is perfectly stable in the dark.

4. Studies using Cavette Flasks

It is proposed to first use cavette flasks (figure 5) to investigate the release of cyanide from sodium nitroprusside during analysis. Solutions of sodium nitroprusside at pH 7.2, in isotonic buffer or erythrocytes, in the light or dark will be analysed for cyanide release. Cyanide will be expected to be released from solutions in the light but not in the dark. A biochemical reaction may be the reason, if substantial cyanide is detected from the mixtures of sodium nitroprusside and erythrocytes in the dark. Not only is this series of experiments an investigation of the photo-initiated decomposition of sodium nitroprusside but it is the investigation of the efficienty of an analytical technique. Analysis is by the colorimetric technique previously discussed (Appendix II) as the trapping solution has a small volume (0.3ml). All sodium nitroprusside solutions were rigorously protected from the light in the studies reported hereafter.

It is necessary to have some understanding of the efficiency of the analytical technique. This was done using two controls. The first, the diffusion of cyanide from aqueous isotonic buffer and, the second, the diffusion of cyanide from erythrocytes (table 10).

The first control (isotonic buffer media) indicates that in a two hour period allowed for diffusion only approximately three quarters of the cyanide in solution actually diffuses to the trapping solution. The second control (isotonic buffer - erythrocytes) show that in the two hour

Diffusion of Hydrogen Cyanide from Solutions

Moles of Potassium Cyanide In	$[CN^-]_t$/ M	Medium for Study	Moles of Hydrogen Cyanide Out	% Cyanide Detected
4.39×10^{-6}	1.44×10^{-3}	Isotonic Buffer	3.45×10^{-6}	78.6
4.39×10^{-6}	1.44×10^{-3}	Isotonic Buffer	3.45×10^{-6}	78.6
4.39×10^{-6}	1.09×10^{-3}	Erythrocytes	2.61×10^{-6}	59.5
4.39×10^{-6}	1.02×10^{-3}	Erythrocytes	2.44×10^{-6}	55.6
9.33×10^{-6}	1.57×10^{-3}	Erythrocytes	4.69×10^{-6}	50.3
9.33×10^{-6}	1.57×10^{-3}	Erythrocytes	4.69×10^{-6}	50.3

Isotonic Buffer medium : 12ml Isotonic Buffer/KCN sol; 1ml 15% H_2SO_7

Erythrocytes : 8ml Isotonic Buffer/KCN sol; 4ml Erythrocytes 1ml 15% H_2SO_7

(table 10)

period allowed little over a half of the cyanide present diffuses into the trapping solution.

These results are poor for an analytical technique. Previous results recorded using this technique[21] made no mention of its efficiency or indicated that the data had been corrected to compensate for it. There is no indication that the technique was calibrated using cyanide solutions. It was previously believed that the volatility of hydrogen cyanide is such that after two hours all the cyanide had diffused from solution. Hydrogen cyanide is extremely soluble in water and has a boiling point of $26^{\circ}C$[34], which although not far, is above room temperature. These two facts explain why hydrogen cyanide is not as volatile as was

previously claimed and why the results are low. In biological media there is the added complication that hydrogen cyanide reacts with haemoglobin to form metcyanohaemoglobin. If the pH is not low enough this compound will not totally dissociate producing a further depression in the percentage of the cyanide detected.

The decomposition of sodium nitroprusside in isotonic buffer was investigated to discover the maximum amount of cyanide, which could be photolytically produced and detected during analysis. Three hours were allowed to elapse in these experiments; a one hour photolytic incubation followed by a two hour analysis (in photolytic conditions). Commercially bought cavette flasks are pyrex, which has the ability to filter light of wavelengths below 325 nm. It was of some interest to discover whether a higher conversion could be produced using soda glass flasks. The results are shown in table 11.

The solutions left in the dark produce little or no cyanide. The cyanide which is produced can be attributed to experimental error or a very slow thermodynamically forbidden but kinetically possible reaction. This reaction is no biological danger and is definitely not the reason for cyanide poisoning. The liver would be able to cope perfectly with this rate of cyanide release (approximately 0.2% in 3 hours). The solutions left in the light produced approximately ten to fifteen times the amount of cyanide compared to those left in the dark. The results in table 11 are not compensated for the poor efficiency of the technique as the cyanide production is low (all the values should be multiplied by 1.3). The difference between pyrex glass and soda glass is quite marked; soda glass produces four times the amount of cyanide. Little

Photolytic Decomposition of Sodium Nitroprusside in Isotonic Buffer

Number of Grammes of Sodium Nitroprusside In	Number of Moles of Complexed Cyanide	Conditions of Reaction	$[CN^-]x10^{-4}/M$	Number of Moles of Cyanide Out	%
0.00570	$9.6x10^{-5}$	Dark	0.3	$9.0\ x10^{-8}$	0.1
0.00570	$9.6x10^{-5}$	Dark	0.2	$6.0\ x10^{-8}$	0.1
0.0298	$5.0x10^{-4}$	Dark	0.0	0.0	0.0
0.00298	$5.0x10^{-4}$	Light/Pyrex	1.47	$4.4\ x10^{-7}$	0.9
0.00298	$5.0x10^{-4}$	Light/Pyrex	1.47	$4.4\ x10^{-7}$	0.9
0.00596	$1.0x10^{-4}$	Light/Pyrex	3.3	$9.9\ x10^{-7}$	1.0
0.00596	$1.0x10^{-4}$	Light/Pyrex	3.4	1.0_2x10^{-6}	1.0
0.0298	$5.0x10^{-4}$	Light/Pyrex	8.6	$2.6\ x10^{-7}$	0.1
0.0298	$5.0x10^{-4}$	Light/Pyrex	7.5	$2.3\ x10^{-7}$	0.1
0.0298	$5.0x10^{-4}$	Light/Soda	68.8	2.0_1x10^{-6}	0.4
0.0298	$5.0x10^{-4}$	Light/Soda	46.0	$1.38x10^{-6}$	0.3

Sodium Nitroprusside was dissolved in 12ml of Isotonic Buffer and analysed in the normal fashion after a 1 hour incubation period

(table 11)

cyanide is produced during analysis due to photolysis.

The decomposition of sodium nitroprusside in the presence of erythrocytes was investigated, to discover the amount of cyanide that could be produced photolytically. Notice that the mixtures kept in the dark will monitor the presence of any biochemical reaction. The experiments were again of a three hour period (one hour incubation; two

hours for analysis), using both soda and pyrex glass flasks. The results are shown in table 12.

Photolytic Decomposition of Sodium Nitroprusside in the presence of Erythrocytes

Number of Grammes of Sodium Nitroprusside In	Number of Moles of Complexed Cyanide	Conditions of Reaction	$[CN^-]x10^{-4}/M$	Number of Moles of Cyanide Out	%
0.00358	$6.00x10^{-5}$	Dark	1.07	$3.22x10^{-7}$	0.5
0.0143	$2.40x10^{-4}$	Dark	1.90	$5.70x10^{-7}$	0.2
0.0286	$4.80x10^{-4}$	Dark	1.90	$5.70x10^{-7}$	0.1
0.0298	$5.00x10^{-4}$	Dark	4.05	$1.21x10^{-6}$	0.2
0.0298	$5.00x10^{-4}$	Dark	3.65	$1.10x10^{-6}$	0.2
0.00358	$6.00x10^{-5}$	Light/Pyrex	2.87	$8.62x10^{-7}$	1.4
0.0143	$2.40x10^{-4}$	Light/Pyrex	4.70	$1.41x10^{-6}$	0.6
0.0298	$5.0\ x10^{-4}$	Light/Pyrex	4.05	$1.4\ x10^{-6}$	0.2
0.0298	$5.0\ x10^{-4}$	Light/Pyrex	4.05	$1.21x10^{-6}$	0.2
0.0298	$5.0\ x10^{-4}$	Light/Soda	2.73	$8.2\ x10^{-7}$	0.2
0.0298	$5.0\ x10^{-4}$	Light/Soda	5.95	$1.79x10^{-6}$	0.4

Sodium Nitroprusside was dissolved in 8ml of Isotonic Buffer and added to 4ml of erythrocytes. The solutions were analysed in the normal fashion after the 1 hour incubation.

(table 12)

Within experimental error there is no difference between the results obtained in the light or dark using pyrex or soda glass. There is a

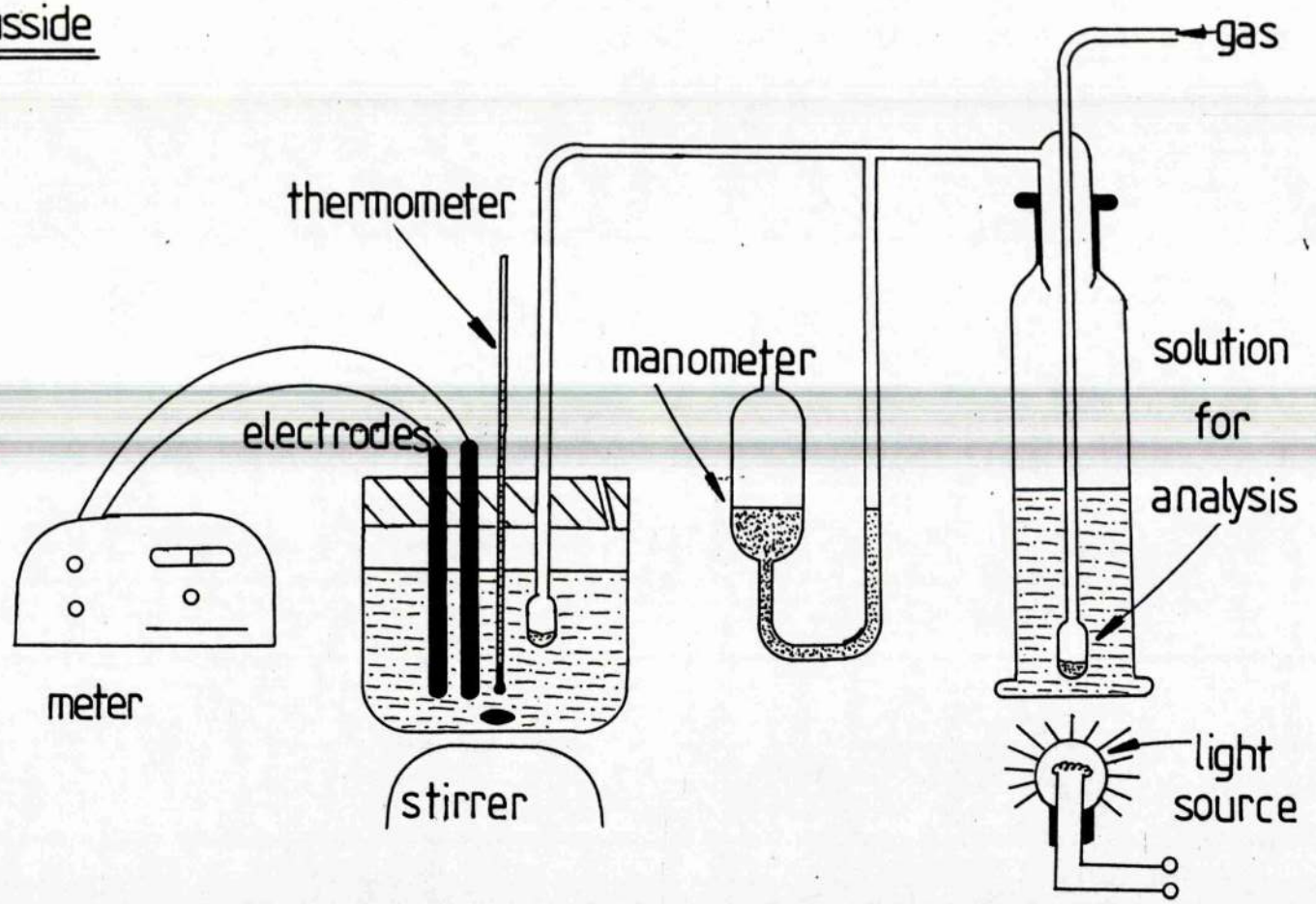
simple explanation for this, namely the main absorption band (Söret band) in haemoglobin absorbs strongly at 400 nm; this is the required wavelength for the primary photochemical step (figure 11). A reduced quantum yield would be expected to such an extent that the flasks in table 12 could be treated as if they were in the dark. The release of cyanide from sodium nitroprusside is higher in the presence of erythrocytes than in simple solution; this release of cyanide could be due to a biochemical reaction.

By keeping the sodium nitroprusside dose constant (0.0286g) and increasing the amount of erythrocytes used (table 13) in the total volume of 12ml. More cyanide should be produced (higher conversion) as the amount of erythrocytes present increases. This experiment was conducted in total darkness. Table 14 shows that no upward trend is observed and there is no evidence for a biochemical reaction producing substantial amounts of cyanide from sodium nitroprusside.

As an analytical technique cavette flasks are very poor; coupled with the inadequate controls and standardisation employed by previous workers, results obtained using this technique cannot be relied on to produce reproduceable results. The results obtained using cavette flasks agree with the previous studies (electrode and spot tests) reported here that there is no substantial decomposition of sodium nitroprusside to cyanide in the presence of human erythrocytes.

The Apparatus used to measure Photolytic Decomposition of Sodium Nitroprusside

(Figure 12)



Decomposition of Sodium Nitroprusside in varying amounts of Erythrocytes in the Dark

Number of mls of Erythrocytes	Number of mls of Isotonic Buffer	$10^{-4}[CN^-]_t/M$	Number of mls of Cyanide	%
4	8	1.90	5.70×10^{-7}	0.1
6	6	1.68	5.02×10^{-7}	0.1
7	5	3.12	9.37×10^{-7}	0.2
8	4	1.45	4.35×10^{-7}	0.1
9	3	3.50	1.05×10^{-6}	0.2
10	2	2.42	7.27×10^{-7}	0.2
11	1	2.02	6.08×10^{-7}	0.1

Total Darkness for all preparations

Number of grammes of Sodium Nitroprusside added to all flasks = 0.0286g

100% Cyanide production as a number of moles = 4.8×10^{-4}

(table 13)

5. Studies using Bubbler Units

It is proposed to use the apparatus first shown in figure 6 in a modified state to examine the behaviour of theoretical therapeutic sodium nitroprusside solutions in the period after they were prepared and before they were administered. The apparatus can also be used to prove conclusively that there is no reaction of sodium nitroprusside in the presence of haemoglobin.

Figure 12 shows the modified apparatus (shown previously in figure 6) to measure photolytic cyanide release from sodium nitroprusside. Rather than rely on a colorimetric procedure (used for the cavette flasks) the

cyanide sensitive electrode is used (Appendix III). The trapping solution was a more concentrated solution of sodium hydroxide (1.0 M), which was required for accurate measurements. A manometer is attached to the gas flow to maintain a steady stream for continuity. The flow rate was held at 0.383 litres of nitrogen per minute or 10 cm of carbon tetrachloride. A light source was positioned under the reaction vessel to effect the photolytic decomposition, this again caused a certain amount of heating ($\sim 20^{\circ}C$) which required the distance from the bulb (a standard 100W light bulb) to be carefully controlled (12 inches). Reactions which were in the dark were foil covered and heated by the same bulb. Since the technique can be used to follow the production of cyanide with different conditions over a period of time simple determination of the cyanide production is not the only information to be gained.

Swinehart states that the aquopentacyanoferrate(III) is the primary product of the photo decomposition of sodium nitroprusside (figure 11). A sample of this compound was prepared[35] in order that its behaviour can be compared to sodium nitroprusside. Since the primary photochemical step is pH independant the rate of evolution of cyanide from sodium nitroprusside and aquopentacyanoferrate(III) should be similar.

5.1 Cyanide Release from Sodium Nitroprusside and Aquopentacyanoferrate(II

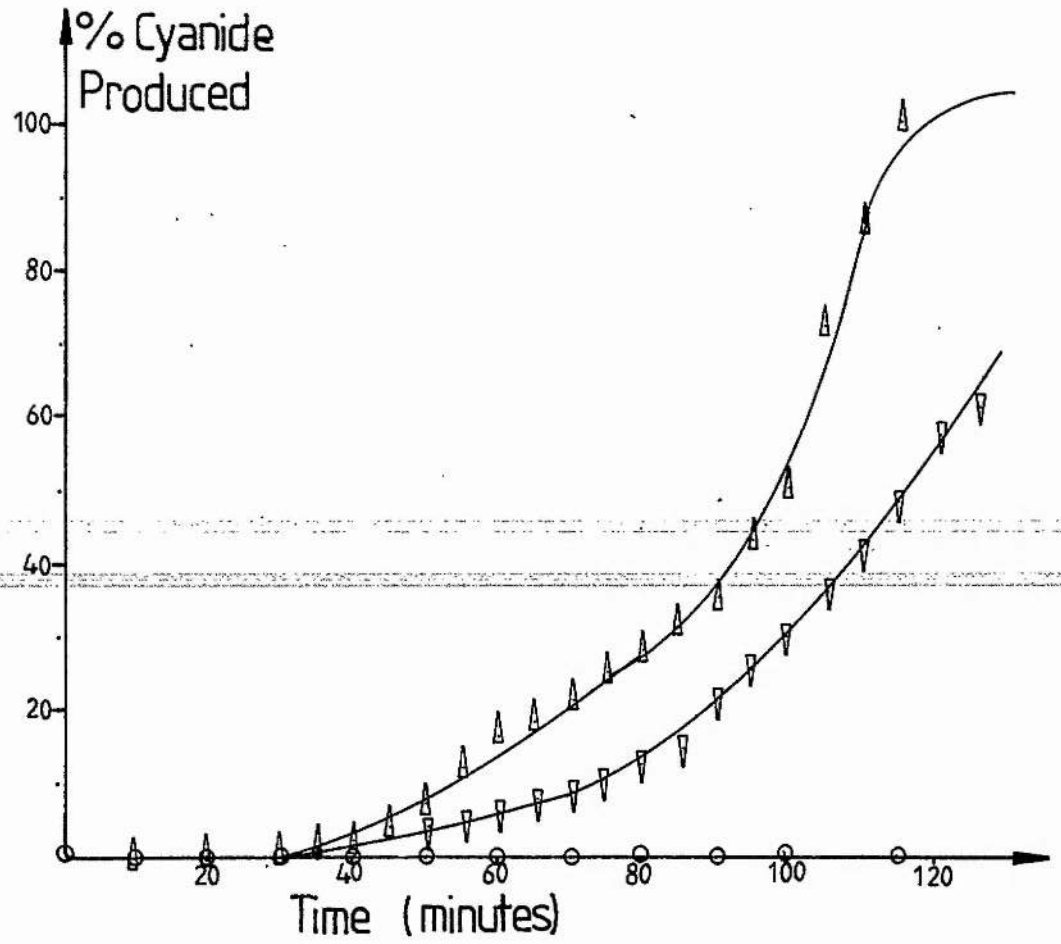
Sodium nitroprusside has been shown by simple experiments to be labile (with respect to cyanide) in the light but stable in the dark. The primary photochemical step which is rapid and pH independant can be investigated using low pH's. During photolysis the nitrosyl group is

ejected when a photon is absorbed leaving the pentacyanoferrate(III) species. Regardless of which ligand (H_2O,CN^-) replaces the nitrosyl group at the iron, the complex is now d^5 substitution labile. The cyanide groups will begin to exchange rapidly and can be trapped as hydrogen cyanide using strong acid. If the cyanide is removed as fast as the d^5 complex is formed the photolytic step becomes rate determining; this will occur at low pH's. Using bubbler units at low pH (2 and 4) the time taken to record 100% decomposition of sodium nitroprusside (as cyanide formed) is the time required to totally convert the d^6 substitutio inert species to the d^5 substitution labile species. It is intended to monitor the cyanide release from a fixed amount of sodium nitroprusside at pH 2 and 4 to investigate the primary photochemical step.

An equimolar amount of aquopentacyanoferrate(III) is treated in exactly the same fashion as the sodium nitroprusside solutions except in the dark. If this is the primary product in the photolysis scheme its reaction profile should match that of the corresponding sodium nitroprusside solution. Figure 13 and table 15 show the results gained at pH 2 and figure 14 and table 16 the results at pH 4.

A control using sodium nitroprusside in the dark was used to show that there is no chemical reaction taking place.

