Kinetics and mechanism of the denitrosation of a nitrosamine and a trionitrite

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"قُلْ إِنَّ صَلَاتِي وَنُسُكِي وَمَحْيَايَ وَمَمَاتِي لِلَّهِ رَبِّ الْعَالَمِينَ"

KINETICS AND MECHANISM OF THE DENITROSATION OF A NITROSAMINE AND A THIONITRITE

by

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Dedicated

to my parents

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ABSTRACT

The work described in this thesis is concerned with the denitrosation of N-methyl-N-nitrosoaniline (NMNA) and a thionitrite (derived from N-acetyl D,L-penicillamine).

The effect of addition of various nucleophiles on the denitrosation reaction of NMNA was examined extensively at three different acidities in the presence of high concentrations of sodium azide to cut out the reverse reaction. The values of $k_1[H^+]$ (which were obtained from the double reciprocal plots) are constant at any one acidity for different nucleophiles and increase with acidity as expected. The slopes increase as the acidity decreases. The ratio k_{-1}/k_2 is nearly the same for the same nucleophiles at different acidities, and [illegible] reactivity of nucleophiles is found as expected.

An [illegible] was also carried out of the denitrosation of a thionitrite. The general form for the rate of equation was established and the relative reactivity of different nitrite traps measured. Nucleophilic catalysis was observed for the following nucleophiles in increasing order of reactivity $H_2O < Cl^- < Br^- < SC(NH_2)_2 < SCN^-$. The reaction mechanism is similar to that proposed for the denitrosation of nitrosamines and also of alkyl nitrites. The thionitrite is approximately 10^6 less reactive than a corresponding alkyl nitrite; this is probably due to the much smaller basicity of the sulphur protonation site compared with oxygen.

CONTENTS

CHAPTER 1

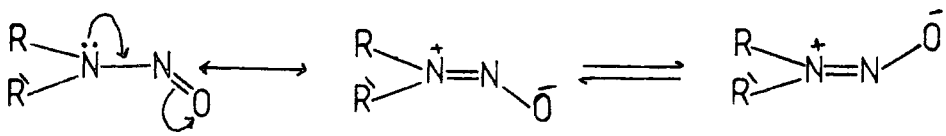
INTRODUCTION

1.0 Introduction

Organic compounds can contain many types of nitroso groups such as -O-N=O nitrites, -S-N=O thionitrites, $\geq$C-N=O C-nitroso compounds and $\geq$N-N=O N-nitrosamines.

In this chapter N-nitrosamines and thionitrites will be discussed in detail. N-nitrosamines have been known since the nineteenth century. They are important in two ways: first, they were used a great deal in industry and second, the carcinogenicity of these compounds was such that they were considered to be of public health interest, mainly as an industrial hazard.

1.1 Structural aspects:



[R-N=N-OH] if R' = H

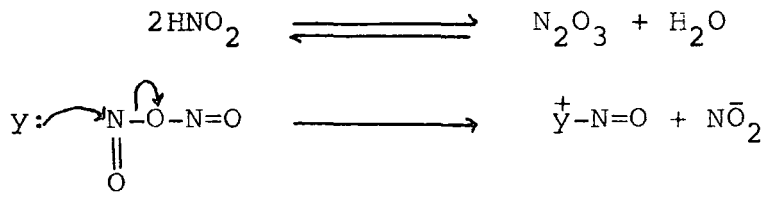
Scheme 1

N-nitrosamines have the nitroso group attached directly to the amine nitrogen. When one of the substituents is hydrogen the equilibrium favours the tautomeric diazoic acid.[1] There are reported cases of stable primary N-nitrosamines, in those instances these N-nitrosamines exist as the tautomeric N-nitrosamines.[2] It has been demonstrated that the α-hydrogen

atoms of N,N-dialkylnitrosamines are magnetically nonequivalent as a result of the large contribution of the dipolar mesomeric structure.[3] These findings confirmed the suggestion that N-nitrosamines are polar.

1.2. Preparation of N-nitrosamines

The nature of the reaction of aromatic amines with nitrous acid is dependent upon whether the amine is primary, secondary or tertiary. It effectively involves the attachment of the nitrosonium ion ($\overset{+}{N}$=O) to a nucleophilic centre which may be the amino nitrogen atom or the aromatic ring. Nitrous acid is normally generated by the addition of sodium nitrite to an aqueous acid solution on the amine, the actual nitrosating species is the nitrous acid anhydride N_2O_3 or $H_2\overset{+}{N}O_2$ cleaves during the reaction with the nucleophilic amine.[4]

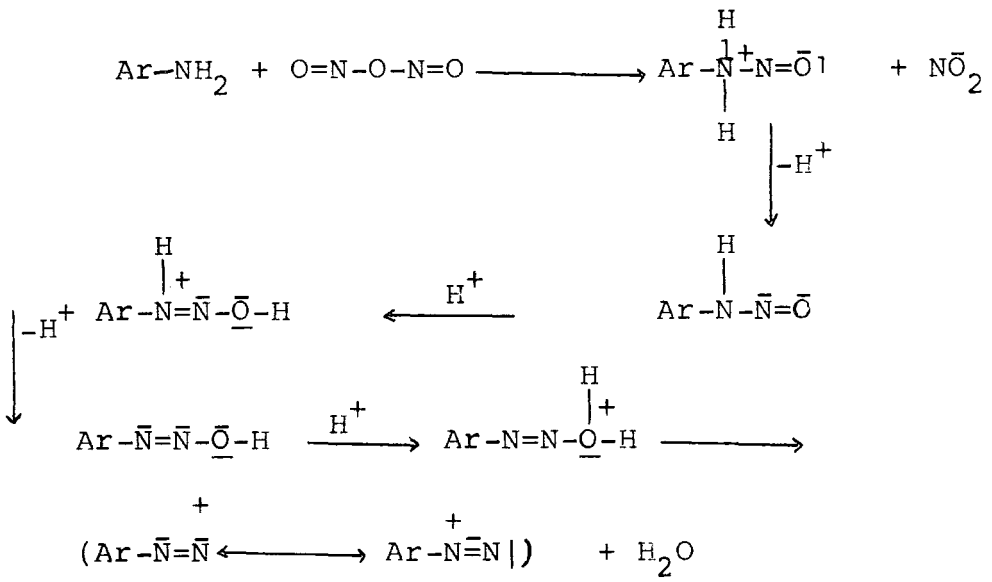


Scheme 2

1.2.a. Reaction of Nitrous Acid with Primary Amines

Primary aromatic amines may be distinguished from the analogous aliphatic amines by the fact that diazonium ions are formed which are relatively stable at temperature $0^{o}c$. In aliphatic amines further reactions occur to yield alkenes and alcohols.[5]

1.2.a. i. Mechanism of Diazotization:



Scheme 3

Taylor[6] showed that the reaction of primary amines with nitrous acid follows that rate equation

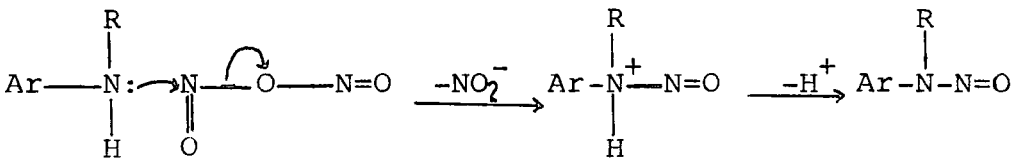
$$\mathcal{V} = k\ [RNH_2]\ [HNO_2]^2$$

where the bracketed expressions refer to the actual concentration of the reactant, and this observation was confirmed by Schmid and Muhr.[7] Hammett suggested that the amine performs a nucleophilic attack on dinitrogen trioxide present in equilibrium with the nitrous acid, this attachment of the nucleophile could lead to the conjugated acid of nitrosamine, then proton transfers to the diazohydroxide which react with acid to give the diazonium ion.[8] This ion is stabilized by conjugation with the aromatic ring.[9]

1.2.b. Reaction of Nitrous Acid with Secondary Amines

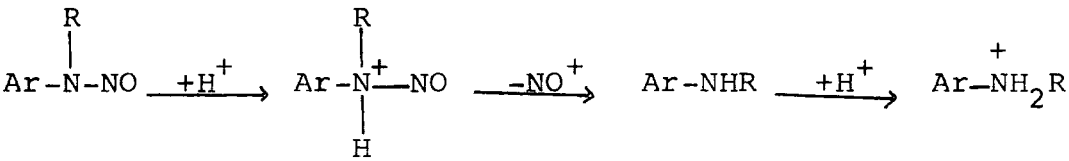
When secondary amines are treated with nitrous acid,

the initial electrophilic attack of the nitrosating agents is followed by the loss of a proton to give an N-nitrosamine. This reaction can be reversible



Scheme 4

The protonation of the amino nitrogen can be achieved under strongly acidic condition and such protonation leads to the loss of the nitrosonium group.[4]



Scheme 5

The reaction of the amines with nitrous acid may be accomplished with dialkyl, diaryl or alkylaryl amines, and even with mono -N- substituted amides [RCONHR].

Other nitrosating agents have been identified kinetically, e.g. nitrosyl chloride NOCl, when the reaction is carried out in the presence of chloride ions.

1.2.b.i. Fischer-Hepp Rearrangement

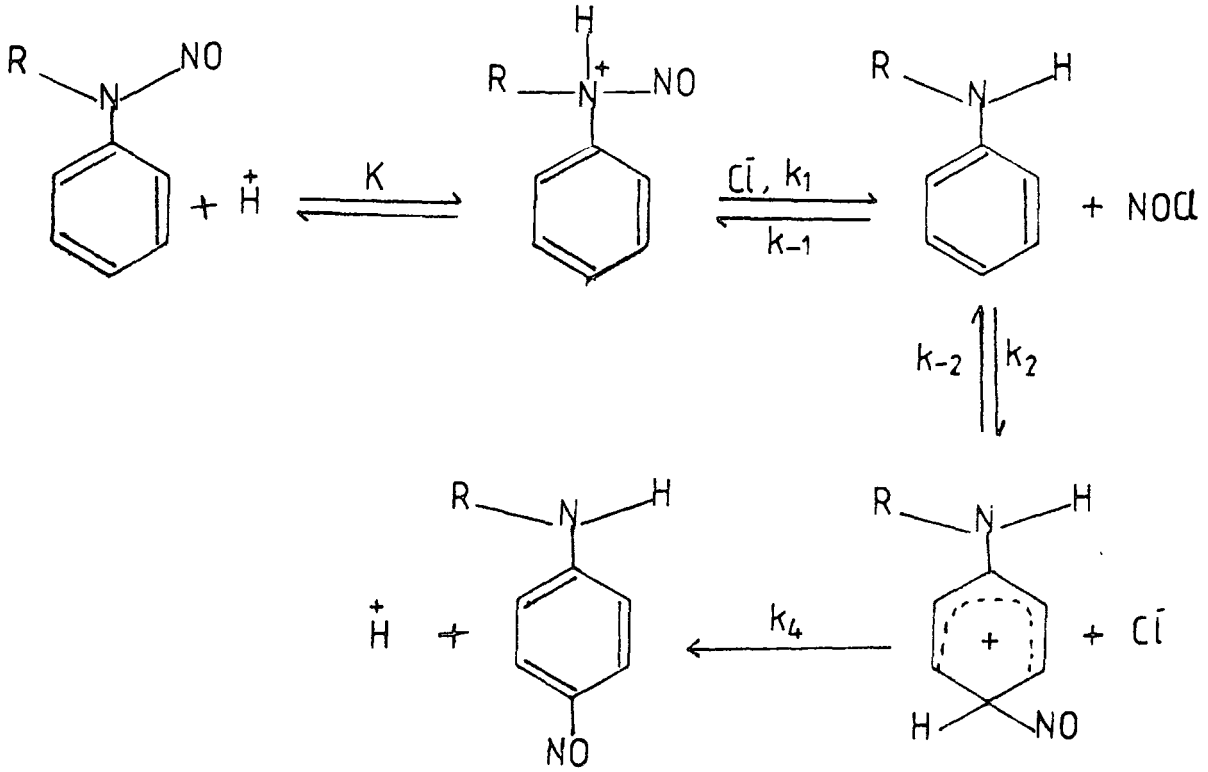
Nitrosation of secondary aromatic amines generally occurs at nitrogen and it is reversible. For aromatic compounds in acid solution the N-nitroso compounds rearrange to the para-nitroso isomer, this isomerization is known as Fischer-Hepp

rearrangement. The rearrangement is found to give best yields in hydrochloric or hydrobromic acids, whereas in sulphuric acid the yields are low and with nitric acid no rearrangement occurs.[10]

1.2.b.ii Inter- and Intramolecular Mechanisms:

There are two theories of the mechanism of the rearrangement, intermolecular and intramolecular reaction.

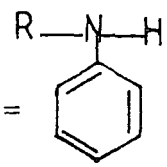
Intermolecular Mechanism: (See Scheme 6)



$NOCl + X \xrightarrow{k_3}$ Various products

y^- = Nucleophile, e.g. Cl^-, Br^-

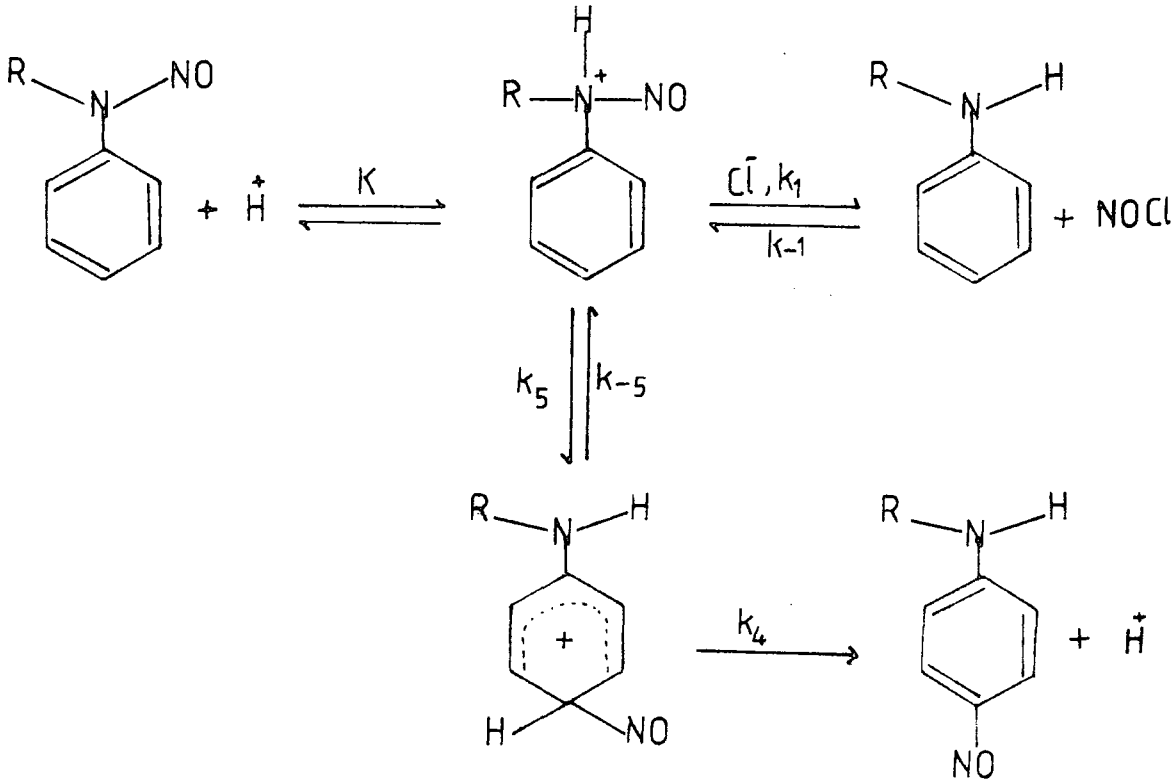
X = Nitrite trap, e.g. hydrazine, urea

C = 

Scheme 6

This mechanism proposes that in the absence of nitrite traps, N-nitroso aromatic compounds undergo denitrosation in the presence of hydrochloric acid to form the corresponding secondary amine and nitrosyl chloride (NOCl). These then react to form p-C-nitroso compound.

