N-NITROSATION BY NITRIC OXIDE

A Thesis Submitted

BY

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ABSTRACT

The chemistry of Nitric oxide (NO) has been reviewed with particular emphasis to its reactions in solution.

N-nitrosamines form rapidly from NO and secondary amines (R_2NH) only in the presence of certain catalysts under anaerobic neutral or acidic conditions at 25°C.

The combination of NO and iodine is a powerful nitrosating agent in neutral conditions. The maximum yield of N-nitrosamine is dependent on $[I_2]$, and is formed within ca. 5-30 min, depending on the solvent. The mechanism is discussed in terms of the rate limiting formation of nitrosyl iodide (NOI) principally from NO and I_2. In acidic aqueous-EtOH I_2 acts as a catalyst and reaction involves the fast formation of NOI from neutralisation of HI by NO to NOI, N_2O and H_2O. Hydrogen iodide itself, and HI released from the solvolysis of metal iodides also react by similar pathways.

N-nitrosamine formation is promoted by Ag^{I}-salts in EtOH at 25°C. Evidence favours a ligand induced disproportionation of Ag^{I} by the amine to Ag^{0} and an Ag^{II}-amine-complex. Product formation occurs via an oxidation of the bound amine to a dialkylamino radical which couples with NO.

Copper(I)- and Copper(II)-salts catalyse N-nitrosamine formation from NO in dioxan at 25°C, and catalysis appears to involve metal bound amine ligands. Homolysis of N-nitrosodiphenylamine is a convenient source of NO, and transnitrosation is also catalysed by these salts.

N-nitrosamines are decomposed by γ-radiation in neutral anaerobic aqueous solution at ambient temperature. Sodium nitrate and nitrite protect N-nitrosamine from decomposition, and in the presence of secondary amines, give both N-nitroso- and N-nitroamines *via* N_2O_4 and N_2O_3 intermediates.

ACKNOWLEDGEMENTS

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J.R. Outram.

THIS IS DEDICATED TO MY

MUM AND DAD,

FOR EVERYTHING.

CONTENTS

CHAPTER 1

INTRODUCTION

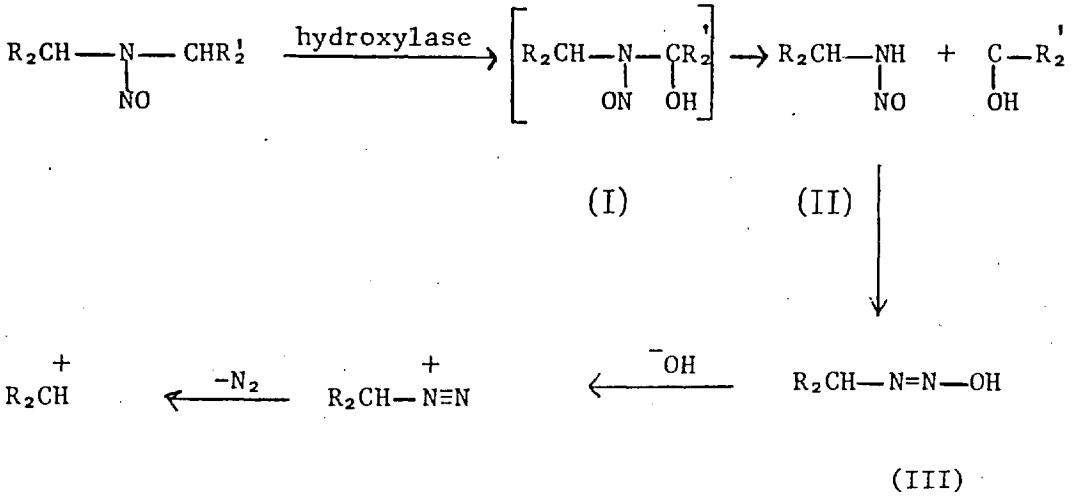
1.1 N-nitrosamines.

1.1 (1) Biological Aspects.

Since Magee and Barnes[1] discovered that rats fed N-nitrosodimethylamine suffered a high incidence of liver tumours, many structurally related compounds have been shown to exhibit powerful carcinogenic properties in animal species.[2-4] For example, both N-nitrosodimethyl- and N-nitrosodiethyl-amine show acute toxicity leading to serious tissue damage, especially of the liver, kidneys, bladder and lungs of hamsters, rabbits, mice and dogs, by either direct administration or inhalation of the vapour.[3] Even in low doses, N-nitrosamines are cumulative toxins, and have been reported to activate weaker carcinogens, resulting in their being implicated in human cancer.[5,6] Tumour induction is believed to result from the alkylation of nucleic acids, leading to atypical base pairings. As there are many excellent reviews on the formation,[7] occurrence,[8-11] and biological properties of N-nitrosamines[2-4] and on the alkylation hypothesis of tumour induction,[3,12,13] only the more salient aspects will be discussed here.

The accepted mode of action for dialkyl-N-nitrosamines is an initial oxidative metabolic activation, with the ultimate formation of an alkylating agent, which reacts with cellular components. Druckrey and his colleagues,[14] suggested that the initial step of metabolic activation (Scheme I.1) was an enzyme mediated α-hydroxylation to give hydroxyalkyl intermediate (I), which decomposes to give an

aldehyde or ketone and an unstable primary N-nitrosamine(II). The latter tautomerises to give diazohydroxide(III) which breaks down into either diazoalkane or a free carbonium ion.



Scheme I.1

Scheme I.1 applies to N-nitrosamines containing α-hydrogen atoms and support for this comes from correlations of the degree of carcinogenicity with α-substituents.[15-17] Recently, Baldwin[18] prepared α-acetoxy-N-nitrosopyrrolidine and reported that this compound required no metabolic activation, the assumption being that hydrolysis to the α-hydroxy compound has taken place during the mutagenicity tests.

Alkylation of nucleophilic sites in DNA, RNA and proteins by N-nitroso carcinogens has been demonstrated, but there is conflicting evidence regarding the alkylating entity.[3,12] There is some evidence that the O-6 alkylation of guanosine is important for N-nitrosamines.[19-21]

1.1 (2) Occurrence.

N-nitrosamines can derive from dialkyl, aryl-alkyl, diaryl and heterocyclic amines. Some amines are themselves toxic but usually there is no potential danger unless a nitrosating agent is present. Nitrosatable amines are widespread and they occur naturally in humans.[22,7] For example, piperidine, an amine found in the brain, can be formed by either the decomposition of proteins or by bacterial mediated coversion of lysine. The amount of piperidine excreted in human urine daily is approximately 1.24 mg.[22] Amines themselves are found in food,[8] fish,[10] spices[22] and are used widely in industry. Di-, and tri-ethanol-amines are used in the manufacture of pesticides, surface active agents and as solvents for dyes, penetrating agents for wood, paper, in waxes, polishes and toilet goods. Morpholine also, is used as a cheap solvent for resins, waxes, as a corrosion inhibitor and in local anaesthetics or antiseptics.[23]

N-nitrosamines have found use in rocket fuels, insecticides and as solvents in the fibre industry.[24] N-nitrosodiethanolamine is a gasoline and lubricant additive.[23] Most of these uses are now under review because of the apparent health risks.

With the advancement in analytical techniques, capable of detecting trace amounts, it is not surprising that N-nitrosamines have been detected in human urine,[25] faeces and blood,[26] in foodstuffs preserved by nitrite, in beer, drugs, pesticides, tobacco smoke (reviewed by Gough[10]),cosmetics,[27] cutting fluids,[28] air,[29] and water.[30]

N-nitrosamines have been reported to form in vivo (i.e., stomach) after the ingestion of nitrite and amines,[7] and also from NO_3^- and piperidine in a reaction mediated by bacteria from the gastrointestinal

tract.[31] Nitrate can be reduced to nitrite by the bacteria in human saliva[32] and by environmental denitrificants.[33] Consequently, N-nitrosopiperidine has been identified in acidic urine from NO_3^- and E. Coli infected bladders,[34] N- nitrosodimethylamine from NO_3^- with psuedomonas herbicola and acidic dimethylamine.[35]

1.1.3 (I). Formation of N-nitrosamines.

The formation of N-nitrosamines in aqueous solution is generally presumed to require acidic conditions (pH < 5) and nitrous acid.[7] These reactions involve a nitrosating agent NOX such as H_2ONO^+, $NONO_2(N_2O_3)$ and the unprotonated amine.[36,7] At moderate pH, the nitrosating agent is N_2O_3, derived from the dimerisation of HONO (pKa = 3.13 at 25°C) after protonation of nitrite.[36,7] At lower pH, H_2ONO^+ is the reactive nitrosating agent. The rate of reaction can, however, be catalysed by certain anions, SCN^- [37], and halide ions[36] by forming NO-X species which are more reactive than N_2O_3. The nitrosating agent is derived from the attack of the anion on the nitrous acidium ion $HONO^+$ (Equation 1.1).

$$NO_2^- + H^+ \rightleftharpoons HONO \overset{HX}{\rightleftharpoons} H_2ONO^+ + X^- \rightleftharpoons NO\text{-}X + H_2O \qquad 1.1$$

In its simplest form, the N-nitrosamine results from the initial nucleophilic attack of the amino-N- lone pair electrons on the nitrosating agent followed by deprotonation to the product (Equation 1.2) (where RR' = alkyl or aryl).

$$RR'\text{-}NH + NOX \longrightarrow \left[RR'\text{-}\overset{+}{N}(H)\text{-}N{=}O \right] \longrightarrow R_2NNO + HX \qquad 1.2$$

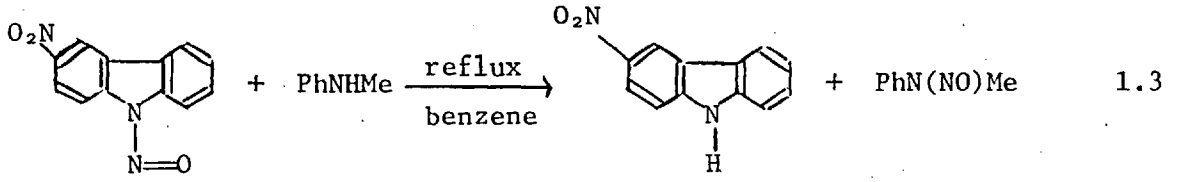
Since only the unprotonated amine reacts, it is desirable to carry out the reaction, especially with the more basic amines, in weakly acidic media. The acidity, however, affects the concentration of both free amine and nitrosating agent and no nitrosation above pH 7.5 has been observed for aqueous nitrite. The kinetics of nitrosation in aqueous acidic nitrite media has been reviewed by Ridd,[38] who has shown also, that similar equations apply to the nitrosation of secondary amines and to the diazotisation of primary amines.[39] Mirvish[7] has recently discussed the effect of pH and the basicity of dialkyl-amines on the rate of N-nitrosamine formation.

Challis and Kyrotopoulos[40] however, have shown that N-nitrosamines form over a much wider range of pH from nitrosyl gases such as N_2O_3, N_2O_4. Earlier, White and Feldman[41] reported rapid N-nitrosamine formation from N_2O_4 in organic solvents, with competitive N-nitroamine formation increasing with decreasing temperature ($\sim -70^{\circ}C$). Gas phase nitrosation from nitrogen oxides has also been reported in connection with tobacco smoke studies.[42] The possibility of these reactions being important in aqueous media (other than N_2O_3 from acidified nitrite) had not been considered, presumably because they were expected to undergo rapid hydrolysis to nitrite and nitrate. Challis and Kyrotopoulos,[40] showed that the formation of N-nitrosamines from dissolved nitrogen oxides in neutral and alkaline aqueous conditions (pH 6-14) at $25^{\circ}C$, was extremely rapid for example, with 400 fold excess N_2O_3, N_2O_4 over 2×10^{-3} M piperidine, 45-65% nitrosation ca. 3 min. Acidic conditions generally inhibited the reaction by converting the amine into its unreactive conjugate acid. Apart from the observations by Challis and Kyrotopoulos, Keefer and Roller[43] showed that formaldehyde

or chloral catalysed the nitrosation of secondary amines from nitrite between pH 6-11, but these reactions are very slow, with typically 1% N-nitrosodiethylamine after 17 hours at pH 6.4 and 24°C. Lijinsky observed that the nitrosation of dimethylamine at pH 7-11, 100°C, was also accelerated by formaldehyde.[7] Tertiary amines also react with nitrosating agents such as N_2O_3 to form N-nitrosamines. The accepted mechanism involves nitrosative dealkylation leading to the secondary amine which reacts further, the significance of these reactions have been discussed by Mirvish.[7]

1.1 3 (II) Transnitrosation.

This process, in which the nitroso group from one N-nitrosamine is transferred to another amine, may in principle, proceed under neutral conditions. Bumgardner[44] reported the transfer of the nitroso group of N-nitroso-3-nitrocarbazole to N-methylaniline under reflux in benzene, to form N-methyl-N-nitrosoaniline and 3-nitrocarbazole (Equation 1.3).



1.3

Transnitrosation at this temperature can be interpreted in terms of the homolysis of the N-N bond of the nitrosocarbazole and reaction involving a free radical chain.[45]

N-nitrosamines can be denitrosated to the parent amine in acidic media, thus,under heterolytic conditions, transnitrosation *via* nitrous acid would be expected since N-nitrosamine formation itself, is reversible. In the presence of halogen acids, NOX can act as the transferring agent and this has been demonstrated for the reaction of N-nitrosodiphenylamine and N_3^-.[46,47] The products, diphenylamine, N_2 and N_2O are those expected since NO^+ reacts irreversibly with N_3^- to give N_2O and N_2.[48] The reaction proceeds *via* an initial, fast reversible protonation at the N-atom, followed by the rate limiting attack of X^- at the nitroso N-atom to give the parent amine and NO-X (Scheme 1.2). NOX is removed irreversibly by its reaction with N_3^-. The reaction rate is sensitive to X^-, the rate being increased by added Cl^-, Br^-, I^-.[47]

$$Ph_2N\text{-}N{=}O \overset{H^+}{\rightleftharpoons} \left\{ Ph_2\overset{+}{N}HNO \right\} \overset{X^-}{\rightleftharpoons} Ph_2NH + NOX$$

$$NO\text{-}X + N_3^- \rightleftharpoons N_3\text{-}NO \longrightarrow N_2O + N_2$$

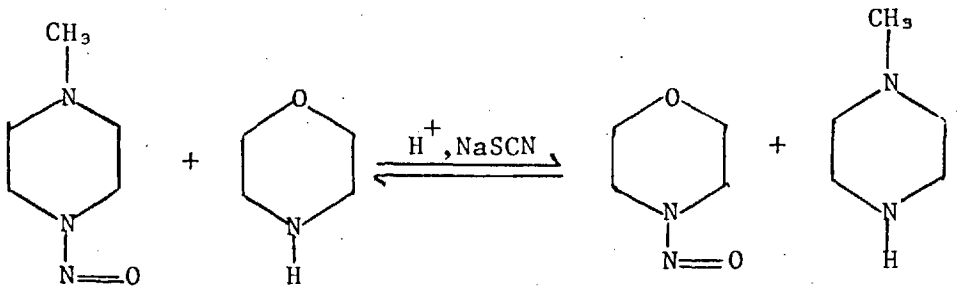
Scheme 1.2

Challis and Osborne[46] showed that the nitroso group of N-nitrosodiphenylamine could be transferred in acidic 50% (v/v) aqueous-ethanol at 25°C, directly to N-methylaniline. The reaction was not catalysed by Cl^- and the suggested mechanism involved nucleophilic attack of the amine on the protonated N-nitrosamine (Equation 1.4).

$$Ph_2N\text{-}NO \overset{H^+}{\rightleftharpoons} Ph_2\overset{+}{N}HNO \overset{PhNHMe}{\rightleftharpoons} Ph_2NH + \left\{ PhNHMe\overset{+}{N}O \right\} \longrightarrow PhN(NO)Me + \overset{+}{H} \qquad 1.4$$

Williams[47] observed the same reaction with aniline and suggested that direct transnitrosation, without the intermediacy of nitrosyl species (NOX), may occur in connection with the Fischer-Hepp rearrangement.

The indirect transnitrosation of aliphatic N-nitrosamines has also been reported,[49,50] for example, the reaction between N-methyl N-nitrosopiperazine and morpholine[50] in 3M HCℓ (Equation 1.5). The rate of transnitrosation was catalysed by SCN^-, Br^-, but no reaction was observed in $HClO_4$ or in aprotic solvents.



1.5

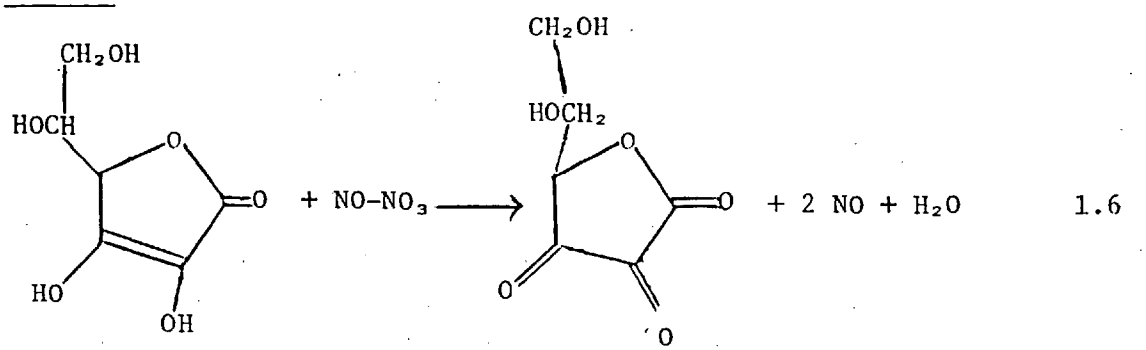
Transnitrosation between seemingly non-carcinogenic N-nitrosamines and other amines has recieved much attention since it is possible that they may act as proximate carcinogens.

However, the reactions are not restricted only to N-nitrosamines; Challis and Shuker[51] have recently shown that alkyl nitrites bearing electronegative substituents can also transnitrosate to a variety of amines in alkaline media. These reactions involve the direct nucleophilic attack of the amine on the alkylnitrite.

1.1.4 Inhibition of N-nitrosamine formation.

Phenols and ascorbic acid compete with the amine for NOX reagents and hence inhibit N-nitrosamine formation.[52] Phenols inhibit by either

the reduction of nitrite to the unreactive NO or by formation of C-nitroso compounds.[53] Ascorbic acid reacts more rapidly with NOX reagents derived from acidified nitrite than amines giving dehydroascorbic acid, NO and H_2O,[54] (Equation 1.6), and hence have been observed to inhibit N-nitrosamine formation both *in vitro* and *in vivo*.[7]



1.6

Mirvish,[7] has suggested that ascorbate should be added to formulations of nitrosatable drugs, because of the low reactivity of NO towards amino groups. Under aerobic conditions, however, evolved NO reacts rapidly with O_2 to give initially NO_2 and subsequently N_2O_3, N_2O_4, hence it is not surprising that a vast excess of ascorbate is sometimes required to block N-nitrosamine formation, and that in some cases, increased N-nitrosamine formation is observed.[55]

1.2. Nitric Oxide. (NO)

Nitric oxide is a colourless gas (bp. -151.74°), it is a major pollutant - 10^6 tons are produced daily,[56] associated with fossil fuel combustion,[56] the exhaust gases of combustion engines[57] and smoking.[58] It is not surprising therefore that NO has been identified in human blood.[59]

1.2 (2) Preparation.

The gas can be prepared by the reduction of nitric acid (Equation 1.7) or by the reaction of aqueous acidic solutions of nitrite and iodide ions (Equation 1.8).[60]

$$HNO_3 + 3Cu \longrightarrow 3Cu(NO_3)_2 + 4H_2O + NO \qquad 1.7$$

$$2HNO_2 + 2KI + 2HX \longrightarrow I_2 + 2H_2O + 2KX + 4NO \qquad 1.8$$

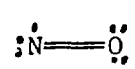
Dry NO can be prepared by the fusion of potassium nitrate and nitrite in the presence of chromium salts[60] (Equation 1.9).

$$KNO_{3(\ell)} + KNO_{2(\ell)} + Cr_2O_{3(s)} \longrightarrow 2K_2CrO_{4(s)} + 4NO \qquad 1.9$$

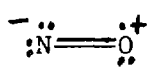
1.2 (3) Properties.

Nitric oxide is a free radical containing eleven valency electrons. The molecule differs uniquely from other free radicals by its lack of dimerisation and chemical inactivity.

In terms of valence bond theory, NO can be represented by structures (I. (I)), (I. (II)).



I. (I)



I. (II)

Structure (I) is more favourable than (II) because of the unfavourable charge distribution, and this is confirmed by the small dipole moment

of 0.16D.[61] More recently, analysis of hyperfine structure by microwave spectroscopy indicated that the odd electron is 65% located on N and 35% on O.[62]

In terms of molecular orbital (MO) theory, the electronic configuration reveals that the odd electron occupies the $2\pi^*$ anti-bonding orbital, giving a total bond order of 2.5. The descriptions adequately explain the observed bond length of NO, 1.15Å, which is intermediate between the ideal double bond length of 1.19Å and a triple bond length of 1.06Å.[62]

At 20°C, the magnetic susceptibility of NO corresponds to one unpaired electron. The unpaired electron has an orbital momentum of 1 and a spin momentum of $\frac{1}{2}$; NO can therefore give an excited state that is paramagnetic or a ground state that is diamagnetic. The energy difference between the two states is only ca. 1.48 kJ mol^{-1}, and so the distribution is dependent on temperature.[61] Nitric oxide shows no marked tendency to dimerise, ΔG, for dimerisation is ca. 8 kJ mol^{-1} at 300°K and 1 atm.,[63] and within the temperature range 273-121.7° K there is no evidence of NO association at 1 atm.[63] At high pressures and low temperatures slight association to give N_2O_2 is observed.

X-ray and magnetic susceptability has shown that NO exists entirely as the dimer in the liquid or solid state, but the weakness of the N-N bond (bent ON-NO) even in the solid is shown by its bond length of 2.18 Å compared with 1.47 Å for the single N-N bond in hydrazine.[64]

Nitric oxide reacts rapidly with O_2 to form NO_2 and it has often been mistakenly concluded that reactions carried out in open vessels were reactions of NO. The oxidation of NO by O_2 is shown (Equation 1.10). The initial step is believed to be the formation of N_2O_2 which then reacts

with O_2.[65]

$$2NO \rightleftharpoons N_2O_2 \xrightarrow{O_2} 2NO_2 \qquad 1.10$$

The rate of oxidation follows third order rate laws, and decreases with increasing temperature and it is therefore dependent on the equilibrium constant for the dimerisation of NO.

1.2.(4) Reactions of NO.

1.2. 4.(I). As a Free Radical.

Nitric oxide behaves chemically as a free radical, and has been used extensively in the gas phase as an inhibitor of radical chain reactions which have been reviewed by Williams.[62] In all cases, NO bonds with radicals at its N-atom although Modica[66] has claimed kinetic support for an oxyimido configuration when F_2C: combines with NO.

There is evidence that NO is also capable of H-abstraction from stable molecules or radicals, for example, 1,3- and 1,4-cyclohexadiene reacts in the gas phase at 306-359°C with NO to give NOH and radical intermediates.[67] NO will also abstract H from HI to form NOH and NOI under both thermal[68] and photolytic[69] conditions in the gas phase. However, the thermal reaction between HI and NO proceeds slowly even at 90°C.[68] The product NOI has been identified by IR spectroscopy at 77°K from the photolysis of HI in the presence of NO.[69]

Nitric oxide also reacts with molecular halogens to form nitrosyl halides in gas phase reactions under thermal[70,71] and photolytic conditions.[72,73] All molecules are reactive and their stability decreases in the order NOF > NOCℓ > NOBr > NOI, consequently, NOCℓ and NOBr always contain decomposition products, e.g., NO + $\frac{1}{2}$ $Cℓ_2$ (0.5%) and

$NO + \frac{1}{2} Br_2$, (7%) at 25°C and 1 atm.[74] The compound NOI is formed transiently and is too unstable for isolation, but Ridd and his co-workers[75] have suggested the intermediacy of NOI in the iodide catalysed acid nitrosation. Nitrous acid and iodide under acidic conditions have been shown, however, to give NO and I_2 (Equation 1.11) and the reaction is believed to proceed *via* NOI.[76]

$$H_2ONO^+ + I^- \longrightarrow NO\text{-}I \rightleftharpoons NO + \tfrac{1}{2} I_2 + H_2O \qquad 1.11$$

Reactions of NO in solution have not be extensively studied, but at 1 atm. pressure and 25°C, NO will abstract HO· from 0.5 M t-butyl-hydroperoxide in benzene yielding t-$BuONO_2$ and t-BuONO, and also reacts with t-butylhypochlorite giving NOCℓ and t-butylnitrite[77] (Equations 1.12 and 1.13).

$$t\text{-}BuO\text{-}C\ell + NO \xrightarrow{25^\circ C,\ benzene} t\text{-}BuO\cdot + NOC\ell \qquad 1.12$$

$$t\text{-}BuO\cdot + NO \longrightarrow t\text{-}BuONO \qquad 1.13$$

The authors also reported that NO reacts with N-chloropiperidine, or *N*-bromophthalimide to form *N*-nitrosamines under the same conditions. The photolytic and metal catalysed reactions of *N*-chloroamines with NO to form *N*-nitrosamines are well established.[78,79]

NO is reported to react directly with hydroxylamine in alkali to give N_2O and N_2, although the mechanism is not clear, the reactive intermediate is believed to be nitrosyl-hydroxylamine.[80]

Drago[81] has reported that NO will react slowly with diethylamine in methanol at 35°C and ether at -78°C, to form the salt $[Et_2\overset{+}{N}H_2.Et_2NN_2O_2^-]$, but the reaction is thought to proceed *via* dimeric NO and not NO itself. Brackman and Smit[82] found evidence that the same reaction in

MeCN and MeOH was catalysed by cupric salts, but required a more complex mechanism to explain their kinetic observations.

