Kinetic and mechanistic studies involving the nitroso group

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KINETIC AND MECHANISTIC STUDIES INVOLVING THE NITROSO GROUP

by

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A thesis submitted for the degree of Doctor of Philosophy of the University of Durham.

September 1982

MEMORANDUM

The work for this thesis has been carried out in the Department of Chemistry at the University of Durham between October 1979 and May 1982. It is the original work of the author unless otherwise stated. None of this work has been submitted for any other degree.

ACKNOWLEDGEMENTS

I would particularly like to express my gratitude to Dr D.L.H.Williams, my supervisor, for his guidance and encouragement during the period in which this work was carried out.

In addition, I would like to thank Dr M.R.Crampton, Dr G.Stedman and Dr G.Kohnstam for helpful discussion. Mr R.N.Brown for maintenance of the spectrophotometers and Mr C.Greenhalgh for assistance in the use of the computer. I would also like to mention my collegues; Tom Meyer, Elaine Aldred and Marion O'Neill.

I also thank the Science and Engineering Research Council for a research studentship.

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ABSTRACT

Nitrosation is a major area of work in physical organic chemistry, which is of interest as an academic study in its own right but is also of wider interest owing to the carcinogenicity of some N-nitroso compounds. The present work has been concerned with some of the reactions involving the nitroso group at nitrogen, oxygen and sulphur sites. The approach has been to determine rate constants for the reactions involved and to use these to establish mechanism and relative reactivities. The work covers four areas;

(i) Denitrosation of N-nitrosoamines in solvents other than water. It has been found that some solvents do enhance the rate of denitrosation relative to that in water.

(ii) Diazotisation of aromatic amines. This reaction proceeds via the formation of an N-nitroso intermediate. The relative reactivities of a number of nitrosating species in this reaction have been established.

(iii)Nitrosation of thiols. Few kinetic studies have been made of S-nitrosation (compared to N-nitrosation), here the rates of reaction of some thiocarboxylic acids toward nitrosating species have been determined and comparisons with other substrates drawn.

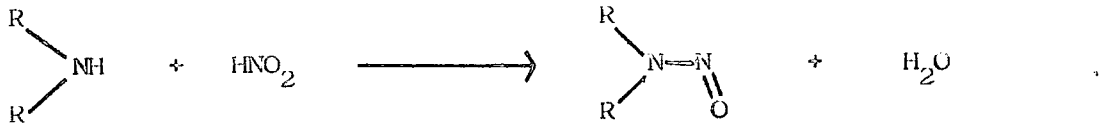
(iv) Nitrosation of alcohols. The kinetics of nitrosation of alcohols has already been studied in some detail, and here the aim has been to use this reaction to throw some light on the nature of the intermediate in aqueous nitrosation.

CHAPTER ONE

Denitrosation of N-nitroso compounds

Introduction

N-nitrosoamines are formed by the reaction of a secondary amine and nitrous acid. This was a standard test for secondary amines as the product of the reaction is usually an oily orange-yellow liquid.

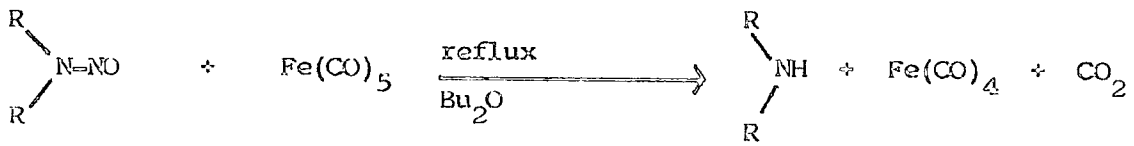


The carcinogenic properties of N-nitroso compounds are well known, and many reviews on the subject have been written.[1] Denitrosation is essentialy the reverse of the above reaction, the conversion of an N-nitrosoamine to the corresponding secondary amine. This is a useful reaction as it provides a means of destroying these potentially toxic compounds. The mechanism of the reaction has been studied in depth and a review of the previous studies is given below. This is followed by an account of the present work, a study of the effect of changing the solvent on the rate of the denitrosation reaction.

1.1 Qualitative aspects of denitrosation

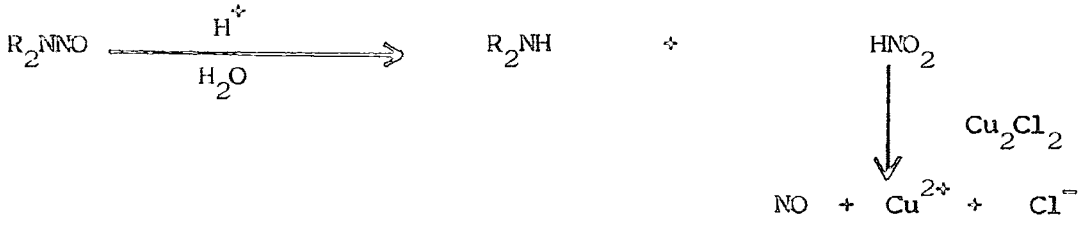
Denitrosation of an N-nitrosoamine affords a method of synthesising a secondary amine. Most synthetic methods involve the reduction of the nitroso group, simple alkyl nitrosoamines can be reduced to the corresponding hydrazine with zinc in acetic acid. Compounds with substituents larger than propyl require more vigorous conditions, giving the secondary amine as the main product.

A more recent method is to reduce with iron pentacarbonyl,[2] this involves hydrogen abstraction from the solvent as shown below.

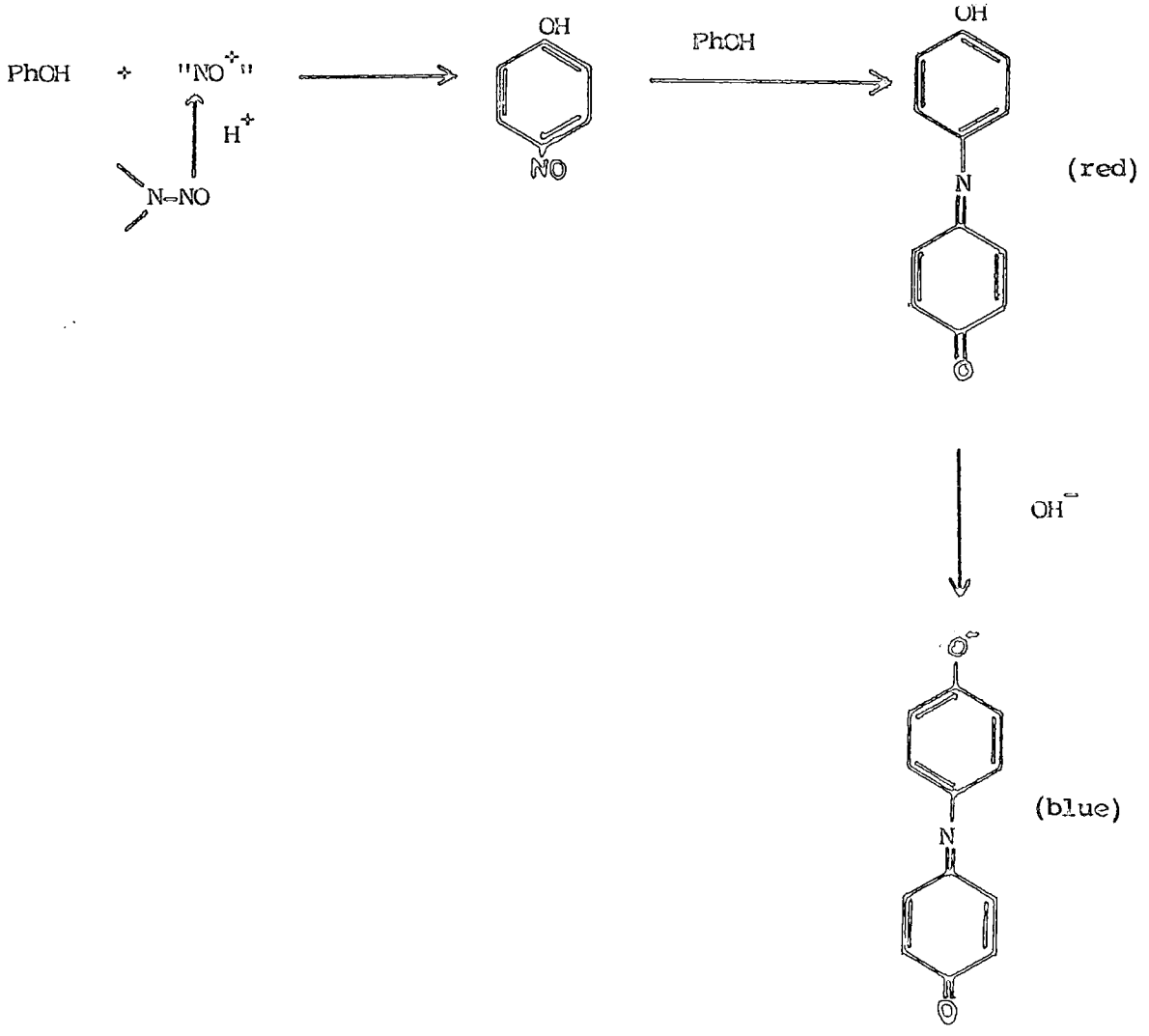


Some nitrosoamines can be reduced photochemically.[3] Denitrosation occurs quantitatively in concentrated mineral acid provided that the formed nitrous

acid is removed using a nitrite 'trap'. In some early studies[4] cuprous chloride was used in this way, and in later work urea, hydrazine and other compounds have been used.

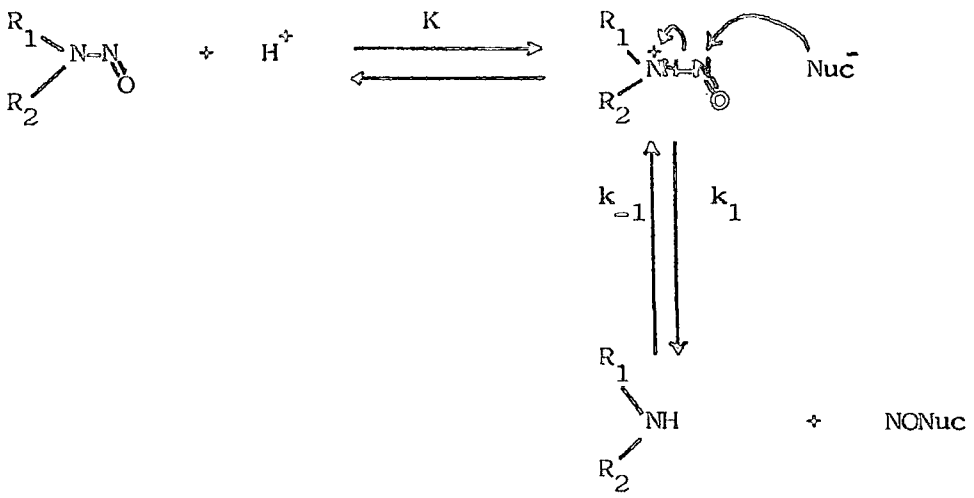


In the absence of a nitrite trap, aromatic N-nitrosoamines also undergo rearrangement to give the C-nitroso compound as a side product. The nitrous acid liberated during denitrosation forms the basis of the Liebermann test which is given by nitrosoamines on warming with sulphuric acid in the presence of phenol. A red colour is formed which undergoes a change to blue on addition of base.



1.2. The mechanistic scheme

The first kinetic studies of denitrosation were carried out by Russian workers.[5] They found that the rate of denitrosation of a nitrosoamine was greater in HCl than in H_2SO_4. It was suggested that a hydrogen bonded complex was formed between the acid and the nitrosoamine, followed by either unimolecular scission to give $NO^{\oplus}$ or nucleophilic attack on the hydrogen bonded complex. This theory was not based on any experimental evidence and recent kinetic studies have shown that this is not the case. The reaction has now been shown to proceed via nucleophilic attack on the protonated nitrosoamine as shown in the scheme below.



$$NONuc + \text{'trap'} \xrightarrow{k_2} \text{Various products}$$

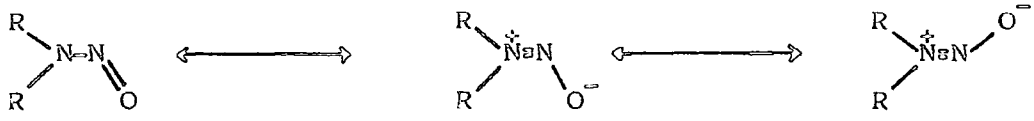
$Nuc^{\ominus} = I^{-}, Br^{-}, Cl^{-}, SCN^{-}$, Thiourea, H_2O.

Trap = Sulphamic acid, hydrazine, hydrazoic acid, ascorbic acid etc.

Although this scheme shows the site of protonation of the N-nitrosoamine as the amino nitrogen atom, there are other protonation sites. Owing to their low basicity, pK_a values for N-nitroso compounds are few. Evidence suggests that of the possible conjugate acid species, that which corresponds to the O-protonated form is the more stable.

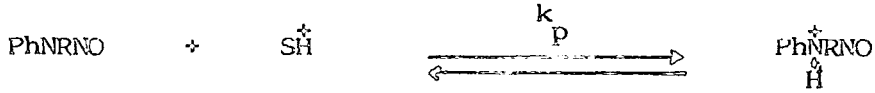
The other possible sites are the nitrogen atoms, as shown below.

M.O. calculations[52] show that the species with the protonated amino group has a stabilisation energy of 67 kJ mol^{-1} greater than the O-protonated species. ^1H n.m.r.[53,54] studies show that on protonation ~~of the methyl groups~~ in dimethyl nitrosoamine $(CH_3)_2NNO$ in $HSO_3F.SbF_5$ (magic acid) the methyl groups remain inequivalent, which is consistent with the O-protonated form. Other n.m.r. studies[9] show the existance of several protonated species in solution though it is not clear as to what they are. Further support for O-protonation is given by the fact that N-nitrosoamines do have considerable dipolar character, and O-complexes have been isolated.[8] Some isomeric forms are shown.

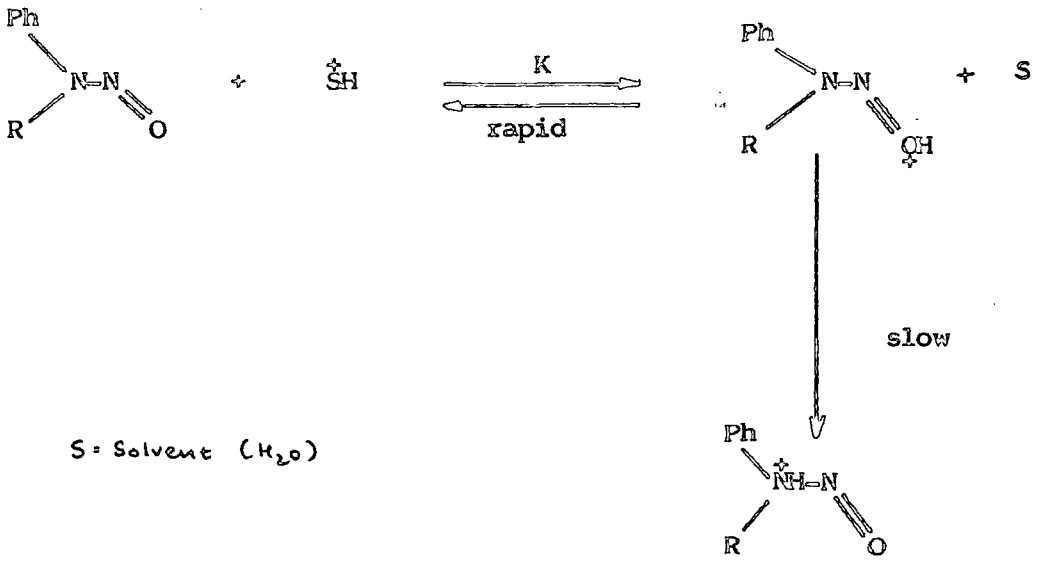


It appears that for denitrosation, protonation on the amino nitrogen atom is necessary and so a small concentration of this species must be present in solution.

Values of the forward rate constant (k_p) for the protonation step in the denitrosation mechanism have been obtained for a series of N-substituted nitrosoamines[6] and have been found to be less than expected for direct N-protonation, and with very little difference in magnitude between the nitrosoamines.



This evidence suggests that N-protonation may occur via an O-protonated intermediate.



The denitrosation reaction is usually observed spectrophotometrically by following the disappearance of the nitrosoamine in the u.v. region. The observed rate constant is given by;

$$\text{Rate} = k_0 [R_1R_2NNO]$$

the rate can also be defined according to the above scheme as;

$$\text{Rate} = k_1 [R_1R_2\overset{+}{N}HNO][Nuc] - k_{-1}[R_1R_2NH][NONuc]$$

if a steady state is applied to NONuc we get the expression;

$$[NONuc] = \frac{k_1 [R_1R_2\overset{+}{N}HNO][Nuc]}{k_{-1}[R_1R_2NH] + k_2[trap]}$$

Making the assumption that the N-nitrosamine acts as a Hammett base:

$$[R_1R_2\overset{+}{N}HNO] = Kh_0 [R_1R_2NNO]$$

$K = 1/K_a$ for the nitrosoamine. It is also assumed that only a small concentrat-ion of the nitrosoamine is protonated and that proton transfer is rapid.

The expression for the observed rate constant becomes:

$$k_0 = \frac{k_2 k_1 Kh_0 [Nuc][trap]}{k_{-1}[R_1R_2NH] + k_2[trap]}$$

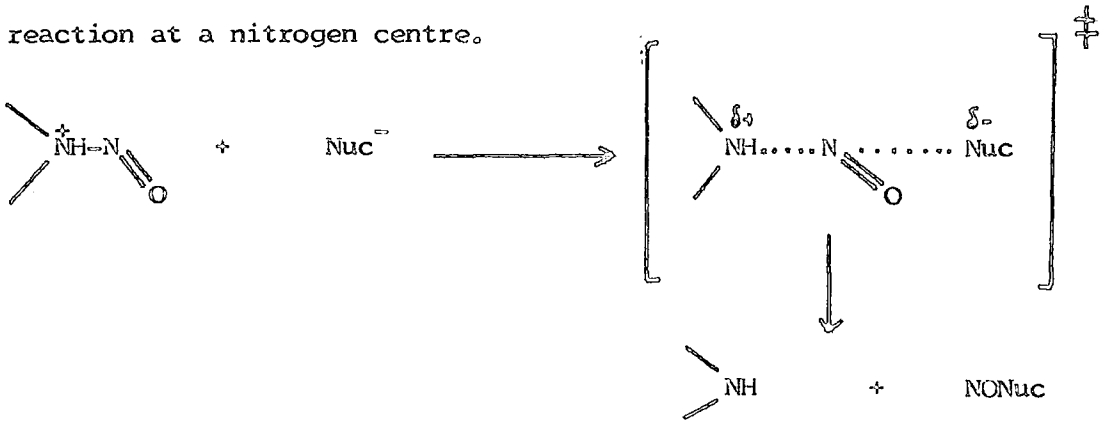
If the trap is in sufficient excess, the condition $k_2[trap] \gg k_{-1}[R_1R_2NH]$ prevails and the reaction becomes zero order in added trap. The observed rate constant is then given by $k_0 = k_1 Kh_0[Nuc]$. It is usual to study the reaction under these conditions as it is effectively irreversible. There are many instances in which the above rate expression is found to apply.

1.3. The rate limiting step

Since the reaction between the nitrite species and the trap should be rapid and irreversible, the rate limiting step for denitrosation is expected to be that of nucleophilic attack on the protonated N-nitrosoamine, however there are instances in which proton transfer to the N-nitroso compound may be rate limiting. Both possibilities are considered below.

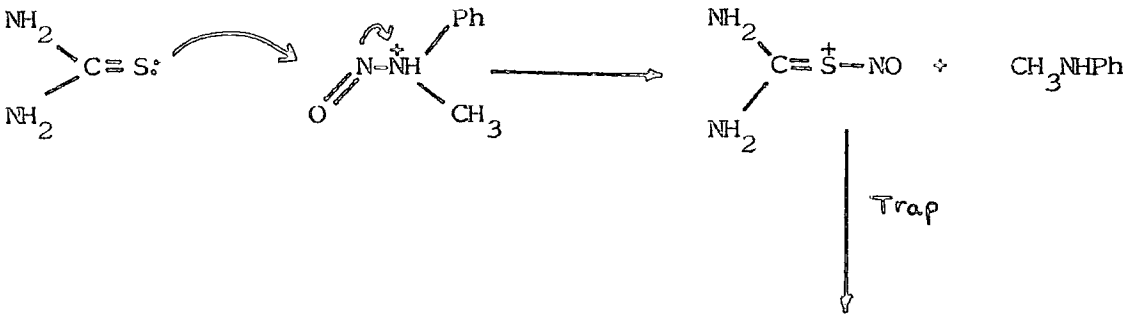
(a) Rate limiting nucleophilic attack

The k_1 step in the scheme for denitrosation is an S_N2 reaction at a nitrogen centre.



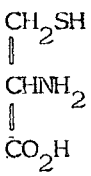
There is the possibility of formation of an intermediate between the N-nitrosoamine and the nucleophile, but there is no evidence for this at present.

The value of k_1 is found to be fairly sensitive to the nature of the nucleophile and shows a reasonably good Swain-Scott[10,11] relationship, using Pearson[9] 'n' values. The solvent isotope effect for denitrosation of N-methyl N-nitrosoaniline (NMNA) is $k_{D_2O}:k_{H_2O}$ :: 2-3 as expected for a rapid pre-equilibrium step forming a small concentration of the conjugate acid. The order of reactivity of nucleophiles toward NMNA has been established as $SC(NH_2)_2 \approx I^- > SCN^- > Br^- > Cl^- > H_2O$ which is the normal sequence for nucleophiles in an S_N2 reaction at a carbon centre. Studies have been made[13] of the reactivity of various sulphur containing nucleophiles toward nitrosoamines. Thiourea is a highly reactive nucleophile, forming an S-nitroso adduct which then undergoes reaction with the trap to give various products.

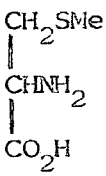


Thiourea + various products

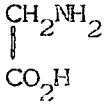
It has been found that for a number[13] of sulphur containing nucleophiles belonging to the amino acids, the S-alkylated species were the most nucleophilic toward nitrosoamines. Cysteine has a reactivity similar to that of chloride ion, S-methyl cysteine is similar to bromide ion, alanine is completely inert.



cysteine

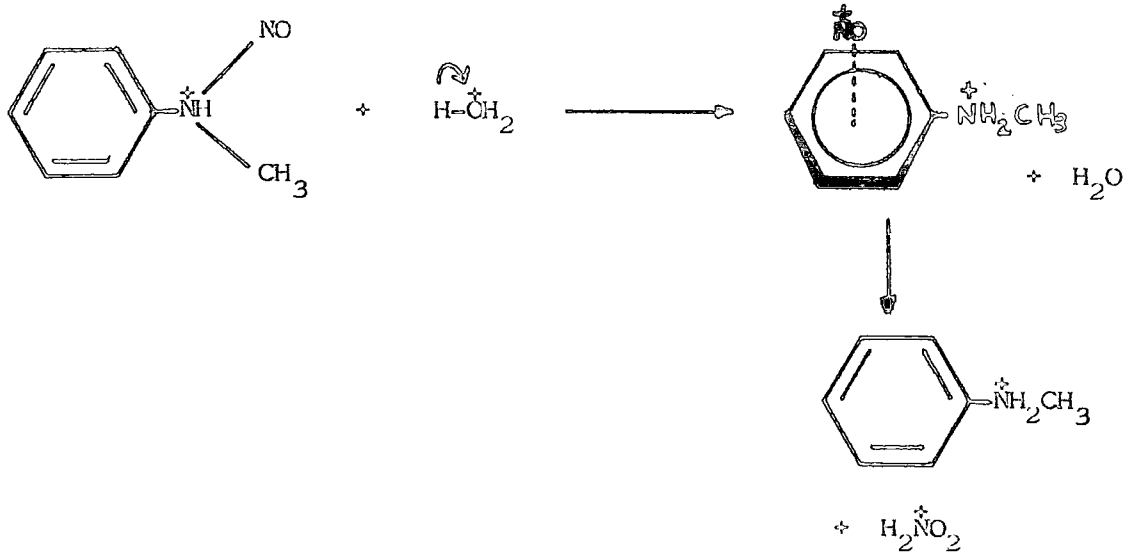


S-methyl cysteine



Alanine

If denitrosation is carried out in sulphuric acid solution in the absence of added nucleophile but in the presence of a sufficient excess of nitrite trap, the reaction proceeds slowly and addition of HSO_4^- does not alter the rate of reaction[12], ruling this out as an effective nucleophile. At higher acidities the rate-acidity profile changes slope suggesting a change in mechanism. At lower acidities water probably acts as the nucleophile, whereas at higher acidities a mechanism involving the interaction of the hydroxonium ion $H_3\overset{+}{O}$ with the ring to form (for aromatic nitrosoamines) a π-complex probably applies.



This closely resembles the mechanism proposed for the nitrosation of aromatic amines by the nitrous acidium ion in 1-3M perchloric acid[14].

(b) Rate limiting protonation of the nitrosoamine

The change in the rate limiting step from nucleophilic attack on the nitrosoamine to the protonation step can occur under various conditions. At high nucleophile concentrations denitrosation is seen to become zero order in added nucleophile.[15] This may be explained with reference to the

scheme below, where at significant excess of nitrite trap (e.g. N_2H_4) the attack by the nucleophile is irreversible and $k_0 = k_2[Nuc]\ [R_1R_2\overset{+}{N}HNO]$.

Applying a steady state to the protonated nitrosoamine we obtain an expression:

$$[R_1R_2\overset{+}{N}HNO] = \frac{k_1[\overset{+}{S}H][R_1R_2NNO]}{k_{-1}[S] + k_2[Nuc]}$$

k_0 is given by:

$$k_0 = \frac{k_1\ [\overset{+}{S}H]k_2[Nuc]}{k_{-1}[S] + k_2[Nuc]}$$

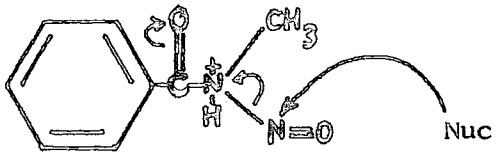
$$R_1R_2NNO + \overset{+}{S}H \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} R_1R_2\overset{+}{N}HNO + S$$

$$R_1R_2\overset{+}{N}HNO \xrightarrow[Nuc]{k_2} R_1R_2NH$$

S = solvent (H_2O or EtOH)

Clearly, if $k_2[Nuc] \gg k_{-1}[S]$ then k_0 will become independent of added nucleophile and the rate limiting step will become the protonation of the nitrosoamine. There is a similar change in the rate limiting step on changing the reaction medium from water to ethanol, as will be discussed later. When an N-nitroso compound has an electron withdrawing group adjacent to the nitroso function, the denitrosation of this compound is also found to be zero order in added nucleophile. This is due to two effects, firstly the decreased bascicity which reduces the equilibrium concentration of the protonated N-nitroso compound and secondly the increased

reactivity toward the nucleophile due to the electron withdrawing substituent which increases the value of $k_{-1}[S]$.



This is observed for N-methyl N-nitrosotoluene p-sulphonamide[16] and for N-nitroso -amides.[17] Another compound which behaves in a similar manner is important as a potential carcinogen, N-acetyl N-nitrosotryptophan.[18] However, this undergoes a more complex mechanism[19] for denitrosation than encountered in the other compounds.

1.4. The effect of alkyl substitution

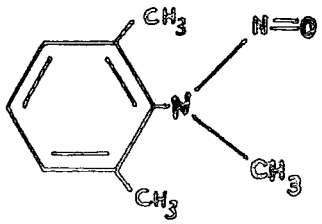
Studies[20] of the effect of various N-alkyl substituents on the denitrosation of PhNRNO by halide ions in acid solution gives the reactivity sequence Me $\ll$ Et $\sim$ n-Pr $\lesssim$ i-Pr . The parent amines have an increasing pK_a which parallels the increasing reactivity of the nitrosoamine. According to the reaction scheme

$$PhNRNO + H^{+} \underset{}{\overset{K}{\rightleftharpoons}} PhN^{+}HRNO \xrightarrow[k_2]{Br^{-}} PhNHR + NOBr$$

NOBr $\xrightarrow{\text{trap}}$ decomposition products

k_2K is a measure of the overall reactivity of the nitrosoamine. The range of reactivity covers a factor of 10 which is quite high considering the small range of pK_a values for the parent amines. It is also observed that the reactivity of N-t-butyl N-nitrosoaniline decreases with increasing size of the nucleophile. For the larger R groups it would be expected that the

value of K would increase, but that k_2 would also decrease. Clearly the effect on K outweighs that on k_2, and may be a combination of both steric and electronic effects. In the case of the t-butyl compound, there is a specific steric effect on the approach by halide ions such as iodide. Some studies of the effect of ring-methyl substitution have been made.[21] 2 and 3 methyl substituted N-methyl N-nitrosoaniline gave a rate enhancement for denitrosation compared to NMNA. The 2,6 disubstituted compound is only very slightly reactive toward nucleophiles due to steric inhibition.



There is a correlation between the inactivity of this species to nucleophilic reagents and its mutagenic properties as it is found to be non-carcinogenic.[23]

1.5. Denitrosation at high acidities

As explained above, if the denitrosation reaction is carried out in a sufficient excess of nitrite trap, the rate becomes zero-order in added trap and the rate constant is given by $k_0 = k_1 K h_0 [Nuc]$. In aqueous solutions containing up to 5M HCl a plot of log k_0 vs $-H_0$ is linear with a slope of c.a. 1.0. However,[22] at higher acidities the rate-acidity profile levels off to zero slope if hydrazoic acid or hydrazine is present as a nitrite trap. This is understood to be due to the complete protonation of the nitrosoamine, allowing the pK_a of the nitrosoamine to be estimated. For NMNA it appears to be c.a. -2. If sulphamic acid or hydroxylamine are used as traps the slope actually becomes negative at high acidities (up to 10M HCl). This is thought to be due to the added effect of the protonation of the trap which renders it ineffective.

$$NH_2SO_3H \xrightarrow{H^+} \overset{+}{N}H_3SO_3H$$

$$\overset{+}{N}H_3CH \xrightarrow{H^+} \overset{+}{N}H_3\overset{+}{C}H_2$$

1.6. The nitrite trap

The trap ensures irreversible denitrosation. The requirements of a good trap are that it should be nucleophilic toward the nitrosyl species but not toward the nitrosoamine, and that the nitrosation of the trap be rapid and irreversible, e.g.

$$HN_3 + NOX \longrightarrow N_2 + N_2O + H^+ + X^-$$

Several studies of the direct nitrosation of trap species have been made.[24,25] The relative efficiencies of a series of nitrite traps can be established using a kinetic method based upon the scheme for the denitrosation mechanism. If the corresponding secondary amine is added to the reaction, the observed rate will decrease. Since k_0 is given by the expression:

$$k_0 = \frac{k_2 k_1 Kh_0 [Nuc][Trap]}{k_{-1}[R_1R_2NH] + k_2[trap]}$$

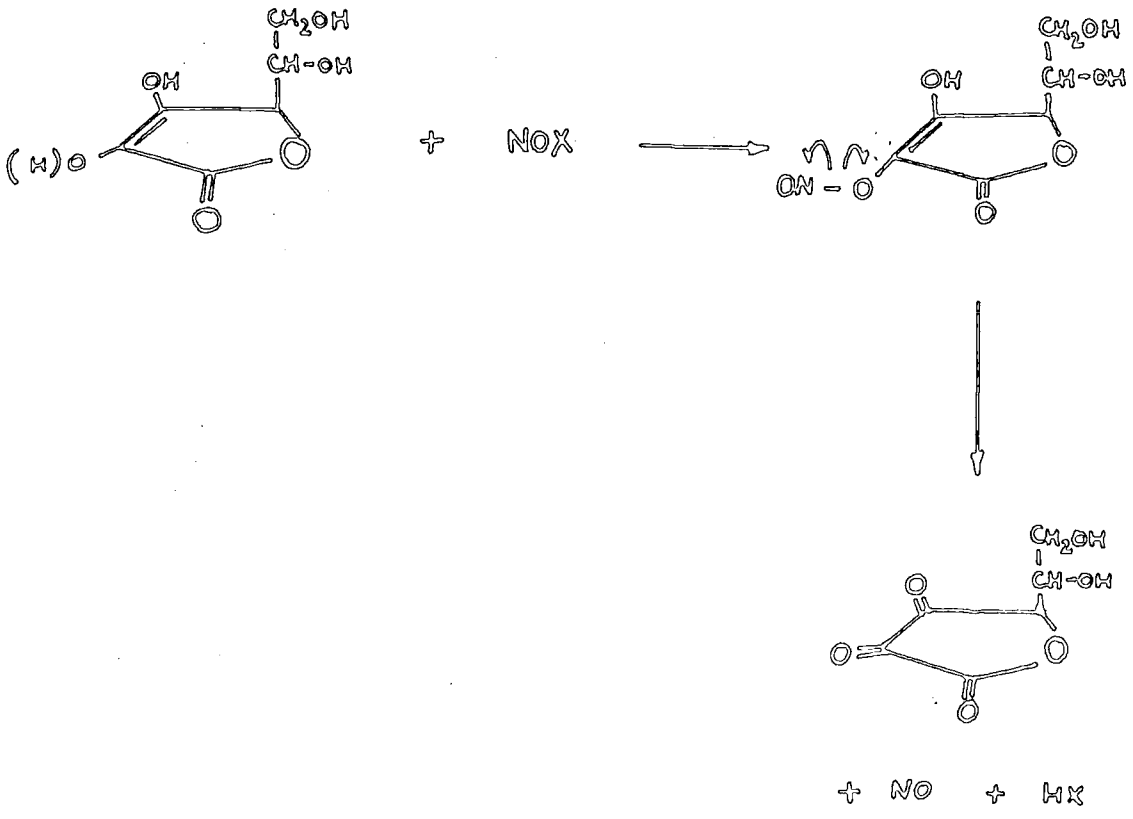
Therefore,

$$k_0^{-1} = \frac{k_{-1}[R_1R_2NH]}{k_2 k_1 Kh_0 [Nuc][trap]} + \frac{1}{k_1 Kh_0 [Nuc]}$$

So a plot of k_0^{-1} against $[R_1R_2NH]$ yields the ratio $k_2:k_{-1}$. This is a measure of the efficiency of the nitrite trap. Using this method the following sequence of trap efficiencies has been obtained.[26]

$$HN_3 \gg NH_2\overset{+}{N}H_3 > NH_2SO_3H > \overset{+}{N}H_3OH > \text{Urea}$$

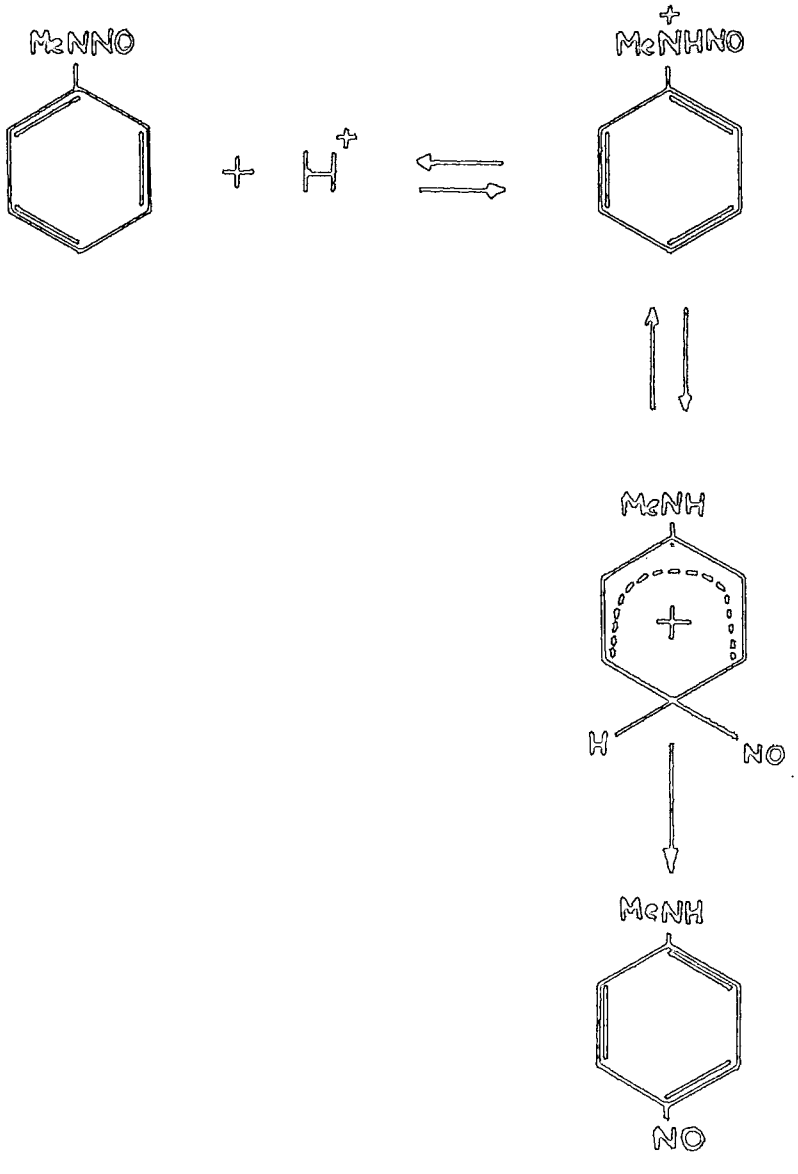
This reactivity sequence is obtained at one specific acidity and may not apply under other conditions. A change in acid concentration will affect not only the protonation equilibria of the trap but also that of the secondary amine. A change of halide concentration will also affect the $k_2:k_{-1}$ ratio. Since nitrite traps may be of some value in reducing the levels of N-nitroso compounds formed in-vivo it is important to know the acid conditions under which they will be most effective. Sulphamic acid, a good trap at a pH of less than 2 actually seems[87] to stimulate nitrosoamine formation above pH 4. A wide range[86] of compounds reduce HNO_2 to NO under mildly acidic conditions e.g. SO_2, bisulphate anion, ascorbic acid, thiols, dihydroxyphenols and some natural and synthetic antioxidants. These have been found to inhibit the formation of nitrosoamines both in-vivo and in-vitro. Ascorbic acid is an exceptionaly good trap as it is effective over a wide range of acidity. The free acid and the ascorbate anion both rapidly reduce NOX to NO.[87]



Kinetic studies compare the efficiency of sulphamic acid with ascorbic acid under various conditions.[90] Ascorbic acid is more effective at lower acidities.