Intramolecular Mechanism: (see Scheme 7)



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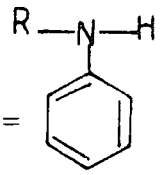
C = 

Scheme 7

Here it is suggested that the protonated compounds can transfer the $\overset{+}{N}O$ group to the para ring position without its becoming free,[9] then the intermediate compound loses a proton to form p-C-nitroso compounds. This means that the denitrosation and rearrangement occur at the same time.

The difference between the two mechanisms can be shown as follows.[11]

1) Reaction at high [X]:

There are a number of species which react rapidly and irreversibly with nitrous acid or derivatives of nitrous acid (e.g. nitrosyl chloride). These are referred to as nitrite traps X. Examples are hydrazoic acid, hydrazine, hydroxylamine, sulphamic acid, ascorbic acid and urea.

At high concentration of nitrite trap, when $k_3[X] \gg k_{-1}[C]$ the rate equations become

$ko = k_1[\bar{Y}]Kho$ (a) intermolecular

$$k_o = \frac{k_4 k_5 Kho}{k_4 + k_{-5}} + k_1[\bar{Y}]Kho \quad \text{(b) intramolecular}$$

It is expected by mechanism (b) that the ratio % rearrangement: % denitrosation should be constant at any one acidity, nucleophile concentration and should be independent of [X]. Mechanism (a) predicts that the % rearrangement should decrease towards zero as [X] concentration is increased.

Table (1) presents the results of the experiments in 2.75M H_2SO_4, at different concentrations of nitrite trap and at various nitrite traps. The reaction is zero-order in [X] and the % rearrangement is constant.

TABLE 1

Added X	[X]/M	$10^4 ko/S^{-1}$	% Rearrangement
HN_3	6.53×10^{-4}	0.65	21
HN_3	16.3×10^{-4}	0.67	21
NH_2SO_3H	3.1×10^{-3}	0.65	21
NH_2SO_3H	7.8×10^{-3}	0.64	22
$CO(NH_2)_2$	0.10	0.62	21
NH_2OH	2.58×10^{-3}	0.62	20
NH_2NH_2	1.56×10^{-3}	0.66	20

2) Reaction at high [C].

At high concentration of added N-methylaniline [C], $k_{-1}[C] \gg k_3[X]$. then mechanism (b) reduced to

$$ko = \frac{k_4 k_5 Kho}{k_4 + k_{-5}}$$

ko should be independent of the concentration of added halide ions,[12] as is found experimentally.

Earlier it was found that the reaction of N-nitrosodiphenylamine in ethanol has no chloride ion catalysis in the rearrangement reaction,[10] as predicted by the above equation, no free nitrosating agent (NOY) is lost by reaction with X, so ko for intramolecular mechanism should become independent of [C], [$\bar{Y}$] and [X].

The intermolecular equation at the same limiting case becomes (from steady-state treatments on reaction intermediates).

$$ko = k_1[\bar{Y}]Kho \left[1 - \frac{k_{-1}[C]}{k_3[X] + [k_{-1} + k_2^1][C]} \right]$$

It is apparent that ko should never be independent of [Y]; this is incompatible with experiment observation, also when $k_{-1}[C] \gg k_3[X]$. the ko should tend towards zero. This is because the rate of C-nitrosation $k_2[C]$ is smaller than the rate of N-nitrosation $k_{-1}[C]$, and because $k_2^1 < k_2$.

where $k_2^1 = k_2 - \frac{k_{-2}k_2[\bar{Y}]}{k_{-2}[\bar{Y}]+k_4}$

1.2.c. Reaction of Nitrous Acid with Tertiary Amines:

There was a belief that tertiary amines do not react with aqueous nitrous acid. The collected reports of the cleavage of tertiary amines with nitrous acid lead to a clear generalization, that the reaction between tertiary amines with aqueous nitrous acid undergo dealkylation to form a carbonyl compound, a secondary nitrosamine and nitric oxide.[13] According to one view, the amine is first oxidized to an aminium ion radical, which then fragments to secondary amine and methylene cation radical, which react with water and further oxidize (by nitrosonium ion) to form aldehydes or ketones and NO.[14]

$$R_2N\text{-}CHR'_2 + NO^+ \longrightarrow R_2\dot{N}^+\text{-}CHR'_2 + NO$$

$$R_2\dot{N}^+\text{—}CHR'_2 \longrightarrow R_2NH + R'_2\dot{C}^+$$

$$R_2NH + HNO_2 \longrightarrow R_2N\text{-}NO$$

$$R'_2\dot{C}^+ + H_2O \xrightarrow{NO^+} R'_2C{=}O + NO$$

Scheme 8

1.3. Carcinogenic Effects:

Until now N-nitrosamines have not been directly associated with human cancer because no population groups had been identified that were inadvertently exposed. Many N-nitroso compounds are powerfully carcinogenic in experimental animals but there is no firm proof that they are also carcinogenic in man.

N-nitrosamines can be formed by the interaction of nitrite and secondary or tertiary amines; it is not clear whether such formation actually takes place in vivo in human ingestion of a normal diet containing necessary precursor.[15] Some workers have suggested that the concentration of volatile nitrosamine in the blood may increase after a meal.[16] Many types of nitrosable amines are to be found in the environment, including a number of amino acids such as proline, hydroxy proline, tryptophan, sarcosine and arginine; some of them can react to give N-nitroso derivatives. Amines are found in fish products, cereals, tobacco, dyes, drugs, pesticides and many other organic materials, also the formation of amines has been indicated during food cooking processes as well as commercial synthesis, amines also occur from cooking plants and during organic decomposition.[17]

The great source of nitrite for formation of N-nitroso compounds in the stomach is cured meats, the concentration of nitrite will be highest because of the rapidity of ingestion of them.[18]

The concentration of nitrite in saliva can be quite high some time after eating a meal high in nitrite, containing vegetables such as spinach, but the secretion of saliva is low.[16]

Other sources of nitrite is a reduction of nitrite by bacteria, whic promotes the synthesis of N-nitroso compounds from nitrite and secondary amines.[18] Also the rate of formation of N-nitrosamine can be proportional to the square of nitrite concentration.[17] If the reagent is N_2O_3, atmospheric gases such as NO, NO_2 are available for secondary nitrosamine formation.

There are physiological compounds which can stimulate or inhibit the formation of nitrosamines; thiocyanate can enhance several hundred fold the rate of nitrosation of secondary amine at acid pH. It occurs in normal humans in saliva and gastric juices, particularly in the case of the smoker; it is to be found in vegetables such as cabbage and cauliflower.[16] Ascorbic acid and glutathione are inhibitors.

Under all these conditions, suitable gastric pH temperature and catalysis, the reactions do take place. So several amines form carcinogenic N-nitrosamines which are commonly ingested by humans, including a variety of components of food, food additives, drugs and agricultural chemicals.

1.3.a. Formation of N-nitroso Compounds in Foodstuffs

There is a large volume of literature referring to this topic. McPherson et al[19] tested 250 samples of various foods such as cured meat products, fried bacon, cooked-out bacon fats, baby foods and different varieties of alcoholic beverages which were analysed for the presence of volatile nitrosamines. N-nitrosopyrrolidine was found in fried bacon and cooked-out bacon fats. NDMA (N-nitroso-dimethylamine) was obtained in the skim milk powder. N-nitrosopyrrolidine has been found in cooked bacon but not in raw bacon.[20] Lijinsky[21] discussed the cooking of food which might produce free amino acids such as proline and arginine; this avoids a source of

nitrosable secondary amines such as pyrrolidine and piperidine. Others[22] reported that at high temperatures, N-nitrosamines are formed by amino acids with nitrite (as a food additive) in the presence of rice starch. The level of amine increased during storage even under freezing conditions. This agreed with Marquardt,[23] who found that Japanese raw fish treated with nitrite produced mutagenic compounds, whereas hot dogs and beef did not. Also, it has been found that covering the fish with aluminium foil or broiling in an electric range is effective in decreasing NDMA formation during cooking.[24] Chinese foods were also considered and vegetables, fish and shellfish; NDMA and NPYR (N-nitrosopyrrolidine) were found. 209 samples of cheese have been tested and 48 samples are shown as positive for NDMA, also 215 samples of bottled, canned and tap beer were analysed and it was found that 66% contained NDMA. In smoked beer they found a maximum value of NDMA.[25]

Many animal experiments have been carried out to establish the effectiveness of these nitrosamines on the main organs.[25] Several nitrosamines have been tested in a number of species; each N-nitroso compound was given as multiple doses during six months or more. The experiments have been carried out on a rat, Syrian golden hamster, European hamster and guinea pig. The results are listed in Table 2.

1.3.b. Formation of N-nitroso Compound in Tobacco Smoke

Tobacco smoking is correlated with cancer of the mouth, larynx, lungs, oesophagus, pancreas, kidneys and urinary bladder. Tobacco chewing can also cause oral cavity and

oesophageal cancer.[26] The nitrosamines are derived from the tobacco alkaloids such as nicotine (which occurs in general in concentration of 1-2 per cent in commercial tobacco products) and nornicotine. These two tobacco alkaloids could be precursors to N-nitroso nornicotine (NNN), formed during smoking. To indicate that, nicotine or nornicotine was added to the cigarettes, after analysing the smoke NNN was increased in concentration. The yields of volatile N-nitrosamines in cigarette smoke are dependent upon the nitrite content of the tobacco and on the protein content. Non-volatile nitrosamines from smoke may be removed by cellulose acetate tips. Other nitrosamines may be found in cigarettes such as NNK [4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone], NNA [4-(N-methyl-N-nitrosamino)-4-(3-pyridyl)butanal], NPY [N-nitrosopyrrolidine] and NAB [nitrosoanabasine].[27]

1.3.c. Formation of N-nitroso Compounds in Cosmetics:

There is limited information about the carcinogenic potency of N-nitrosodiethanolamine (NDELA) and its ability to penetrate the skin. Di and triethanolamines are common precursors of cosmetic formulation.[17] Under certain conditions, such as the presence of micro-organisms, oxygen, certain trace metals, reagents containing carbonyl groups, temperature and pH, in the presence of diethanolamine (DELA), NDELA might be formed.

Fan et al[24] have reported on the presence of N-nitrosodiethanolamine in a variety of cosmetics, body lotions and hair shampoos. Others[17] assumed that NDELA is formed after the cosmetic has been completely formulated, thus it might be expected that industrial workers who are involved in the

Table 2

Carcinogenicity of Nitrosamines in Different Species

Compound	Species and Organs Affected Cumulative dose in g/kg[a]			
	Rat	Syrian hamster	European hamster	Guinea Pig
2,6-Dimethyl nitroso morpholine	oesophagus (0.5)	pancreas (1)	pancreas (8)	liver (1)
2,6-Dimethyldinitroso-pip erazine	oesophagus (0.8)	lung (3)	lung (2)	liver (4)
Nitrosoheptamethyl eneimine	lung oesophagus(0.7)	forestomach oesophagus(2)	lung (0.7)	no tumours (3.6)
Nitrosomethyl dodecylamine	bladder (2.4)	bladder (0.3)	bladder (18)	liver (8)
Nitrosomethylurethane	forestomach (0.1)	oesophagus forestomach(0.1)		pancreas (1)
Nitrosomethyl. diethyl urea.	brain spinal cord (1.0)			no tumours (1.3)

a = cumulative dose = does giving rise to tumours in 50%, or more, of treated animals

packing of the final products could be exposed to a varying amount of NDELA. Recent studies[29] compared N-nitroso amine levels in food and cosmetics, they found that high concentrations occurred in cosmetics.

1.3.d. Formation of N-nitroso Compounds in Other Consumer Products

Many pharmaceutical products on the market contain amines or amine derivatives, and several drugs have formed N-nitroso compounds when nitrosated in vitro and/or in vivo.[30] Dimethylnitrosamine has been present in all 68 samples of aminopyrine. They suggest that dimethylnitrosamine (DMN) might form in various drugs by the in situ reaction of aminopyrine with nitrogen oxides in the air or by the synthetic process. Recent studies show the formation of malignant tumours after feeding animals aminopyrine with sodium nitrite which form DMN.

It has been reported[30] that N-nitroso impurities are present in several herbicide formulations used by both home gardeners and farmers. Most of the herbicides which they examined were formulated as dimethylamine salt. It is not known if the DMN and DPN (dipropylnitrosamine) were present at the time of manufacture, or if they formed during storage. The volatile nature of these materials suggests inhalation and skin contact as the main way of absorption. Either the regular spraying of crops and land add a wide area for a hazard exposure, also workers exposed to a large amount of the aqueous solutions of herbicide might be at risk.

Workers in the rubber industry show an increased incidence of tumours of the bladder. Organic accelerators, carbon

black reinforcing agents, organic anti-oxidants, and miscellaneous groups which include plasticisers, softeners, dyes tuffs, these are four main groups of rubber additives.[30] Gorrod et al have tested four derivatives of nitrosamine; the tumours were seen in rats treated with N-methyl-N-4-dinitrosoaniline and N-nitroso-2,2,4-trimethyl-1-,2-dihydroquinoline.

1.4. Thionitrites

Thionitrites are derivatives of thiols, and they have nitroso groups attached to the sulphur atoms (RSNO). The bond between sulphur and nitroso groups is not as strong as in N-nitrosamines, so many of the thionitrites are unstable, probably due to homolytic fission.

The preparation of thionitrites have been described. In 1919,[31] triphenylmethyl thionitrite was prepared from triphenylmethanthiol with nitrous acid. It is green in colour, crystalline at room temperature and stable for several days if it is stored in a refrigerator. Zwet and Koogman,[31] have studied the thermal decomposition of Ph_3CSNO; the product of this decomposition was the disulphide and the colour turned to yellow, due to formation of NO_2. Also they found that, after decomposition of Ph_3CSNO, it was possible to obtain this thionitrite again by storing it in the dark at $0^{o}c$.

In 1926, t-butyl and ethyl thionitrite were made. The tertiary compounds are more stable than the secondary and primary. The perfluorination of the alkyl nitroso-compounds stabilizes the thionitrite, but the stability of thionitrites is decreased with increasing negative inductive effect of R.

The strong negative inductive effect in thionitrite caused the formation of disulphide, but in N-nitroso compounds it does not form NN dimerisation.

Trifluoromethyl thionitrite, CF_3SNO, which is a red gas boiling at about -3^oC was made in 1969[32] from $Hg(SCF_3)_2$ with NOCl and also by the reaction of CF_3SH with alkyl nitrites or NOCl, all at low temperatures. It gives the disulphide $CF_3S \cdot SCF_3$ and NO, when it decomposes.

It has also been found[33] that both arene and alkane-thiols react rapidly with an equimolar amount of N_2O_4 at -10^oC in an inert solvent such as CCl_4, hexane, ether or acetonitrile, to form corresponding thionitrite. Their decomposition was faster at higher concentration in solution and gave nitric oxides and corresponding disulphide, which is formed by the intermolecular reaction of thionitrite with athiyl by homolytic fission of the sulphur-nitrogen bond. Oae[34] has recently carried out reactions of thionitrite with other thiols and sulphinic acids to yield the corresponding unsymmetrical disulphide or thiolsulphonates in a good yield.

The reaction of a thionitrite with an alcohol gave O-nitroso compounds and with secondary amines N-nitroso compounds were formed but only slowly and in very low yield. It has been shown that thionitrites are photochemically unstable, so it is possible to add thiyl radicals ($R\dot{S}$) to double bonds in olefins,[35,36,37] and to dienes[38] to give sulphur containing C-nitroso compounds.

Finally, Field[39] has prepared N-acetyl-S-nitroso, D,L, penicillamine from N-acetyl-D,L penicillamine with nitrous acid. This thionitrite is the most stable so far found (9.5

months). It forms deep green crystals, and gives the disulphide 100% and NO after refluxing it in methanol for two hours. Also it decomposes in solution in light. This compound has no carcinogenic effects.[40] For other thionitrites there are no studies about their carcinogenicity. Others[16] have said that thiols remove nitrite from the environment and form S-nitroso thiols, which are relatively stable, but they become less so as the pH rises.