Challis and Krytopoulos[83] recently reported that NO itself does not react with secondary amines to produce N-nitrosamines and this confirmed a previous observation that NO does not react with Ph_2NH at 25°C.[84] The reaction of various amines with NO in MeCN at 25°C produced only 25% N-nitrosamine over 72 hours, the rate reaction was found to be independent of amine basicity. The zeroth order plots indicated that O_2 was leaking into the reaction vessel, and thus previous suggestions[85,86] that NO itself effects the nitrosation of amines should be treated cautiously because O_2 reacts rapidly with NO to form NO_2 with the eventual formation of N_2O_3, N_2O_4, which are known to be effective nitrosating agents.[40]

1.2.4.2. Nitric Oxide as NO^+.

The nitrosonium ion is a relatively stable molecule, loss of the π^* electron occurs easily and this is reflected by the low ionisation potential ($NO \longrightarrow NO^+ + e^-$, 9.25 eV).[87] This low value is expected since NO^+ is isoelectronic with CO and N_2, and its formation results in an increase in the bond order.

The existence and chemistry of NO^+ is well reviewed,[38,60] but experimentally, when N_2O_3, N_2O_4 are dissolved in conc. H_2SO_4, the ionic salt $NO^+HSO_4^-$ can be isolated.

1.2.5.1. Metal Nitrosyls.

The versatility of NO as a coordinating ligand arises from its unusual electronic structure and it is best regarded as a 3 electron donor. It forms nitrosyl complexes with many transition metals but not with non-transition elements or electron acceptors. Mononitrosyls

(i.e. those compounds containing only one NO group) can be conveniently prepared by the action of gaseous NO on the metal halide (Equation 1.14), cyanides (Equation 1.15) or by displacement of CO from carbonyl complexes (Equation 1.16).

$$CuCl_2 + NO \xrightarrow{EtOH,\ 25^oC} [CuCl_2.NO] \qquad 1.14$$

$$[Fe(CN)_6] + NO \xrightarrow{H_2O,\ H^+,25^oC} [Fe(CN)_5NO]^{2-} \qquad 1.15$$

$$Fe_3(CO)_{12} + 6NO \xrightarrow{petrol,\ 25^oC} 3[Fe(NO)_2(CO)_2] + 6CO \qquad 1.16$$

There are many reviews[88,89] which exhaustively list the wide range of transition metals that form nitrosyl complexes, and their preparation and properties will not be discussed here.

1.2.5.2. Bonding of Coordinated NO.

Sidwick regarded[90] nitrosyl complexes as derivatives of NO^+ and NO^-, and this classification is still in current usage. X-ray crystallography has revealed that NO can combine with transition metals by three principal modes;[56,91]

(I) As a 3e-donor, where transfer of one electron to the metal reduces its valency by one and produces NO^+, where the metal-NO (M-$\hat{N}O$) bond angle is close to 180° (the so-called linear or NO^+ mode) Figure 1.1.

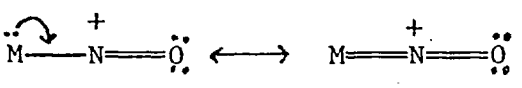


Figure 1.1

The N-atom is sp hybridised so donation of the lone pair of electrons to the metal and acceptance of electrons into the π^* antibonding molecular orbital of N, from the full d-atomic orbitals of the metal, gives multiple bonding character. This description is in good agreement with the short M-N bond length compared to other M-N bonds (Table 1.1) that are observed.

(II) As electron acceptor to form NO^-, with $M-\widehat{NO}$ bond angles of $120\text{-}130^0$, (the bent nitrosyls). The bent nitrosyls (Figure 1.2) are of similar geometry to nitrosyl halides and organic nitroso compounds where the X-N bond is also bent.

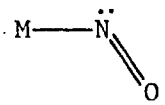
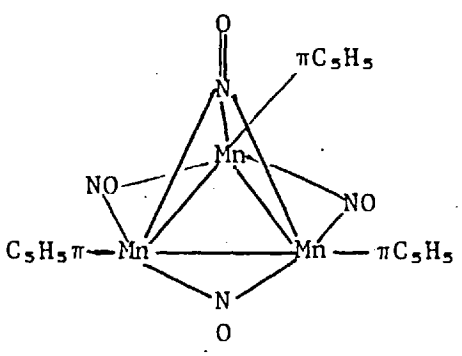


Figure 1.2

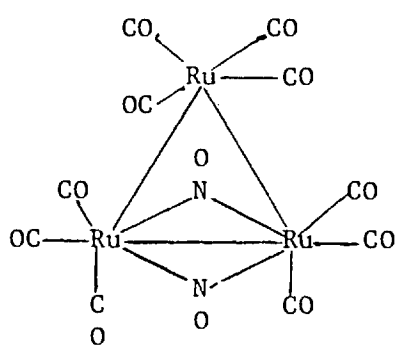
The N-atom is sp^2 hybridised, and formally there is a covalent bond between M-N. The complexes have a range of polarities and longer bond lengths (Table 1.1).

(III) Bridging Nitrosyls. This type of bonding is rare but is exemplified by two nitrosyls $[Mn_3(\pi\text{-}C_5H_5)_3(NO)_4]$[92] structure 1.III and $[Ru_3(NO)_2(CO)_{10}]$[93] structure 1.IV, where NO forms bridges between 2 or 3 metal atoms.



1. III

$(Mn)_3(\pi\text{-}C_5H_5)_3(NO)_4$



1. IV

$Ru_3(CO)_{10}(NO)_2$

TABLE 1.1

SOME PHYSICAL PROPERTIES OF COORDINATED NITRIC OXIDE COMPLEXES.

NO^+-Nitrosyls.	M-N/Å.	M-$\hat{N}$-O°.	ν_{NO} cm^{-1}.
$[Fe(CN)_5NO]^{2-}$	1.63	178	1939
$[V(CN)_5NO]^{3-}$	1.66	176	1530
$[Ir(NO)(PPh_3)_3]^{4+}$	1.67	180	1615
$[Co(diars)_2NO]^{2+}$	1.68	179	1852
$[RuCl_5NO]^{2-}$	1.74	177	1930
$[Ru(NH_3)_5NO]^{3+}$	1.80	167	1925
$[Mn(CO)_4NO]$	1.80	180	1763
$[Os(NO)(Co)(PPh_3)_2]^{2+}$	1.89	177	1750
NO^--Nitrosyls			
$[Co(en)_2ClNO]^+$	1.82	124	1611
$[Co(NH_3)_5NO]^{2+}$	1.87	119	1620
$[IrCl_2(NO)PPh_3]^+$	1.94	123	1560
$[IrCl(NO)(CO)PPh_3]^+$	1.97	124	1680
$[Co(NH_3)_6]^{3+}$	1.97		
$[Co(en)_3]^{3+}$	1.96		
$[Co(N_3)(NH_3)_5]^{2+}$	1.94		

Data taken from reference 93.

en = ethylenediammine.

PPh_3 = triphenylphosphine.

diars = diarsine.

Recent work suggests that Ag^+ attached to O atoms of an exchange zeolite forms two types of Ag-NO complexes depending on the experimental conditions. Below 50 Torr, the Ag-NO complex gave $\underline{\nu}_{NO}$ 1884 cm^{-1}, indicative of $[Ag^{I}(NO)]^+$ structure, whereas at pressures greater than 300 Torr, NO forms a bridge between 2 Ag^+ centres.[94]

In 1958, Lewis and Wilkinson[95] proposed that the IR stretching frequency of coordinated NO, $\underline{\nu}_{NO}$, could be correlated with the type of bonding. When NO loses the π^* electron, the $\underline{\nu}_{NO}$ increases from 1880 cm^{-1} (gaseous NO) to 2200-2300 cm^{-1} (in NO^+ salts). On coordination with metals containing d-electrons, synergistic π back bonding reduces ν_{NO}, and characteristically, ν_{NO} lies within the range 1045-2000 cm^{-1}. The majority of ν_{NO} are between 1750-1900 cm^{-1}, the so-called NO^+ region, while those within 1045-1650 cm^{-1} characterise the NO^- region. (Organic nitroso compounds, with R-NO bent normally absorb in the 1500-1600 cm^{-1} region).

Although ν_{NO} can give a rough guide to the reactivity of coordinated NO, it is misleading however, to describe all linear complexes as derivatives of NO^+ from only IR data. The two limiting ground states, linear or bent, are dictated by four factors - (I) the coordination number, (II) the coordination geometry (III) the number of electrons occupying the d-orbitals of the metal and π^* molecular orbitals of NO and (IV), the nature of the highest occupied MO.[96]

1.2.5.3. Nucleophilic Attack on Coordinated NO^+ Groups.

The ability of metal nitrosyls to act as nitrosating agents is well established.[97,98] Historically, the analysis of groups such as SH^-, thiammine and indoles, involved complexation with nitroprusside, $[Fe(CN)_5NO]^{2-}$ (ν_{NO} 1925-1944 cm^{-1}), resulting in a variety of characteristic colours.[57]

Many of these reactions have now been attributed to nucleophilic attack on the coordinated NO^+, and have been reviewed by Swinehart.[98] The reaction of nitroprusside with HO^-, however, requires further comment: the nitrosyl reacts in alkali to give $[Fe(CN)_5NO_2]^{4-}$ and eventually $[Fe(CN)_5H_2O]^{3-}$ and NO_2^- (Equation 1.17).

$$[Fe(CN)_5NO]^{2-} + 2OH \rightleftharpoons [Fe(CN)_5NO_2]^{4-} \overset{H_2O}{\rightleftharpoons} [Fe(CN)_5H_2O]^{3-} + NO_2^- \quad 1.17$$

The rate of hydrolysis of the nitroprusside is 10^6 x slower than that of the hydrolysis of free NO^+. This large difference was ascribed to enhanced stability of the nitrosyl, which makes it more selective and hence a good source of NO^+.

Nitroprusside and $[Ru(bipy)_2C\ell(NO)]^{2+}$ both react with N_3^-, NH_3, hydroxylamine giving N_2O and N_2 and with secondary amines to give N-nitrosamines, and so behave chemically as NO^+.

Bottomley[99] regarded several metal nitrosyls as potential electrophiles, susceptible to nucleophilic attack and suggested that this property can be loosely related to the direct magnitude of ν_{NO}. This property is probably restricted to six coordinate nitrosyls having $\nu_{NO} > 1886\ cm^{-1}$ and it may exclude most metal nitrosyls apart from those which can be considered to approach true ionic character (Table 1.2). For example, complexes of metals with high positive charge and little back bonding, such as $[Ir^{III}(NO)C\ell_5]^{2-}$, give rise to a high residual positive charge on NO and reaction with NH_3 to give N_2 occurs instantaneously at 25°C.[100] However, no reaction of $[Os^{III}(NH_3)_4(NO)OH]^{2+}$ is observed, the ligands (other than NO) are all σ donors without

TABLE 1.2

THE RELATIONSHIP OF ν_{NO} OF METAL NITROSYLS, TOWARDS NUCLEOPHILIC ATTACK. (TAKEN FROM REFERENCE 57).

Nitrosyl Complex.	ν_{NO} cm^{-1}.	Nucleophile.	Product.
$[IrCl_5(NO)]^-$	2006	HO^-	$[IrCl_5NO_2]^{3-}$
		NH_3	$[IrCl_5(NH_3)]^{2-}$
		N_3^-	$[IrCl_5(H_2O)]$, $N_2 + N_2O$
		NH_2OH	$[IrCl_5(H_2O)]$, N_2O
$[IrCl_3(NO)(PPh_3)]ClO_4$	1945	EtO^-	$[IrCl_3(EtONO)PPh_3]ClO_4$
$[Fe(CN)_5NO]^{2-}$	1938	HO^-	$[Fe(CN)_5NO_2]^{4-}$
$[RuCl(bipy)_2(NO)]^{2+}$	1927	HO^-	$[RuCl(bipy)_2NO_2]$
		$ArNH_2$	$[RuCl(bipy)_2(NNAr)]^{2+}$
		$ArNMe_2$	$[RuCl(bipy)_2\{(ON)ArNMe_2\}$
		N_3^- aq.	$[RuCl(bipy)_2H_2O]^+$, N_2, N_2O
$[Ru(NH_3)_5NO]^{3+}$	1925	HO^-	$[Ru(NH_3)_5NO_2]^{2+}$
$[RuCl_5NO]^{2-}$	1886		No Reaction
$[Os(NH_3)_5NO]^{3+}$	1885		No Reaction
$[Os(NH_3)_4(OH)NO]^{2+}$	1883		No Reaction

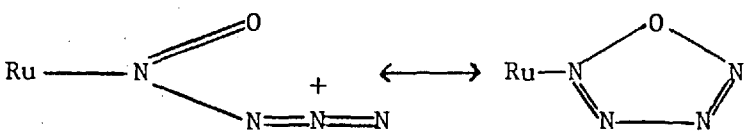
PPh_3 - Triphenylphosphine

bipy - Bipyridyl

π-acceptor properties. Thus, even though there is a high positive charge on the metal, back bonding of some electrons reduces ν_{NO} and consequently, the reactivity towards nucleophiles.

Bottomley's relationship is not always followed, thus, cis-$[Ru(NH_3)_4Br(NO)]Br_2$, ν_{NO} 1902 cm^{-1}, only converts to trans-$[Ru(NH_3)(OH)(NO)]Br_2$ even when heated in alkaline solution.[91] On the other hand, $[Pt(NH_3)_3NO]X_2$, $[Pt(NH_3)_3NOSO_4]X$ and $[Pt(NH_3)_2(NOSO_4)(HSO_4)]$, which all have ν_{NO} 1713-1810 cm^{-1}, all react to give NO_2^- with aqueous alkali.[91]

Azide ion reacts with metal nitrosyls having ν_{NO} > 1886 cm^{-1}, with the formation of N_2O and N_2. The nitrosyl-azide intermediate Ru-NO-N_3 (Equation 1.18) has been reported to have been isolated from the reaction of $[Ru(bipy)_2Cl(NO)]^{2+}$ and N_3^-.[57] The products N_2O



1.18

and N_2 arise from the displacement by excess azide, hence, establishing that N_2O formation is not due to the disproportion of NO. The reaction of $[Ru(bipy)_2Cl(NO)]^{2+}$ with N_3^- (Equation 1.19) is analogous to the nitrosation of $[Co(NH_3)_5N_3]^{2+}$ with free NO^+ (Equation 1.20), and free NO^+ and N_3^-.[101]

$$[Ru(bipy)_2Cl(NO)]^{2+} + N_3^- \xrightarrow{H_2O} [Ru(bipy)_2Cl(H_2O)]^+ + N_2 + H_2O \qquad 1.19$$

$$[Co(NH_3)_5N_3]^{2+} + NO^+ \xrightarrow{H_2O} [Co(NH_3)_5H_2O]^{3+} + N_2 + N_2O \qquad 1.20$$

The reaction in MeCN leads to the same product but with different coordinating ligands (Equation 1.21), but unlike H_2O, the organic

$$[Ru(bipy)_2C\ell(NO)]^{2+} + N_3^- \xrightarrow{MeCN} Ru(bipy)_2C\ell(MeCN)]^{+} + N_2O + N_2 \qquad 1.21$$

molecule is labile and can be displaced.[102] Hydroxide ion also reacted with the ruthenium nitrosyl to give $Ru\text{-}NO_2^-$, and the reaction is reversible in the presence of acid.[103]

The reaction of alcohols with $[IrC\ell_3(NO)PPh_3]^+ \nu_{NO}$ 1945 cm^{-1}, to give a RONO- complex can also be reversed to give starting materials by acid.[104] Hence, the reactions of coordinated NO^+, are analogous to the behaviour of both free NO_2^- and alkylnitrites in acidic media. In the presence of NO, $CoC\ell_2$ also nitrosates alcohols and alkoxides,[105] but the reaction proceeds <u>via</u> reductive nitrosylation, (Equation 1.22), the cobalt dinitrosyl [IV] has ν_{NO} 1876, 1792 cm^{-1} which may be considered as derivatives of NO^+ and NO^- (Where L = tetramethylethylenediammin

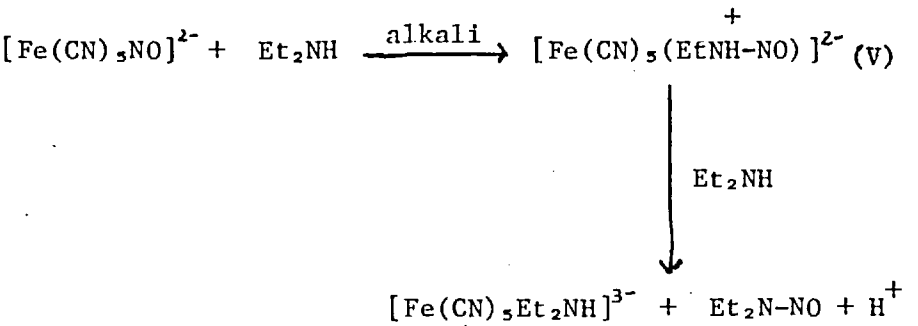
$$NEt_3 + CoC\ell_2 + 2L + 3NO \xrightarrow[MeOH]{25^oC} \underset{(IV)}{CoC\ell(NO)_2L} + MeONO + Et_3N.HC\ell \qquad 1.22$$

There are relatively few examples of amines reacting with coordinated NO^+ leading to either diazonium ion or <u>N</u>-nitrosamine formation. Bowden and his co-workers[106,107] prepared the arenediazonium complex by the reaction of $ArNH_2$ with $[Ru(bipy)_2C\ell NO]^{2+}$ (Equation 1.23).

$$[Ru(bipy)_2C\ell NO]^{2+} + ArNH_2 \xrightarrow[MeCN]{25^oC} [Ru(bipy)_2C\ell NNAr]^{2+} + H_2O \qquad 1.23$$

The reaction mechanism was shown very elegantly using ^{15}NO to involve NO^+ and the intermediate diazonium salt could be treated with KI

or β-naphthol to give iodobenzene or the diazo compound. Secondary and tertiary aromatic amines reacted with the same nitrosyl[107] to give C-nitroso-products, but in these cases, it is unknown whether the initial step is direct C-nitrosation or N-nitrosation followed by the Fischer-Hepp rearrangement. N-nitrosamine formation has, however, been reported for the reaction of nitroprusside[108] and the copper-nitrosyl[109,82] with secondary amines. Maltz,[108] reported the unique nitrosation of amines with $[Fe(CN)_5NO]^{2-}$ in alkaline media up to pH 12.7, above this pH, nitroprusside is converted into the NO_2^- salt by hydrolysis. Primary amines were deaminated to alcohols and N_2 via the diazonium salt (generated in alkaline media); secondary amines, such as diethylamine gave N-nitrosamines, while aromatic secondary amines or tertiary amines did not react. The yields of product showed dependence on the initial amine concentration, and required that a two-fold excess over the nitroprusside was present. The reaction mechanism involved initial attack of the amine, for example, Et_2NH, on the nitroprusside complex, (Scheme 1.3) (which reflects the dependence on amine basicity and indicates why aromatic amines did not react), to form the protinated N-nitrosoamine complex (V), in the case of diethylamine. Displacement of the N-nitrosoamine by another mole of amine gave H^+ and the iron-amine-cyanato complex.



Scheme 1.3.

Cu^{II}-halides in ethanol form violet nitrosyls[110] when in contact with gaseous NO. These complexes have ν_{NO} 1824-1830 cm^{-1}, are diamagnetic and so may formally be regarded as $[Cu^{+}(NO^{+})]X_2$. Generally, these complexes are too unstable for isolation, although Frazer[111] showed that the nitrosyl was formed in solvents with donor properties. In ethanol, the violet nitrosyl slowly dissociates into a colourless ion, with the formation of ethylnitrite [111] (Equation 1.24). The formation of ethylnitrite implies that nucleophilic attack on coordinated NO^{+} was possible.

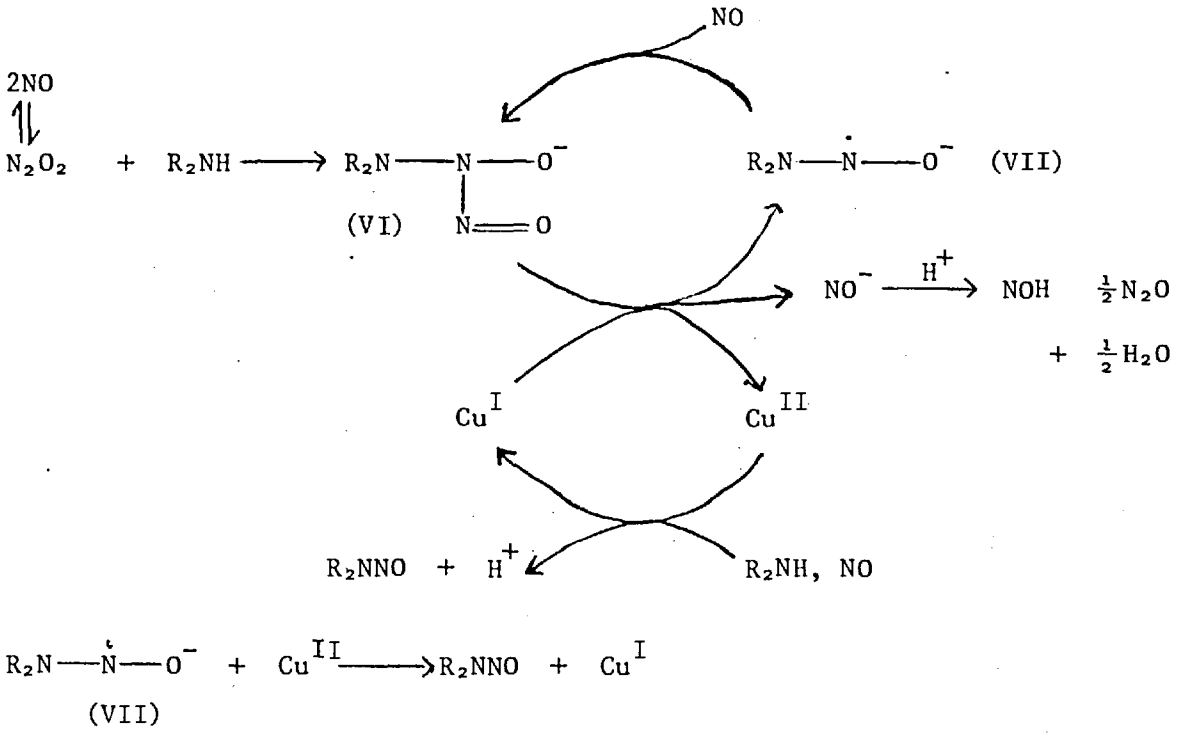
$$CuX_2 + NO \rightleftharpoons [CuX_2^{-}NO^{+}] \underset{}{\overset{EtOH}{\rightleftharpoons}} CuX + EtONO + HX \qquad 1.24$$

Doyle and his colleages[109] found that N_3^{-} reacted with the nitrosyl giving, N_2O, N_2 and Cu(I), whereas primary aliphatic amines when complexed to $CuC\ell_2$ were oxidatively deaminated to geminal dihalides (Equation 1.25).

$$ArCH_2CH_2\text{-}NH_2.CuC\ell_2 + [CuC\ell_2.NO] \xrightarrow[MeCN]{25^{o}C} ArCH_2CHC\ell_2 + N_2O + N_2 \qquad 1.25$$

These results suggested that the low ν_{NO} (due to extensive back bonding from d-electrons) does not reflect the eletrophilic character of $Cu(NO)C\ell_2$ as predicted by Bottomley.[99]

Earlier, Brackman and Smit[82] observed that the copper-nitrosyl could nitrosate secondary amines and alcohols. Their mechanism (Scheme 1.4) for the nitrosation of secondary amines is complicated, but it now appears that the diffusion of nitric oxide into the solvent may have been the rate-limiting under their conditions. They found that $CuC\ell_2$ behaved catalytically, Cu(I) predominated during the course of reaction



$$R_2N—\dot{N}—O^- \text{ (VII)} + Cu^{II} \longrightarrow R_2NNO + Cu^{I}$$

Scheme 1.4

and that N_2O was formed. From these observations, they suggested that after the rapidly established pre-equilibrium, dimeric NO reacted slowly with the amine (dimethylamine) to give the Drago salt[81] (VI).