1.7. Rearrangement

If NMNA is allowed to stand in acid solution without a trap present, a significant amount of rearrangement to the C-nitroso compound will take place. The mechanism of the reaction has been extensively investigated[28] and it has been established that the so-called Fischer-Hepp rearrangement proceeds via an intramolecular mechanism rather than an inter-molecular mechanism as was first proposed. The reaction is zero order in added nucleophiles and is believed to occur via the formation of a σ-complex.



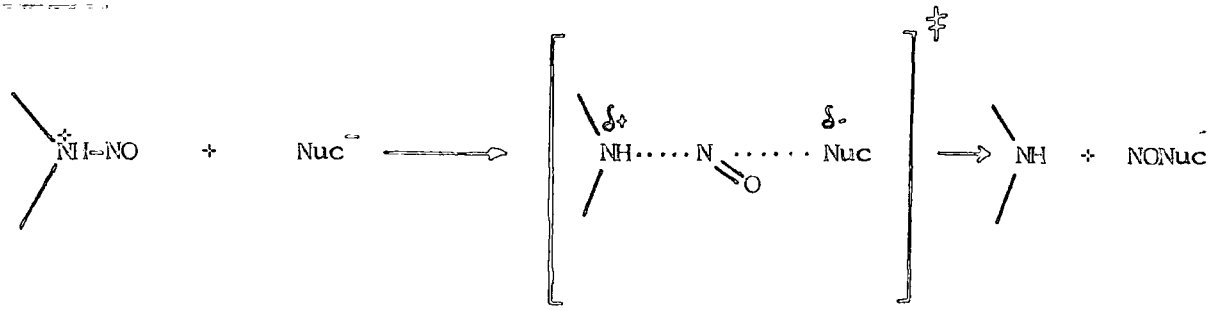
1.8. The effect of solvent on denitrosation

In ethanol[29] it has been found that the rate of denitrosation of NMNA in the presence of hydrogen chloride and ascorbic acid (as a nitrite trap) is much greater than the rate in aqueous solution at a corresponding acidity. On increasing the amount of ethanol in aqueous solution there is no apparent change in the observed rate constant up to 95% (v/v) ethanol. At higher ethanol concentrations there is a considerable increase in the rate constant, up to forty-fold. In anhydrous ethanol there is no nucleophilic catalysis and the solvent isotope effect ($k_{EtOH}:k_{EtOD}$) = c.a. 3 which indicates that proton transfer to the nitrosoamine is rate limiting. Ascorbic acid is shown to be an effective nitrite trap although in the absence of trap the reaction produced more denitrosation (as opposed to rearrangement) than in aqueous solvent. The explanation of these observations must be based on the solvent effect on the overall rate of denitrosation. According to the mechanistic scheme for denitrosation the rate is given by;

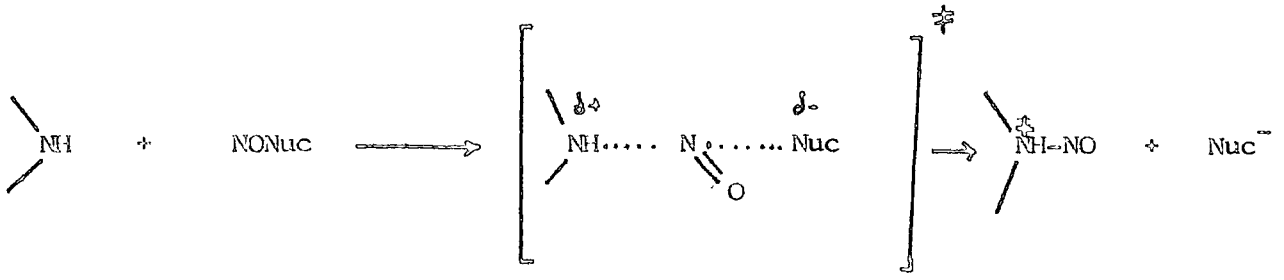
$$\text{Rate} = k_1 [R_1R_2\overset{+}{N}HNO][Nuc] - k_{-1}[NONuc][R_1R_2NH]$$

A simple theory that implies a rational explanation is that of Hughes and Ingold[30] who explain solvent effects in terms of relative solvation of the transition state and of the reactants. This is dependent upon the charge type of the reaction. The k_1 and k_{-1} steps for the denitrosation mechanism are both S_n2 reactions at nitrogen centre, but are of different charge types. For the k_1 step there is a decrease in charge on forming the transition state. A highly polar solvent would tend to inhibit the formation of the products and so the reaction would be slower. The opposite would be true of the k_{-1} step.

k_1 step



k_{-1} step



Clearly then, the rate of denitrosation would be faster in a less polar solvent. Ethanol is less polar than water as it has a dielectric constant of 24.3 compared to water which has a value of 78.4 (both at 25°C), and the observed rate enhancement bears this argument out. Although the dielectric constant is a convenient means of expressing the polarity of the solvent it is not always a reliable means of expressing the ionizing power of the solvent with respect to rate data. More satisfactory measurements of solvent ionizing power have been devised, based upon solvolysis rates[31] and spectroscopic data.[32-34]

Ethanol, like water, is a hydroxylic solvent in which hydrogen bonding is extensive. The sequence of S_n2 reactivity for nucleophiles in hydroxylic solvents is commonly observed as $I^- > SCN^- > Br^- > Cl^-$. This is in fact the opposite of what would be expected in terms of the density of charge on the anionic species. The chloride ion is small, and therefore highly charged and should be a more powerful nucleophile than the diffusely charged iodide ion. The observed sequence of reactivity is largely due to solvation by hydrogen bonding, the chloride ion being the most heavily solvated.

1.9. Denitrosation in aqueous acetic acid solution

An analytical technique for the detection of nitrosoamines has been developed[35] which involves denitrosation in aqueous acetic acid by hydrogen bromide. It was thought to be of some interest to study denitrosation in this solvent.

Glacial acetic acid is a poor solvent due to its low polarity (dielectric constant = 6). Many salts are insoluble in this medium, and exten-

-sive ion-pairing occurs with salts having small anions. The solvent has been used for many studies of S_n1 solvoyses to show the existance of ion-pairs as intermediates in this reaction[36]. No studies of denitrosation in glacial acetic acid have been attempted here, in fact very few studies of S_n2 reactions have ever been done in this solvent. A range of solvents using different proportions of acetic acid to water were used.

1. 80% AcOH-water (v/v)
2. 2:1 AcOH-water (v/v)
3. 1:2 AcOH-water (v/v)
4. 10% AcOH-water (v/v)

In 80% acetic acid, the denitrosation of NMNA was carried out in the presence of sulphuric acid and bromide ion, using sodium azide as the nitrite trap. At a concentration of 5×10^{-3}M NaN_3 the reaction was zero order in added trap. This is approximately the same concentration necessary to produce a zero order dependancy in water. The results are given in table 1, fig 1. As a result 5×10^{-3}M NaN_3 was used in all the subsequent runs.

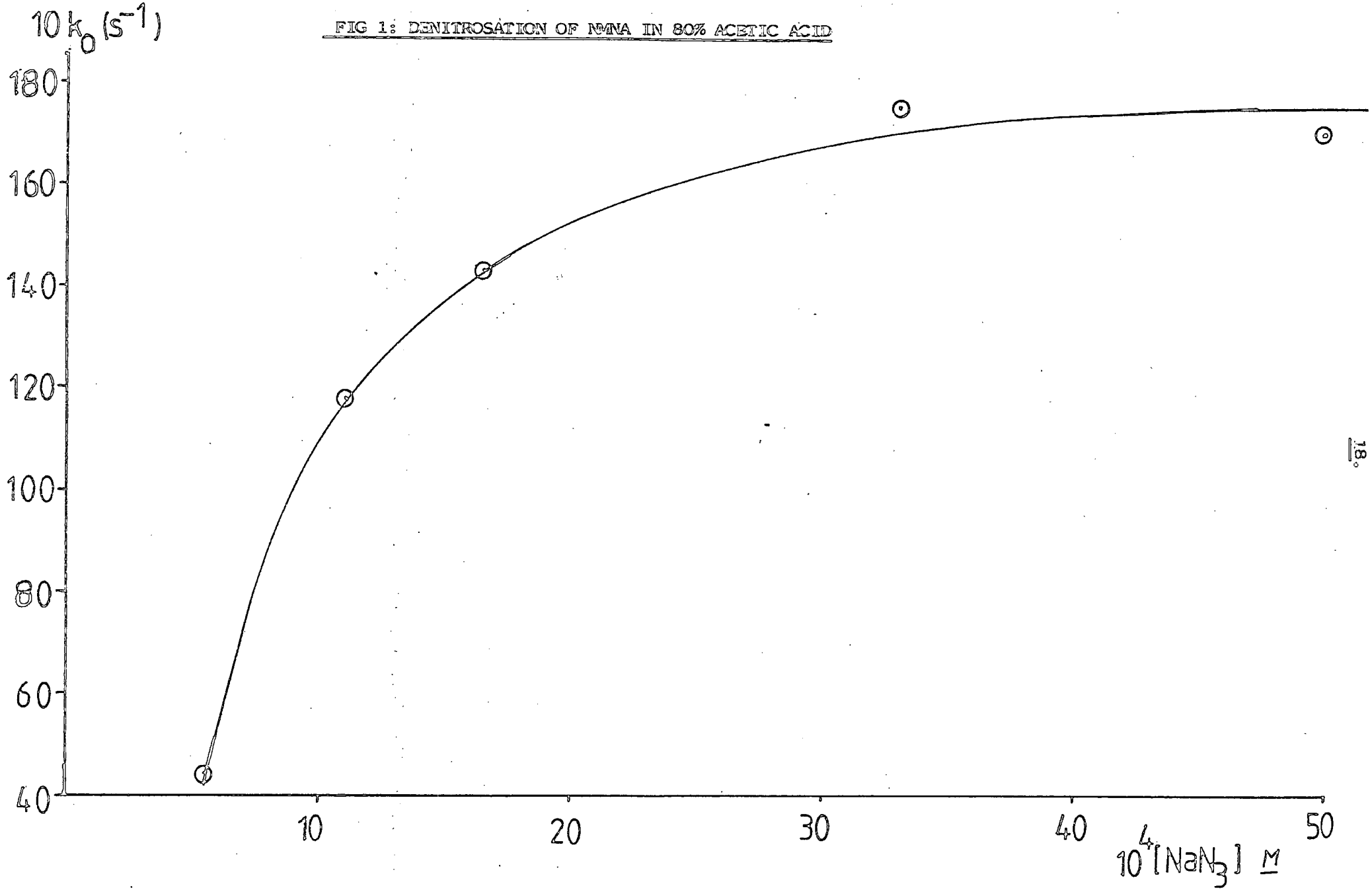
TABLE 1

$10^4[NaN_3]$ (M)	$10^4 k_0 (s^{-1})$
5.55	42.3
11.1	118
16.6	143
33.2	175
50.0	170

$[NMNA] = 1 \times 10^{-4}$M $[LiBr] = 0.05$M

$[H_2SO_4] = 0.4$M 80%(v/v) AcOH-H_2O

FIG 1: DENITROSATION OF NMNA IN 80% ACETIC ACID



In each of the four solvents, the effect of added thiourea, thiocyanate, bromide and chloride was observed. All rate constants were obtained by following the disappearance of the nitrosoamine at 270nm, at a temperature of $31^{0}C$. The results are given in tables 2-5.

Most of the plots of observed rate constant (k_0) against nucleophile concentration are linear. The rate expression $k_0 = k_1 K h_0^x [Nuc]$, given by the mechanistic sheme for denitrosation, applies here. The value of x in water is c.a. 1.0, however in these solvents the value of x may be significantly altered. The value of k_1K is a measure of the reactivity of the nucleophile in each case. Since h_0^x is not known in each solvent, absolute values of k_1K cannot be determined. An attempt was made to measure an acidity function in these solvents but the major difficulty was in determining the hydrogen ion concentr-ation. By measuring the slope of the k_0 vs $[Nuc]$ plot it is possible to obtain a value of k_1K relative to chloride ion which is the least reactive nucleophile in each solvent. Table 6 shows the collected data.

TABLE 2

Effect of added bromide ion

(a) 80%(v/v) AcOH-water

$10^4 k_0 (s^{-1})$	$[Br^-]$(M)
163	0·0125
276	0·025
361	0·0375
448	0·050

(b) 2:1(v/v) AcOH-water

$10^4 k_0 (s^{-1})$	$[Br^-]$(M)
2·63	0
54·3	0·013
88·2	0·027
116	0·04
219	0·05
271	0·075
319	0·083
327	0·10

(c) 1:2(v/v) AcOH-water

$10^4 k_0 (s^{-1})$	$[Br^-]$(M)
55·2	0·25
91·2	0·5
147	0·75
146	0·80
155	1·00

(d) 10%(v/v) AcOH-water

$10^4 k_0 (s^{-1})$	$[Br^-]$(M)
13·7	0·25
25·7	0·50
40·5	0·75
51·5	0·875
64·2	1·00

Concentrations of other reagents;

$[NMNA] = 1·1 \times 10^{-4}$M

$[NaN_3] = 5 \times 10^{-3}$M

$[H_2SO_4] = 1·1$M (exept in 80% acetic acid = 0·6M)

bromide was added as the lithium salt

TABLE 3

The effect of added chloride ion in aqueous acetic acid

(a) 80% AcOH (v/v)

$10^4 k_0$ (s^{-1})	$[Cl^-]$
12·04	0·05
23·90	0·10
32·30	0·15
42·75	0·20

(b) 2:1 AcOH (v/v)

$10^4 k_0$ (s^{-1})	$[Cl^-]$
2·63	0
4·15	0·10
8·18	0·375
9·18	0·50

(c) 1:2 AcOH-water (v/v)

$10^4 k_0$ (s^{-1})	$[Cl^-]$ (M)
1·04	0·50
2·49	0·75
3·89	1·00

Concentrations of other reagents used;

$[NMNA] = 1·1 \times 10^{-4}$ M

$[NaN_3] = 5 \times 10^{-3}$ M

$[H_2SO_4] = 1·1$M (except for 80% HOAc = 0·6M)

Chloride ion was added as the lithium salt.

TABLE 4

Effect of added thiocyanate ion

(a) 80%(v/v) AcOH-water		(b) 2:1(v/v) AcOH-water	
$10^4 k_0 (s^{-1})$	$10^4[SCN^-]$ (M)	$10^4 k_0 (s^{-1})$	$10^4[SCN^-]$ (M)
19	25	2·63	0
34	50	39·8	50
50	75	60·0	75
62	100	70·7	100
(c) 1:2(v/v) AcOH-water		(d) 10%(v/v) AcOH-water	
$10^4 k_0 (s^{-1})$	$10^4[SCN^-]$ (M)	$10^4 k_0 (s^{-1})$	$10^4[SCN^-]$ (M)
19·9	25	14·1	25
36·6	50	24·6	50
62·5	75	42·4	75
74·6	100	56·3	100

Concentrations of other reagents;

$[NMNA] = 1{\cdot}1 \times 10^{-4}$ M

$[NaN_3] = 5 \times 10^{-3}$ M

$[H_2SO_4] = 1{\cdot}1$M

SCN^- added as the sodium salt.

TABLE 5

Effect of added thiourea

(a) 80%(v/v) AcOH-water		(b) 2:1 (v/v) AcOH-water	
$10^4 k_0 (s^{-1})$	10^4[thiourea](M)	$10^4 k_0 (s^{-1})$	10^4[thiourea]
20·2	25	21·9	25
28·6	35	44·3	50
38·3	50	69·1	75
42·9	60	76·9	100
53·9	75		
(c) 1:2(v/v) AcOH-water		**(d) 10%(v/v) AcOH-water**	
$10^4 k_0 (s^{-1})$	10^4[thiourea] (M)	$10^4 k_0 (s^{-1})$	10^4[thiourea]
29·7	21·7	36·9	25
53·9	43·4	82·5	50
84·4	65·2	98	75
94·4	86·9	119	100

Concentrations of other reagents;

[NPNA] ≈ $1·1 \times 10^{-4}$M

[NaN_3] ≈ 5×10^{-3}M

[H_2SO_4] ≈ 1·1M (except in 80% acetic acid ≈ 0·6M)

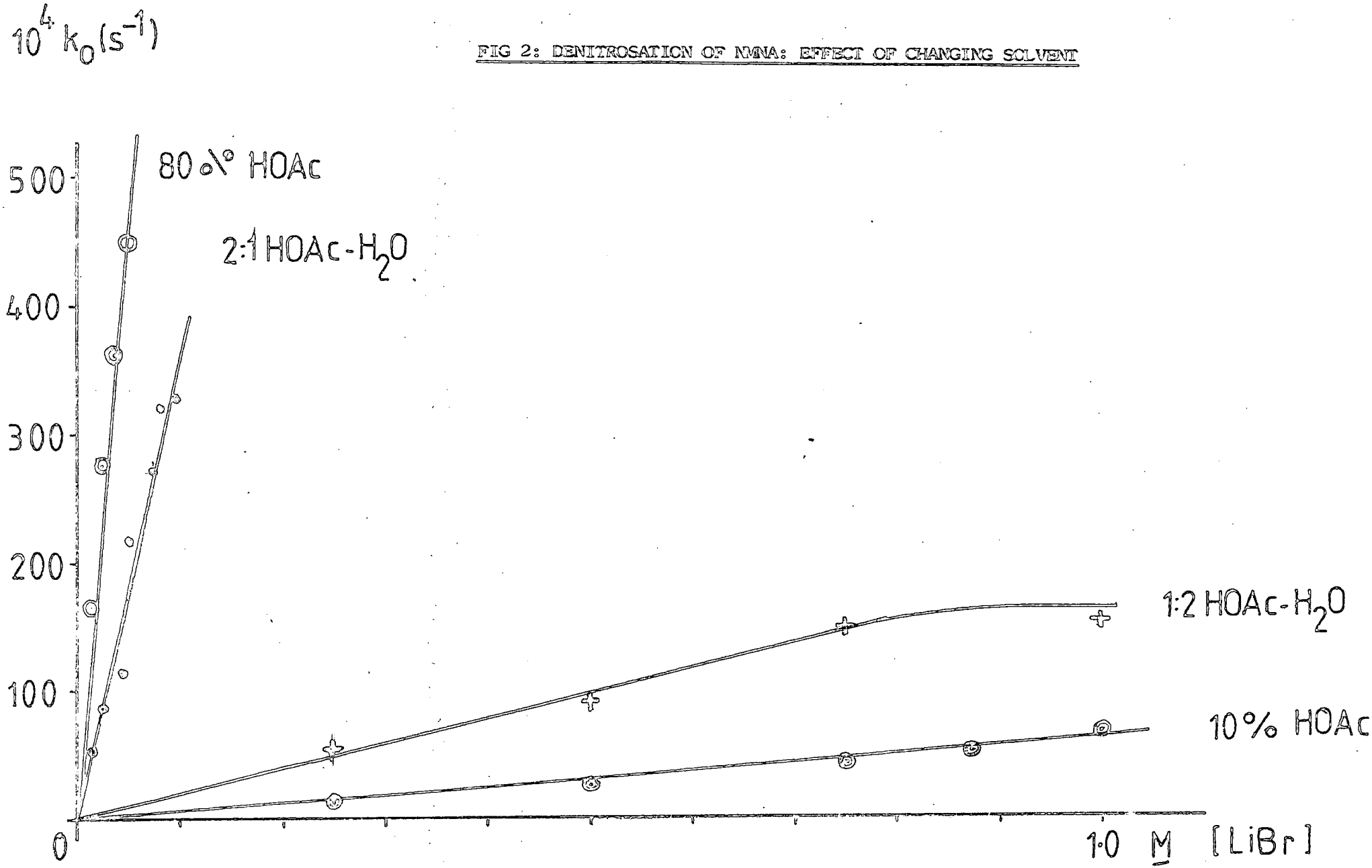
TABLE 6

SOLVENT	NUCLEOPHILE	SLOPE	REL. k_1K
80% HOAc	Cl^-	2.2×10^{-2}	1.00
	Br^-	1.33	60
	✲ SCN^-	0.315	14
	Thiourea	0.68	31
2:1 HOAc:H_2O	Cl^-	1.27×10^{-3}	1.00
	Br^-	0.32	251
	SCN^-	0.68	536
	Thiourea	0.77	606
1:2 HOAc:H_2O	Cl^-	3.5×10^{-4}	1.00
	Br^-	0.021	61
	SCN^-	0.68	1946
	Thiourea	0.98	2800
10% HOAc †	Br^-	6×10^{-3}	1.00(1.0)
	SCN^-	0.56	93(100)
	Thiourea	1.48	247(236)

✲ As a first approximation, the slope has been corrected for a different acid concentration, as this series of runs was carried out using a higher acid concentration than the rest.

† Chloride ion catalysis in this solvent was very slow, and so k_1K values are relative to bromide ion. Figures in brackets refer to relative reactivities in water.

FIG 2: DENITROSATION OF NMNA: EFFECT OF CHANGING SOLVENT

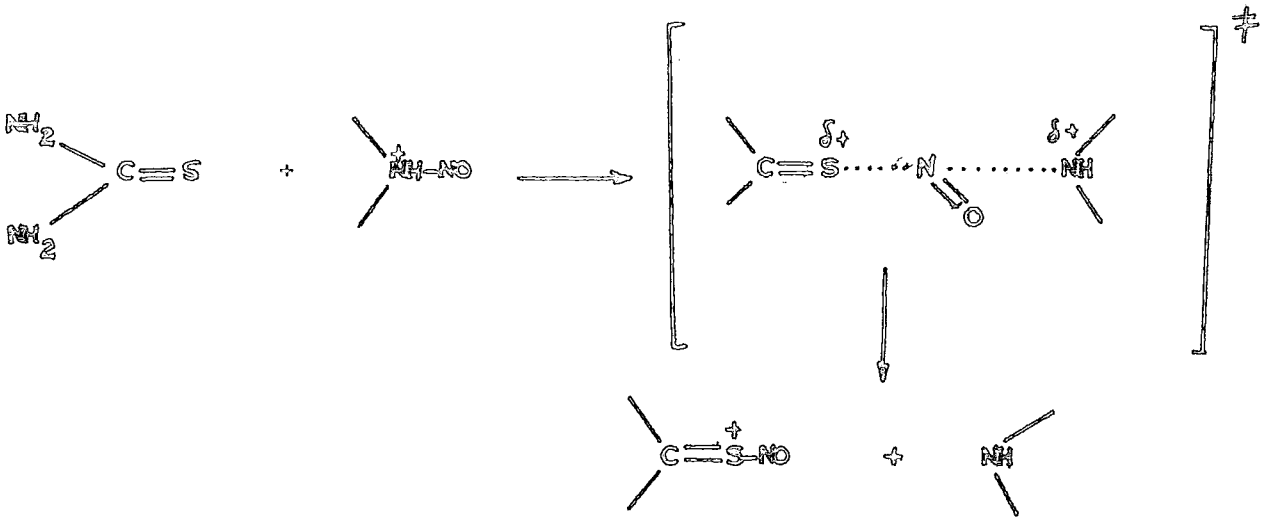


Examination of the collected values gave the following conclusions.

(i) The normal sequence of nucleophilic reactivity for a hydroxylic solvent is observed in all solutions except 80% acetic acid, here the sequence becomes $Br^- \gg$ thiourea $\gg SCN^- \gg Cl^-$ i.e. bromide ion surpasses thiourea in reactivity. The difference in reactivity for the various nucleophiles in 80% acetic acid is not very marked, though as the amount of water in the solvent increases the differences in reactivity become much more appreciable. In 10% acetic acid solution the relative reactivities are almost exactly the same as for water solvent.

(ii) As the concentration of acetic acid in the solvent is increased, the effect of added bromide ion becomes more pronounced. The effectiveness of chloride ion also undergoes some enhancement at higher acetic acid concentrations. The effectiveness of thiourea as a nucleophile is virtually unchanged by a change in the composition of the solvent. The same is true of thiocyanate ion. So at high concentrations of acetic acid bromide ion overtakes thiourea in terms of nucleophilic reactivity. The effect of changing solvent on nucleophilic reactivity of bromide ion is shown on fig2.

Any attempt at rationalising these results must be based upon consideration of the solvation of the reactants and the transition state because the rate limiting step is the nucleophilic attack on the protonated nitrosoamine. According to the Hughes-Ingold theory, there should be an increase in the overall rate of reaction as the amount of acetic acid in the solvent is increased, because of the decrease in polarity in the solvent. 10% acetic acid has a dielectric constant of 83 (higher than water), this drops steadily[37] down to a value of 27 for 80% acetic acid. The overall increase in rate constant is observed for bromide and chloride but not for thiourea or thiocyanate catalysis. The reason for the comparitively small change in the reactivity of thiourea may be due to the solvation of the transition state. Thiourea is neutral species and so there will be only a dispersal of charge on forming the transition state. Consequently only a very slight increase in the overall rate of reaction would be expected.



The explanation for thiocyanate does not seem so clear. It may be that some specific solvent effect is taking place. Hydrogen bonding interactions would not explain the failure of this anion to increase in re-activity at high acetic acid concentrations, as the effect would be even greater for the smaller, less polarisable bromide ion. The same is true for ion-pairing effects at high acetic acid concentrations, as the effect would be even greater for lithium bromide. It is known[38,39] that thiocyanogen $(SCN)_2$ exists in a stable form in glacial acetic acid and there is the possibility that this molecule may be formed at high acetic acid strength though this seems unlikely to occur to the extent that would affect the concentration of free thiocyanate ion as it is known that $(SCN)_2$ cannot be prepared in any quantity in acetic acid containing traces of water.[38] Another, more plausible possibility is that the pK_a for the formation of HSCN is significantly altered in high acetic acid concentration to render the formation of thiocyanic acid in much greater concentrations, hence reducing the concentration of free thiocyanate anion. A discussion of the protonation of thiocyanate ion is given at the end of this chapter.

The above results show clearly that denitrosation is compari-tively rapid and irreversible if carried out in solutions of sulphuric acid dissolved in acetic acid with low water content. The presence of bromide ion produces a considerable catalytic effect. The denitrosation of NMNA in

the presence of HBr in 60% acetic acid gave a quite rapid reaction (table 7).

TABLE 7

[HBr] (M)	$10^4 k_0 (s^{-1})$
0.492	124
0.737	273
0.982	500
1.474	920

$[NMNA] = 1 \times 10^{-4} M$ $[NaN_3] = 2 \times 10^{-2} M$

1.10 Denitrosation of NMNA in DMSO and DMSO-water solvent

Since the rate limiting step in aqueous denitrosation of NMNA is nucleophilic attack on the protonated nitrosoamine by an S_N2 reaction, it was thought to be of interest to study the reaction in DMSO (dimethyl sulphoxide) as a large number of bimolecular reactions have been studied in this solvent[40-42] and in many cases the observed rates have been considerably larger than that observed in water solvent.

DMSO is a fairly polar solvent (ε = 46.6 at 25°C) according to the arguments laid out earlier (ignoring specific solvent effects characteristic of DMSO) denitrosation in DMSO should be faster than in water which is even more polar (ε = 78.4) though the difference will not be as marked as in the case of ethanol which is much less polar than water. However, for many reactions of the same charge type as the denitrosation reaction there is a rate enhancement of many factors of ten on going from water to DMSO which could not be accounted for by the modest change in solvent polarity involved. The reason for the rapid reactions in DMSO is due to a certain degree of de-solvation of the reactants in this solvent. DMSO is

known as a dipolar aprotic solvent owing to its lack of hydrogen bonding character together with its appreciable polarity. An anionic species such as chloride ion which is heavily solvated by hydrogen bonding in water is effectively de-solvated in DMSO and is much more reactive. Large anions are solvated by dipolar interactions so the combined solvation effects reverse the reactivity sequence given in water to $Cl^- \gg Br^- \gg SCN^- \gg$ thiourea. Other dipolar aprotic solvents include dimethyl formamide (DMF), acetonitrile, nitromethane and sulpholane.

On this basis it might be expected that the denitrosation step in DMSO would be so rapid that the protonation would become rate limiting as in ethanol. The effect of increasing the concentration of DMSO in DMSO-water solvent for the denitrosation of NMNA (with hydrogen chloride and a high concentration of sulphamic acid as a nitrite trap) is to increase the rate considerably as shown in table 8 and fig 3. NMNA gives a peak at 272nm in DMSO (extn. coeff. = 6800 $l mol^{-1} cm^{-1}$). The reaction was followed at 300nm due to interference by the trap at the maxima for the nitrosoamine.

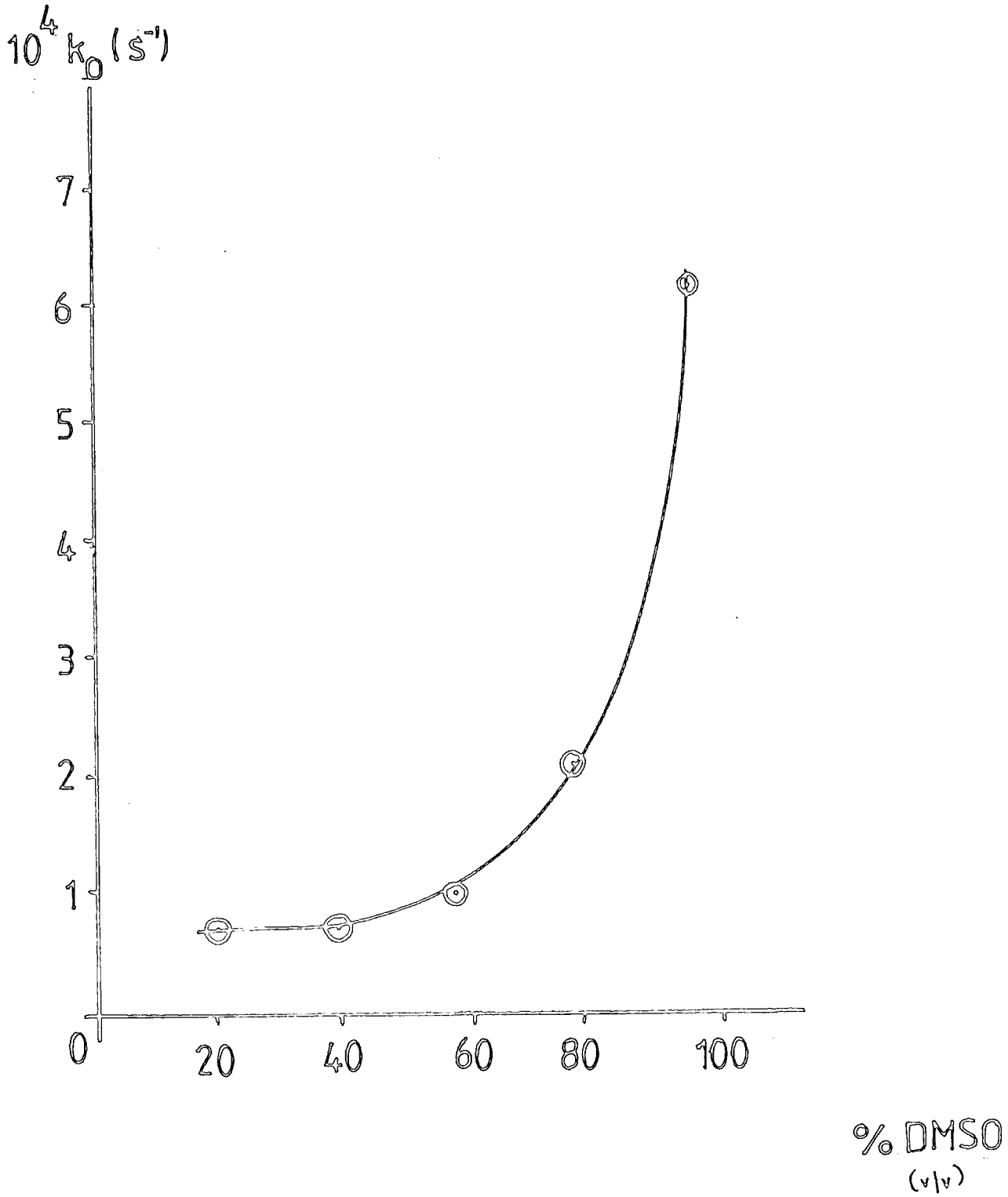
TABLE 8

%(v/v) DMSO in aqueous solvent	$10^4 k_0 (s^{-1})$
20	0·68
40	0·69
60	0·97
80	2·08
100	6·10

$[NMNA] = 1{\cdot}3 \times 10^{-4} M$ $[\text{sulphamic acid}] = 0{\cdot}2M$

$[HCl] = 1{\cdot}20M$

FIG 3: DENITROSATION OF NMNA: EFFECT OF DMSO AS A SOLVENT



Variation of the HCl concentration in DMSO was found to give a linear relationship between log k_0 and $-H_0$ with a slope of unity (fig 4). The acidity function data was taken from the literature.[43] The slope of unity suggests that chloride ion catalysis is not contributing to the reaction as expected if protonation is rate limiting. The results are shown in table 9.