1.5 Mechanism of Denitrosation:

There are two classes of organic N-nitroso compounds, namely N-nitrosoamines and N-nitrosamides; the two classes differ in their stabilities, their basicities and the mechanism of their carcinogenicities.[17]

Recently[41,42,43] studies on the mechanisms of denitrosation of N-nitrosoamides in acid solution have been reported. It has been established[41,42,43] that the decomposition of nitroso amides in acid solution produce either denitrosation or deamination. The deamination reaction occurs at low acid concentration, and proceeds through the O-conjugate acid formed in a rapid pre-equilibrium step. Denitrosation predominates at high acidity and is believed to involve rate limiting protonation of the amino nitrogen atom, followed by decomposition to give ($\overset{+}{N}O$) and the corresponding amide.

$$RR'NNO + H^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} RR'\overset{+}{N}HNO$$

$$RR'\overset{+}{N}HNO + \bar{Y} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} RR'NH + NOY$$

$$NOY + X \xrightarrow{k_3} \text{decomposition products}$$

Scheme 9

1.5.a. Effect of Added Nitrite Traps

Reversible reaction of denitrosation can be prevented by adding nitrite traps which act as inhibitors. The denitrosation of nitrosoamides are irreversible in presence or absence of nitrite trap,[41,42,43,44] but the denitrosation of N-nitrosoamines (N-methyl-N-nitrosoaniline and N-nitrosodiphenylamine) were reversible, with the equilibrium lying well over to the nitrosation side. When $k_3[X] \gg k\text{-}2[\text{amine}]$ the overall first order rate constant k_o is given by the following equation:

$$k_o = \frac{k_1}{k_{-1}} k_2 [H^+] [\bar{Y}]$$

For each nitrosamine the concentration of [X] required to suppress the reverse reaction will depend upon the rate of N-nitrosation of the corresponding amine, and will therefore range from one nitrosamine to another. The efficiencies of the various traps have been established (by a kinetic analysis method[42,43]) as $HN_3 \backsim NH_2NH_2 > NH_2SO_3H > (NH_2)_2CO$.

1.5.b Acid Catalysis:

The denitrosation of nitrosoamides has been shown[42,43,44] to be first order in acid concentration and may occur either by a) unimolecular reaction by loss of $N\overset{+}{O}$ from the protonated nitrosoamide, or b) bimolecular reaction between the hydrogen bonded complex of the protonated form and a nucleophile, or c) bimolecular reaction between the protonated form and the nucleophile.

Denitrosation of N-nitrosoacetamide[41] was more strongly acid catalysed than deamination; also general acid catalysis was found to be operative [k (H_2SO_4) > k ($HClO_4$)]. In aqueous buffer solution Bronsted general acid catalysis was observed for N-nitroso-2-pyrrolidone.[42] For N-methyl-N-nitroso-p-toluenesulphonamide (MNTS), acid catalysis was observed for the dichloroacetic acid reaction, and there were slightly different reactivities shown by different acids, the reaction with $HClO_4$ + NaCl was faster than for HCl and H_2SO_4.[43] Similar behaviour was found for denitrosation of MNTS in ethanol solvent as in water and the reactivity in water was greater by a factor Ca. 10^2.[44]

1.5.c Nucleophile Catalysis:

Nucleophile catalysis plays an important role in the denitrosation reaction. In acid solution, nucleophile ions attack the protonated form of the N-nitrosocompound to form the corresponding amine and a nitrosating agent (NOY), the latter reacting with a nitrous acid trap to give various products. N-nitrosoamides show no halide ion catalysis.[41,42,43,44] The reactivity of nucleophiles towards N-nitrosoamine in acid solution has been examined and the following sequence was established quantitatively $H_2O < Cl^- < Br^- < SCN^- < SC(NH)_2$ I^-[46,47].

The general expression for k_o from Scheme 9 is as follows:

$$k_o = \frac{k_1 k_2 [H^+][\bar{Y}]}{k_{-1} + k_2[Y]} \qquad \text{if } k_3[X] \gg k_{-2}[R^1 R^{11} NL]$$

Nucleophilic catalysis by $[\bar{Y}]$ will only be observed if $k_{-1} \gg k_2[\bar{Y}]$ so that k_o becomes $k_1k_2[H^+][\bar{Y}]/k_{-1}$. At the other limiting condition of $k_{-1} \ll k_2[\bar{Y}]$ k_o reduces to $k_1[H^+]$ and no nucleophilic catalysis should occur. Acid catalysis should be present under all conditions. Curved plots of k_o vs $[\bar{Y}]$ have been observed for the reaction of N-nitroso-diphenylamine[48] and N-methyl-N-nitrosoaniline.[49] This behaviour is consistent with scheme 9 and a change from one limiting form to another. In general the reciprocal form should show a linear dependence of $(k_o)^{-1}$ upon $(\bar{Y})^{-1}$ at constant acidity.

$$k_o^{-1} = k_{-1}/k_1k_2\,[H^+][\bar{Y}] + 1/k_1[H^+]$$

In ethanol solution no nucleophilic catalysis is observed for NMNA or NNDA under any conditions and it has been argued that here $k_{-1} \ll k_2[\bar{Y}]$. A gradual change in the solvent composition from ethanol to water produces a decrease in the rate constant for denitrosation of NMNA and nucleophilic catalysis gradually become evident.[44]

When full nucleophilic catalysis occurs ($k_o \propto [Y^-]$) there is a good correlation between the reactivity and the Pearson nucleophilicity parameter. This was found for N-methyl-N-nitrosoaniline (slope 1.41)[47], and also for N-nitrosodiphenylamine (slope 0.95).[50] The latter is the more reactive (by a factor of about 100) and the lower slope is to be expected from reactivity-selectivity arguments.

1.5.d Solvent Isotope Effect

This has been determined for the denitrosation reaction in deuterium oxide for both nitrosoamines and nitrosoamides. For reaction in sulphuric acid, the ratio $(k_o)D_2O : (k_o)H_2O$ was found[46,47] to be, 2.9 for NMNA, and 2.0 for the NNDP reaction, under conditions where nucleophilic catalysis is also observed. These results are consistent with a rapid equilibrium protonation of the nitrosoamines, followed by rate determining nucleophilic attack. The reaction of MNTS was slower in deuterium oxide by a factor of 1.5[43] as was the denitrosation of nitrosamides.[41,42] Similarly in ethanol solution reaction was faster in EtOH than in EtOD by factor between 2.6 and 3.8.[44] It appears that where nucleophilic catalysis occurs a normal solvent isotope effect is also found ($k_{D_2O} > k_{H_2O}$) whereas when there is no nucleophilic catalysis the reverse is true. This is consistent with the mechanism outlined in scheme 9 and is associated with the two limiting forms $k_{-1} \gg k_2[\bar{Y}]$ and $k_{-1} \ll k_2[\bar{Y}]$.

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CHAPTER 2

KINETICS AND MECHANISM OF THE DENITROSATION OF N-METHYL-N-NITROSOANILINE

Denitrosation of N-methyl-N-nitrosoaniline takes place in acid solution and with an efficient nucleophile which forms (NOY) nitrosyl halide this can be removed as it is formed by nitrite traps.[1] The results are consistent with the scheme (1) below.

$$N + H^+ \rightleftharpoons {}^{+}\!NH \qquad \text{Rapid equilibrium}$$

$$\overset{+}{N}H + \bar{Y} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} NMA + NOY$$

$$NOY + X \xrightarrow{k_3} \text{Various Decomposition Products}$$

N = $PhN(Me)NO$

$\overset{+}{N}H$ = $Ph\ \overset{+}{N}H(Me)NO$

NMA = Ph NMeH

$\bar{Y}$ = Nucleophiles such as $\bar{C}l$, $\bar{B}r$, $\bar{S}CN$, $SC(NH_2)_2$ and $\bar{I}$

X = Nitrite traps such as HN_3, NH_2NH_2, NH_2SO_3H, NH_2OH and $CO(NH_2)_2$

Scheme 1

To ensure complete denitrosation, $k_3[X]$ must be significantly (10 or 20 times) greater than k_{-2} [NMA]. This can readily be achieved for many of the nitrite traps used.

The reaction shows first order behaviour in $[\bar{Y}]$ for a range nucleophiles and correlations with the Pearson nucleophilicity parameter n have been established.[2,3] However, at high $[\bar{Y}]$, it had been noticed[4,5] that the reaction loses its first-order dependence upon $[\bar{Y}]$ i.e. k_o vs $[\bar{Y}]$ plots

become curved. Challis and Osborne[6] had noticed this effect also, and it has been found that nitrosoamides[7] and a nitrososulphonamide[8,9] all show[10] no nucleophilic catalysis. It was the aim of this work to examine this aspect further. At high $[\bar{Y}]$ it is possible that $k_2[Y^-]$ competes effectively with the deprotonation of $[\overset{+}{N}H]$, in the first step the scheme (1) has to be modified as below (scheme 2).

$$N + H^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \overset{+}{N}H$$

$$\overset{+}{N}H + Y^- \xrightarrow{k_2} NMA + NOY \xrightarrow{} \text{Removed}$$

Scheme 2

We assume that sufficient nitrite trap is present so that the overall reaction is irreversible. A steady-state treatment on $\overset{+}{N}H$ can then be carried out.

The first order rate constant k_o is defined by

$-d[\text{Nitrosoamine}]/dt = k_o [\text{Nitrosoamine}]$.

$$\frac{d[\overset{+}{N}H]}{dt} = 0$$

Rate of formation of $[\overset{+}{N}H] = k_1[N][H^+]$

Rate of reaction of $[\overset{+}{N}H] = k_{-1}[\overset{+}{N}H] + k_2[\overset{+}{N}H][Y^-]$

$\therefore k_1[N]NH^+] = k_{-1}[\overset{+}{N}H] + k_2[\overset{+}{N}H][\bar{Y}]$

$$\therefore [\overset{+}{N}H] = \frac{k_1[N][H^+]}{k_{-1} + k_2[\bar{Y}]}$$

Overall reaction rate $= k_2[\overset{+}{N}H][\bar{Y}]$

$$= \frac{k_2[Y^-]k_1[N][H^+]}{k_{-1} + k_2[Y^-]}$$

$$= k_o[N]$$

where k_o is the observed first order rate constant

$$\therefore k_o = \frac{k_1[H^+]\ k_2\ [Y^-]}{k_{-1} + k_2[Y^-]} \qquad (1)$$

This can be used experimentally by using equation (1) in the reciprocal form.

$$\frac{1}{k_o} = \frac{k_{-1}}{k_1[H^+]k_2[Y^-]} + \frac{1}{k_1[H^+]} \qquad (2)$$

A plot of $(k_o)^{-1}$ vs $[Y^-]^{-1}$ should be linear with slope equal $k_{-1}/k_1[H^+]k_2$ and intercept = $1/k_1[H^+]$.

2.1 Effect of Added Nitrite Trap (X)

Denitrosation of N-methyl-N-nitrosoaniline is reversible, a nitrite trap is added to capture any free nitrosating agent (NOY) from the equilibrium system, so this nitrite trap suppresses the reversible reaction. For studying the catalysis of the denitrosation of NMNA by various nucleophiles, we have to work at high concentrations of nitrite trap to ensure that $k_3[X] \gg k_{-2}[NMA]$.

The sequence of different reactivities of nitrite traps using NMNA has been found to be[11,12] $HN_3 > NH_2SO_3H > PhNH_2 > NH_2OH > CO(NH_2)_2$. This means various nitrite traps remove the nitrosating agent NOY by different rates.

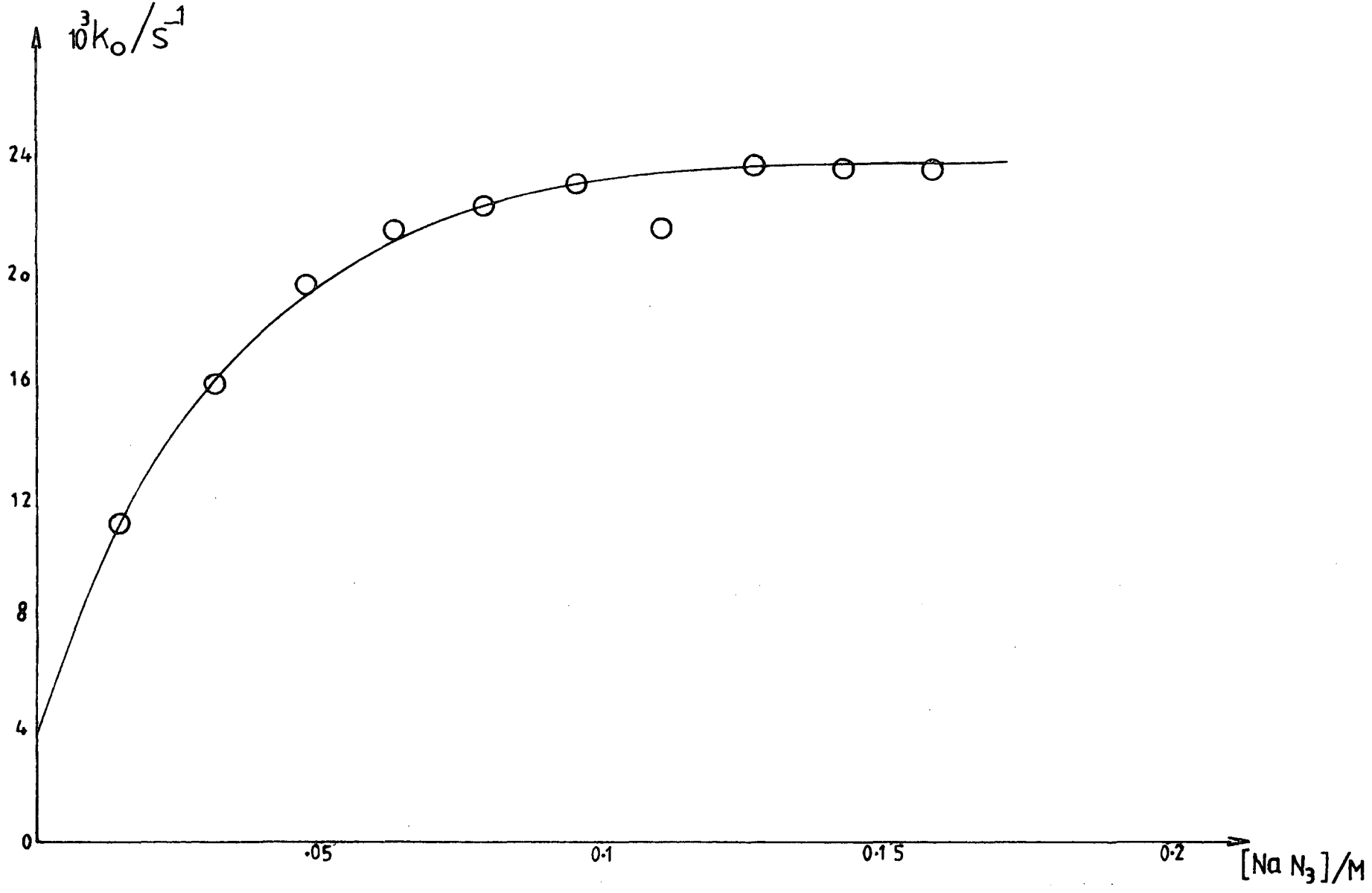
Recently others[13] have studied the relative efficiencies of different nitrite traps at different acidities and at different bromide ion concentration, the same sequence of

reactivity of nitrite traps was obtained for many of the traps although the ratios k_2/k_{-1} depend to some extent on $[H^+]$ and $[B\bar{r}]$.

For all the nitrite traps, the limiting value at high concentration was easily reached for N-methyl-N-nitrosoaniline, but for N-nitrosodiphenylamine it was not possible. This is because of the different basicities of the free base of the two products of denitrosation, diphenylamine and N-methyl-aniline. Diph enylamine has a very much lower basicity than N-methylaniline, so the reverse reaction (N-nitrosation is faster for NNDA than for NMNA). This means that the denitrosation of NNDA needs[11] higher concentration of nitrite trap to reach the limiting condition. The results have been obtained for denitrosation of N-nitroso-diphenylamine in sulphuric acid in the presence of sodium azide Figure (1) for this reaction shows k_o levelling off and the reaction becoming zero-order in added NaN_3. The limit occurs at about 0.16M added NaN_3.[11]

Our work was carried out for the denitrosation of N-methyl-N-nitrosoaniline (3.32×10^{-4}M) in sulphuric acid (.36 - 1.72M) containing various nucleophiles in the presence of sodium azide, the limiting value for sodium azide was 1.93×10^{-3}M. Incidentally, when we were using sulphamic acid as a nitrite trap with SCN^- the results were not at all as expected and there was evidence of interference by the sulphamic acid.