The copper nitrosyl reacted with the amine to give N-nitrosamine H^+ and Cu^I, which was continually oxidised by (VI) to Cu^{II} and complex (VII). The anion radical (VII) could react with Cu^{II} to give N-nitrosamine, but in the presence of NO, regeneration of (VI) was most likely. Essentially, the reaction can be abbreviated to (Equation 1.26).

$$Cu^{II} + 2NO \xrightarrow{R_2NH} Cu^{II} + R_2NNO + NOH \qquad 1.26$$

1.2.5.4. Electrophilic Attack.

There is clear evidence that in some metal nitrosyls, the bound NO behaves as NO^-. Normally, protonation of the coordinated NO has been accepted as indicative of the presence of NO^-.[56] The few examples known are shown in Equations 1.27, and 1.28.

$$OsCl(NO)(CO)L_2 + HCl \rightleftharpoons OsCl(HNO)(CO)L_2 \qquad 1.27$$

ν_{NO} 1600 cm^{-1}

$$[Co(diars)_2NO]^{2+} \xrightleftharpoons{HBr} [Co(diars)_2(HNO)(Br)]^{2+} \qquad 1.28$$

1.2.5.5. Reduction Reactions.

The nitrosyl ligand can, in principle, also be reduced to NO, NO^-, N_2 and NH_3. For example, Cr^{2+} has been used to reduce the coordinated nitrosyl group of $[Ru(NH_3)_5NO]^{3+}$ to NH_3 (Equation 1.29).[56,57]

$$[Ru(NH_3)_5NO]^{3+} + 6Cr^{2+} + 5H^+ \longrightarrow [Ru(NH_3)_6]^{2+} + 6Cr^{3+} + H_2O \qquad 1.29$$

Also, the nitrosyl complexes of ruthenium, rhodium and iridium have been reduced to N_2O by CO.

1.2.5.6. Disproportionation.

Disproportionation was mentioned briefly in the reductive nitrosylation of methanol by $CoCl_2$; Rhodium(III) catalyses the disproportionation of NO in ethanol to give EtONO and N_2O.[56] The Scheme can best be depicted in terms of NO^- assisted attack of NO^+ on ethanol (Equation 1.30).

$$[RhIII(NO^-)(NO^+)] + EtOH \longrightarrow Rh(EtONO) + NOH \qquad 1.30$$

1.3. Concluding Remarks.

Although NO is a major pollutant,[56,57] reports of its chemical inactivity in solution have led to very little research into its chemistry. However, the better understanding of bonding in metal nitrosyls, especially in those cases where NO^+ formation is important, has increased the predictive powers of its reactivity substantially.

The fate of NO^+, generated under very mild conditions, has not been hitherto, extensively investigated with respect to N-nitrosamine formation. Clearly, the ubiquitous nature of N-nitrosamines cannot be accounted for only by acidified nitrite. The results that follow show unequivocally that NO, by suitable activation, is capable of readily converting amino compounds into carcinogenic N-nitrosamines under neutral conditions in solution and is therefore, of relevance in the elucidation of human exposure to N-nitrosamines.

CHAPTER 2

THE FORMATION OF N-NITROSAMINES IN SOLUTION FROM GASEOUS NITRIC OXIDE IN THE PRESENCE OF IODINE.

2.1. Introduction.

Halide ions ($C\ell^-$, Br^-, I^-) have been observed to catalyse the diazotization of aniline,[75] and the formation of N-nitrosamine from secondary amines[7] from the reaction with acidified nitrite. The general reaction mechanism (Scheme 2.1) involves attack of the halide ion on the nitrous acidium ion H_2ONO^+, resulting in the formation of

$$HNO_2 + H^+ \; Hal \rightleftharpoons NO^+ - Hal + H_2O$$

$$NO^+ - Hal + R'RNH \xrightarrow{slow} R'\overset{+}{R}NH - NO + Hal$$

$$\downarrow$$

Products

Scheme 2.1

a nitrosyl halide (NOCℓ, NOBr, NOI), which then reacts directly with the unprotonated amino compound to give the corresponding products. For Br^-, I^- catalysis, the formation of both NOBr and NOI can be made rate-limiting.[75] It was reported recently, however, that nitrous acid (HONO) oxidises I^- to I_2, with the reduction of HONO to NO.[76] These products were believed to form from the decomposition of the NOI intermediate. This is in good agreement with previous observations that the stability of the nitrosyl halide decreases in the order NOCℓ > NOBr > NOI and these NOX species decompose to varying extent

into NO and X_2 at $25^{o}C$, 1 atm.[74] The entity NOI is not isolable even at $77^{o}K$[69] and decomposes readily into NO and I_2.

The thermal [68] and photolytic[69] studies of the reaction between NO and I_2 in the gas phase, have shown that it is possible to form NOI, but the direct interaction of NO with I_2 has not been previously considered as a source of NOI in solution.

2.2. Results and Discussion.

2.2.1. Reactions in EtOH.

Under anaerobic conditions, and in the absence of iodine, N-nitrosamine formation from NO in EtOH and other solvents at 25°C was exceedingly slow. Typically, with 0.01 M piperidine, morpholine and N-methylpiperazine only 5% N-nitrosamine was formed on standing overnight. Generally, the small amounts of N-nitrosamine were found immediately after injection of the amine into the reaction solution, but thereafter, its concentration increased slowly or not at all over the next 2-3 days. NO itself, is a poor nitrosating agent for molecular amines, presumably because it is unable to abstract the amino-H-atom to generate the dialkylamino radical ($R_2\dot{N}$) which might be expected to combine further with NO (Equation 2.1). The relative stabilities of $R_2\dot{N}$ and $\dot{N}O$ make the first step of Equation 2.1 very endothermic.

$$R_2NH + NO \longrightarrow (NOH) + R_2\dot{N} \xrightarrow{NO} R_2N\text{-}NO \qquad 2.1$$

Any reaction in the absence of catalysts probably results from the adventitious leakage of air into the reaction vessel. This would produce either N_2O_3 or N_2O_4, both of which have recently been shown to be powerful nitrosating agents.[40]

N-nitrosamine formation was very much faster in the presence of elemental iodine. Reaction was usually complete in ca. 20 minutes, and, oxygen apart, iodine appears to be one of the best promoters of nitrosation by NO. Unfortunately, these reactions did not lend themselves to precise kinetic measurements because of the difficulties both

in controlling the NO concentration in solution and in devising an artifact-free quenching procedure. Mechanistic deductions can be drawn, however, from the variation of % Reaction with respect to time of g.l.c., injection typified by Figure 2.I,for 0.01 M N-methylpiperazine in EtOH saturated with NO and containing various deficient concentrations of iodine. (At the end of reaction, when maximum yield is obtained, the iodine colour is completely lost). The initial concentration of dissolved NO was found to be ca. 2.2×10^{-2} M (v. Experimental 2.4.(VI))and since the reactions were carried out under an atmosphere of NO, it should be maintained throughout the reaction by absorption from the gaseous phase.

Similar plots were obtained for the nitrosation of morpholine and piperidine, and variations in the maximum yield of the corresponding N-nitrosamine and its time of formation with initial concentrations of both iodine and amine (in the case of morpholine), are summarised in Table 2.I. These results show several important characteristics. Thus, the maximum yield of N-nitrosamine is dependent only on the initial $[I_2]$, but is virtually independent of both the basicity (reactivity) and initial concentration of the amine substrate. Figure 2.2 shows the independence of amine basicity on the rate of reaction under standard conditions of ca. 2.5×10^{-3} M I_2 and 0.01 M amine in ethanol at 25°C. This observation implies that the amine is not involved in the rate-limiting step since the time required to reach the maximum yield is about the same for all reactions. The relationship between the maximum yield and initial $[I_2]$ is quantified by Figure 2.3, which shows that 2 moles of N-nitrosamine are produced by each mole of iodine. Thus, the reaction rate probably follows Equation 2.2, although proof of the kinetic order for NO and I_2 requires

Figure 2,(I).

The effect of iodine on the formation of N-methyl-N-nitroso piperazine from initial 0.01 M N-methylpiperazine and initial saturated (ca. 2.2 x 10^{-2} M) NO in EtOH at 25°C.

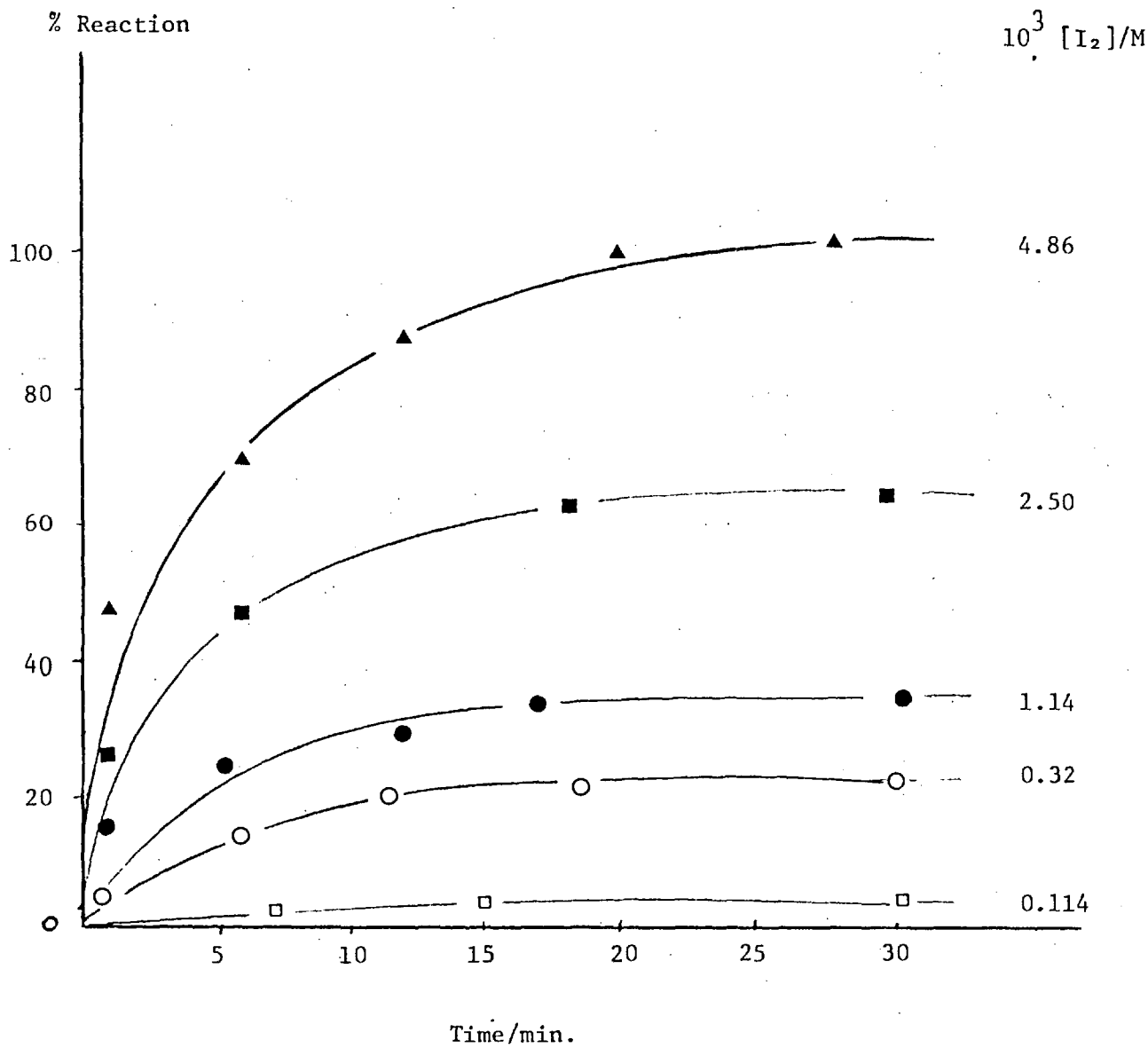


Table 2.1.

Limiting yields and time of N-nitrosamine formation from secondary amines, NO and I_2 in EtOH at 25°C. Initial [NO] ca. 2.2×10^{-2} M.

10^2[Amine]/M	$10^3[I_2]$/M	Limiting Yields (%)[a]	Time (min)[b]
1.0 Piperidine	0	4	1200
"	0.53	15	20
"	1.05	20	20
"	2.46 (2.64)[c]	44 (48)	20 (20)
"	2.45[d]	45	20
"	4.70	57	25
1.0 N-Methylpiperazine	0	4	80
"	0.32	14	18
"	1.14	34	17
"	2.50 (2.37)[c]	49 (60)	21 (18)
"	4.86	100	20
1.0 Morpholine	0	4.3	450
"	1.15	22	20
"	2.60 (2.63)[c]	54 (48)	22 (20)
"	5.15	58	25
0.50 Morpholine	2.51	46	22
2.0 Morpholine	2.46	25	20

[a] Based on initial [Amine]

[b] Time to reach limiting yields

[c] Duplicate experiments in parenthesis

[d] I_2 and NO in EtOH left standing for 40 hrs. before addition of piperidine.

Figure 2.2.

The rate of N-nitrosamine formation from deficient 2.5-2.74 x 10^{-3} M I_2 in the presence of initial 0.01 M piperidine (▲), morpholine (●), N-methylpiperazine (■), with initial saturated (ca. 2.2 x 10^{-2} M) NO in EtOH at 25°C.

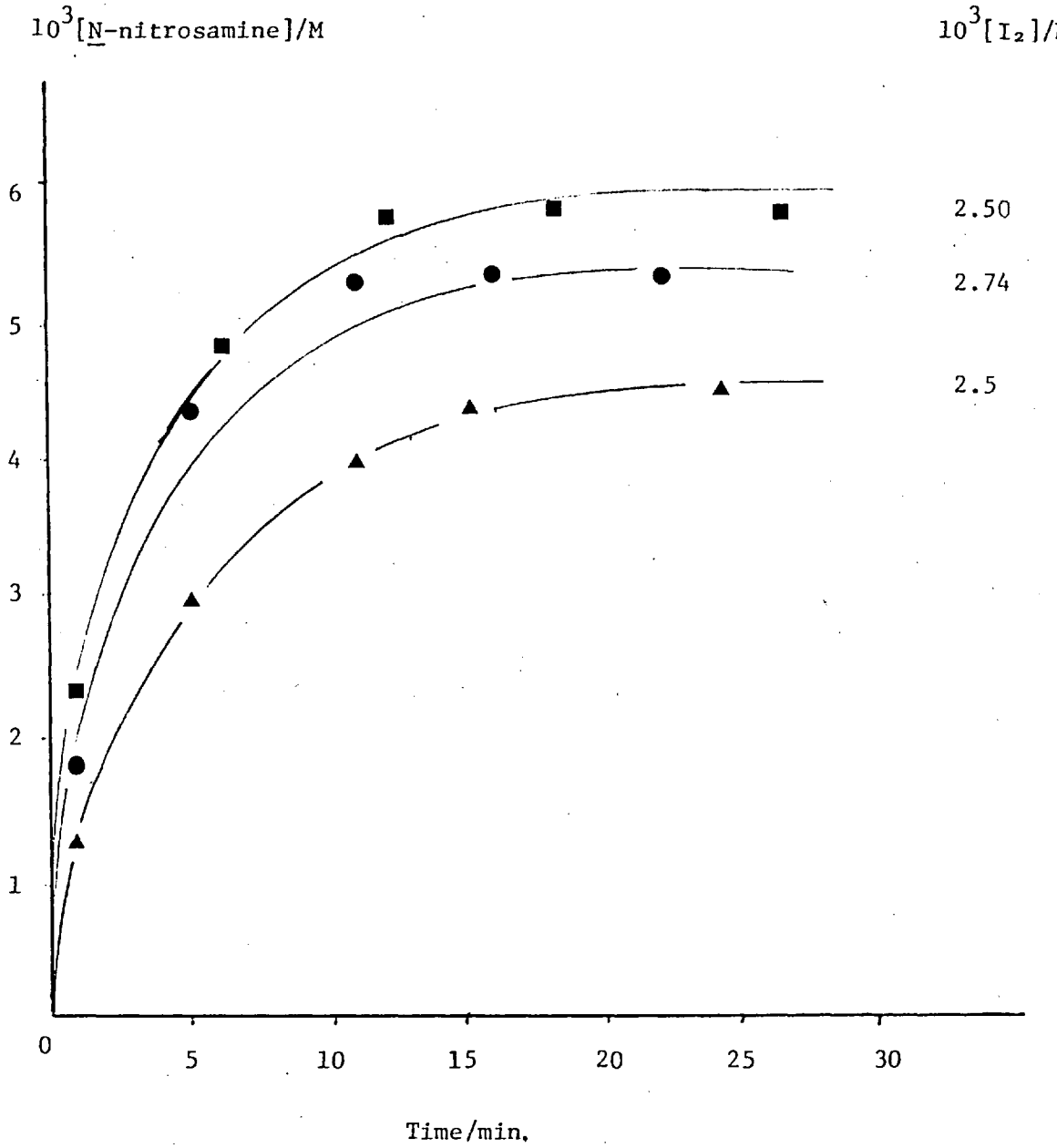
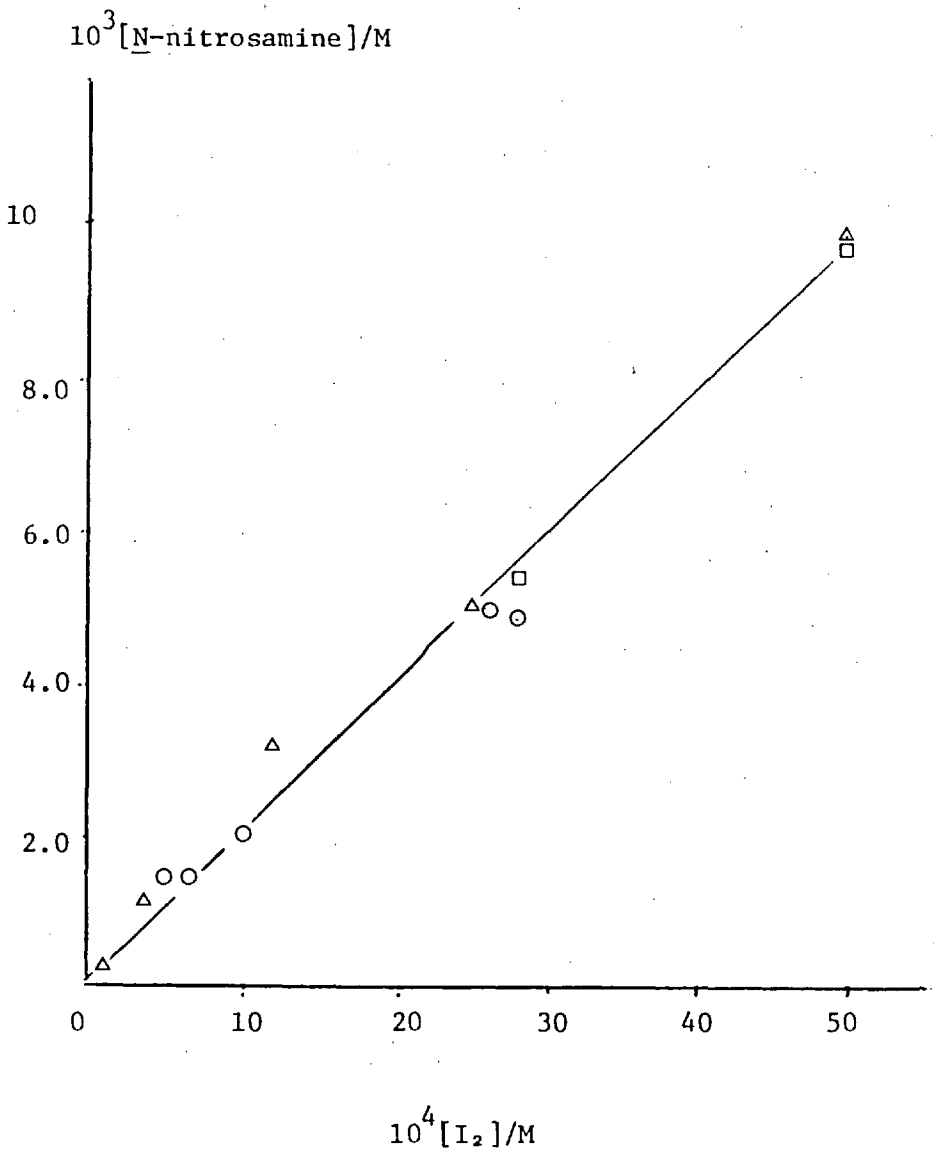


Figure 2.3.

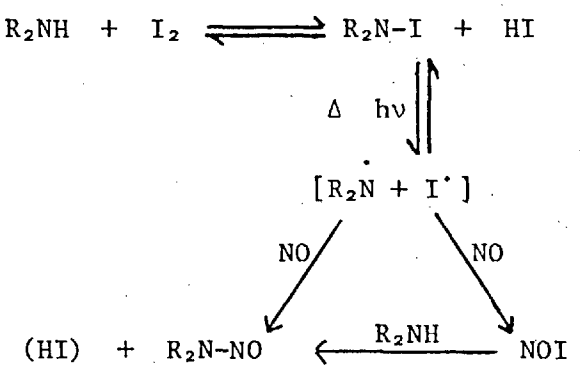
Dependence of limiting N-nitrosamine yields on the initial iodine concentration for the reaction of excess piperidine (O), morpholine (□) and N-methylpiperazine (△) with saturated NO (ca. 2.2 x 10^{-2} M) in EtOH at 25°C.



further experiments.

$$\text{Rate} = \underline{k}\,[NO][I_2]^{\frac{1}{2}} \qquad 2.2$$

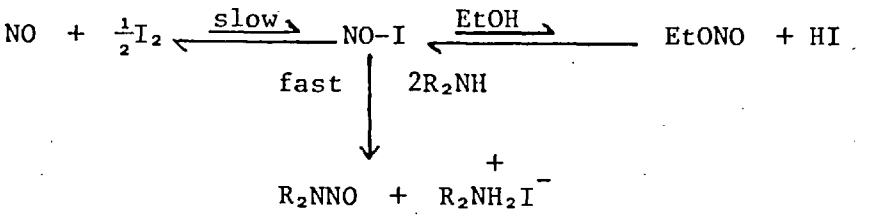
Although N-iodoamines may be expected to react with NO under thermal conditions,[112] these pathways (e.g., Scheme 2.2) cannot apply to the reaction of NO and I_2, because both rates and yields



Scheme 2.2

are independent of the basicity and concentration of the secondary amine.

These observations also rule out reactions via charge transfer complexes, which are known to form readily from secondary amines and iodine in organic solvent[113]. Further, no products typical of dialkylamino radical intermediates, such as imines and tetra-alkyl hydrazines, could be detected in the reaction solutions. Therefore, the preferred mechanism (Scheme 2.3) is one involving rate-limiting formation of nitrosyl-iodide (NOI), principally from NO and iodine, followed by rapid reaction of this reagent with the amine.



Scheme 2.3

Scheme 2.3: Mechanism for the formation of N-nitrosamines from NO and secondary amines in the presence of iodine.

Two aspects of Scheme 2.3 require further comment. The first concerns the release of HI as a by-product which, as the reaction proceeds, will both acidify the solution and lead to the formation of tri-iodide ($I_2 + HI \rightleftharpoons HI_3$). The second concerns the formation of ethyl nitrite (EtONO) which could be the effective nitrosating agent in the presence of HI.[114] The production of HI must also lead to amine cation ($R_2NH_2I^-$) formation, and inter alia, a reduced yield of N-nitrosamine. This consequence is not clearly evident in Table 2.I because excess amine was employed, but it may explain the slight reduction in the maximum yields of N-nitrosamine with increasing basicity of the amine (i.e., piperidine (pK_A 11.22)[115] < morpholine (8.33)[115] < N-methylpiperazine (9.8, 5.11)[115]). The effect is apparent, however, for reactions carried out with an excess of I_2 over amine. These additional results are summarised in Table 2.2 . Significantly, the maximum yield of N-nitrosopiperidine does not normally exceed 50% of the initial [piperidine] as required for the mechanism in Scheme 2.3. Further, the addition of small amounts of NaOH or NaOEt to the solution allows the reaction to proceed almost to completion. For N-methylpiperazine, however, N-nitrosamine formation is virtually complete without the addition of

Table 2.2

Limiting yields for N-nitrosamine formation from secondary amines and NO with excess I_2 in EtOH at 25°C. Initial $[I_2]$ = 0.99-1.04 x 10^{-2} M, [NO] ca. 2.2 x 10^{-2} M.