TABLE 9

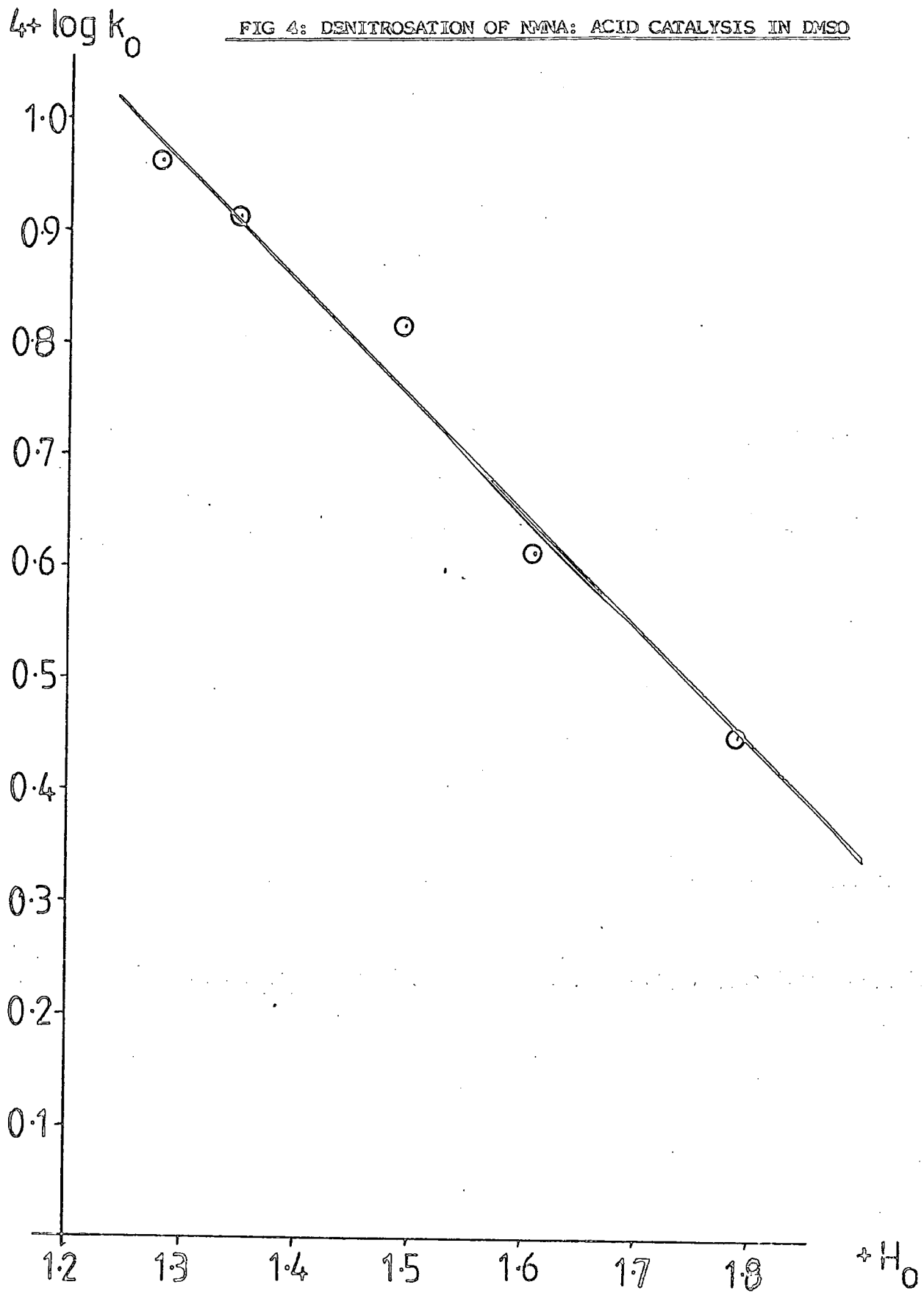
[HCl] (M)	$10^4 k_0 (s^{-1})$	$4 + \log k_0$	$-H_0$
0·536	2·82	0·45	1·79
0·804	4·11	0·614	1·61
1·132	6·55	0·816	1·495
1·698	8·19	0·913	1·35
2·13	9·17	0·962	1·278

[NMNA] = 1×10^{-4} M [Sulphamic acid] = 0·2M

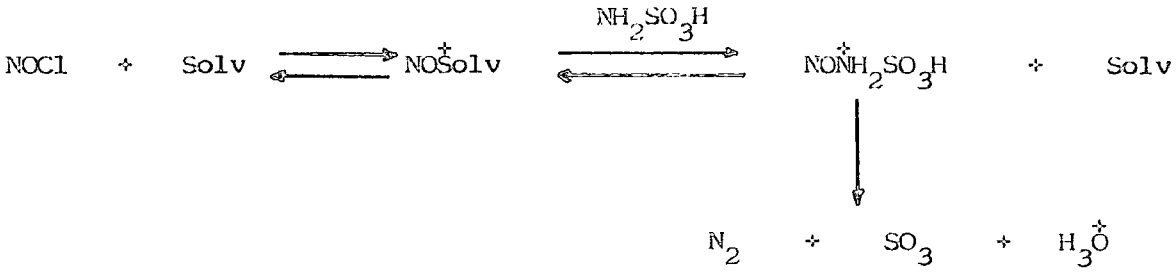
Slope of log k_0 vs $-H_0$ plot = 1·035 ± 0·03

It was not possible to confirm that the reaction was zero-order in added chloride ion or any other nucleophile owing to the low solubility of these reactants in acidified DMSO. It was found that sulphamic acid made little difference to the reaction rate, and this led to doubt as to the effectiveness of this reagent as a trap in this solvent. However, the individual rate constants in table 9 gave reasonably good Guggenheim plots (see chapter 5) which suggest that if the trap is not effective then the denitrosation reaction in this solvent must be largely irreversible and can proceed without a trap being present. There was no evidence for the formation of a significant amount of rearrangement product.

FIG 4: DENITROSATION OF NMNA: ACID CATALYSIS IN DMSO



The direct nitrosation of sulphamic acid by nitrous acid was carried out in 90% DMSO by following the disappearance of nitrous acid at 380nm and 31°C. An observed rate constant of $2{\cdot}08 \times 10^{-3} s^{-1}$ was obtained with 0·85M H_2SO_4, 0·048M sulphamic acid and 2×10^{-3}M nitrous acid. Under the same conditions in water the rate constant was $6{\cdot}71 s^{-1}$, so a change in solvent in this case produced a difference in rate of c.a. 3000. The reason for the much slower reaction in DMSO is due to the inefficiency of sulphamic acid as a trap in this solvent. It has been shown[92] that in water, owing to the low bascicity of sulphamic acid there is no direct reaction with nitrosyl halides in aqueous solution, only the nitrous acidium ion reacts.



Solv = H_2O or DMSO

In comparisón to the nitrous acidium ion the equilibrium concentration and reactivity of $NO\text{-}\overset{+}{O}{=}SC(CH_3)_2$ would be small. Therefore the reaction would tend to be slower in the less polar, less nucleophilic solvent DMSO. To see if other traps were effective in the denitrosation reaction carried out in this solvent, sodium azide was used and found to be completely ineffective in 90% DMSO and direct nitrosation produced a very slow, reversible reaction. Hydrazoic acid is known to react readily with nitrosyl halides in aqueous solution and so the explanation for the lack of reactivity of HN_3 towards NOCl in DMSO may be simply due to the polarity of the solvent, which according to the Hughes-Ingold model would retard the rate of reaction in comparisin to water.

1.11 Protonation of nucleophilic species

It is necessary to state that in any kinetic studies involving thiocyanate ion in acid solution it is necessary to consider the formation of thiocyanic acid according to the equilibrium

$$H^{+} + SCN^{-} \rightleftharpoons HSCN$$

As this is of relevance to other areas of the present work it will be discussed in some detail. Clearly, significant protonation of thiocyanate ion would alter the concentration of the nucleophile and a correction based upon the pK_a of HSCN would have to be applied to the results.

Gorman and Connel[45] state that HSCN is a strong acid and that the pK_a is probably less than 3. Edwards[46] reports an estimated pK_a of -0.74 but does not state its source. A more detailed study of the formation of thiocyanic acid is provided by Boughton and Keller[47] who have determined a pK_a value based upon thermodynamic data at various temperatures for the formation of HSCN. Their value, $pK_a = 0.97 \pm 0.7$ at $25^{0}C$ is in agreement with the value of 0.85 obtained by Suzuki[48], however they make it clear that the acidity is probably too high for their technique (potentiometric titration) and that the values may be rather high. Stedman[49] has obtained a value of $pK_a = -1.84$ at $25^{0}C$. This was acheived by solvent extraction and measuring partition coefficients. Studies of u.v. absorbance of HSCN confirms the value. Measuremants using the H_- acidity function yield a value of -1.38. A more recent value[50], obtained by raman spectroscopy gives a pK_a of -1.1 ± 0.3 at $20^{0}C$ in aqueous solvent.

There is therefore a plethora of values for the pK_a of HSCN. Since the most conventional and trustworthy techniques were used by Stedman and co-workers, the value of $pK_a = -1.84$ seems the most reliable. Under typical reaction conditions, the concentration of free thiocyanate ion can be calculated using this value.

$$[SCN^-] = \frac{[SCN^-]_{total}}{1 + \frac{[H^+]}{K_a}}$$

If $K_a = 69$ ($pK_a = -1.84$), and $[H^+] = 2.0M$, and $[SCN^-]$ total $= 10^{-2}M$
Then $[SCN^-] = 9.72 \times 10^{-3}M$. So, for the conditions encountered in this work it is unlikely that protonation of thiocyanate will be significant, although it should be stressed that the values of K_a and $[H^+]$ in non-aqueous solvents may be sufficiently altered to cause more extensive protonation.

Thiourea will also undergo protonation in acidic solution though this will be almost entirely due to N-protonation under the conditions used here. Only S-protonation would affect the nucleophilicity of thiourea and virtually all the positive charge reside on the amino nitrogen atom (see page 4). There is n.m.r. evidence[51] for significant S-protonation but only in concentrated, almost anhydrous acid solutions.

1.12. Summary

(1). For denitrosation of N-methyl N-nitrosoaniline, the effect of changing the solvent from water to DMSO is to increase the rate of reaction. This is due to the change in dielectric constant of the solvent.

(2). A very considerable increase in rate is observed for bromide ion catalysed denitrosation on increasing the proportion of acetic acid in aqueous acetic acid solvent, a similar increase in rate is observed for chloride ion catalysis but virtually no increase in rate is observed for thiourea or thiocyanate ion catalysed denitrosation.

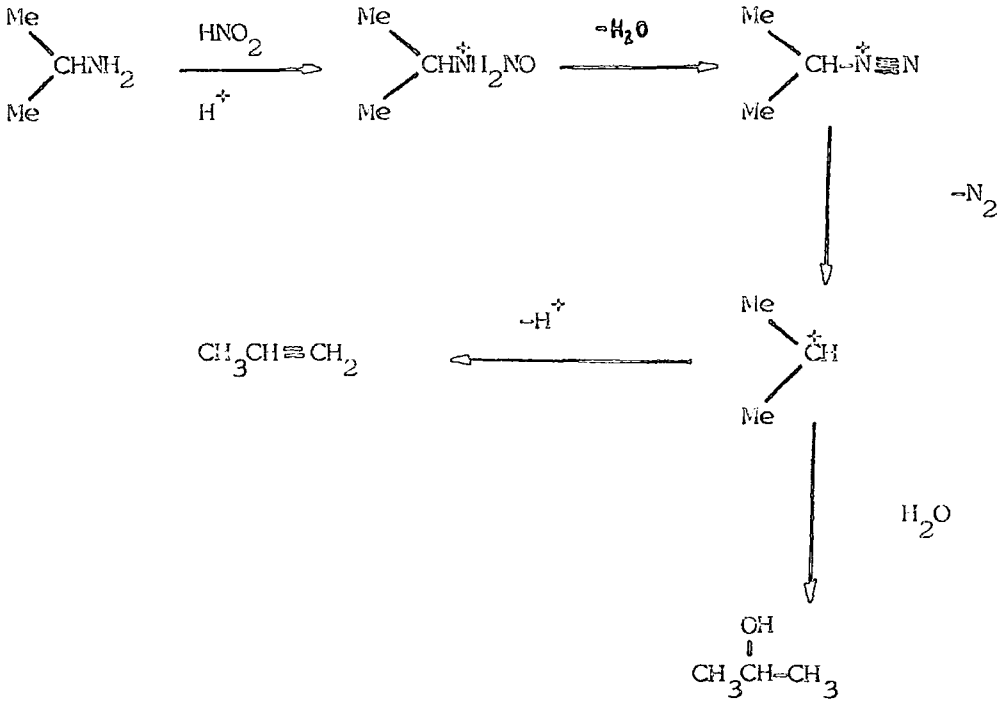
(3). Nitrite traps that are very effective in water (e.g. sulphamic acid and azide ion) are quite unreactive in DMSO solvent.

CHAPTER TWO

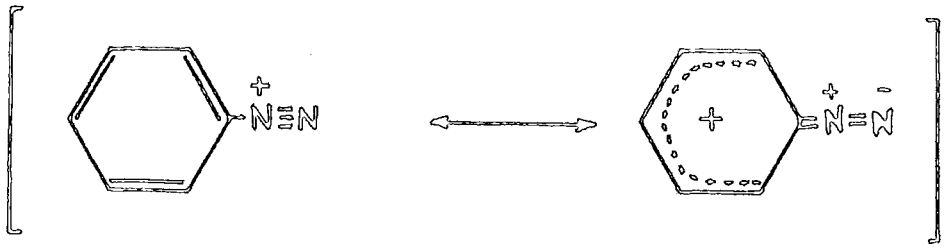
Diazotization of aromatic amines.

2.1 Introduction

Primary aliphatic amines react with nitrous acid to form various products of deamination e.g. alcohols and alkenes. The reaction proceeds via a diazonium ion as intermediate.



With secondary amines, the N-nitrosoamine formed in the first step is sufficiently stable for the reaction to stop at this point. Tertiary amines sometimes undergo a nitrosative dealkylation reaction to give an N-nitrosoamine as the main product. The diazonium species formed by an aliphatic amine is very unstable, though the aromatic diazonium ion is very much more stable owing to the aromatic π-orbital system which re-duces the leaving group ability of nitrogen.



For aniline, the diazonium ion is quite stable in aqueous solution at low concentrations. Attempts to isolate solid diazonium salts generally result in decomposition, although solid fluoroborates $ArN_2^+BF_4^-$ have been isolated. The diazonium cation is quite reactive and can undergo coupling reactions with other aromatic species to give intensely coloured compounds which are used as dyes. In solutions of PhN_2^+, unreacted aniline can undergo a coupling reaction with the diazonium ion if the concentration of aniline is sufficiently high, therefore it is necessary to carry out diazotization at an acidity high enough to ensure that the concentration of free aniline is low.

The diazotization of aniline was first studied in 1849 by Wurtz[55] who obtained phenol as the final product of the reaction.

$$PhNH_2 + HNO_2 \xrightarrow{HCl} PhN_2^+Cl^- \xrightarrow{H_2O} PhOH + N_2$$

Griess[56] isolated the diazonium salt in 1861. Since then the reaction has been studied in great detail and has been the subject of extensive reviews.[57] The initial nitrosation step is rate limiting under certain conditions and governs the rate of reaction. The primary nitrosoamine formed (in the case of aniline) is unstable and is only detected at low temperatures.[58] It undergoes rapid proton transfer to the solvent followed by a tautomeric shift and loss of water.

$$ArNH_2 \underset{H^+}{\overset{HNO_2}{\rightleftharpoons}} ArN^+H_2NO \longrightarrow ArN{=}N{-}OH \longrightarrow ArN_2^+ + H_2O$$
$$+ H^+$$

Molecular nitrous acid is not a nitrosating agent itself but forms several nitrosating species depending upon the conditions in solution. At low acidity, nitrous anhydride (N_2O_3) is the effective nitrosating agent.

$$2HNO_2 \rightleftharpoons N_2O_3 + H_2O$$

At higher acidities an equilibrium is set up with eitheir the nitrous acidium ion ($H_2NO_2^+$) or the nitrosonium ion (NO^+) as the nitrosating agent.

$$HNO_2 + H^+ \rightleftharpoons H_2NO_2^+ \rightleftharpoons NO^+ + H_2O$$

At moderate acidities and in the prescence of halide ion or pseudo-halide species, a nitrosyl compound NOX is formed in solution and this acts as a nitrosating agent.

$$H^+ + X^- + HNO_2 \rightleftharpoons NOX + H_2O$$

These mechanisms will now be discussed in a little more detail.

2.2 Diazotization in acid solution

The equilibrium constant for the formation of nitrous anhydride in water at 25°C has been measured[59,60] as $K = 0.21 mol^{-1}$. Subsequent kinetic studies were made on the diazotization of ring-substituted anilines. The kinetic form of the reaction having been established as $Rate = k[ArNH_2][N_2O_3]$. Using the K value obtained previously, the bimolecular rate coefficients for a number of aromatic amines were of the order of 10^6-10^7 $lmol^{-1}s^{-1}$. However, a much more recent value of K has been reported[63], and this is considerably smaller ($K = 3.03 \times 10^{-3} lmol^{-1}$). This new value now makes the bimolecular rate constants obtained for the diazotisation of aniline derivatives by N_2O_3 of the order of 10^8-10^9 $lmol^{-1}s^{-1}$, which is close to the diffusion controlled limit for bimolecular encounter. The nitrosation step is assumed to have the stochiometry;

$$ArNH_2 + N_2O_3 \rightleftharpoons ArNH_2NO^+ + NO_2^-$$

The free amine is the reactive species. At lower acidities ($< 0.01M$ HCl), or with very basic amines, the concentration of free amine becomes comparable to that of the total amine concentration. Under these conditions the

nitrosation step becomes rapid and the rate is independent of the free amine concentration.[64] The formation of N_2O_3 is now rate limiting. At acidities above 0.1M perchloric acid the rate of reaction increases due to the formation of a new, more reactive species. Larkworthy[62] has measured k values in the range 0.1-0.5M $HClO_4$ according to the rate expression;

$$\text{Rate} = k\,[ArNH_2][\,HNO_2]$$

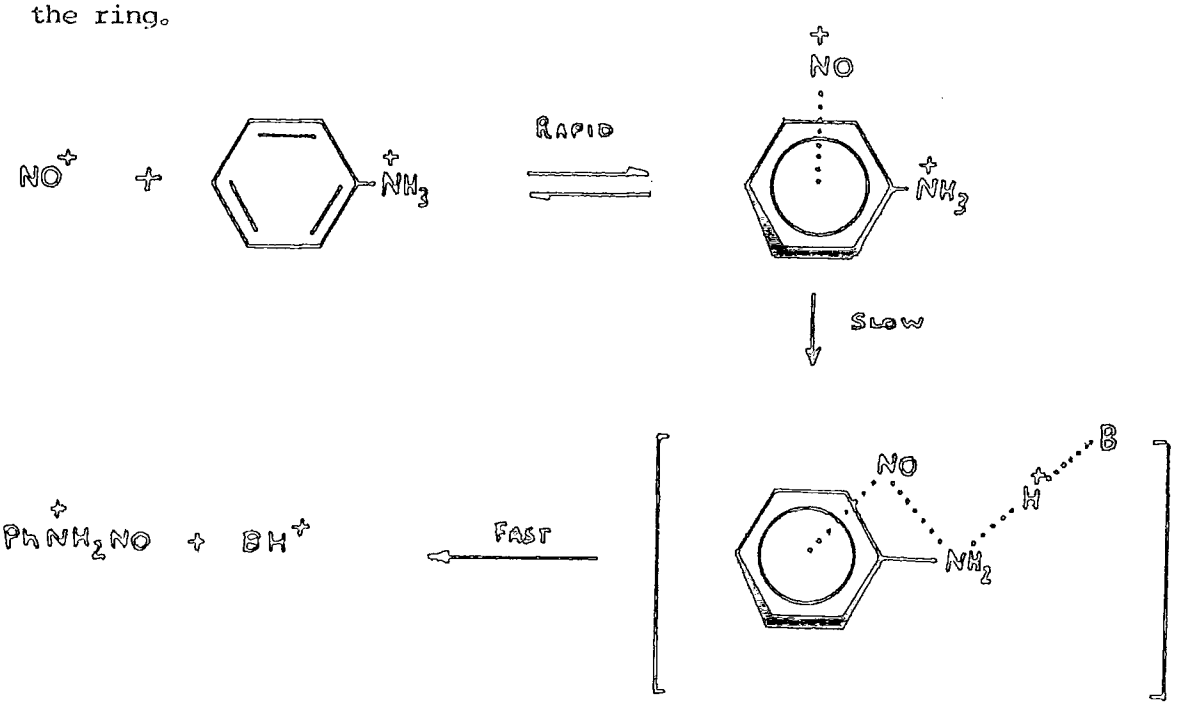
The values do not vary by any appreciable amount with the bas icity of the amine indicating that the nitrosating species is very reactive and indiscriminate toward the various amines. On the other hand, the rate constants obtained for nitrous anhydride showed a considerable variation from one amine to another, and the weakly basic amines such as p-nitroaniline failed to react. At higher acidities this species reacted readily. The nitrosating species at higher acidities is believed to be the nitrous acidium ion ($H_2NO_2^+$) or the nitrosonium ion (NO^+). There is some debate as to the exact identity of the nitrosating species, many workers have considered that $H_2NO_2^+$ is the reactive species for moderate acidities with NO^+ being predominant at higher acidities ($\geqslant$ 3M $HClO_4$). This question will be discussed in more detail in chapter four. The nitrosation step in 0.1-0.5M $HClO_4$ is presumably;

$$H_2NO_2^+ \;+\; ArNH_2 \rightleftharpoons Ar\overset{+}{N}H_2NO \;+\; H_2O$$

The equilibrium constant for the formation of the nitrous acidium ion is unknown and so absolute rate constants for the bimolecular nitrosation step are not available although it is believed that these will approach the diffusion controlled limit. This seems likely considering that the rates for nitrosation via N_2O_3 approach the same limit. As the acidity is increased further (to 3M $HClO_4$), the rate expression becomes[65,66];

$$\text{Rate} = k\,[Ar\overset{+}{N}H_3][\,HNO_2]$$

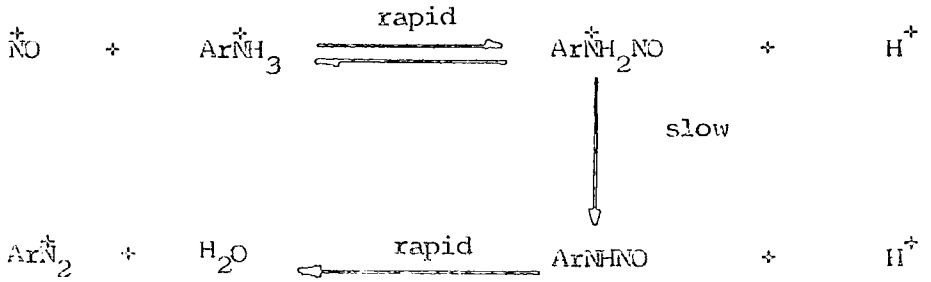
signifying that the protonated amine is now the reactive species, the nitros-ating species, most probably NO^+ under these conditions, is believed to attack the ring.



At high acid concentrations[67] (60% H_2SO_4), virtually all the nitrous acid is present as NO^+. The rate of diazotization of aniline is found to decrease with increasing acidity in this region according to the expression;[68]

$$\text{Rate} = k[\,Ar\overset{+}{N}H_3][HNO_2]\,h_0^{-2}$$

It is difficult to postulate a reaction mechanism that fits the above expression although it seems likely that rapid nitrosation is followed by slow proton transfer to the medium.



In support of this, a large deuterium isotope effect ($k_H:k_D = 10$) is observed at this acidity.

2.3. Diazotization by nitrosyl halide and pseudo-halide species

At moderate acidities (0.1M H^+) solutions of nitrous acid give, in the presence of halide ion or species such as SCN^- and thiourea, nitrosyl compounds (NOX) according to the equilibrium;

$$HNO_2 + H^+ + X^- \underset{}{\overset{K}{\rightleftharpoons}} NOX + H_2O$$

NOX acts as a nitrosating agent.

$$ArNH_2 + NOX \rightleftharpoons ArN\overset{+}{H}_2NO + X^-$$

The rate expression was established by Schmid[69] (1937).

$$\text{Rate} = k\,[ArNH_2][H^+][X^-][HNO_2]$$

As Hammett[70] pointed out, this corresponds to;

$$\text{Rate} = k\,[ArNH_2][NOX]$$

It is neccessary to establish accurate values of K, the equilibrium constant of formation for the nitrosyl species, before the bimolecular rate constants for the nitrosation step can be determined. K values have been determined for several species in water at 25°C.

TABLE 10

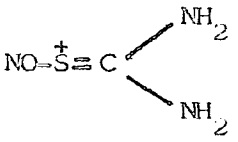
X	$K(l^2mol^{-2})$	Ref
Thiourea	5×10^3	71
Thiocyanate	32†	72
Bromide	5.1×10^{-2}	73
Chloride	1.1×10^{-3}	74

†Determined at 20°C, but from thermodynamic data available[72] the calculated value at 25°C is not significantly different.

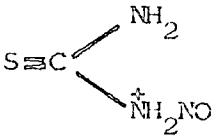
For thiourea and thiocyanate there is the possibility of N- as well as S-nitrosation. The large K values, however suggest the formation of a 'soft' S-nitroso adduct rather than a 'hard' N-nitroso compound.

e.g. NO-S-C≡N rather than NO-N=C=S

and,



rather than



The effect[75] of adding various nucleophiles on the observed rate constant for the nitrosation of morpholine is to catalyse the reaction due to the formation of NOX species. The sequence of catalysis is, thiourea ≫ SCN^- ≫ Br^- ≫ Cl^-, this is due to the large difference in the equilibrium constant of formation of the NOX species, for thiourea K is c.a. 10^6 times greater than chloride. This sequence does not tell us the actual reactivity of NOX toward the amine. The bimolecular rate constants have been determined[75] for morpholine and the following trend in reactivity is obtained; NOCl ≫ NOBr ≫ NOSCN ≫ $NO\text{-}\overset{+}{S}{=}C(NH_2)_2$.

The K value for the formation of NOCl obtained by Schmid and co-workers is criticised by Bailyss and Watts[76] on two points; firstly, that the method used by Schmid assumed that all the nitrous acid was present as NOCl, however spectrophotometric evidence shows that a considerable amount of NO^+ may have been present under the reaction conditions used. Secondly, that the wavelength at which Schmid measured the absorbance due to NOCl did not correspond to the maximum of the NOCl absorption, and that the NOCl transition overlaps with HNO_2 transition, so a reliable

extinction coefficient cannot be estimated. It seems that, noting these difficulties, the estimated K value of Schmid is the best available and seems to fit in well with the expected trends of reactivity. It has been shown[77] that nitrosyl iodide is an effective nitrosating agent for the diazo-tisation reaction, but a K value for the formation ofm this species is not available, as will be discussed in section 2.6.

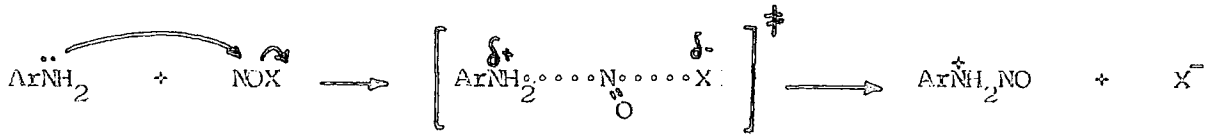
Schmid[78] has obtained bimolecular rate coefficients for nitrosation of aromatic amines by nitrosyl chloride at 25°C. These were of the order of $10^9 lmol^{-1}s^{-1}$ which is close to the diffusion controlled rate limit, and it was assumed that there was little discrimination between different substrate amines. An indirect method of measuring the relative reactivity of substituted anilines toward NOX (X= Cl^- or SCN^-) has been adopted[79] which is based upon the mechanism for denitrosation of N-methyl N-nitrosoaniline as described in the previous chapter. The aniline derivative is used as a nitrite trap according to the scheme below.

$$PhNMeNO + H^+ \underset{}{\overset{K}{\rightleftharpoons}} PhN^+HMeNO \underset{k_{-1}}{\overset{X^-, k_1}{\rightleftharpoons}} PhNHMe + NOX$$

$$NOX + ArNH_2 \xrightarrow{k_2} ArN_2^+$$

A plot of k_0^{-1} against concentration of added N-methylaniline yields the ratio $k_{-1}:k_2$ which gives the relative reactivity of $ArNH_2$ toward NOX. Values of $(k_2)_R:(k_2)_H$ are collected for a series of anilines with R substituents. A Hammett correlation[80] can be applied in such a case, a plot of $\log(k_2)_R:(k_2)_H$ against σ_p is linear with a slope of -3.1 for NOCl and -3.6 for NOSCN. This shows quite a large range of reactivity between the different amines and argues against a diffusion controlled rate of reaction where there would be little discrimination in reactivity toward even the less basic amines.

Using stopped-flow spectrophotometry it is possible to get quite accurate rate constants for diazotigation in solution. A series of substituted anilines[81] have been diazotised with NOCl and NOBr using this technique and bimolecular rate constants for the nitrosation stage have been obtained. These rate constants show that NOCl is more reactive than NOBr toward all the substituted anilines studied and, contrary to the findings of Schmid[78], there is some discrimination in reactivity between the amines of lower basicity, for the more reactive i.e. more basic amines the rate constants level off as they approach the diffusion controlled limit (see fig 9). The bimolecular rate constants for the nitrosation of aniline by NOSCN and $NO\text{-}\overset{+}{S}{=}C(NH_2)_2$ have been measured[75], also by stopped-flow technique. The results show an order of reactivity towards aniline of the various electrophilic species of NOCl $\gg$ NOBr $\gg$ NOSCN $\gg$ $NO\text{-}\overset{+}{S}$. This being the same as for morpholine. This sequence may be explained using the hard-soft acid-base theory[85], if we consider the nitrosation of the free amine as an S_N2 type reaction.



According to the general classification of acids and bases, aniline would be considered 'borderline' between being a 'hard' and 'soft' base and the above reaction would proceed the most rapidly if the leaving group were approximately of the same degree of 'hardness'. This is the case with NOCl, down the series, the leaving groups get progressively softer and therefore NOX becomes less reactive with respect to the amine. The sequence NOCl $\gg$ NOBr can also be explained upon purely electronegative grounds.

The bimolecular rate coefficient for the reaction of NOBr and NOSCN with aniline was determined[75] at 0^0C and 30^0C and the activation energy determined[75] as E_a = 12kJmol^{-1} for NOBr and 48kJmol^{-1} for NOSCN. Since a reaction might be considered diffusion controlled

if $E_a < 20 kJmol^{-1}$ it seems that NOBr is close to the encounter limit but NOSCN is not.

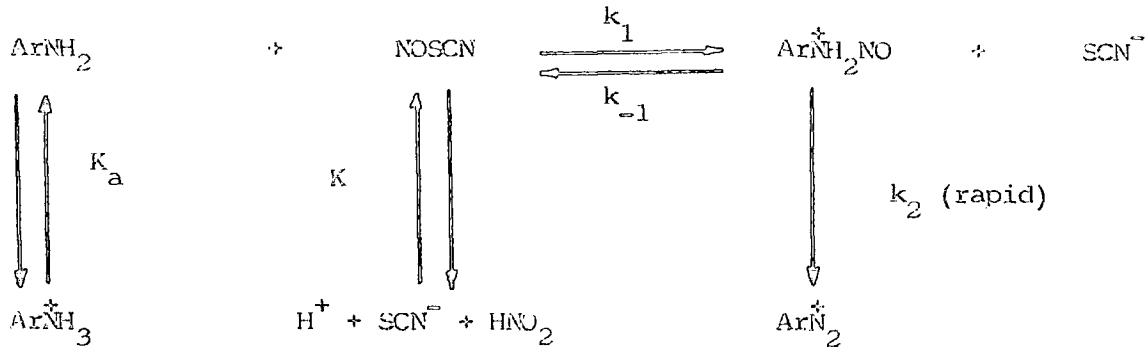
2.4. Diazotisation of aromatic amines by nitrosyl thiocyanate

The above observation concerning the activation energy for NOSCN suggests that this species may show a high degree of selectivity toward various substituted anilines owing to it's lower reactivity, and in the present work bimolecular rate constants for nitrosation of a series of substituted anilines have been determined using stopped-flow spectrophotometry. Previously, only indirect rate constants have been obtained for such a series of amines and only for aniline has a stopped-flow study been applied.

The reactions have been carried out in sulphuric acid solution with conditions adjusted so that the total amine concentration was always at least ten-fold in excess of the total nitrite concentration, to ensure first-order kinetics in the individual runs. The observed rate constant (k_0) is defined as;

$$-\frac{d[HNO_2]}{dt} = k_0[HNO_2]$$

Ideally, the reaction should therefore be followed by observing the disappearance of nitrous acid (which has an absorbance comprising several peaks around 370nm). In order to keep the condition that the total amine concentration must be in at least ten-fold excess over nitrite, rather strong solutions of amine would be required which is not possible for some of the less soluble amines. It is more satisfactory to follow the appearance of the diazonium ion ArN_2^+ at a particular wavelength and work at low nitrite concentration. The mechanistic scheme for the overall reaction is given below;



The rate of reaction is given by;

$$\text{Rate} = k_1 [ArNH_2][NOSCN] - k_{-1}[Ar\overset{+}{N}H_2NO][SCN^-]$$

Applying a steady state to $Ar\overset{+}{N}H_2NO$,

$$[Ar\overset{+}{N}H_2NO] = \frac{k_1 [ArNH_2][NOSCN]}{k_{-1}[SCN^-] + k_2}$$

Therefore,

$$k_0 = \frac{k_1 k_2 [ArNH_2] K [H^+][SCN^-]}{k_{-1}[SCN^-] + k_2}$$

Since, $[ArNH_2]_{total} \cong [Ar\overset{+}{N}H_3]$, under the acid conditions used.