The two sets of data show that (using these particular conditions) the primary photochemical step is complete in two hours. There is a slight difference between the result at pH 2 and pH 4; this can be attributed to experimental error. Slight differences in the light intensity, differences in the temperature, and the flow rate which constantly required attention will be important here. The results also



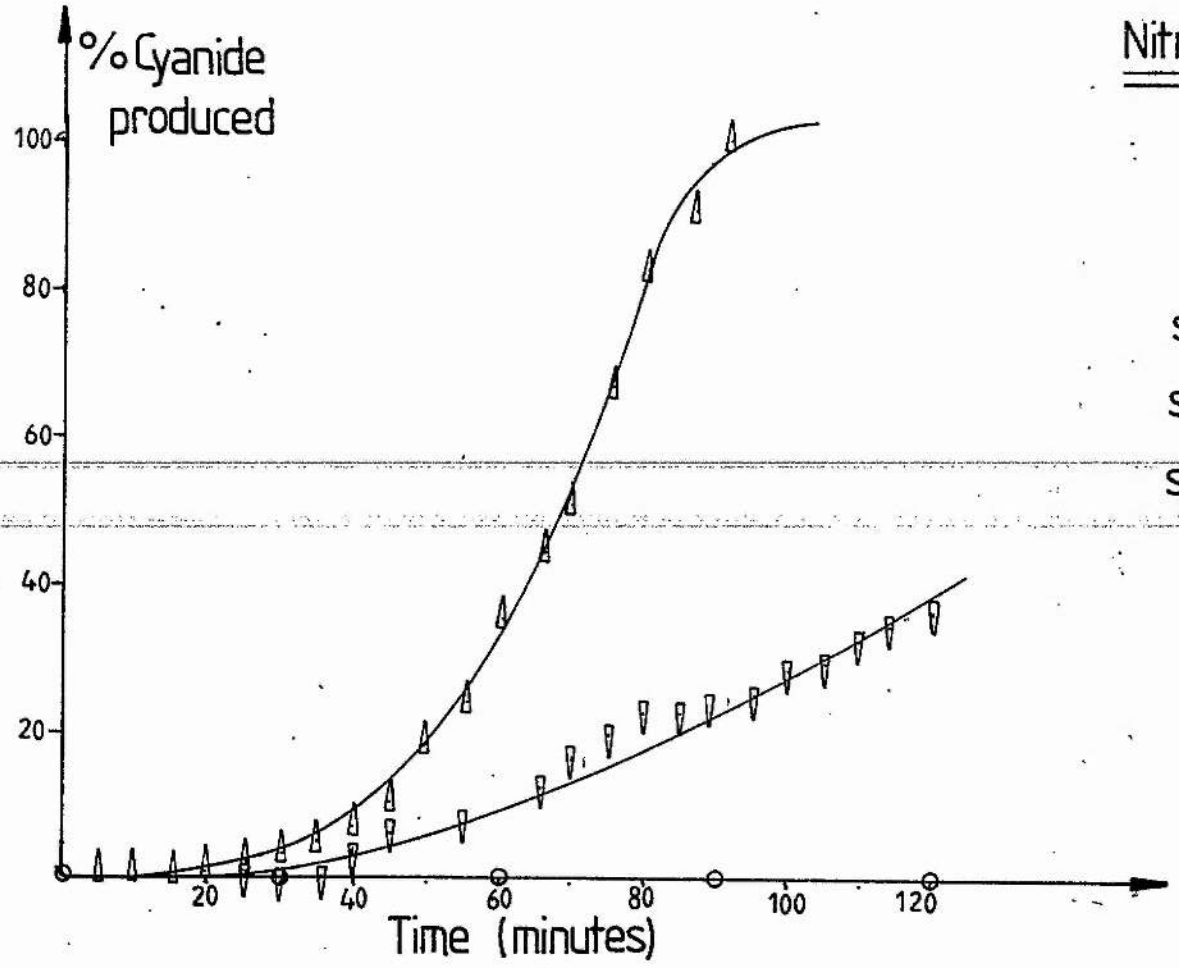
Cyanide Release from Sodium Nitroprusside at pH 2

- ⊙ sodium nitroprusside /dark
- sodium nitroprusside /light
- sodium aquopentacyano-ferrate(III) /dark

100% in 115 min

(Figure 13)

Cyanide Release from Sodium Nitroprusside at pH 4



sodium nitroprusside /dark

sodium nitroprusside /light

sodium aquopentacyano ferrate(III) /dark

100% in 90 min

(Figure 14)

0.0298g of sodium nitroprusside in 100ml of solvent

0.0279g of sodium aquopentacyanoferrate(III) in 100ml of solvent

HCl/KCl buffer at pH 2.0; gas flow rate 0.383 litre/minute

The light source was a 100W bulb at 12 inches

Cyanide concentrations measured in 25ml of 1.0 M sodium hydroxide

(table 15)

Cyanide Release from Sodium Nitroprusside and Aquopentacyanoferrate(III) at pH 2

Time	$Na_2Fe(CN)_5NOH_2O$ (Dark)			$Na_2Fe(CN)_5NOH_2O$ (Light)			$Na_2Fe(CN)_5OH_2$ (Dark)		
Min	-mVx100 Reading	No of Moles $x10^{-4}$	%	-mVx100 Reading	No of Moles $x10^{-4}$	%	-mVx100 Reading	No of Moles $x10^{-4}$	%
0	0.7045	0.001	0.0	0.8065	0.001	0.0	1.0635	0.005	0.1
5	-	-	-	0.9030	0.002	0.0	1.1150	0.006	0.1
10	0.9545	0.003	0.1	0.9655	0.003	0.1	1.1800	0.009	0.2
15	-	-	-	1.0325	0.004	0.1	1.2545	0.013	0.2
20	1.0180	0.004	0.1	1.0755	0.005	0.1	1.3315	0.018	0.3
25	-	-	-	1.1625	0.008	0.2	1.3940	0.051	1.0
30	1.0915	0.006	0.1	1.2530	0.013	0.3	1.5045	0.083	1.6
35	-	-	-	1.4180	0.057	1.1	1.5735	0.138	2.7
40	1.1515	0.008	0.2	1.5500	0.102	2.0	1.6195	0.169	3.3
45	-	-	-	1.7280	0.226	4.4	1.6935	0.192	3.7
50	1.2165	0.011	0.2	1.8335	0.361	7.1	1.7340	0.230	4.4
55	-	-	-	1.9570	0.620	12.2	1.7645	0.265	5.1
60	1.2700	0.014	0.3	2.0210	0.828	16.2	1.8120	0.327	6.3
65	-	-	-	2.0510	0.953	18.7	1.8680	0.420	8.1
70	1.3195	0.017	0.3	2.0865	1.107	21.7	1.9000	0.483	9.3
75	-	-	-	2.1145	1.260	24.8	1.9296	0.550	10.6
80	1.3590	0.020	0.4	2.1425	1.421	28.0	1.9680	0.658	12.7
85	-	-	-	2.1690	1.586	31.2	2.0000	0.757	14.6
90	1.3735	0.022	0.4	2.1950	1.789	35.2	2.0920	1.129	21.8
95	-	-	-	2.2375	2.185	43.0	2.1275	1.325	25.6
100	1.3840	0.023	0.5	2.2740	2.564	50.4	2.1645	1.571	30.2
105	-	-	-	2.3595	3.712	73.0	2.2040	1.861	35.9
110	-	-	-	2.3960	4.399	86.5	2.2355	2.141	41.3
115	1.4100	0.026	0.5	2.4325	5.163	101.5	2.2700	2.500	48.2
120	-	-	-	2. -	-	-	2.3100	2.979	57.4
125	-	-	-	-	-	-	2.3225	3.195	61.6

(table 15)

0.0298g of sodium nitroprusside in 100ml of solvent

0.0279g of sodium aquopentacyanoferrate(III) in 100ml of solvent

phthalate buffer at pH 4.0; gas flow rate 0.383 litre/minute

The light source was a 100W bulb at 12 inches

Cyanide concentrations measured in 25ml of 1.0 M Sodium hydroxide

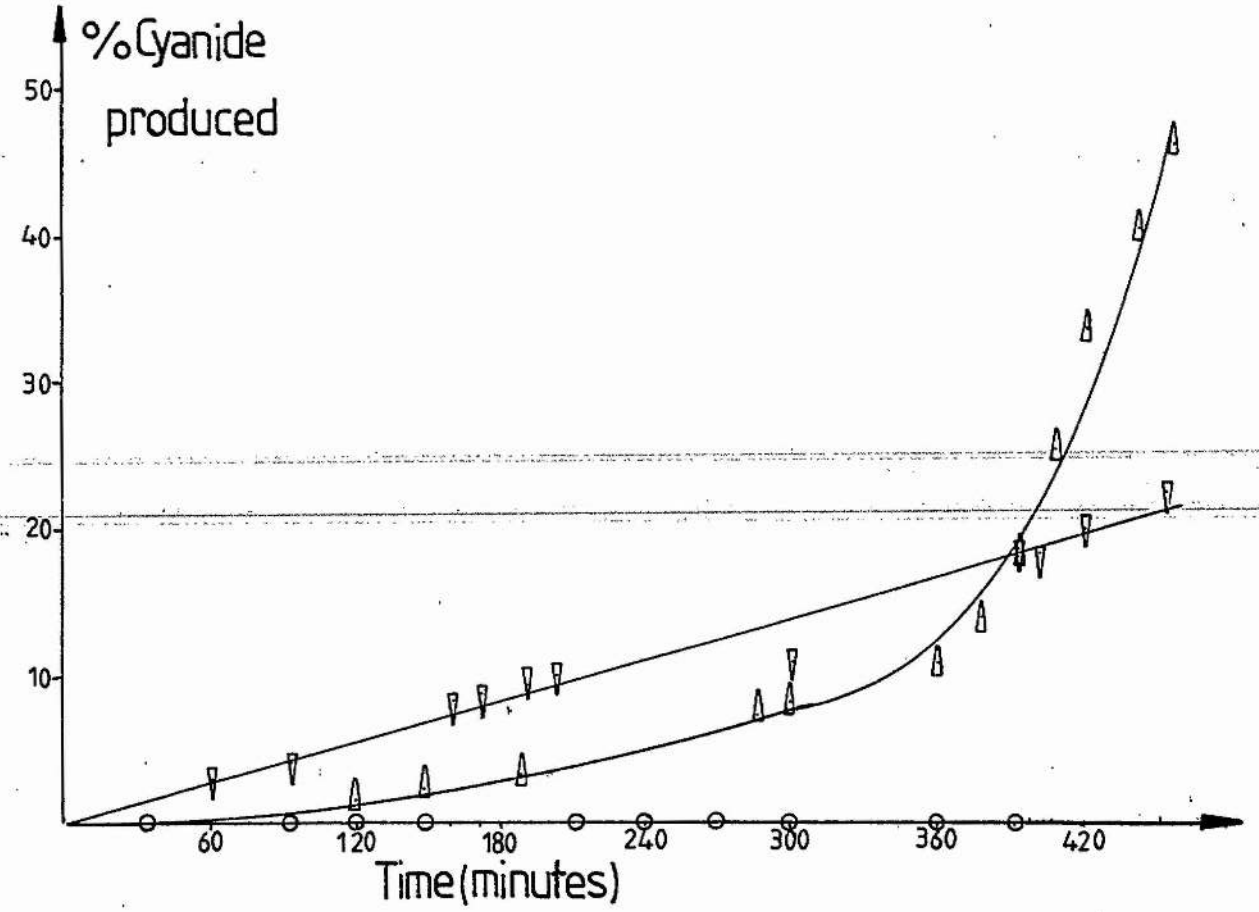
(table 16)

Cyanide Release from Sodium Nitroprusside and Aquopentacyanoferrate(III) at pH 4

Time	$Na_2Fe(CN)_5NO$ (Dark)			$Na_2Fe(CN)_5NO$ (Light)			$Na_2Fe(CH)_5OH_2$ (Dark)		
Min	-mVx100 Reading	No of Moles $x10^{-4}$	%	-mVx100 Reading	No of Moles $x10^{-4}$	%	-mVx100 Reading	No of Moles $x10^{-4}$	%
0	0.4260	0.000	0.0	0.8375	0.002	0.0	0.9665	0.003	0.1
5	-	-	-	1.1045	0.006	0.1	0.9945	0.004	0.1
10	-	-	-	1.1960	0.009	0.2	1.0600	0.005	0.1
15	-	-	-	1.3025	0.016	0.3	1.1530	0.008	0.2
20	-	-	-	1.4285	0.059	1.2	1.2550	0.013	0.2
25	-	-	-	1.5525	0.103	2.1	1.3570	0.020	0.4
30	0.6710	0.001	0.0	1.6510	0.159	3.3	1.4610	0.068	1.3
35	-	-	-	1.7540	0.254	5.2	1.5795	0.115	2.2
40	-	-	-	1.8215	0.327	6.7	1.6545	0.162	3.2
45	-	-	-	1.9005	0.487	10.0	1.7085	0.206	4.0
50	-	-	-	2.0335	0.879	18.0	-	-	-
55	-	-	-	2.1010	1.187	24.3	1.8430	0.376	7.3
60	0.8400	0.002	0.0	2.1840	1.718	35.2	-	-	-
65	-	-	-	2.2420	2.163	44.3	1.9610	0.632	12.3
70	-	-	-	2.2680	2.487	50.9	2.0240	0.836	16.2
75	-	-	-	2.3280	3.259	66.7	2.0650	1.012	19.6
80	-	-	-	2.3770	4.061	83.1	2.0950	1.152	22.4
85	-	-	-	2.4040	4.488	91.8	2.0990	1.175	22.8
90	0.9170	0.002	0.1	2.4420	5.373	109.0	2.0990	1.175	22.8
95	-	-	-	-	-	-	2.1220	1.299	25.2
100	-	-	-	-	-	-	2.1490	1.464	28.4
105	-	-	-	-	-	-	2.1590	1.540	29.9
110	-	-	-	-	-	-	2.1825	1.684	32.7
115	-	-	-	-	-	-	2.1920	1.770	34.4
120	0.9840	0.003	0.1	-	-	-	2.2000	1.843	35.8

(table 16)

Cyanide Release from Sodium Nitroprusside at pH 7·2



○ sodium nitroprusside / dark

△ sodium nitroprusside / light

▽ sodium aquopenta-cyanoferrate(III) / dark

100% in approx 10 hours

(Figure 15)

0.0144g of sodium nitroprusside in 100ml of solvent

0.0140g of sodium aquopentacyanoferrate(III) in 100ml of solvent

isotonic buffer at pH 7.2; gas flow rate 0.383 litre/minute

The light source was a 100W bulb at 12 inches

Cyanide concentration measured in 25ml of 1.0 M sodium hydroxide

(table 17)

Cyanide Release from Sodium Nitroprusside and Aquopentacyanoferrate(III) at pH 7.2

Time	$Na_2Fe(CN)_5NO$ (Dark)			$Na_2Fe(CN)_5NO$ (Light)			$Na_2Fe(CH)_5OH_2$ (Dark)		
Min	-mVx100 Reading	No of Moles $x10^{-4}$	%	-mVx100 Reading	No of Moles $x10^{-4}$	%	-mVx100 Reading	No of Moles $x10^{-4}$	%
0	0.7450	0.001	0.0	0.6380	0.000	0.0	1.0685	0.005	0.0
30	0.7780	0.001	0.0	0.7835	0.001	0.0	1.3470	0.019	0.4
60	-	-	-	1.2190	0.011	0.2	1.6440	0.154	3.1
90	0.6955	0.001	0.0	1.3650	0.021	0.4	1.7060	0.204	4.1
120	0.6780	0.001	0.0	1.4700	0.071	1.5	-	-	-
150	0.7720	0.001	0.0	1.5470	0.100	2.1	1.8405	0.372	7.4
160	-	-	-	-	-	-	1.8565	0.397	7.9
170	-	-	-	-	-	-	1.8785	0.437	8.7
190	-	-	-	1.6500	0.159	3.3	1.8900	0.464	9.2
200	-	-	-	-	-	-	1.9080	0.502	10.0
210	0.9615	0.003	0.1	-	-	-	-	-	-
240	1.0525	0.005	0.1	-	-	-	-	-	-
270	1.1070	0.006	0.1	-	-	-	-	-	-
285	-	-	-	1.8320	0.353	7.3	-	-	-
300	1.0890	0.006	0.1	1.8585	0.399	8.3	1.9440	0.589	11.7
360	1.1675	0.008	0.2	1.9155	0.517	10.7	-	-	-
375	-	-	-	1.9755	0.678	14.0	-	-	-
390	1.1920	0.009	0.2	2.0345	0.871	18.0	-	-	-
400	-	-	-	-	-	-	2.0400	0.906	18.0
405	-	-	-	2.1180	1.273	26.3	-	-	-
410	-	-	-	-	-	-	2.0560	0.943	18.7
420	-	-	-	2.1720	1.618	33.5	2.0660	1.012	20.1
435	-	-	-	2.2140	1.957	40.5	-	-	-
450	-	-	-	2.2425	2.229	46.1	2.0880	1.118	22.2

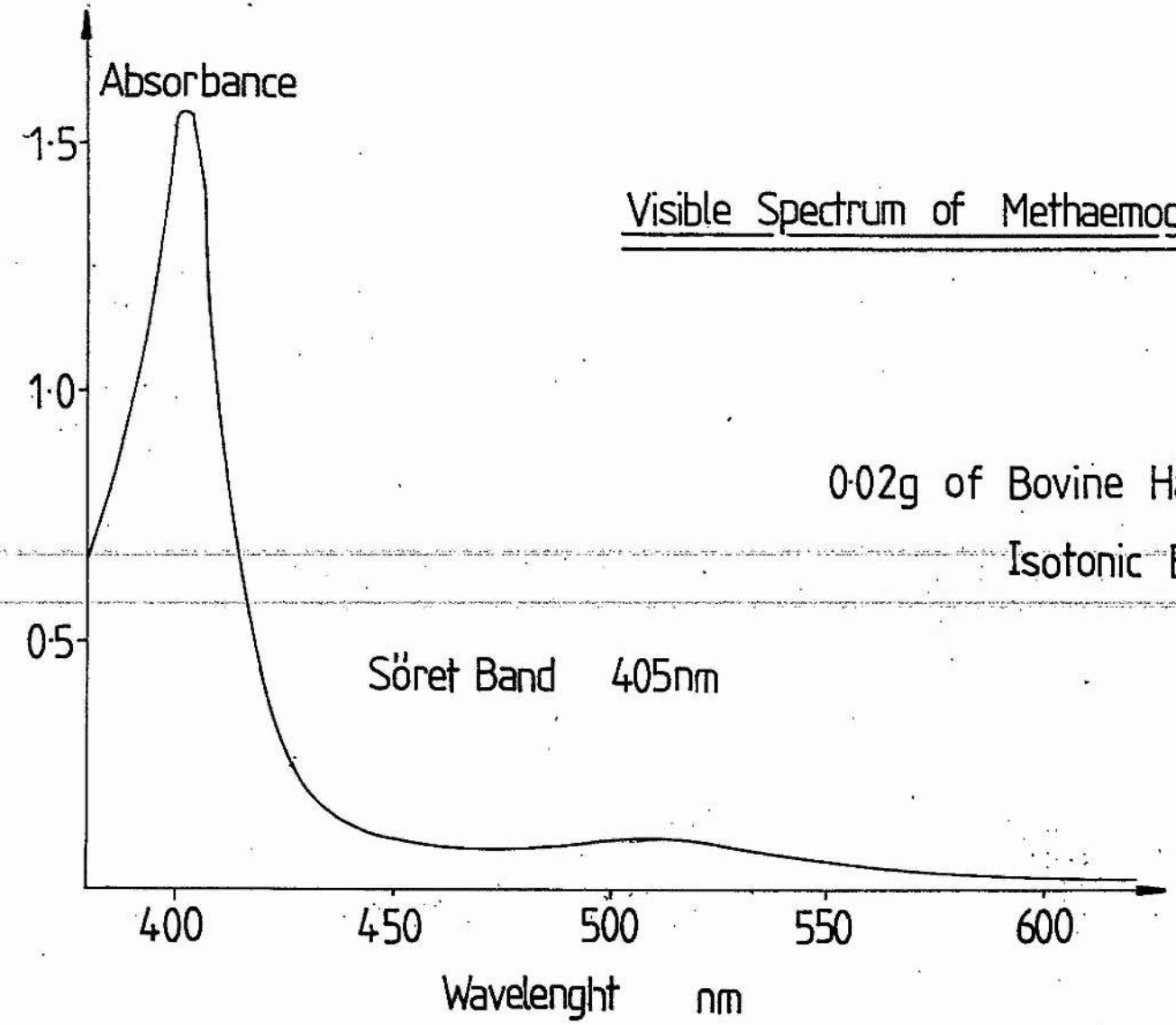
(table 17)

show that sodium nitroprusside is stable over the same period in the dark; there is no thermal excitation of electrons producing a similar dissociative process to that observed photolytically. The aquation of the pentacyanoferrate(III) species as the product of the primary photochemical step would seem to be a gross oversimplification. It is not within the scope of this study to discuss excited states and their lifetimes and the aquopentacyanoferrate(III) will still be referred to as the product of the primary photochemical step.