10^3 [Amine]/M	Initial Limiting yield (%)[a]	Added base[b] (µl 4 M NaOH)	Final Yield[c] (%)
1.25 Piperidine	16	100 (0.01)	100
2.5 "	14	100 (0.01)	99
3.75 "	21	-	-
5.0 "	29	50 (0.005)	94
10.0 "	42	50 (0.005)	95
15.0 "	47	-	-
5.0 Morpholine	65	100[d] (0.01)	89
10.0 "	64	100[d] (0.01)	93
10.0 "	58[e]	100[d] (0.01)	87
10 N-Methylpiperazine	100	-	-
10 "	100[e]	-	-

[a] Based on initial [Amine]

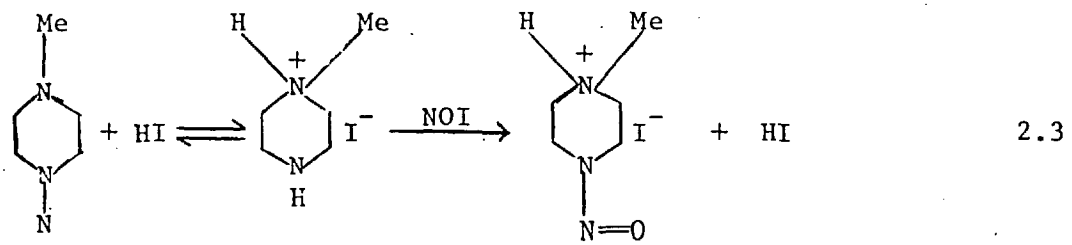
[b] Added after ca. 15 min. Molar concn. in solution in parenthesis

[c] After addition of base, based on initial [Amine]

[d] µl 4.04 M NaOEt

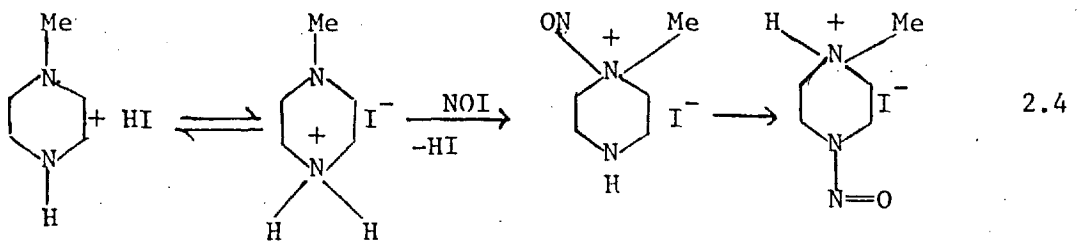
[e] With 5 x 10^{-3} M iodine.

NaOH. This amine is dibasic (pK_A 9.8, 5.11) so it may protonate at the tertiary N-atom, but still react with NOI at the secondary position (Equation 2.3), or form a quarternary nitrosonium salt which then under-



2.3

goes an intramolecular transnitrosation (Equation 2.4).



2.4

In any event, under equivalent reaction conditions, free base concentrations will be higher for N-methylpiperazine than either piperidine or morpholine.

Examination of the reaction solutions by h.p.l.c. showed that low concentrations of ethyl nitrite were present. Attempts to quantify the amount were largely unsuccessful probably because this compound is very volatile (b.p., $17^{o}C$[116]). The equilibrium between n-butyl nitrite and NO (Equation 2.5) gave more reproducible results but significant errors in estimating the n-BuONO by h.p.l.c. assay combined with complications arising from the additional equilibrium between

$$\text{n-BuONO} + 5/4\ \text{HI} \rightleftharpoons \text{n-BuOH} + \text{NO} + {}^{I}2/4 + {}^{HI}3/4 \qquad 2.5$$

iodine and tri-iodide ion [$I_2 + I^- \rightleftharpoons I_3^-$], which is included in Equation 2.5, prevented calculation of equilibrium constants. This process was examined, however, to give some idea of the extent of alkyl nitrite formation under kinetic conditions. Measurements were taken both of the dissociation of n-BuONO in the presence of HI (by u.v.) and for the formation of n-BuONO from NO plus iodine in n-BuOH (using h.p.l.c.). Addition of HI (actually KI plus HCℓ) to equimolar amounts of n-BuONO in solvent EtOH produced the characteristically strong absorptions attributed to I_3^- (λ_{max} (H_2O) 288, 353 nm, log ε 4.60, 4.42[117]). The I_3^- concentrations, assuming λ_{max} is independent of solvent and HI_3 is completely dissociated, (Table 2.3) are not quantitative (i.e., $[I_3^-] < [HI]/3$).

Independent measurements, however, showed that not all the iodine would be converted into I_3^-, even in the presence of HI (Table 2.4). When adjusted for this factor, the results in Table 2.3 indicate that ca. 16% n-BuONO remains in equilibrium with NO, I_2 and I_3^-. This figure agrees qualitatively (± 5%) with direct h.p.l.c. estimates of n-BuONO formed in NO saturated n-BuOH containing 0.01 M I_2, although these measurements were also uncertain owing to extraneous n-BuONO formation on the h.p.l.c column. This was apparent from the analyses of NO in n-BuOH containing no added I_2. It probably arises from the oxidation of NO to NO_2 by adventitious oxygen. Data werecorrected for this effect (v. Experimental 2.4.IV). The most reliable estimate of EtONO formation, therefore, comes indirectly from the limiting yields of N-nitrosamine. As noted above, these reflect the extent of amine protonation and inter alia, the acidity of the reaction solutions. For the data in Table 2.2, the limiting yields of N-nitrosopiperidine before the addition of base decreases from ca. 42-14.5% with decreasing initial piperidine

Table 2.3

Equilibrium between HI plus n-BuONO and I_3^- in EtOH at 25°C.

10^5 [n-BuONO]/M	10^5 [HI][a]/M	OD(λ 290 nm)[b]	10^6 $[I_3^-]$/M
1	1	0.01	0.25
2	2	0.02	0.50
3	3	0.085	2.2
6	6	0.255	6.38
8	8	0.465	11.6
10	10	0.625	15.6

[a] From equimolar KI and HCℓ

[b] 10 mm cell.

Table 2.4

Equilibrium between I_3^- and I_2 in EtOH at 25°C and influence of added HI.

10^5 $[I_2]/M$	10^5 $[HI]/M$	OD (λ 290 nm)[a]	10^5 $[I_3^-]/M$
2	-	0.16	0.4
5	-	0.64	1.6
10	-	1.00	2.5
20	-	1.18	2.95
10	2.5	1.46[b]	3.65
10	5.0	1.80[b]	4.5
10	7.5	2.04[b]	5.1
10	10.0	2.32[b]	5.8
10	12.5	2.58[b]	6.4

[a] In 10 mm cell

[b] Reaction solutions diluted volumetrically by 2 for on-scale reading.

concentration, and the deficiency below 50% should equal the amount of HI produced by the reaction of NOI with the solvent. This corresponds to 8±2% of the initial iodine concentration. For example, with 0.01 M I_2 and 0.01 M piperidine, the limiting yield of N-nitrosopiperidine (Table 2.2) = 4.2×10^{-3} M. Thus, the amount of I_2 reacting to produce HI is given by $(0.01 - 2 \times 0.0042)/2 = 0.0008$ M, i.e., 8%. Since two moles of HI arise from each mole of I_2, this agrees well with the estimate based on the dissociation of n-BuONO. Hence, the conclusion is that under kinetic conditions, less than 10% of the initial iodine had reacted with the solvent. There was no apparent change in the maximum yield of N-nitrosopiperidine (Table 2.2) when a solution of NO and I_2 in EtOH was left to stand for 40 hours.

Ethyl nitrite presumably arises from nucleophilic attack by the solvent on NOI, in competition with the secondary amine. Most alkylnitrites, however, are ineffectual nitrosating agents under non-acidic conditions,[114,51] so the likelihood of N-nitrosamines arising from the ethyl nitrite in the presence of either excess amine (Table 2.1) or added NaOH (Table 2.2) seems remote. This conclusion was confirmed by observing that 0.011 M piperidinium hydrochloride with 0.0063 M n-BuONO and 0.0078 M KI in EtOH at 25°C gave only 5×10^{-4} M N-nitroso-piperidine (ca. 5% reaction) over a period of 24 hours; also, when 0.0175 M n-BuONO and 0.01 M HCℓ in EtOH under anaerobic conditions was left to stand for 10 min , then 0.01 M piperidine added, only 1-5% N-nitrosopiperidine over 24 hours was obtained. If, however, 0.01 M KI is added to the above solution of n-BuONO and HCℓ, I_2/I_3^- is formed immediately and on addition of piperidine, 30% N-nitrosopiperidine forms after only 35 min. The observation suggests that the combination of NO and I_2 is unique for the nitrosation of amines in alcohols, and this presumably arises

from the relative positions of the associated equilibria and also from how the reagent is stored. With n-BuONO and HCℓ, (Equation 2.5) the equilibrium lies well over to the left hand side, i.e. the formation of n-BuONO from NOCℓ and n-BuOH. (The decomposition of

$$\text{n-BuONO} + \text{HC}\ell \rightleftharpoons \text{n-BuOH} + \text{NOC}\ell \quad \ldots\ldots 2.6$$

MeONO in HCℓ is reported to be 7% at 25°C[118]). This is in good agreement with the observation related to the inhibition of NO^+BF_4 (reported later, Chapter 4), and NOCℓ, N_2O_3 nitrosation of piperidine with alcohols.[119] NOCℓ is, however, attacked by I^- to give nitrosyl-iodide, which exists almost exclusively as NO and I_2 (Equation 2.7), and hence shifts the equilibirum to the right.

$$\text{n-BuONO} + \text{HC}\ell \rightleftharpoons \text{n-BuOH} + \text{NOC}\ell \xrightleftharpoons{I^-} C\ell^- + \text{NOI} \rightleftharpoons \text{NO} + \tfrac{1}{2}I_2 \quad \ldots.2.7$$

2.2.2 Reactions in Acetonitrile.

Examination of the reactions in solvent MeCN confirmed that ethylnitrite is incidental to the formation of N-nitrosamines from the NO-iodine reagent. The change of solvent gave much faster reactions with limiting yields of N-nitrosamine reached in less than 5 min. Data summarised in Table 2.5 shows that with excess iodine over amine, this limiting yield is ca. 50% for piperidine, 66% for morpholine and 100% for N-methylpiperazine. These differences are probably related to protonation of the amine substrate by the HI produced as a byproduct. As in EtOH, addition of NaOH to the reaction mixture increased the yields of N-nitrosopiperidine and N-nitrosomorpholine to ca. 100%.

Table 2.5

Formation of N-nitrosamines from secondary amines, NO and I_2 in MeCN at 25°C. Initial [NO] ca. 1.4×10^{-2} M, $[I_2] = 10^{-2}$ M.

10^3 [Amine]/M	Initial Limiting Yield[a] (%)	Added Base[b] (µl 4 M NaOH)	Final Yield[c] (%)
10 Piperidine	75	50 (0.005)	95
5 "	50	100 (0.01)	94
2.5 "	56	-	-
1.25 "	50	-	-
5 Morpholine	66	100 (0.01)	100
5 N-Methylpiperazine	100	-	-

[a] Based on initial [Amine] and reached after ca. 5 min.

[b] Added after ca. 20 min. Molar concentration in reaction solution in parenthesis.

[c] After addition of base, based on initial [Amine].

2.2.3. Reactions in Aqueous-EtOH.

Results summarised in Table 2.6 and depicted in Figures 2.4 and 2.5, show that the addition of up to 50% (v/v) H_2O to solvent EtOH progressively reduces the limiting yield of N-nitrosamine. This reduction is more marked for N-nitrosopiperidine than N-methylpiperazine. The presence of H_2O must result in competitive hydrolysis of NOI (Equation 2.8) to produce 2 moles of relatively strong acids (i.e, HNO_2 and HI). Thus protonation of the amine substrate will be more

$$NO + \tfrac{1}{2}I_2 \rightleftharpoons NOI \xrightleftharpoons{H_2O} HNO_2 + HI \qquad \ldots\ldots 2.8$$

extensive in aqueous-ethanol than in pure EtOH, which will lower the limiting yield of N-nitrosamine. The effect should be stronger for the more basic amine (e.g., piperidine) as is observed experimentally. Further, Equation 2.8 will be largely irreversible under reaction conditions because of neutralization by the amine substrate [e.g., $HNO_2 + HI + 2R_2NH \rightleftharpoons R_2NH_2^+I^- + R_2NH_2^+NO_2^-$). This exacerbates the drop in the amount of product by consuming the iodine promoter and is probably the prime factor influencing the yield of N-methyl-N-nitrosopiperazine. Addition of H_2O produces only a slight reduction in the rate of N-nitrosamine formation, Figures 2.4, 2.5 and, as in EtOH, these rates are virtually the same for both amines. Both observations suggest that the formation of the NOI reagent from NO and iodine remains rate-limiting as shown in Scheme 2.3. Independent measurements (v. Experimental 2.4.VI) showed that H_2O progressively decreased the concentration of dissolved NO in the solvent and this would account for the slight reduction in reaction rates.

Table 2.6

Effect of added H_2O on the nitrosation of piperidine and N-methylpiperazine by [NO] and [I_2] in EtOH at 25°C : Initial [Amine] = 10^{-2} M

10^3 [I_2]/ M	(v/v)H_2O/%	Maximum Yield[a] (%)	Time[b] min.
Piperidine			
2.46 (2.64)[d]	0	48 (44)	20 (20)
2.52 (2.40)[d]	2.5	48 (40)	20 (23)
0	10	1	200
2.45	10	32	33
2.44 (2.45)[d]	25	25	28 (25)
2.66 (2.66)[d]	25	23 (11)[c]	25 (27)
2.44 (2.45)[d]	50	16 (13)	25 (20)
N-Methylpiperazine			
2.37	0	63	18
2.44	2.5	52	20
2.39	10	46	20
2.45 (2.50)[d]	25	45 (45)	25 (21)
2.49 (2.40)[d]	50	36 (33)	25 (22)

[a] Based on initial [Amine]

[b] Time required to reach maximum yield.

[c] With 2 x 10^{-2} M Piperidine.

[d] Duplicate experiments in parenthesis.

Figure 2.4.

The effect of added H_2O on the nitrosation of piperidine by saturated NO and 2.4 - 2.5 x 10^{-3} M I_2 in EtOH at 25°C. Initial [piperidine] = 0.01 M.

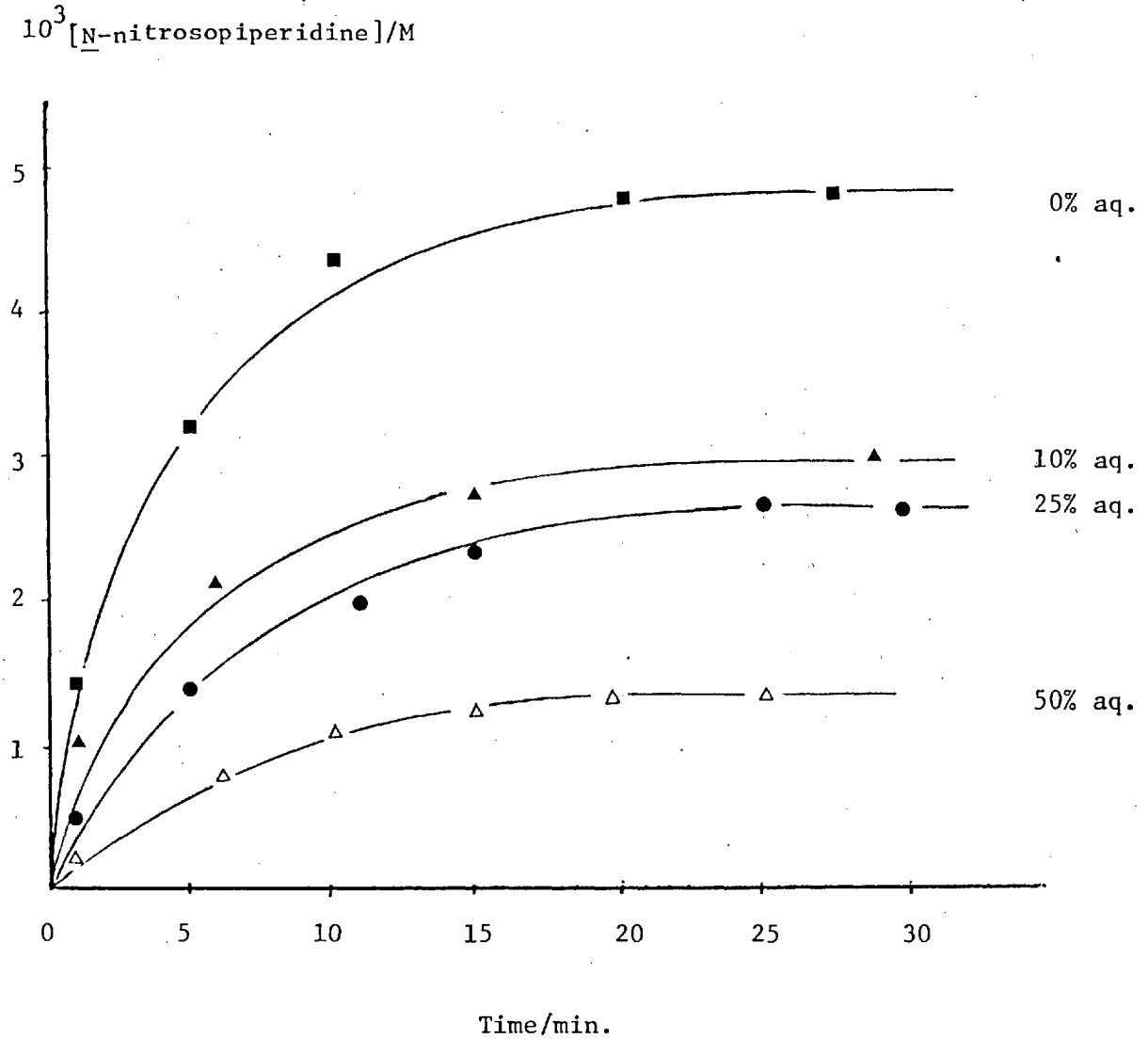
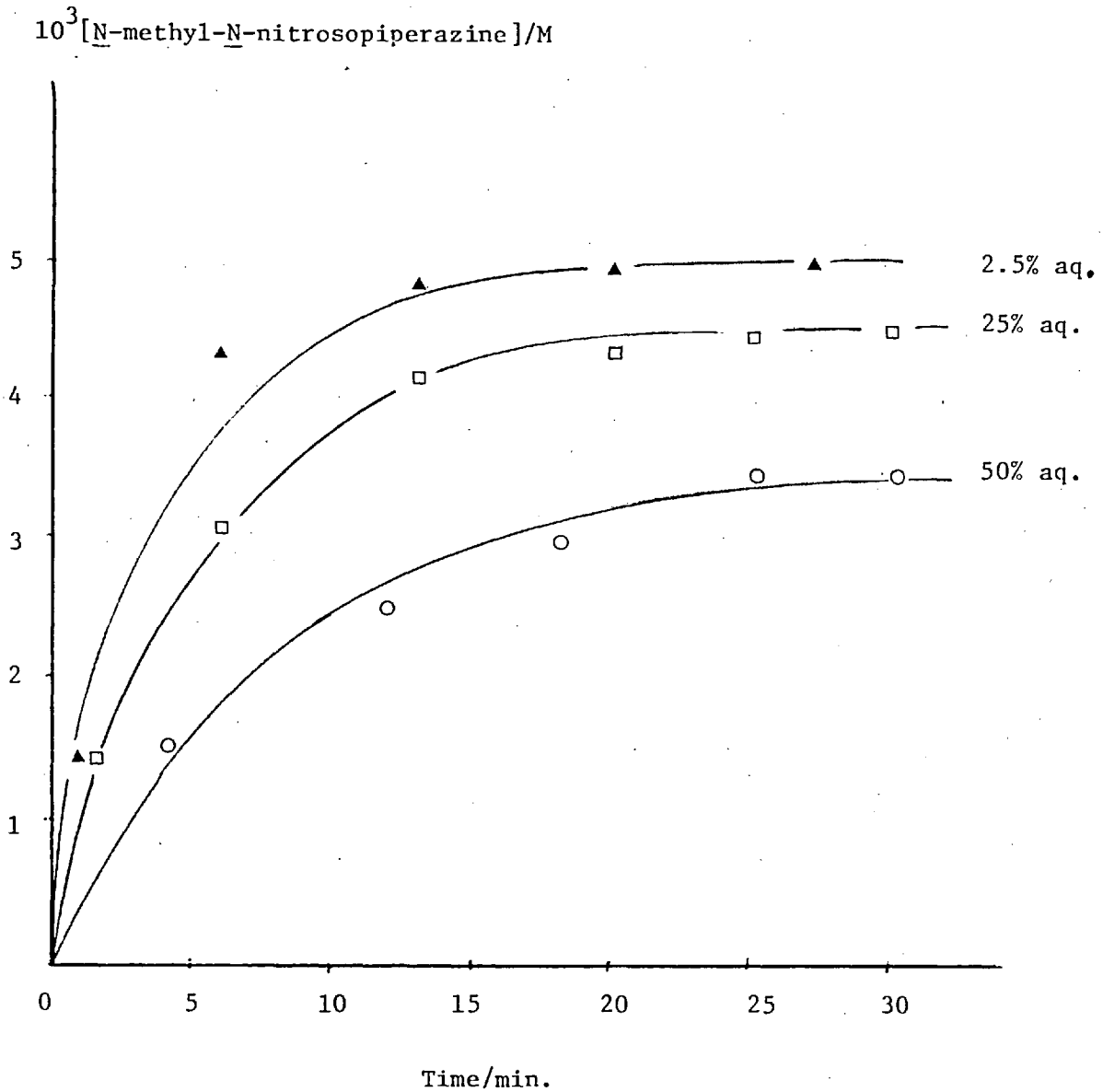


Figure 2.5.

The effect of added H_2O on the nitrosation of N-methylpiperazine by saturated NO and 2.40 - 2.50 x 10^{-3} M I_2 in EtOH at 25°C. Initial [N-methylpiperazine] = 0.01 M.



In view of much recent interest in the formation of N-nitrosamines under gastric conditions,[120] the influence of added mineral acid (HCℓ, $HClO_4$) was also examined for the reactions in 25% (v/v) aqueous-EtOH. For piperidine or morpholine, a severe inhibitory effect was apparent: for example, reaction of 10^{-2} M amine with 2.5×10^{-3} M I_2 and 1.2×10^{-2} M NO (saturated) gave insignificant amounts of the corresponding N-nitrosamine over 60 min. in the presence of 0.02 M HCℓ, 0.024 M $HClO_4$ and 0.014 M H_2SO_4, compared to at least 25% reaction over 20 min. in their absence. Under acidic conditions, both morpholine and piperidine should be extensively protonated and therefore quantitative. In contrast, addition of acid facilitated the nitrosation of N-methylpiperazine (e.g., 2.5×10^{-3} M I_2 and 1.2×10^{-2} M NO in the presence of 2.4×10^{-2} M $HClO_4$ or 1.4×10^{-2} M H_2SO_4, 10^{-2} M N-methylpiperazine gave quantitative yields). The findings for HCℓ additions are summarised in Table 2.7. The data gave reasonably linear pseudo first order plots for additions of greater than 5×10^{-3} M HCℓ. The k_o (observed rate) are quoted for plots of $\log[(\%\ \text{reaction})_\infty - (\%\ \text{reaction})_t]$ versus time, but arediscussed in Chapter 3.

The k_o values show the optimum is ca. 0.01-0.02 M HCℓ where the experimental pH = 2.42-2.15 (pH measured in 25%(v/v)aqueous-EtOH using electrodes calibrated in wholly aqueous standard buffers). Closer scrutiny of Table 2.7 shows that HCℓ has a dual effect: it makes the yield of N-methyl-N-nitrosopiperazine quantitative and therefore independent of initial $[I_2]$, yet progressively reduces k_o. Thus, the reactions are relatively slow at high acidity, but proceed to completion (100% N-methyl-N-nitrosopiperazine) overnight. The kinetic effect is clearly linked to the protonation of N-methyl piperazine. The

Table 2.7

Effect of added HCℓ on the nitrosation of N-methylpiperazine in 25% v/v aqueous EtOH at 25°C. Initial [N-methylpiperazine] = 10^{-2} M ; [NO] ca. 1.2×10^{-2} M ; $[I_2]$ $2.4 - 2.5 \times 10^{-3}$ M.

10^2 x [HCℓ]/M	Experimental pH[a]	Yield (%)[b]	10^4 k_o[c] sec^{-1}
0	8.10	46 (49)[d]	-
0.25	-	46	-
0.50	2.70	68	-
0.75	-	70[d]	5.0
1.0	2.42	75 (76)[d,f]	5.37
2.0	2.15	76 (80)[d,f]	7.95
2.0	2.15	5[e]	-
3.0	2.03	63[f]	3.65
4.0	1.86	19[f]	1.8
5.0	1.77	5.5[f]	0.4
7.0	1.65	0.5[f]	-

[a] pH measured in 25%(v/v)aqueous-EtOH, using electrodes calibrated in wholly aqueous standard buffers.

[b] After 25 min. reaction time based on initial [N-methylpiperazine].

[c] 1st order plot of $\log_{10}((\%\ \text{Reaction})_\infty - (\%\ \text{Reaction})_t)$

[d] Duplicate reactions in parenthesis.

[e] In absence of added I_2.

[f] 100% overnight.

quantitative yields, however, show that in the presence of HCℓ, iodine is a catalyst, rather than a promoter, and therefore, implies the formation of NOI via additional pathways to those shown in Scheme 2.3. These may be the reaction of HI with either HNO_2 (i.e., reversal of Equation 2.8) or NO (Equation 2.9)). Independent experiments

$$2NO + MI \rightleftharpoons (NOH) + NOI \qquad 2.9$$

reported later (Chapter 3) show that NO is a powerful nitrosating agent when used in conjunction with HI or metal iodides. Further, the catalysis by HCℓ cannot be explained simply in terms of either the regular acid-catalysed nitrosation by HNO_2 itself, or by NOCℓ, because much slower reactions prevail in the absence of iodine (v. Table 2.7).