And, $[NOSCN] = K[HNO_2][H^+][SCN^-]$

So, $$k_0 = \frac{k_1 k_2 [A]_t K K_a [SCN^-]}{k_{-1}[SCN^-] + k_2}$$

The assumption is made that the nitrous acid is predominantly present as free nitrous acid and not as NOSCN in any appreciable quantity.

The thiocyanate ion catalysed diazotisation of a series of substituted anilines was studied and the plots of k_0 vs $[SCN^-]$ (Fig 5 & 6) all showed a certain degree of curvature. At low thiocyanate concentration we have the condition $k_2 \gg k_{-1}[SCN^-]$ and so, $k_0 = k_1 K K_a [SCN^-][A]_t$. This refers to the linear part of the plot. The opposite condition prevails at high $[SCN^-]$ i.e. $k_{-1}[SCN^-] \gg k_2$ and $k_0 = (k_1 k_2 / k_{-1})[A]_t K K_a$ so the rate becomes zero order in thiocyanate ion. Since the experimental data shows that the conditions are intermediate between these two extremes, the second order rate constant for nitrosation of $ArNH_2$ is best obtained by taking a double-reciprocal plot of k_0^{-1} against $[SCN^-]^{-1}$ where, according to the rate expression obtained previously;

$$k_0^{-1} = \frac{k_{-1}}{k_1 k_2 K K_a [A]_t} + \frac{1}{k_1 K K_a [SCN^-][A]_t}$$

In addition to a value for k_2, the ratio $k_{-1}:k_2$ can be obtained from such a plot, this is a measure of the nucleophilicity of SCN^- toward the protonated nitrosoamine. The value of this ratio should be higher for the less basic substrates as electron withdrawing groups will tend to activate the nitrosoamine toward denitrosation. The results are given in the following tables. A 'weighted' linear regression analysis has been applied to the double reciprocal plots (see chapter five). For aniline, the rate constamt for the nitrosation step was determined also by varying the concentration of aniline, assuming that under the conditions used, k_0 is given by $k_0 = k_1 K K_a [SCN^-][A]_t$ i.e. having fixed thiocyanate concentration.

TABLE 11: ANILINE

$[H_2SO_4] = 2{\cdot}83M$ $[A]_t = 5{\cdot}95 \times 10^{-3}$ M $[NaNO_2] = 2{\cdot}5 \times 10^{-4}$ M

$[SCN^-]$(M)	$[SCN^-]^{-1}$	$k_0(s^{-1})$	k_0^{-1}
2×10^{-3}	500	1·50	0·667
5×10^{-3}	200	4·055	0·247
1×10^{-2}	100	7·745	0·129
$1{\cdot}5 \times 10^{-2}$	66·67	10·109	0·099
$2{\cdot}0 \times 10^{-2}$	50	11·49	0·087

320nm. 25°C.

slope = $1{\cdot}17 \pm 0{\cdot}04 \times 10^{-3}$

intercept = $0{\cdot}022 \pm 0{\cdot}004$

$k_0 = 1{\cdot}746 \times 10^8$ $lmol^{-1}s^{-1}$

$[H_2SO_4] = 0{\cdot}198$ M $[SCN^-] = 1 \times 10^{-4}$ M $[NaNO_2] = 2{\cdot}5 \times 10^{-4}$ M

$[A]_t$	$k_0(s^{-1})$
$2{\cdot}5 \times 10^{-3}$	2·72
5×10^{-3}	5·98
$7{\cdot}5 \times 10^{-3}$	7·67
$1{\cdot}0 \times 10^{-2}$	10·35

slope = 1035 ± 29

$k_1 = 1{\cdot}12 \times 10^8$ $lmol^{-1}s^{-1}$

TABLE 12: p-TOLUIDINE

$[H^+] = 0.285$ M $\qquad [A]_t = 6 \times 10^{-3}$ M

$[NaNO_2] = 2.5 \times 10^{-4}$ M.

$[SCN^-]$ (M)	$[SCN^-]^{-1}$	$k_0(s^{-1})$	k_0^{-1}
0.02	50	8.15	0.123
0.017	58.82	8.31	0.12
0.012	83.33	5.85	0.171
0.010	100	5.45	0.183
0.005	200	2.78	0.359
0.002	500	1.25	0.80

320 nm.

25°C.

SLOPE = $1.536 \pm 0.034 \times 10^{-3}$

intercept = 0.0378 ± 0.002

$k_1 = 4.077 \times 10^8$ lmol^{-1}s^{-1}

TABLE 13: p-ANISIDINE

$[H^+] = 0.285$ M $[A]_t = 4.61 \times 10^{-3}$ M $[NaNO_2] = 2.5 \times 10^{-4}$ M

$[SCN^-]$ (M)	$[SCN^-]^{-1}$	$k_0(s^{-1})$	k_0^{-1}
0.02	50	6.87	0.14
0.016	62.5	6.32	0.16
0.01	100	4.02	0.24
0.005	200	2.21	0.45
0.002	500	0.88	1.14

340 nm
25°C.

SLOPE = $2.135 \pm 0.028 \times 10^{-3}$

intercept = 0.028 ± 0.002

$k_1 = 7.398 \times 10^8 \text{ lmol}^{-1}\text{s}^{-1}$

TABLE 14: p-AMINOBENZOIC ACID

$[H^+] = 0.285$ M $\quad [A]_t = 6 \times 10^{-3}$ M $\quad [NaNO_2] = 2.5 \times 10^{-4}$ M

$[SCN^-]$(M)	$[SCN^-]^{-1}$	$k_0(s^{-1})$	k_0^{-1}
0.02	50	10.05	0.099
0.017	58.82	8.57	0.117
0.012	83.33	7.02	0.142
0.010	100	5.88	0.17
0.005	200	3.50	0.286
0.002	500	1.80	0.555

330 nm, 25°C.

SLOPE = $1.086 \pm 0.028 \times 10^{-3}$

INTERCEPT = 0.051 ± 0.003

$k_1 = 1.261 \times 10^6 \text{ l mol}^{-1} \text{s}^{-1}$

TABLE 15: p-CHLOROANILINE

$[H^+] = 0.285$ $[A]_t = 6 \times 10^{-3}$ M $[NaNO_2] = 2.5 \times 10^{-4}$ M

$[SCN^-]$(M)	$[SCN^-]^{-1}$	$k_0(s^{-1})$	k_0^{-1}
0.02	50	19.66	0.059
0.017	58.82	16.05	0.062
0.012	83.33	11.60	0.086
0.01	100	10.25	0.0976
0.005	200	8.15	0.122
0.002	500	2.98	0.335

330 nm, 25°C.

SLOPE = $5.449 \pm 0.28 \times 10^{-4}$

INTERCEPT = 0.033 ± 0.003

$k_1 = 9.13 \times 10^7$ $lmol^{-1}s^{-1}$

TABLE 16: m-ANISIDINE

$[H^+] = 0.285$ M $\quad [A]_t = 6 \times 10^{-3}$ M $\quad [NaNO_2] = 2.5 \times 10^{-4}$ M

$[SCN^-]$(M)	$[SCN^-]^{-1}$	$k_0(s^{-1})$	k_0^{-1}
0.02	50	2.43	0.411
0.016	62.5	2.27	0.44
0.01	100	1.717	0.582
0.005	200	0.953	1.049
0.002	500	0.446	2.242

350 nm, 25°C

Slope = $3.144 \pm 0.53 \times 10^{-3}$

Intercept = 0.329 ± 0.006

$k_1 = 2.625 \times 10^7$ $lmol^{-1}s^{-1}$

FIG 5: DIAZOTISATION OF AMINES BY NOSCN

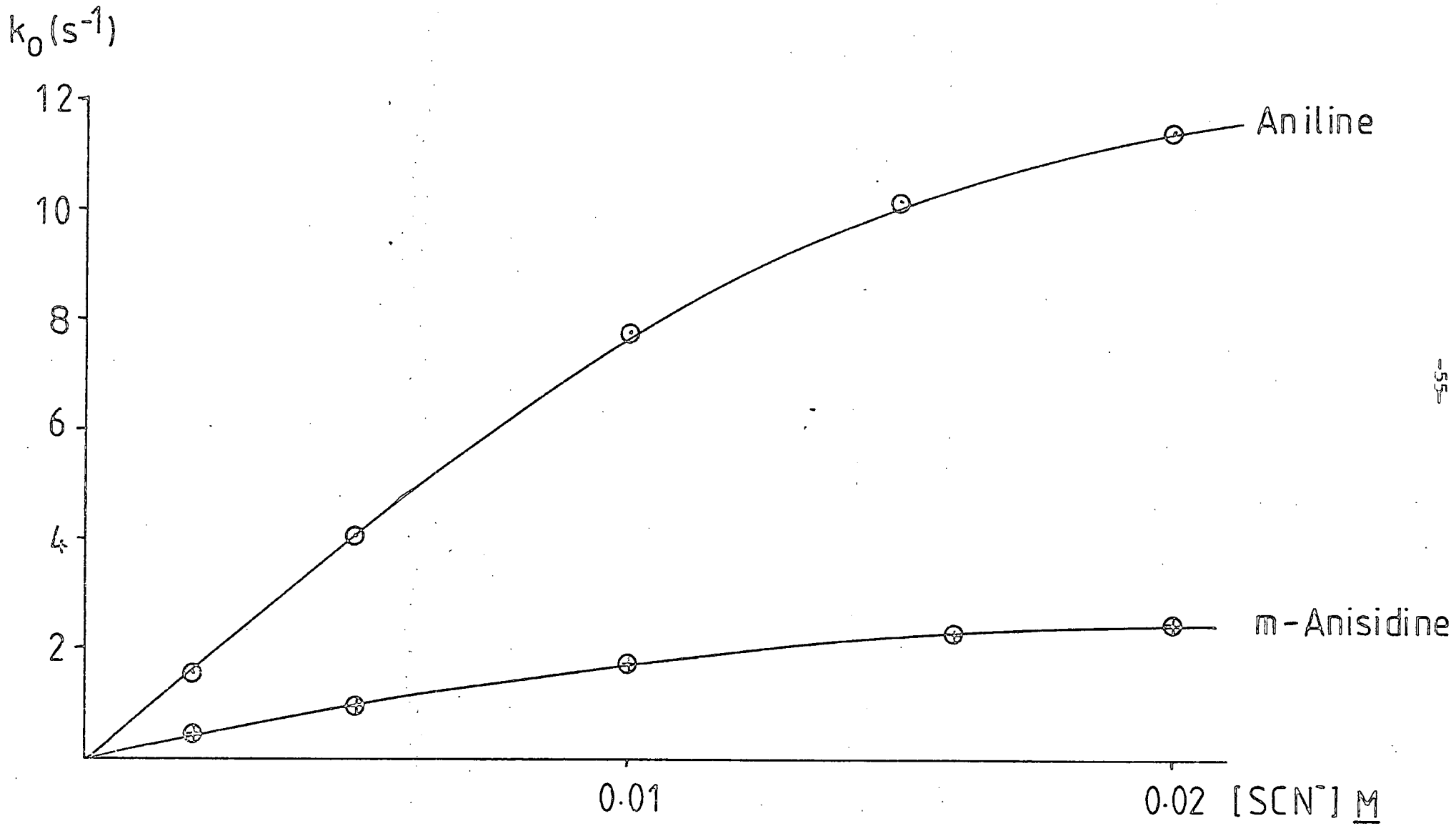
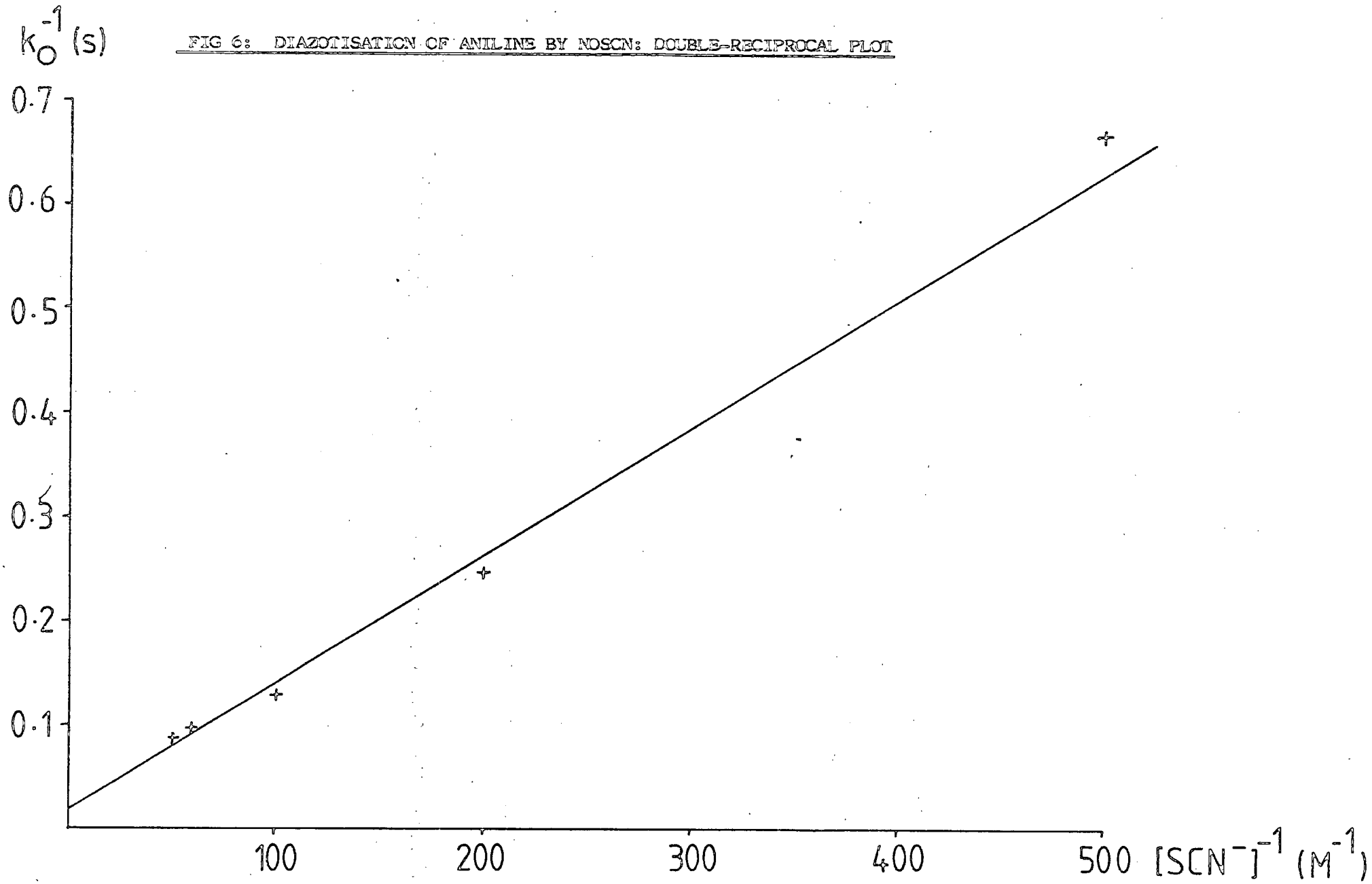


FIG 6: DIAZOTISATION OF ANILINE BY NOSCN: DOUBLE-RECIPROCAL PLOT



The collected results are given in the table below

TABLE 17

Amine*	$10^{-8}k_1$ † ($1mol^{-1}s^{-1}$)	log k_1	$k_{-1}:k_2$
-H (4·59)	1·75	8·24	19
m-OMe (4·20)	0·26	7·42	80
p-OMe (5·36)	7·39	8·87	13
p-Cl (3·98)	0·95	7·96	60
p-CH_3 (5·08)	4·08	8·61	25
p-CO_2H (2·42)	0·013	6·10	47

*pK_a values are given in brackets and are obtained from ref 82.

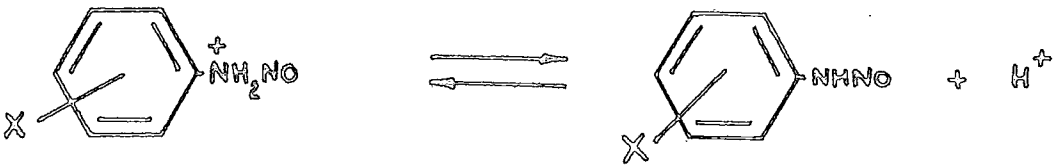
† at 25°C.

The bimolecular rate constants are plotted on a Brönsted-type plot (fig 9) of log k_1 against pK_a for the aromatic amine. As can be seen, the values of the rate constants do not level off toward the diffusion controlled limit for the more basic amines, as observed for NOCl and NOBr. The actual rate constants are numerically smaller than these more reactive nitrosyl compounds by a factor of 10^1-10^2 whereas NOCl and NOBr do not differ greatly in reactivity near the diffusion controlled limit. The $k_{-1}:k_2$ ratio's do not fit in exactly to the expected trend, though these values are based upon the intercepts of the double-reciprocal plots and as such are open to considerable error. The values, however, do indicate that the less basic amines (e.g. p-aminobenzoic acid)give N-nitroso compounds that are generally more reactive toward thiocyanate ion than those that are more basic (e.g. p-toluidine).

An attempt was made to diazotise p-nitroaniline. Earlier work[81] has shown that this substrate, on nitrosation, gives a second (slower) relaxation. This is observed to some extent for all substrates with electron withdrawing groups, but for p-nitroaniline this second relaxation is so marked that it overlaps the first (rapid) relaxation and a reliable value of k_0 cannot be obtained. The nature of this second process is not at all clear. It has been shown[81] that it is not a coupling reaction between the diazonium ion and excess amine, and it has been found to be first order in acid concentration. It is thought that this may well be the tautomeric shift as given by;

$$ArNHNO \longrightarrow ArN_2CH$$

This is because it is possible that for the less basic amines the direct formation of ArN_2^+ may not necessarily be observed, only the initial step (below) followed by the slower, acid catalysed, step.



2.5. Diazotization of aromatic amines by nitrosyl iodide

For the diazotization of aromatic amines by nitrosyl halides it is possible, under certain conditions for the rate of reaction to be zero-order in added amine. This can be achieved by adjusting the acid concentration or the basicity of the amine. Hughes and Ridd[77] found that for NOCl this was not possible, for NOBr this was possible using a weakly basic amine such as o-chloroaniline and for NOI the reaction was zero-order in aniline. Under the conditions where the reaction is zero-order in amine, the reaction is governed by a purely inorganic process i.e. the formation of the nitrosyl halide.

Considering the nitrosation of aniline by nitrosyl iodide in terms of the following mechanism;

$$H^{+} + I^{-} + HNO_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} NOI + H_2O$$

$$ArNH_2 + NOI \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} Ar\overset{+}{N}H_2NO + I^{-}$$

$$Ar\overset{+}{N}H_2NO \xrightarrow{k_3} Ar\overset{+}{N}_2$$

This is virtually the same as the scheme applied to the nitrosation of amines by NOSCN so we obtain the expression;

$$\text{Rate} = \frac{k_2 k_3 [ArNH_2][NOI]}{k_{-2}[I^{-}] + k_3}$$

Applying a steady state to NOI

$$k_0 = \frac{k_2 k_3 [ArNH_2]\, k_1 [H^{+}][I^{-}]}{(k_{-2}[I^{-}] + k_3)(k_{-1} + k_2[ArNH_2])}$$

If $k_{-1} \ll k_2 [ArNH_2]$ and $k_3 \gg k_{-2} [I^-]$

$$k_0 = k_1 [H^+][I^-]$$

These conditions are met, according to Hughes and Ridd[77] at low iodide and acid concentration with aniline as the substrate. They obtain a value of $k_1 = 1370\ l^2 mol^{-2} s^{-1}$ at 0^0C. The same applies for bromide ion catalysis of the nitrosation of o-chloroaniline. Clearly, if the equilibrium constant of formation of the nitrosyl species is known, then the value of k_{-1} i.e. the rate of hydrolysis of NOX can be determined. Values of k_{-1} of 1.8×10^6 s^{-1} and $5.3 \times 10^4 s^{-1}$ have been obtained for NOCl and NOBr respectively (the former value is obtained from the nitrosation of azide where the formation of NOCl is rate limiting). A value of k_{-1} is not given because there is no value for the equilibrium constant of formation of NOI in the literature. Beck[83] and co-workers have established that NOI undergoes decomposition to form iodine at high iodide concentration. A mechanistic scheme is proposed;

$$H^+ + HNO_2 + I^- \underset{}{\overset{K}{\rightleftharpoons}} NOI + H_2O$$

$$I^- + NOI \longrightarrow I_2^- + NO$$

$$I_2^- + H^+ + HNO_2 \xrightarrow{\text{rapid}} NO + I_2 + H_2O$$

$$2NOI \longrightarrow I_2 + 2NO$$

Since Hughes and Ridd[77] have established that the rate of nitrosation of aniline is governed by the rate of formation of NOI at low acid concentrations (2×10^{-3} M $HClO_4$), it should be possible to see a first order dependency on aniline concentration by increasing the acidity. In the present work this has been observed in $\sim 0.2M$ H_2SO_4. (table 18 and fig 7). From the slope (100), a value of k_2K (where K is the equilibrium constant of formation of

NOI, which is unknown) is obtained since we make the assumption that $k_2[ArNH_2] \gg k_{-1}$ and thus obtain the expression;

$$k_0 = k_2K[H^+][I^-][ArNH_2]$$

The k_2K value is $1{\cdot}55 \times 10^{10}$ $l^3mol^{-3}s^{-1}$. This value is somewhat larger than that obtained by varying the iodide concentration.

TABLE 18

$[Aniline]_{total}$ (M)	k_0 (s^{-1})
$2{\cdot}5 \times 10^{-3}$	1·11
$5{\cdot}0 \times 10^{-3}$	1·20
$1{\cdot}0 \times 10^{-2}$	1·57
$2{\cdot}0 \times 10^{-2}$	2·75

$[H_2SO_4]$ = 0·198 M $[KI] = 1 \times 10^{-3}$M $[NaNO_2] = 2{\cdot}5 \times 10^{-5}$ M

It will be noticed that there is a positive intercept on this plot. In the absence of added amine a non-first order kinetic process was observed and this is thought to be due to the formation of iodine and I_3^- by the decomposition of NOI shown in the above scheme. In fact, the u.v. spectrum of the reaction mixture after the reaction had gone to completion showed a maximum at 288nm and at 353nm which is characteristic of the equilibrium[84];

$$I_2 + I^- \rightleftharpoons I_3^-$$

Owing to the uncertainty over the K value for the formation of NOI it is not possible to obtain the bimolecular rate constants for the nitrosation of substituted anilines, but values of the relative rate constants (relative to aniline) can be determined and some idea of the degree of discrimination

of NOI toward the different amines obtained. This will give an indication of the reactivity of this electrophile. In order to minimise the formation of iodine by decomposition of NOI, runs were carried out by varying iodide concentration not exeeding 1×10^{-3} M iodide. The value for k_2K being determined for each amine in the same way as the thiocyanate catalysed re-action. Double-reciprocal plots were only used when there was a certain amount of curvature in the k_o vs $[I^-]$plot.

TABLE 19 (Aniline)

320nm, 25°C. $[PhNH_2]_t = 1 \times 10^{-2}$ M $[H^+] = 1{\cdot}6 \times 10^{-2}$ M

$[NaNO_2] = 2{\cdot}5 \times 10^{-4}$ M

$[KI]$ (M)	$k_o(s^{-1})$
2×10^{-4}	0·195
4×10^{-4}	0·447
6×10^{-4}	0·819
1×10^{-3}	1·460

Slope = 1221 ± 22

$k_2K = 4{\cdot}43 \times 10^9$ $l^3 mol^{-3} s^{-1}$

TABLE 20: m-Anisidine

350 nm, 25°C $[ArNH_2]_t = 3{\cdot}09 \times 10^{-3}$ M

$[H^+] = 0{\cdot}195$ M $[NaNO_2] = 2{\cdot}5 \times 10^{-4}$ M

$[KI]$ (M)	$[KI]^{-1}$	$k_o(s^{-1})$	k_o^{-1}
1×10^{-3}	10^3	0·41	2·44
$7{\cdot}5 \times 10^{-4}$	$1{\cdot}33 \times 10^3$	0·34	2·94
5×10^{-4}	2×10^3	0·24	4·17
$2{\cdot}5 \times 10^{-4}$	4×10^3	0·14	7·14

Slope = $1.57 \pm 0.01 \times 10^{-3}$

Intercept = 0.91 ± 0.04

$k_2K = 3.27 \times 10^{9}\ l^{3}mol^{-3}s^{-1}$

TABLE 21: p-Aminobenzoic acid

$[ArNH_2]_t = 4.73 \times 10^{-3}$ M $[H^+] = 0.452$ $[NaNO_2] = 2.5 \times 10^{-4}$ M

340 nm , 25^0C

$[KI]$ (M)	$[KI]^{-1}$	$k_0(s^{-1})$	k_0^{-1}
1×10^{-3}	10^3	0.885	1.13
7.5×10^{-4}	1.33×10^3	0.839	1.19
5×10^{-4}	2×10^3	0.685	1.46
2.5×10^{-4}	4×10^3	0.375	2.67

Slope = $5.31 \pm 0.16 \times 10^{-4}$

Intercept = 0.51 ± 0.04

$k_2K = 1.05 \times 10^{8}\ l^{3}mol^{-3}s^{-1}$

TABLE 22: p-chloroaniline

$[ArNH_2]_t = 6.0 \times 10^{-3}$ M $[H^+] = 0.452$ $[NaNO_2] = 2.5 \times 10^{-4}$ M

$[KI]$ (M)	$k_0(s^{-1})$
1×10^{-3}	1.35
7.5×10^{-4}	1.28
5×10^{-4}	0.69
2.5×10^{-4}	0.39

Slope = 1360 ± 96

$k_2K = 2.16 \times 10^9\ l^3 mol^{-3} s^{-1}$

TABLE 23: p-anisidine

$[ArNH_2]_t = 3.94 \times 10^{-3}$ M $\qquad [H^+] = 0.40$ M

$[NaNO_2] = 2.5 \times 10^{-4}$ M

$[KI]$ (M)	$[KI]^{-1}$	$k_0 (s^{-1})$	k_0^{-1}
1×10^{-3}	1×10^3	0.58	1.72
7.5×10^{-4}	1.33×10^3	0.56	1.78
5×10^{-4}	2×10^3	0.40	2.50
2.5×10^{-4}	4×10^3	0.18	5.55

Slope = $1.33 \pm 0.04 \times 10^{-3}$

Intercept = 0.109 ± 0.11

$k_2K = 4.37 \times 10^{10}\ l^3 mol^{-3} s^{-1}$

A Hammett correlation[80] can be applied to these results as shown in fig 8 . The slope is ≃ -3.6 and shows that there is a considerable degree of selectivity of NOI toward the different substrates, as is observed for NOSCN (see reference 79).

FIG 7: DIAZOTISATION OF ANILINE BY NCI

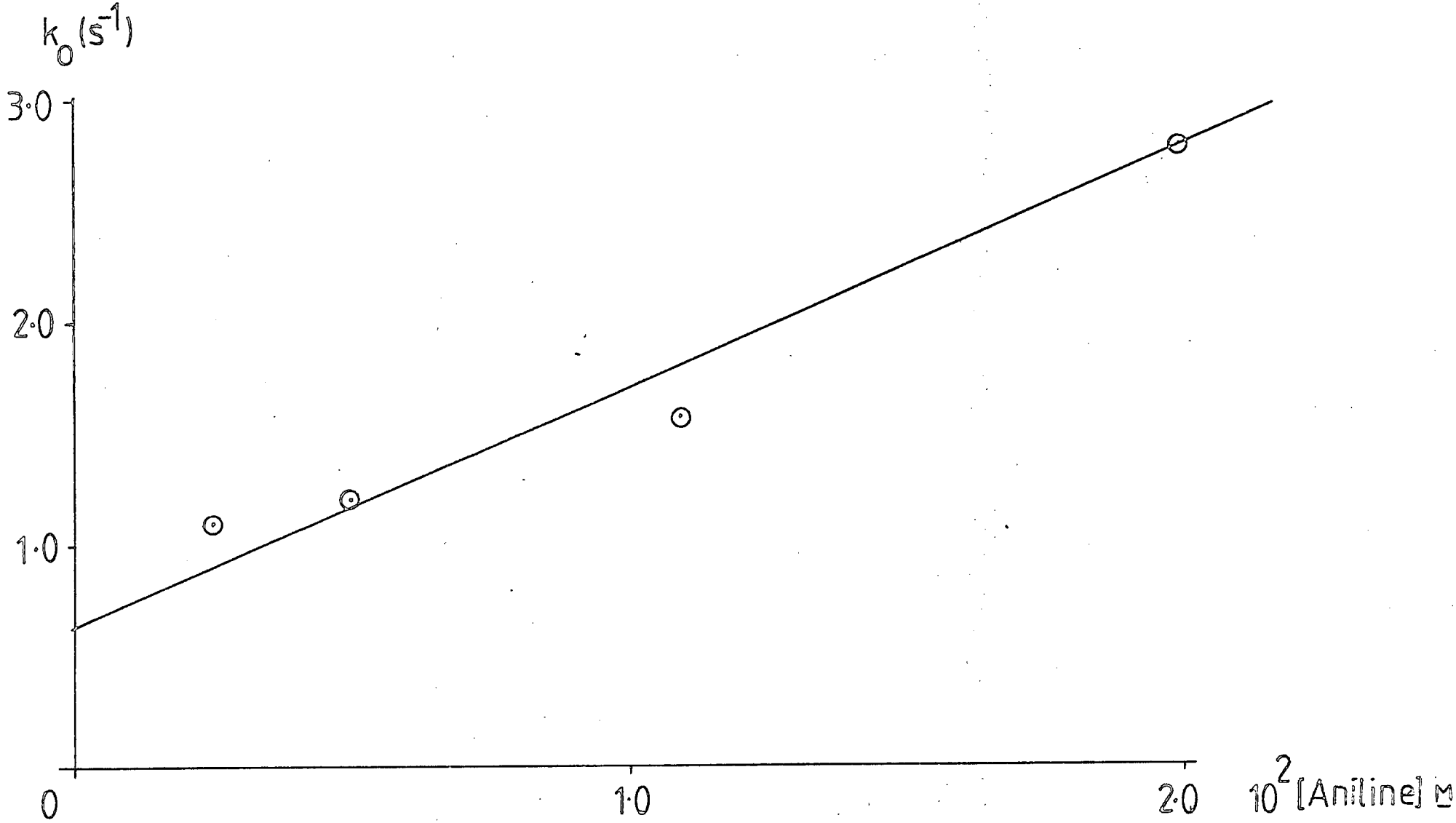
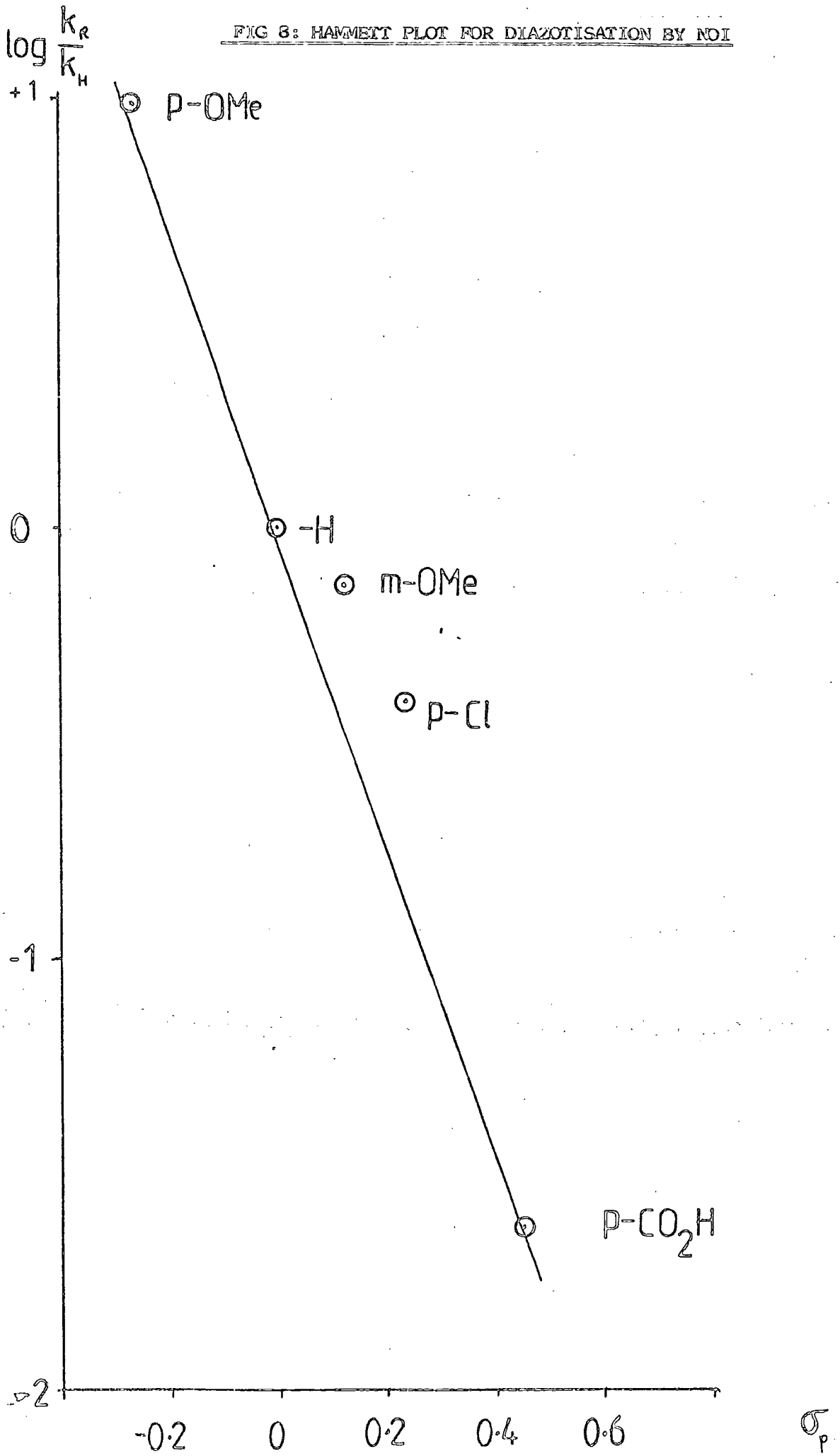


FIG 8: HAMMETT PLOT FOR DIAZOTISATION BY NOI



2.6. Diazotisation by the S-nitroso adduct of thiourea

Several kinetic studies have been made of the nitrosation of thiourea and these will be discussed under the heading of S-nitrosation in the next chapter. The S-nitroso adduct of thiourea is the reactive species and has a large equilibrium constant of formation[71] ($K = 5 \times 10^3\ l^2 mol^{-2}$ at 25^0C). The stoichiometry for the nitrosation reaction is simple:

$$ArNH_2 + NO\text{-}\overset{+}{S}{=}C(NH_2)_2 \rightleftharpoons Ar\overset{+}{N}H_2NO + S{=}C(NH_2)_2$$

Since the K value is so large, a considerable amount of nitrite will be present as the S-nitroso adduct, unlike the situation for diazotisation by other nitrosyl species. It is therefore necessary to modify the rate expression.