FIGURE 1



2.2 Effect of added Nucleophiles ($\bar{Y}$)

Denitrosation of N-methyl-N-nitrosoaniline at low nucleophile concentration and in water solvent, has been shown to involve rate-determining attack by a nucleophile at the nitroso nitrogen atom of the protonated form of the nitrosoaniline; this step, normally reversible cam be examined kinetically, if there is present sufficient excess nitrite trap $k_3[X] \gg k_{-2}$ [NMA] which removes the free nitrosating agent (NOY) as soon as it is formed.

At higher $[Y^-]$ values curved plots of k_o vs $[Y^-]$ have been observed. This can be explained if in scheme 2 the rate of step k_{-1} is comparable with that of k_2. Quantitatively this means (via equation 2) that $(k_o)^{-1}$ should be proportional to $[Y^-]^{-1}$ at constant acidity. This enables $k_1[H^+]$ and k_{-1}/k_2 to be obtained from the intercept and slope of the $(k_o)^{-1}$ vs $[Y^-]^{-1}$plot.

We set out to examine this relation (equation 2) more widely for each of the nucleophiles $B\bar{r}$, SCN^-, $SC(NH_2)_2$ and I^-, (containing varying amounts of them) at each of these acidities (.36-1.72M H_2SO_4) and at high concentration of sodium azide (1.93×10^{-3}M).

Good straight lines were obtained in each case. The results are summarized in Tables 1 to 12. Figure (2) illustrates (k_o vs $SC(NH_2)_2$) the lines for thiourea at different acidities (.36, .74 and 1.55M H_2SO_4).

Previously[1] the reaction was found to be first order in $[\bar{Y}]$ for $[Y^-] = C\bar{l}$, $B\bar{r}$, $S\bar{CN}$, $SC(NH_2)_2$. At high $[Y^-]$ in other cases[4] k_o levelled off at high $B\bar{r}$ using N-nitrosodiphenylamine.

$Ph(Me)N.NO = 3.32 \times 10^{-4} M$, $NaN_3 = 1.93 \times 10^{-3} M$.

Table 1

$[Br^-]$ M	$10^4 k_o$ S^{-1}
1.29	25.4
1.50	60.4
1.78	38.8
2.14	48.6
2.28	51.4
2.42	54.0
2.57	65.7
2.71	60.5
2.85	65.0

Variation of k_o with $[Br^-]$ at .64M H_2SO_4

Table 2

$[Br^-]$ M	$10^4 k_o$ S^{-1}
1.29	44.9
1.50	53.0
1.78	79.0
2.14	90.5
2.28	96.8
2.42	100.0
2.57	106.0
2.71	113.0
2.85	118.0

Variation of k_o with $[Br^-]$ at .943M H_2SO_4

Table 3

$[Br^-]$ M	$10^4 k_o$ S^{-1}
1.29	193
1.50	167
1.78	252
2.14	271
2.28	322
2.42	337
2.57	240
2.71	362
2.85	429

Variation of k_o with $[Br^-]$ at 1.72M H_2SO_4

$Ph(Me)N.NO = 3.32 \times 10^{-4}$ M, $NaN_3 = 1.93 \times 10^{-3}$ M

Table 4

$10^3[S\bar{C}N]$ M	$10^4 k_o$ S^{-1}
30.0	35
40.0	43
50.0	50
100.0	67
200.0	94
300.0	104
400.0	93
500.0	117
598.0	121
799.0	128

Variation of k_o with $[S\bar{C}N]$ at .36M H_2SO_4

Table 5

$10^3[S\bar{C}N]$ M	$10^4 k_o$ S^{-1}
10.0	27
20.0	54
30.0	72
40.0	94
50.0	106
63.2	126
126.0	178
190.0	193
253.0	224
316.0	239

Variation of k_o with $[S\bar{C}N]$ at .74M H_2SO_4

Table 6

$10^3[S\bar{C}N]$ M	10^4 k_o S^{-1}
10.0	80
20.0	153
30.0	212
40.0	272
50.0	313
63.2	366
126	491
190	561
253	621
316	691

Variation of k_o with $[S\bar{C}N]$ at 1.55M H_2SO_4

Ph(Me) N.NO = 3.32×10^{-4}M, NaN_3 = 1.93×10^{-3}M

Table 7

$10^3[SC(NH_2)_2]$M	$10^4 k_o$ S^{-1}
2.04	9.36
3.06	13.4
4.08	17.2
5.11	19.7
10.0	30.8
20.0	55.2
30.0	68.5
40.0	78.6
50.0	87.5

Variation of k_o with $[SC(NH_2)_2]$ at .36M H_2SO_4

Table 8

$10^3[SC(NH_2)_2]$M	$10^4 k_o$ S^{-1}
1.02	10
2.04	20
3.06	30
4.08	38
5.10	43
20.0	119
30.0	154
40.0	164
50.0	196

Variation of k_o with $[SC(NH_2)_2]$ at .73M H_2SO_4

Table 9

$10^3[SC(NH_2)_2]$M	$10^4 k_o$ S^{-1}
1.02	29
2.04	57
3.06	81
5.10	128
10.0	192
20.0	336
30.0	337
40.0	520
50.0	557

Variation of k_o with $[SC(NH_2)_2]$ at 1.55M H_2SO_4

$Ph(Me)N.NO = 3.32 \times 10^{-4}M$, $NaN_3 = 1.93 \times 10^{-3}M$

Table 10

$10^3[\bar{I}]$ M	$10^4 k_o$ S^{-1}
1.0	5.22
2.0	8.10
3.0	12.1
4.0	14.9
5.0	18.3
10.0	26.9
20.0	52.0
30.0	62.3
40.0	75.3
50.0	79.7

Variation of k_o with $[\bar{I}]$ at .36M H_2SO_4

Table 11

$10^3[\bar{I}]$ M	$10^4 k_o$ S^{-1}
1.0	12.3
2.0	18.7
3.0	26.8
4.0	32.5
5.0	34.9
10.0	60.1
20.0	101
30.0	161
40.0	146
50.0	220

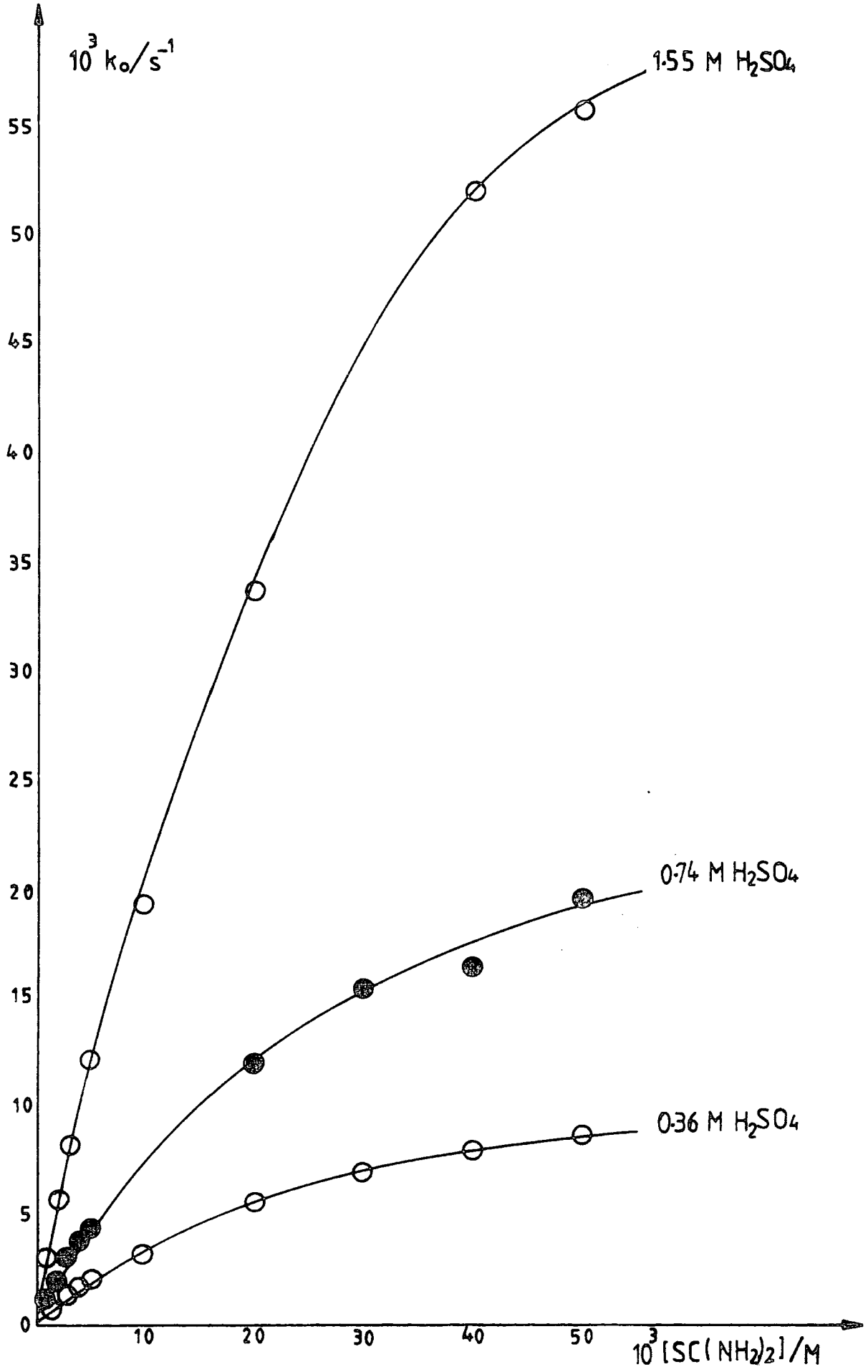
Variation of k_o with $[\bar{I}]$ at .74M H_2SO_4

Table 12

10^3 $[\bar{I}]$M	$10^4 k_o$ S^{-1}
1.0	23.0
2.0	48.3
3.0	74.0
4.0	105
5.0	104
10.0	170
20.0	276
30.0	391
40.0	472
50.0	570

Variation of k_o with $[\bar{I}]$ at 1.55M H_2SO_4

FIGURE 2


$10^3 k_o/s^{-1}$
1·55 M H_2SO_4
0·74 M H_2SO_4
0·36 M H_2SO_4
0
5
10
15
20
25
30
35
40
45
50
55
10
20
30
40
50
$10^3[SC(NH_2)_2]/M$

Also it has been found that the same effects are obtained at high [$SC\bar{N}$] and $SC(NH_2)_2$ for denitrosation of N-methyl-N-nitrosoaniline.[5] This finding agrees with our results for $SC\bar{N}$ and $SC(NH_2)_2$. It appears that at high [$\bar{Y}$] the other limiting form of equation(1) begins to be evident, i.e. $k_o = k_1[H^+]$, first-order in [H^+] and zero-order in [$\bar{Y}$].

Others[14] have found out that the denitrosation of aromatic nitroso amine in presence of hydrochloric acid is faster than in sulphuric acid. Also it was claimed that two mechanisms were operating, one involving nucleophilic attack by anion HSO_4^- or $C\bar{I}$ on a hydrogen bond between nitroso-amine and acid, the other mechanism requires unimolecular fission of such complex to give the secondary amine and free $N\overset{+}{O}$, but[4] the fast protonation is followed by rate-determining nucleophilic attack by a halide ion. The nitrosyl halide is removed effectively by reaction with nitrite trap. There is no evidence of direct reaction between nitrite trap and the protonated nitrosoamine.[1]

The figures 3 to 5 illustrate the relationship between $(k_o)^{-1}$ and $[Y^-]^{-1}$, and the results are summarized in Tables 13 to 16 for each nucleophile at different acidities.

The slopes ($k_{-1}/k_1k_2[H^+]$) increase as the acidity decreases as expected and the slopes have the smallest values for iodide and thiourea, and the biggest for bromide at the same acidity. This is due to the fact that iodide and thiourea are expected to be the most powerful nucleophiles followed by $SC\bar{N}$ and $B\bar{r}$.

It has been found that the following order of reactivity of nucleophiles was established quantitatively[12,1]

$H_2O < C\bar{I} < B\bar{r} < SC\bar{N} < SC(NH_2)_2 < \bar{I}$.