2.2.4 Inhibition by NaN_3 and Thiourea.

Both these compounds are known to inhibit conventional nitrosation reaction by competing for the reagent and undergoing rapid deamination.[48] Results summarised in Table 2.8 show they have similar effect on the nitrosation of piperidine by NO and I_2. This supports the conclusion that neither N-iodoamines nor amine/iodine charge transfer complexes are involved in N-nitrosamine formation.

2.3 Conclusion.

Nitric oxide appears to be an ineffectual nitrosating agent in the absence of catalysts. Its deamination of NH_3, particularly at elevated temperatures and pressures is well known,[121] and of hydroxylamine[80] under alkaline conditions. Two other applications of NO to the diazotization of aromatic amines may be influenced by the presence of oxygen.[85,86] NO at atmospheric pressure under anaerobic conditions

Table 2.8

Inhibition of N-nitrosopiperidine formation in EtOH from NO and iodine at 25°C by added NaN_3 and thiourea. Initial [Piperidine] = 10^{-2} M [NO] ca. 2.2×10^{-2} M.

10^3 [inhibitor]/M	10^3 [I_2]/M	Limiting Yield[a] (%)	Time[b] mins.
Thiourea			
0	2.46	44	20
1.06	2.44	30	20
2.50	2.40	6	80
NaN_3			
0[c]	3.10	48	23
2.50[c]	3.16	40	20
5.63[c]	3.23	22	20
0[d]	2.45	26	25
3.23[d]	2.55	8	80

[a] Based on initial [Piperidine]

[b] Time to reach limiting yield.

[c] In 2.5% aqueous-EtOH.

[d] In 25% aqueous-EtOH.

form Drago salts slowly with secondary amines.[81]

Nitrosyl-iodide is a much more powerful nitrosating agent and its reactions have previously been investigated in connection with the diazotization of aromatic amines by HNO_2 uder acidic conditions in the presence of iodide salts.[75] Further, there is much evidence to suggest that nitrosyl-iodide forms transiently in the gaseous phase from NO and I_2 atoms.[70-73] It is therefore, surprising that the combination of NO and I_2 has not previously been used to effect nitrosation in solution.

All the experimental findings are consistent with the mechanism for N-nitrosamine formation outlined in Scheme 2.3 . The conclusion that the generation of NOI is rate-limiting is consistent with the known properties of this entity. Thus, its reaction with aromatic amines in acidic aqueous solutions is believed to occur on encounter,[38] and the same conclusion should apply to the more basic (and therefore, more reactive) aliphatic amines. Further, the formation of NOI from NO and I_2 is thermodynamically unfavourable. This is evident from examination of the equilibrium concentration of alkyl nitrites in the reaction solutions, studies of the formation of NOI in aqueous media,[76] and the standard redox potentials for both $NO^+ + e^- \rightleftharpoons NO\uparrow$ [E_o = 1.46 eV in H_2O at 25°C][122] and $I_2 + 2e^- \rightleftharpoons 2I^-$ [E_o = 0.621 eV in H_2O at 25°C].[122] Further, in the gaseous phase ΔG^o_{298} is ca. 35.84 kJ $mole^{-1}$ for the formation of NOI from NO and I_2 atoms.[72]

Both the yield of N-nitrosamine and its rate of formation are dependent on the solvent. Little reaction was evident in pure H_2O, because iodine is insoluble. Also, NO is less soluble in H_2O than in EtOH, and, as noted previously, this contributes to the inhibitory effect with mixed solvents. The faster rates in solvent MeCN compared

to EtOH are more difficult to explain. It must relate, however, to the iodine, because the concentration of NO in MeCN is about half that in EtOH (v. Experimental 2.4.VI). Tri-iodide ion formation ($I_2 + I^- \rightleftharpoons I_3^-$) is bound to be less extensive in the aprotic MeCN and the free iodine concentration therefore larger. Further, iodine is known to interact by charge transfer with both MeCN [123] and EtOH,[124] which may further influence the above equilibrium and also alter the oxidation potential of iodine. The kinetic solvent effect is therefore consistent with rate-limiting NOI formation, where either the free iodine concentration is higher in MeCN, or iodine is more readily reduced by NO.

2.4 EXPERIMENTAL.

2.4.1. Instrumentation.

Ultra-violet spectra were recorded on Pye-Unicam SP1800 or SP800 A spectrophotometers, thermostatted at 25°C, with 1 cm pathlength quartz cells. Infra-red spectra were recorded on Perkin-Elmer 257 or 457 spectrophotometer, using sodium chloride discs unless otherwise stated. Gas-liquid chromatography analyses were carried out on either Perkin-Elmer F11 (fid) and Perkin-Elmer F33 (fid) instruments. High pressure liquid chromatography assay were carried out using a Waters Associates 600 pump attached to a Cecil CE212 spectrophotometer . pH determinations were made on Radiometer type 26 pH meter fitted with a G202C glass electrode and a calomel electrode. Calibration was at 25°C using standard phosphate buffers. Refractive indices were measured on Hilger-Watts M46-M1L8 instrument. Microanalysis and mass spectra were

carried out in the Department of Chemistry, Imperial College. Weighings were carried out on Gallenkamp Mettler H20. Deionised water was obtained by passing distilled water through an Eigastat column.

2.4 II. Reagent, Substrates and Products.

AnalaR MeOH (99.4%), EtOH (99.8%), KI, sulphanilamide, N-1-naphthy - lethylene diamine dihydrochloride and volumetric standard HCℓ, H_2SO_4 were used without further purification. Deionised water was degassed by boiling and cooling under N_2. n-Butanol (b.p. 116°C/760 mm) and cyclohexane (b.p. 80°C/760 mm) were dried over anhydrous K_2CO_3 and sodium wire, respectively, and then fractionally distilled. MeCN (b.p., 82°C/760 mm) was heated under reflux with P_2O_5 prior to fractional distillation and storage over 4A molecular sieves.

Nitric oxide (BoC 99%) was purified by passage through two wash bottles containing 30% aqueous KOH and one containing a saturated aqueous HCℓ solution of sulphanilamide. The gas was then passed through two drying towers (30 cm) containing both NaOH pellets and anhydrous $CaCℓ_2$.

Iodine (BDH, Sublimed) was recrystallised from benzene and then sublimed. Reagent grade piperidine (p.b. 105°C/760 mm), morpholine (b.p. 127.5°C/760 mm) and N-methylpiperazine (b.p. 138°C/760 mm) were dried and distilled over solid KOH.

n-Butyl nitrite was prepared from acidified nitrite and n-BuOH and purified following a literature procedure.[125]

n-BuONO b.p., 77°C/760 mm, (lit[125] 76.5/760 mm); n_D^{22} = 1.3760, (lit[126] n_D^{20} = 1.3762); ν_{max}^{film} 1608, 1650 cm^{-1} (NO), lit[126] ν_{max}^{film}

1608, 1648 cm^{-1} (NO)). λ_{max}^{BuOH} 357 nm (log ε = 1.90) (lit[126] λ_{max}^{BuOH} 357 nm (log ε = 1.90).

N-nitrosamines were prepared from the reaction of acidified nitrite and amine as described in Vogel.[127] The products were purified by fractional distillation under reduced pressure.

N-nitrosopiperidine, yellow oil, b.p. 109°C/20 mm, (lit[128] 109°C/ 20 mm). N-nitrosomorpholine, yellow-green solid m.p. 28°C , b.p., 83°C/2.5 mm (lit[128] m.p., 29°C, b.p. 96 °C/ 6mm). N-methyl-N-nitrosopiperazine, yellow oil, b.p., 74°C/1.5 mm (lit[128] 76°C /1.5 mm).

2.4. III Reaction Methods.

2.4.III(a) N-nitrosamine Formation.

The nitrosation reactions (which is referred to throughout this Thesis for other catalysed reactions) were carried out in a 50 ml. three necked flask, fitted with Subaseal stopper, a gas inlet and an aspirator. The catalyst was weighed into the flask and the solvent (40 ml) added via a 50 ml. burette. The solution was then carefully degassed by either several freeze-thaw cycles under vacuum or by passing purified N_2 (white spot N_2, passed through Feiser's solution,[129] concentrated H_2OS_4, and finally, through a drying tower (100 cm) containing both NaOH pellets and anhydrous $CaC\ell_2$) for ca. 100 min. The latter procedure did not cause any significant loss of solvent. The flask was then placed in a water bath thermostatted at 25°C ± 0.5°C and the contents stirred magnetically. Purified NO was passed into the reaction vessel for ca. 15 min. The reaction was initiated by injecting neat amine into the reaction flask via the Subaseal stopper with a microlitre syringe. At timed intervals, 1-5µl aliquots of the reaction solution were withdrawn via the Subaseal stopper and analysed by g.l.c. immediately.

2.4. III(b) Product Analysis.

The g.l.c. analysis for N-nitrosamines was carried out on a Perkin-Elmer F11 (fid) or Perkin Elmer F33 (fid) (fitted with a Nitrogen specific detector) instruments. The operating conditions and columns used, together with the retention times of the products are shown below:-

Perkin-Elmer F11

Column : 2 metre, $\frac{1}{8}$" o.d., stainless steel column packed with Antarox Co-990 on chromosorb W Dmcs (80-100 mesh).

Conditions : Column temperature = 180°C ; injection temp. = 200°C. N_2 = H_2 = Air = 30 p.s.i.

Compound	Retention Time (min.)
N-nitrosopiperidine	3.0
N-nitrosomorpholine	3.5
N-methyl-N-nitrosopiperazine	4.0

Perkin-Elmer F33 (N- specific detector)

Column : 2 metre $\frac{1}{8}$" o.d., stainless steel column packed with 8% Carbowax 20 M + 2% KOH on Chromosorb W (80-100 mesh)

Conditions : Column temperature = 180°C, injection temp. = 225°C. N_2 = 41 p.s.i.; Air = 3 p.s.i.; H_2 = 18 p.s.i.

Compound	Retention Time (min)
N-nitrosopiperidine	3.6
N-nitrosomorpholine	4.3
N-methyl-N-nitrosopiperazine	4.7

The instruments were calibrated with standard solutions of the N-nitrosamine dissolved in the appropriate solvent to show that the concentrations were linearly related to the peak height and reproducible for any given sample to better than ± 8%. Within the chosen sensitivity range, the concentration of N-nitrosamines are linearly related to the peak height, for example 1.42, 2.86, 4.31, 5.74 x 10^{-3} M N-methyl-N-nitrosopiperazine in EtOH gave peak heights of 16(16), 29(32.5) 43.5(45) 65(66) for the Antarox column, and where the peak heights in brackets correspond to duplicate analyses one-week later. The instruments were calibrated daily with 50% and 100% reaction standards to allow for changes in the column performance.

n-BuONO Standard.

[n-BuONO] used as standard for h.p.l.c. determinations and other reactions was estimated using the modified Shinn's method described by Kershaw and Chamberlin.[130] The method involves the diazotization of sulphanilamide in 5N HCℓ with n-BuONO, and coupling with N-1-naphthyl-ethylenediamine to produce an azo dye ($\lambda_{max}^{H_2O}$ 541 nm, log ε = 4.73).

The light absorbance is proportional to [n-BuONO] according to the Beer-Lambert Law. Typically, 0.1-0.5 ml of n-BuONO standard was added to 4 ml of acidic 0.5% sulphanilamide in a 25 ml. volumetric flask. After ca. 3 min , 4 ml. 0.1% N-1-napthylethylenediamine in H_2O was added. The solution was made up to standard volume , and λ_{max} at 541 nm recorded on Pye-Unicam SP800A.

2.4. IV. Equilibrium Between n-BuONO and HI.

The dissociation of n-BuONO was determined by adding equimolar amounts of KI and HCℓ to 10^{-4} M n-BuONO in solvent EtOH under anaerobic conditions in a glove box. This immediately produced a highly coloured solution indicative of the formation of I_3^- (λ_{max}(H_2O) 288, 353 nm, log ε 4.60, 4.42[117]). The solutions were transferred to a tightly stoppered cuvette, and the absorption was measured at 290 nm using a Pye-Unicam SP1800 spectrophotometer. Independent checks established that the absorptions at this wavelength of n-BuONO (log ε 1.7) and I_2 (log ε 1.98) were insignificant.

The related equilibrium between I_2 and I_3^- was examined similarly by preparing solutions of I_2 in EtOH and adding equimolar concentrations of KI and HCℓ in an anaerobic glove box. The concentration of I_3^- was determined spectrophotometrically as above.

2.4. V. Estimation of n-BuONO.

The formation of n-BuONO from saturated solutions of NO in n-BuOH and I_2 was examined by a procedure analogous to that employed for the formation of N-nitrosamines. The amount of n-BuONO R_F 2 min. was estimated by h.p.l.c. assay using a Waters Associates 600 pump

fitted with a 70 cm x 2.1 mm i.d. stainless steel column packed with C_{18} porasil/microbondapack-CN attached to a Cecil CE212 spectrophotometer. The eluent was degassed 1% aqueous-MeOH, analytical samples were injected directly on to the column via a septum, and the absorption of the eluent was monitored at 230 nm. The instrument was calibrated with standard solutions of n-BuONO in n-BuOH and peak heights gave concentrations reproducible to ± 5%. Analyses of NO in n-BuOH showed that n-BuONO was formed in the absence of I_2. This was attributed to the oxidation of NO to NO_2 by adventitous oxygen followed by rapid reaction with the solvent. NO_2 has been shown to be a powerful nitrosating agent.[40] The concentration of n-BuONO produced in the absence of I_2 was substracted from the reaction solutions.

For example:-

$[I_2]$/M	10^3[BuONO]/M	10^3[Δ BuONO]/M[a]	% Equilibrium[b]
0	5.0		
0.01	6.45	1.45	14.5
0.01	6.75	1.75	17.5
0.01	6.20	1.20	12.0
0.02	8.10	3.10	15.5
0	7.59		
0.01	9.18	1.60	16.0

[a] $\Delta\ BuONO = [BuONO_{I_2} - BuONO_{Blank}]$.

[b] $\frac{\Delta\ [BuONO]}{[I_2]} \times 100$ = % equilibrium, but since

$I_2 + 2NO \rightleftharpoons 2NOI \xrightleftharpoons{BuOH} 2\ BuONO + 2HI$; % change in $[I_2]$ = $\frac{\%\ equilibrium}{2}$

2.4.VI Concentration of Dissolved NO.

These were ascertained by two methods for pure EtOH and MeCN The first, based on the procedure of Walters and Taylor,[131] involved coupling with alkaline sulphite. Here, a small quantity (usually 2 µl) of the NO solution was injected through a Subaseal stopper into a degassed solution of 10^{-2} M Na_2SO_3 in 0.025% KOH contained in a 10 mm cuvette. The absorbance at λ_{max} 258 nm (log ε 3.81) was read against a sulphite solution reference. The second procedure involved direct measurement of the u.v. absorption of the solution containing NO at λ_{max} 325 nm (log ε 1.83). Only the second procedure was used for the aqueous-EtOH solutions. The concentrations found are summarised below, where the figure in parenthesis refers to the direct u.v. estimation.

Solvent	10^2[NO]/M
EtOH	2.1 (2.18)
10% aq.-EtOH	(1.85)
25% " "	(1.2)
50% " "	(0.92)
MeCN	1.4 (1.13)

2.4. VII (i) Typical Reactions.

(1) Initial $[I_2] = 4.97 \times 10^{-3}$ M, Initial [N-methylpiperazine] = 10^{-2} M, in EtOH at 25°C, initial saturated (ca. 2.2×10^{-2} M) NO.

time min.	Attenuation	Peak Height	10^3[N-methyl-N-nitroso-piperazine]/M
0.5	x 100	41	5.16
6		56	7.05
12		72	9.07
20		79	9.95
27		79	9.95
∞		79	9.95

Injection sample 3 µl.

3 µl 5.1×10^{-3} N-methyl-N-nitrosopiperazine gave peak heights at attenuation x 100 of 43, 38, 40, 42, = av. = 40.5 on the Antarox column under standard operating conditions.

(ii) Initial $[I_2] = 2.64 \times 10^{-3}$ M, initial [piperidine] = 10^{-2} M in EtOH at 25°C, initial saturated NO (ca. 2.2×10^{-2} M)

time min.	Attenuation	Peak Height	10^3[N-nitrosopiperidine]/M
1	x 200	15	1.36
5		34	3.08
10		45	4.07
15		46	4.17
20		52	4.71
24		52	4.71
28		53	4.8
34		54	4.89
∞		53	4.8

Injection sample = 3 µl

3 µl 4.8 x 10^{-3} M N-nitrosopiperidine at attenuation x 200 gave peak heights 50, 53, 53 on Antarox column under standard operating conditions.

(iii) Initial [I_2] = 2.60 x 10^{-3} M. Initial [Morpholine] = 10^{-2} M in EtOH at 25°C. Initial saturated (ca. 2.2 x 10^{-2} M) NO.

Time min.	Attenuation	Peak Height	10^{-3}[N-nitrosomorpholine]/M
1	x 100	18	1.5
5		44	3.67
11		53	4.42
16		54	4.5
22		62	5.17
27		66	5.5
32		64	5.3
37		61	50.8
∞		61	50.8

Injection sample = 3 µl

3 µl 5.0 x 10^{-3} M N-nitrosomorpholine at attenuation x 100 gave peak height 60, 60, 60 on Antarox column under standard operating conditions.

CHAPTER 3

THE FORMATION OF N-NITROSAMINES IN SOLUTION FROM GASEOUS NITRIC OXIDE IN THE PRESENCE OF HI AND METAL IODIDES.

3.1 (1) Results and Discussion.

In Chapter 2, it was suggested that the formation of N-nitrosamines by NO and iodine in neutral solutions involved reaction between nitrosyl-iodide (NOI) and the unprotonated secondary amine (Scheme 2.3). In 25% (v/v) aqueous-ethanol, small amounts of added mineral acid either HCℓ or H_2SO_4, were observed to severely inhibit the reactions with piperidine (pK_A 11.22[115]) and morpholine (pK_A 8.33[115]). This was attributed to the extensive protonation of both amines. For N-methyl-piperazine (pK_A 9.8, 5.11[115]), however, the reaction rate increased and passed through a maximum in ca. 0.01-0.02 M HCℓ. At higher acidities, the rate was slower, but the yields of N-methyl-N-nitrosopiperazine were quantitative irrespective of the initial iodine concentration. The kinetic effect was related to protonation of the amine substrate, but the quantitative yields implied that iodine acted as a catalyst rather than as a promoter in the presence of HCℓ. The change of mechanism with acidity suggested that NOI may, in principle, result from the interaction of NO and HI (Equation 3.1) in addition to that with I_2 (Scheme 2.3).

$$2NO + HI \rightleftharpoons (NOH) + NOI \qquad \ldots\ldots\ldots 3.1.$$

Other explanations, such as additional reaction paths involving either the nitrous acidium ion (H_2^+ONO) or nitrosyl chloride ($NOC\ell$), can be excluded because much slower reactions were apparent in the absence of elemental iodine.

3.1 (2) Reactions of N-methylpiperazine with NO in the presence of HI.

A direct test of the above explanation was to examine reactions using HI in place of I_2. For practical reasons, these reactions were carried out in 25% (v/v) aqueous-EtOH with HI being generated *in situ* from KI and mineral acid. The reaction solutions were carefully degassed to avoid both the generation of I_2 and any catalysis of N-nitrosamine formation by adventitious oxygen. On passing NO, however, a brown colouration developed within *ca.* 10-15 min. with strong uv absorptions at λ_{max} = 288 and 353 nm, characteristic of I_3^-.[117] This is not unexpected, because the equilibrium $HI + I_2 \rightleftharpoons HI_3$ was observed previously with NO and I_2 in EtOH (Chapter 2). Analysis of the gaseous products by infrared (v. Experimental 3.3) showed the presence of N_2O at concentrations of *ca.* 20-30% of the initial KI. These findings are consistent with the equilibrium described by Equation 3.2.

$$2NO + \tfrac{5}{2}\,HI \rightleftharpoons N_2O + H_2O + \frac{I_2}{2} + \frac{HI_3}{2} \qquad \text{.....} \quad 3.2.$$

Thus, passing NO into the reaction solution has a dual effect of reducing its acidity by converting HI into H_2O and generating I_2 in equilibrium with HI_3. Visual examination indicated that the oxidation of HI (Equation 3.2) was considerably faster than N-nitrosamine formation. This was confirmed by observing similar rates of N-methyl-N-nitrosopiperazine formation irrespective of whether the reaction was initiated by adding amine to the solution of KI, HCℓ and NO in 25% (v/v) aqueous EtOH or by adding HCℓ to the solution of KI, amine and NO, cited in Table 3.3 as reverse addition.

Results summarised in Tables 3.1 - 3.3 and Figures 3.1 - 3.5 show unequivocally that HI catalyses the formation of N-methyl-N-nitroso-piperazine. Very little reaction is evident (Table 3.3) in the absence of either KI or HCℓ compared to substantial amounts after short times in their presence. It was not always possible to calculate accurate rate coefficients from the experimental data. This stems from the inability to control the concentration of NO and the complex inter-related equilibria between the reagents. There is strong evidence that the diffusion of NO into solution is rate-limiting for some of the fastest reactions.

Sensible kinetic dependencies were obtained when NO was passed continuously while the reaction proceeded. This applies to the data in Table 3.1 some of which is plotted in Figures 3.1 - 3.5. These reactions were carried out with similar concentrations (10^{-3} to 10^{-2} M) of KI and N-methylpiperazine with low amounts of HCℓ in 25% aqueous-EtOH at 25°C. Significantly, plots of $\log_{10}$ (N-methyl-N-nitrosopiperazine) versus time are reasonably linear (Figures 3.2 - 3.4), so the reaction must follow pseudo first order kinetics (Equation 3.3).

$$\text{Rate} = \underline{k}_o\,[\text{N-methylpiperazine}] \qquad \ldots\ldots \quad 3.3$$

This conclusion is confirmed in Table 3.1 by the constancy of k_o ($=(0.54 \pm 0.06) \times 10^{-3}$ sec^{-1}) for reaction of different, initial [N-methylpiperazine] with 10^{-3} M HI in the presence of 10^{-2} M excess HCℓ. The data in Table 3.1 shows further, that $\underline{k}_o$ has a first order dependence on initial [KI] with no excess HCℓ.

Table 3.1

Rate of formation of N-methyl-N-nitrosopiperazine from N-methyl-piperazine (NMP), KI, in acidic media with a constant excess of NO in 25%(v/v) aqueous-EtOH at 25°C.

10^3[KI]/M	10^3[N MP]/M	10^3[HCℓ]/M	10^3[ΔHCℓ]/M[a]	$10^4 k_o$[b] sec^{-1}	$10^7 k_2$[c] $1\ mol^{-1}\ sec^{-1}$
1.0	10	11	0	2.9	2.9
5.0	10	15	0	14.0	2.8
10.0	10	20	0	26.9	2.7
1.0	5	6	0	2.5	2.5
1.0	2.5	3.5	0	2.8	2.8
10.0	5	15	0	22.3	2.2
1.0	10	21	10	5.4 (5.4)	5.4
1.0	5	16	10	5.95	6.0
1.0	2.5	13.5	10	4.9	4.9
5.0	10	20	5	14.2	2.8

[a] ΔHCℓ = excess HCℓ over [KI] + [N-MP].

[b] pseudo first order rate constant

[c] $k_2 = \frac{k_o}{[KI]}$

Table 3.2

Rate of formation of N-methyl-N-nitrosopiperazine from N-methyl-piperazine (NMP), I_2 in acidic media, with constant excess of NO in 25% (v/v) aqueous-EtOH at 25°C.

$10^3[I_2]/M$	$10^3[NMP]/M$	$10^3[HCl]/M$	$10^3[\Delta HCl]/M$ [a]	$10^4 k_o$ sec^{-1} [b]
0.5	5.0	5.0	0	3.8
0.5	10	10	0	4.2
2.5	10	10	0	14.0
5.0	10	10	0	26.9

[a] See Table 3.1 (page 70)

[b] Pseudo first order rate constant.

Table 3.3

Yields, reaction times and initial rates for N-methyl-N-nitroso-piperazine formation from N-methylpiperazine (N-MP) NO and KI under acidic conditions in 25% (v/v) aqueous-EtOH at 25°C. Initial saturated [NO] ca. 1.2×10^{-2} M.