Since, $[\text{Total nitrite}] = [HNO_2] + [NOX]$

$$K[H^+][X^-] = \frac{[NOX]}{[T.N.] - [NOX]}$$

$$= \frac{1}{\left\{\frac{[T.N.]}{[NOX]} - 1\right\}}$$

Since, rate $= k_1[ArNH_2][NOX] - k_{-1}[Ar\overset{+}{N}H_2NO][X^-]$ (according to the mechanistic scheme described for the thiocyanate catalysed reaction)

$$k_0 = \frac{k_1 K_a [ArNH_2]_t}{[H^+]} \left\{ \frac{1}{\frac{1}{K[H^+][X^-]} + 1} \right\}$$

In this case X^- refers to any nucleophile, though of course thiourea is a neutral species. It is also necessary to correct for the difference betweem the total concentration of thiourea and the free thiourea (i.e. that which is not bound up as the nitroso adduct). We define K as;

$$K = \frac{[NOX]}{[H^+]\{[T.N.] - [NOX]\}\{[X^-]_t - [NOX]\}}$$

Solving this expression for NOX involves solving a quadratic equation. It is found that under the conditions used in the present work the concentration of thiourea bound up as nitrite is insignificant compared to the total.
It has been established[86] that the observed rate constant for the diazotisation of aniline becomes zero-order in thiourea at high thiourea concentration as would be expected. As for the work described earlier the bimolecular rate constants for nitrosation in a series of substituted anilines has been determined by varying the amine concentration. For aniline, two sets of runs were performed. The first was carried out at low thiourea concentration where the rate expression defined above applies and the second at higher thiourea concentration where the term in brackets, i.e.

$$\left\{ \frac{1}{\frac{1}{K[H^+][X^-]} + 1} \right\}$$

is approximately 1.0, that is, $[X^-]$ is large. The two results are in reasonable agreement as would be expected.

TABLE 24: ANILINE

(a) $[\text{thiourea}] = 1 \times 10^{-3}$M $\quad [NaNO_2] = 1 \times 10^{-4}$M

$[H^+] = 0.50$ M

$[A]_t$ (M)	k_o (s^{-1})
1.06×10^{-3}	0.06
2.12×10^{-3}	0.108
3.18×10^{-3}	0.165
3.50×10^{-3}	0.178

Slope = 49.15 ± 0.49

$k_1 = 1.34 \times 10^6$ $lmol^{-1}s^{-1}$

(b) $[\text{thiourea}] = 1 \times 10^{-2}$M $\quad [NaNO_2] = 5 \times 10^{-4}$M $\quad [H^+] = 0.22$M

$[A]_t$ (M)	k_o (s^{-1})
6×10^{-3}	0.75
1.2×10^{-2}	1.44
1.8×10^{-2}	2.23
2.4×10^{-2}	3.09
3.0×10^{-2}	4.09

Slope = 131 ± 2

$k_1 = 1.05 \times 10^6$ $lmol^{-1}s^{-1}$

TABLE 25: p-chloroaniline

$[NaNO_2] = 1 \times 10^{-5}$ M $[H^+] = 0.50$ M $[\text{thiourea}] = 1 \times 10^{-3}$ M

$[A]_t$ (M)	k_0 (s^{-1})
5×10^{-4}	0.032
1×10^{-3}	0.068
2×10^{-3}	0.138
3×10^{-3}	0.228

Slope = 70.6 ± 0.2

$k_1 = 4.72 \times 10^5$ $lmol^{-1}s^{-1}$

TABLE 26: p-toluidine

concentrations of other reagents as in table 25

$[A]_t$ (M)	k_0 (s^{-1})
3×10^{-3}	0.225
2×10^{-3}	0.154
1×10^{-3}	0.079
5×10^{-4}	0.039

Slope = 75 ± 0.4

$k_1 = 6.31 \times 10^6$ $lmol^{-1}s^{-1}$

TABLE 27: p-anisidine

$[NaNO_2] = 1 \times 10^{-4}$ M $[H^+] = 0.23$ M $[\text{thiourea}] = 1 \times 10^{-3}$ M

$[A]_t$ (M)	k_0 (s^{-1})
2×10^{-3}	0.287
1×10^{-3}	0.155
5×10^{-4}	0.079
2.5×10^{-4}	0.036

Slope = 142.1 ± 1.8

$k_2 = 1.40 \times 10^7$ $lmol^{-1}s^{-1}$

TABLE 28: p-aminobenzoic acid

concentrations of other reagents as in table 27

$[A]_t$ (M)	k_0 (s^{-1})
4×10^{-3}	0.662
3×10^{-3}	0.505
1×10^{-3}	0.201
5×10^{-4}	0.109

Slope = 209 ± 8.0

$k_1 = 2.36 \times 10^4$ $lmol^{-1}s^{-1}$

TABLE 29: m-anisidine

This reaction was followed by observing the dissappearance of the S-nitroso adduct of thiourea at 420 nm.

$[NaNO_2] = 5 \times 10^{-4}$ M $[H^+] = 0.201$ M $[\text{thiourea}] = 1 \times 10^{-2}$ M

$[A]_t$ (M)	k_0 (s^{-1})
1.52×10^{-2}	2.89
4.56×10^{-3}	0.82
2.74×10^{-3}	0.43
1.21×10^{-3}	0.25
9.12×10^{-3}	1.62

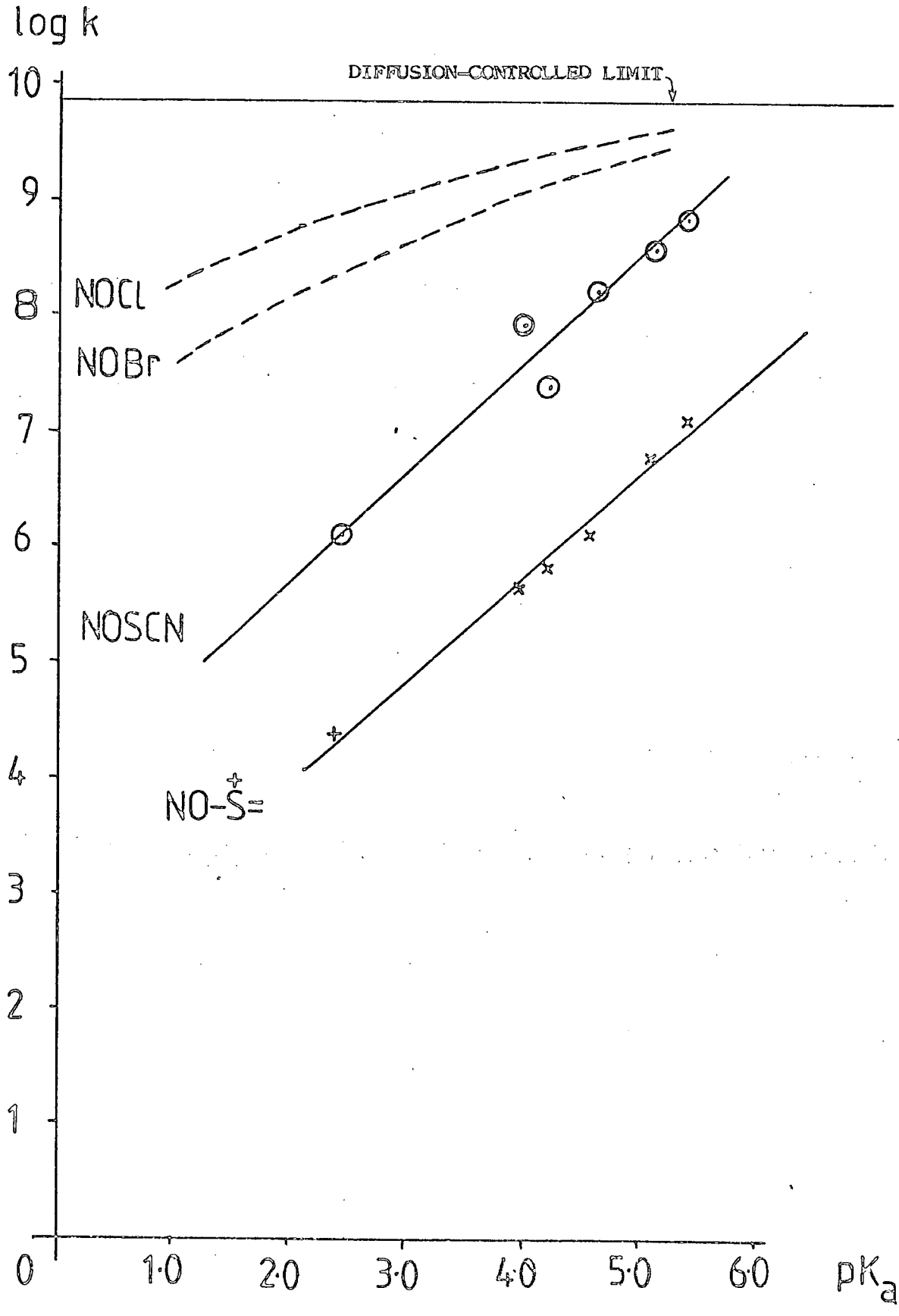
Slope = 190.48 ± 2.02

$k_1 = 6.64 \times 10^5$ $lmol^{-1}s^{-1}$

The collected results are given below; and are plotted on the Brönsted curve (fig 9).

Amine	pK_a	log k_1
-H	4.59	6.127
p-Cl	3.98	5.674
p-Me	5.08	6.80
p-OMe	5.36	7.146
m-OMe	4.20	5.822
p-CO_2H	2.42	4.373

FIG 9: BRONSTED-TYPE PLOT FOR NITROSATION OF AROMATIC AMINES



The combined data for diazotisation of aromatic amines by nitrosyl halides shows quite clearly the reactivity sequence NOCl $\gg$ NOBr $\gg$ NOSCN $\gg$ NO-$\overset{+}{S}<$, there being a difference of more than 10^3 in the rate of nitrosation over the reactivity range. For the S-nitroso adduct of thiourea and for NOSCN there is considerable discrimination in reactivity between the different amines with no sign of levelling off with amines of high basicity as is observed for the more reactive NOCl and NOBr species.

2.7. Summary

(1). Diazotisation of aromatic amines is catalysed by thiocyanate ion, due to the formation of nitrosyl thiocyanate. Since the equilibrium constant for the formation of NOSCN is known, absolute second order rate coefficients for the nitrosation of the amine can be determined from the overall rate of diazotisation of the amine. The resulting rate data shows that the second order rate constants are well below the diffusion controlled rate limit and there is considerable discrimination by NOSCN toward amines of different basicity.

(2). The above comments apply equally to diazotisation in the presence of thiourea. The S-nitroso adduct of thiourea is less reactive towards amines than NOSCN and the reactivity sequence NOCl $\gg$ NOBr $\gg$ NOSCN $\gg$ NO-$\overset{+}{S}<$ can be said to apply to the diazotisation reaction.

(3). As in the case ofdiazotisation by NOCl and NOBr, for amines that contain electron withdrawing groups a second, slower relaxation was observed along with the formation of the diazonium species. This was observed particularly in the case of p-nitroaniline diazotisation by NOSCN and NOI.

(4). For iodide catalysed reaction, absolute second order rate constants for the nitrosation step could not be obtained owing to the fact that the equilibrium constant for formation of NOI is not known. However, using a Hammett (σ-ρ) correlation it was possible to show that there is some

discrimination in reactivity toward amines of different basicity. By comparisin with $\sigma\cdot\rho$ data obtained for the diazotisation of aromatic amines by NOSCN and NO-$\overset{+}{S}<$ it seems that the degree of discrimination shown by NOI is comparable to these other electrophiles.

(5). In the iodide catalysed reaction, it was found that as the concentration of added iodide ion increased the nitrosation step was accompanied by the formation of I_2 and I_3^- due to a side reaction between NOI and I^-. Any rate data was taken at low iodide concentration.

CHAPTER THREE

Nitrosation of Sulphur compounds.

3.1. The Chemistry of Thionitrites

The first recorded[93] reaction between a thiol and nitrous acid was that involving thiophenol, the product being a disulphide. The reaction presumably occurs via the formation of a thionitrite.

$$PhSH + HNO_2 \longrightarrow PhSNO + H_2O \longrightarrow PhSSPh + NO$$

A wide range of nitrosating agents have been used for this reaction, including nitrous anhydride[94] (N_2O_3) and dinitrogen tetraoxide[95] (N_2O_4). Thionitrites have a characteristic intense colouration, e.g. the thionitrite of penicillamine is green, that of n-Alkyl thiols is red. Phillipe and Moore[96] have studied the infra-red spectra of thionitrites and established that an -N=O stretch at c.a. 1530 cm^{-1} and an -S-N stretch at 630 cm^{-1} are characteristic frequencies. This is supported by the i.r. spectrae of a wide range of thionitrites recorded recently by Oae[95]. Alkyl thionitrites are usually only observed as coloured inter-mediates in the oxidation of thiols to disulphides. Thionitrites containing more bulky substituents e.g. Ph_3CSNO seem to be more stable. This particular thionitrite is quite stable at room temperature over a period of several days and weeks.[97] The most stable thionitrite yet to be isolated is the derivative of (±)-2-Acetylamino-2-carboxy-1,1-dimethylethanethiol (i.e. N-acetyl penicill-amine).[97]

$$HSCMe_2CH(NHAc)CO_2H \xrightarrow{HNO_2} ONSCMe_2CH(NHAc)CO_2H$$

The X-ray structure of this compound has been established, and is the first compound to yield parameters for the -S-NO group. Although the compound will keep indefinitely if refrigerated it will give the disulphide by refluxing in methanol. The mechanism of nitrosation of thiols has been studied by electron spin resonance[100] and this has revealed that the reaction seems to have a radical component to the mechanism.

$$RSH + NOX \rightleftharpoons RSNO + HX$$

$$RSNO \rightleftharpoons RS^{\circ} + NO^{\circ}$$

$$RSNO + NO^{\circ} \xrightarrow[\text{trapping}]{\text{spin}} RSN(N{=}O)O^{\circ}$$

X can be R_2N i.e. a nitrosoamine, or Cl i.e. NOCl.

Thionitrites can also undergo transnitrosation e.g. with secondary amines to give N-nitrosoamines,[94] however it has not been established whether this occurs directly, or via the initial hydrolysis of RSNO.

$$RSNO + R_2NH \longrightarrow R_2NNO + RSH$$

Similarly, thionitrites can undergo denitrosation[101] in strongly acidic (1-4M HCl) solutions.

$$RSNO + HCl \rightleftharpoons RSH + NOCl \xrightarrow{\text{Trap}} \text{removed}$$

A nitrite trap must be present in sufficient excess to remove NOCl because the reaction is reversible and lies well over to the side of the thionitrite. The first kinetic study of S-nitrosation was made on the direct nitrosation of n-butyl mercaptan.[98]

$$RSH + HNO_2 \xrightarrow{k} RSNO + H_2O$$

This work established that the formation of thionitrite was favoured and the rate law was given as;

$$\text{Rate} = k[H^{+}][HNO_2][RSH].$$

Stedman[114] has made kinetic measurements on the nitrosation of cysteine, apart

from this few kinetic studies have been done on the nitrosation of thiols.

3.2. Oxidation of thiols by nitrosation

Most thionitrites are unstable species and in the presence of excess thiol are readily oxidised to give a disulphide. This affords a convenient synthetic route to form -S-S- bridges. Oae[95] has utilised this method to synthesise unsymmetrical disulphides.

$$PhSH \quad + \quad N_2O_4 \Longrightarrow PhSNO \xrightarrow{Me_2CHSH} PhSSCHMe_2$$

Thiols can form thionitrites by reaction with N-nitrosoamines, this may have some importance in terms of nucleic acid chemistry where sulphur bridges play an important part. It has been shown[99] that cysteine can be oxidised to cystine in the presence of a nitrosoamine.

$$R\text{-}N(NO)CH_3 \quad + \quad \begin{matrix} CO_2H \\ | \\ CH\text{-}NH_2 \\ | \\ CH_2SH \end{matrix} \longrightarrow R\text{-}NHCH_3 \quad + \quad \left(\begin{matrix} CO_2H \\ | \\ CH\text{-}NH_2 \\ | \\ CH_2 \end{matrix} \right)_2$$

Some detailed work has been done[103] on the nitrosation of thiophenol by nitric oxide and dinitrogen tetraoxide. Nitrosation by NO gas was found to give quantitative yields of the disulphide when the reaction was carried out in aqueous solution, and was catalysed by addition of base. Nitrosation by NO_2 was exothermic and unlike the NO reaction, some over-oxidation products were given (e.g. sulphoxides, sulphones etc) though the disulphide was formed in good yield. Green[104] has shown that glutathione and cysteine can "protect" antibacterial macrophages in the lung from poisoning by cigarette smoke. These macrophage contain active enzymes such as 6-phosphate dehydrogenase which would be rendered inactive by disulphide formation due to the oxides of nitrogen which are present in tobacco smoke in high concentrations (up to 80 p.p.m.). The presence of cysteine or glutathione may be protective due to "sacrificial" oxidation of these species.

3.3. Nitrosation of thiourea

Wexner[105,106] studied the reaction between thiourea and nitrous acid and found that at low and high acidities the reaction had a different stochiometry. At low acidities;

$$HNO_2 + CS(NH_2)_2 \longrightarrow H^+ + SCN^- + N_2 + 2H_2O$$

At higher acidities;

$$2H^+ + 2HNO_2 + 2CS(NH_2)_2 \longrightarrow (NH_2)_2\overset{+}{C}-S-S-\overset{+}{C}(NH_2)_2 + 2NO + 2H_2O$$

For the latter reaction, X-ray crystallography[107] has confirmed the structure of the dithioformamidium ion. In both cases, the S-nitroso adduct of thiourea $(NH_2)_2C=\overset{+}{S}-NO$ is observed as a yellow intermediate species having a maximum absorbance at 420 nm. Stedman[71] and co-workers have measured the rates of the foreward and backward reactions for the formation of this species in acid sol-ution,

$$H^+ + HNO_2 + CS(NH_2)_2 \underset{}{\overset{K}{\rightleftharpoons}} (NH_2)_2C=\overset{+}{S}-NO + H_2O$$

A value for the equilibrium constant of formation of the species obtained from this rate data is $5 \times 10^3 l^2 mol^{-2}$ at 25^0C. The same workers have gone on to suggest a mechanism (based upon kinetic evidence) for the reaction at low and high acidity.[108] At lower acidities, it appears that S-nitrosation will make the nitrogen protons on thiourea more acidic, giving a sufficient concentration of the conjugate base to allow for a reaction in which there is slow migration of the nitroso group to the nitrogen atom.

$$(NH_2)_2C=\overset{+}{S}-NO \rightleftharpoons (NH_2)(\bar{N}H)(\overset{+}{S}NO) + H^+$$

$$\downarrow \quad \text{slow rearrangement}$$

$$NH_2CS-NHNO$$

$$\downarrow \quad \text{rapid}$$

$$H^+ + CNS^- + N_2 + H_2O \longleftarrow NH_2CS-N=N-OH$$

This ties in with the observed rate law, and also agrees with the suggestion[109] that in the nitrosation of amides, initial nitrosation of the carbonyl oxygen is followed by transfer to the amino group. Some ^{15}N n.m.r. studies[110] have suggested that direct N-nitrosation may occur. At higher acidities, the kinetics of formation of the dithioformamidium ion have been studied.[108] Since thiols react directly with thionitrites to give disulphides, it seems likely that an analogous reaction takes place here.

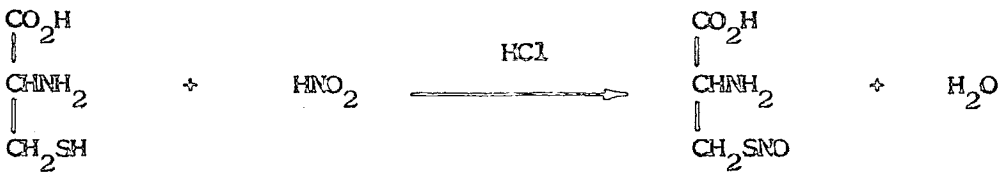
$$(NH_2)_2CS \quad + \quad (NH_2)_2C\overset{+}{S}NO \rightleftharpoons \left[(NH_2)_2C{-}S{-}S{-}C(NH_2)_2\right]^{+\cdot} \quad + \quad NO^{\cdot}$$

The radical cation can undergo various pathways to give the dithioformamidium ion. Other kinetic studies of the oxidation of thiourea to the disulphide include oxidation by Cr(VI).[111]

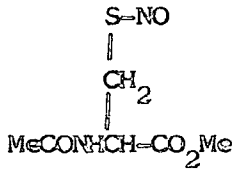
3.4. Nitrosation of Cysteine

It is known that cysteine $HSCH_2CH(NH_2)(CO_2H)$ acts as a nitrite scavenger in cured meats[112], being the most reactive meat protein residue toward nitrite. It has also been shown that S-nitroso cysteine is an effective nitrosating agent.[113] Some protein-bound studies of the nitrosation of cysteine residues have made.[113]

In mildly acidic solutions cysteine exists in the zwitterionic form, $HSCH_2CH(CO_2^-)NH_3^+$. The amino group is very unreactive in such conditions and there is no clear evidence of N-nitrosation taking place in the presence of nitrous acid.[116] An S-nitroso compound is formed, on nitrosation, which undergoes oxidation to give the disulphide under certain conditions. The study of the nitrosation of cysteine may be of biological importance as cysteine residues play an important part in the structure of peptides. The reaction between cysteine and nitrous acid was studied by Stedman[114] who found that formation of the S-nitroso compound was as far as could be detected irreversible.



The solid S-nitroso derivative of cysteine has been isolated,[113,117] although this compound is unstable. It is a yellow species in solution with a broad absorbance around 330 nm. Bonnett[117] has isolated the nitrosation product of N-acetyl cysteine methyl ester which has similar physical characteristics to the S-nitroso derivative of cysteine and is equally unstable.



In this present work, the kinetics of nitrosation of L-cysteine have been studied in aqueous solution at 25°C. The reaction is rapid and is followed by observing the formation of the thionitrite at 330 nm. using a stopped-flow spectrophotometer. The results are given in table 30. The individual runs gave good, first order, plots (for $\log(A_t - A_\infty)$ vs t). The data confirms the rate expression established by Stedman[114]

$$\text{Rate} = k_3 [\text{Cysteine}][H^+][HNO_2]$$

From a plot of the observed rate constant (k_0) vs [Cysteine], a value for the third order rate constant (k_3) of 448 $l^2 mol^{-2} s^{-1}$ is obtained. This is in good agreement with a value of 456 $l^2 mol^{-2} s^{-1}$ obtained by Stedman.[114] k_3 can also be determined by plotting k_0 vs $[H^+]$. This has a small positive intercept, and this is probably due to general acid catalysis by the carboxyl group in the thiol. There is therefore an additional $k_2'[RSH]$ term, and so k_0 is given by;

$$k_0 = k_3[RSH][H^+] + k_2'[RSH]$$

TABLE 30

[Cysteine] (M)	[$NaNO_2$] (M)	[Br^-] (M)	[H^+] (M)	k_o (s^{-1})
1.3×10^{-2}	1×10^{-5}	0	2.06×10^{-2}	0.179
1.0×10^{-2}	"	"	"	0.135
5×10^{-3}	"	"	"	0.068
1×10^{-2}	"	"	6.18×10^{-2}	0.320
"	"	"	4.12×10^{-2}	0.205
"	"	"	1.24×10^{-2}	0.098
"	"	0.50	2.06×10^{-2}	0.20
"	"	0.25	"	0.163
"	"	0.10	"	0.151

TABLE 31

[M.S.A.] (M)	[$NaNO_2$] (M)	[Br^-] (M)	[H^+] (M)	k_o (s^{-1})
1×10^{-3}	1×10^{-5}	0	2.06×10^{-2}	0.032
1×10^{-3}	1×10^{-4}	"	"	0.030
1×10^{-2}	1×10^{-5}	"	"	0.308
5×10^{-3}	"	"	"	0.145
"	"	"	4.12×10^{-2}	0.271
"	"	"	1.24×10^{-2}	0.105
"	"	"	8.24×10^{-3}	0.067
"	"	0.50	2.06×10^{-3}	0.215
"	"	0.40	"	0.197
"	"	0.25	"	0.176

M.S.A. = Mercaptosuccinic acid

FIG 10: NITROSATION OF CYSTEINE

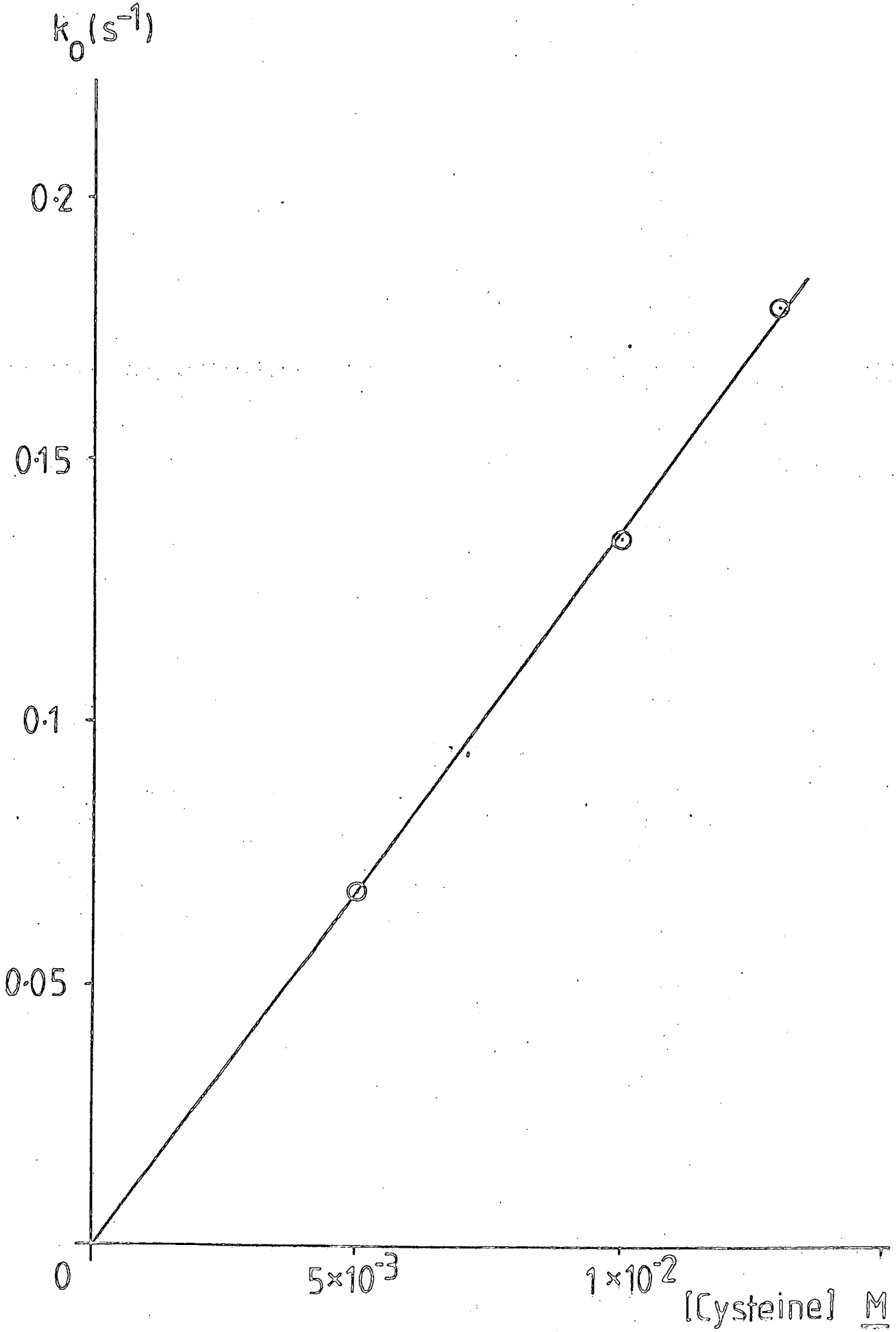
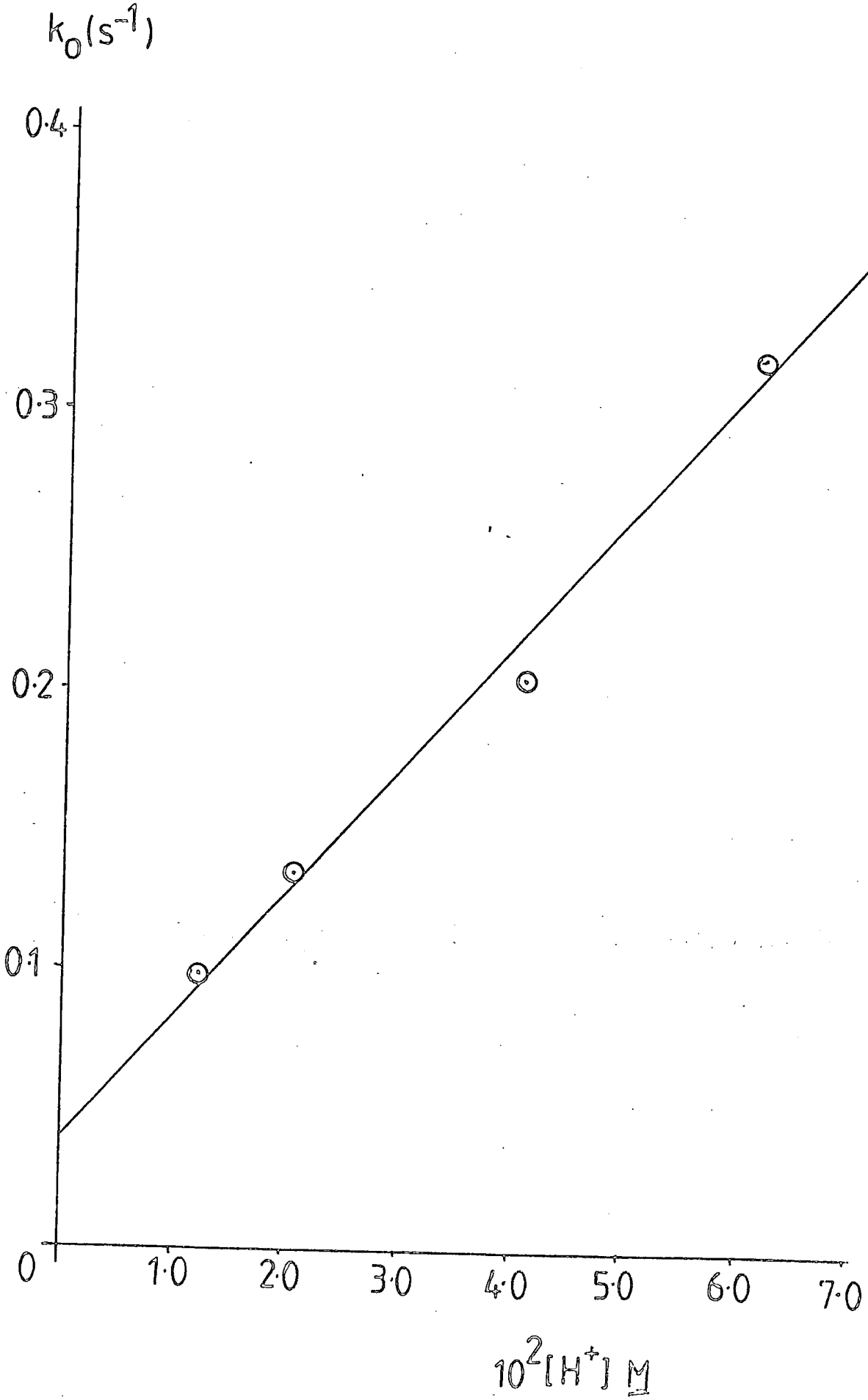


FIG 11: NITROSATION OF CYSTEINE



In this case, $k_3 = 438\ l^2 mol^{-2} s^{-1}$ and $k_2' = 4.1\ l mol^{-1} s^{-1}$. The value of k_3 obtained here is in close agreement to the value obtained by varying [RSH]. The bromide ion catalysed reaction was studied for the first time, the results being included in table 30. The rate expression obtained from these results is of the form;

$$\text{Rate} = k[Br^-][HNO_2] + k'[HNO_2]$$

Now, for substrates such as aniline, the mechanistic scheme for nitrosation by NOBr is given by;

$$SH + NOBr \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \overset{+}{S}\text{-}NO \; (H) + Br^-$$

$$\overset{+}{S}\text{-}NO \; (H) \xrightarrow{k_p} SNO + H^+$$

S = RNH, RO etc.

Since the rate of disappearance of SH is given by:

$$\text{Rate} = k_2[NOBr][SH] - k_{-2}[\overset{+}{S}HNO][Br^-]$$

Applying a steady state to $\overset{+}{S}HNO$

$$[\overset{+}{S}HNO] = \frac{k_2[NOBr][SH]}{k_{-2}[Br^-] + k_p}$$

$$\text{So,} \quad \text{Rate} = \frac{k_p k_2[NOBr][SH]}{k_{-2}[Br^-] + k_p}$$

If $k_p \gg k_{-2}[Br^-]$, as would be expected for a rapid proton transfer to the solvent, then;

$$\text{Rate} = k_2[NOBr][SH]$$
$$= k_2K[Br^-][H^+][HNO_2][SH]$$
$$= k[Br^-][HNO_2]$$

The term $k[Br^-][HNO_2]$ in the observed kinetic form, therefore seems to correspond to the above mechanism and on this basis if we use the value of K (equilibrium constant for the formation of NOBr), we obtain a value for k_2 of $1{\cdot}20 \times 10^4$ $lmol^{-1}s^{-1}$. The significance of this result will be discussed later by comparing it with data for other thiocarboxylic acids.

3.5. Nitrosation of Mercaptosuccinic acid

Since it has been observed[97] that the N-acetyl derivative of penicillamine forms a stable thionitrite, it follows that other thiocarboxylic acids may form relatively stable products on nitrosation and react rapidly with nitrosating agents. It has been the aim of the present work to study the nitrosation of several such compounds.

The direct nitrosation of mercaptosuccinic acid $(HO_2C)CH(SH)CH_2(CO_2H)$, was studied by observing the formation of the S-nitroso compound at 330 nm by stopped-flow spectrophotometry, exactly as for cysteine (at 25^0C). The results (table 31) show the same kind of rate law as for cysteine. From the variation of thiol concentration , a value of 1456 $l^2mol^{-2}s^{-1}$ is obtained for k_3. By varying the acid concentration, k_3 = 1208 $l^2mol^{-2}s^{-1}$ and k_2' is 4·6 $lmol^{-1}s^{-1}$. The rate constant for bromide catalysed reaction is $2{\cdot}63 \times 10^4$ $lmol^{-1}s^{-1}$.

If thiocyanate is used as the nucleophile, there is a tendancy for the plot of k_0 vs $[SCN^-]$ to curve off at high $[SCN^-]$ as shown in table 32 and in figure 12 . There may be several reasons why this levelling off may take place at high thiocyanate concentrations, these will be considered in turn;

TABLE 32

[KCNS] (M)	$[KCNS]^{-1}$	k_o (s^{-1})	k_o^{-1}
0		0·161	
0·01	100	0·55	1·181
0·02	50	1·30	0·766
0·10	10	3·69	0·271
0·20	5	6·45	0·155
0·60	1·67	13·37	0·075
1·20	0·83	19·49	0·051

$[H^+] = 2·3 \times 10^{-2}M$ $[NaNO_2] = 1 \times 10^{-4}M$ $[MSA] = 5 \times 10^{-3}M$

1.) Considering the high ionic strength of the solution (up to 1·2 $moll^{-1}$) there is the possibility of a kinetic salt effect operating. However it is difficult to see how this would affect the rate of nitrosation which is essentially a reaction between two neutral molecules (RSH and NOSCN). The reverse reaction (denitrosation) between $R\overset{+}{S}HNO$ and SCN^- would be retarded in a solution of increasing ionic strength. This, therefore does not seem to be a plausible explanation for the observed decrease in rate at high thiocyanate concentration.