At physiological pH (7.2) using the same conditions the cyanide release is slower (table 17, figure 15). But as was previously the primary photochemical step is a constant process. In the first two hours all the sodium nitroprusside will have been photochemically transferred into the aquopentacyanoferrate(III). The cyanide release is slow because iron(III) salts have high formation constant (table 2); there is no longer the driving force of the high hydrogen ion concentrations. The medium will contain iron cyanide salts of varying stoichiometry. The release of cyanide from these solutions is governed by a series of equilibria shown below.

$$Fe^{III}(CN)_x^{y-} \rightleftharpoons_{Fe^{III}} CN^-_{(aq)} \overset{H^+}{\rightleftharpoons} HCN_{(aq)} \rightleftharpoons HCN_{(g)} \qquad (1)$$

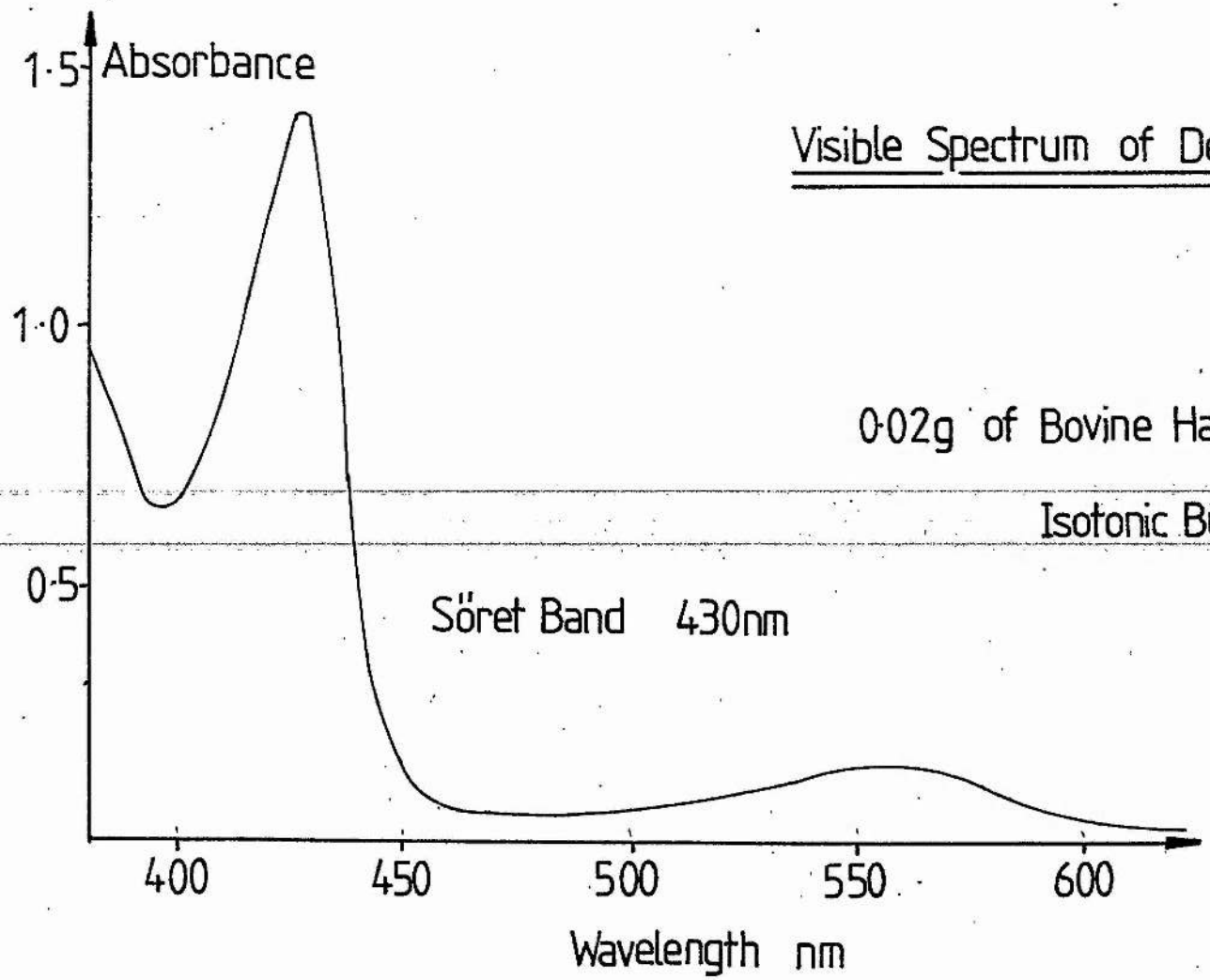
From a medical aspect these results are extremely important. Although the direct light intensities may not be high in the operating theatre, the complex will still decompose. This will produce toxic solutions containing cyanide and labile iron(III) cyanide salts; it is no surprise that patients exhibit signs of cyanide poisoning. If the



Visible Spectrum of Methaemoglobin

0·02g of Bovine Haemoglobin in Isotonic Buffer (100ml)

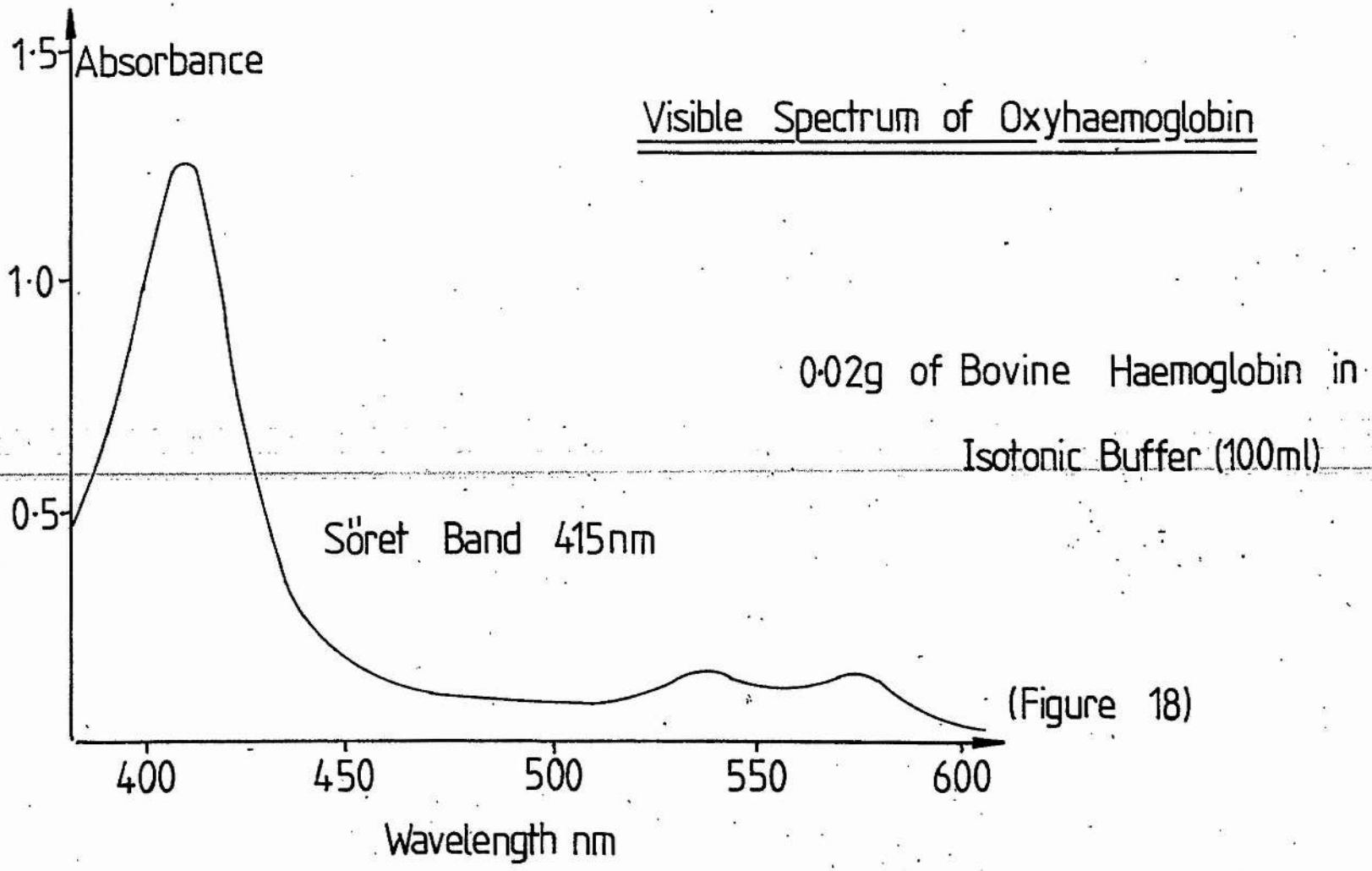
(Figure 16)



Visible Spectrum of Deoxyhaemoglobin

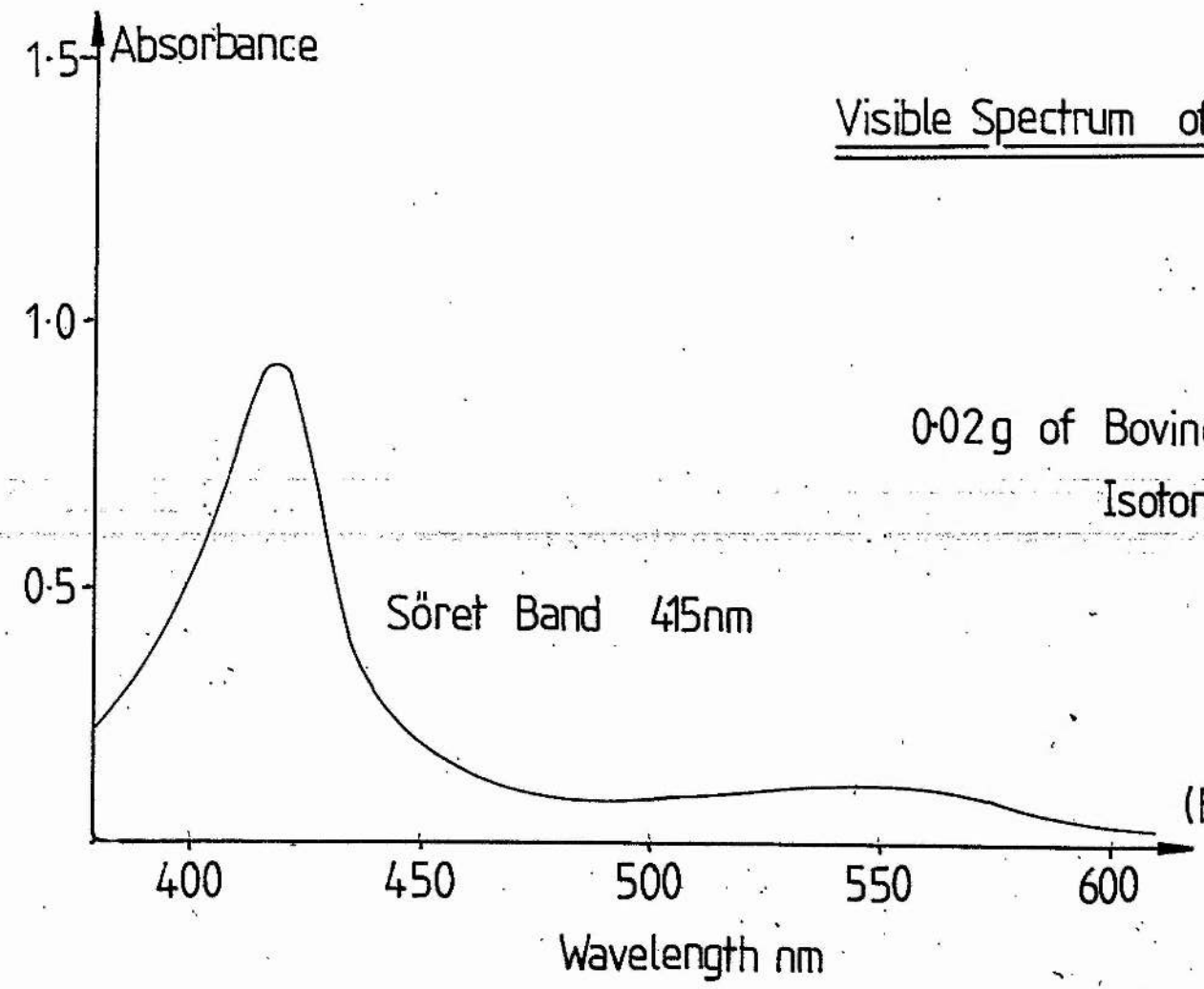
0·02g of Bovine Haemoglobin in Isotonic Buffer (100ml)

(Figure 17)


Visible Spectrum of Oxyhaemoglobin
1·5
Absorbance
1·0
0·5
0·02g of Bovine Haemoglobin in
Isotonic Buffer (100ml)
Söret Band 415nm
400
450
500
550
600
Wavelength nm

(Figure 18)

Visible Spectrum of Metcyanohaemoglobin



0·02g of Bovine Haemoglobin in Isotonic Buffer (100ml)

(Figure 19)

solutions are exposed to high light intensities during the operation, the concentration of sodium nitroprusside will steadily fall. The anaesthetist will be required to steadily increase the dosage to compensate for what he believes to be an immune reaction. If the solutions are particularly old, there will be no sodium nitroprusside left in the solutions and they will have no hypertensive action; the patients will be observed to resist the treatment.

5.2 Can cyanide release be accelerated in the presence of haemoglobin?

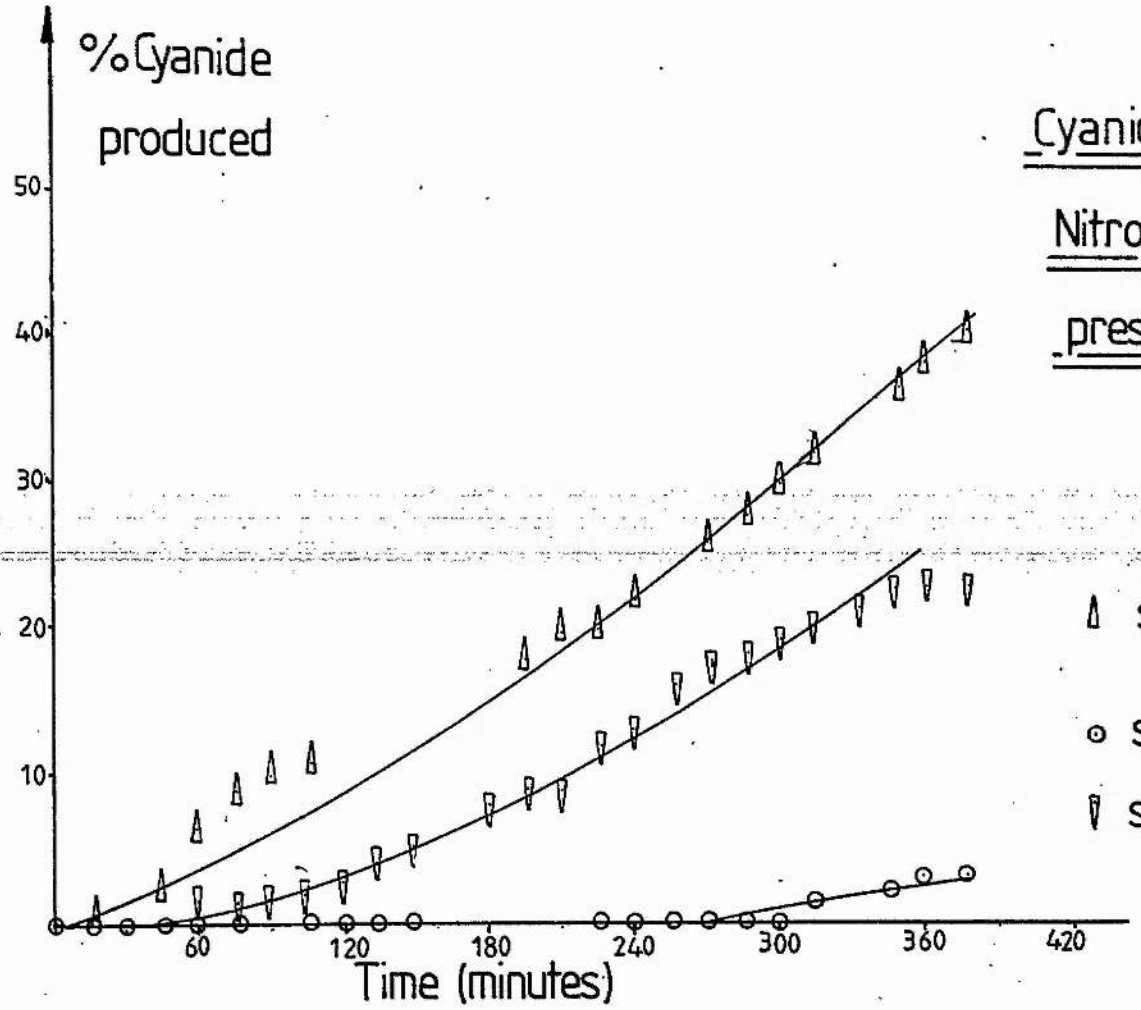
It was decided to show once again that sodium nitroprusside was stable in the presence of haemoglobin using the bubbler units. Bovine haemoglobin (solid form) was used in these experiments since the human blood would denature before the experiments had been completed. Bovine haemoglobin obtained through commercial outlets is a mixture of methaemoglobin and deoxyhaemoglobin and it was necessary to convert it to the form required chemically. Figures 16, 17, 18, and 19 show the spectra of methaemoglobin, deoxyhaemoglobin, oxyhaemoglobin and metcyanohaemoglobin.

It was now possible to study the effects if any of deoxyhaemoglobin and oxyhaemoglobin on sodium nitroprusside in the light and the dark. Unfortunately the dithionite used in the preparation of the haemoglobin species caused complications. During the analysis the dithionite decomposes to produce SO_2 and SO_3, these species are carried by the gas into the sodium hydroxide where they are trapped. This produces a "psuedo"-cyanide reading similar to those encountered during the electrode studies. To compensate for this effect a dithionite in

haemoglobin solution was treated independantly to observe the "psuedo"-cyanide readings. The cyanide values attributed to this effect were calculated and subtracted from the values obtained in sodium nitroprusside solutions to give the "corrected" values.

Haemoglobin in the oxygenated form was studied first. To prevent any natural deoxygenation of this species, the solution was purged with oxygen rather than nitrogen. Table 18 and figure 20 shows the photolytic reaction of sodium nitroprusside in isotonic buffer (light) and the reaction of sodium nitroprusside in an oxyhaemoglobin mixture in both the light and the dark.

The results show that there is no release of cyanide from sodium nitroprusside in the presence of oxyhaemoglobin in the dark. In the light there is a release of cyanide; this is not due to any biological reaction but the photolytic reaction. There is a reduced quantum yield comparing the sodium nitroprusside in isotonic buffer with the sodium nitroprusside in haemoglobin solution, this is due to the soret band (ca 400 nm figures 16-19) filtering the light. There was no change in the u.v.-visible spectrum of the oxyhaemoglobin (figure 18) solution during analysis; that is there was no cyanide absorption by the haemoglobin. This is what would be expected as high partial pressures of oxygen are used to remove species like cyanide and carbon monoxide from poisoned haemoglobin.