10^2[N-MP]/M	10^3[KI]/M	10^3[HCℓ]/M	Yield[a] %	Time/[b] min.	10^4 k_o sec^{-1} [c]
1.0		20	5 (5)[d]	100	
1.0	0.70		3	100	
1.0	2.50		12	100	
1.0	1.0	20	50 (50)[d,f]	25	5.4
1.0[e]	1.0	20	50[f]	26	5.4
1.0	2.56	20	55[f]	23	5.8
1.0	5.30 (5.08)	20	50[f] (50)[d,f]	15 (15)	11.9 (12.2)[d]
1.0[e]	5.30	20	50[f]	15	11.5
1.0	10.4 (10.0)	20	85[f] (82)[d,f]	24	26.9
0.5	0.79	20	150[f]	40	3.0
1.0	0.86	20	70[f]	42	3.7
2.0	0.84	20	14	30	0.9
4.0	0.80	20	10	105	
1.0	5.0 (5.1)	10	40[f]	15	4.1 (3.7)[d]
1.0	5.1 (5.30)	20	50[f]	15	12.0 (11.9)[d]
1.0	5.0	30	23[f]	15	2.5 (2.3)
1.0	5.0	40	10[f]	15	0.9
1.0	5.2	50	2.5[f]	15	0.4
1.0	5.4	60	0	15	

[a] Yield based on initial [N-MP]

[b] Time to reach yield

[c] Pseudo first order plot, initial rates, ca. initial 10% reaction

[d] Duplicate reaction in parenthesis

[e] Reverse addition

[f] 100% overnight

Figure 3.1

The dependence on [KI] and [NO] on the nitrosation of 10^{-2} M N-methylpiperazine with 2 x 10^{-2} M HCℓ in 25% (V/V) aqueous-EtOH at 25°C. Open characters initial saturated [NO] ca. 1.2 x 10^{-2} M. Closed characters [NO] excess.

● ○ [KI] = 10^{-2} M
▲ △ [KI] = 5 x 10^{-3} M
■ □ [KI] = 1 x 10^{-3} M

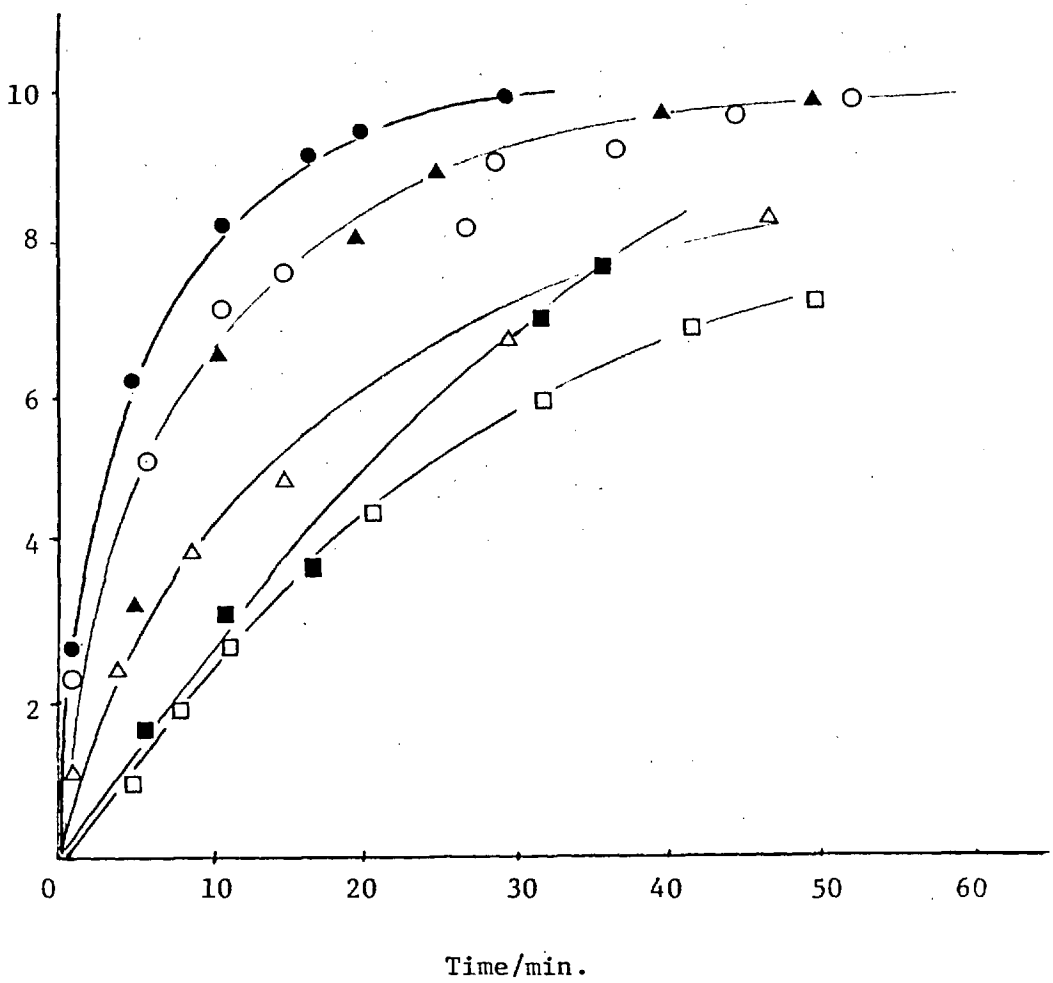


Figure 3.2

Dependence on [NO] for the rate of N-methyl-N-nitrosopiperazine formation. Initial [KI] = 10^{-2} M [HCℓ] = 2 x 10^{-2} M, [N-methyl-piperazine] = 10^{-2} M in 25% (v/v) aqueous-EtOH at 25°C. (●), (○) duplicate run - [NO] bubbling through. (■) initial saturated [NO] = 12. x 10^{-2} M. (R_∞ = 10^{-2} M N-methyl-N-nitrosopiperazine, R_t = % reaction log ($\%R_\infty - \%R_t$) at time t.)..

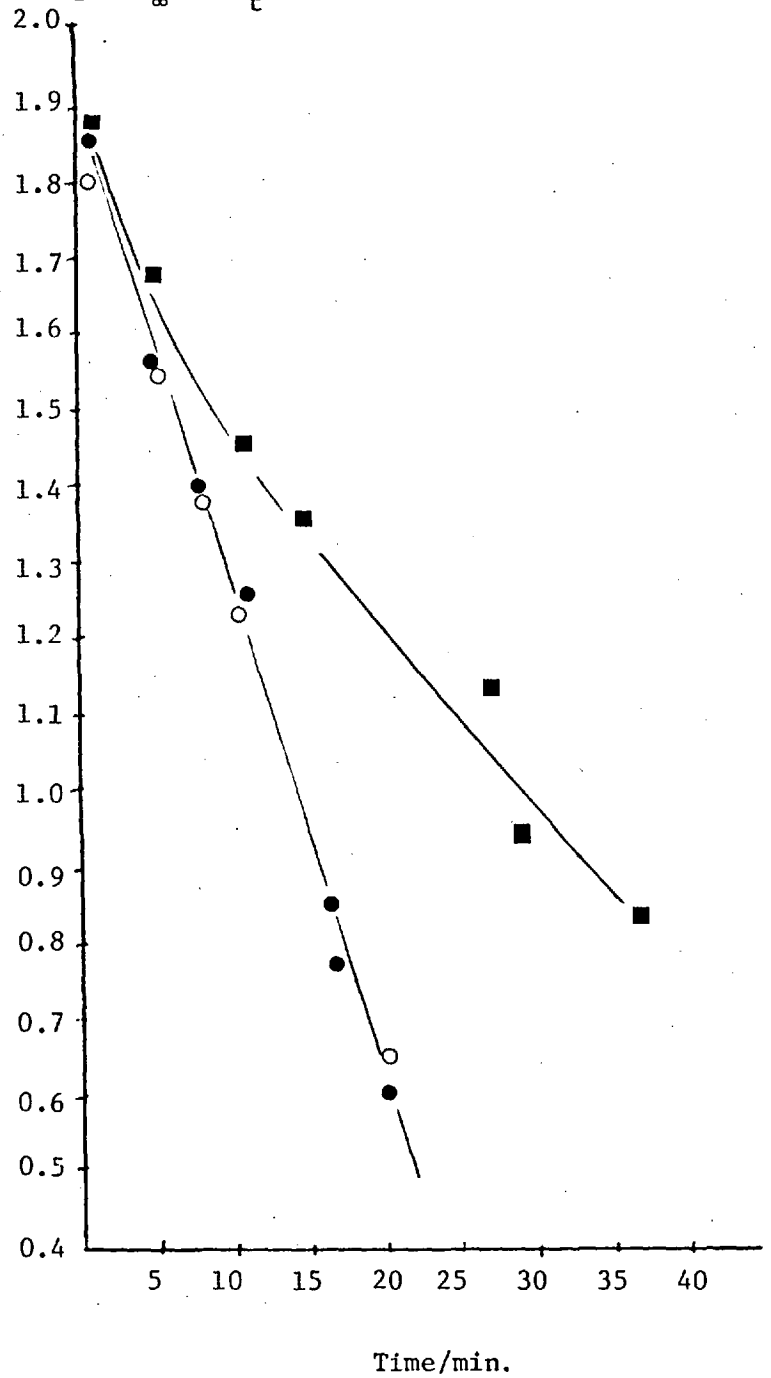


Figure 3.3

Dependence on [NO] for the rate of N-methyl-N-nitrosopiperazine formation. Initial [KI] = 5×10^{-3} M, [HCℓ] = 2×10^{-2} M, [N-methylpiperazine] = 10^{-2}M, in 25% (v/v) aqueous-EtOH at 25°C. $R_\infty = 10^{-2}$ M, N-methyl-N-nitrosopiperazine R_t = % yield at time t.

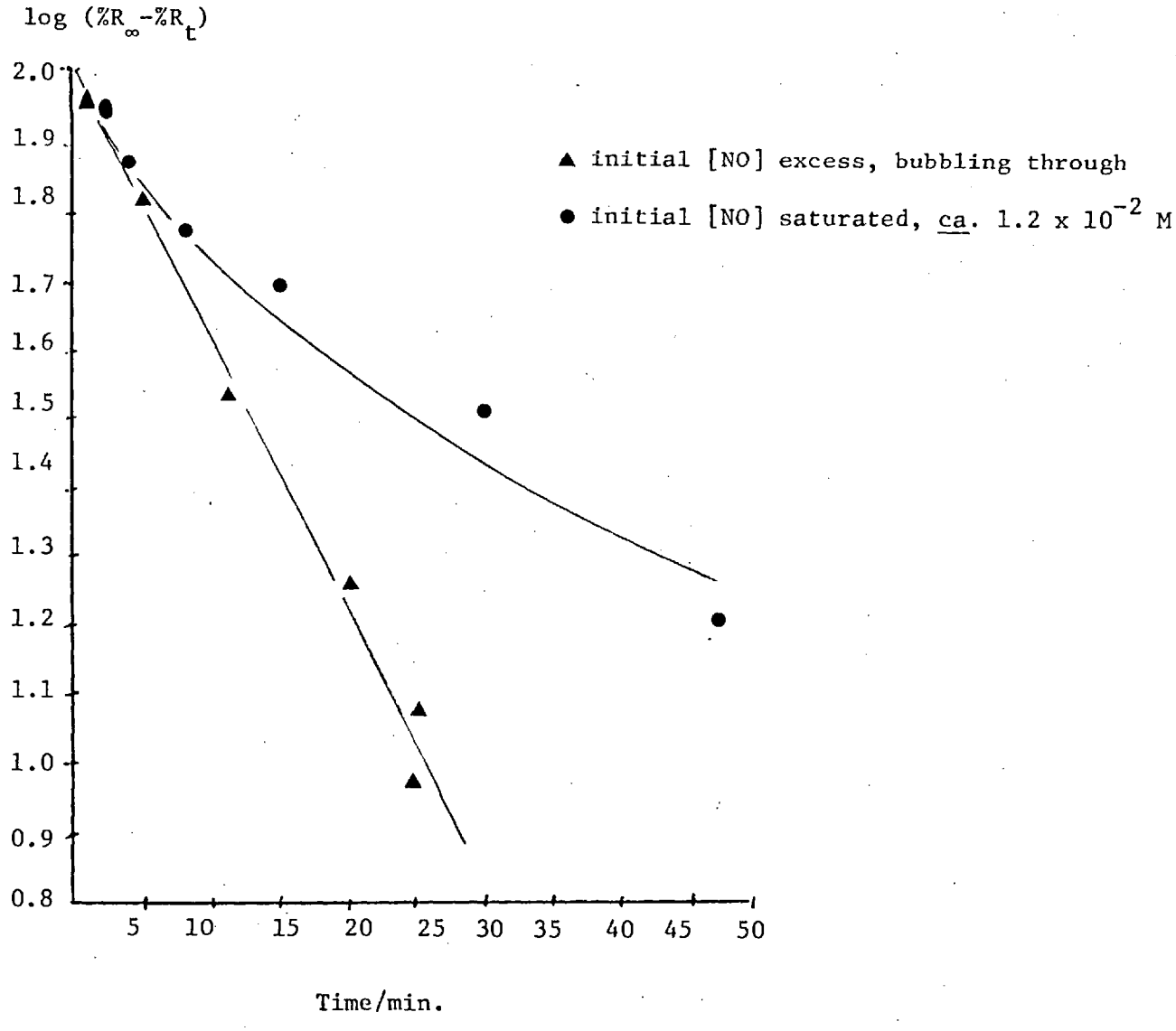


Figure 3.4

Dependence on [NO] for the rate of N-methyl-N-nitrosopiperazine formation. Initial [KI] = 10^{-3} M, HCℓ = 0.02 M, [N-methylpiperazine] = 10^{-2} M, in 25% (v/v) aqueous-EtOH at 25°C. R_∞ = 10^{-2} M N-methyl-N-nitrosopiperazine, R = % reaction at time t. min.

▲ Initial [NO] excess

● Initial [NO] saturated ca. 1.2 x 10^{-2} M

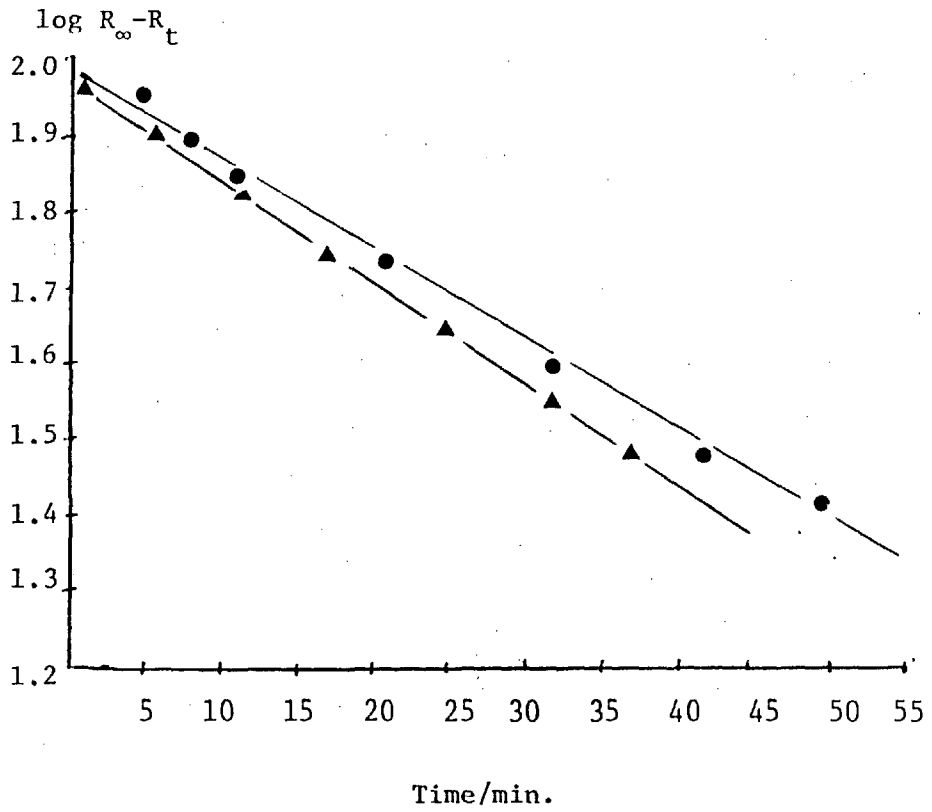
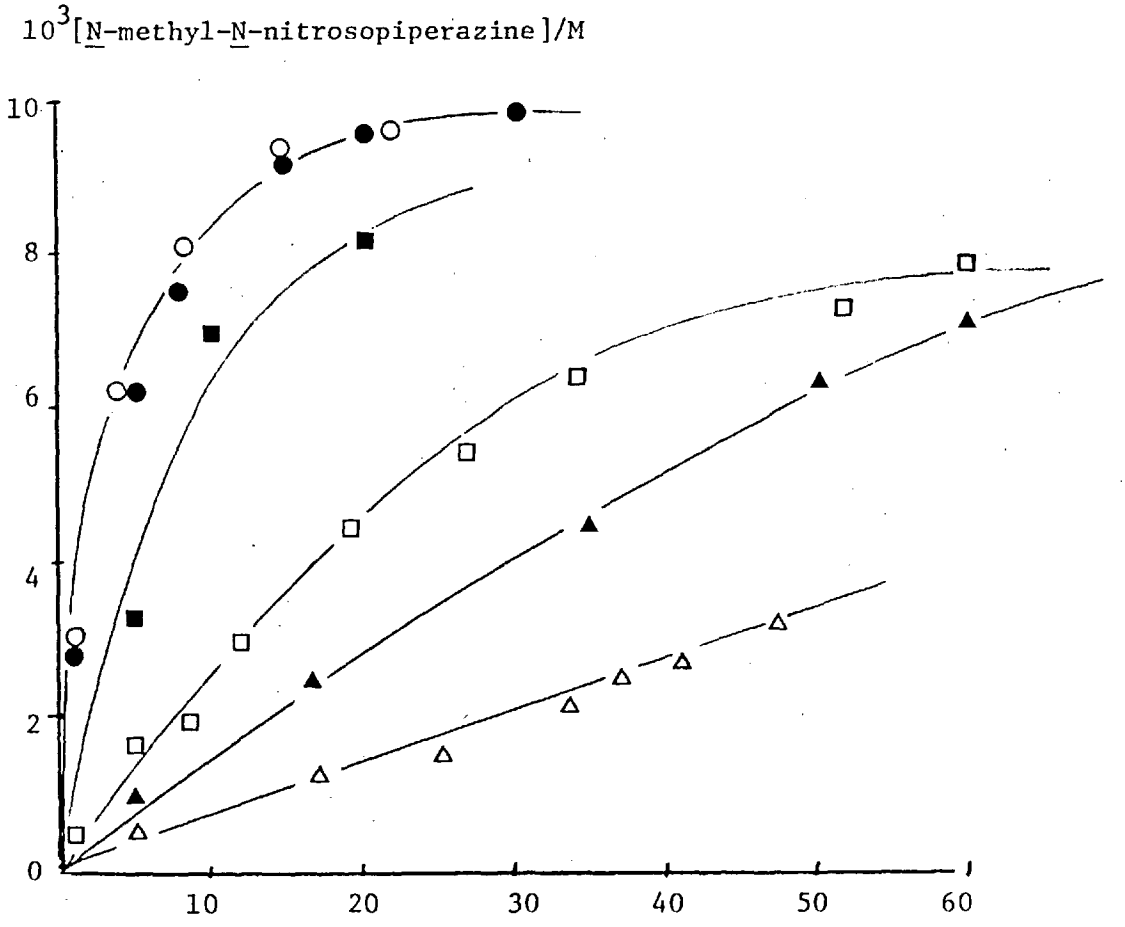


Figure 3.5

The dependence on [KI], [I_2] on the nitrosation of N-methylpiperazine (NMP) with no excess HCℓ in 25% (v/v) aqueous-EtOH at 25°C. [NO] excess.

10^2[KI]/M	10^2[NMP]/M	10^2[HCℓ]/M	10^3[I_2]/M	
1.0	1.0	2.0		●
	1.0	1.0	5	○
0.5	1.0	1.5		■
0.1	1.0	1.1		▲
0.1	0.5	0.6		△
	1.0	1.0	0.5	□



Thus, under acidic conditions, the full rate expression is probably given by Equation 3.4, which points to nitrosation

$$\text{Rate} = k_3[\text{N-methylpiperazine}][\text{HI}][\text{NO}] \quad \ldots\ldots\ldots \quad 3.4$$

by nitrosyl iodide (NOI) as found previously for catalysis by elemental iodine, although proof of the kinetic order for NO awaits experiments where the concentration of this reagent can be varied.

The observation of a first-order dependence on HI and the pseudo first-order kinetics for reaction with equimolar amounts of N-methylpiperazine and HI show that HI is not consumed during the reaction, i.e., its effect is catalytic.

A simplified mechanism, omitting equilibria between I_2 and HI_3 ($I_2 + HI \rightleftharpoons HI_3$), between NOI and the solvent ($EtONO + NOI \rightleftharpoons EtONO + HI$) and the protonation of N-methylpiperazine is given in Scheme 3.1. [The existence of these equilibria and their effect on the rates of N-methyl-N-nitrosopiperazine formation from NO in EtOH were discussed previously (Chapter 2). EtONO formation is not extensive (ca. 10%) and NOI appears to form predominantly from the interaction of NO and I_2. Also, the formation of N-methyl-N-nitroso-piperazine is not inhibited (as in the present case) by up to 1 mole of mineral acidand presumably because the reaction proceeds readily at the unprotonated N-atom of the N-methylpiperazine cation (Equation 2.3).]

The overall stoichiometry shows that HI (or I_2) is not consumed, which accounts for the pseudo first-order kinetics (Figures 3.1 - 3.4), when the concentration of dissolved NO is maintained by bubbling the gas into solution throughout the reaction.

$$HI + NO \xrightarrow{\text{fast}} (NOH) + \tfrac{1}{2} I_2$$

$$NO + \tfrac{1}{2} I_2 \xrightarrow{k_a} NOI$$

$$R_2NH + NO\text{-}I \xrightarrow{k_b} R_2NNO + HI$$

$$R_2NH + 2NO \xrightarrow{HI} (NOH) + R_2NNO$$

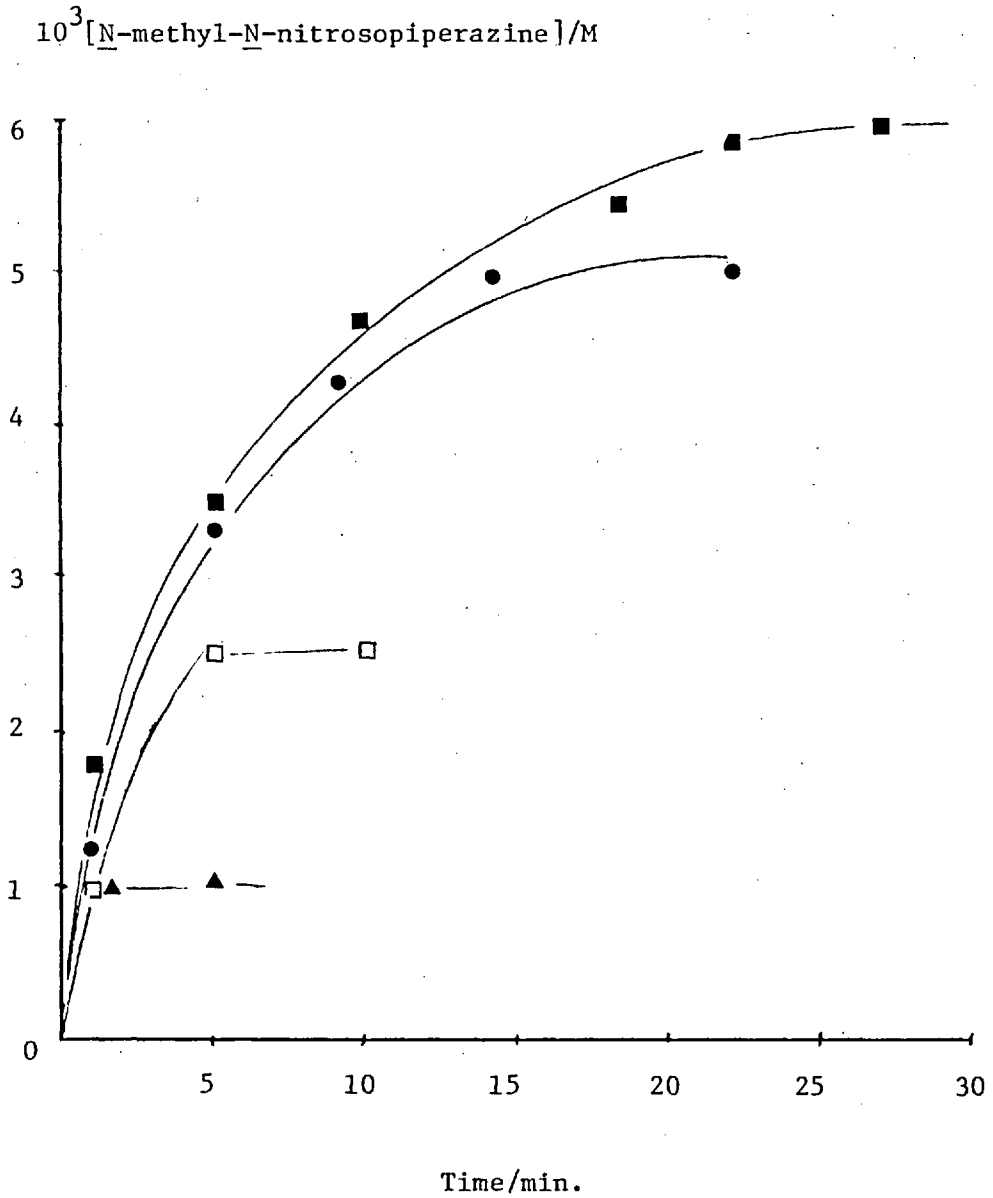
Scheme 3.1 : Mechanism and overall stoichiometry for N-nitrosamine formation from NO and HI with secondary amines (e.g., N-methylpiperazine).