2.) The acid concentration may be altered at high $[SCN^-]$ owing to the formation of thiocyanic acid (HSCN). A calculation based upon the pK_a value for this species (see chapter one) shows that under the conditions in this set of runs the amount of H^+ 'bound' up as HSCN is negligible compared to the total acid concentration. So this will not satisfactorily explain the observed data.

3.) Since the observed rate constant is defined as the rate at which free nitrous acid is used up in the reaction it follows that if a considerable concentration of HNO_2 is present as NOSCN, then the rate expression must be altered to take account of the fact. This is the case in the nitrosation of

aniline by the S-nitroso adduct of thiourea (see chapter two) where the equilibrium constant for the formation of the nitrosating species is so large that a significant amount of HNO_2 is 'bound' up as the S-nitroso adduct. At high thiocyanate ion concentration a similar situation may arise, and so we may use the same sort of expression as derived previously for thiourea (see page 67)

$$[NOSCN] = \frac{[HNO_2]_{xs}}{\frac{1}{K[H^+][SCN^-]} + 1}$$

At low SCN^- this becomes, $[NOSCN] = K[H^+][SCN^-][HNO_2]_{xs}$

Under the conditions used i.e. at high $[SCN^-]$, the more complex expression applies. If we say that the rate expression for the nitrosation of RSH by NOSCN is given by;

$$\text{Rate} = k[RSH][NOSCN]$$

Under these conditions,

$$\text{Rate} = \frac{k[RSH][HNO_2]_{xs}}{\frac{1}{K[H^+][SCN^-]} + 1}$$

The discussion of this relationship will be continued after considering the fourth possible case.

4.) Reffering to the above scheme for the nitrosation of the substrate SH by NOBr (in this case we can replace NOBr by NOSCN), it can be seen that at high Br^- concentration there may be some reversibility in the reaction due to competition between the denitrosation of $\overset{+}{S}HNO$ and proton loss to the solvent. This is what is probably observed at high bromide concentration in the diazotis-ation of aniline, although no kinetic isotope studies have been made as yet. If we get the condition $k_p \ll k_{-2}[Br^-]$ and the rate becomes zero order in

bromide ion. This might provide an explanation for the decrease in the rate at high thiocyanate concentration in the nitrosation of mercaptosuccinic acid. Clearly we have two possible conditions;

i) $k_p \gg k_{-2}[SCN^-]$

So,

$$\text{Rate} = \frac{k_2[RSH][HNO_2]_{xs}}{\left\{\frac{1}{K[H^+][SCN^-]} + 1\right\}}$$

Under this condition the reaction is irreversible and the decrease in rate at high thiocyanate concentration could only be attributed to the increasing amount of nitrous acid being formed as NOSCN. Now, the rate is given by;

$$\text{Rate} = k_0[HNO_2]_{xs}$$

If a double-reciprocal plot is now taken,

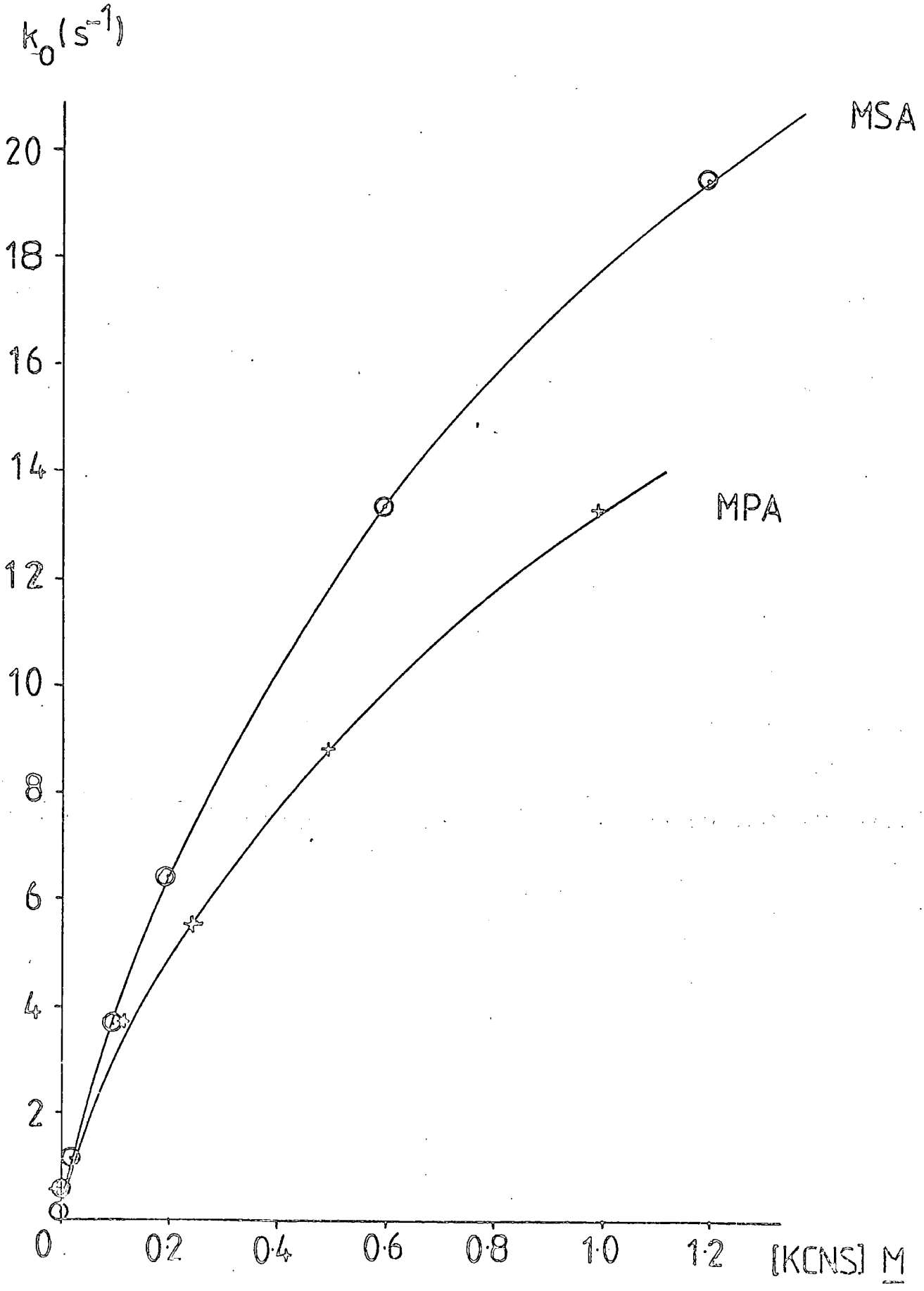
$$k_0^{-1} = \frac{1}{k_2[RSH]} + \frac{1}{k_2[RSH]K[H^+][SCN^-]}$$

This should give a straight line with a posotive slope and intercept. From the data in table 32 this is in fact what is observed. The slope has a value of 0·0171 ± 0·0003 and the intercept 0·044 ± 0·014. The k_2 value obtained from the intercept is $4{\cdot}54 \times 10^3$ $lmol^{-1}s^{-1}$.

ii) $k_p \ll k_{-2}[SCN^-]$

$$\text{Rate} = \frac{k_p k_2[RSH]}{k_{-2}[SCN^-]} \times \left\{\frac{1}{\frac{1}{K\ H^+[SCN^-]} + 1}\right\}$$

FIG 12: NITROSATION OF THIOLS BY NOSCN



A double reciprocal plot would not yield a linear relation, however the following expression would be obtained;

$$k_0^{-1} = \frac{k_{-2}}{k_p k_2 [RSH]} \left\{ \frac{1}{K[H^+]} + [SCN^-] \right\}$$

Examination of the experimental data shows that this expression is not followed over any of the concentration range for thiocyanate ion. This suggests that the explanation for the decrease in rate at high thiocyanate concentration may be solely due to the formation of NOSCN in significant quantities and not due to a change in the rate limiting step. To test this further, the solvent isotope effect for the nitrosation of mercaptosuccinic acid at 1·0M SCN^- is $k_{D_2O} : k_{H_2O}$ = 1·11. This suggests that proton transfer to the solvent from $R\overset{+}{S}HNO$ is rapid compared to the attack by SCN^- on $R\overset{+}{S}HNO$ and that the condition $k_p \gg k_{-2}[SCN^-]$ holds under these conditions.

An attempt was made to isolate the thionitrite, several grammes of the thiocarboxylic acid were dissolved in a solution of sodium nitrite. Concentrated sulphuric acid was added dropwise and the solution turned deep red. The thionitrite was extracted in ether and on removing the solvent on a rotary evaporator, the cherry red compound rapidly decomposed giving off brown fumes, presumably NO_2.

3.6. Nitrosation of 3-mercaptopropanoic acid

The nitrosation of another thiocarboxylic acid was studied. 3-mercaptopropanoic acid ($HSCH_2CH_2CO_2H$) forms a thionitrite with a maximum absorbance at 320 nm. This substrate was subjected to the same kinetic studies as cysteine and mercaptosuccinic acid. The results are given in table 33 and refer to runs at 25°C. The kinetic data from the k_0 vs [RSH] plot gives $k_3 = 4·66 \times 10^3\ l^2 mol^{-2} s^{-1}$. By varying the acidity, $k_3 = 4·35 \times 10^3\ l^2 mol^{-2} s^{-1}$ and $k_2' = 7·6\ l mol^{-1} s^{-1}$. From the k_0 vs $[Br^-]$ plot, $k_2 = 4·5 \times 10^5 l mol^{-1} s^{-1}$.

TABLE 33

$[RSH]$ (M)	$[NaNO_2]$ (M)	$[H^+]$ (M)	$[Br^-]$ (M)	$k_0(s^{-1})$
1×10^{-3}	1×10^{-5}	$2{\cdot}06 \times 10^{-2}$	0	0·093
5×10^{-3}	"	"	"	0·480
1×10^{-2}	"	"	"	0·959
5×10^{-3}	"	$4{\cdot}12 \times 10^{-2}$	"	1·015
"	"	$3{\cdot}3 \times 10^{-2}$	"	0·777
"	"	$1{\cdot}02 \times 10^{-2}$	"	0·258
"	"	$2{\cdot}06 \times 10^{-2}$	0·50	1·674
"	"	"	0·25	1·112
"	"	"	0·10	0·743

RSH = 3-mercaptopropanoic acid

TABLE 34

$[KCNS]$ (M)	$[KCNS]^{-1}$	$k_0(s^{-1})$	k_0^{-1}
0	-	0·54	-
0·25	4	5·48	0·182
0·125	8	3·66	0·273
0·50	2	8·74	0·114
1·00	1	13·22	0·076

$[H^+]$ = 0·023 M $[NaNO_2]$ = 1×10^{-4} M $[RSH]$ = 5×10^{-3} M

As for mercaptosuccinic acid, large concentrations of thiocyanate ion are needed before the reaction shows any sign of becoming zero-order in added SCN^-. The double reciprocal plot shows, as for mercaptosuccinic acid, a linear relationship giving a k_2 value of $3{\cdot}51 \times 10^3$ $lmol^{-1}s^{-1}$ (slope = $0{\cdot}0277 \pm 0{\cdot}0008$, intercept = $0{\cdot}057 \pm 0{\cdot}004$). The solvent isotope effect at 1·0M SCN^- is $k_{D_2O}:k_{H_2O} = 1{\cdot}25$ so this seems to suggest the same conclusion as for mercaptosuccinic acid i.e. that the decrease in rate is due to the formation of NOSCN rather than a change in the rate limiting step.

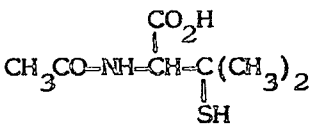
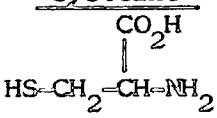
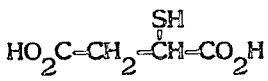
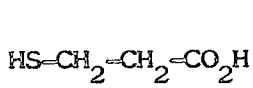
An attempt was made to isolate the thionitrite. A quantity of the thiol i.e. several grammes were added to a solution of sodium nitrite and acidified. The solution turned deep red and was extracted into ether. On removing the solvent a cherry red liquid remained which gave an i.r. spectrum with peaks at 2560 cm^{-1}, 1750 cm^{-1}, 1530 cm^{-1} (-N=O str), 1400 cm^{-1} and 630 cm^{-1} (-S-N str). The red liquid was refluxed in methanol and gave off brown fumes, although the colour persisted. On adding aniline the colour disappeared probably due to the formation of the disulphide.

$$2\ RSNO + 2\ PhNH_2 \longrightarrow 2\ PhN_2^+ + RSSR + H_2O$$

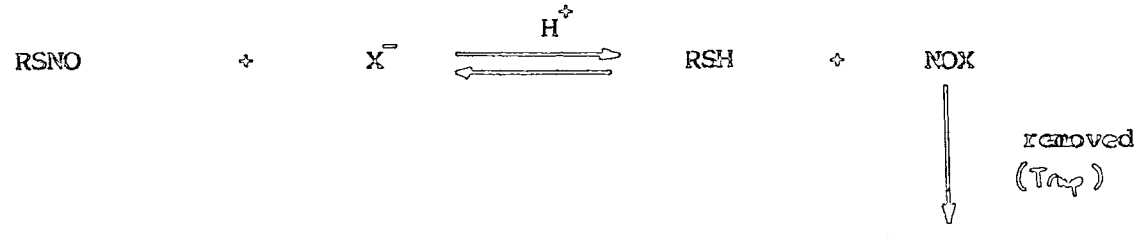
3.7. Discussion

The collected data for the nitrosation of the various thiols are given in table 25, together with some data for N-acetyl D,L-penicillamine at 31^oC, obtained by previous workers.[115] The magnitude of k_2 (for NOBr) is well below the diffusion controlled rate-limit, unlike the case for the nitrosation of aniline by NOBr. The nucleophilicity of sulphur is greater than that of nitrogen, although the nucleophilicity of the thiol will be dependent upon the nature of the R group (aniline and thiophenol both have a Pearson[91] 'n' value of 5·70). In all cases, nitrosation proceeds rapidly and irreversibly, this is in contrast to the nitrosation of alcohols (see chapter four) where the reaction shows a substantial degree of reversibility.

TABLE 35

THIOL	$k_3(HNO_2)$ $l^2 mol^{-2} s^{-1}$	$k_2(NOSCN)$ $l mol^{-1} s^{-1}$	$k_2(NOBr)$ $l mol^{-1} s^{-1}$
N-acetyl penicillamine 	840	3×10^3	$1{\cdot}4 \times 10^5$
Cysteine 	448	-	$1{\cdot}2 \times 10^4$
Mercaptosuccinic acid 	$1{\cdot}46 \times 10^3$	$4{\cdot}54 \times 10^3$	$2{\cdot}63 \times 10^4$
3-mercaptopropanoic acid 	$4{\cdot}66 \times 10^3$	$3{\cdot}51 \times 10^3$	$4{\cdot}53 \times 10^5$

For the penicillamine derivative, the reverse reaction (denitrosation) has been studied[101] in high acid concentration (1-4 M HCl).



X^- = Cl^-, Br^-, SCN^- etc.

Owing to the low basicity of the thionitrite, the reaction proceeds very slowly and the equilibrium is very much in favour of the thionitrite. In

the presence of a nitrite trap such as sodium azide or sulphanic acid the reaction can be made to proceed irreversibly provided the trap is in sufficient excess. The reaction is found to be first order in RSNO and acid-catalysed, being proportional to h_0. The reaction was catalysed by halide ions, thiocyanate and thiourea as for the denitrosation of N-nitrosoamines. In the absence of nitrite traps, RSNO was shown to act as a nitrosating agent toward amines (e.g. N-methyl 4-nitroaniline), where the mechanism involves prior denitrosation of RSNO yielding a free nitrosationg species (e.g. HNO_2 or NOCl), which effects the amine nitrosation. For comparison, the rate constants of nitrosation by NOBr (at 25^0C) of some amines, thiols and alcohols are given in table 36.

TABLE 36

Substrate	k_2 ($l mol^{-1} s^{-1}$)
MeOH	2×10^4
Cysteine	1.2×10^4
3-mercaptopropanoic acid	4.53×10^5
$C_6H_5NH_2$	1.7×10^9

For reasons explained above (i.e. difference in nucleophilicity) there is a difference of several orders of magnitude between the k_2 values for the nitrosation of aniline and other substrates. However, the overall reactivity does not show such a marked difference because aniline will be extensively protonated in acid solution, whereas the thiols and alcohols will be present almost entirely in the free form. Consequently, the observed rate constants for the nitrosation of the various species are not markedly different.

It has been observed [115] that for the N-acetyl derivative of pen-icillamine the rate of nucleophile catalysed reaction becomes zero-order with respect to iodide and thiocyanate at high nucleophile concentration. This is not observed for the Br^- or Cl^- catalysed reaction. In the case of SCN^-

however, the concentrations involved are too low to cause a decrease in rate due to the formation of NOSCN (as is the case for mercaptosuccinic acid and 3-mercaptopropanoic acid). The only plausible explination is that the rate of denitrosation of $R\overset{+}{S}HNO$ exceeds that of proton loss to the solvent at high $[SCN^-]$ and $[I^-]$. Since these two nucleophiles are more reactive than Cl^- or Br^- it follows that this effect should be seen in the case of the former nucleophiles rather than the less reactive Cl^- and Br^-. So for nitrosation of N-acetyl penicillamine by NOSCN or NOI it seems quite possible that there is a change in the rate limiting step on going from low to high anion concentration. According to the scheme laid out earlier we go from the condition $k_p \gg k_{-2}[SCN^-]$ to $k_p \ll k_{-2}[SCN^-]$ and in the latter case the rate is zero order in SCN^- (or I^-) To test this hypotheeis, a kinetic isotope study has been carried out for the iodide catalysed nitrosation of N-acetyl penicillamine at 25^oC. The results are given in table 37 below.

TABLE 37

[KI] (M)	$k_o(s^{-1})$
0	1·95
0·005	(2·32)
0·01	2·46 (1·98)
0·02	3·44
0·03	4·21
0·05	4·81 (1·87)

$[RSH] = 2{\cdot}4 \times 10^{-3}$ M $[NaNO_2] = 1 \times 10^{-4}$ M

$[H^+ \text{ or } D^+] = 0{\cdot}01$ M

Reaction followed at 338 nm. Figures in brackets refer to runs in D_2O.

Clearly there is a considerable difference in the rate constant between the reaction in water and D_2O at high iodide concentration. This indicates that

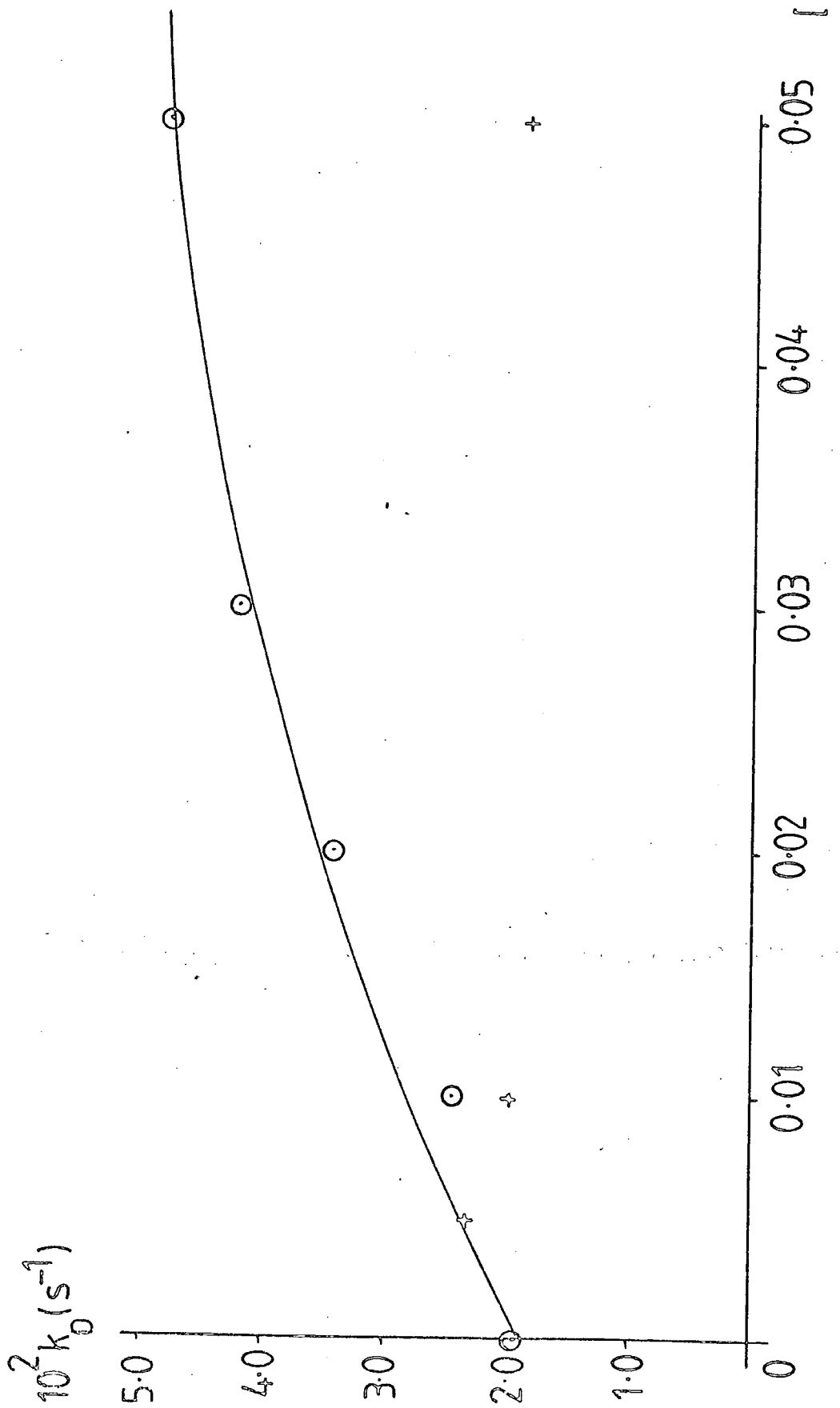
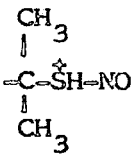
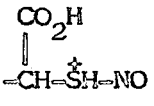


FIG 13: NITROSATION OF N-ACETYL PENICILLAMINE BY NOI

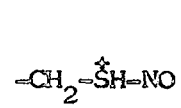
proton transfer to the solvent from $R\overset{+}{S}HNO$ must be rate limiting and that the condition $k_p \ll k_{-2}[I^-]$ applies. As explained earlier, a similar effect is not observed for mercaptosuccinic acid and 3-mercaptopropanoic acid so there must be a structural characteristic involved in N-acetyl penicillamine which causes this to happen. An explanation might be based upon consideration of steric and electronic effects in the various protonated thionitrites.



N-acetyl penicillamine



Mercaptosuccinic acid



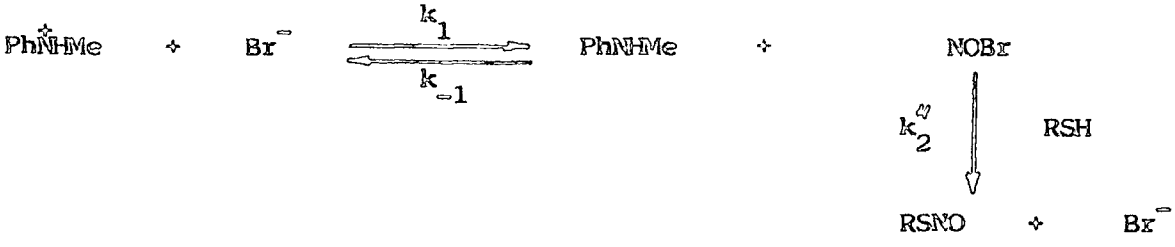
3-mercaptopropanoic acid

Considering the electronic effects, the pres ence of carboxylic groups adjacent to the thiol group in the succinic and propanoic residues will enhance proton transfer to the solvent, wheraes the presence of methyl groups adjacent to the -SNO function in penicillamine will make proton transfer relatively slow. Steric effects would also be important here as approach of a water molecule would be hindered by the methyl groups in the case of penicillamine. Steric hindrance is less of a problem in the case of the other two thiocarboxylic acids. So, for the penicillamine derivative, the condition $k_p \ll k_{-2}[SCN^-]$ is reached more readily upon increasing the thiocyanate concentration. The condition does not seem to be reached at all in the case of the other two thiols.

3.8. The use of thiocarboxylic acids as nitrite traps

It is clear from the above discussion that these thids react irreversibly and rapidly with nitrosyl halides to form thio-nitrites which appear to be relatively stable in solution. It is known[13] that thiols are not very reactive towards nitrosoamines in a direct reaction e.g. cysteine has a nucleophilicity approximately the same as that of chloride ion toward N-methyl N-nitrosoaniline. Provided the thiol does not significantly catalyse the denitrosation, acting as a nucleophile it is of interest to study the efficiency of these compounds as nitrite traps in the denitrosation of

nitrosoamines. In order to determine the efficiency of the particular thiol i.e. the reactivity toward the formed nitrosyl species in the denitrosation re-action, the method described in chapter one is employed. From the overall (observed)rate constant for denitrosation, the ratio $k_2'':k_{-1}$ can be obtained by adding increasing amounts of PhNHMe.



$k_2'':k_{-1}$ is a measure of the effectiveness of RSH in 'savenging' NOBr and hence making denitrosation irreversible.

(a) N-acetyl D,L-penicillamine

The reaction was followed by observing the disa-ppearance of the nitrosoamine at 270 nm on a conventional u.v. spectrophotometer All runs were made at 31°C. The results are in table 37. These results show the following;

i) The denitrosation reaction is zero order in added penicillamine at concentration greater than 8×10^{-4} M. Good, first order plots for each individual rate constant were obtained under such conditions.

ii) In the presence of a sufficient excess of sodium azide to ensure that all the NOBr was being effectively removed from the reaction, addition of penicill-amine had little effect on the rate and so the added penicillamine does not appear to show any nucleophilic activity toward the nitrosoamine.

iii) The effect of adding N-methylaniline (NMA) was minimal, and this suggests that the NOBr is being trapped out very effectively by the thiol. The $k_2'':k_{-1}$ ratio was estimated as 695 although with such a small slope this is subject to considerable errors. Even so, it seems that N-acetyl penicillamine is an exceptionally good trap. Under similar conditions, sodium azide (considered to be an effective trap) gives a ratio of 32.

TABLE 38

10^4 [RSH] (M)	[NMA] (M)	[NaN_3] (M)	$10^4 k_o (s^{-1})$	k_o^{-1}
0·768	-	-	*	-
3·84	-	-	20·8	-
1·152	-	-	14·9	-
2·15	-	-	20·9	-
11·5	-	-	23·0	-
7·68	-	-	23·9	-
3·6	$7·5 \times 10^{-2}$	-	15·9	629
"	5×10^{-2}	-	19·4	515
"	$2·5 \times 10^{-2}$	-	21·3	469
"		-	21·8	459
	-	4×10^{-3}	18·7	-
-	-	$1·6 \times 10^{-2}$	18·5	-
-	-	2×10^{-2}	19·1	-
4·81	-	"	19·7	-
9·62	-	"	23·7	-

* this run did not give a first order relationship btween absorbance and time

[H_2SO_4] = 2·43 M [NMNA] = 1×10^{-4} M [NaBr] = 0·1M

RSH = N-acetyl penicillanine

(b) Mercaptosuccinic acid

The effectiveness of this compound as a trap was studied in the same way as penicillamine. The results are given in table 39 .

TABLE 39

10^3 [RSH]	10^2 [NMA]	10^4 $k_0(s^{-1})$	k_0^{-1}
0·024	-	8	-
0·238	-	11·9	-
0·475	-	12·9	-
1·190	-	12·9	7·75
"	0·794	10·1	9·90
"	0·529	10·9	9·17
"	1·32	8·23	12·10

$[H_2SO_4]$ = 1·95 M $[NMNA] = 1 \times 10^{-4}$ M [NaBr] = 0·1M

RSH = Mercaptosuccinic acid

For the k_0^{-1} vs [NMA] plot;

Slope = $3·27 \pm 0·11 \times 10^4$

Intercept = $7·57 \pm 0·09 \times 10^2$

$k_2'' : k_{-1} = 19·4$

With sodium azide present in sufficient excess to remove all the NOBr formed, the addition of mercaptosuccinic acid provides no further rate increase and so it appears that the thiol acts only as a trap and does not react directly with the nitrosoamine as a nucleophile. The value of the above ratio shows that under these conditions mercaptosuccinic acid is less effective as a nitrite trap than penicillamine. As explained in chapter one, the ratio $k_2'' : k_{-1}$ above is liable to vary with acid concentration and halide concentration, so all these runs have been done under approximately the same conditions, as

this enables a meaningful comparison of the effectiveness of the traps to be made. In addition, for mercaptosuccinic acid, the effect of varying acid concentration and halide concentration on the $k_2'':k_{-1}$ ratio was studied. The results are given in table 40. For the sake of clarity, the individual runs have not been included - only the ratio is given.

TABLE 40

$[H_2SO_4]$ (M)	[NaBr] (M)	$k_2'':k_{-1}$
1·95	0·1	19·4
1·05	0·1	5·3
3·27	0·1	133
2·11	0·1	25·5
2·11	0·2	11·6
2·11	0·4	2·7

$[M.S.A.] = 1·2 \times 10^{-3}$ M $[NMNA] = 1 \times 10^{-4}$ M

The results show the same trends as other nitrite traps, i.e. that as the acidity increases, so the $k_2'':k_{-1}$ ratio increases. However, for the thiol the effect is much more pronounced. The reason for the increase in the value of the ratio is that the added N-methylaniline becomes increasingly protonated and so $k_{-1}[NMA]$ decreases. Against this is the increasing protonation of the trap, however for the thiol there will be very little protonation and this may explain the effect of added acid in creating a very large increase in the $k_2'':k_{-1}$ ratio. It should be stressed that the increase in this ratio is not due to the increasing effectiveness of the trap itself, but simply the removal of PhNHMe from the reaction by protonation. The effect of added bromide ion, in certain cases, decreases the value of $k_2'':k_{-1}$ (see chapter one). If we refer to the scheme on page 35, it can be seen that k_2'' is a composite rate constant and is in

fact given by;

$$k_2'' = \frac{k_p k_2}{k_{-2}[Br^-] + k_p}$$

Now, for mercaptosuccinic acid it has been shown that under the conditions used, $k_p \gg k_{-2}[Br^-]$, giving a k_2'' independent of $[Br^-]$. Therefore the decrease in the $k_2'':k_{-1}$ ratio must be due to the nitrosation of N-methylaniline i.e. the k_{-1} step.

(c) 3-mercaptopropanoic acid

The results are given in table 41. The $k_2'':k_{-1}$ value of 592 shows that this substrate appears to act as a very good nitrite trap.

TABLE 41

$10^4[RSH]$	$10^2[NMA]$ (M)	$10^4 k_0 (s^{-1})$	$10^{-2} k_0^{-1}$
0.718*	-	5.89	-
1.077*	-	7.45	-
1.795*	-	7.7	-
3.59*	-	7.1	-
1.79	8.93	8.08	12.38
"	3.57	12.0	8.33
"	1.79	13.15	7.60
"	0.71	12.70	7.87

Slope = $6.36 \pm 0.44 \times 10^3$

Intercept = $6.74 \pm 0.21 \times 10^2$

$k_2'':k_{-1} = 592$

For runs (*), $[HCl] = 2.49$ M others, $[H_2SO_4] = 2.11$M

$[NMNA] = 1 \times 10^{-4}$M $[NaBr] = 0.1$ M

The thiol did not show any nucleophilic activity toward the nitrosoamine.

(d) L-Cysteine

The results are given below in table 42.

TABLE 42.

10^4 [RSH] (M)	[NMA] (M)	$10^4 k_0 (s^{-1})$	k_0^{-1}
0·384	-	not first order	-
1·15	-	17·8	-
1·54	-	17·8	-
1·92	-	15·9	629
3·84	-	14·7	-
19·2	-	16·1	-
0 (76.9 N_3^-)	-	17·2	-
1·92	$6·8 \times 10^{-3}$	7·05	$1·42 \times 10^3$
"	$1·7 \times 10^{-2}$	2·82	$3·55 \times 10^3$
"	$3·4 \times 10^{-2}$	1·41	$7·09 \times 10^3$

Slope = $1·95 \pm 0·04 \times 10^5$

Intercept = 359 ± 77

$k_2'' : k_{-1} = 9·6$

The collected data is given below;

THIOL	$k_3(HNO_2)$ $l^2 mol^{-2} s^{-1}$	$k_2(NOSCN)$ $l mol^{-1} s^{-1}$	$k_2(NOBr)$ $l mol^{-1} s^{-1}$	$k''_2 : k_{-1}$
N-acetyl penicillamine (31^0C)	840	3×10^3	1.4×10^5	695
Cysteine	448	-	1.2×10^4	10
Mercaptosuccinic acid	1.46×10^3	4.54×10^3	2.63×10^4	19
3-mercaptopropanoic acid	4.6×10^3	3.51×10^3	4.53×10^5	592
Sodium azide*			220 (Ref 137)	32

* Included for comparison purposes, the $k''_2 : k_{-1}$ value was obtained from reference 27, the conditions being approximately the same as those for the thiol runs. Examination of the above results show that under certain conditions, the thiols make extremely effective nitrite traps in comparisin to other reagents hithero used as nitrite traps. At high acidities, mercaptosuccinic acid is particularly effective because, unlike many traps (e.g. sulphamic acid, hydrazine) it does not undergo extensive protonation.

The above rate constants do not all fit in to a consistent pattern. For example, 3-mercaptopropanoic acid is more reactive toward NOBr than N-acetyl penicillamine yet the latter seems to be the more effective nitrite trap. This may prove to be a trivial comparisón however, because of the considerable margin for error when measuring a $k''_2 : k_{-1}$ ratio of such a size. Under these conditions only a very small change in rate constant is observed as large amounts of N-methylamine are added to the denitrosation reaction.

In another example, it is difficult to see why cysteine is less reactive toward nitrous acid than mercaptosuccinic acid when one considers that the latter has a carboxyl group adjacent to the thiol group. Also, it can be seen that mercaptosuccinic acid and 3-mecaptopropanoic acid both show the same reactivity toward NOSCN, but show different reactivities toward NOBr. A steric factor may operate here as mercaptosuccinic acid is a considerably more bulky compound than 3-mercaptopropanoic acid.

3.9 Summary

1.) The rate of nitrosation of the thiols studied here are several orders of magnitude less than that of aniline and of the same order of magnitude as several alcohols and carbohydrates. This could be explained by comparing the nucleophilic reactivity of the various species toward an electrophilic reagent e.g. NOBr.