FIGURE 3

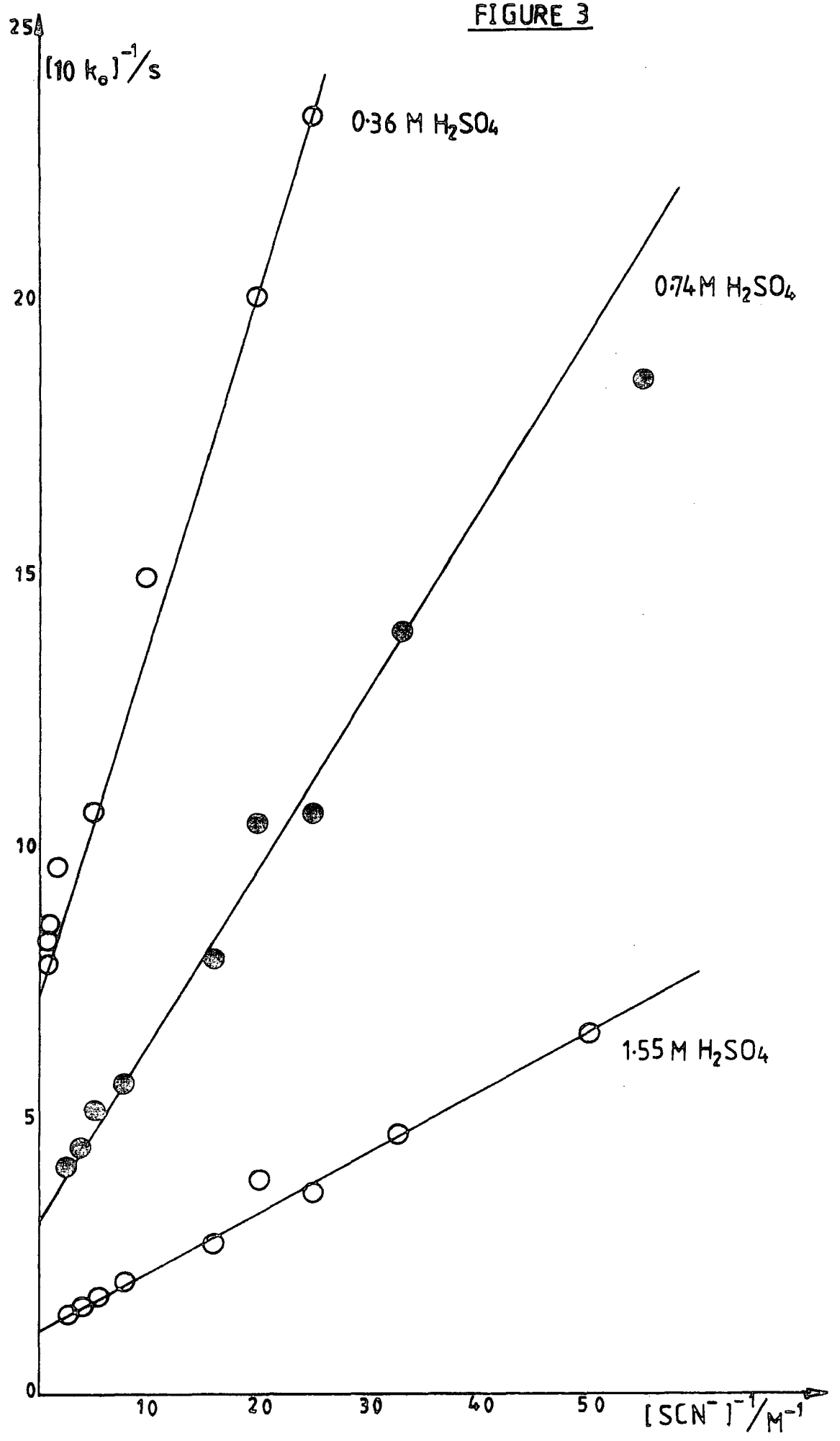

25
20
15
10
5
0
$[10\ k_o]^{-1}/s$
0·36 M H_2SO_4
0·74 M H_2SO_4
1·55 M H_2SO_4
10
20
30
40
50
$[SCN^-]^{-1}/M^{-1}$

FIGURE 4

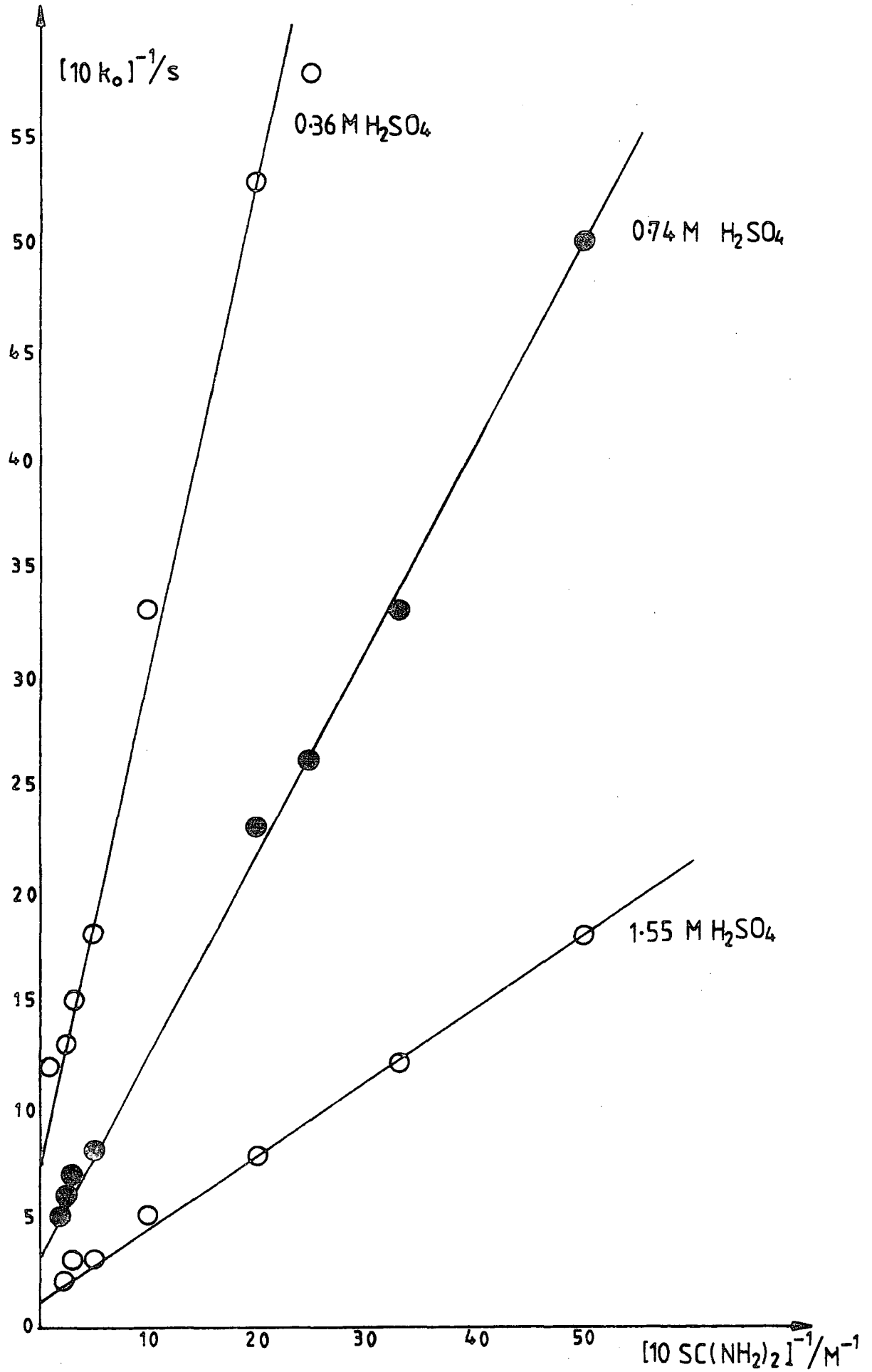

[10 k$_o$]$^{-1}$/s
0·36 M H_2SO_4
0·74 M H_2SO_4
1·55 M H_2SO_4
55
50
45
40
35
30
25
20
15
10
5
0
10
20
30
40
50
[10 SC(NH_2)$_2$]$^{-1}$/M^{-1}

FIGURE 5

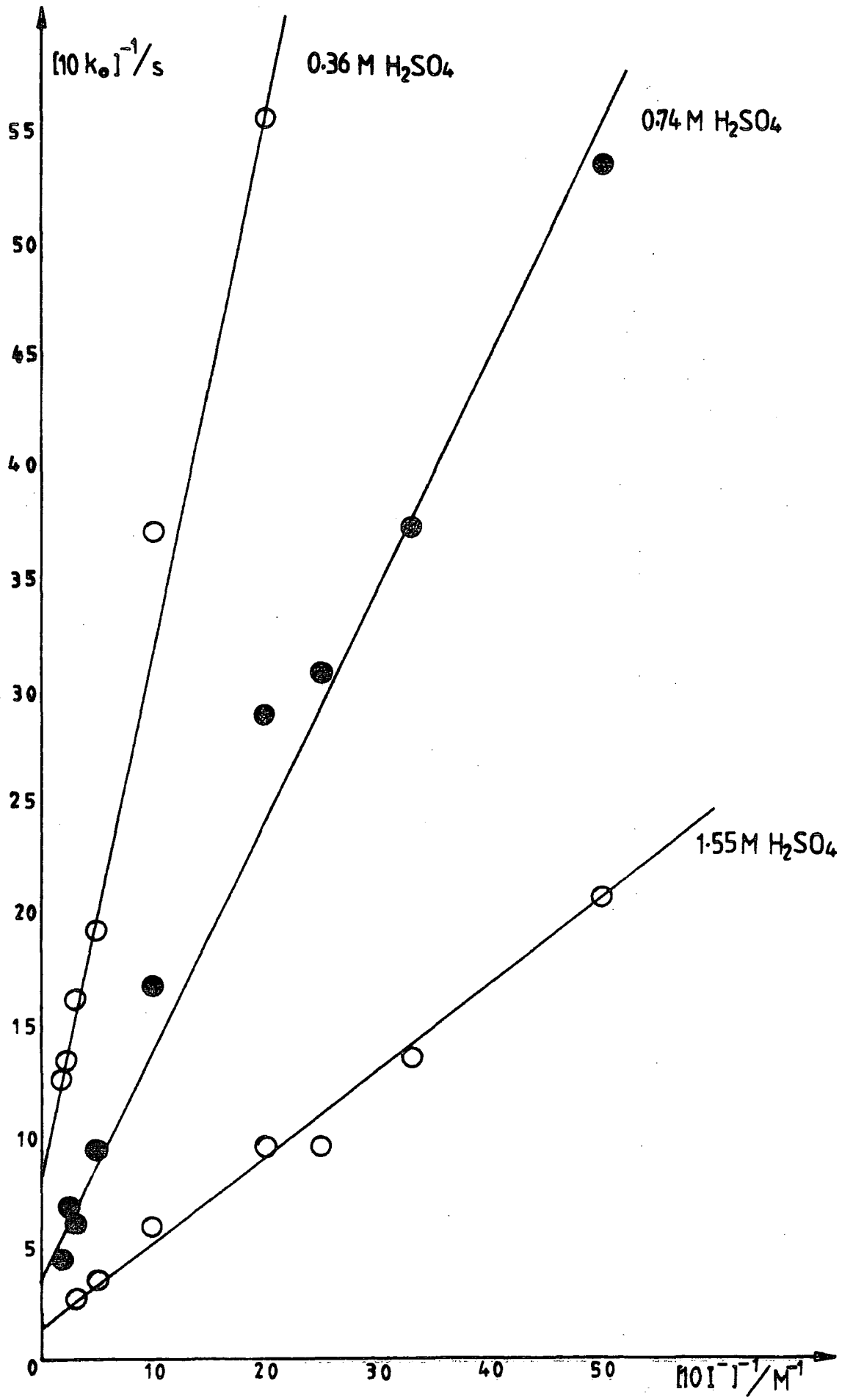

[10 k₀]⁻¹/s
0.36 M H₂SO₄
0·74 M H₂SO₄
1·55 M H₂SO₄
55
50
45
40
35
30
25
20
15
10
5
0
10
20
30
40
50
[10 I⁻]⁻¹/M⁻¹

Table 13

Acid Concentration	Values for Bromide ion
.64 M H_2SO_4	Slope = 351
	$1/k_1[H^+]$ = 40
	$k_1[H^+]$ = 2.5 x $10^{-2}/S^{-1}$
	k_{-1}/k_2 = 8.75
.94M H_2SO_4	Slope = 189.6
	$1/k_1[H^+]$ = 21.5
	$k_1[H^+]$ = 4.6 x $10^{-2}/S^{-1}$
	k_{-1}/k_2 = 8.81
1.72M H_2SO_4	Slope = 58.3
	$1/k_1[H^+]$ = 6.7
	$k_1[H^+]$ = 14.9 x $10^{-2}/S^{-1}$
	k_{-1}/k_2 = 8.71
	Av.of k_{-1}/k_2 = 8.76

Values of slopes, intercepts and ratio k_{-1}/k_2 for $[B\bar{r}]$ in different acidities, Ph(Me)N.NO = 3.32 x 10^{-4}M NaN_3 1.93 x 10^{-3}M

Table 14

Acid Concentration	Values for Thiocyanate ion
.36M H_2SO_4	Slope = 6.55 $1/k_1[H^+]$ = 72 $k_1[H^+]$ = $1.39 \times 10^{-2}/S^{-1}$ k_{-1}/k_2 = 9.10×10^{-2}
.74M H_2SO_4	Slope = 3.15 $1/k_1[H^+]$ = 32 $k_1[H^+]$ = $3.13 \times 10^{-2}/S^{-1}$ k_{-1}/k_2 = 9.85×10^{-2}
1.55M H_2SO_4	Slope = 1.08 $1/k_1[H^+]$ = 11.2 $k_1[H^+]$ = $8.93 \times 10^{-2}/S^{-1}$ k_{-1}/k_2 = 9.64×10^{-2} Av.of k_{-1}/k_2 = 9.53×10^{-2}

Values of slopes, intercepts and ratio k_{-1}/k_2 for $SC\bar{N}$ in different acidities, Ph(Me)N.NO = 3.32×10^{-4}M, NaN_3 = 1.923×10^{-3}M.

Table 15

Acid Concentration	Values for thiourea
.36M H_2SO_4	Slope = 2.19 $1/k_1[H^+]$ = 75 $k_1[H^+]$ = $1.33 \times 10^{-2}/s^{-1}$ k_{-1}/k_2 = 2.92×10^{-2}
.74M H_2SO_4	Slope = .967 $1/k_1[H^+]$ = 31 $k_1[H^+]$ = $3.23 \times 10^{-2}/s^{-1}$ k_{-1}/k_2 = 3.12×10^{-2}
1.55M H_2SO_4	Slope = .341 $1/k_1[H^+]$ = 11 $k_1[H^+]$ = $9.09 \times 10^{-2}/s^{-1}$ k_{-1}/k_2 = 3.10×10^{-2} Av.of k_{-1}/k_2 = 3.05×10^{-2}

Values of slopes, intercept and ratio k_{-1}/k_2 for $SC(NH_2)_2$ at different acidities, $Ph(Me)N.NO = 3.32 \times 10^{-4}M$, $NaN_3 = 1.93 \times 10^{-3}M$

Table 16

Acid Concentration	Values for Iodide ion
.36M H_2SO_4	Slope = 2.32 $1/k_1[H^+]$ = 80 $k_1[H^+]$ = $1.25 \times 10^{-2}/S^{-1}$ k_{-1}/k_2 = 2.88×10^{-2}
.74M H_2SO_4	Slope = 1.09 $1/k_1[H^+]$ = 35 $k_1[H^+]$ = $2.86 \times 10^{-2}/S^{-1}$ k_{-1}/k_2 = 3.09×10^{-2}
1.55M H_2SO_4	Slope = .376 $1/k_1[H^+]$ = 13 $k_1[H^+]$ = $7.69 \times 10^{-2} S^{-1}$ k_{-1}/k_2 = 2.90×10^{-2} Av.of k_{-1}/k_2 = 2.95×10^{-2}

Values of slopes, intercept and ratio k_{-1}/k_2 for $[I^-]$, at different acidities, Ph(Me)N.NO = 3.32×10^{-4}M, NaN_3 1.93×10^{-3}M

The reaction of denitrosation of N-nitrosamine with nucleophiles can be compared with conventional S_N2 substitution at saturated carbon. The Pearson n values for these nucleophiles[2] are [F^- 2.7, Cl^- 4.37, Br^- 5.79, I^- 7.42, SCN^- 6.70 and $SC(NH_2)_2$ 7.27]. It was found[1] that the log k_1K values obtained for denitrosation of N-methyl-N-nitrosoaniline by various nucleophiles correlated very well with n Pearson values.[2] These were originally determined from rate constants for conventional substitution at saturated carbon atom. The slope of the line is 1.33. A similar correlation has been found[12] for the same nitrosoamine with slope equal to 1.4. The slope of this correlation for NMNA was larger than the slopes generally found for the conventional S_N2 at saturated carbon. It was shown that the reactivity of nucleophile is important for attack at the nitroso-nitrogen atom. Figure 6 shows this correlation. Table 17 presents the results of the Pearson nucleophilicity parameter and 4 + log k_1K. The values k_1K are obtained from slope of straight line of correlation of k_o vs $[Y^-]$, which was measured at 1.55 M H_2SO_4 for all nucleophiles except for Br^- at 1.72 M H_2SO_4. This compared[4] with the correlation which was obtained for N-nitrosodiphenylamine with these nucleophiles, but there was some deviation for iodide and thiourea which could be due to a steric effect.

Table 17

Nucleophile	4 + log k_1^K	'n'
Bromide	2.09	5.79
Thiocyanate	3.83	6.70
Thiourea	4.41	7.27
Iodide	4.42	7.42

Values of k_1 $[H^+]$ obtained from the double reciprocal plots are constant, within the experimental error at any one acidity for the different nucleophiles, and increased with acidity as expected.

The ratio k_{-1}/k_2 is nearly the same for the same nucleophiles at different acidities. These values of the ratio are smallest for iodide, and thiourea, increasing for thiocyanate and bromide. The ratio $k_2[I^-]/k_2[SC(NH_2)_2]$ equal av.1.03, that indicates they have nearly the same reactivity, then the ratio $k_2[SC(NH_2)_2]/k_2[SCN^-]$ equal av. 3.1, it means that thiourea is about three times more powerful a nucleophile than SCN^-, and the ratio $k_2[SCN^-]/k_2[B\bar{r}]$ equal av. 92, it means that SCN^- is about 92 times more powerful a nucleophile than $B\bar{r}$. These are the ratios expected at least qualitatively from the nucleophilicities.