Cyanide Release from Sodium Nitroprusside at pH 7·2 in the presence of Oxyhaemoglobin

△ sodium nitroprusside/ light/ O_2

○ sodium nitroprusside/dark/ O_2/HbO_2

▽ sodium nitroprusside/light/O_2/HbO_2

(Figure 20)

The Photolysis of Sodium Nitroprusside in the Presence of Oxyhaemoglobin at pH 7.2

Time	Sodium Nitroprusside (Light)			Oxyhaemoglobin (Dark)	
Min	-mVx100 Reading	No of Moles $x10^{-4}$	%	-mVx100 Reading	No of Moles $x10^{-4}$ Due to Volatile Sulphur
0	0.8045	0.001	0.1	0.5043	0.000
15	1.1825	0.009	0.4	0.8250	0.001
30	-	-	-	1.0340	0.004
45	1.4260	0.058	2.5	1.2150	0.011
60	1.6660	0.154	6.6	1.3230	0.020
75	1.7200	0.217	9.2	1.4100	0.055
90	1.7430	0.238	10.1	1.5555	0.104
105	1.7540	0.252	10.7	1.6400	0.151
120	-	-	-	1.6830	0.183
135	-	-	-	1.7020	0.198
150	-	-	-	1.7100	0.208
180	-	-	-	1.7100	0.208
195	1.8685	0.420	17.9	1.7100	0.208
210	1.8800	0.459	19.5	1.7100	0.208
225	1.8830	0.464	19.7	1.7100	0.208
240	1.9135	0.512	21.8	1.7100	0.208
255	-	-	-	1.6990	0.204
270	1.9510	0.607	25.9	1.6990	0.204
285	1.9650	0.645	27.5	1.6990	0.204
300	1.9815	0.699	29.7	1.6990	0.204
315	1.9970	0.749	31.9	1.6990	0.204
330	-	-	-	1.6990	0.204
345	2.0280	0.853	36.3	1.6990	0.204
360	2.0380	0.888	37.8	1.6990	0.204
375	2.0480	0.943	40.1	1.6990	0.204

0.0144g of sodium nitroprusside in 100ml of solvent.
0.02g of bovine haemoglobin in 100ml of solvent; oxygen atmosphere
isotonic buffer at pH 7.2; gas flow rate 0.383 litre/minute

The Photolysis of Sodium Nitroprusside in the Presence of Oxyhaemoglobin at pH 7.2

Time	Sodium Nitroprusside Oxyhaemoglobin (Light)				Sodium Nitroprusside Oxyhaemoglobin (Dark)			
Min	-mVx100 Reading	No of Moles $x10^{-4}$ Calculated	No of Moles $x10^{-4}$ Actual	%	-mVx100 Reading	No of Moles $x10^{-4}$ Calculated	No of Moles $x10^{-4}$ Actual	%
0	0.8455	0.002	0.001	-	0.7915	0.001	0.001	-
15	0.9255	0.003	0.001	-	0.8775	0.002	0.000	-
30	1.0635	0.005	0.000	-	1.2545	0.013	0.009	0.4
45	1.1770	0.009	-	-	-	-	-	-
60	1.2520	0.013	-	-	1.3885	0.049	0.029	1.2
75	1.3210	0.017	-	-	1.5265	0.091	0.034	1.4
90	-	-	-	-	1.6270	0.144	0.040	1.6
105	1.4200	0.057	-	-	1.7000	0.198	0.047	1.9
120	1.4550	0.067	-	-	1.7525	0.251	0.068	2.8
135	1.4970	0.081	-	-	1.7955	0.305	0.107	4.3
150	1.5290	0.093	-	-	1.8160	0.333	0.125	5.1
180	-	-	-	-	1.8630	0.407	0.199	8.1
195	-	-	-	-	1.8780	0.441	0.233	9.4
210	-	-	-	-	1.8710	0.424	0.217	8.8
225	1.6470	0.157	-	-	1.9115	0.507	0.301	12.2
240	1.6620	0.168	-	-	1.9205	0.528	0.322	13.1
255	1.6825	0.179	-	-	1.9485	0.601	0.396	16.1
270	1.7020	0.200	-	-	1.9570	0.626	0.421	17.1
285	1.7190	0.217	0.013	0.5	1.9695	0.658	0.454	18.4
300	1.7320	0.228	0.024	1.0	1.9750	0.671	0.467	18.9
315	1.7405	0.237	0.033	1.4	1.9820	0.692	0.488	19.9
330	-	-	-	-	1.9920	0.727	0.523	21.2
345	1.7620	0.259	0.054	2.2	2.0020	0.757	0.553	22.4
360	1.7785	0.281	0.077	3.3	2.0020	0.757	0.553	22.4
375	1.7860	0.290	0.086	3.6	2.0020	0.757	0.553	22.4

The light source was a 100W bulb at 12 inches

Cyanide concentration measured in 25ml of 1.0 M sodium hydroxide

(table 18)

$$(CN)_5 Fe\,NO^{2-} + HbO_2 \xrightarrow{dark/O_2} \text{No reaction}$$

$$(CN)_5 Fe\,NO^{2-} + HbO_2 \xrightarrow{light/O_2} HbO_2 + 5CN^- + Fe^{III}(salts)$$

$$(CN)_5 Fe\,NO^{2-} + Hb \xrightarrow{dark/N_2} MetHb + (CN)_5 FeNO^{2-}$$

$$(CN)_5 Fe\,NO^{2-} + Hb \xrightarrow{light/N_2} MetcyanoHb + 4CN^- + Fe^{III}(salts)$$

HbO_2 ≡ Oxyhaemoglobin

Hb ≡ Deoxyhaemoglobin

MetHb ≡ Methaemoglobin

MetcyanoHb ≡ Metcyanohaemoglobin

The "Reactions" of the Nitroprusside anion in Blood

(Figure 21)

Deoxyhaemoglobin in the deoxygenated form was treated in a similar manner except nitrogen was used to prevent formation of oxyhaemoglobin. Table 20 shows the results obtained.

The dithionite in solution causes widely erratic results. The cyanide concentrations rise to impossible levels (100% plus) and then fall again (50%). No information can be gained from this particular study.

By observing the u.v.-visible spectra of solutions interesting information can be gained. In the dark the u.v. spectrum of the solution is initially that shown in figure 17 (deoxyhaemoglobin), this changes slowly to the spectra of methaemoglobin (figure 16). There is no metcyanohaemoglobin production and therefore no cyanide production (from sodium nitroprusside) in the dark. However in the light the spectrum of deoxyhaemoglobin (figure 17) slowly changes to metcyanohaemoglobin. Again cyanide is produced only in the light. These results are shown in figure 21.

There is no evidence for a biochemical degradation of sodium nitroprusside to iron salts and cyanide.

Interaction of Sodium Nitroprusside and Deoxyhaemoglobin

Time	Sodium Nitroprusside (Light)			Deoxyhaemoglobin (Dark)	
Min	-mVx100 Reading	No of Moles $x10^{-4}$	%	-mVx100 Reading	No of Moles $x10^{-4}$ Due to Volatile Sulpher
0	0.8045	0.001	0.1	0.9290	0.003
15	1.1825	0.009	0.4	1.2750	0.014
30	-	-	-	1.5810	0.121
45	1.4260	0.058	2.5	1.7445	0.242
60	1.6660	0.154	6.6	1.8205	0.340
75	1.7200	0.217	9.2	1.9040	0.502
90	1.7430	0.238	10.1	1.9475	0.601
105	1.7540	0.252	10.7	2.0865	1.118
120	-	-	-	2.0700	1.032
180	-	-	-	2.0300	0.871
195	1.8685	0.420	17.9	2.0300	0.871
210	1.8800	0.459	19.5	2.0300	0.871
225	1.8830	0.464	19.7	2.0300	0.871
240	1.9135	0.512	21.8	2.0300	0.871
255	-	-	-	2.0300	0.871
270	1.9510	0.607	25.9	2.0300	0.871
285	1.9650	0.645	27.5	2.0300	0.871
300	1.9815	0.699	29.7	2.0300	0.871
315	1.9970	0.749	31.9	2.0300	0.871
330	-	-	-	2.0300	0.871

Interaction of Sodium Nitroprusside and Deoxyhaemoglobin

Time	Sodium Nitroprusside Deoxyhaemoglobin (Dark)				Sodium Nitroprusside Deoxyhaemoglobin (Light)			
Min	-mVx100 Reading	No of Moles $x10^{-4}$ Calculated	No of Moles $x10^{-4}$ Actual	%	-mVx100 Reading	No of Moles $x10^{-4}$ Calculated	No of Moles $x10^{-4}$ Actual	%
0	0.5600	-	-	-	0.8795	-	-	-
15	-	-	-	-	2.0410	0.906	0.892	38.0
30	2.1600	1.524	1.403	59.7	2.1930	1.753	1.632	69.5
45	-	-	-	-	2.2400	2.184	1.942	82.6
60	2.2000	1.843	1.503	64.0	2.2600	2.390	2.050	87.2
75	-	-	-	-	2.2650	2.463	1.961	83.5
90	2.2180	1.684	1.083	46.1	2.3390	3.392	2.791	118.7
105	-	-	-	-	2.3220	3.292	2.174	92.5
120	2.2000	1.843	0.811	34.5	2.3220	3.292	2.260	96.2
180	2.1740	1.635	0.764	32.5	2.2740	2.513	1.642	69.9
195	-	-	-	-	2.2670	2.463	1.592	67.7
210	-	-	-	-	2.2670	2.463	1.592	67.7
225	-	-	-	-	2.2670	2.463	1.592	67.7
240	2.1790	1.684	0.813	34.6	2.2480	2.274	1.403	59.7
255	-	-	-	-	2.2480	2.274	1.403	59.7
270	2:1855	1.719	0.848	36.1	2.2360	2.141	1.270	54.0
285	2.1855	1.719	0.848	36.1	2.2360	2.141	1.270	54.0
300	2.1855	1.719	0.848	36.1	2.2235	2.017	1.146	48.8
315	2.1855	1.719	0.848	36.1	2.2235	2.017	1.146	48.8
330	2.1855	1.719	0.848	36.1	2.2235	2.017	1.146	48.8

0.0144g of sodium nitroprusside in 100ml of solvent

0.02g of bovine haemoglobin in 100ml of solvent; nitrogen atmosphere

isotonic buffer at pH 7.2; gas flow rate 0.383 litre/minute

The light source was a 100W bulb at 12 inches

Cyanide concentration measured in 25ml of 1.0 M sodium hydroxide

(table 19)

Concluding Remarks

Sodium nitroprusside has been proved to be totally stable in the presence of haemoglobin in all its forms in the dark. We are not qualified to extend this study to experiments on animals and it is therefore recommended that a re-examination of the in-vivo results is made, to show that in the absence of light falling on solution (before, during, and after experimentation) no cyanide is released.

Using the information collected in chapter I; there is no mild chemical reaction which changes the oxidation state of the iron from II to III (creating a labile species). All the products of the reactions described in chapter I are d^6 substitution inert. It would be extremely surprising if a reaction existed in the human body which can effect this change in oxidation state. An explanation would also be required of why the iron in haemoglobin is not also totally in the iron(III) oxidation state. (Binding of oxygen to iron(III)-haemoglobin species is irreversible; respiration would be impossible.)

It has been shown that the analytical procedures are not the main cause of cyanide production and the cyanide detected in solution. The solutions are well protected by the media (haemoglobin; Söret bands ca 400 nm) from the light. The majority of the cyanide is formed in solution in the pre-operative and pre-experimental stages, where it is believed that the nitroprusside solutions are allowed to stand in the light (of varying intensities and varying lengths of time). In communications with Ninewells it was said to be normal procedure for the sodium nitroprusside solutions to be prepared well in advance ($\sim$2 hours) of the operation with no precautions in the intervening time to prevent

photolysis. The erratic behaviour of the physiological responses of the patients to the complex is not surprising. The behaviour can be attributed to bad handling techniques before the operation proceeds. It is suggested that if the complex is to be used, it is rigorously protected from light. As a partial solution to the problem, packaging could be developed which has a strong absorption at 300 nm-500 nm to compete with the $d_{xy}d_{xz} \longrightarrow \pi^*NO$ ca 400 nm which is responsible for the photodecomposition. The problem of the photolytic degradation cannot totally be eradicated only minimised. This situation is not satisfactory for a complex which is being administered therapeutically and it is suggested that a non-cyanide based replacement for sodium nitroprusside be found.

EXPERIMENTAL

Materials

Bovine haemoglobin, chloramine T, dilydrogen sodium phosphate, disodium hydrogen phosphate, malonitrile, 3-methyl-1-phenyl-2-pyrazol-5-one, potassium cyanide, potassium hydrogen phthalate, pyridine, sodium nitroprusside and sulphuric acid were all bought commercially. Isotonic buffer, aquopentacyanoferrate(III)[35] and the forms of haemoglobin[36] (methaemoglobin, deoxyhaemoglobin, oxyhaemoglobin and metcyanohaemoglobin) were prepared by documented methods. Human erythrocytes were acquired from John Reglinski and Dr. A. R. Butler.

Isotonic buffer is the common name for 0.020 M phosphate buffer in 0.9% sodium chloride solution. This should have a pH of 7.2 with an ionic strength approximately equal to that of the blood stream. This is important as biological components like erythrocytes are irreparably damaged if they are not in solutions at this pH and ionic strength. The phosphate used is two parts sodium dilydrogen phosphate to three parts disodium hydrogen phosphate in 9% NaCl total phosphate concentration 20 M.

Sodium aquopentacyanoferrate(III) was prepared from sodium nitroprusside via the aquopentacyanoferrate(II) species[37]. Sodium nitroprusside (0.3g) was dissolved in 50ml of water. This was added to a solution of hydroxylamine (0.1g) in 50ml of sodium carbonate (0.1 M). After one hour, ethanol (500ml) was added and a yellow-green oil formed. The ethanol layer was decanted off and more ethanol added, this procedure was repeated until the oil became a green powder (aquopentacyanoferrate(II)). The powder was redissolved in water and precipitated with ethanol and dried in a desiccator.

The total product is dissolved in 10ml of water and treated with excess bromine. This forms the intensely blue bispentacyanoferrate(III) species. The excess bromine is allowed to evaporate off and the solution is treated with ethanol (100ml) which forms a blue oil. Further treatment with 100ml aliquots of ethanol produces a stick blue material which can be further dried in a desiccator.

An approximately 5×10^{-3}M solution of the blue dimer is treated with sodium hydroxide (0.25 M) and allowed to stand for 1-2 hours or until the deep blue solution turns pale yellow. Careful treatment with ethanol produces a yellow solid from solution which has the formula $Fe(CN)_5OH^{3-}$. This product is redissolved in a minimum amount of water and reprecipitated using ethanol, dried in a desiccator and stored in the dark. This hydroxy-complex is converted in solution to the aquo-pentacyanoferrate(III) species by the action of acid just before use. The aquopentacyanoferrate(III) species is extremely unstable in solid form, partially decomposing to iron oxide and cyanide and partially reforming the bispentacyanoferrate(III) species.

The commercially bought haemoglobin was converted using well documented methods to (a) methaemoglobin, (b) deoxyhaemoglobin, (c) oxyhaemoglobin, and (d) metcyanohaemoglobin.

(a) Methaemoglobin: commercially bought bovine haemoglobin (0.02g) was dissolved in 100ml of isotonic buffer and the u.v.-visible spectrum recorded. The solution was treated with potassium chlorate solution (0.1 M) using a dropping pipette, the u.v.-visible spectrum was monitored until the Soret band moved into position (405-407 nm). The solution is methaemoglobin.

(b) Commercially bought bovine haemoglobin (0.02g) was converted by the method in (a) to the methaemoglobin form. It was then purged with nitrogen to remove all the oxygen and treated with a solution of sodium dithionite (0.1 M) dropwise using a dropping pipette. The u.v.-visible spectrum was monitored until the Söret band was in the correct position (430 nm).

(c) Commercially bought bovine haemoglobin (0.02g) was converted by the method in (b) to the deoxyhaemoglobin form. It was then purged with oxygen to oxygenate the haemoglobin. The u.v. spectrum was monitored and the Söret band should be at 412-415 nm.

(d) Commercially bought bovine haemoglobin (0.02g) was converted by the method in (b) to deoxyhaemoglobin and then treated with KCN solution (0.1 M) to form metcyanohaemoglobin. The Söret band is used again to indicate reaction (moving to 414 nm).

Whole blood, which was extracted by the blood transfusion service, was separated into two factions, erythrocytes and plasma, by centrifugation on a MSE centrifuge at 3,000 revs/minute for 30 minutes. The plasma was drawn off and stored, for direct use, in a fridge at 4-5°C. The erythrocytes were washed with isotonic buffer and re-centrifuged at 3,000 revs/minute for 15 minutes. The buffer was removed with a dropping pipette and the cleaning process repeated twice. The erythrocytes should be clean, free of both plasma and haemolysis products (dead cell debre). Plasma can be stored, in solid form, for long periods of time, but the erythrocytes denature rapidly, this is

caused by airborne bacteria. Samples of erythrocytes were prepared just previous to experimentation and kept no longer than 48 hours.

The Electrode

The Corning cyanide sensitive electrode (003.15.005) is effective only in solution at pH 12 and above. At these pH's the cyanide present is expected to be totally ionised with no association.

Aqueous solutions for cyanide analysis are treated with an equal volume of 2.0 M sodium hydroxide (unless they are already at pH 12 or above). The pH was checked, the electrodes inserted and the solution mixed. The electrode is allowed to stabilise, giving a steady, accurate reading. The cyanide concentration is measured as a potential difference on a Beckman research pH meter. The meter is calibrated in aqueous solvent using standard potassium cyanide solutions (Appendix III)

Biological solutions for cyanide analysis are treated with an equal volume of 2.0 M sodium hydroxide. This quenches any reaction and denatures any biological material, releasing any bound cyanide (e.g. metcyanohaemoglobin). Erythrocytes produce excessive cellular debre which is removed using a centrifuge (3,000 revs/minute for 30 minutes) to prevent excessive blockage occurring in the electrode ducts. The pH is checked, the electrodes inserted and the solution mixed. The electrode is allowed time to stabilise, giving a steady, accurate reading. The cyanide concentration is measured as a potential difference. The meter is calibrated in biological material using standard potassium solutions in biological material, see text.

Spot Tests

(a) For sodium nitroprusside[29]: this is done by employing the Legal reaction; the reaction of sodium nitroprusside with malonitrile in alkali solution producing a deep red species. The test solution was prepared by dissolving malonitrile (6.6g) and sodium hydroxide (4.0g) in 250ml of distilled water. This solution decomposes and should be prepared fresh and used within one hour.

(b) For cyanide[31]: this is done by employing Faulkofs technique; this was put on a quantitative scale in Appendix II. Cyanide anion is converted using Chloramine T to cyanogenhalide. These have the ability to split pyridine rings to form glutaconaldehydes. The glutaconaldehyde couples with the pyrazolone to produce a highly coloured species (λmax 615 nm). Two solutions are required

1. Chloramine T 0.1% in 0.75 M sodium dihydrogen phosphate
2. 0.25g of 3-methyl-1-phenyl-2-pyrazol-5-one is dissolved (with difficulty) in 100ml of distilled water at 75°C. This is added to pyridine (20ml) just before the solutions are required.

Both solutions are kept on ice at 0-4°C. The solution being analysed is mixed with a small volume of Chloramine T solution and mixed. Two minutes are allowed to pass before the pyrazolone/pyridine solution is added (approximately 5ml). The solution should turn blue if cyanide is present.

Experiments involving Cavette Flasks

Two different solutions were required in these experiments; one, the trapping solution, and two, the reaction mixtures. The trapping solution was at all times sodium hydroxide (0.3ml, 0.1 M). The reaction mixture had at all times a total volume of 13ml, inclusive of one millilitre of 15% sulphuric acid. Stock concentrated solutions of potassium cyanide and sodium nitroprusside in isotonic buffer were prepared. Greater detail of these solutions is not given as the nature of these experiments requires that solutions be made fresh at the beginning of each experiment. The exact amounts of compounds used are given in the results. Solutions for study were prepared in three ways.

(a) For experiments not involving human erythrocytes: xml of the required isotonic buffer solution (potassium cyanide or sodium nitroprusside) was pipetted onto the base of the cavette flask and made up to 12ml, mixed, stoppered, and treated in the normal manner.

(b) For experiments involving human erythrocytes (except one experiment; pages 73-74): xml of the required isotonic buffer solution (potassium cyanide or sodium nitroprusside) was pipetted onto the base of the cavette flask and made up to 8ml, 4ml of human erythrocytes were added and the "solution" mixed, stoppered, and treated in the normal manner.

(c) For the experiment involving different volumes of human erythrocytes: 1ml of the sodium nitroprusside solution (0.298g in 10ml of isotonic solution) was added to the required volume

of erythrocytes and made up to 12ml with isotonic buffer (table 13). The solution was mixed and treated in the normal manner. This experiment was conducted in total darkness.

The vessels which are allowed to react in the dark were foil wrapped allowing them to be treated in exactly the same manner as those exposed to the light. There was an initial incubation period of one hour in the light. This caused a certain amount of heating ($\sim 10^{\circ}C$) and it was important that the foil wrapped vessels were warmed in a similar manner. After this period had elapsed 15% sulphuric acid (1ml) was added and diffusion allowed to take place for two hours, again in the light.