Thus, under neutral conditions, (Chapter 2), the formation of NOI (Step k_a) is rate-limiting, whereas under acidic conditions (cf. Equation 3.3) its interaction with N-methylpiperazine (Step k_b) is slow. The change in rate limiting step relates to the diminution of unprotonated amine on raising the solvent acidity for which a good precedent exists in the diazotization of aniline by NOI in dilute aqueous acid.[75] This explanation was confirmed by examination of the kinetic order in N-methylpiperazine for reaction in 25% v/v aqueous-EtOH with 0.011 M KI and 0.006 M HCℓ (i.e., under neutral conditions, NO passed into solution before addition of amine) as shown in Figure 3.6. The independence on [N-methylpiperazine] was previously observed and confirmed here for NO and HI, to give H_2O, N_2O and I_2 (Equation 3.1), i.e., the rate-limiting formation of nitrosyl iodide.

Further evidence for the mechanism in Scheme 3.1 was obtained by a direct comparison of reactions carried out using equivalent

Figure 3.6

The formation of N-methyl-N-nitrosopiperazine from 0.011 M KI and 0.006 M HCℓ with excess NO (bubbling through) in 25% (v/v) aqueous-EtOH at 25°C. Initial [N-methyl-piperazine] = 0.02 M (■), 0.005 M (●) 0.0025 M (□), 0.001 M (▲).



concentrations of I_2 in place of HI (Table 3.2). Comparison with results in Table 3.1 shows similar k_o values apply to reactions of either 5×10^{-3} M I_2 or 10^{-2} M (KI + HCℓ) with 10^{-2} M N-methylpiperazine hydrochloride. This agrees with neutralization of 10^{-2} M HI by NO to give 5×10^{-3} M N_2O and 5×10^{-3} M I_2 (in equlibrium with I_3^-) (Equation 3.1). With low (5×10^{-4} M I_2 or 10^{-3} M (KI + HCℓ)) catalyst concentration, however, both the rate of formation of N-methyl-N-nitrosopiperazine and the shape of the respective plots (% reaction versus time) (Figure 3.5) differ. The flattening of the plot and lower rate of N-methyl-N-nitrosopiperazine formation probably arises from the incomplete conversion of HI to I_2 (which is only apparent for slow reactions) and the subsequent inter-related equilibrium with HI_3.

Less coherent kinetics were observed for rapid reactions when the concentration of dissolved NO is not maintained, other than by diffusion from the gaseous phase above the reaction solution. The effect is best observed from the first order kinetic plots (Figures 3.2 - 3.4). These are linear for reactions where the [NO] is maintained during reaction, but curved when only the initial saturated solution (ca. 1.2×10^{-2} M) NO is used. For the slowest reaction (Figure 3.4) the difference is less marked. Nonetheless, these reactions are of considerable interest because of their relevance to environmental situations where the concentration of dissolved NO is likely to be low. It is evident from plots in Figure 3.1, that variation in the yield of N-methyl-N-nitrosopiperazine with time is initially curved, but it approaches linearity as the reaction proceeds.

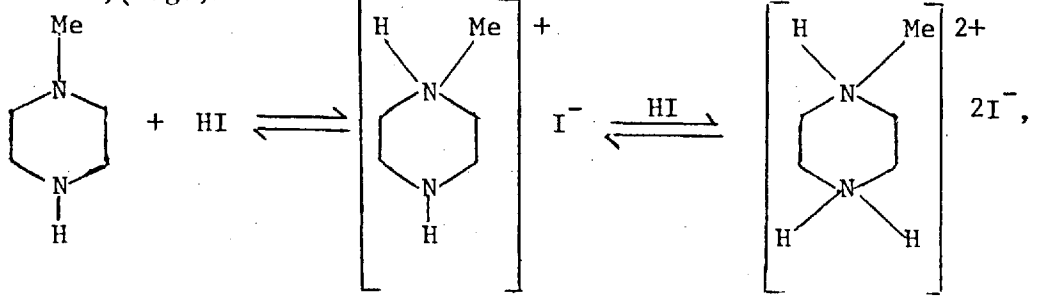
Further, the initial rate (i.e., when the solution is saturated with NO) is comparable to that of the pseudo first order reactions and the subsequent fall off is more noticable for rapid than for slow reactions. Results obtained for these reactions are summarised in Table 3.3. The k_o values are obtained from the initial part of the reaction and yields with their time of formation are given. (In all reaction of KI (10^{-2} - 10^{-3} M) and HCℓ, with 10^{-2} M N-methylpiperazine, quantitative yields are obtained on standing overnight, however, for reactions at high acidity with ca. 5×10^{-3} M KI , the yield after 15 min. is quoted for comparison).

It is apparent that k_o has a reduced, non-linear dependence on initial [KI] and passes through a minimum as either the initial [N-methylpiperazine] or acidity increases. All the findings are consistent with the mechanism outlined in Scheme 3.1, with the added proviso that diffusion of NO into the solution becomes rate-limiting for the faster reactions.

Previous measurements (v. Experimental 2.4.VI) showed the concentration of NO in 25%(v/v) aqueous-EtOH is ca. 1.2×10^{-2} M. Since 2 moles of NO are required to convert HI to NOI (Equation 3.1), the maximum yield of N-methyl-N-nitrosopiperazine would be ca. 6×10^{-3} M without the diffusion of NO from the gaseous phase. Examination of the plots in Figure 3.1 after this concentration is reached, shows that N-methyl-N-nitrosopiperazine continues to form at an approximately constant rate (ca. 10^{-6} moles sec^{-1}) irrespective of the initial [KI]. The apparent decrease from first to zero order dependence on [KI] as reaction proceeds when NO is not passed through continuously is good evidence that the diffusion of NO into solution is kinetically

significant and this is confirmed by the departure from the first order plots (Figures 3.2 - 3.4).

As far as the results in Table 3.3 are concerned, the reduced kinetic dependence on KI results from the deficiency of NO rather than a change of mechanism. The inhibition by excess N-methylpiperazine shows dependence of acid for the reaction and probably arises from its effect on the solvent acidity. A complicated acidity dependence is expected for the reaction because several prototropic equilibria are involved, (e.g.,

(I)  $2I^-$,

(II) $KI + HC\ell \rightleftharpoons HI + KC\ell$, (III) $HI + I_2 \rightleftharpoons HI_3 \rightleftharpoons H^+ + I_3^-$,
(IV) $HI + 2NO \rightleftharpoons NOH + NO\text{-}I$.). It is clear, however, from Table 3.1 and Table 3.3. that the fastest reactions with N-methylpiperazine in 25% (v/v) aqueous-EtOH is obtained with ca. 0.02 M $HC\ell$ (pH 2.1)*. The apparent increased rate with increasing acidity up to ca. 0.02 M $HC\ell$ for ca. 5×10^{-3} M KI and 10^{-2} M N-methylpiperazine (Table 3.3), was also observed for similar reactions of ca. 2.5×10^{-3} M I_2 (Table 2.7).

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* Footnote:

This refers to pH measured in 25% v/v aqueous-EtOH using electrodes calibrated in wholly aqueous buffers.

The k_o values across both sets of data are in fairly good agreement with the conversion of 5×10^{-3} M HI to ca. 2.5×10^{-3} M I_2 by NO. The k_o values show more clearly that the rate of reaction with 2.5×10^{-3} M I_2 (I_2 itself and I_2 derived from HI) is maximum with the addition of ca. 1.5×10^{-2} M HCℓ (after neutralisation of KI) and 10^{-2} M N-methylpiperazine : for example, with 2.5×10^{-3} M I_2 and 10^{-2} M, 2×10^{-2} M HCℓ, k_o is ca. 5.4×10^{-4} sec^{-1} and 7.95×10^{-4} sec^{-1} respectively, while with ca. 5.1×10^{-3} KI and 2×10^{-2} M HCℓ, k_o is ca. $1\,2 \times 10^{-4}$ sec^{-1} in the presence of 10^{-2} M N-methylpiperazine. The k_o values also suggest that at high acidity the rate falls more sharply for HI reaction, but this probably relates to the accuracy of the data.

Since the concentration of free amine is higher at low acidity the effect of increasing rate up to 0.02 M HCℓ probably relates to the concentration of the nitrosating agent i.e., NOI and hence, increasing [HI]. The slower rate at low acidity may be due to prototropic equilibria such as (I) shown above. Further, at higher acidity the concentration of the nitrosating agent derived from HI should be higher, but the reduction in rate reflect extensive protonation of N-methylpiperazine and hence reaction with NOI.

Two additional observations which supports the mechanism of Scheme 3.1 concerns inhibition by both added NaN_3 and excess KI. Thus, 0.012 M NaN_3 virtually blocks the reaction of 0.01 M N-methyl-piperazine with saturated NO in the presence of 0.02 M HCℓ and 7.7×10^{-3} M KI. The yield of N-methyl-N-nitrosopiperazine in the presence of NaN_3 is only 2.5×10^{-4} M (2.5%) after 24 hours compared to

7.1×10^{-3} M (71%) after ca. 40 min. in its absence. NaN_3 is known to consume nitrosating agents (such as NOI) by undergoing rapid deamination[48] (Equation 3.5). Although inhibition by KI

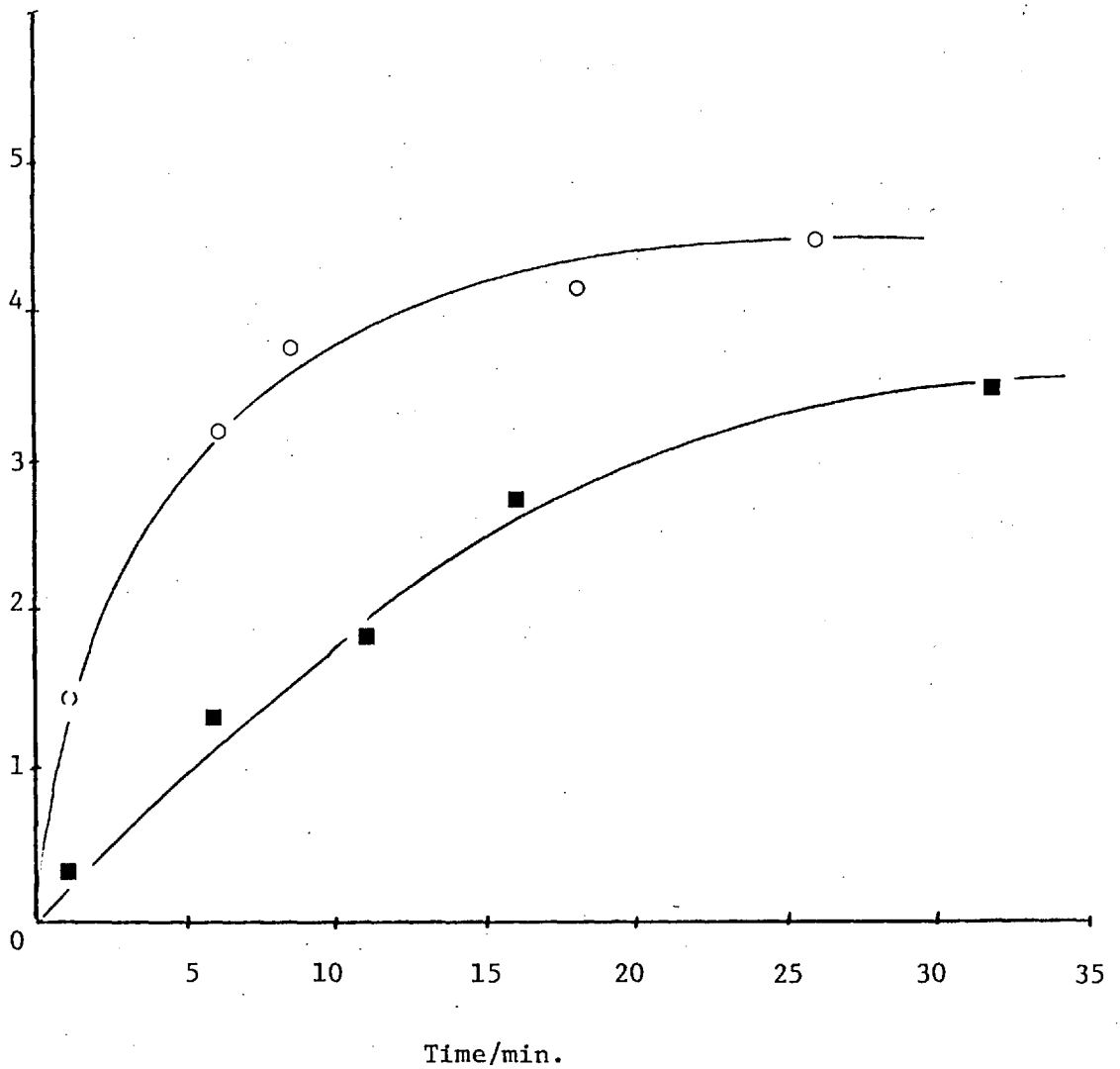
$$N_3^- + NOI \longrightarrow N_2O + N_2 + I^- \quad \ldots\ldots\ldots \quad 3.5$$

is less dramatic (Figure 3.7), it demonstrates the kinetic dependence on $[I_2]$ required by Scheme 3.1. For example, 0.01 M N-methylpiperazine and saturated NO in 5×10^{-3} M HCℓ, yields of N-nitrosamine after 10 min. are 3.9×10^{-3} and 1.8×10^{-3} M in the presence of 5.09×10^{-3} M and 87.7×10^{-3} M KI respectively. Thus excess KI reduces the $[I_2]$ by displacing the equilibrium, $I_2 + I^- \rightleftharpoons I_3^-$ to the right.

Figure 3.7

The effect of excess KI on the nitrosation of 10^{-2} M N-methylpiperazine with 0.005 M HCℓ from initial saturated (ca. 1.2×10^{-2} M) NO in 25% (v/v) aqueous-ethanol at 25°C.

Initial [KI] = 5.09×10^{-3} M (○) 87.7×10^{-3} M (■)



3.1 (3). Reaction of Piperidine and Morpholine with NO in the Presence of HI.

Previous experiments (Chapter 2), showed that the nitrosation of both morpholine and piperidine by NO in the presence of I_2 was severely inhibited by the addition of mineral acid. This was attributed to extensive protonation of these more basic amines (pK_A 8.33 and 11.22, respectively) under the reaction conditions. A similar outcome applies to their reaction in the presence of KI and HCℓ, but only when HCℓ is in excess. For example, 0.01 M morpholine with saturated NO in 25% (v/v) aqueous-EtOH at 25°C in the presence of 0.02 M HCℓ and 7.5×10^{-4} M KI produces only 2.2×10^{-3} M N-nitrosomorpholine (i.e., 22%) over 220 min . With 0.01 M piperidine, the extent of reaction is negligible (Table 3.4). It is clear from Equation 3.1, however, that NO can reduce the acidity of reaction solutions by converting HI to H_2O. It follows that faster N-nitrosation of strongly basic amines by NO should be observed in the presence of equimolar HCℓ and KI. This deduction is confirmed for morpholine and piperidine with saturated NO in 25% (v/v) aqueous-EtOH at 25°C in Figures 3.8 and 3.9, respectively. Thus, both amines give substantial limiting amounts of the corresponding N-nitrosamines after ca. 20 min. although N-nitrosomorpholine continues to form slowly to give up to 85% yield over 24 hours (Table 3.4). The initial yields vary in relation to the amine basicity and the amount of HCℓ and KI added, but their time of formation (ca. 20 min.) is about the same for piperidine, morpholine and N-methylpiperazine (Table 3.4). All of these results are similar to those found previously

Table 3.4

Yields and reaction times of N-nitrosamine formation from NO in 25% (v/v) aqueous-EtOH at 25°C. Initial saturated [NO] ca. 1.2×10^{-2} M.

10^2[Piperidine]/M	10^3[KI]/M	10^3[HCℓ]/M	Yield[a] %	Time/ min.
1.0	0.75	20.0	0	1200
1.0	5.27	5.0	17	19
1.0 (10)	10 (10)	10 (10)	27 (27)[b]	25 (21)
2.0 (20)	20.2 (19)	20 (20)	27 (27)[b]	25 (25)
10^2[Morpholine]/M				
1.0	0.70	20	22[c]	218
1.0	10.0	10.0	52[d]	35
20 (20)	20.1 (18.5)	20 (20)	42 (36)[b,e]	35 (25)
10^2[N-Methylpiperazine]/M				
1.0	0.75	20	75	50
1.0 (1.0)	5.09 (5.0)	5.0 (5.0)	45 (45)	30 (34)
1.0	10.3	10.0	80	33
1.0	87.7	5.0	35[f]	32

[a] Yield based on initial [Amine]

[b] Duplicate reactions in parenthesis

[c] Increased to 42% after 1200 min .

[d] Increased to 85% after 1200 min .

[e] Increased to 50% after 1200 min .

[f] Decreased rate for excess KI

Figure 3.8

The nitrosation of piperidine from equimolar [KI], [HCℓ] in 25% (v/v) aqueous-EtOH at 25°C. Initial [NO] saturated ca. 1.2×10^{-2} M

△ [KI], [HCℓ], [piperidine] = 2×10^{-2} M

□ [KI], [HCℓ], [piperidine] = 10^{-2} M

○ [KI], [HCℓ], = 5×10^{-3} [piperidine] = 10^{-2}M

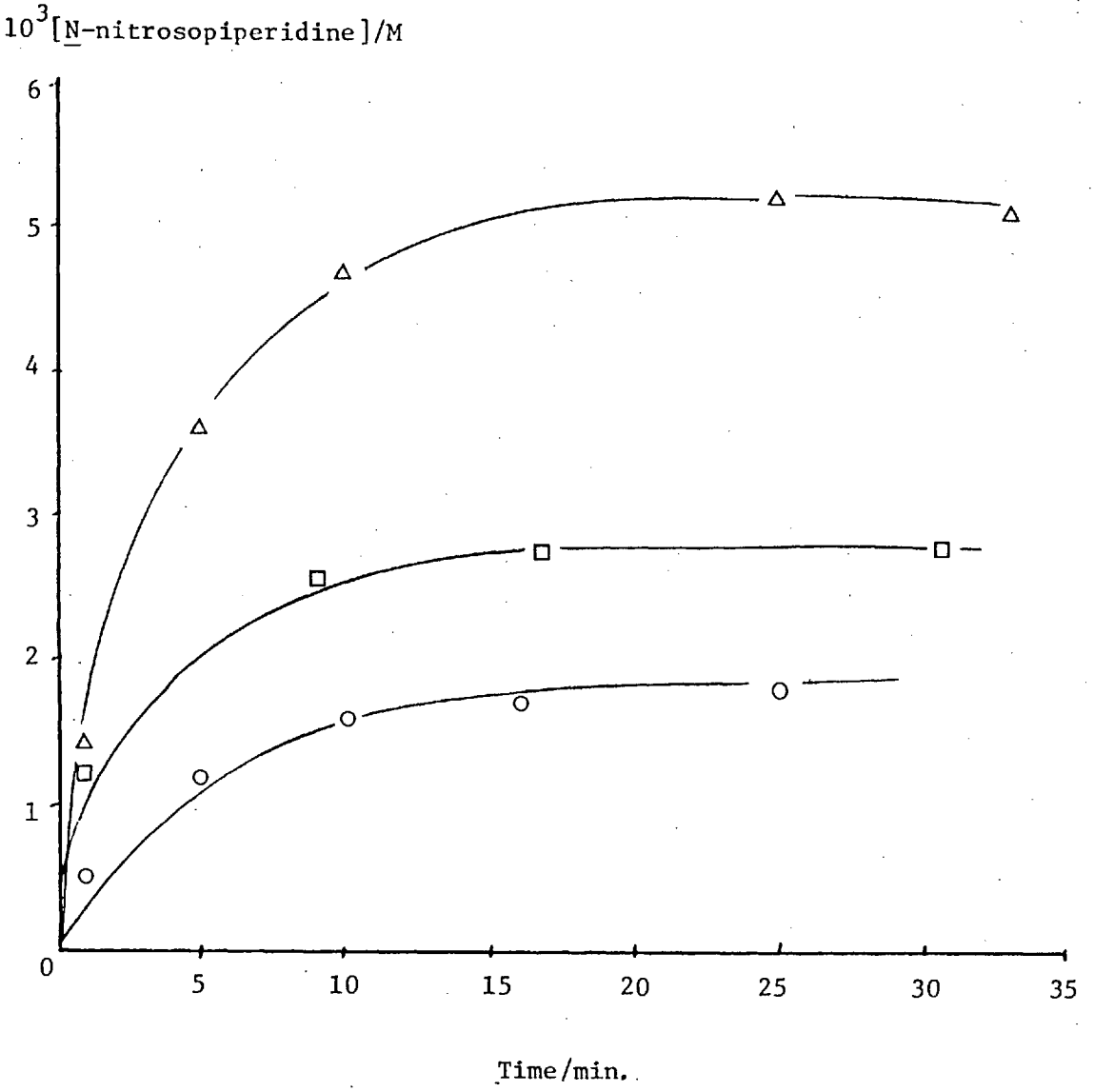
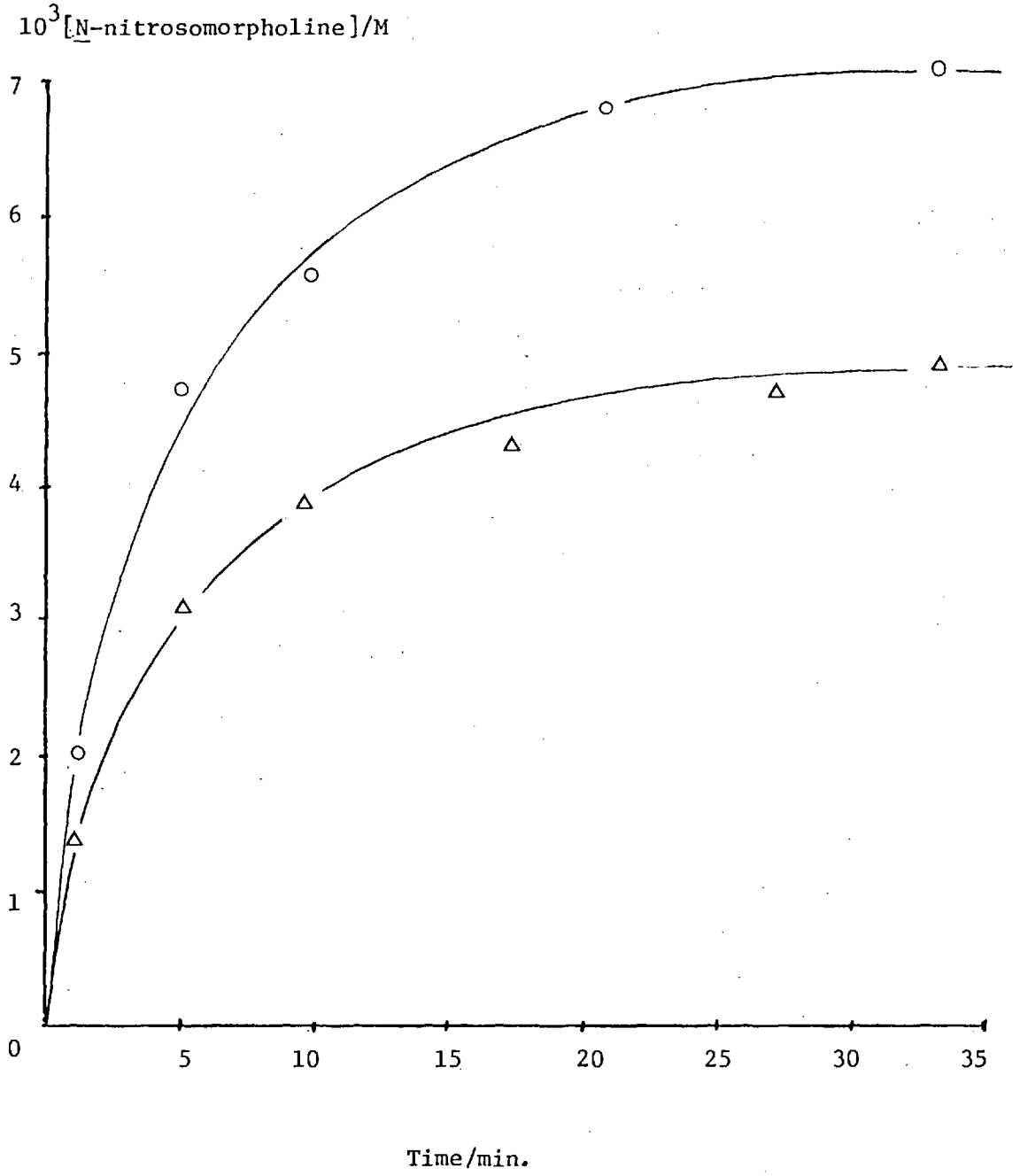


Figure 3.9

The nitrosation of morpholine from equimolar [KI], [HCℓ] in 25% (v/v) aqueous-EtOH at 25°C. Initial [NO] saturated ca. 1.2 x -0^{-2} M. [KI], [HCℓ], [Morpholine] = 2 x 10^{-2} M (○), 10^{-2} M (△)



for comparable reaction by NO and I_2 in neutral 25% (v/v) aqueous-EtOH (Chapter 2). They are consistent with the mechanism in Scheme 3.1, where formation of NOI (Step $\underline{k}_a$) is rate-limiting as expected for neutral conditions. Variations in the initial yields of N-nitrosamine with amine basicity, [HCℓ] and [KI], however, imply that a proportion of the amine substrate is protonated. This probably arises from concurrent hydrolysis of NOI to form HNO_2 (Equation 3.6), which, unlike HI is not neutralised by the passage of NO.

$$NO + \tfrac{1}{2} I_2 \rightleftharpoons NOI \xrightleftharpoons{H_2O} HNO_2 + HI \qquad \ldots\ldots\ldots \quad 3.6$$

3.1.(4). N-Nitrosamine Formation from NO in the Presence of Metal Iodides and Related Compounds.