FIGURE 6

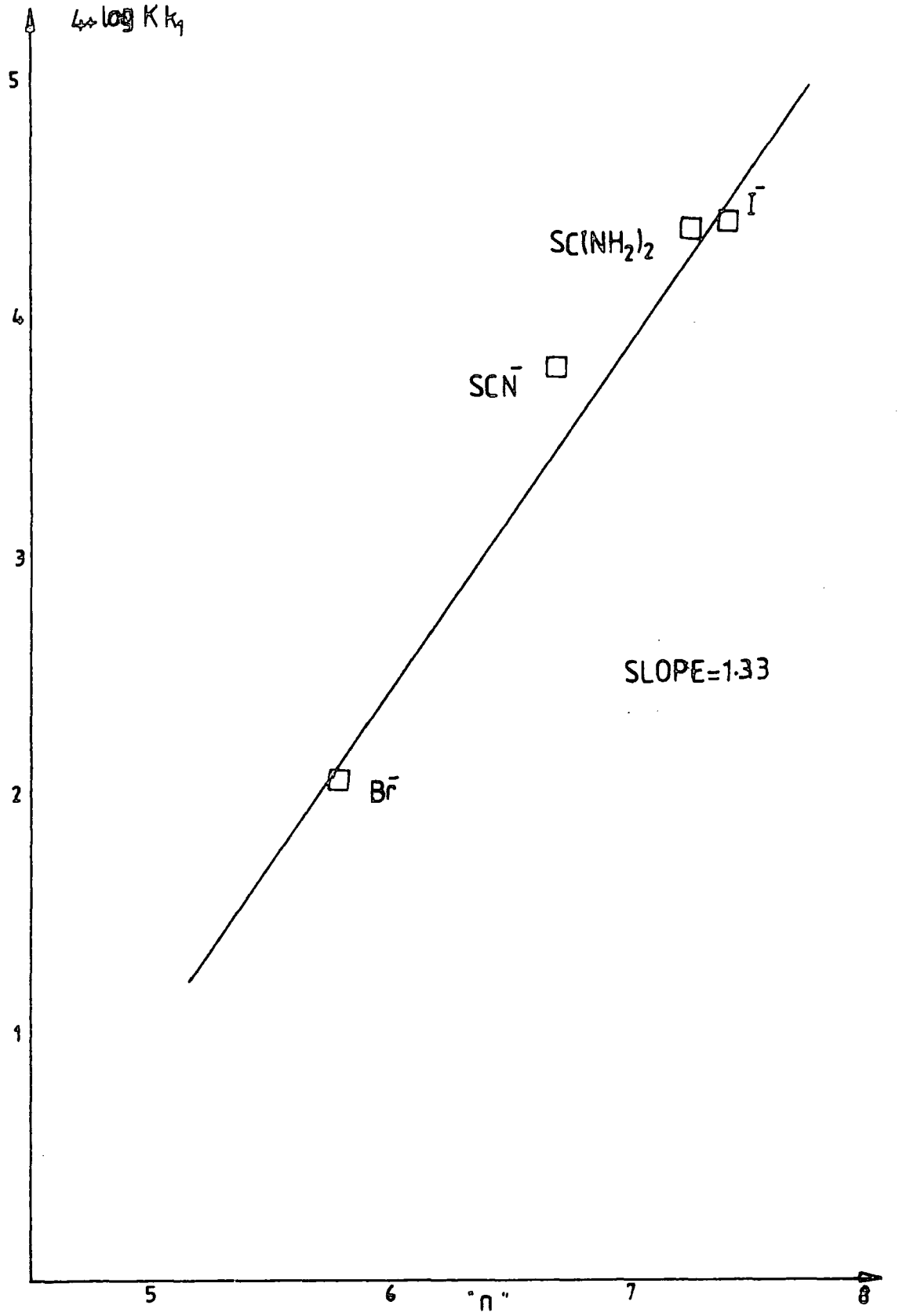


Table 18

$k_2[\bar{I}] / k_2[SC(NH_2)_2]$	1.03
$k_2[SC(NH_2)_2]/k_2[S\bar{C}N]$	3.10
$k_2[SCN^-]/k_2[B\bar{r}]$	92.0

2.3 Acid Catalysis

Denitrosation occurs in sulphuric acid at high concentration of nitrite trap, containing no added nucleophiles. In this case water behaves as a nucleophile in the rate-determining step, but at a much smaller rate constant than the reaction in the presence of nucleophile.

Denitrosation in hydrochloric acid was much faster than in sulphuric acid. It was found[1] that log k_o is proportional to the ho$[Cl^-]$; this was interpreted as rate-determining nucleophilic attack by chloride ion on the protonated form of nitrosoamine. Other results[11] were obtained for the reaction of NMNA in sulphuric acid containing different nucleophiles, the reaction was very sensitive to the nature of the nucleophile.

The results for denitrosation in sulphuric acid (.36 - 1.72M) are shown in Table 19 where the concentration of N-methyl-N-nitrosoaniline is 3.3×10^{-4}M, NaN_3 1.9×10^{-3}M and in the presence of various nucleophiles such as $B\bar{r}$, SCN^-, $SC(NH_2)_2$ and I^-.

Figure 7 shows the plots of $k_1[H^+]$ versus $[H^+]$ and $k_1[H^+]$ against $M(H_2SO_4)$. Both of them show marked upward curvature. But the plot of $k_1[H^+]$ against ho is linear over the whole acid range studied, with slope 2.9×10^{-2}, showing that the Hammett acidity function reasonably expresses the

protonation of this nitrosamine.

Table 19

$10^2 k_1 H^+$	M H^+	M H_2SO_4	ho
1.32	o.470	o.36	o.51
2.50	o.830	o.64	1.00
3.07	o.955	o.74	1.20
4.60	1.21	o.94	1.70
8.57	2.02	1.55	4.20
14.93	2.27	1.72	5.34

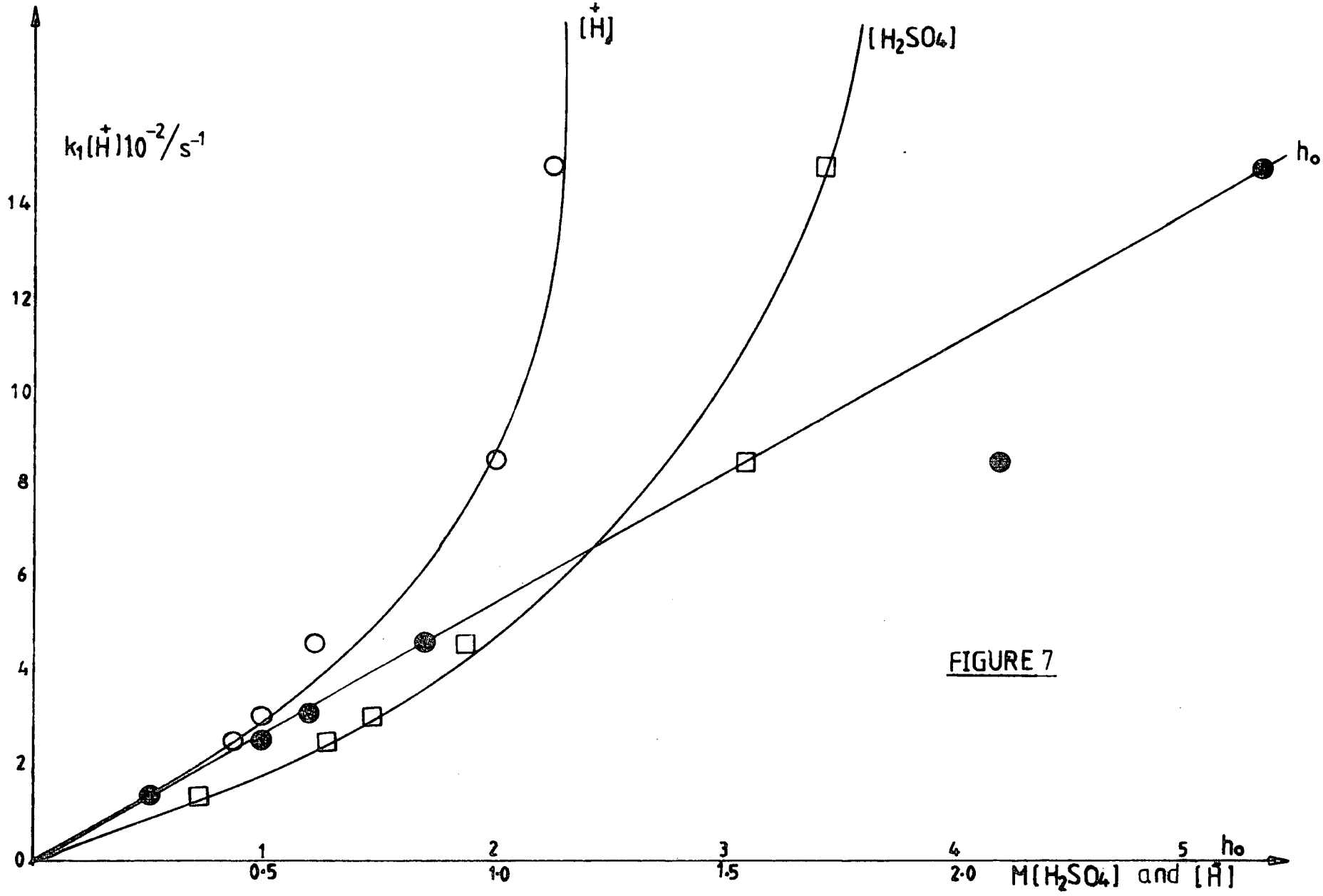

$k_1[\dot{H}]10^{-2}/s^{-1}$
14
12
10
8
6
4
2
0
$[\dot{H}]$
$[H_2SO_4]$
h_o
1
0·5
2
1·0
3
1·5
4
2·0
5
h_o
$M[H_2SO_4]$ and $[\dot{H}]$

FIGURE 7

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Chapter 3

KINETICS AND MECHANISM OF THE DENITROSATION OF N-ACETYL-S-NITROSO-D,L-PENICILLAMINE

N-acetyl-S-nitroso-D,L-penicillamine is a deep green solid; it was prepared from the thiol and nitrous acid as described by Field et al.[1] It is said to be relatively stable as a solid (9.5 months), whereas the colour of thionitrite solutions (RSNO/MeOH) disappeared slowly and gave disulphide on heating or on exposure to light.

The aim of this work was to examine in more mechanistic detail the chemistry of thionitrites which have not been previously investigated, particularly with respect to the trans-nitrosation reactions involving amines. The stable thionitrites may have some important role in reacting with ingested nitrite derived for example from foods and naturally occurring waters.[2]

The kinetics and mechanism were studied for the denitrosation reaction of N-acetyl-S-nitroso-D,L -penicillamine brought about by various nucleophilic compounds. Experimentally, reaction was marked by following the disappearance of the absorbance peak of 338nm due to the thionitrite (ϵ = 1044) in acid solution.

A possible mechanism is given below, by analogy with earlier work involving denitrosation of nitrosamines.[3,4]

$$RSNO + H^+ \rightleftharpoons R\overset{+}{S}HNO$$

$$R\overset{+}{S}HNO + Br^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} RSH + NOBr$$

$$NOBr + HN_3 \xrightarrow{k_2} N_2 + N_2O + Br^- + H^+$$

Scheme (1)

There are two possible sites for the protonation of the thionitrite at the sulphur and nitrogen atoms. Sulphur is the most likely site, since it has been shown[5] by n.m.r. that protonation of thioacetamide occurs on the sulphur atom, and also the same is true for thiourea, N-methylthiourea and thioacetanilide. Janssen[6] has found that the protonation of thioamides is also on sulphur atom by using u.v. and i.r. methods.

In this mechanism (scheme 1) protonation occurs initially at the sulphur atom in a rapid equilibrium, followed by nucleophilic attack on the nitroso group forming the thiol and nitrosyl bromide which react rapidly and irreversibly with hydrazoic acid to form N_2, N_2O, Br^- and H^+.

3.1 Effect of Added Nitrite Trap (X):

Denitrosation of the thionitrite occurred in acid solution. The reaction was reversible with equilibrium lying well over to the side of thionitrite. It had previously been found that when alcohols undergo O-nitrosation,[7,8] the reaction is reversible with equilibrium constant around 1-2 e.g. for methanol. In the present case of the thionitrite, it appears that the equilibrium constant for the formation of the thionitrite is much larger. No denitrosation of RSNO was observed in the absence of nitrite traps. This means that the system cannot be treated kinetically as a reversible process, as for the alcohols case. It is more convenient in this case to study the denitrosation of RSNO and the nitrosation of RSH separately. The latter reaction has been studied at low acidity,[9] where it is essentially irreversible, this work reports the results of the denitrosation process

which can only be studied at relatively high acidities.

Since the denitrosation of the thionitrite is reversible (the same as the denitrosation of many nitrosoamines) a well-known method is used, by adding a nitrite trap. Such traps have been used before in the nitrosoamine denitrosations[3,10] (HN_3, $NH_2\overset{+}{N}H_3$, NH_2SO_3H, $\overset{+}{N}H_3 \cdot OH$ and $CO[NH_2]_2$).

From scheme 1, using a steady state treatment for the reactive NOBr intermediate the following equations apply.

$$k_1[R\overset{+}{S}HNO][B\bar{r}] = k_{-1}[RSH][NOBr] + k_2[NOBr][HN_3]$$

$$\therefore [NOBr] = \frac{k_1[R\overset{+}{S}HNO][B\bar{r}]}{k_{-1}[RSH] + k_2[HN_3]}$$

It is assumed that the initial protonation is rapid, reversible, only proceeds to a very small extent and can be represented by the Hammett acidity function h_o, and equilibrium constant K.

$$\therefore [R\overset{+}{S}HNO] = [RSNO]\, h_o\, K$$

$$\therefore [NOBr] = \frac{k_1 K h_o [RSNO][B\bar{r}]}{k_{-1}[RSH] + k_2[HN_3]}$$

Now the overall rate of reaction can be expressed by $k_2[NOBr][HN_3]$

$$\therefore \text{Rate} = \frac{k_2 k_1 K h_o [RSNO][B\bar{r}][HN_3]}{k_{-1}[RSH] + k_2[HN_3]}$$

If we define a first order rate constant k_o by $-d[RSNO]/dt = k_o[RSNO]$

$$\text{then } k_o = \frac{k_2 k_1 Kh_o [B\bar{r}][HN_3]}{k_{-1}[RSH] + k_2 [HN_3]} \qquad (1)$$

Similar expression apply for other nucleophiles and also other nitrite traps.

Figure (1) shows the dependence of the observed first order rate constant k_o upon the concentration of added sodium azide for reaction in 3.1M H_2SO_4 containing 0.2M sodium bromide. It is apparent that k_o increases at first with increasing sodium azide, then levels off and becomes zero-order in added NaN_3 at about 1.75×10^{-2}M. At this concentration, the reverse reaction is completely suppressed. At high $[HN_3]$ the limiting case $k_2[HN_3] \gg k_{-1}[RSH]$ applies and k_o becomes $k_1 Kh_o[B\bar{r}]$. The results of k_o vs $[HN_3]$ are given in Table (1).

Sulphamic acid was also used as a nitrite trap for the thionitrite reaction, and similar behaviour was found. The limiting condition was not achieved so readily since sulphamic acid is known[10] to be less effective than hydrazoic acid as a nitrite trap. The results of k_o vs $[NH_2SO_3H]$ are given in Table (2).

In general when $k_2[HN_3] \gg k_{-1}[RSH]$ does not apply, a requirement for constant values of k_o during any one of kinetic run is that [RSH] is virtually constant. This can be achieved in practice by arranging that it is in ten-fold excess over the initial thionitrite concentration by addition of [RSH].