After the two hour period has elapsed the trapping solution is analysed quantitatively using Faulkofs technique (Appendix II). 0.125ml of the trapping solution is removed using an automatic dispenser and placed in a cuvette. Chloramine T solution (1.5ml, 0.1% in 0.75 M sodium dihydrogen phosphate) is added to the cuvette, mixed, and allowed to stand for two minutes. 1.5ml of pyrazolone/pyridine solution (0.25g of pyrazolone in 100ml of water and 20ml of pyridine) is added and the rise in absorbance at 615 nm is monitored by a Pye-Unicam SP-8,100.

The concentration of cyanide in solution is determined using the calibration chart which was produced using standard potassium cyanide solutions (Appendix II).

Studies using Bubbler Units

Studies using these units were carried out at three pH's

(a) pH 2 using KCl/HCl buffer with either 0.0298g of sodium

nitroprusside or 0.0279g of aquopentacyanoferrate(III).

(b) pH 4 using potassium hydrogen phthalate buffer with either 0.0298g of sodium nitroprusside or 0.0279g of aquopentacyanoferrate(III).

(c) pH 7.2 using isotonic buffer. There are two types of study carried out at this pH; photolytic studies in isotonic media not involving haemoglobin and, photolytic studies in isotonic media involving haemoglobin. All studies used 0.0144g of sodium nitroprusside in the appropriate experiment. In the study not involving haemoglobin 0.0144g of aquopentacyanoferrate(III) was used. In the studies involving haemoglobin 0.02g of the bovine haemoglobin was dissolved in the isotonic buffer (100ml) and converted to the form required using the appropriate method.

Again reactions which were to be in the dark were foil wrapped in order that they could experience the same heating effect from the bulb (100W). In all cases 100ml of buffer was used, the compound was introduced into solution via a weighing boat which was immersed in the solution. The stoppers were fitted firmly and the gas flow turned on. The cyanide electrode is used to monitor the cyanide concentration (Appendix III). The gas flow is monitored using a carbon tetrachloride manometer.

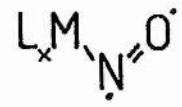
CHAPTER III

Some Notional Analogues of Sodium Nitroprusside

INTRODUCTION

Lewis[1] states that there are five different ways a nitric oxide molecule can bind to a metal, these are shown

(a)



(bent)

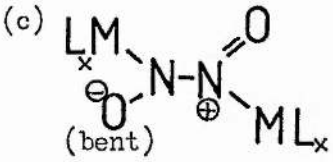
by donation of two electrons from a neutral NO to give paramagnetic species. There is very little evidence for the existence of metal complexes with this bonding type.

(b)

(linear)

by donation as in (a) but with coupling of the unpaired electron on the NO group with an unpaired d electron of the metal to give a -bond (nitroprusside type).

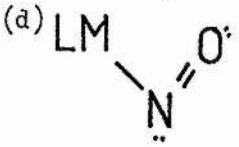
(c)



(bent)

by transfer of an electron from the metal to NO and donation of two electrons from the $[NO]^-$ ion.

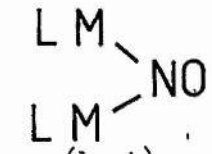
(d)



(bent)

the NO group is bonded at some angle; the electrons of the multiple bond in the NO may be regarded as to some extent taking part in bonding.

(e)



(bent)

the NO group is bonded in a bridging position.

These structures show that metal nitrosyl complexes may be cationic ($[NO]^+$), anionic ($[NO]^-$) or nearly neutral depending on the nature and valence state of the metal and on the ligands present in the same complex. Lewis[1] again has shown that the cationic $[NO]^+$ stretching frequency falls in the range between 1 940 cm^{-1} and 1 575 cm^{-1} whereas $[NO]^-$ stretching

frequency is observed in the range 1 200 cm^{-1} and 1 040 cm^{-1}; this leaves the region 1 575 cm^{-1}-1 200 cm^{-1} for neutral species.

Sodium nitroprusside has, as Lewis[1] quite correctly forecasts, an extremely well developed and reactive positive nitrosyl group. By simply replacing the central metal ion in the nitroprusside anion with other transition metals and balancing the charges one creates a series of compounds worthy of study, namely: $(V(CN)_5NO^{3-})^{2}$; $(Cr(CN)_5NO^{3-})^{3}$; $(Mn(CN)_5NO^{3-})^{4}$; $(Co(CN)_5NO^{3-})^{5}$; and $(Mo(CN)_5NO^{4-})^{6}$. These are all notional analogues of sodium nitroprusside which have been previously prepared. Interest in the compounds has to date been, in the main, confined to the structure of these complexes rather than the chemistry. The infra-red stretching frequencies of the nitrosyl and cyanide groups

Data for the Various Nitrosylpentacyanometallates

Compound	V CN cm^{-1}	V NO cm^{-1}	M-N-O angle	Oxidation state	Configuration
$V(CN)_5NO^{3-}$	2095	1575	180^{o} [7]	I	d^4
$Cr(CN)_5NO^{3-}$	2137	1645	180^{o} [8]	I	d^5
$Mn(CN)_5NO^{3-}$	2129	1730	180^{o} [9]	I	d^6
$Co(CN)_5NO^{3-}$	2205cm^{-1}	1114cm^{-1} 1052,971cm^{-1}	Bent [10]	III	d^6
$Mo(CN)_5NO^{4-}$	2130cm^{-1}	1595cm^{-1}	180^{o} [11]	0	d^6
$Fe(CN)_5NO^{2-}$	2152	1925	178^{o} [12]	II	d^6

(table 1)

are listed along with the metal nitrosyl bond angle determined by X-ray

analysis in table 1. All but one have linear metal-nitrosyl groups with a nitrosyl stretching frequency comparable with what Lewis states we should expect for $[NO]^+$. Bonding in four of the complexes, just as with sodium nitroprusside is expected to be of type (b), the cobalt complex is bonding type (c) (this will be discussed later).

It is proposed to test Lewis's theory that these groups are $[NO]^+$ and that they react as such. This would prove that they are true chemical analogues of sodium nitroprusside. The nitroprusside anion reacts with neucleophiles like hydroxide, hydrogen sulphide, thiols, and amines both primary and secondary and is unreactive with electrophiles. We would expect similar behaviour from other metal nitrosyl complexes with bonding type (b). If any of the complexes are true analogues of nitroprusside they must be viewed as potential hypertensive agents, this possibility can only be tested using animals. The fact that they are cyanide based is from a chemical point of view irrelevant as the toxicity of nitroprusside anion is due to its photoinstability rather than a biological reaction which was previously believed to exist.

RESULTS AND DISCUSSION

Potassium Nitrosylpentacyanovanadate(II)

This complex was first prepared by Griffiths[2] in 1959 and was formulated as $V(CN)_5NO^{5-}$ with a reported nitrosyl stretching frequency of 1 575 cm^{-1}. Lewis's[1] theory fixes the complex as $[(CN)_5V^{(-I)}-NO^{+}]^{5-}$. Manoharan[13] produced an electronic study of nitrosylpentacyanometallates and his data for the vanadium complex are shown in table 2. He favours a $[(CN)_5V^{I}-NO^{-}]^{5-}$ formulation in contrast to Griffiths who favours the $[NO]^{+}$. He does not entertain the idea of the formulation $[(CN)_5V^{o}-NO]^{5-}$ with a neutral nitrosyl group as this produces a zero oxidation vanadium atom. The compound is air stable and recoverable from aqueous solution using ethanol, which would be surprising properties for a zero oxidation species.

Ultra-violet Data for Nitrosylpentacyanovanadate(I)-Anion

Observed cm^{-1}	Calculated energy cm^{-1}	ε max	Wavelength nm	Band Assignment
12 900	9 200	1.15	775	$d_{xy} \longrightarrow \pi^*NO$
21 160	19 000	36.5	472	$d_{xy} \longrightarrow d_{x^2-y^2}$
32 470	30 100	1000	308	$d_{xz}d_{yz} \longrightarrow \pi^*NO$
37 470	23 260	5200	266	$d_{xy} \longrightarrow \pi^*CN$

(table 2)

But the compound on re-investigation by Vannerberg[7] for crystalographic reasons was shown to be $V(CN)_5NO^{3-}$; contamination of the compound with potassium cyanide being to blame for the previous

misleading analytical data. Vannerberg used hand picked crystals for a re-appraisal of the nitrosyl stretching frequency achieving an accurate value of 1 530 cm^{-1}. This makes the compound $[(CN)_5V^{II}\text{-}NO]^{3-}$ with a neutral nitrosyl group (bonding type (a)).

It was found to be impossible to prepare analytically pure potassium nitrosylpentacyanovanadate(II) by the method of Griffiths[2] in quantity; this difficulty had already been encountered by Vannerberg[7], who circumvented it by growing small crystals in mixed solution. This was impossible on a preparative scale; washing the complex with clean, dry, warm methanol to remove excess potassium cyanide was successful but the complex was found to be extremely unstable in the light, decomposing to potassium vanadate and potassium cyanide. Due to the lack of pure complex, in depth kinetic studies would be futile, but spot tests and simple experiments should indicate whether the complex exhibits nitroprusside-type chemistry.

(a) Reactions in Acid

There is a reaction between the nitrosylpentacyanovanadate(II) anion and H_3O^+ which is observed as the appearance of a shoulder at 250 nm-300 nm in the u.v.-visible spectrum or as a yellowing of solution visually. This reaction produces dioxovanadium(V) cation (shoulder 250 nm-300 nm at pH 2-3), hydrogen cyanide and nitrite. Attack of the proton could be at either the cyanide ligands or the nitrosyl group.

(b) Reactions in Base

There is a reaction between the nitrosylpentacyanovanadate(II) anion

and OH^- which is observed as the appearance of a peak at 270 nm (pH 12-13) in the ultra-violet. This reaction produces potassium vanadate, potassium cyanide and potassium nitrite. The reaction is most probably a straightforward dissociation of the cyanides from the vanadium atom centre (the complex is substitution labile). The reasons for this argument are that attack of hydroxide on the nitroprusside anion results in an increase absorption at 394 nm ($d_{xy}d_{yz} \longrightarrow \pi NO^*$ band assignment). The reaction of the nitrosylpentacyanovanadate(II) anion with hydroxide does not result in an increase absorption in its respective ultra-violet band position 308 nm.

(c) Reactions with Amines

The complex is unreactive to amines in aqueous solution at pH 10-11. The base reaction mentioned above does occur and at high amine concentration this is a rapid reaction.

(d) Reaction with Hydrogen Sulphide

There is a reaction between hydrogen sulphide and the impure nitrosylpentacyanovanadate(II) anion, which is observed as the appearance of a strong band in the ultra-violet (300 nm-370 nm). Müller[14] reports a yellow complex $K_4V(CN)_6NO$ which has the characteristic band at 300-370 nm, which is produced in solution by the reaction of ammonium metavanadate, potassium cyanide, hydroxylamine hydrochloride and hydrogen sulphide. To get Müller's product from the nitrosylpentacyanovenadate(II) anion, which is an intermediate in the Müller preparation, requires reaction with impurities in solution (potassium cyanide). It

would be interesting to see what reaction occurs if pure complex could be prepared.

(e) Reaction with Cysteine Hydrochloride

There is no evidence for a reaction between cysteine hydrochloride and the nitrosylpentacyanovanadate(II) anion. But cysteine hydrochloride reacts in acidic media with the dioxovanadium(V) cation to form cystine and cysteine reacts in basic media with the vanadate anion to form cystine. If the nitrosylpentacyanovanadate(II) anion is added to an acid, neutral, or basic solution of cysteine hydrochloride a reaction occurs, but that of the relevant oxovanadyl species produced in solution.

Nitrosylpentacyanovanadate Anion - Cysteine Interaction

$$(CN)_5VNO^{3-} \xrightarrow[\text{medium}]{\text{acid}} VO_2^+ + \text{cysteine} \xrightarrow{O_2} \text{cystine}$$

$$(CN)_5VNO^{3-} \xrightarrow[\text{medium}]{\text{basic}} VO_4^{3-} + \text{cysteine} \xrightarrow{O_2} \text{cystine}$$

(figure 1)

(f) Photochemical Reactions

The complex, as stated previously, is unstable as a solid in light. In solution at pH 7.2 it decomposes to produce cyanide. What is important from a therapeutic point is that it is also unstable in the dark at pH 7.2 with respect to potassium cyanide and vanadate.

The formulation[7] of the complex was $[(CN)_5V^{II}\text{-}NO]^{3-}$ and this is what the chemistry indicates. The nitrosylpentacyanovanadate(II) anion will react with both strong nucleophyls (OH^-,SH^-) and strong electrophiles (H^+).

From a therapeutic point of view the compound is of no value. It is extremely toxic, having labile cyanide groups and does not exhibit nitroprusside-type chemistry.

Potassium Nitrosylpentacyanochromate(I)

This compound was first prepared by Griffiths[3] in 1959 and was formulated as $[(CN)Cr^{I}\text{-}NO^{+}]^{3-}$, bonding type (b). Formulation of the complex as an $[NO]^{+}$ species is confounded by the fact that it is paramagnetic[8], considerable neutral character would be expected, $[(CN)_5Cr^{II}\text{-}NO]^{3-}$, bonding type (a). Manoharan[13] produced the electronic data for this complex which are shown in table 3. His conclusion is that the nitrosyl group is more positive than the respective vanadyl complex, with the possibility that it is a d^5NO^{+} species.

U.V. Data for the Nitrosylpentacyanochromate Anion

Observed cm^{-1}	Calculated energy cm^{-1}	ϵ max	Wavelength nm	Band Assignment
13 700	12 660	8	730	$d_{xz}d_{yz} \longrightarrow d_{xy}$
15 300	13 890	1.5	653	$d_{xy} \longrightarrow \pi^*NO$
22 200	26 550	72	450	$d_{xy}d_{yz} \longrightarrow \pi^*NO$
27 320	28 260	59	366	$d_{xy} \longrightarrow d_{x^2-y^2}$
37 300	37 420	1100	268	$CN \longrightarrow \pi^*CN$
43 480	35 680	3600	229	$d_{xy} \longrightarrow \pi^*CN$

(table 3)

The acid hydrolysis of the complex has been studied by polarographic[15] and ESR[16] techniques. The results are shown in figure 2. The aquation is quite simple; with the axial cyanide being the more labile and the nitrosyl group remaining until the last. The four equatorial cyanides have no set pattern for aquation after the

departure of the axial cyanide.

Acid Hydrolysis of the Nitrosylpentacyanochromate(I) Anion

$$(CN)_5CrNO^{3-} + 3H_3O^+ \longrightarrow (CN)_2Cr(H_2O)_3NO + 3HCN$$

This Reaction occurs between pH 7 and 2.

$$(CN)_5CrNO^{3-} + 5H_3O^+ \longrightarrow (H_2O)_5CrNO^{2+} + 5HCN$$

$$(H_2O)_5CrNO^{2+} + H_2O \longrightarrow Cr(H_2O)_6^{3+} + NO^- \qquad pH\ 2 >$$

(figure 2)

The compound was found to be completely inert to hydroxide, amines, hydrogen sulphide and thiols (in basic solution). It is extremely photo-stable as a solid but in solution rapidly decomposes to give hydrogen cyanide, which can be driven off using a nitrogen stream and frittered disc.

The compound was tested for hypertensive activity at Ninewells Hospital in Dundee. This involved the use of animals and was impossible in the chemistry department in St. Andrews. The results were negative - potassium nitrosylpentacyanochromate(I) has no therapeutic value. This is not surprising as it shows no nitroprusside-type chemistry.

Formulation of the complex using Lewis criteria[1] (νNO 1645cm^{-1} NO^+) is not effective as the species does not react with nucleophiles, it is also paramagnetic and a possible better formulation would be $[(CN)_5Cr^{II}\text{-}NO]^{3-}$.

Potassium Nitrosylpentacyanomanganate(I)

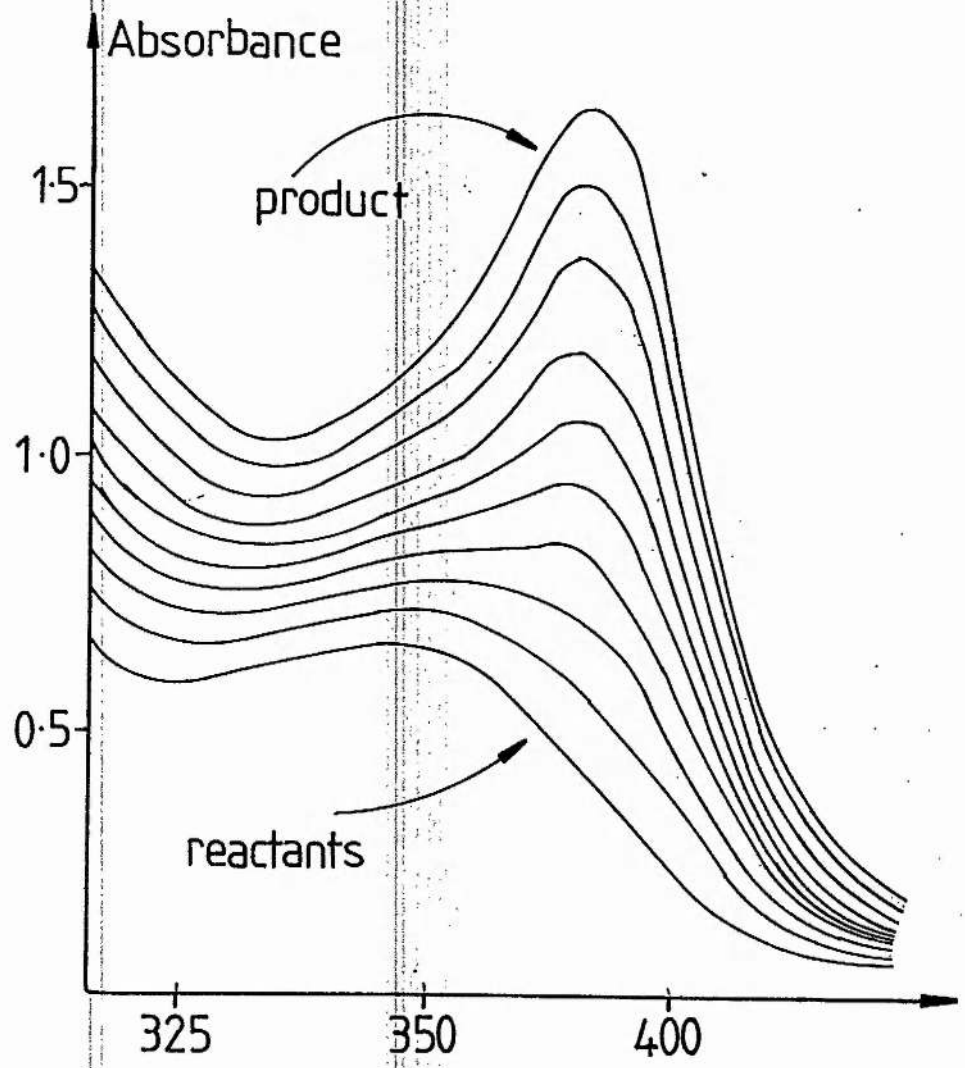
This compound was first prepared by Manchot[17] in 1926, but it was Lewis[1] in 1958 who recorded the nitrosyl stretching frequency at 1 730 cm^{-1}, formulating the complex as NO^{+}. Manoharan's[13] electronic and molecular orbital study (table 4) agrees with Lewis.

U.V. Data for the Nitrosylpentacyanomanganate Anion

Observed cm^{-1}	Calculated energy cm^{-1}	ε max	Wavelength nm	Band Assignment
18 520	14 700	22.2	540	$d_{xy} \longrightarrow \pi^{*}NO$
24 690	24 200	66	405	$d_{xz}d_{yz} \longrightarrow \pi^{*}NO$
28 980	26 500	111	345	$d_{xy} \longrightarrow d_{x^2-y^2}$
37 850	37 770	1000	264	$d_{xz}d_{yz} \longrightarrow d_{z^2}$
42 550	41 490	4500	255	$d_{xz}d_{yz} \longrightarrow d_{x^2-y^2}$
45 450	40 470	5000	220	$d_{xy} \longrightarrow \pi^{*}CN$

(table 4)

This compound reacts with acid and alkali; these reactions have been reported by Cotton[18] but little investigated. Cotton states that the product of reaction with acid is the nitrosylpentacyanomanganate(II) ($Mn(CN)_5NO^{2-}$) anion and that it is a direct result of action of a proton at the nitrosyl group. This statement goes unsupported, but if action were at the cyanide groups hydrogen cyanide gas would be expected. In alkali Cotton simply states that manganese dioxide is the product.