2.) The overall reactivity of thiols toward nitrosating agents is similar to that of aniline under approximately the same conditions, though this is due to the large difference in basicity between the two species i.e. aniline is virtually completely protonated in acid solution compared to the thiol which is mostly present in the free form.

3.) For thiols containing electron withdrawing groups adjacent to the thiol group, zero-order dependence upon halide ion was not obtained at high halide concentration (unlike aniline and N-acetyl penicillamine) this was attributed to a steric and electronic effect on the acidity of the protonated thionitrite.

4.) The thionitrites were all observed as stable species in solution, giving broad absorptions in the region 300-350 nm. They all proved difficult to isolate and to characterise owing to their instability.

5.) The thiocarboxylic acids were found to make effective nitrite traps in the denitrosation of N-nitroso N-methylaniline, especially at high acidities where they were not prone to undergo protonation (rendering them unreactive toward nitrosating species such as NOBr).

CHAPTER FOUR

The Nitrosation of Alcohols

4.1. Introduction

Alcohols react with nitrous acid reversibly to give an alkyl nitrite.[118]

$$ROH + HNO_2 \rightleftharpoons RONO + H_2O$$

The rate of the forward reaction is so rapid that until recently it has been possible only to measure it by indirect methods,[119] although now it can be obtained directly by stopped-flow spectrophotometry.

Alkyl nitrites are stable compounds if kept dry, though they have been used in aqueous solution as nitrosating agents. Since alkyl nitrites undergo rapid hydrolysis in water this indicates that the alkyl nitrite may not be acting as a nitrosating agent itself but may hydrolyse to nitrous acid which then forms a nitrosating agent. The acid catalysed hydrolysis of alkyl nitrites is particularly rapid[120] and this would support such a view. In addition, it has been found that the nitrosation of sulphanilamide[121] by cyclo-hexyl nitrite occurs via prior hydrolysis to give nitrous acid. Some work[122] has also been done in alkaline solution which suggests that alkyl nitrites containing β-electron withdrawing groups rapidly give nitrosation of secondary amines. A study of the nitrosation of anilines by propyl nitrite[123] in acidified propan-1-ol shows that there is virtually no reaction in the absence of halide ions or thiourea and that the reaction is readily catalysed by these species. This suggests that propyl nitrite does not act directly as a nitrosating agent but must undergo prior denitrosation to form the nitrosating agent. The rates of acid-catalysed hydrolysis of alkyl nitrites in water have been measured and a mechanism has been proposed[124] in which there is rapid protonation followed by rate limiting denitrosation.

$$RONO + H_3O^+ \underset{}{\overset{\text{rapid}}{\rightleftharpoons}} RO(H)^+-NO + CH_2$$

$$RO(H)^+-NO \xrightarrow{\text{rate limiting}} RCH + H_2NO_2^+$$

The reaction is catalysed by halide ions which act as nucleophiles. Studies of the nitrosation of alcohols by nitrosyl chloride[125,126] have yielded values for the equilibrium constant of the reaction;

$$ROH + NOCl \underset{}{\overset{K}{\rightleftharpoons}} RONO + HCl$$

the effect of electron withdrawing groups in the alcohol is to shift the equilibrium to the right by enhancing the basicity of the alcohol. Steric factors do not appear to play a major part in determining the value of K, this is probably due to the small size of NOCl.

More recent sudies[115] have provided the rate constants for the forward reaction (k_2) at 25°C, according to the scheme below;

$$MeOH + NOX \underset{}{\overset{k_2}{\rightleftharpoons}} MeO\overset{H}{\overset{|}{N}}O + X^- \quad ; \quad MeO\overset{H}{\overset{|}{N}}O \rightleftharpoons MeONO + H^+$$

For $X = Cl^-$, $k_2 = 2{\cdot}1 \times 10^5 \text{ lmol}^{-1}\text{s}^{-1}$. For $X = Br^-$, $k_2 = 2{\cdot}0 \times 10^4 \text{ lmol}^{-1}\text{s}^{-1}$. For comparison, if we consider the nitrosation of aniline, we get a k_2 value of $2{\cdot}2 \times 10^9 \text{ lmol}^{-1}\text{s}^{-1}$ for $X = Cl^-$, this illustrates that aniline is considerably more reactive than methanol. However the overall (observed) rate constants for the nitrosation of methanol and aniline are of the same order of magnitude because aniline is extensively protonated in acid solution.

A kinetic study[127] of the reaction between methanol and nitrous acid has yielded k_1 and k_{-1} values for the reaction;

$$MeOH + HNO_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} MeONO + H_2O$$

Since the observed rate constant is defined as;

$$\text{Rate} = k_0 [HNO_2]$$

Assuming $[HNO_2] \ll [MeOH]$, i.e. under first order conditions;

$$k_0 = k_f + k_r$$

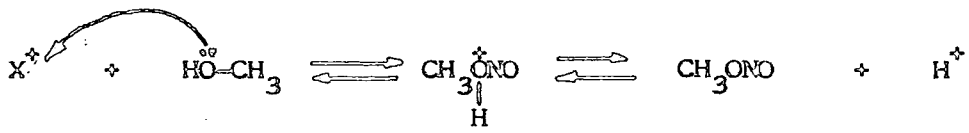
$$k_f = k_1[\text{MeOH}] \quad \overset{k_{-1}}{\longleftarrow}$$

$$k_r = k_{-1}$$

So,

$$k_0 = k_1[\text{MeOH}] + k_{-1}$$

Both the forward and reverse reactions were subject to acid catalysis which is consistent with a mechanism involving rate limiting nitrosation by the nitrous acidium ion $H_2N\overset{+}{O}_2$ or the nitrosonium ion $N\overset{+}{O}$.



$X^+ = H_2N\overset{+}{O}_2$ or $N\overset{+}{O}$.

The rate of the forward reaction is observed to be given by;

$$\text{Rate} = k_3[\text{MeOH}][H^+][HNO_2]$$

k_3 has a value of 700 $l^2 mol^{-2} s^{-1}$ at 25^oC. The value of the rate constant for the bimolecular reaction between MeOH and X^+ is unobtainable as a value for the pK_a of nitrous acid is unknown. Other alcoholic substrates have been subjected to the same kinetic study[115] and the same rate expressions apply in each case. The values of k_3 differ for each alcohol (though they are of the same order of magnitude), this suggests that the bimolecular nitrosation step must have a rate constant that is well below the diffusion-controlled limit as there is some discrimination between the various substrates.

The equilibrium constants for the overall non-halide ion catalysed reaction i.e. $k_1:k_{-1}$ were determined for a series of alcohols and the following sequence obtained in order of decreasing equilibrium constant; $CH_3OH > EtOH > i\text{-}PrOH > t\text{-}BuOH$. This series is due almost entirely to a decrease in the magnitude of the forward rate constant for the nitrosation reaction, and can only be attributed to steric effects as the electronic effects would give the opposite trend.

4.2. The intermediate in aqueous nitrosation

In aqueous acid solution, nitrous acid forms a nitrosating species which could be either $H_2NO_2^+$, the nitrous acidium ion or NO^+, the nitrosonium ion. There is no direct evidence for the existence of $H_2NO_2^+$, but there is spectroscopic evidence[67] for the existence of nitrous acid as NO^+ in quantitative amounts in 60% H_2SO_4. At lower acidities the identity of the nitrosating species is not clear, although it is generally assumed that at high acidities (e.g. > 3M acid) the nitrosating agent is the NO^+ ion. Some evidence for the nitrous acidium ion as the reactive species at low acid concentrations is given in a study[128] of the reaction between hydrazoic acid and nitrous acid.

$$HNO_2 + H^+ + N_3^- \xrightarrow{\text{slow}} H_2O + N_3NO$$

$$N_3NO \xrightarrow{\text{rapid}} N_2 + N_2O$$

^{18}O enriched water was used, and the formed nitrous oxide was not found to have any ^{18}O incorporated. If the reaction had taken place via NO^+, the rate of exchange between water and NO^+ would have been equal to the rate of nitrosation and the formed nitrous oxide would be expected to have the same isotopic composition as the solvent. As this was not the case, this suggests that $H_2NO_2^+$ was the reacting species. It is worth comparing this to an analogous situation i.e. the nitration of aromatic substrates (e.g. mesitylene). It is generally accepted that the nitronium ion NO_2^+ is the reactive species rather than $H_2NO_3^+$

$$H_2NO_3^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} H_2O + NO_2^+ \xrightarrow[\text{ArH}]{k_2} ArNO_2 + H^+$$

Rate measurements[129] show that k_2 is large (diffusion-controlled) and that $k_{-1} \gg k_1$, so that the reaction can be made zero-order in ArH with formation of NO_2^+ rate limiting. The half-life of the NO_2^+ ion is calculated to be $\sim 10^{-6}$s This corresponds to a diffusion controlled reaction with the aromatic species. Ridd[57] estimates the half life of NO^+ to be $\sim 10^{-10}$s. This may be too short

for the species to be considered a nitrosating agent under these conditions. Benton & Moore[130] have studied the nitrosation of hydrogen peroxide to give pernitrous acid as an intermediate and nitric acid as the product.

$$HNO_2 + H_2O_2 \longrightarrow H_2O + HOONO \longrightarrow HNO_3$$

They have suggested a mechanistic scheme as follows;

$$H^+ + HNO_2 \underset{}{\overset{K}{\rightleftharpoons}} H_2NO_2^+$$

$$H_2NO_2^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} NO^+ + H_2O$$

$$NO^+ + H_2O_2 \xrightarrow{k_2} HNO_3 + H^+$$

or,

$$H_2NO_2^+ + H_2O_2 \xrightarrow{k_2'} HNO_3 + H^+$$

Since, rate $= k_0 [HNO_2]$, then two expressions for k_0 could be obtained depending on which species was the nitrosating agent. If the nitrous acidium ion is the reactive species, then;

$$k_0 = k_2' K [H^+][H_2O_2]$$

If the nitrosonium ion is the reactive species, then;

$$k_0 = \frac{k_2 k_1 K [H^+][H_2O_2]}{k_{-1}[H_2O] + k_2[H_2O_2]}$$

The latter expression would mean that at high $[H_2O_2]$ the rate could become zero order in substrate if $k_2[H_2O_2] \gg k_{-1}[H_2O]$. This could apply to any substrate and the rate constant for which zero-order conditions are achieved would, at constant acidity, be the same for each substrate. It is found that the reaction does tend to become zero-order at high concentrations of H_2O_2 (4 $moll^{-1}$) and this might suggest that NO^+ is the nitrosating species. However,

there are two possible objections to such a conclusion. The first is that the value of the limiting rate constant was obtained as c.a. 600 $lmol^{-1}s^{-1}$ for the nitrosation of hydrogen peroxide at $0^{o}C$, this is in agreement with the earlier work of Anbar & Taube[131] but is about three times the measured rate of exchange of ^{18}O between $N\overset{*}{O}$ and H_2O at this temperature. Secondly, the concentration of hydrogen peroxide used is so great that H_2O_2 could be considered a co-solvent and a medium effect may play a part in the rate of nitrosation "levelling" off at high concentrations of peroxide. In the present work it has been the aim to clarify the situation by carrying out the nitrosation of a series of alcohols at high alcohol concentration and at different acidities to see if the rate constant at which zero-order conditions is obtained (the limiting rate constant) is independent of the nature of the alcohol, which according to the Benton and Moore work should be the case.

4.3. Nitrosation of alcohols at high alcohol concentration

All the reactions were studied by observing the disappearance of nitrous acid by stopped-flow spectrophotometry at $25^{o}C$ unless otherwise stated. The runs gave good, first order plots and over a long period of time showed reversibility (due to hydrolysis of the alkyl nitrite).

The results are given in tables 43-48. These results show that for each alcohol, the ratio($\lim k_o : [H^+]_{xs}$) is the same at different acidities, but condradictory to the observations of Benton and Moore this ratio differs from one alcohol to another (see fig 14). This suggests that the levelling off in rate at high alcohol concentration may not be due to nitrosation by $N\overset{*}{O}$ but may be due to the nature of the alcohol itself i.e. a medium effect. This seems likely considering the high concentrations of alcohol used. To study this further, an inert solvent,(tetrahydrofuran) was added. This is unreactive toward nitrosation and does not protonate to any significant extent under these conditions.[132] Upon adding this solvent to a reaction mixture, the rate of nitrosation of ethanol decreased by c.a. 40% when THF was added in a concentration similar to that needed to produce zero-order kinetics for ethanol (see table 49)

This lends further weight to the argument that the zero-order conditions observed by Benton and Moore for the nitrosation of hydrogen peroxide may be due to a solvent effect and not due to $N\dot{O}$ as the nitrosating agent. This does not rule out the nitrosonium ion as the nitrosating agent nor does it confirm that the nitrous acidium ion is the effective nitrosating agent under these conditions, but it does show that the existance of the ~~nitrosonium~~ nitrous acidium ion as a nitrosating species cannot be discounted.

TABLE 43

$[NaNO_2]$ = 0·04 M Observed at 386nm

$[MeOH]$ (M)	$[H^+]$ (M)	$k_0(s^{-1})$
0·50	$6·2 \times 10^{-2}$	46·94
1·0	"	62·83
2·0	"	72·53
3·0	"	78·60
4·0	"	79·18
0·5	0·164	149·4
1·0	"	177
2·0	"	200
3·0	"	206·2
0·5	$4·8 \times 10^{-2}$	43·83
1·0	"	52·45
2·0	"	62·24
3·0	"	61·54
4·0	"	61·99
0·5	"	3·61*
1·0	"	4·73*
2·0	"	5·90*
3·0	"	6·91*
4·0	"	7·48*
6·0	"	8·15*
8·0	"	8·76*

* = studied at 0^oC.

TABLE 44

Methanol

$[H^+]_{xs}$ (M)	limiting $k_0(s^{-1})$	lim $k_0 : [H^+]_{xs}$
6.2×10^{-2}	79	1274
0.164	208	1268
4.8×10^{-2}	62	1292
" (0^oC)	9.0	187

The energy of activation is calculated to be 67 $kJmol^{-1}$.

TABLE 45

Observed at 290 nm. All measurements at 25^oC. $[NaNO_2] = 1 \times 10^{-2}$ M

[EtOH] (M)	$[H^+]$ (M)	$k_0(s^{-1})$
0.1	4.7×10^{-2}	29.95
0.5	"	36.01
1.0	"	41.16
1.5	"	42.78
2.0	"	45.95
2.5	"	46.47
0.2	1.4×10^{-2}	11.82
0.5	"	13.65
1.0	"	14.15
1.5	"	14.21
0.2	7.5×10^{-2}	44.86
0.5	"	51.40
1.0	"	60.81
2.0	"	69.73
2.5	"	67.06

TABLE 46

Ethanol

$[H^+]_{xs}$	Limiting $k_o(s^{-1})$	lim $k_o : [H^+]_{xs}$
4.7×10^{-2}	46.5	989.4
1.4×10^{-2}	14.2	1014
7.5×10^{-2}	68.4	977

TABLE 47

Studied at 285 nm and 25°C. $[NaNO_2] = 1 \times 10^{-2}$M

[i-propanol] (M)	$[H^+]$ (M)	k_o (s^{-1})
0.20	4.7×10^{-2}	27.01
0.50	"	30.4
1.0	"	30.31
2.0	"	32.19
0.2	0.104	56.38
0.5	"	62.90
1.0	"	70.13
1.5	"	70.04
0.20	7.0×10^{-2}	41.65
0.50	"	43.20
1.0	"	42.02
1.5	"	45.80
2.0	"	46.05

TABLE 48

i-propanol

$[H^+]_{xs}$	lim $k_0(s^{-1})$	lim $k_0 : [H^+]_{xs}$
4.7×10^{-2}	32	681
0.104	70	673
7×10^{-2}	46	657

TABLE 49

Nitrosation of ethanol (0.5 M) at 0.07 M H^+ and 25°C

[THF] (M)	$k_0(s^{-1})$
0	54.3
0.5	48.8
1.0	38.2
1.5	34.7
1.75	31.4

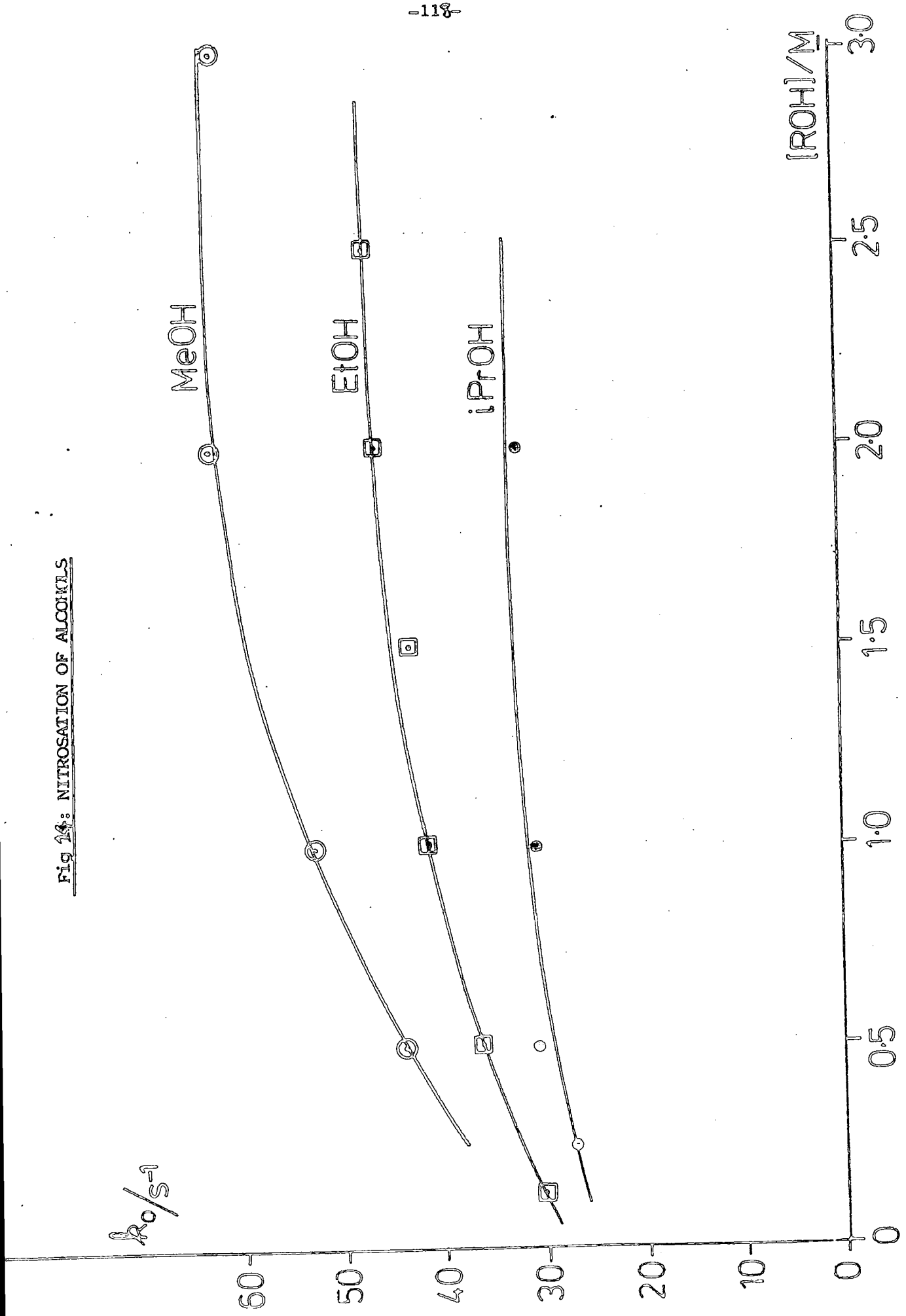
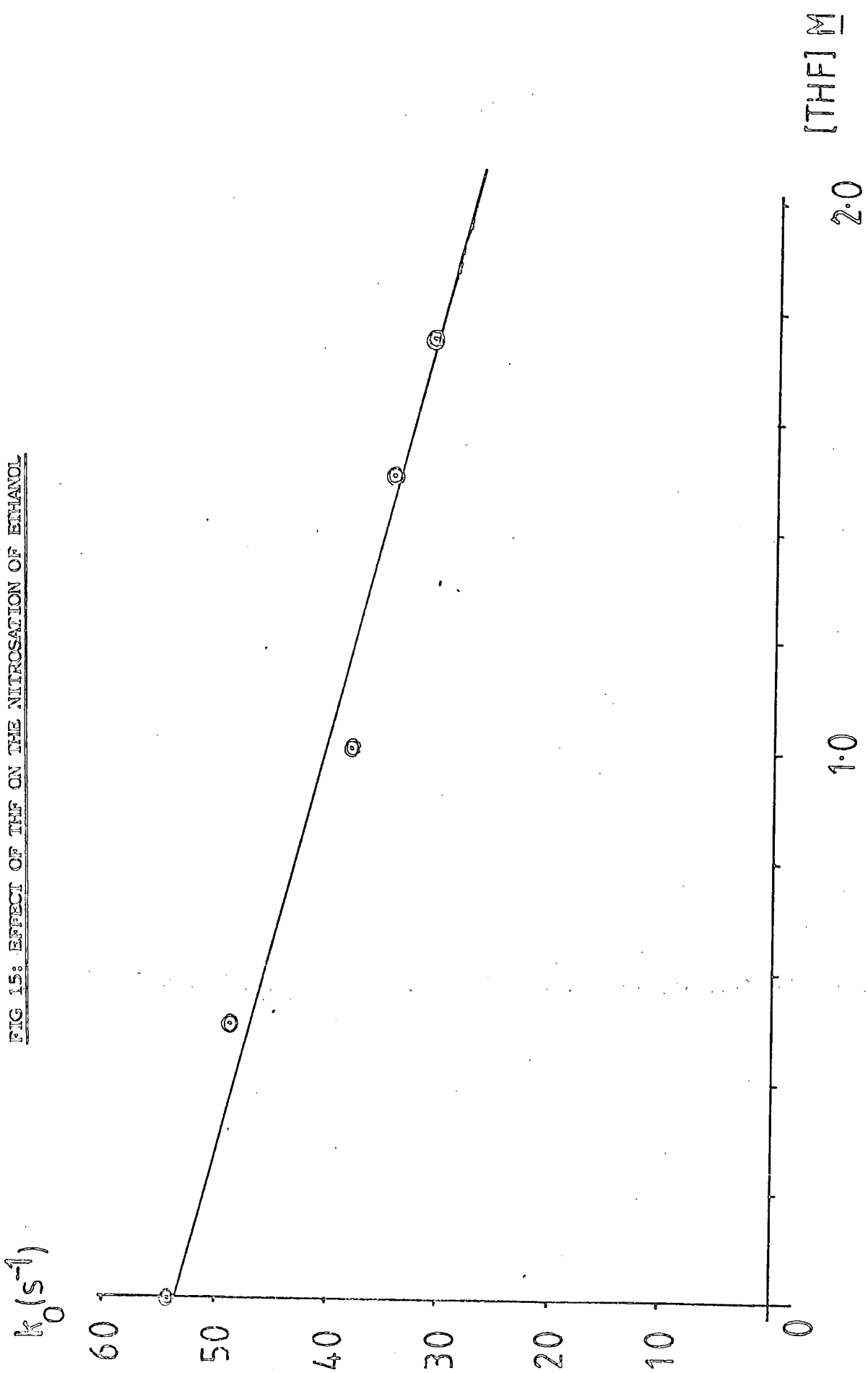


Fig 14: NITROSATION OF ALCOHOLS

FIG 15: EFFECT OF THF ON THE NITROSATION OF ETHANOL



CHAPTER FIVE

Experimental Details

5.1. Reagents used

In this work, all the compounds have been used directly as the analytical grade reagent except in the following cases.

(i) DMSO solvent. This was used as a medium for denitrosation in chapter one. The solvent is very hygroscopic and was distilled under reduced pressure over calcium hydride and stored in a desiccator before use.[133]

(ii) N-Methyl N-nitrosoaniline. This was prepared by the method of Vogel[134] i.e. by direct nitrosation of N-methyl aniline. Owing to the potentially carcinogenic properties of this compound, great care has been taken in handling it. Disposable rubber gloves were used, and any solutions which may have contained the compound were disposed of in a denitrosation mixture containing sulphuric acid, halide and a suitable nitrite trap e.g. hydrazine.

(iii) para-Substituted anilines. These were used for the studies in chapter two. They were obtained as the commercially produced compound and purified before use eitheir by distillation under reduced pressure or by recrystallisation from ethanol or ethanol-water mixture as appropriate.

(iv) The solutions of hydrogen chloride in DMSO were prepared by bubbling HCl gas from a cylinder through sulphuric acid (for drying purposes) and then through a large, round-bottomed flask to prevent suck-back, and finally into the DMSO. The acidity being determined by titration in the normal way.

5.2. Determination of observed rate constants

In this work, the conditions under which the reactions have been studied ensure first order kinetics, i.e. in a reaction $A \longrightarrow B$, one reactant (A) is in large excess over the other (B), and the observed rate constant is given by the rate of disappearance of the less concentrated reactant (B). The rate constants have been determined by measuring the change of absorbance in the u.v.-visible region of B (or in some cases, one of the products) with time, giving an exponential relationship as expected for a first order kinetic process. Absorbance measurements bear a direct relationship to concentration of substrate according to the Beer - Lambert relationship;

$$a = \varepsilon cl$$

a = absorbance ε = extinction coefficient l = path length

For a reaction, $A \longrightarrow B$

$$\bar{B}_t = \bar{A}_0 - \bar{A}_t$$

Where $\bar{B}_t$ = concentration of B at time t, etc.

Now, $\bar{A}_0 = \bar{B}_\infty$ and $\bar{A} = \bar{B}_0$

$$a_0 = \varepsilon_A \bar{A}_0$$

a_0 = absorbance at time t = 0.

$$a_t = \varepsilon_A \bar{A}_t + \varepsilon_B \bar{B}_t$$

$$= \varepsilon_A \bar{A}_t + \varepsilon_B (\bar{A}_0 - \bar{A}_t)$$

$$a_\infty = \varepsilon_A \bar{A}_t - \varepsilon_B \bar{A}_t$$

$$\bar{A}_t = \frac{(a_t - a_\infty)}{(\varepsilon_A - \varepsilon_B)}$$

Now, for a first order reaction,

$$k_0 = \frac{1}{t} \ln \frac{\bar{A}_0}{\bar{A}_t}$$

Since, $a_0 = \varepsilon_A \bar{A}_0$ and $a_\infty = \varepsilon_B \bar{A}_0$

$$\bar{A}_0 = (a_0 - a_\infty)/(\varepsilon_A - \varepsilon_B)$$

So,

$$k_0 = \frac{1}{t} \ln \frac{(a_0 - a_\infty)}{(a_t - a_\infty)}$$

So if we determine a_0, a_∞ and a_t we obtain an 'instantaneous' value for k_0 at time $t = t$. Since, from the above expression;

$$\ln (a_t - a_\infty) = -k_0 t + \ln(a_0 - a_\infty)$$

a plot of $-\ln(a_t - a_\infty)$ vs t will give the observed rate constant from the slope. Some examples from actual kinetic runs are given below.

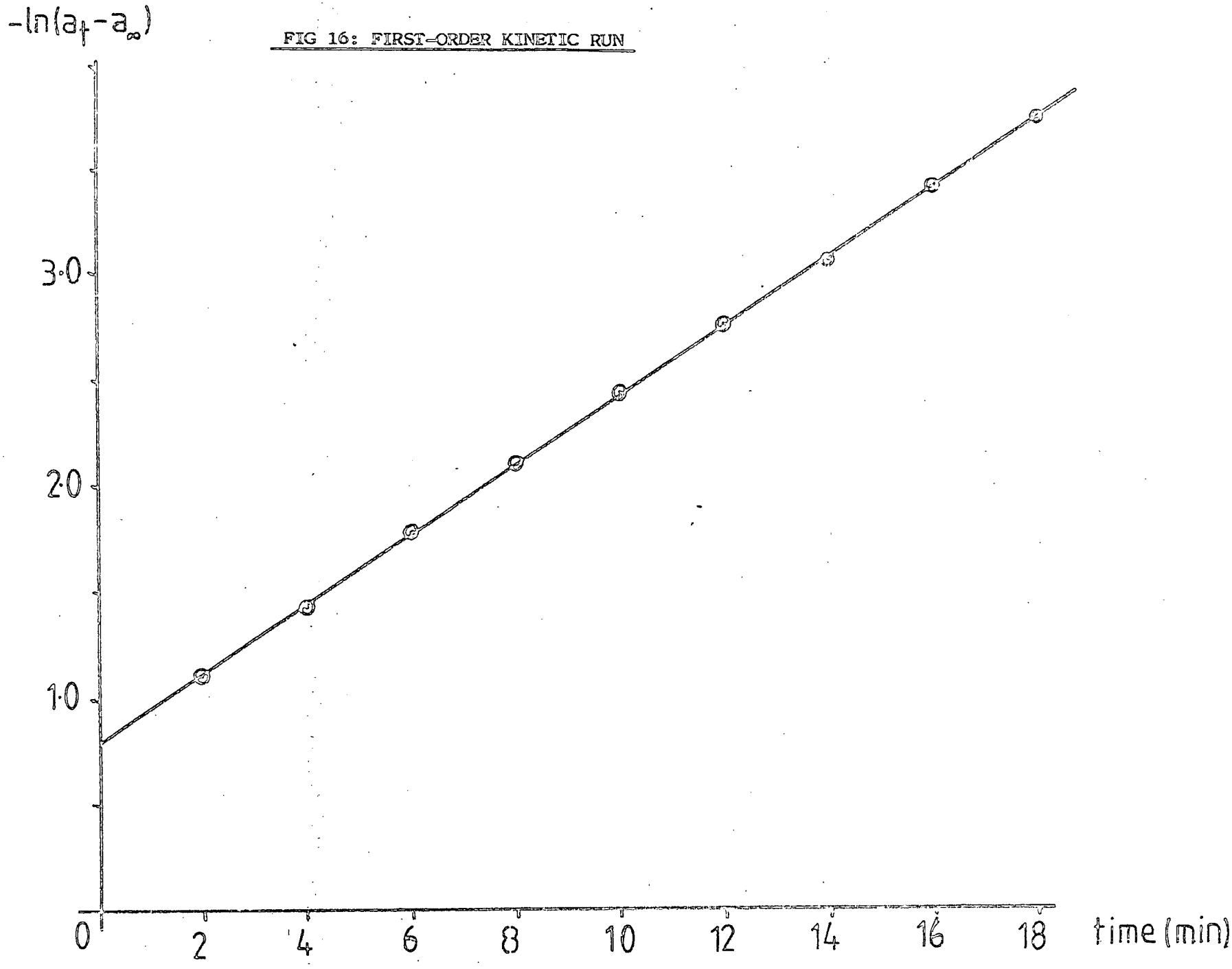
Example 1

Denitrosation of N-methyl N-nitrosoaniline in a solution of 10%(V/V) acetic acid-water, with 0·5M Br^-, 1·1M H_2SO_4 and 5 x 10^{-3}M NaN_3. Absorbance measurements made on a 'Beckmann' type 25 recording spectrophotometer. See fig 16 and page 20.

Time (mins)	a_t	$-\ln(a_t - a_\infty)$	$10^3 k_0 (s^{-1})$
0	0·813	0.959	
2	0·76	1·11	2·83
4	0·67	1·43	2·75
6	0·602	1·76	2·75
8	0·552	2·10	2·77
10	0·518	2·43	2·77
12	0·493	2·76	2·76
14	0·477	3·06	2·73
16	0·463	3·41	2·75
18	0·454	3·73	2·74
∞	0·43	-	-

This gives $k_0 = (2·76 \pm 0·03) \times 10^{-3}$ sec^{-1}

FIG 16: FIRST-ORDER KINETIC RUN



The individual rate constants at each time interval are not normally calculated, but have been shown here to give an impression of the error margin obtained in a run, generally about ± 1%. Rate constants are reproducible to within ± 5% for repeated runs on the Beckmann instrument.

Example 2

The direct nitrosation of methanol by nitrous acid at 25°C. 4M methanol, 0·048M H^+, 0·04M $NaNO_2$. This is a much more rapid reaction than the first example, and is followed on a stopped-flow spectrophotometer. The absorbance is given as a voltage change on an oscilloscope screen. There is a background voltage (5V) across the photomultiplier and a linear relationship between absorbance and voltage is only given if the voltage change is less than 10% of the background voltage. Therefore concentrations are adjusted so that a voltage change of less than 0·5V is observed. Owing to the errors in measuring fast reactions, the individual runs are repeated several times and an average obtained. The rate constants are calculated in exactly the same way as for the first example, a plot of $-\ln(V_t - V_\infty)$ vs time being made, where V = voltage. The rate constants for this example have been calculated on a computer.

RUN 1:

V(mV)	300	270	220	180	150	135	75
t(ms)	0	5	10	15	20	25	∞

$k_0 = 61{\cdot}7\ s^{-1}$

RUN 2:

V(mV)	360	285	235	190	160	140	75
t(ms)	0	5	10	15	20	25	∞

$k_0 = 59{\cdot}6\ s^{-1}$

RUN 3:

V(mV)	360	295	240	200	165	145	90
t(ms)	0	5	10	15	20	25	∞

$k_0 = 64{\cdot}5\ s^{-1}$

RUN 4:

V(mV)	375	300	245	200	175	155	95
t(ms)	0	5	10	15	20	25	∞

$$k_0 = 62{\cdot}2\ s^{-1}$$

Average $k_0 = 62{\cdot}0 \pm 1{\cdot}4\ s^{-1}$

Example 3

A few kinetic runs in this work have involved rather slow reactions in which it is difficult to estimate accurately the absorbance at infinity. Some of these runs have been carried out on a Beckmann spectrophotometer at fixed wavelength and others on an SP-800 instrument by scanning the spectrum between 200-300nm at fixed time intervals using an automatic timer. An alternative kinetic method is applied to these slow reactions- the Guggenheim[135] method, which does not require an absolute estimate of the infinity value. Absorbance measurements are taken at times t_1, t_2, t_3 etc and at times $(t_1 + \Delta), (t_2 + \Delta), (t_3 + \Delta)$ etc where Δ is approximately two half-lives. At time t, the absorbance is at a_t and at time $(t + \Delta)$ the absorbance is a'_t.

For a first order reaction A ——> B;

$$\bar{A}_t = \bar{A}_0 e^{-kt}$$

$$\therefore (a_t - a_\infty) = (a_0 - a_\infty)e^{-kt}$$

and,
$$(a'_t - a_\infty) = (a_0 - a_\infty)e^{-k(t+\Delta)}$$

$$\therefore (a_t - a'_t) = (a_0 - a_\infty)e^{-kt}(1 - e^{-k\Delta})$$

$$\therefore \ln(a_t - a'_t) = -k_0 t + \ln(a_0 - a_\infty)(1 - e^{-k\Delta})$$

$$= -k_0 t + \text{constant}$$

So a plot of $\ln(a_t - a'_t)$ vs t will give k_0. Some data is given below for the de-nitrosation of N-methyl N-nitrosoaniline in DMSO solvent. [HCl] = 1·12M, [NMNA] = $1{\cdot}3 \times 10^{-4}$M, [sulphamic acid] = 0·2M.