FIGURE 1

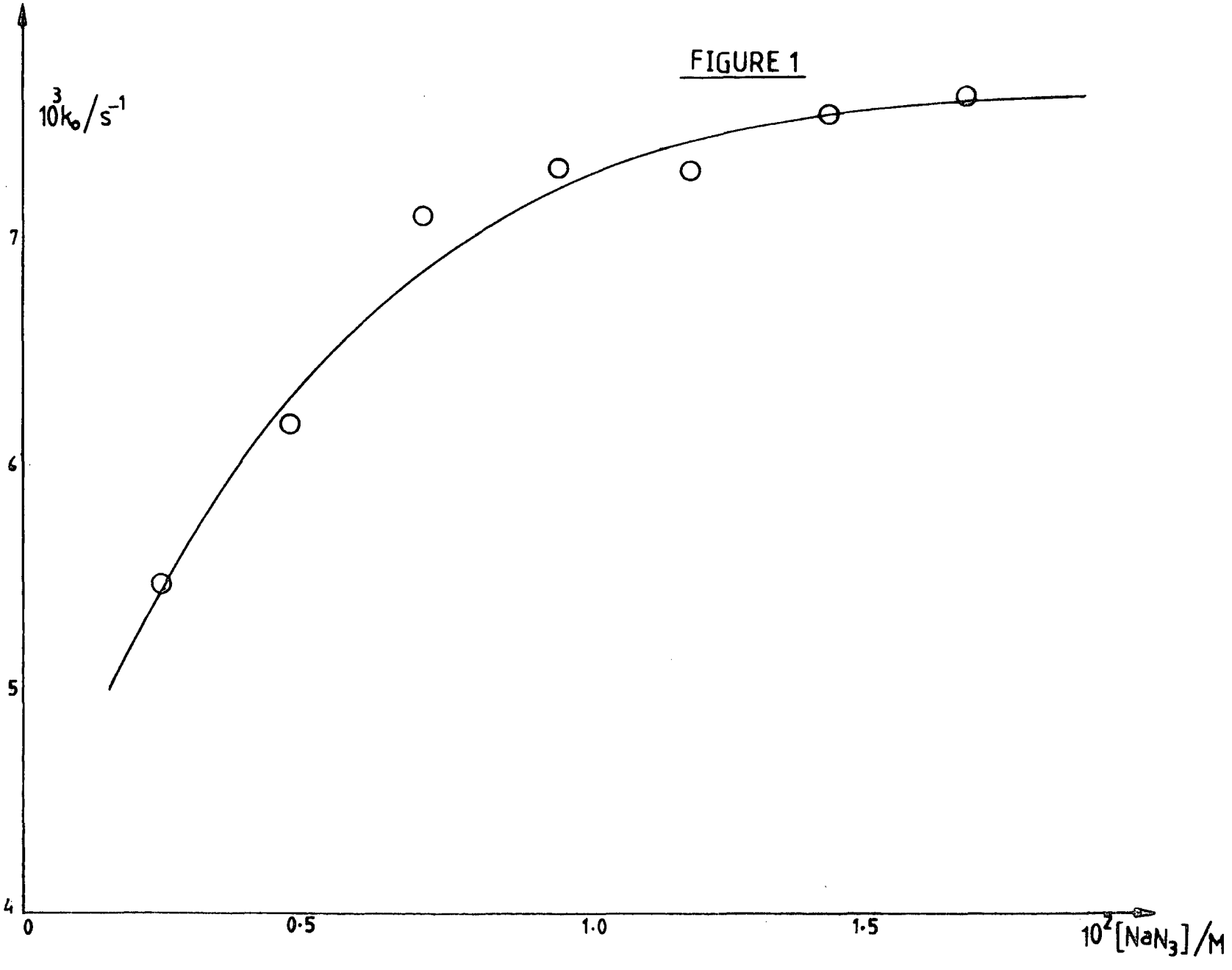


Table 1

10^2 [HN_3]/M	10^3 k_o S^{-1}
0.251	5.48
0.483	6.18
0.724	7.12
0.966	7.34
1.21	7.32
1.45	7.60
1.69	7.74

Variation of k_o vs [HN_3] at 3.1M H_2SO_4 and .2M NaBr

Table 2

10^2 [NH_2SO_3H]/M	10^3 k_o S^{-1}
4.75	2.00
9.50	2.88
19.0	3.98
28.5	4.95
38.0	4.72
47.5	5.74

Variation of k_o vs [NH_2SO_3H] at 3.3M H_2SO_4 (without NaBr)

Some experiments were carried out for the denitrosation of thionitrite (1.4×10^{-3}M) in sulphuric acid (2,3,3.7M) containing N-acetyl-penicillamine (1.43×10^{-2}M) with the presence of sodium azide.

The aim of these experiments was to establish the general form of the equation for k_o and also to determine if possible the relative reactivities of different nitrite traps. A series of kinetic runs was done at constant added [RSH], whilst varying the [HN_3].

Tables 3-5 summarize the results of k_o vs [HN_3], and the results of k_o vs [NH_2SO_3H] are given in Table (6), the latter experiments were carried out in 3.3M H_2SO_4.

To get the reactivity of the different nitrite traps towards the free nitrosating agent, it needs the reciprocal form of equation (1) given as equation (2).

$$k_o^{-1} = \frac{k_{-1}[RSH]}{k_2[HN_3]k_1K\ h_o[B\bar{r}]} + \frac{1}{k_1\ K\ h_o[B\bar{r}]} \qquad (2)$$

The double reciprocal plot $[k_o]^{-1}$ vs $[HN_3]^{-1}$ should be linear with a positive slope and intercept. Such plots are shown in Figure (2) for reaction at three different acid concentrations for the solvent-promoted denitrosation. Tables 7-9 show the results of $[k_o]^{-1}$ vs $[HN_3]^{-1}$ and $[k_o]^{-1}$ vs $[NH_2SO_3H]^{-1}$ given in Table 10.

Table 3

$10^2[NaN_3]/M$	$10^3\ k_o\ S^{-1}$
0.444	0.134
0.888	-
1.33	0.408
1.78	0.378
2.22	0.415

Variation of k_o with $[NaN_3]$ at 2M acid

Table 4

$10^2[NaN_3]/M$	$10^3\ k_o\ S^{-1}$
0.444	0.327
0.888	0.530
1.33	0.740
1.78	0.967
2.22	1.15

Variation of k_o with $[NaN_3]$ at 3M acid

Table 5

$10^2[NaN_3]/M$	$10^3\ k_o\ S^{-1}$
0.444	0.966
0.888	1.38
1.33	2.18
1.78	2.76
2.22	2.90

Variation of k_o with $[NaN_3]$ at 3.7M acid

RSNO = 1.4×10^{-3}M, penicillamine 1.4×10^{-2}M

Table 7

$[NaN_3]^{-1}$	$10^3[k_o]^{-1}$
225	7.47
113	-
75.2	2.45
56.2	2.6
45.1	2.41

Variation of $[k_o]^{-1}$ with $[NaN_3]^{-1}$ at 2M acid

Table 8

$[NaN_3]^{-1}$	$10^3[k_o]^{-1}$
225	3.06
113	1.89
75.2	1.35
56.2	1.03
45.1	0.870

Variation of $[k_o]^{-1}$ with $[NaN_3]^{-1}$ at 3M acid

Table 9

$[NaN_3]^{-1}$	$10^3[k_o]^{-1}$
225	1.04
113	0.725
75.2	0.459
56.2	0.362
45.1	0.345

Variation of $[k_o]^{-1}$ with $[NaN_3]^{-1}$ at 3.7M acid

RSNO = 1.4×10^{-3}M, penicillamine 1.4×10^{-2}M

RSNO = 1.4 x 10^{-3}M , penicillamine 1.4 x 10^{-2}M

Table 6

$[NH_2SO_3H]$/M	$10^3 k_o$ S^{-1}
0.102	0.448
0.204	0.809
0.306	0.964
0.408	1.23
0.510	1.48

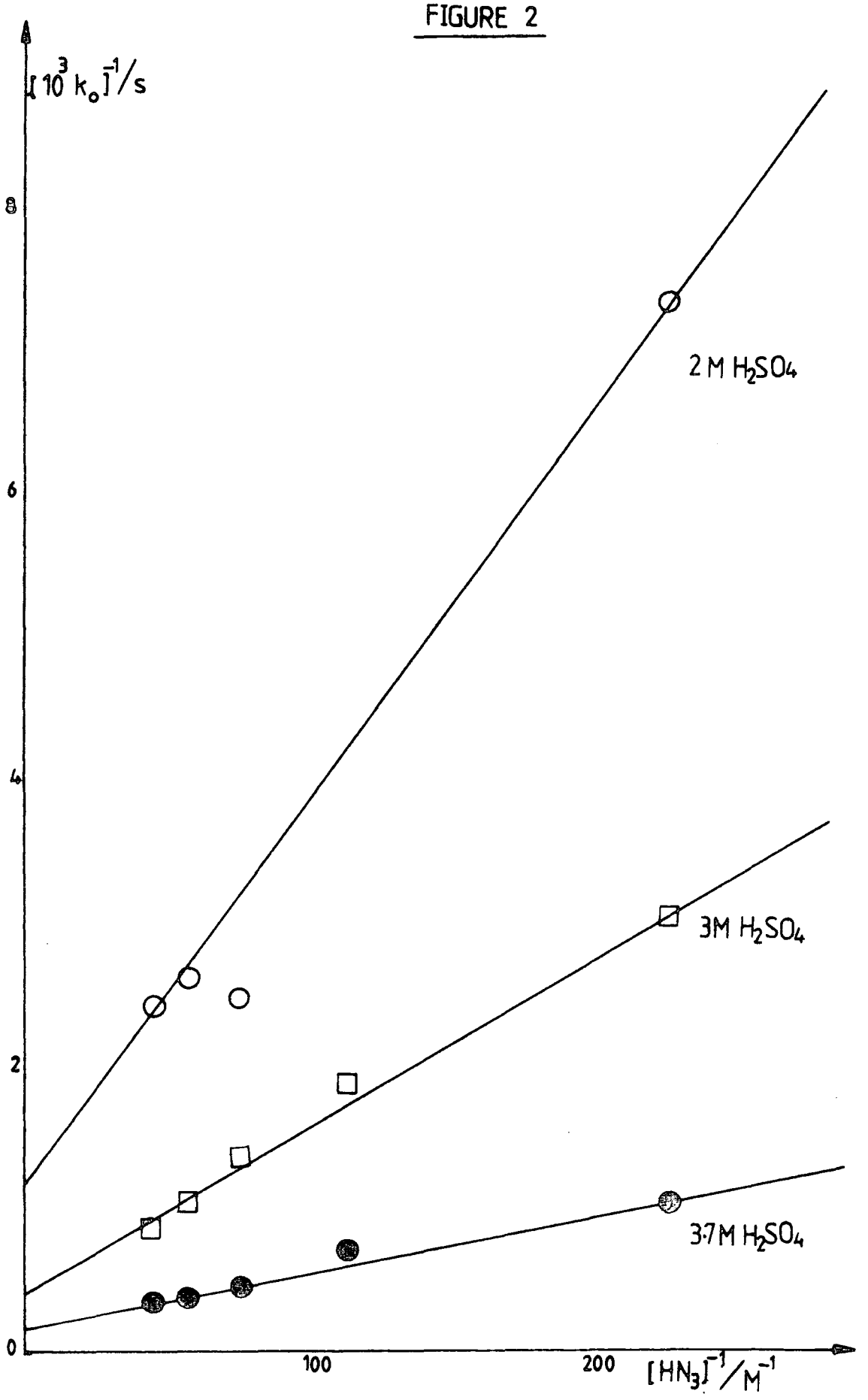
Variation of k_o with $[NH_2SO_3H]$ at 3.3M acid

Table 10

$[NH_2SO_3H]^{-1}$/M	$10^3 [k_o]^{-1}$
9.80	2.23
4.90	1.24
3.27	1.04
2.45	0.812
1.96	0.676

Variation of $[k_o]^{-1}$ with $[NH_2SO_3H]^{-1}$ at 3.3M acid

FIGURE 2



The values of $1/k_1Kh_o$ obtained from the intercept, and the rate constant ratio k_{-1}/k_2 in each case, since k_{-1}/k_2 = slope/intercept [RSH].

Table 11

Results from the double reciprocal plots

Trap	$[H_2SO_4]/M$	$k_1 K h_o$	k_{-1}/k_2
HN_3	2.0	9.1×10^{-4}	1.8
HN_3	3.0	2.5×10^{-3}	2.1
HN_3	3.7	6.2×10^{-3}	1.7
NH_2SO_3H	3.3	3.9×10^{-3}	55

Table 11 gives the values of k_{-1}/k_2 and k_1Kh_o. The slopes decrease as the acidity increase , as expected, since the slope gives $k_{-1}/k_2[HN_3]k_1Kh_o$. The value of k_1Kh_o (derived from the intercepts) increases with increasing acidity, but the ratios k_{-1}/k_2 are constant (within experimental error) at different acidity about 1.9. This shows that N-acetyl-penicillamine is slightly more reactive than HN_3 towards free nitrous acid. For NMNA and under different conditions Williams[11] found the corresponding values for different nitrite traps to be HN_3 31, NH_2NH_2 21, NH_2SO_3H .59, $^+NH_3OH$ .03 and $CO(NH_2)_2$ .009. Thus it can be seen that azide is the most powerful trap and the urea is the least reactive.

Thiols (RSH) might be used as a nitrite trap, since it removes nitrite from the environment and forms S-nitroso thiols in a very efficient reaction.

Table 11 gives also the results which were obtained for sulphamic acid at one acidity 3.3M H_2SO_4 the k_1Kh_o value of 3.9×10^{-3} S^{-1} agrees very well with the value 3.8×10^{-3} S^{-1} from the HN_3 results for this acidity. The ratio k_2 $[HN_3]/k_2[NH_2SO_3H]$ equals $\sim$ 29 which means that sodium azide is about 29 times more powerful as a nitrite trap. This result agrees quite well with an earlier report[11] of $\sim$ 40 obtained from kinetic measurements of denitrosation of nitrosoamines at higher acidity (4.8M H_2SO_4).

3.2. The Effect of Added Nucleophile ($\bar{Y}$):

At the limiting case of $k_2[X] \gg k_{-1}[RSH]$ the reaction is not reversible and it should be possible to examine the effect of different nucleophiles on the denitrosation reaction. Experiments were carried out with $C\bar{l}$, $B\bar{r}$, $S\bar{C}N$ and $SC(NH_2)_2$ all in the presence of a large excess of added sodium azide (1.75×10^{-2}M) at 3.1M H_2SO_4. For each nucleophile ($\bar{Y}$) straight lines were found for the plots of k_o vs $[Y^-]$ as shown in Figure 3, underlining the first order dependence on $[Y^-]$. All gave a common intercept at $[Y^-] = o$ which represents the solvent-promoted reaction. The data are given in Tables 12-15.

The slopes of each line represent k_1Kh_o and are measures of k_1 since K and h_o are constant for the series. Table 16 shows the values obtained.

Table 12

$[Cl^-]$/M	$10^3 k_o$ s^{-1}
0·0	4.21
0.087	4.87
0.173	5.38
0.260	6.17
0.346	6.06

Variation of k_o with $[Cl^-]$/M

Table 13

$[Br^-]$/M	$10^3 k_o$ s^{-1}
0.05	5.02
0.10	5.51
0.20	7.33
0.30	8.34
0.40	9.91

Variation of k_o with $[Br^-]$/M

Table 14

$[SCN^-]$/M	10^3 $k_o s^{-1}$
0.0	4.60
0.05	6.35
0.10	7.59
0.15	9.62
0.20	11.36
0.25	12.95

Variation of k_o with $[SCN^-]$/M

Table 15

$[SC(NH_2)_2]$/M	$10^3 k_o s^{-1}$
0.025	5.30
0.05	5.82
0.075	6.58
0.10	7.42
0.125	7.95

Variation of k_o with $[SC(NH_2)_2]$/M

thionitrite 1.4×10^{-3}M, sodium azide 1.75×10^{-2}M

3.1M H_2SO_4

FIGURE 3

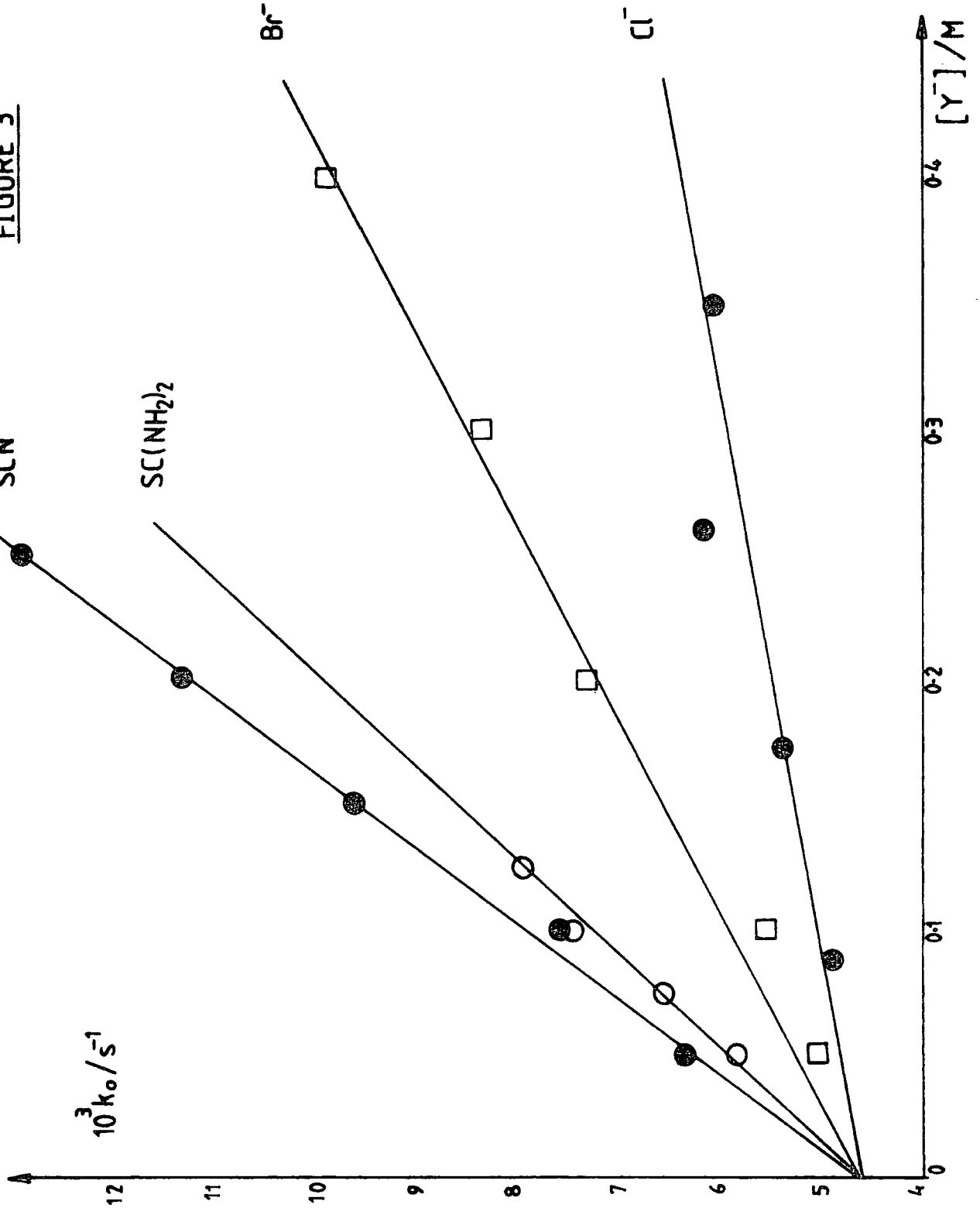


Table 16

The slopes of plots of k_o vs $[Y^-]$

Nucleophile	slope of k_o vs $[Y^-]$ $1\ mol^{-1}\ s^{-1}$
Cl^-	4.6×10^{-3}
Br^-	13.0×10^{-3}
$SC[NH_2)_2$	27×10^{-3}
SCN^-	34×10^{-3}

It is clear from Table 16 that the values increase in the expected order [12] of nucleophilicity, the slope has the smallest value for chloride ion and the biggest for thiocyanate ion. It is expected thiourea should be more reactive than thiocyanate ion, perhaps this reversal of SCN^- and $SC(NH_2)_2$ is due to a steric effect for the reaction of a tertiary nitrosothiol and thiourea.