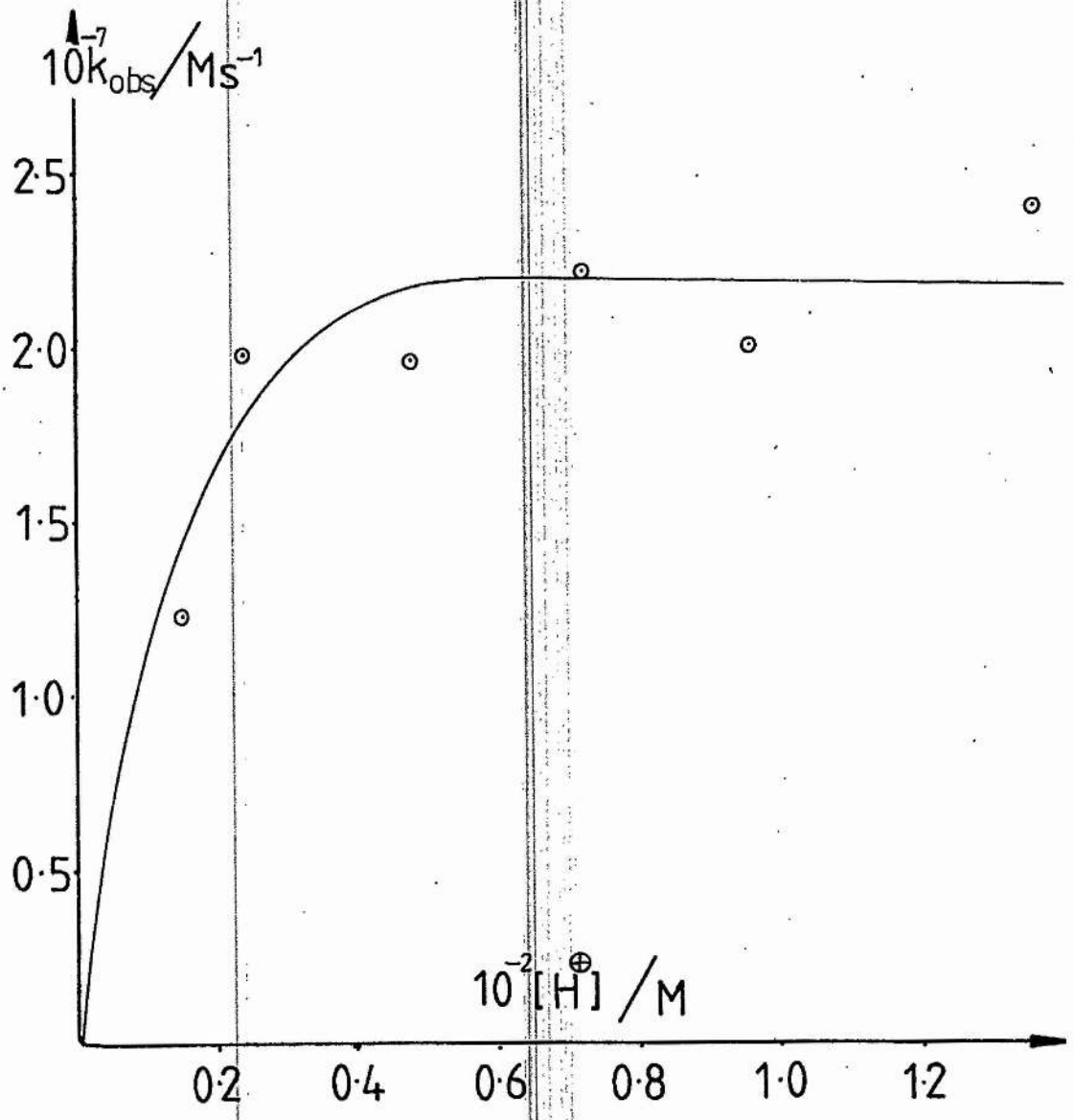


$[(CN)_5MnNO^{3-}] = 5{\cdot}0 \times 10^{-3}$ M

Reading every two minutes

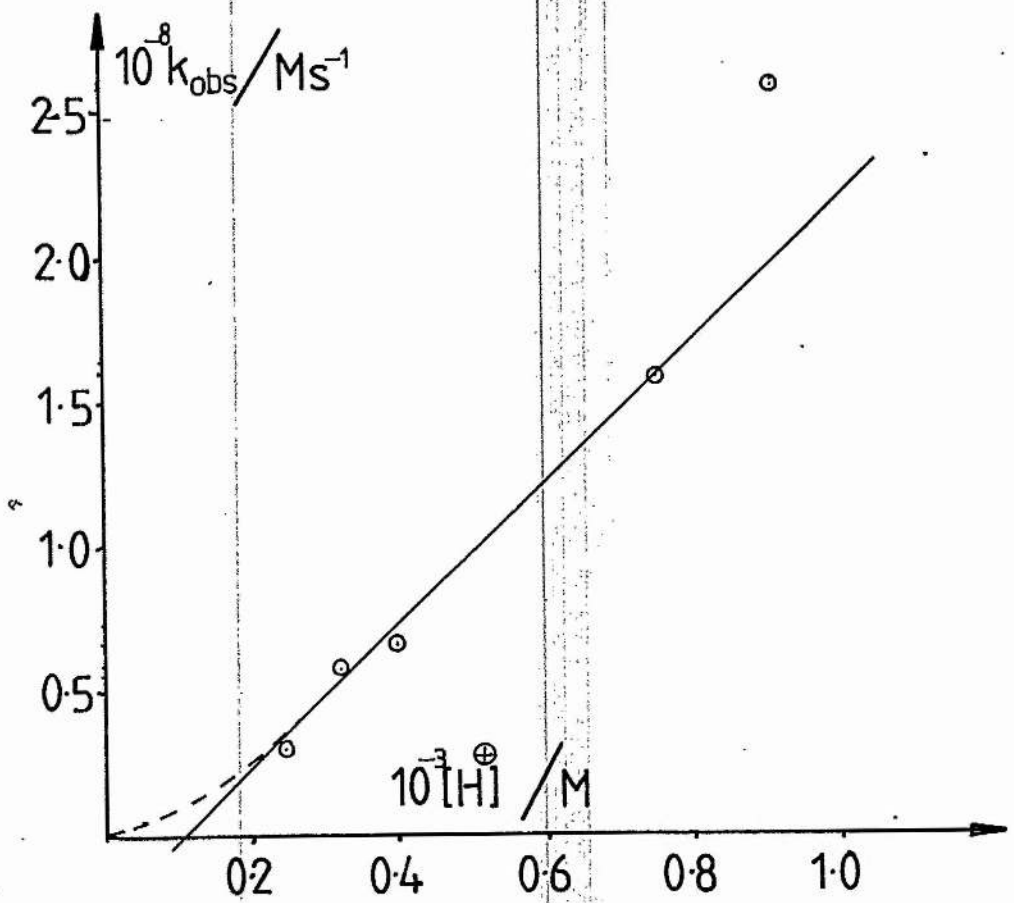
The Reaction of Nitrosylpentacyanomanganate(I) in Acidic Media

(Figure 3)



Plot of the Kinetic Data for the Production of Nitrosylpentacyanomanganate(II) between pH2 and 3

(Figure 4)



Rate Constant = $2{\cdot}5 \times 10^{-5} s^{-1}$

Intercept = $-2{\cdot}5 \times 10^{-9} Ms^{-1}$

Plot of the Kinetic Data for the Production of Nitrosylpentacyanoferrate(II) between pH3 and 4

(Figure 5)

Reaction Acidic Medium

The reaction of the nitrosylpentacyanomanganate(I) anion in acidic solution to produce the nitrosylpentacyanomanganate(II) anion is shown as a spectral change, figure 3, as a change in absorbance at 384 nm.

The kinetics were studied at 384 nm using phthalate buffer in the regions pH 2-3 and pH 3-4. The data is shown in table 5 and was curiously zeroth law in the appearance of product.

Kinetic Data for the
Nitrosylpentacyanomanganate(I) Anion Reaction
in Acidic Media

pH of solution	1.870	2.022	2.136	2.312	2.628	2.820
$10^{-3}[H^+]/M$	13.5	9.51	7.31	4.88	2.36	1.52
$10^{+7}k_{obs}/Ms^{-1}$	2.41	2.00	2.22	1.96	1.98	1.22

Concentration of manganate(I) complex = 1.43×10^{-3} M

Phthalate/HCl buffer

384 nm; 302 K; 1.0 M Ionic Strength (KCl)

pH of solution	3.608	3.495	3.400	3.123	3.035
$10^{-4}[H^+]/M$	2.47	3.21	4.00	7.53	9.22
$10^{+8}k_{obs}/Ms^{-1}$	0.302	0.573	0.684	1.61	2.58

Concentration of manganate(I) complex = 1.43×10^{-3} M

Phthalate/HCl buffer

384 nm; 302 K; 1.0 M Ionic Strength (KCl)

(table 5)

Scheme 1

$$(CN)_5MnNO^{3-} + H_3O^+ \xrightarrow[\text{unidirectional}]{\text{fast } k_1} (CN)_5MnNOH^{2-} + H_2O$$

$$(CN)_5MnNOH^{2-} \xrightarrow[k_2]{\text{slow, rate determining}} (CN)_5MnNO^{2-} + \tfrac{1}{2}H_2$$

Reaction of Nitrosylpentacyanomanganate(I) in Acidic Solution

The buffer was observed to be operating perfectly and, between pH 3 and 4, the reaction was first order in hydrogen ion concentration, and, between pH 2 and 3, the reaction was zeroth order in hydrogen ion concentration. A mechanism has been postulated (scheme 1) where there is a rapid undirectional reaction of the original nitrosylpentacyano-manganate(I) anion with acid to produce an intermediate; this reacts further in a slow rate determining step producing the nitrosylpentacyano-manganate(II) anion and hydrogen.

The compound (product) is extremely air unstable and a good sample was difficult to prepare. It was finally isolated as a cobalt salt for analytical purposes. But the calculation of zeroth law rate constants requires an accurate extinction co-efficient of the pure product which was found to be impossible. Manoharan[13] gives a value (ϵ max 1,700 385 nm) which was subsequently used to give the values shown in table 5.

Figure 5 shows a negative intercept, which is impossible. Three different explanations can be given for this.

(a) Manoharan's extinction co-efficient is low; producing a mathematical translation of the graphical results producing a negative intercept. If his preparation was also contaminated by the air reaction this would explain why he should have a low result.

(b) There could be a slow reaction at neutral pH's where the kinetics would probably change. This claim is impossible to prove as the reaction at this pH would be far too long for study and, the compound would probably react with low concentrations of hydroxide at this pH.

(c) The reaction put forward in scheme 1 is incorrect; figure 4 could be interpreted as a catalytic process (similar to enzymes).

Reaction in Alkali

Cotton[18], in his simple study, mentioned that the nitrosylpentacyanomanganate(I) anion reacted with hydroxide to produce MnO_2. This reaction was found to be on a stopped-flow scale, but the manganese-dioxide produced coated the glass and produced floculants. Attempts to keep the manganese dioxide in solution using KCl and KF electrolytes failed and kinetic studies were impossible. Using the cyanide sensitive electrode on a basic solution of the nitrosyl complex produced no substantial evidence for cyanide production. Only 10% of the cyanide in the complex was detected in solution, whereas 95% of the manganese was accounted for as MnO_2.

The nitrosylpentacyanomanganate(I) anion does not react with amines, hydrogen sulphide, and thiols.

Photochemistry of the Nitrosylpentacyanomanganate(I) Anion

The compound is unstable in its solid form; a brown mixture (MnO_2) results from prolonged exposure to light. In solution at pH 7.2 the complex is also photo labile with respect to potassium cyanide but in the dark is stable. (There may be a slow reaction with OH^- and/or H^+ in the dark but neither results in the production of cyanide anion.) Stability in the dark is to be expected as the complex is d^6 low spin

substitution inert. The photolability is due to the two bands (table 4) $d_{xy} \longrightarrow \pi^*NO$ at 540 nm and $d_{xz}d_{yz} \longrightarrow \pi^*NO$ at 405 which would be expected to produce NO similar to the nitroprusside anion (figure 6).

The Photo Decomposition of Nitrosylpentacyano; Manganate and Ferrate Anions

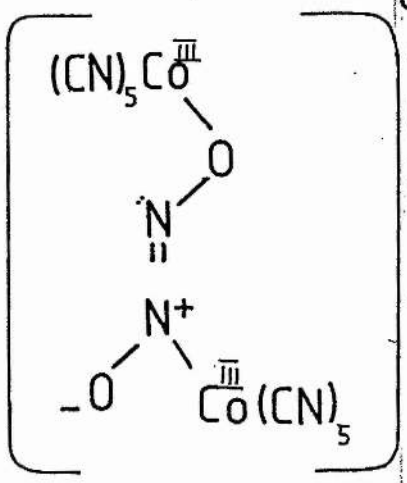
$$(CN)_5Mn^{I}NO^{3-} \xrightarrow{h\nu} (CN)_5Mn^{II3-} + NO$$

d^6 substitution inert $\qquad$ d^5 substitution labile

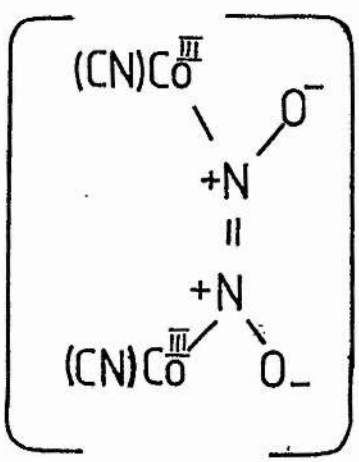
$$(CN)_5Fe^{II}NO^{2-} \xrightarrow{h\nu} (CN)_5Fe^{III2-} + NO$$

(figure 6)

The compound was tested for hypertensive activity at Ninewells Hospital in Dundee. The results were negative and nitrosylpentacyano-manganate has no therapeutic value. Although this complex exhibits nitroprusside-type chemistry it does not react with amines and thiols, which are believed to be the important groups for hypertensive activity (as mentioned previously).



SKEW (A)



CIS (B)

The Two Possible Structures for the Hyponitritobispentacyanocobaltate (III) anion

(Figure 7)

Potassium Hyponitritobispentacyanocobaltate(III)

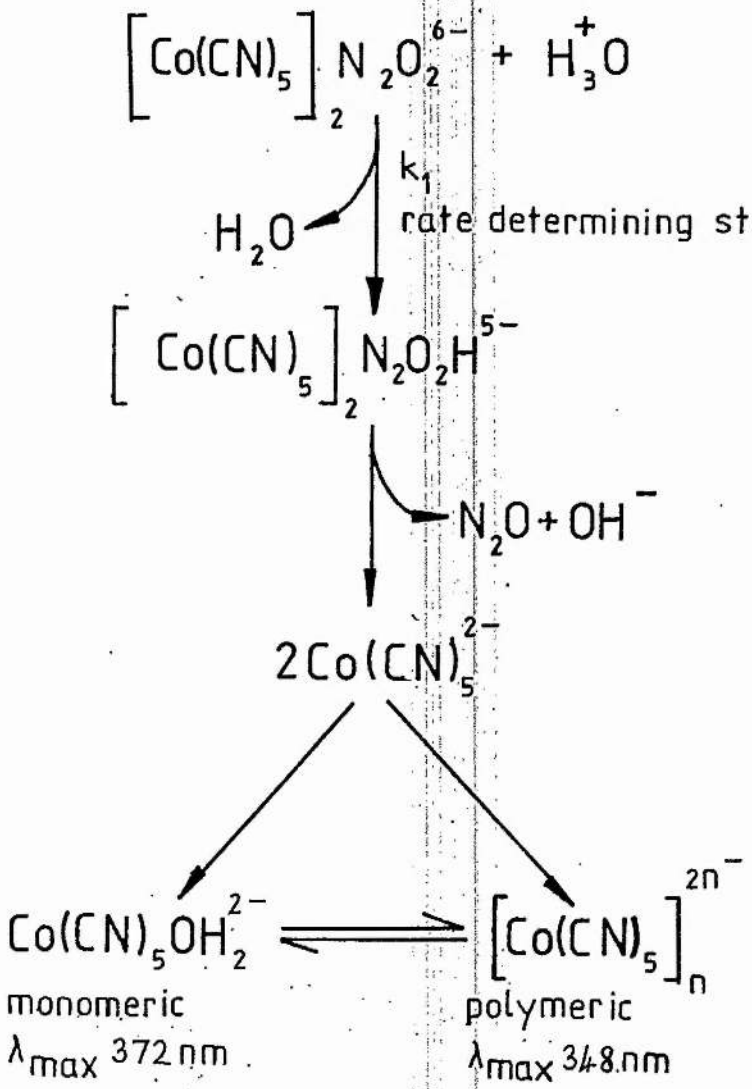
This compound was prepared in the belief that it was a true nitrosylpentacyanometallate but in fact it is a hyponitrito complex. This fact is proven by the infra-red spectrum[5], which does not show a nitrosyl stretching frequency in the correct region (1 575 cm^{-1}-2 000 cm^{-1}) but shows three frequencies at 1 130 cm^{-1} (N=N), 1 052 and 971 cm^{-1} (NO). The proposed structures are shown in figure 7.

The trans structure, similar to B, is ruled out as it has been calculated that the frequencies at 1 130 cm^{-1} and 1 050 cm^{-1} would be forbidden. The argument could be resolved by a ^{15}NNMR spectrum, which due to chemical reasons is impossible. This compound was prepared by the method of Nast[5] via a bispentaaminecobalt(II) chloride[19] complex (black form). As was expected, using Lewis[1] bonding theory, this compound is reactive only with acid. Hydroxide, hydrogen sulphide, amines, and ionised thiols are completely unreactive with the complex.