Time (mins)	a_t	$-\ln(a_t - a'_t)$
0	0·870	0·879
2	0·818	0·967
4	0·780	1·016
6	0·727	1·133
8	0·689	1·201
10	0·651	1·287
12	0·621	1·351
14	0·590	1·419
16	0·563	1·496
18	0·537	1·570
20	0·515	1·614
30	0·455	
32	0·438	
34	0·418	
36	0·405	
38	0·388	
40	0·375	
42	0·362	
44	0·348	
46	0·339	
48	0·329	
50	0·316	

From the slope, $k_0 = 6·55 \pm 0·03 \times 10^{-4}$ sec^{-1}

5.3. Experimental determination of rate constants

(a) Conventional u.v. spectrophotometry.

This method was used for the denitrosation of N-methyl N-nitrosoaniline (NMNA). Solutions were made up in eitheir water, DMSO, DMSO- water or acetic acid-water solvents. An appropriate solution (taken from several stock solutions) was made up containing all the reagents except the nitroso--amine. The solution was thermostatted in a water bath at 31^{0}C for 15mins before commencing the reaction by adding the nitrosoamine. 1 ml of NMNA solution was added using an 'Eppendorf' spring-loading pipette with a disposable tip, ensuring rapid and safe delivery from a stock solution of NMNA. The solution was shaken and rapidly transferred to a 1cm silica cuvette and placed in a the thermostatted block of the Beckmann model 25 spectrophotometer. This meant that the first 5-10 seconds of the reaction was missed, but in most cases this was not significant. The reaction was followed by observing the disappearance of the nitrosoamine absorb--ance. In water this absorbs at a maximum of 270nm, and in DMSO and acetic acid there is little shift in the wavelength. In DMSO it was necessary to follow the reaction at 300nm due to interference by a sulphamic acid peak.

(b) Stopped-flow spectrophotometry.

The conventional u.v. spectrophotometer is not suitable for reactions with half-lives much below 10 seconds, nor is it suitable for reactions in which there may only be very small amounts of solution available (e.g. enzyme studies). A more sophisticated technique may be employed here - stopped-flow spectrophotometry. This allows large numbers of runs to be performed consecu. tively with very little mixing time. The reagents are kept in two hypo--dermic syringes which are mounted on a block with a set of parallel guide rods and are pushed manually or automatically to evenly deliver the solutions along a central tube and into a third syringe (fig 17). Before the solutions are pushed in, there will already be a maximum concentration of product (from the previous run) at point P. As the unreacted solutions are pushed in, at point P the

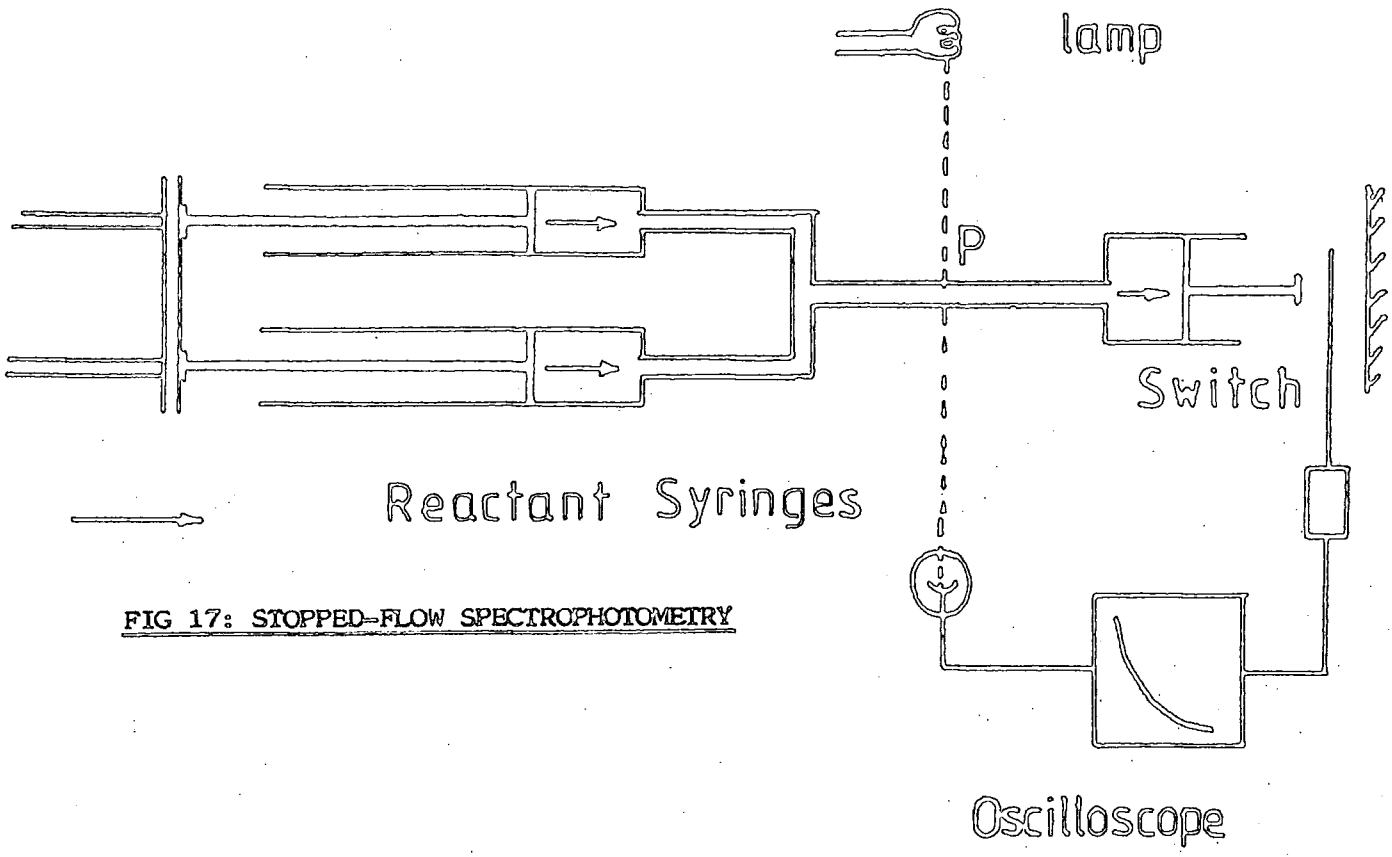
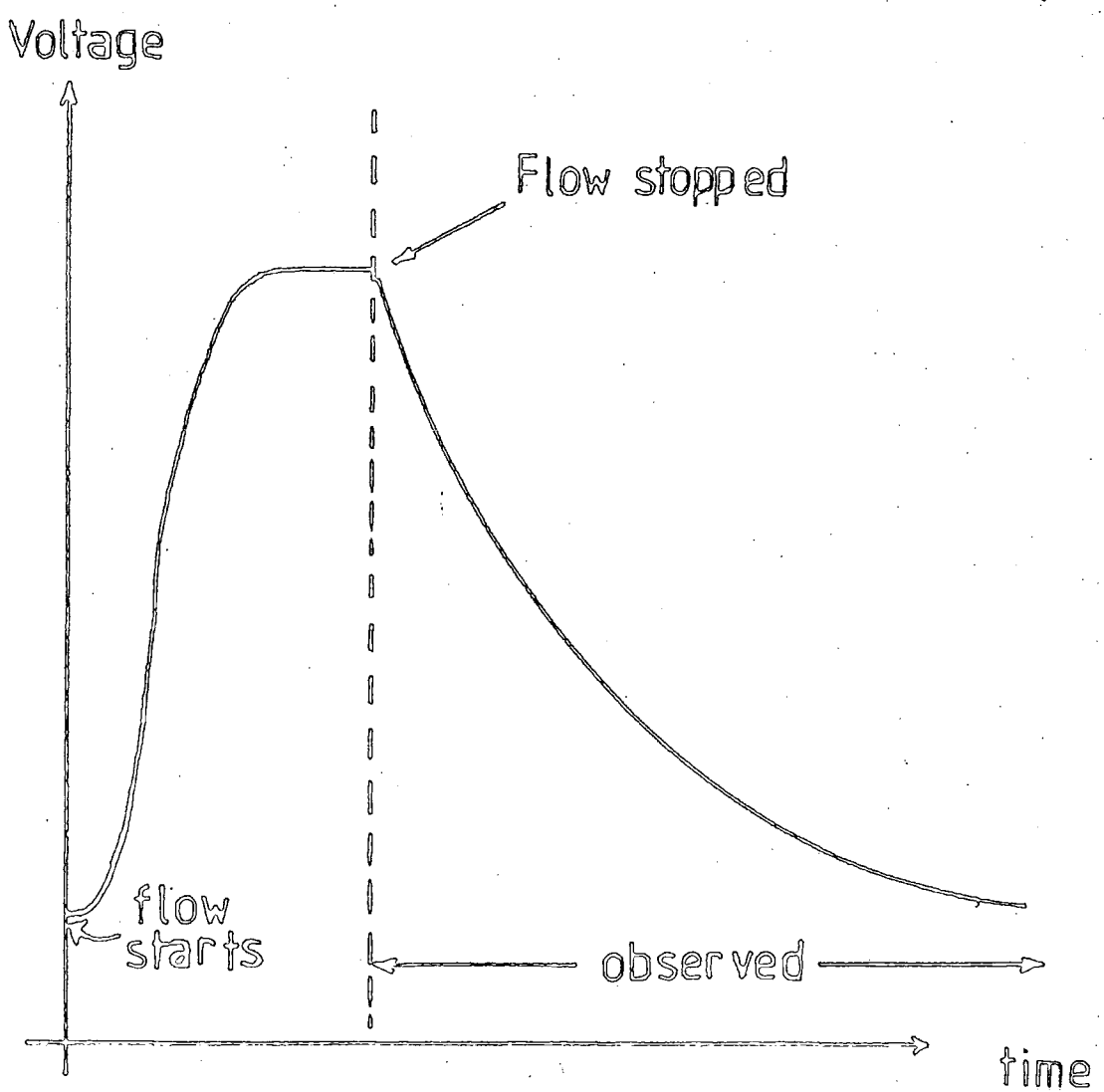


FIG 17: STOPPED-FLOW SPECTROPHOTOMETRY



maximum concentration of product will be replaced by an increasingly weaker solution of product and the voltage across the photomultiplier which monitors the light source that passes through P will increase (if we are looking at the absorbance of the product), i.e. there is a 'concentration jump' and there reaches a point where a steady-state is maintained (see fig 17). If the flow is suddenly stopped then the concentration of product will relax back to it's original value and we see a decay signal on the oscilloscope screen which is triggered to respond as soon as the flow is stopped. Readings are then taken from the oscilloscope trace.

In a typical run, the sodium nitrite solution is kept in one syringe and a solution containing all other reagents is kept in the other. Reagent concentrations are halved when they pass together into the cell. The instrument used in this work was a 'Nortech' SF-3 system with thermostatting to $\pm 0{\cdot}1^{o}C$. The rate constant at $0^{o}C$ (page 113) was determined by injecting liquid nitrogen into a Dewer flask which contains a reservoir in which the cell (at point P along the central tube) is immersed in liquid paraffin which makes a good thermostatting fluid. The correct wavelength is provided by a suitable lamp-filter arrangement, a deuterium lamp being used for u.v. studies (i.e. wavelengths below 340nm), a tungsten lamp is used for higher wavelengths. The wavelengths used in chapter two for the study of the formation of ArN_2^{+} by diazotisation were determined by a previous worker. The wavelength of the S-nitroso compounds studied in chapter three were determined by simply carrying out the nitrosation in a flask and obtaining λ_{max} on a conventional u.v. spectrophotometer.

5.4. Error treatment

(i) The standard error of the mean ($\bar{x}$) of a series of values $x_1, x_2, x_3, \ldots\ldots$ is given by $\sigma_x / \sqrt{n}$, where σ_x is the standard deviation of the mean and n = no of values. The standard deviation is given by;

$$\sigma_x = \sqrt{\frac{n\sum x^2 - \left(\sum x\right)^2}{n(n-1)}}$$

Therefore, for example in a set of rate constants, the mean value is given in the form $\bar{x} \pm (\sigma_x/\sqrt{n})$.

(ii) The least-squares line is the best-fitting line of the form $y = mx + c$ to a given set of data. The slope is given by;

$$m = \frac{\sum xy - \frac{(\sum x)(\sum y)}{n}}{\sum x^2 - \frac{(\sum x)^2}{n}}$$

the intercept is given by; $c = \bar{y} - m\bar{x}$

Where, $\bar{y} = \frac{\sum y_i}{n}$ $\bar{x} = \frac{\sum x_i}{n}$

The error in the intercept and slope is given by measuring the standard deviation in the slopes and intercepts of a series of lines that go through the calculated 'best' intercept or slope at each individual point.

$$\sigma_m^2 = \frac{N\sigma^2}{\Delta}$$

N = no of points $\Delta = N \sum x^2 - (\sum x)^2$

$$\sigma^2 = \frac{1}{N} \sum (mx + c - y)$$

The standard error of the slope is = $\sigma_m / \sqrt{n}$

Similarly, the variance of the intercept is given by;

$$\sigma_i^2 = \frac{\sigma^2 \sum x^2}{\Delta}$$

(iii) Weighted least-squares regression analysis.

In the double reciprocal plots in chapter two and three, the points tend to be 'crowded' at one end of the scale, with one or two points much further out. A small error in this latter point may lead

to a considerable error in the slope and so it is more satisfying to use a 'statistical 'weighting' on the more closely grouped points. This method is called a weighted linear regression analysis[136] and is often used to analyse data from enzyme kinetic experiments.

The slope is given by;

$$m = \frac{\sum w \sum wxy - \sum wy \sum wx}{\sum w \sum wx^2 - (\sum wx)^2}$$

w = weighting factor = $1/y^2$

Intercept (c) = $\bar{y} - m\bar{x}$

$$\bar{y} = \frac{\sum wy}{\sum w} \qquad \bar{x} = \frac{\sum wx}{\sum w}$$

Error in the slope = $\sqrt{(\sigma_s^2)/n}$

where,

$$\sigma_s^2 = \frac{\sigma^2}{\sum wx^2 - \left\{(\sum wx)^2 / \sum w\right\}}$$

and,

$$\sigma^2 = \frac{\sum w (y - c - mx)^2}{n-2}$$

Error in the intercept, = $\sqrt{\sigma_i^2/n}$

$$\sigma_i^2 = \frac{\sigma^2 \sum wx^2}{\sum w \sum wx^2 - (\sum wx)^2}$$

REFERENCES

1. There are many accounts of the carcinogenic properties of N-nitroso compounds. Some are listed here;

(a) G.A.Digonis & C.H.Issidores, Bio-organic Chem. 8, 97, 1979.

(b) P.N.Magee & J.M.Barnes, Brit J. Cancer 10, 114, 1956.

(c) W.Lijinsky & S.S.Epstein, Nature, 21, 225, 1970.

(d) P.D.Lawley & P.Brookes, Biochem J. 89, 127, 1963.

2. H.Alpen & J.T.Edward, Can J. Chem 48, 1543, 1970.

3. E.M.Burgess & J.M.Lavarish, Tet.Lett. 1221, 1964.

4. E.C.S.Jones & J.Kenner, J.C.S., 711, 1932.

5. E.Y.Belyaev, T.I.Nikulicheva & B.A. Porai-Koshits, Zh.Org.Khim. 5, 2141, 1965.

6. T.A.Meyer, Ph.D. thesis, Univ. of Durham, 1981.

7. B.C.Challis & M.R.Osborne, J.C.S.Perkin II, 1526, 1973.

8. G.Büttner et al, Chem Ber, 104, 1118, 1971.

9. W.S.Layne, H.H.Jaffe & H.Zimmer, J.A.C.S. 85, 1816, 1963.

10. C.G.Swain & C.B.Scott, J.A.C.S. 75, 141, 1953.

11. D.L.H.Williams, J.C.S.Perkin II, 128, 1977.

12. I.D.Biggs & D.L.H.Williams, J.C.S.Perkin II, 107, 1975.

13. G.Hallet & D.L.H.Williams, J.C.S. Perkin II, 624, 1980.

14. E.Kalatzis & J.H.Ridd, J.C.S. (B), 529, 1966.

15. T.A.Meyer, G.Hallet & D.L.H.Williams, IARC symp, publication no 31. IARC, Lyon 1980 p31.

16. D.L.H.Williams J.C.S.Perkin II, 1838, 1976.

17. B.C.Challis, Chem.Comms, 627, 1972.

18. D.F.Heath, Biochem J. 85, 72, 1962.

19. As ref 6.

20. I.D.Biggs & D.L.H.Williams, J.C.S.Perkin II, 691, 1976.

21. " " " " 44, 1977.

22. " " " " 601, 1976.

23. W.Lijinsky & H.W.Taylor, Int J.Cancer 16, 318, 1975.

24. J.R.Perrott, G.Stedman & N.Uysal, J.C.S.Dalton, 2058, 1976.

25. J.R.Perrott & G.Stedman, J.Inorg.Nuc.Chem, 39, 325, 1977.

26. D.L.H.Williams J.C.S.Perkin II, 128, 1977.

27. G.Ellison & D.L.H.Williams, J.C.S.Perkin II, 699, 1981.

28. D.L.H.Williams Int.J.Chem.Kinetics, 7, 215, 1975.

29. S.S.Johal, D.L.H.Williams & E.Buncel, J.C.S.PerkinII, 165, 1980.

30. E.D.Hughes & C.K.Ingold, J.C.S., 255, 1935.

31. E.Grunwald & S.Winstein, J.A.C.S. 70, 846, 1948.

32. E.M.Kowoser, J.A.C.S. 80, 3253, 1958.

33. K.Dimroth et al, Ann.Chem. 661, 1, 1963.

34. R.W.Taft et al, Prog.Phys.Org.Chem. 13, 485, 1981.

35. G.Eisenbrand & R.Preussman, Arzneim-Forsch 1513 1970.

36. S.Winstein & G.C.Robinson, J.A.C.S. 80, 169, 1958.

37. J.J.Lagowski, 'The chemistry of non-aqueous solvents' 3, 245, 1970, Academic press.

38. C.E.Vanderzee & A.S.Quist, Inorganic Chem 5, 1238, 1966.

39. W.H.Gardner & H.Weinberger, Inorganic synthesis 1, 84, 1939.

40. A.J.Parker, Chem Rev 69, 1, 1969.

41A.J.Parker, Adv Phys Org Chem 5, 173, 1967.

42. A.J.Parker, Chem Soc Rev 163, 1962.

43. C.Kaladis et al Proc Indian Acad Science. A, 76, 51, 1972.

44. U.S.Dept of Commerce NBS monograph 1968, 105.
Chem Soc Japan Chemical Handbook 1975, 999.

45. M.Gorman & J.Connel, J.A.C.S. 69, 2064, 1947.

46. J.O.Edwards, J.A.C.S. 76, 1540, 1954.

47. J.H.Boughton & R.N.Keller J.inorg & Nuc chem. 28, 2851, 1966.

48. T.Suzuki & H.Hugisuru, Bull Inst Phys Chem Res Tokyo 21, 601, 1942.

49. T.D.B.Morgan, G.Stedman & P.A.E.Whincup J.Chem.Soc. 4813, 1965.

50. A.K.Covington & R.A.Matheson, J.Solution Chem. 5, 781, 1976.

51. T. Birchall & R.J.Gillespie Can J. Chem. 41, 2645, 1963.

52. Y.L.Chow, Acc Chem Res 6, 354, 1973.

53. S.J.Kuhn & J.S.McIntyre, CanJ.Chem. 44, 105, 1966,

54. G.A.Olah, D.J.Donavon & L.K.Keefer. J.Nat Cancer Inst. 54, 465, 1975.

55. A.Wurtz, Annalen 1849, 71, 330.

56. P.Griess, Annalen 1861, 120, 125.

57. In particular, J.H.Ridd Quart rev, 15, 418, 1961.

58. E.Miller & H.Haiß,' Chem Ber, 96, 570, 1963.

59. C.A.Bunton & G.Stedman, J.C.S. 2440, 1958.

60. T.A.Turney J.C.S. 4263, 1960.

61. H.Schmid & C.Essler, Monatsh Chem, 91, 484, 1960.

62. L.F.Larkworthy, J.C.S. 1959, 3116,

63. G.Y.Markovits, S.E.Schwartz & L.Newman, Inorg Chem 20, 445, 1981.

64. E.D.Hughes, C.K.Ingold & J.H.Ridd, J.C.S. 1958, 65.

65. E.Kalatzis & J.H.Ridd , J.C.S.(B) 530, 1966.

66. E.C.R. de Fabrizio, E.Kalatzis & J.H.Ridd, J.C.S.(B), 533, 1966.

67. N.S.Bailyss, R.Dingle, D.W.Watts & R.G.Wilkie, Aust J.Chem 16, 933, 1963

68. B.C.Challis & J.H.Ridd, Proc Chem Soc 245, 1960.

69. H.Schmid, Z.Electrochem. 43, 626, 1937.

70. L.P.Hammett, 'Physical organic chemistry' McGraw-Hill N.Y. 1940 p 294

71. K.Al-Mallah, P.Collings & G.Stedman, J.C.S.Dalton 2469, 1974.

72. G.Stedman & P.A.E.Whincup J.C.S. 5796, 1963.

73. H.Schmid & M.G.Fouad, Monatsch Chem 88, 631, 1957.

74. H.Schmid & A.Maschka Z.Physik.Chem. B, 49, 171, 1941.

75. T.A.Meyer & D.L.H.Williams J.C.S.Perkin II 361, 1981.

76. N.S.Bailyss & D.W.Watts, Aust J.Chem, 9, 319, 1956.

77. E.D.Hughes & J.H.Ridd J.C.S. 82, 1958.

78. H.Schmid & H.Hallaba Monatsch 87, 560, 1956.

79. D.L.H.Williams J.C.S.Perkin II 502, 1977.

80. Ref 70, p 355.

81. M.R.Crampton, J.T.Thompson & D.L.H.Williams J.C.S.Perkin II 18, 1979.

82. D.D.Perrin "Dissociation constants of organic bases in aqueous solution" Butterworths. London. 1965.

83. L.Dozsa, I.Szilassy & M.T.Beck Inorg Chim Acta 17, 147, 1976.

84. A.D. Awtrey & R.E. Connick J.A.C.S. 73, 1842, 1955.

85. R.W.Alder, R.Baker & J.M.Brown "Mechanism in Organic Chemistry" Wiley 1978 p206-207.

86. T.A.Meyer, Ph.D.thesis (Durham) 1981.

87. D.Ziebarth & B.Teichmann I.A.R.C. symposium 31, 231, 1980.

88. M.L.Dougless et al J.Soc Cosmet Chem 29, 581, 1978.

89. H.Dahn & L.Loewe Helv Chem Acta 43, 294, 1960.

90. D.L.H.Williams Food Cosmet Toxicol. 16, 365, 1978.

91. R.G.Pearson, H.Sobel & J.Songstad J.A.C.S. 90, 319, 1968.

92. M.N.Hughes J.Chem Soc (A) 902, 1967.

93. H.S.Tasker & H.O.Jones J.C.S. 95, 1910, 1917.

94. S.Oae et al J.C.S.PerkinI 913, 1978. 95. S.Oae Chem Lett 8, 893, 1977.

96. R.J.Phillipe & H.Moore J.Mol.Spect. 6, 492, 1961.

97. L.Field et al Chem Comms 249, 1978.

98. G.Kreize & J.Winkler Ber 96, 1203, 1963.

99. U.Schulz & McCalla Can J.Chem 47, 2021, 1969.

100. G.C.Young & A.Joshi J.Phys Chem 84, 228, 1980.

101. S.S.Al-Kaabi , D.L.H.Williams, R.Bonnett & S.L.Ooi J.C.S.Perkin II 227, 1982

102. Same as 101.

103. A.Pryor et al J.Org Chem 47, 156, 1982.

104. G.M.Green Science 162, 810, 1968.

105. A.E.Werner J.C.S. 101, 1912, 2180.

106. A.E.Werner & M.E.Coade J.C.S. 101, 2180, 1912.

107. O.Foss, J.Johnsen & O.Tvedten Acta Chem Scand 12, 1782, 1958.

108. P.Collings, M.Garkey & G.Stedman J.C.S.Dalton 331, 1981.

109. B.C.Challis & J.Challis "Chemistry of Amides" (ed J.Zabricky) Interscience London 1970 p 782.

110. J.W.Lawn & S.M.S.Chauhan Chem Comms 675, 1981.

111. M.A.Olatunji & A.McAuley J.C.S.Dalton 682, 1975.

112. R.G.Cassens et al Food Prod Dev 8, 50, 1974.

113. M.J.Dennis, R.C.Massey & D.J.McWeeny J.Sci Food Agric 31, 1195, 1980.

114. P.Collings, K.Al-Mallah & G.Stedman J.C.S.Perkin II 1734, 1975.

115. S.E.Aldred, D.L.H.Williams & M.Garley J.C.S.Perkin II 777, 1982.

116. Some evidence is given in : W.C.J.Ross Ann N.Y.Acad Sci 68, 669, 1957.

117. R.Bonnett & P.Nicolaidou J.C.S.Perkin I 1969, 1979.

118. A.H.Blatt (Ed) Org Synth Coll 2, 108, 204, 363, 1943.

119. H.Schmid & P.Riedl Monatsch 98, 1783, 1967.

120. S.Oae, N.Asai & K.Fujimori J.C.S. Perkin II 571, 1978.

121. A.J.Shenton & R.M.Johnson Int J Chem Kinetics 4, 235, 1972.

122. B.C.Challis & D.E.G.Shuker Chem Comms 315, 1979.

123. S.E.Aldred & D.L.H.Williams J.C.S.Perkin II 1021, 1981.

124. A.D.Allen J.C.S. 1954, 1968.

125. A.Dalq & A.Bruylants Tet Letts 377, 1975.

126. V.Napoleone & Z.A.Schelly J.Phys Chem 84, 17, 1980.

127. S.E.Aldred & D.L.H.Williams J.C.S. Chem Comms 73, 1980.

128. C.A.Bunton & G.Stedman J.C.S. Perkin II 3467, 1959.

129. J.W.Chapman & A.N.Strachan Chem Comms 293, 1974.

130. D.J.Benton & P.Moore J.Chem Soc (A) 3179, 1970.

131. M.Anbar & H.Taube J.A.C.S. 76, 6243, 1954.

132. E.M.Arnet & C.Y.Wu J.A.C.S. 84, 1684, 1962.

133. C.D.Ritchie, G.A.Shiner & V.G.Badding J.A.C.S. 89, 2063, 1967.

134. A.I.Vogel 'Textbook of practical organic chemistry' 572, Longmans 1966.

135. J.W.Moore & R.G.Pearson 'Kinetics & Mechanism' 3rd Edn, 71, Wiley 1981.

136. D.V.Roberts 'Enzyme Kinetics'. Cambridge press 1977. Appendix.

137. M.E.O'Neill, Unpublished work.

LECTURES AND SEMINARS

Lectures given to the Durham university chemical society 1979-82

Oct 18, 1979 Dr G.Cameron (Aberdeen): "Synthetic Polymers".

Oct 25, 1979 Prof P.Grey (Leeds): "Oscillatory Combustion reactions".

Nov 1, 1979 Dr J.Ashby (I.C.I. Toxicological laboratory): "Does Chemically-Induced cancer make sense"?

Nov 8, 1979 Prof J.H.Turnbull (R.M.C. Shrivenham): "Luminescence of drugs".

Nov 15, 1979 Prof E.A.V. Ebsworth (Edinburgh): "Stay still you brute:The shape of simple silyl complexes."

Jan 24, 1980 Prof R.J.P.Williams (Oxford): "On first looking into bilogy's chemistry". Liversidge lecture.

Feb 14, 1980 Prof G.Gamlen (Salford): "A yarn with a new twist-Fibres and their uses".

Feb 23, 1980 Dr M.L.H.Green (Oxford): "Synthesis of highly reactive organic compounds using metal vapours".

Feb 28, 1980 Prof S.F.A.Kettle (East Anglia): "Molecular shape, structure and chemical blindness".

March 6, 1980 Prof W.D.Ollis (Sheffield): "Novel molecular rearrangements".

Oct 16, 1980 Dr D.Maas (Salford): "Reactions a go-go".

Oct 23, 1980 Prof T.M.Sugden (Cambridge): "Reactions in flames".

Oct 30, 1980 Prof N.Grassie (Glasgow): "Inflarmability hazards in commercial polymers".

Nov 6, 1980 Prof A.G.Sykes (Newcastle): "Metalloproteins: An inorganic chemists approach".

Nov 13, 1980 Prof N.N.Greenwood (Leeds): "Metalloborane chemistry".

Dec 4, 1980 Rev. R.Lancaster: "Fireworks".

Jan 22, 1981 Prof E.A.Dawes (Hull): "Magic and mystery through the ages".

Jan 29, 1981 Mr H.J.F.MacLean (I.C.I. Agricultural division): "Managing the chemical industry in the 1980's".

Feb 5, 1981 Prof F.G.A.Stone (Bristol): "Chemistry of carbon to metal triple bonds".

Feb 12, 1981 Dr I.Fleming (Cambridge): "Some uses of silicon compounds in organic synthesis".

Feb 12, 1981 Prof W.P.Jencks (Bradeis, U.S.A.): "When is an intrmediate not an intermediate".

May 1, 1981 Prof M.Gordon (Essex): "Do scientists have to count?".

Oct 22, 1981 Dr P.J.Corish (Dunlop): "What would life be like without rubber".

Oct 29, 1981 Miss J.M.Cronyn (Durham): "Chemistrty in Archeology".

Nov 12, 1981 Prof A.I.Scott (Edinburgh): "An organic chemist's view of life through the N.M.R. tube".

Nov 19, 1981 Prof B.L.Shaw (Leeds): "Big rings and metal-carbon bond formation."

Dec 3, 1981 Dr W.O.Ord (Northumbria water authority): "The role of the scientist in a regional water authority".

Jan 28, 1982 Prof I.Fells (Newcastle): "Balancing the energy equations".

Feb 11, 1982 Dr D.W.Turner (Oxford): "Photoelectrons in a strong magnetic field".

Feb 18, 1982 Prof R.K.Harris (East Anglia): "N.M.R. in the 1980's".

Feb 25, 1982 Prof R.O.C.Norman (York): "Turning points and challenges for the organic chemist".

March 4, 1982 Dr R.Whyman (I.C.I. Runcorn): "Making metal clusters work".

Research Colloquia given to the Department of Chemistry 1979-82

21 Nov, 1979	Dr J.Miller (Bergen): "Photochemical reactions of Ammonia".
28 Nov, 1979	Dr B.Cox (Stirling): "Macrobycyclic Cryptate complexes".
5 Dec , 1979	Dr G.C.Eastmond (Liverpool): "Synthesis and properties of some multicomponent polymers".
12 Dec, 1979	Dr C.I.Ratcliffe: "Rotor motions in solids".
19 Dec, 1979	Dr K.E.Newman (Lausanne): "High pressure n.m.r. for fast reaction mechanisms".
6 Feb, 1980	Dr J.M.E.Quirke (Durham): "Degredation of chlorophyl-A in sediments".
14 May 1980	Dr R.Hutton (Water associates, U.S.A.): "Recent developments in HPLC".
21 May 1980	Dr T.W.Bentley (Swansea): "Medium & Structural effects in solvolytic reactions".
10 July, 1980	Prof P.des Marteau (Heidelburg): "Developments in Organonitrogen fluorine chemistry".

Oct 7, 1980 Prof T.Fehler (Notre Dame, U.S.A.): "Mettaloboranes-cages or co-ordination compounds".

Oct 15, 1980 Dr R.Alder (Bristol): "Medium ring bicyclic molecules".

Nov 12, 1980 Dr M.Gerlock (Cambridge): "Magnetochemistry is about chemistry".

Nov 19, 1980 Dr T.Gilchrist (Liverpool): "Nitroso-olefins as synthetic intermediates".

Dec 3, 1980 Dr J.A.Connor (Manchester): "Thermochemistry of transition metal compounds".

Dec 18, 1980 Dr R.F.Evans (Adelaide): "The Australian journal of failed chemistry".

Feb 18, 1981 Prof S.F.A.Kettle (East Anglia): "Variations of the molecular dance at the crystal ball".

Feb 25, 1981 Dr K.Bowden (Essex): "Transmission of polar effects of sub-stituents".

Mar 4, 1981 Dr S. Craddock (Edinburgh): "Pseudo-linear pseudohalides".

Mar 11, 1981 Dr J.F.Stddart (I.C.I.): "Stereochemistry of synthetic molecular receptors".

Mar 18, 1981 Dr P.J.Smith (Int.Tin research inst): "Organotin compounds".

April 9, 1981 Dr W.H.Meyer (Zurich) "Properties of aligned polyacetylene".

May 6, 1981 Prof M.Szwarz "Ions and ion-pairs".

June 10, 1981 Dr J.Rose (I.C.I.) "New engineering plastics".

June 17, 1981 Dr P.Moreau (Montpellier): "Perfluoro-organometallic chemistry".

June 26, 1981 Prof A.P.Schaap (U.S.Office of Naval research): "Molecular dynamics in molecular crystals".

Oct 14, 1981 Prof E.Kluk (Katowice): "Chemiluminescence & Photooxidation".

Oct 28, 1981 Dr R.J.H.Clark (University college, London): "Resonance Raman spectroscopy".

Nov 6, 1981 Dr W.Moddeman (Monsato labs, St Louis, Missouri): "High energy materials".

Nov 18, 1981 Prof M.J.Perkins (Chelsea college): "Spin trapping and Nitroxide radicals".

Nov 25, 1981 Dr M.Baird (Newcastle): "Intramolecular reactions of carbenes and carbenoids".

Dec 2, 1981 Dr G.Beamson (Durham): "Photoelectron spectroscopy in a strong magnetic field".

Nov 30, 1981 Dr B.T.Heaton (Kent): "N.M.R. studies of carbonyl clusters".

Jan 20, 1982 Dr M.R.Bryce (Durham): "Organic metals".

Jan 27, 1982 Dr D.L.H.Williams (Durham): "Nitrosation & nitrosoamines".

Feb 3, 1982 Dr D.Parker (Durham): "Modern methods of determining enantiomeric purity".

Feb 10, 1982 Dr D.Pethrick (Strathclyde): "Conformation of small and large molecules".

Feb 17, 1982 Prof D.T.Clark (Durham): "Studies of surfaces by ESCA".

Feb 24, 1982 Dr L.Field (Oxford): "Application of N.M.R. to biosynthetic studies on penicillin".

Mar 3, 1982 Dr P.Bamfield (I.C.I. Organics): "Computer aided design in synthetic organic chemistry".

Mar 17, 1982 Prof R.J.Haines (Cambridge/Natal): "Clustering around Ru, Fe and Rh".

April 7, 1982 Dr A. Pensak (Dupont, USA): "Computer aided synthesis".

May 5, 1982 Dr G.Tennant (Edinburgh): "The aromatic nitro group in hetero-cyclic reactions".

May 7, 1982 Dr C.D.Garner (Manchester): "Molybdenum centres in enzymes".

May 26, 1982 Dr A.Welch (Edinburgh): "Conformation and distortion in Carbometalloboranes".

Induction course for research students: October 1979

1. Safety in the laboratories

2. Electrical equipment

3. Chromatographic service

4. Analytical service

5. Mass spectrometry

6. N.M.R. spectrometry

7. Glassblowing