However, it is clear that the rate-determining stage in this sequence is the attack of the nucleophile on the protonated form of the thionitrite. No evidence of a change to an earlier rate-determining step (protonation) was found contrasting with the behaviour of the alkyl nitrites,[9] and in some cases of the nitrosamines.

3.3 Acid-Catalysis:

In the absence of added nucleophiles (Cl^-, Br^-, SCN^- and $SC(NH_2)_2$) denitrosation of thionitrite occurs as expected from scheme (1) and equation (3), but with a much smaller rate constant. As expected the reaction is strongly acid-catalysed. In aqueous sulphuric acid the reaction was carried out with initial thionitrite 1.4×10^{-3}M and

sodium azide 1.75 x 10^{-2}M, over a range of acidities; the results are given in Table 17.

$$k_o = k_1 Kho\ [Y^-] \qquad (3)$$

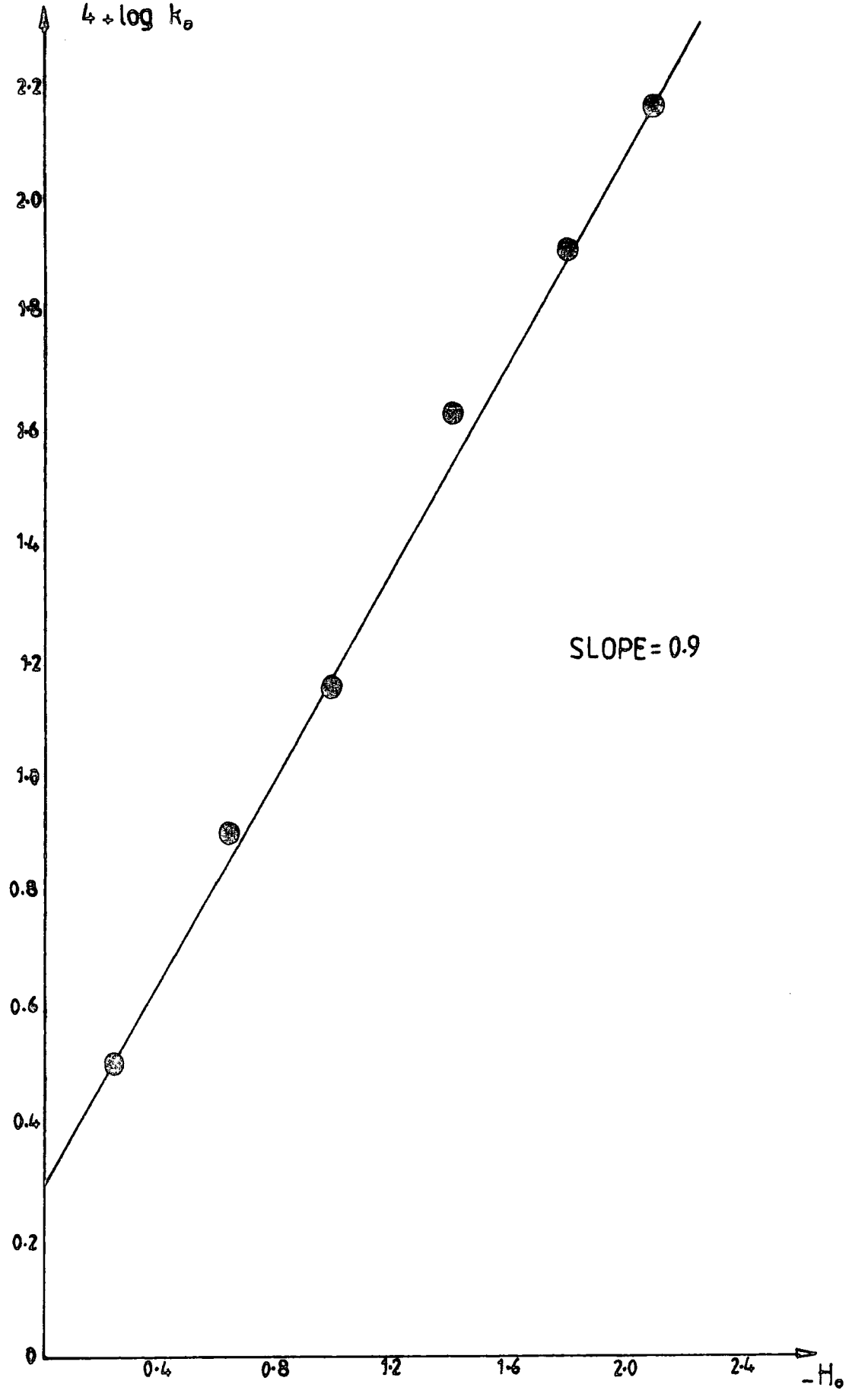
Table 17

Acid-catalysis in the denitrosation of RSNO

$[H_2SO_4]$M	$10^4\ k_o\ s^{-1}$	$4 + \log k_o$	$-H_o$
.943	3.21	.506	.245
1.58	7.98	.902	.635
2.20	14.4	1.15	.995
3.08	42.1	1.63	1.41
3.77	81.7	1.91	1.80
4.35	14 3	2.16	2.10

The plot $4+\log k_o$ vs the Hammett acidity function -Ho is shown in figure 4; it gives a good line of slope 0.9 which shows that the protonation of the thionitrite is well expressed by this acidity function. Comparison of these results with those for denitrosation of methylnitrite,[7] shows that the alkyl nitrite undergoes denitrosation approximately 10^6 times more rapidly than does RSNO. This effect may be due in part to a steric effect, since the comparison is between a primary and tertiary system, but it is likely that the major effect lies in the size of the equilibrium constants for protonation of RONO and RSNO.

FIGURE 4


4 + log k_θ
2·2
2·0
1·8
1·6
1·4
1·2
1·0
0.8
0.6
0.4
0.2
0
0·4
0·8
1·2
1·6
2·0
2·4
$-H_\theta$
SLOPE = 0·9

The pKa values of these nitroso compounds are not known although they are expected to be very low. It is possible to compare O vs S-protonation in other compounds. The values for diethylether and diethylsulphide are, for example, -2.39 and -6.8 respectively.[14] Generally S-compounds are much less basic than there O-counterparts. This effect arises from the electronegativity differences (O > S) and the bond strength (O-H and S-H).

3.4 Product analysis:

It was possible to find out the product of denitrosation of thionitrite. To confirm the presence of these two products (nitrous acid and N-acetyl penicillamine) we have to determine each of them separately. For nitrous acid, this is possible by measurement of the total amount of HNO_2 which is released from a known initial substrate concentration of thionitrite. This can be achieved by adding N-methyl-4-nitroaniline (in the absence of nitrous trap) which should give the corresponding nitrosoamine.

In practice, excess of N-methyl-4-nitroaniline was added to the mixture containing thionitrite 1.65×10^{-4}M in acid solution (1M H_2SO_4) and sodium bromide. At the end of this reaction, the u.v. spectra were recorded for aliquots from the reaction mixture. It was found that N-nitroso-N-methyl-4-nitroaniline is built up at 310nm. By knowing the extinction coefficient for this nitrosoamine (ϵ = 14326), one can get the concentration of N-nitroso compound which was 1.18×10^{-4}M (72%). The same experiment done in the presence of sodium azide, gave no nitrosoamine at all, and the amine peak in the u.v. spectrum remains unchanged. This

can be readily explained by the reaction between nitrosyl bromide and hydrazoic acid (as is clear from scheme 1), to form N_2, N_2O, $B\bar{r}$ and $\overset{+}{H}$. These gases can be seen as bubbles inside the cell during any kinetic run.

This reaction shows that the nitrosation of amines occurs by prior denitrosation of thionitrite followed by rapid nitrosation of amines. Thus in common with amines generally and also alkyl nitrites, direct nitrosation of amines does not seem to occur, but rather can only be achieved by attack by relatively strong non-basic nucleophile such as $C\bar{l}$, $B\bar{r}$, $SC\bar{N}$ and $SC(NH_2)_2$ in these strongly acid solutions.

The other product N-acetyl-penicillamine, which appears at 260nm (ϵ = 346) was difficult to determine quantitatively because of its relative instability, presumably to oxidation in these solutions. Nevertheless its presence could be detected qualitatively in the reaction solutions.

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CHAPTER 4

Experimental Techniques

1. Denitrosation of N-methyl-N-nitrosaniline: experimental details

a. Materials:

N-methyl-N-nitrosaniline (NMNA) was prepared by direct nitrosation of N-methylaniline, using sodium nitrite and hydrochloric acid.[1]

Other materials were obtained commercially (NaN_3, KBr, KCNS, NaI, $SC(NH_2)_2$ and H_2SO_4) and were used without purification, except for KCNS which was dried in the oven. Distilled water was used to make up volumetric stock solutions.

Our work was carried out at three different concentrations of sulphuric acid. The final acid strength of the solutions were determined by titrating them against standard aqueous sodium hydroxide solution using phenol red indicator. The strength of the acids were 1.55, .74 and .36M. The concentration of NMNA in the kinetic runs was 3.32×10^{-4}M and sodium azide typically was 1.9×10^{-3}M. Stock solutions of KBr were 2, 2.5, 3 and 4M, KSCN .28 and 1.77M, $SC(NH_2)_2$ .279 and 2.81M and NaI 2.8×10^{-2} and .28M.

b. Preparation of Reaction Mixtures:

The desired quantities of all the reactants were mixed together in the appropriate solvent except for the N-methyl-N-nitrosaniline solution. This mixture was then placed in the constant temperature water-bath kept at $31^{\circ}C$.

The following table shows the quantities of reactants in typical reaction mixtures (total volume 28 ml).

H_2SO_4	Sodium azide	Nucleophile	Water	NMNA
20 ml	2 ml	1 ml	4 ml	1 ml
20 ml	2 ml	2 ml	3 ml	1 ml
20 ml	2 ml	3 ml	2 ml	1 ml
20 ml	2 ml	4 ml	1 ml	1 ml
20 ml	2 ml	5 ml	O	1 ml

c. Kinetic Measurements:

The kinetic measurements were carried out on Beckman Model 25 recording spectrophotometer, which was thermostatically kept at 31^{o}c electrically.

After ten minutes $1cm^3$ of stock solution of the nitrosoamine was added to the reaction mixture, the solutions mixed and some transferred to a 1cm silica cell, and placed in the sample-block of the spectrophotometer. The other cell containing water (as a solvent) was placed in the reference position.

The reaction was followed by the disappearance of the nitrosoamine peak at fixed wavelength for the KSCN, KBr and NaI experiments at 275nm and at 300nm for the $SC(NH_2)_2$ work. The disappearance of absorbance with time was followed for at least two half lives; the infinity value was determined in each case after a ten half-lives. Good first order behaviour was found, as shown by the linear plots of ln $(A-A_\infty)$ against time.

2. Denitrosation of N-acetyl-S-nitroso-D,L-penicillamine (Thionitrite) Expermental details:

a. Preparation of Thionitrite:

N-acetyl-D,L-penicillamine (5mmol) was dissolved in methanol: 1M HCl (1:1,20ml), then 1 ml of Conc.

sulphuric acid was added gradually to the reaction mixture with vigorous stirring for 20 minutes. The solution was then treated with sodium nitrite solution (.69 g [10mmol] in 10 ml of water) at 25°C. Deep green crystals (with red reflections) were observed immediately. After a further 15 minutes, the green crystals were filtered off and washed thoroughly with water, and were dried under reduced pressure (61% yield).[2] It was examined by elemental analysis [Found: N,12.4; C, 38.31; H, 5.42; S, 16.8%, $C_7H_{12}O_4N_2S$, calculated, N,12.7; C, 38.1; H, 5.46; S,14.6%].

Other materials were obtained commercially (nucleophiles, nitrite traps compound and solvents).

b. Preparation of Reaction Mixtures:

The same procedure which was described before was used here to prepare the reactions mixture.

The following table shows the quantities of reactants in the reaction mixture (this table was used to find out the limiting value for the nitrite trap) total volume 20 ml.

H_2SO_4	penicillamine	nitrite trap	water	thionitrite
10 ml	2 ml	1 ml	6 ml	1 ml
10 ml	2 ml	2 ml	5 ml	1 ml
10 ml	2 ml	3 ml	4 ml	1 ml
10 ml	2 ml	4 ml	3 ml	1 ml
10 ml	2 ml	5 ml	2 ml	1 ml
10 ml	2 ml	6 ml	1 ml	1 ml
10 ml	2 ml	7 ml	0 ml	1 ml

In the following table, these quantities were used to get the reactivity of nucleophile towards thionitrite.

H_2SO_4	Sodium azide	nucleophile	water	thionitrite
10 ml	2 ml	0	7 ml	1 ml
10 ml	2 ml	1 ml	6 ml	1 ml
10 ml	2 ml	2 ml	5 ml	1 ml
10 ml	2 ml	3 ml	4 ml	1 ml
10 ml	2 ml	4 ml	3 ml	1 ml
10 ml	2 ml	5 ml	2 ml	1 ml

c. Preparation of thionitrite solution:

Since stock solutions of the thionitrite in dioxan (or methanol) were unstable over a period of time, each kinetic run was started by weighing approximately .0065 g of the solid thionitrite, adding 1ml dioxan (for ease of solubility) and then immediately addition of the acid solution containing the appropriate nucleophile and trap concentrations.

The concentration of thionitrite in the run was $\sim$ 1.4×10^{-3}M, in 3M H_2SO_4, and the stock solutions of sodium azide was 5.07×10^{-2}M, sulphamic acid 1.9M, sodium chloride 1.72M, sodium bromide 2M, KSCN 2M and thiourea .5M.

d. Kinetic Measurements:

The kinetic measurements were carried out on a Pye-Unicam SP-8-100 recording spectrophotometer at 31^{o}c. The reaction was followed by the disappearance of the thionitrite at fixed wavelength (338nm). Good first order behaviour was found, again from the linear plots of ln (A-Aoo) against time.

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