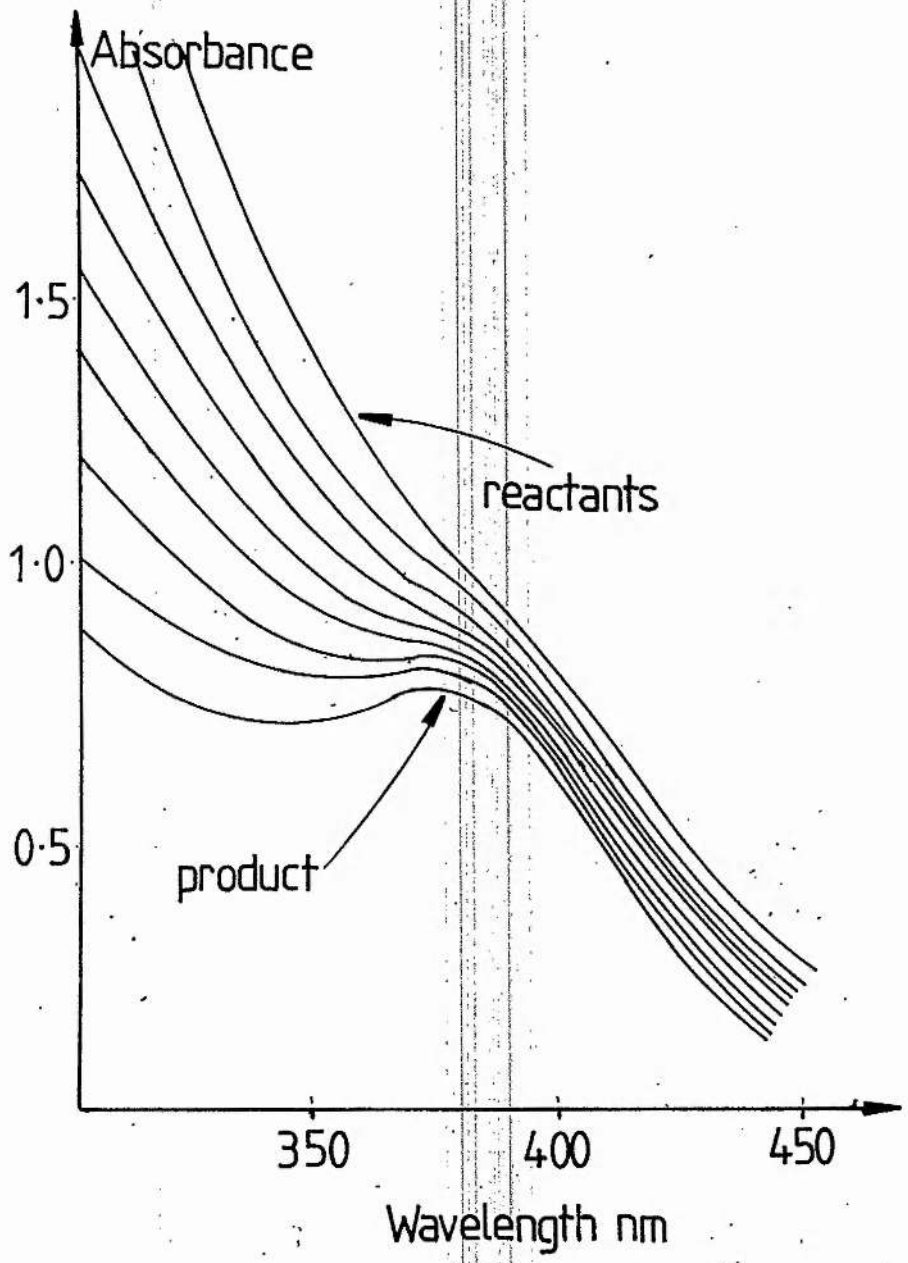
Reaction Acidic Media

The reaction of hyponitritobispentacyanocobaltate(III) anion with acid to produce the aquopentacyanocobaltate(III) anion is shown in figure 8 as the appearance of a strong absorption maximum at 370 nm from under a shoulder in the u.v.-visible spectrum. The product was isolated as a silver salt and a potassium salt from aqueous solution. It was clearly identifiable as the aquo complex from the elemental analysis of the silver salt (potassium salts do not analyse well). The u.v. spectrum was identical to the u.v. spectrum of the aquopentacyanocobaltate anion reported, produced by a different reaction[20] (in both

Scheme 2



Reaction of the Hyponitritobispentacyanocobaltate(III) Anion in Acidic Media

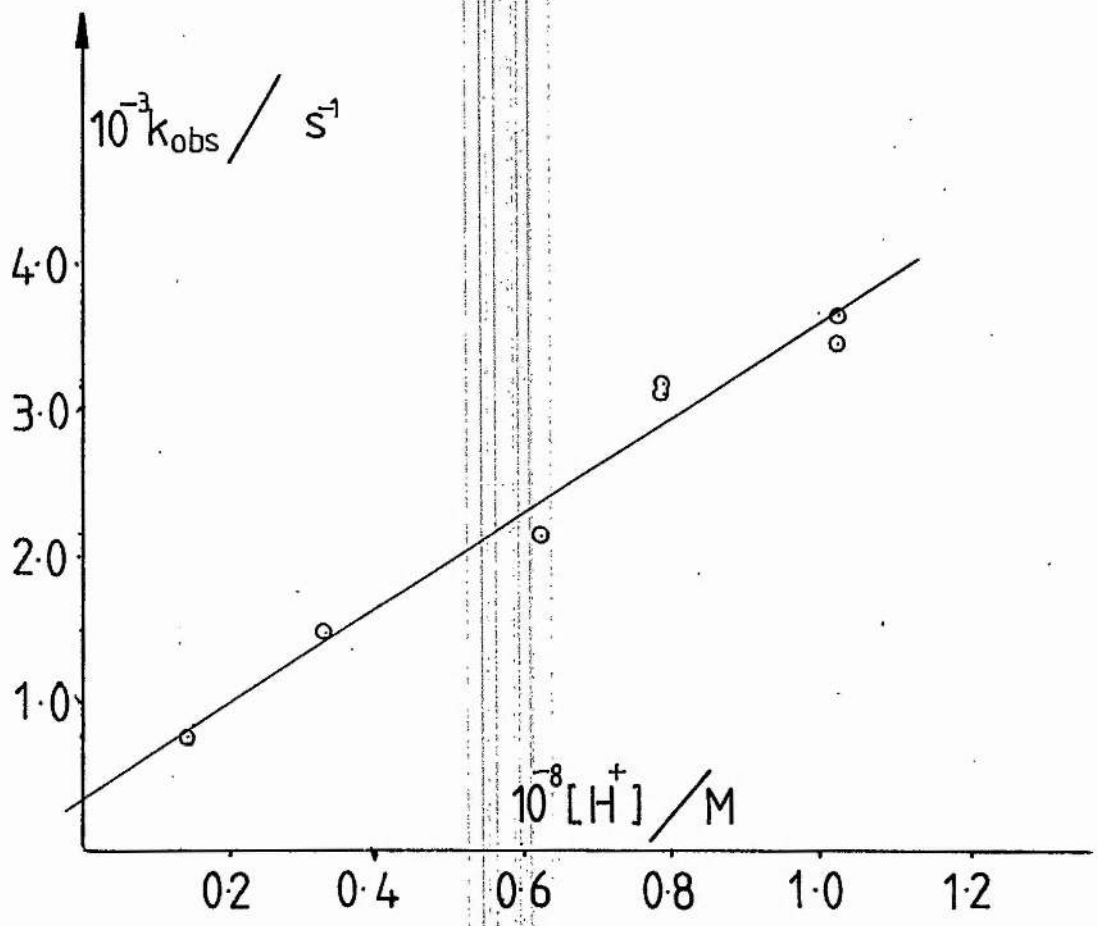


Reading every two minutes

$[((CN)_5Co)_2N_2O_2^{6-}] = 1{\cdot}6 \times 10^{-3}\,M$

The Reaction of the Hyponitritobispentacyanocobaltate(III) Anion in Acidic Medium

(Figure 8)



Rate Constant = $3{\cdot}3 \times 10^{5}\ M^{-1}s^{-1}$

Intercept = $3{\cdot}5 \times 10^{-4}\ s^{-}$

Plot of the Kinetic Data for the Reaction of the Hyponitritopentacyanocobaltate anion in Acidic Medium

(Figure 9)

monomeric and polymeric form). The kinetics of the reaction were studied at 290 nm where a greater change in absorbance occurred compared to 370 nm. The reaction was good first order in the appearance of product and table 6 shows the data obtained between pH 8-9 in borax buffer.

Kinetic Data for the Reaction of the
Hyponitritobispentacyanocobaltate(III) Anion
in Acidic Medium

pH	7.99	8.10	8.15	8.20	8.47	8.03
$10^{-9}[H^+]M$	10.33	7.91	7.11	6.24	3.36	1.46
$10^{+3}k_{obs}/s^-$	3.47	3.41	2.77	2.18	1.50	0.78
	3.75	3.28	2.79	2.16	1.49	0.74

Concentration of cobaltate species = 4.76×10^{-4} M

290 nm; 303 K; 1.0 M Ionic Strength (KCl)

(table 6)

The results indicate that the rate determining step is the reaction of one acid moiety with a metal complex species. Scheme 2 shows the proposed system; very little information can be gained from these particular kinetic results except that the "nitrosyl" species is highly reactive to hydrogen ions and Lewis bonding theory would seem to be appropriate in this case.

As was mentioned previously a ^{15}NNMR spectrum would resolve the structure, except this compound is unstable (with respect to hydrogen ions) even at pH 12. This would make long term fourier transform analysis impossible. Since the compound is unstable at pH 7.2 it is of no therapeutic value.

Potassium Nitrosylpentacyanomolybdate(C)

This compound was first prepared by Hofman in 1896 but like many authors he had difficulty elucidating the correct formula. Hieber[6] in 1948 had the correct formula, but Griffiths[3] in 1958 contradicted him giving his own interpretation $K_4[Mo^{II}(OH)_2(CN)_5NO]$. A bigger dispute is the position of the nitrosyl stretching frequency. Griffiths gives a value of 1 595 cm^{-1}, Vannerberg 1 620 cm^{-1}, and Riley[21] 1 455 cm^{-1}. Using Lewis's theory Griffiths and Vannerberg would formulate their complex as $[(CN)_5Mo^{0}\text{-}\overset{+}{N}O]^{4-}$ whereas Riley would formulate his as $[(CN)_5Mo^{I}\text{-}NC]^{4-}$. This difficulty of achieving both the correct formula and pure product not to mention why the nitrosyl frequency has a wide range of values can be explained by the compound's chemistry.

A reaction of this complex in concentrated hydrochloric acid has been reported and is shown in figure 10. This reaction was reported for preparative purposes and on further investigation it was found that the cyanide ligands were labile in solution even at high pH (11-12).

Reaction of the Nitrosylpentacyanomolybdate Anion in Acid [22]

$$(CN)_5MoNO^{4-} + 5HCl \longrightarrow (Cl)_5MoNO^{4-} + 5HCN$$

(figure 10)

Just as with nitrosylpentacyanochromate(I) the axial cyanide is believed to be the most labile. Aquation is still possible at high pH due to the equilibria shown in figure 11. At pH's less than 9.0 the cyanide

anion will be driven off as hydrogen cyanide gas.

Consequences of Aquation of the
Nitrosylpentacyanomolybdate(0) Anion

$$(CN)_5MoNO^{4-} \underset{CN^-}{\overset{H_2O}{\rightleftharpoons}} (CN)_4Mo(H_2O)NO^{3-} \underset{CN^-}{\overset{H_2O}{\rightleftharpoons}} (CN)_xMo(H_2O)_{5-x}NO$$

but

$$H_3O^+ + CN^- \rightleftharpoons HCN + H_2O$$

$$K_a = [H_3O^+][CN^-] / [HCN][H_2O]$$

$$= 6.3 \times 10^{-10}$$

$$pK_a = 9.2$$

At pH's less than 9.2 the equilibrium will favour the right hand reaction producing hydrogen cyanide, which should diffuse from solution. (figure 11)

During the preparation; it is recommended that the product be washed in demineralised water (pH 5-7); this will introduce impurities into the complex due to decomposition. The randomness of the data (NO stretching frequencies) mentioned previously can be attributed to this fact.

Attempts to record the kinetic events failed, the reaction was too fast for conventional kinetics and the solutions were too caustic for the stopped-flow apparatus. There is also the problem that the compound is contaminated with potassium hydroxide which cannot be washed out for fear of decomposing the starting material.

There is no evidence for reactions of the complex with hydroxide amines or thiols and it is obvious that this compound will be of no therapeutic use.

From the chemistry reported here it would seem that Riley's[21] product is probably the purest of the four workers. It displays a neutral to negative nitrosyl group.

Conclusions

Lewis's theory about nitrosyl stretching frequencies is in the light of this work chemically optimistic. His ranges are shown in figure 12.

Nitrosyl Stretching Frequencies

	Lewis range of frequencies	This study
νNO^+	1 940 cm^{-1}-1 575 cm^{-1}	1 940 cm^{-1}-1 750 cm^{-1}
νNO	1 575 cm^{-1}-1 200 cm^{-1}	1 750 cm^{-1}-1 200 cm^{-1}
νNO^-	1 200 cm^{-1}-1 040 cm^{-1}	1 200 cm^{-1} and below

(figure 12)

This study has shown that only the nitrosylpentacyanoferrate(II) anion and the nitrosylpentacyanomanganate(I) anion exhibit what could be described as reactions of a well developed positive nitrosyl group. The group of anions nitrosylpentacyanovanadate(I), nitrosylpentacyanochromate(I), and nitrosylpentacyanomolybdate(0) show reactions associated with cyanide groups (exchange reactions) and aquation in acidic medium. In the latter two cases the integrity of the nitrosyl group in strongly acidic and strongly basic medium suggests it is chemically neutral (or has little developed charge). The hyponitrito complex has an acidicly active nitrito group and is classed as an anionic species as Lewis suggests. By classing the chromium, vanadium, and molybdenum complexes as neutral nitrosyl complexes we immediately re-assess the oxidation start of the metal atom centre as Chromium II (d^4); Vanadium II (d^3),

and Molybdenum I (d^5).

Unfortunately, none of the complexes under study here have any therapeutic value. What has been shown is that a compound with a nitrosyl stretching frequency less than 1 750 cm^{-1} would not be expected to show nitroprusside-type chemistry or have any hypertensive action. It is therefore suggested that in the search for a replacement for nitroprusside only complexes with a nitrosyl stretching frequency in excess of 1 750 cm^{-1} be studied.

EXPERIMENTAL

Materials

Ammonia, ammonium vanadate, analer hydrochloric acid, borax, chloramine T, chromium trioxide, cobalt(II) chloride, cysteine hydrochloride, diethylamine, diethylether, ethanol, ethylamine, glacial acetic acid, hydrogen sulphide, hydroxylamine hydrochloride, manganous acetate, 3-methyl-1-phenyl-2-pyrazol-5-one, molybdenum trioxide, nitric oxide, potassium carbonate, potassium cyanide, potassium hydrogen phthalate, potassium hydroxide, pyridine and silver nitrate were all bought commercially.

The potassium salts of the following anions, nitrosylpentacyanovanadate(I), nitrosylpentacyanochromate(I), nitrosylpentacyanomanganate(I) hyponitritobispentacyanocobaltate(III), and nitrosylpentacyanomolybdate(0) were prepared by documented methods.

Potassium nitrosylpentacyanovanadate(I) was prepared by the method of Griffiths[2]. Ammonium vanadate (5g) was dissolved in a cooled solution of potassium hydroxide (15g in 50ml of water) and treated with excess of potassium cyanide (15g in 100ml of water). The solution was then filtered and hydroxylamine hydrochloride (8g) was added, whereupon the solution became deep red. It was refluxed and stirred for 30 minutes or until no more ammonia was evolved. Care was taken to prevent crystalisation. The cooled and filtered solution was slowly added to alcohol, and the resulting orange oil separated, washed with alcohol, dissolved in water (7ml), and again precipitated with alcohol. The oil was then triturated with warm absolute alcohol and ether whereupon it quickly crystalised. This procedure was repeated and the bright orange crystals were collected and washed with dry methanol (to

remove traces of potassium cyanide) and dried under reduced pressure.

Potassium nitrosylpentacyanochromate(I) was prepared by the method of Griffiths[3]. Chromium trioxide (7g) was added to a cold saturated solution of potassium hydroxide (20g) with ice cooling. Saturated aqueous potassium cyanide (35g) was then added and the mixture filtered. Hydroxylamine hydrochloride (8g) was then added to the filtrate and the solution was heated (steam bath) for two hours, and then filtered and cooled. The filtrate is poured with stirring into ethanol (250ml). The precipitate was dissolved in the minimum quantity of water and the compound again precipitated with ethanol and dried in a desiccator under reduced pressure.

Potassium nitrosylpentacyanomanganate(I) was prepared by the method of Blanchard[4]. Manganous acetate (19.6g in 25ml of water) was added to potassium hydroxide solution (5.2g in 25ml) and treated with excess potassium cyanide (26g in 25ml) in a bubbler unit. Nitric oxide gas was passed through the solution (frittered disc). This was maintained for four and one half hours, by which time it is estimated that approximately 500ml of nitric oxide will have reacted.

The green precipitate which forms at first ($K_5Mn(CN)_6$) gradually disappears and the solution becomes a deep red-purple, resembling the colour of permanganate. There is also a small amount of light coloured residue ($Mn(OH_2)$). The nitric oxide is removed and the solution poured into a beaker. Manganous acetate (28g) is added, then glacial acetic acid (40ml) is added slowly with stirring. The mixture is stirred for one half hour and the rose coloured precipitate collected on a filter and washed with water.

The precipitate was digested with potassium carbonate (16g in 15ml of water) warming gently. This was poured on a filter and the residues washed with water alcohol (1:3). Acetic acid is added to the filtrate carefully until evolution of carbon dioxide ceased. Alcohol (100-150ml) was added and a powder formed. Digestion with potassium carbonate was repeated and the precipitate collected on a filter and washed with alcohol and dried under vacuum.

Potassium hyponitritobispentacyanocobaltate(III) was prepared via the pentamminecobalt(II) chloride by the method of Nast[5]. Cobalt(II) chloride six hydrate (25g in 50-60ml) was cooled in an ice bath; ammonia (0.88,150ml) was added slowly with stirring. Any pink precipitate which formed is removed by filtration and discarded. This solution was passed via a funnel into an air free reaction vessel. Nitric oxide gas was passed through the solution for three to four hours. The solution was filtered, under nitrogen, producing black shiny crystals (pentammine-cobalt(II) chloride), which were washed using alcohol and dried under vacuum in a desiccator.

Pentamminecobalt(II) chloride (20g in 50ml of distilled water) are mixed with potassium cyanide (30g in 50ml of distilled water) and cooled in an ice bath with stirring. After the evolution of ammonia the solution is a red-black colour. The excess ammonia is removed by suction of air through the solution for one half hour. The solution was filtered and added to methanol (500ml) with vigorous stirring. The product precipitates as a golden powder. The compound was redissolved and reprecipitated, washed with ethanol and dried under vacuum in a desiccator.

Potassium nitrosylpentacyanomolybdate(0) was prepared using a modified method of Hieber[6]. Molybdenum trioxide (10g) was dissolved with potassium hydroxide (20g) in a minimum amount of water. A saturated solution of potassium cyanide (40g) was added and the solution mixed. Hydroxylamine hydrochloride (7.5g) was added and the solution refluxed for 90 minutes. The solution changes colour from red through yellow, red to purple, at which point the product begins to precipitate down. Concentrated potassium hydroxide (50%, 50ml) is added and the solution allowed to cool. The product precipitates out and is filtered. The compound is unstable in solid form in contact with air and is stored under concentrated potassium hydroxide (20 M). The product is insoluble in this solution.

Reactivity of the Complexes

All the complexes were investigated to discover if they reacted with acid, alkali, amines (ethylamine, diethylamine) and thiols (hydrogen sulphide and cysteine). The investigations were conducted spectrophotometrically using a Unicam SP 800. A reaction was said to occur if there is a change in the u.v.-visible spectrum (200 nm-850 nm) of the original complex. Standard solutions of the complexes were prepared, normally 10^{-4}M, 10^{-3}M, and 5×10^{-3}M. These three concentrations were capable of recording all the chemical events for the nitrosyl species under study. (Above 5×10^{-3}M a reaction is clearly visible with the naked eye.) The u.v.-visible spectra of these solutions were recorded, care being taken to observe any reaction which occurs on dissolution. (Reactions of this type occur with the hyponitritopentacyanocobaltate(III)

anion and the nitrosylpentacyanomolybdate(0) anion on dissolution due to the pH.) The standard solutions were doped with a small volume (~0.1ml) of concentrated reagent and the u.v.-visible spectrum monitored for change (indication of a reaction); care is taken not to confuse a reaction with a dilution effect. The amines increase the pH of solution, due to buffering and the thiols must be dissolved in solution of alkali pH (~8) to produce a reactive species. It is important that any reaction of these species must not be confused with a base reaction. The nitrosylpentacyanomolybdate(0) and hyponitritobispentacyanocobaltate(III) anions are extremely reactive in neutral or acid media. Studies using amines and thiols on these species were carried out in sodium hydroxide (1.0 M).

Kinetic Studies

The kinetic studies of the reactions of acid with the nitrosylpentacyanomanganate(I) and hyponitritobispentacyanocobaltate(III) anions were carried out on a Pye-Unicam SP 8-100 spectropholometer at 384 nm and 290 nm respectively.

The same technique was employed for both studies. A fixed amount of complex was weighed directly into a dry standard flask (25ml). Buffer was added (~20ml) and the compound quickly dissolved; the flask was made up to the mark. Cuvettes were filled and the reaction monitored; an accurate pH was recorded using the remaining solution with an added check on buffering.

In the study of the hyponitritobispentacyanocobaltate(III), the rate constants were calculated using the method of Kedzy[22] and Swinbourne[23] (Appendix I).

Products

(a) Potassium nitrosylpentacyanovanadate(I) in acidic media

The reaction of the nitrosylpentacyanovanadate(I) anion in hydrochloric acid gives a yellow powder, which had no carbon, nitrogen or hydrogen present, but was identifiable using spectroscopic techniques as dioxovanadium(V) chloride.

Potassium nitrosylpentacyanovanadate(I) (0.3g) was dissolved in a minimum amount of water and treated with hydrochloric acid (0.1 M,10ml). The solution was allowed to stand for five minutes after which it was added to methanol (50ml) and cooled in an ice bath. A yellow solid precipitated out and was collected on a filter, washed with ethanol and ether and dried in a desiccator under vacuum.

Infra-red stretching frequencies (ν max) 3 500 cm^{-1}-3 200 cm^{-1} (H_2O) medium, 1 600 cm^{-1} (H_2O) medium, 950 cm^{-1} (VO) strong, 825 cm^{-1} (VO) strong, and 750 cm^{-1} (VO) strong. The ultra-violet spectrum of the product (10^{-3}M,pH 2.0) is exactly the same as a solution of dioxovanadium(V) chloride; a shoulder at 275 nm extending into the visible region.

(b) Potassium nitrosylpentacyanovanadate(I) in basic medium

The reaction of the nitrosylpentacyanovanadate(I) anion in potassium hydroxide gives a white powder, which had no carbon, nitrogen or hydrogen present, but was identified using spectroscopic techniques as potassium vanadate(V).

Potassium nitrosylpentacyanovanadate(I) (0.3g) was dissolved in the minimum amount of water and treated with potassium hydroxide

(10ml,1.0 M). The solution was added to methanol (50ml) and cooled on ice. A white solid precipitated out and was collected on a filter, washed with ethanol and ether, and dried under vacuum in a desiccator.

Infra-red stretching frequencies (ν max) 3 500 cm^{-1}-3 300 cm^{-1} (H_2O) medium, 1 620 cm^{-1} (H_2O) medium, 870 cm^{-1} (VO) medium and 825 cm^{-1} (VO) medium. The ultra-violet spectrum of the product (10^{-3}M,pH 9.5) was exactly the same as a commercial sample of ammonium vanadate; an absorption maximum at 260 nm.

(c) Potassium nitrosylpentacyanomanganate(I) in acidic medium

The reaction of the nitrosylpentacyanomanganate(I) anion in acidic solution gives a product, which on analysis, is consistent with the nitrosylpentacyanomanganate(II) anion. Two samples were prepared, using the method of Cotton[18], a potassium salt and a cobalt(II) salt.

Concentrated nitric acid is added dropwise to a filtered solution of potassium nitrosylpentacyanomanganate(I) (5.0g in 15ml of water). The solution, which turned yellow and a gas was evolved, was allowed to stand for five minutes and then taken to dryness at the water pump on a hot water bath. Acetone (100ml,A.R. grade) was added to the residue, the product being extracted as a yellow solution. The acetone solution was added to dry ether (500ml), whereupon a yellow powder precipitates. This was filtered, but not completely, care was taken to always have a layer of dry ether over the product. All the acetone was washed away and the filter funnel, complete

with product and layer of ether, was transferred to a desiccator. A vacuum applied and a clean dry ether atmosphere produce; the product was dried and special arrangements for elemental analysis made. This compound is very unstable.

Infra-red stretching frequencies (ν max) 3 650 cm^{-1}-3 450 cm^{-1} (H_2O) medium, 2 140 cm^{-1} (CN) strong, 1 900 cm^{-1}-1 850 cm^{-1} (NO^+) strong and 1 600 cm^{-1} (H_2O) medium. Found: C,18.0; H,0.0; N,25.5%. Expected for $K_2[Mn(CN)_5NC].2H_2O$ C,18.2; H,1.2; N,25.5%. The complex has a strong absorption at 384 nm in the visible spectrum. The molar extinction co-efficient was calculated to be 1250 $M^{-1}cm^{-1}$ this is compared to Manoharan's[13] value of 1700 $M^{-1}cm^{-1}$.

Concentrated hydrochloric acid was added dropwise to a solution of potassium nitrosylpentacyanomanganate(I) (1.0g in 5ml of water), whereupon the solution turned yellow. A concentrated solution of cobalt(II) chloride was added and a red precipitate formed. This was collected on a filter and washed with hot water (ca 70°C), ethanol and ether, transferred to a desiccator and dried under vacuum.

Infra-red stretching frequencies (ν max) 3 500 cm^{-1}-3 400 cm^{-1} (H_2O) strong, 2 180 cm^{-1} (CN) strong, 1 880 cm^{-1} (NO^+) strong and 1 600 cm^{-1} (H_2O) medium. Found: Co,16.5; Fe13.2; C,17.0; H,1.4; N,22.3%. Expected for $Co[Mn(CN)_5NO].5H_2O$ Co,16.2; Fe,15.1; C,16.5; H,2.8; N,23.1%.

(d) Potassium hyponitritobispentacyanocobaltate(III) in acidic medium

The reaction of the hyponitritobispentacyanocobaltate(III) anion in acid medium gives a product, which on analysis was constant with the aquopentacyanocobaltate(III) anion. Two samples were prepared: a potassium salt and a silver salt. Potassium hyponitritobispentacyanocobaltate(III) (1.0g) was dissolved in demineralised water (25ml). The solution was allowed to stand (react) for 30 minutes. 15ml of this solution was taken and lyophilised to produce a crude yellow product. This was redissolved in the minimum amount of water and reprecipitated with methanol (50ml), cooling the solution in an ice bath. The product (potassium salt) was filtered, washed with ethanol, ether and dried in a desiccator.
The remaining 10ml of the solution were treated with a concentrated silver nitrate solution to precipitate a yellow-white powder. This was collected, washed with hot water (ca 70°C), ethanol, ether and dried in a desiccator under vacuum.
Data for the potassium salt: infra-red stretching frequencies 3 550 cm^{-1}-3 440 cm^{-1} (H_2O) medium; 2 130 cm^{-1} (CN^-) strong and 1 600 cm^{-1} (H_2O) medium. The ultra-violet spectrum shows an absorption maximum at 372 nm before it is precipitated for the first time and an absorption maximum at 348 nm on redissolution of the product. The relative molar extinction co-efficient were calculated to be 250 $M^{-1}cm^{-1}$ and 291 $M^{-1}cm^{-1}$ respectively. These values compare favourably with Haim[20] and Wilmarth's data

for monomeric aquopentacyanocobaltate(III) (380 nm, 260 $M^{-1}cm^{-1}$) and polymeric aquopentacyanocobaltate(III) (352 nm, 265 $M^{-1}cm^{-1}$), which they observed as the product of a totally different process.

Data for the silver salt: infra-red stretching frequencies 3 550 cm^{-1}-3 450 cm^{-1} (H_2O) weak, 2 160 cm^{-1} (CN) strong and 1 600 cm^{-1} (H_2C) weak. Found: C,14.7; H,0.2; N,16.6%. Expected for $Ag_2[Co(CN)_5OH_2].H_2O$ C,14.2; H,0.4; N,16.6%.

Decomposition of the Complexes

The ability of the complexes to decompose photolytically to produce cyanide was investigated at pH 7.2. Two solutions (100ml,10^{-3}M) of each compound were prepared; one of each was rigorously protected from the light by foil. The solutions were placed in bubbler units and subjected to a 100W bulb at 12 inches. Nitrogen gas was passed through the solution using a frittered disc, to remove the cyanide and then through a trapping solution (sodium hydroxide 0.1 M) again using a frittered disc. The solutions which were protected from the light were subjected to the same procedure, but experiencing only the heating effect from the bulb.

The detection of cyanide in the trapping solution was by the method of Faulkof[25] using chloramine T, and pyridine/3-methyl-1-phenyl-2-pyrazol-5-one solution (Appendix II).

Animal Testing

This was carried out by the Department of Pharmacology and Therapeutics; Ninewells Hospital.

APPENDIX I

Kezdy-Swinbourne method for the determination of a first order rate constant (k)

It is often impracticable to measure the initial concentration or concentration after 'infinite' time of a reactant during a kinetic study and the method outlined below overcomes these problems.

Consider a first-order reaction of which observations $(x_0, x_1, x_2, \ldots\ldots x_n \ldots\ldots x_\infty)$ are taken at times $(t_0, t_1, t_2, \ldots\ldots t_n \ldots\ldots t)$. For a reading (x_n) taken at (t_n)

$$(x_\infty - x_n) = (x_\infty - x_0)\exp.(-kt_n) \qquad (1)$$

Now consider a second series of observations $(x_0, x'_1, x'_2, \ldots\ldots x'_n \ldots\ldots x)$ taken at times $(t_0, t_1 + \Delta t, t_2 + \Delta t_1 \ldots\ldots t_n + \Delta t \ldots\ldots t_\infty)$ where t is a small, constant time interval.

For a reading (x'_n) taken at time $(t_n + \Delta t)$

$$(x_\infty - x'_n) = (x_\infty - x_0)\exp.[-k(t_n + \Delta t)] \qquad (2)$$

Dividing (1) by (2) and rearranging gives

$$(x_\infty - x_n)\exp.(kt_n) = (x_\infty - x'_n)\exp.[k(t_n + \Delta t)] \qquad (3)$$

Therefore,

$$x_n = x_\infty[1 - \exp.(k\Delta t)] + x'_n \exp.(k\Delta t)$$

A straight line is obtained when plotting the observed readings in the first series (x_n) against the corresponding readings in the second series (x'_n), and the rate constant (k) of the reaction can be evaluated from the log of the slope of the line.

For $t=\infty$; $x_n = x'_n = x$, and therefore x is the point on the line at which x_n and x'_n are equal. Also, if the time of commencement of the

reaction is known it is possible to extrapolate back along the line and find the corresponding value of x_0.

The following features should, however, be noted when employing this method:

(a) readings taken towards the end of the reaction are 'telescoped' on the graph and, therefore, are weighted less than earlier readings

(b) the data should be recorded over a period of time greater than $t_{\frac{1}{2}}$ (the half-life of the reaction) and preferably greater than twice this period. The time interval t should be in the range $0.5_{t\frac{1}{2}}$ $t\frac{1}{2}$.

(c) the method is relatively insensitive to deviations from strict first-order law, so an independent check of this is advisable.

APPENDIX II

Detection and Determination of Cyanide using Colorimetric Techniques

This is a sensitive method of measuring cyanide concentrations in solution; developed by M.M. Faulkof, B. Witten and B. Giehauf. It is based upon the ability of cyanogen halide to split pyridine rings to form a glutaconaldehyde derivative. This derivative in turn couples with pyrazolones to form highly coloured compounds.

Cyanide present in solution must first be converted to cyanogen halide using chloramine T.

Solutions required for quantitative analysis and calibration

(a) 0.1% chloramine T solution in 0.75 M sodium dihydrogen phosphate. This solution is cooled on ice.

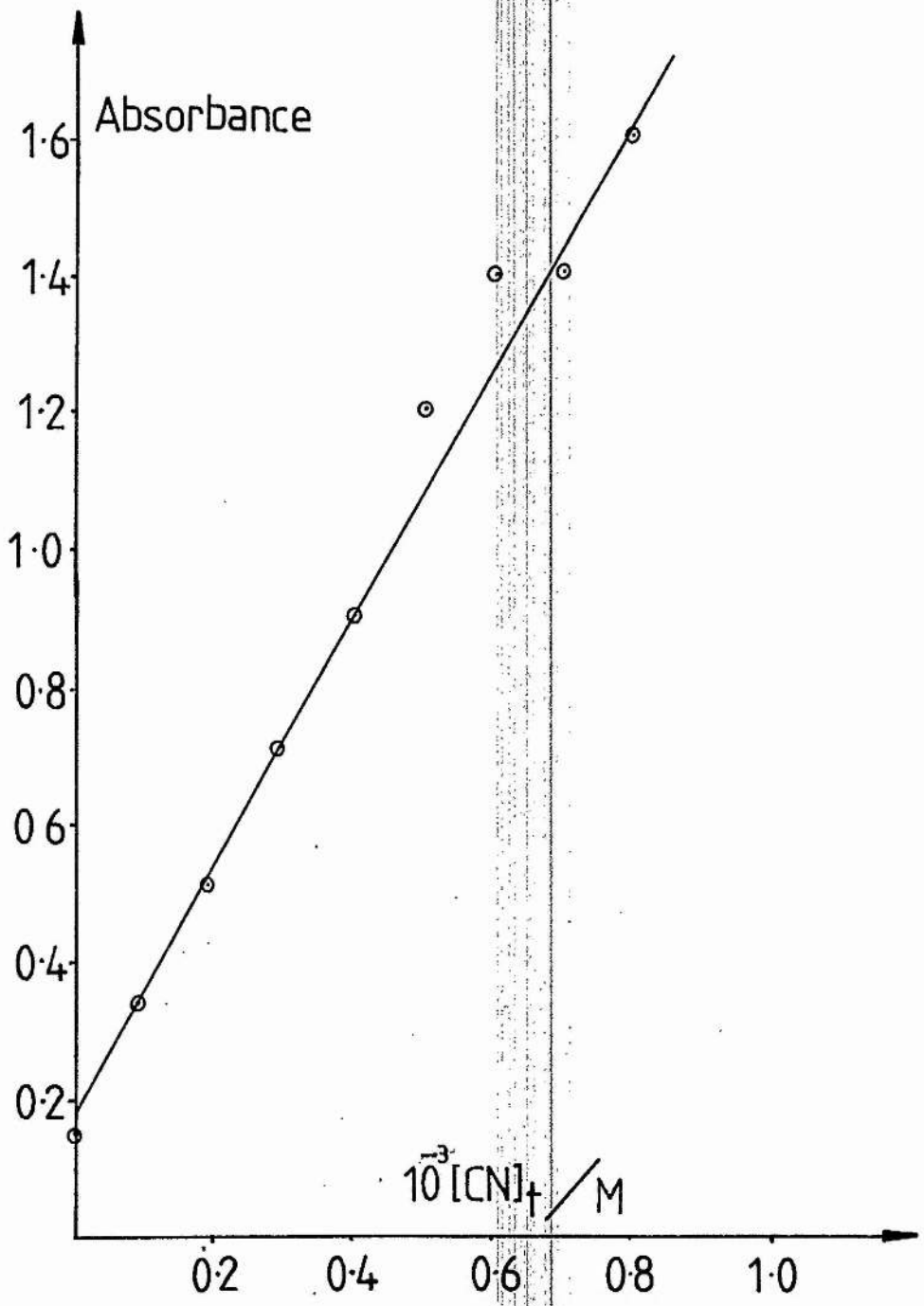
(b) 0.25g of 3-methyl-1-penyl-2-pyrazolin-5-one dissolved in 100ml of deionised water. (This could require heating.)

(c) 20ml of pyridine.

(d) Standard potassium cyanide solution ($0-10^{-3}$M) in sodium hydroxide (0.1 M).

Solutions must be made fresh each day. Solution (b) and (c) are mixed just previous to calibration or analysis and cooled.

For calibration: potassium cyanide solution (0.125ml) is transferred using an automatic dispenser directly into a cuvette. Chloramine T solution (1.5ml) is added and the cuvette shaken and allowed to stand for two minutes. Pyrazolone/pyridine solution (1.5ml) is



Calibration Chart for Colorimetric Cyanide Determination

(Figure A)

added; a blue colour forms and absorption maximum is measured when the reaction is complete (approximately 5 minutes) at 615 nm on a Unicam SP 8-100. Plotting absorption maximum against the original cyanide concentration gives a good straight line.

The same procedure is repeated using solutions for analysis. The original concentration of the solution can be measured directly.

Calibration Data for Faulkof's Technique

$10^{-4}[CN^-]_t$/M	Absorption Maximum
0.00	0.147
1.02	0.345
2.04	0.514
3.06	0.710
4.08	0.900
5.10	1.218
6.12	1.430
7.14	1.430
8.16	1.618

$[CN^-]_t$ = concentration of cyanide in the original solution (table (a))

It is important that a control is used with a solution containing no cyanide, as there is a certain amount of decomposition due to the reactivity of the solutions.

APPENDIX III

The Calibration of the Cyanide Sensitive Electrode in Aqueous Solution

Accurate determination of the cyanide concentration in a solution can only be made if the solution has a pH in excess of 12. Calibration was carried out using standard potassium cyanide solutions in potassium hydroxide (1.0 M). The data and chart are shown below.

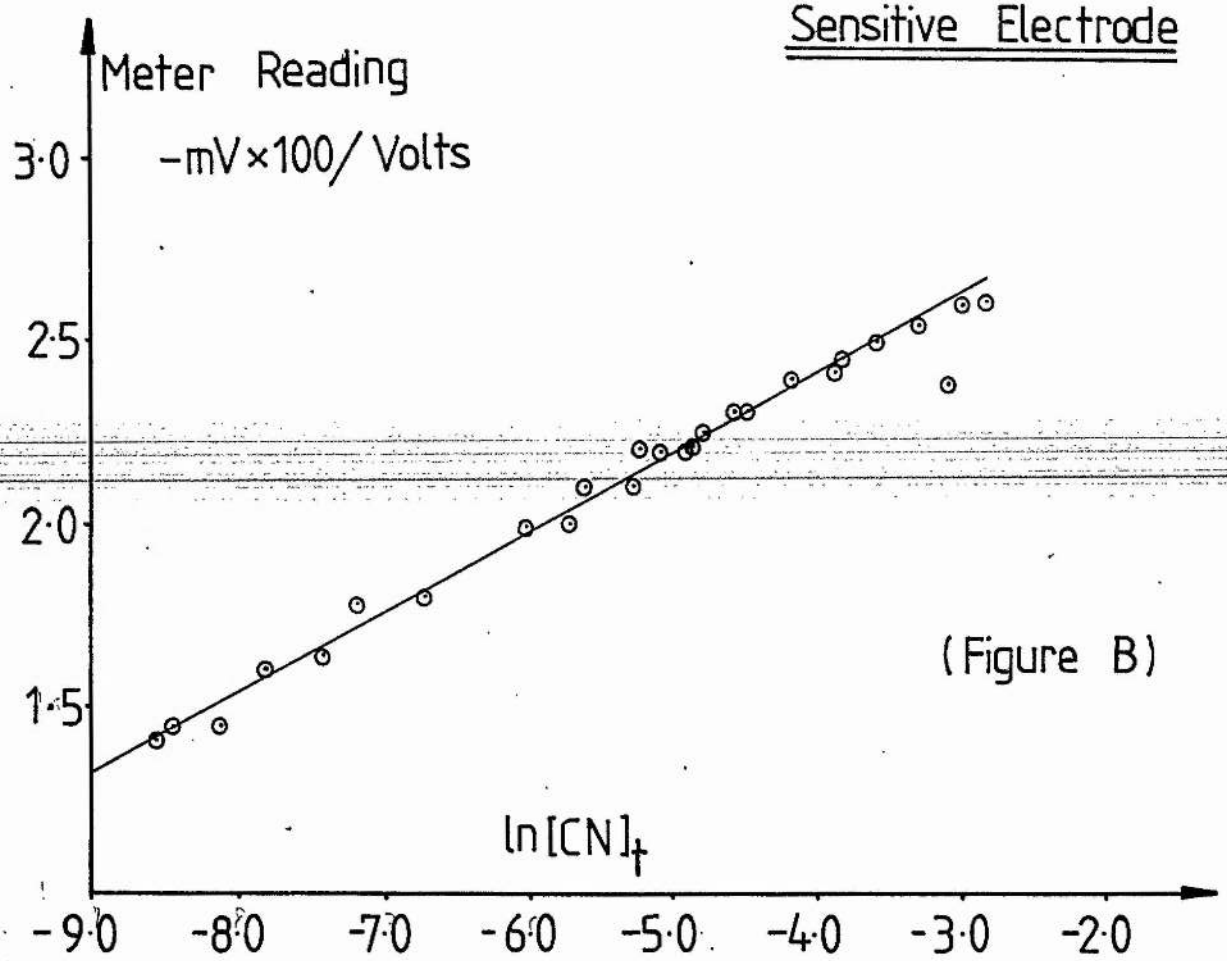
Method

Standard solutions of potassium cyanide were prepared (0.1 M; 0.01 M and 0.001 M).

To a standard flask (25ml) was added potassium hydroxide (10.0ml; 2.5 M) and a set volume of a stock standard potassium cyanide solution. The flask was then made up to the mark. This process is repeated to create a range of standard potassium cyanide solutions at the correct pH.

The solutions cyanide concentration were then measured in order of ascending concentration. The electrode, after insertion into solution, was allowed five minutes to stabilise during which the solution was gently stirred.

Calibration Chart for the Cyanide Sensitive Electrode



(Figure B)

Calibration Data for the Cyanide Sensitive Electrode

$[CN^-]/M$	$-\ln[CN^-]$	Meter Reading -mVx100/Volts
1.89×10^{-4}	8.57	1.418
2.31×10^{-4}	8.37	1.439
2.84×10^{-4}	8.17	1.454
4.23×10^{-4}	7.77	1.597
6.28×10^{-4}	7.37	1.637
7.36×10^{-4}	7.21	1.687
1.20×10^{-3}	6.72	1.792
2.48×10^{-3}	6.00	1.980
3.42×10^{-3}	5.68	2.034
3.51×10^{-3}	5.63	2.058
4.83×10^{-3}	5.33	2.102
5.05×10^{-3}	5.29	2.174
6.28×10^{-3}	5.07	2.170
7.20×10^{-3}	4.93	2.188
7.51×10^{-3}	4.89	2.214
8.50×10^{-3}	4.77	2.244
9.93×10^{-3}	4.61	2.274
1.15×10^{-2}	4.46	2.308
1.52×10^{-2}	4.19	2.381
2.01×10^{-2}	3.91	2.418
2.05×10^{-2}	3.89	2.439
2.75×10^{-2}	3.59	2.516
3.72×10^{-2}	3.29	2.558
4.41×10^{-2}	3.12	2.375
4.99×10^{-2}	3.00	2.580
5.88×10^{-2}	2.83	2.607

(table (b))

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