Passage of NO into clear, neutral solutions of ZnI_2 in EtOH also produces a brown solution characteristic of $I_3^- \rightleftharpoons I_2 + I^-$ with uv. absorptions at λ_{max} = 288 and 353 nm.[117] Addition of secondary amine to these solutions progressively discharges the colour while the corresponding N-nitrosamine forms concurrently. Data summarised in Table 3.5, show that at 25°C these reactions reach completion within ca. 25 min. of adding the amine, irrespective of its structure (basicity) or concentration. Further, with excess amine, the limiting yield of N-nitrosamine depends principally on the initial $[ZnI_2]$. From Table 3.5, approximately 0.43 moles of N-nitrosopiperidine are obtained per mole of ZnI_2. Analysis by i.r. of the gaseous contents of the reaction vessel showed the presence of N_2O at concentrations of ca. 14 - 20% of the initial $[ZnI_2]$. If, at this stage, the colourless reaction solution is heated to 60°C, the brown coloumation reapppears and further N-nitrosamine formation ensues.

Zinc nitrosyl salts are unknown, so these reactions probably proceed by an initial solvolysis of ZnI_2 to release HI (Equation 3.7), which then promotes the formation of N-nitrosamines as described above.

$$ZnI_2 + EtOH \underset{}{\overset{25^\circ}{\rightleftharpoons}} Zn(OEt)I + HI \overset{60^\circ/EtOH}{\rightleftharpoons} Zn(OEt)_2 + HI \quad .... \; 3.7$$

Table 3.5

Limiting yields and times of N-nitrosamine formation from secondary amines, NO and ZnI_2 in EtOH at 25°C. Initial saturated [NO] ca. 2.2 x 10^{-2} M.

10^2[Amine]/M	10^3[ZnI_2]/M	10^3[N-nitrosamine]/M[a]	Time/min.[b]
1.0 Piperidine	2.2	1.2	25
"	4.23	(2.2) 4.4[c]	(25) 100
"	4.7	2.3	21
"	9.2	4.0	22
"	9.4	3.8	25
"	10.5	4.9[d]	23
2.0 Piperidine	4.7	2.2	25
"	9.9	3.5	25
4.0 Piperidine	10.1	4.0	25
1.0 Morpholine	4.9	2.1	25
1.0 N-methylpiperazine	4.8	2.0	24
2.0 Piperidine	10.0	6.8[e]	25
"	9.7	5.0[f]	42
1.0 Piperidine	10.0	0.5[g]	60
"	9.8	0.6[h]	60

[a] Limiting yield

[b] Time to reach limiting yield

[c] After 25 min . 2.2 x 10^{-3} M, heated at 60°C, 4.4 x 10^{-3} after 100 min .

[d] Inverse addition

[e] In 5% aqueous-EtOH

[f] In 25% aqueous-EtOH

[g] In MeCN

[h] In the presence of 0.011 M thiourea.

The stoichiometry of the reaction suggest that solvolysis is incomplete at 25°C, but proceeds further at higher temperatures. From Equation 3.7, ca. 0.5 mole of N-nitrosamine is expected per mole of ZnI_2 at 25°C, but in practice this limiting yield is rarely observed (Figure 3.10). The deficiency may arise because some HI is lost while passing NO through the reaction vessel. In one experiment, (cited as inverse addition in Table 3.5) where ZnI_2 was added after saturation of the reaction solution with NO, the yield of N-nitrosopiperidine at 25°C corresponded to 0.49 mole of ZnI_2.

Support for these mechanistic conclusions comes from less extensive experiments showing inhibition by added thiourea (Table 3.5) and those employing either different solvents (Table 3.5) or other zinc salts (Table 3.6). Thus, N-nitrosopiperidine formation is insignificant in MeCN (ca. 1% reaction over 60 min at 25°C), but more extensive in aqueous-EtOH. ZnI_2 is stable in MeCN, but hydrolyses completely in H_2O to give 2 moles of HI (Equation 3.8).

$$ZnI_2 + H_2O \rightleftharpoons ZnO + 2HI \qquad \ldots\ldots \quad 3.8$$

Hence, more extensive hydrolysis is expected for aqueous-EtOH than EtOH, itself, and, significantly, the limiting yield of N-nitroso-piperidine at 25°C increases from ca. 3.9×10^{-3} M in pure EtOH to ca. 6.8×10^{-3} M and 5.0×10^{-3} M in 5% (v/v) and 25% (v/v) aqueous-EtOH, respectively.* Further, N-nitrosopiperidine formation in pure

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*Footnote: H_2O increases the amount of HI, but inhibits I_2-promoted N-nitrosamine formation (Chapter2). Thus, N-nitrosopiperidine yields should optimise for an intermediate aqueous-EtOH as observed.

Figure 3.10

The effect of $[ZnI_2]$ on the yield of N-nitrosopiperidine from initial 0.01 M piperidine and initial saturated (ca. 2.2×10^{-2} M) NO in EtOH at 25°C.

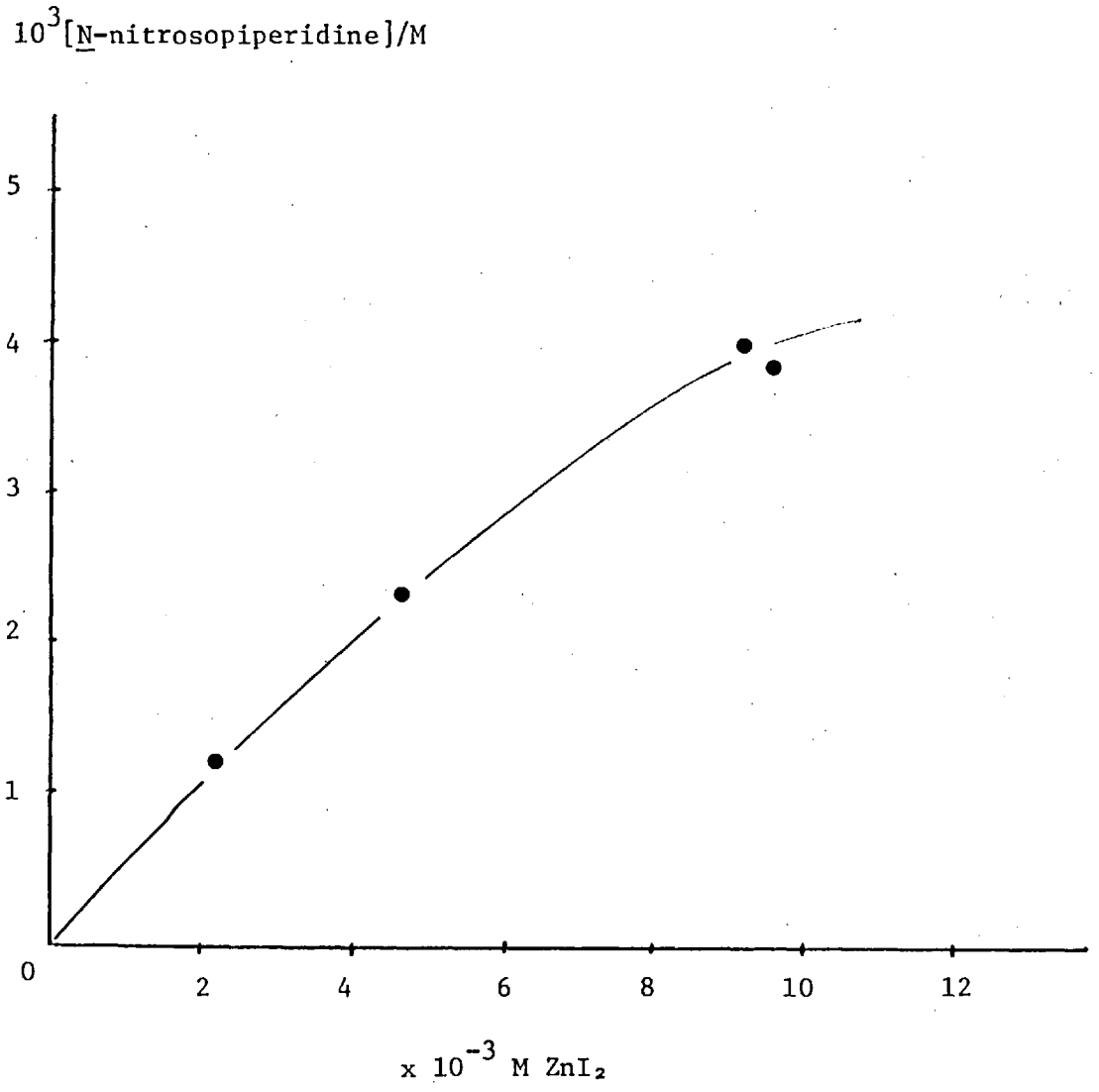


Table 3.6

Formation of N-nitrosamines from secondary amines and NO in the presence of various metal salts or other catalysts in EtOH at 25°C. Initial saturated [NO] ca. 2.2×10^{-2} M.

10^2[Amine]/M	10^3[Catalyst]/M	10^3[N-nitrosamine]/M	Time[a] min.
1.0 Piperidine	7.0 $ZnBr_2$	2.9	220
"	8.0 $ZnCl_2$	0	1440
"	5.33 $Zn(OAc)_2$	0.2	40
"	5.70 $ZnSO_4$	0.6	30
1.0 N-Methylpiperazine	0.64 FeI_2	1.9	22
"	2.6 FeI_2	5.1	30
"	5.1 FeI_2	5.8	20
"	4.9 FeI_2	5.8	90[b]
"	0.46 SnI_4	2.3	20
"	2.5 SnI_4	6.4	38
"	5.6 SnI_4	6.5	40
"	5.0 SnI_4	3.8	30[b]
2.0 Piperidine	5.0 BiI_3	4.5	25[c]
"	9.1 PdI_2	8.0	1440
"	9.0 MnI_2	0.14	60[d]
"	10.0 CdI_2	trace	60
"	10.1 Cu_2I_2	1.0	1440[d]
2.0 N-Methylpiperazine	15 $PhCOCl$ plus 4.7 KI	7.4	30
"	4.85 Me_2SiCl_2 plus 4.7 KI	5.6	30
"	5.3 $SnCl_2$ plus 4.7 KI	5.3	25[d]

[a] Time to reach yield shown

[b] In 25% (v/v) aqueous-EtOH

[c] In 10% (v/v) aqueous-EtOH

[d] In 5% (v/v) aqueous-EtOH.

EtOH is much slower with $ZnBr_2$ in place of ZnI_2 and proceeds to only negligible extents in the presence of either $ZnC\ell_2$, $Zn(OAc)_2$ or $ZnSO_4$ (Table 3.6). Thus release of HI is fundamental to the rapid formation of N-nitrosamines.

The ability of other iodide salts to promote N-nitrosamine from NO was therefore examined. These findings are also summarised in Table 3.6. As expected, substantial reaction applies only to those compounds which readily solvolyse to produce HI. SnI_4, FeI_2, and BiI_3 all dissolved in EtOH to give brown solutions (like ZnI_2) on addition of NO and all readily promote N-nitrosamine formation: CdI_2, MnI_2 and Cu_2I_2, however, are either insoluble or give no evidence of solvolysis and produce little N-nitrosamine. One exception is PdI_2 which promotes N-nitrosamine formation without appreciable solvolysis.

N-nitrosamine formation is also effected by both metal chlorides (e.g., $SnC\ell_2$) and acid chlorides in the presence of KI (Table 3.6). Here, solvolysis of the acid chloride to produce $HC\ell$, and subsequently HI, is probably involved.

3.2. Conclusions.

By confirming that NO is a good nitrosating agent in the presence of HI as well as I_2, these new results explain the I_2-catalysed formation of N-methyl-N-nitrosopiperazine under acidic conditions reported in Chapter 2. They further extend and define, the range of conditions under which carcinogenic N-nitrosamines may form from NO.

It is clear that NO maybe expected to react rapidly with secondary amines under neutral conditions in the presence of a wide range of iodide salts where solvolysis leads to the generation of HI (i.e., in protic solvents). The reactions are inhibited by excess acid to an extent dependent on the amine basicity, but HI itself, is not an inhibitor because it is neutralised by NO. Thus, provided excess iodide is present, the reaction solutions remain neutral and N-nitrosamine formation should proceed until all the amine is consumed. The reactions are solvent dependent insofar as solvolysis of the iodide salt is necessary. Also, no reaction is observed in highly aqueous alkaline media presumably because NOI is rapidly converted to innocuous NO_2^-. Under aqueous acidic conditions, however, diazotization of anilines by NOI is well-known,[75] so N-nitrosamine formation from NO and iodide salts may also be expected. One other important observation relevant to environmental circumstances is that diffusion of NO into solution may be rate-limiting under these conditions.

The general mechanism (Scheme 3.1) is one where solvolysis of the iodide salt to HI followed by its conversion by NO to I_2 precedes N-nitrosamine formation. The last step (i.e., the interaction of secondary amines with NO in the presence of I_2) has been demonstrated

and discussed previously (Chapter 2). One aspect of the present findings that warrants further discussion is the apparent conversion of HI to I_2 by NO. This reaction has been reported under both thermal[68] and photolytic[69] conditions in the gas-phase, but not hitherto, in solution. Although examination of the relevant standard oxidation potentials suggest it is thermodynamically favourable (for $I_2 + 2e^- \rightleftharpoons 2I^-$, E_o = 0.621 eV in H_2O at 25°C, and for $2NO + 2H^+ + 2e^- \rightleftharpoons N_2O + H_2O$, E_o = 1.59 eV in H_2O at 25°C),[122] the thermal gas phase reaction proceeds only slowly even at 90°C.[68] Further, NO is considered to <u>inhibit</u> the gas phase conversion of HI to I_2.[68]

The conversion of HI to I_2 by NO under our conditions was checked by passing NO into an anaerobic solution of 10^{-2} M KI plus 2 x 10^{-2} M HCℓ and examining the solutions by <u>uv</u>. spectrophotometry and by direct titration of I_2. Strong absorption at λ_{max} = 288, 353 nm are characteristic of those reported for I_2 itself (λ_{max} = 288, 353 nm, log ε = 4.60, 4.42[117]).

Visual examination of the rate of appearance of I_2 from HI and NO in 25%(v/v) aqueous-EtOH, H_2O and 25% aqueous MeCN, showed that the reaction <u>is</u> solvent dependent and occurs over a period of several minutes. In aqueous solution, no I_2 was evident over <u>ca</u>. 25 min , which is consistent with expectations that HI is predominantly dissociated (pK_{HI} <u>ca</u>.-9.0 in water at 25°C).[132] In 25% (v/v) aqueous-EtOH, however, the reaction proceeds over <u>ca</u>. 10 min, and in 25%(v/v) aqueous-MeCN over <u>ca</u>. 5 min. Clearly, the reaction of HI and NO shows a solvent dependence where an increase in the [HI] is expected.

Comparison of the uv. absorption of these solutions (after NO was removed), with that of I_2 itself, suggested that in both cases, the reaction is virtually complete over these time periods. Thus, a 20 µl sample of 7.9×10^{-3} M I_2 in 25%(v/v)aqueous-MeCN (diluted in 3.0 ml solvent contained in a 1 cm pathlength cuvette) gave absorbances of 0.43, 0.24 at λ_{max} 288, 353 nm respectively, while the reaction solution (10^{-2} M KI plus 2×10^{-2} M HCℓ) gave absorbances of 0.33, 0.206 under the same conditions and at λ_{max} 288 and 353 nm respectively. Assuming that ε = 40,000 at λ_{max} 288,[117] and that the ε value is not markedly solvent dependent, the $[I_3^-]$ = 1.61×10^{-3} M and 1.23×10^{-3} M for the standard 7.9×10^{-3} M I_2 and the reaction solution respectively. The increase in $[I_3^-]$ for the reaction of HI and NO, suggests that neutralization(Equation 3.1) maybe less than 100% and that residual HI increases $[I_3^-]$ by shifting the equilibrium ($I_2 + HI \rightleftharpoons HI_3$).

Similar results were obtained from the reaction of 10^{-2} M KI and 2×10^{-2} M HCℓ and NO in 25% (v/v) aqueous-EtOH. Thus, 20 µl 8.33×10^{-3} M I_2 in 25% (v/v) aqueous-EtOH (diluted as above) gave absorbances of 0.365, 0.214, while the reaction solution gave 0.51, 0.31, at λ_{max} 288, 353 nm respectively. Hence, the $[I_3^-]$ for the reaction solution (ε = 40,000) is ca. 1.9×10^{-3} M. This value is slightly higher than $[I_3^-]$ estimated from reaction in 25% aqueous MeCN and probably relates to additional equilibria involving the solvent EtOH.

The best evidence for the conversion of HI by NO to I_2 was obtained by direct volumetric analysis. The reaction solutions were titrated against standard sodium thiosulphate. The results confirm the neutralization of HI by NO to I_2 according to Equation 3.1, that the reaction is virtually complete ca. 86 - 87 %. For example, 10.0 ml

of both reaction solutions (25% (v/v) aqueous-EtOH, -MeCN) required 8.70, 8.60 ml 0.01 M sodium thiosulphate for complete loss of I_2 colour, (equivalent to 4.35 - 4.30 x 10^{-3} M I_2). The results also explain the increase of $[I_3^-]$ observed by uv. spectrophotometry, which probably arises from the reaction of unreacted HI and I_2 (HI + I_2 $\rightleftharpoons$ HI_3). Titration of 5 x 10^{-3} M I_2 in 25% (v/v) aqueous-EtOH required 8.40 ml 0.01 M sodium thiosulphate, equivalent to ca. 4.5 x 10^{-3} M I_2 in equilibrium with I_3^-.

Independent experiments showed that the rate of appearance of I_2 is catalysed by I_2 and oxygen, but not light. The effect of oxygen may be the conversion of NO into N_2O_3(N_2O_4) which then reacts directly with HI (or I^-) to give I_2. An added proviso therefore, is that the reactions may be catalysed by adventitious oxygen leading to small amounts of I_2, which then acts as a promoter.

3.3 EXPERIMENTAL

3.3 (a) Reagents, Substrates and Products.

The purification of solvents, amines, NO, the preparation and purification of authentic N-nitrosamines have been described in Chapter 2.4. Here AnalaR KI and standardised volumetric grade NHCℓ, thiosulphate were used without further purification. Reagent grade ZnI_2, $ZnBr_2$, $ZnCl_2$, $Zn(OAc)_2$ $ZnSO_4$, $SnCl_2$, BiI_3, CdI_2, SnI_4, MnI_2, FeI_2 were used without further purification other than vacumm drying at ca. 80°C overnight.

PdI_2 was prepared by the reaction of KI with $PdCl_2$ following a literature procedure.[133] The black PdI_2 was washed with warm H_2O and then vacuum dried at 100°C. Cu_2I_2 was prepared from the reaction of $CuCl_2$ and KI, purified as described in a literature procedure.[134] Reagent grade benzoyl chloride and dimethylsilylchloride were both fractionally distilled and the middle cuts taken.

3.3 (b) I. Reaction Methods.

Procedures for carrying out the reactions, examining the equilibrium between I_2/I_3^-, the metal salts in solution and measuring the concentration of N-nitrosamine were discussed in Chapter 2.4. For N-nitrosation reactions involving HI and NO, the following procedure was adopted for the preparation of the catalyst. KI was dissolved in the calculated volume of water and made up to 10.0 ml. by the appropriate addition of N HCℓ using a graduated pipette. EtOH (30 ml) was added, and the reaction solution degassed as described in Chapter 2.4. Purified NO

was passed into solution for either 15-20 min. for saturated solutions or continuously for excess (no attempt was made to standardise the flow of NO accurately). The reaction was initiated by the addition of neat amine *via* a microlitre syringe and *N*-nitrosamine analysed by g.l.c.

3.3 (b) II. *Estimation of N_2O.*

The gaseous reaction products were examined by i.r. spectroscopy on a Perkin-Elmer 457 spectrophotometer. The reaction vessel was coupled to a simple vacuum manifold bearing two low temperature traps at 77°K, and the i.r. cell (100 mm pathlength, KBr windows, 110 ml. volume, fitted with a stopcock) in sequence. When g.l.c. analysis showed that *N*-nitrosamine formation was complete, the gaseous contents of the reaction flask were swept into the manifold by nitrogen for *ca*. 20 min. This reaction flask was then isolated and the products transferred by trap to trap distillation to the i.r. cell. After equilibration to atmospheric pressure, the i.r. absorption of the content was recorded. N_2O was collected over several fractions by extraction of the gaseous products in the manifold using the i.r. cell under vacuum. (This multiple extraction was necessary because of the large difference in the volume of the manifold and N_2O formed during the reaction). The $[N_2O]$ was estimated from the absorption at 2240 cm^{-1} [135] by comparing the peak height for known additions of authentic N_2O (B.O.C.) into the i.r. cell. (This was done placing the i.r. cell under slight vacuum isolated by stopcock and sealing with a Subaseal stopper. N_2O was added with a 1-5 ml air tight syringe *via* the Subaseal

stopper into the i.r. cell by opening the stopcock, and the cell allowed to attain atmospheric pressure by slowly leaking nitrogen into the i.r. cell). Independent measurements showed that the peak height was proportional (± 10%) to the volume of N_2O added to the i.r. cell.

N_2O was also shown not to arise from NO, N_2O_3 or N_2O_4 contaminants. When NO is passed into the reaction vessel containing the catalyst, some N_2O is expected to be lost and hence erratic recoveries were obtained. Typically, with 0.01 M KI, 0.02 M HCℓ, and 0.0 M N-methylpiperazine, 1.2 - 1.85 ml N_2O was recovered (≡ 13.6 - 22% N_2O based on 10^{-2} M N-nitrosamine formation). N_2O was also recovered from the reaction of NO, N-methylpiperazine in the presence of ZnI_2 and benzoylchloride plus KI. The volume N_2O recovered was ca. 1.2 - 1.5 ml in both cases. With 0.01 M ZnI_2, 0.01 M piperidine, initial saturated NO in EtOH, the maximum yield of N-nitrosopiperidine obtained was 3.8×10^{-3} M, and ca. 1.25 ml N_2O recovered. Thus, since 1 mole N-nitrosopiperidine is equivalent to 0.5 mole N_2O (Equation 3.1) the % recovery N_2O based on the final yield of N-nitrosopiperidine is:-

$$\% \text{ recovery} = \frac{2 \times [N_2O]}{[\text{N-nitrosopiperidine}]} \quad \frac{2 \times 1.4 \times 10^{-3} \text{ M}}{3.8 \times 10^{-3} \text{ M}} \times 100 = 74\%$$

The volume of N_2O was not corrected to S.T.P.

3.3.c.(I) Typical Reactions.

[KI] = 10^{-2} M, [HCℓ] = 2 x 10^{-2} M, [N-methylpiperazine] = 10^{-2} M. in 25%(v/v) aqueous-EtOH at 25°C. NO excess, bubbled through.

Time min.	Attenuation	Peak Height	10^3[N-methyl-N-nitroso-piperazine]/M
1	x 1600	18	2.9
5		39	6.2
9		45	7.1
13		52	8.3
17		56	8.9
21		58	9.2
25		60	9.5
∞ 50	x 1600	63	10.0

Injection sample = 1 µl.
1 µl 10^{-2} M N-methyl-N-nitrosopiperazine gave peak heights at attenuation x 1600 of 63, 63, 63 on 8% carbowax + 2% KOH column on F33 under standard operating conditions (v. Experimental 2.4.III (b)).

3.3.c.II Typical Reactions.

[KI] = 8.6 x 10^{-4} M, [HCℓ] = 2 x 10^{-2} M, [N-methylpiperazine] = 10^{-2} M in 25%(v/v) aqueous-EtOH at 25°C. Saturated NO ca. 1.2 x 10^{-2} M.

Time min.	Attenuation	Peak Height	10^3[N-methyl-N-nitrosopiperazine]/M
1	x 3200	7	.7
6		24	2.4
11		34	3.4
21		46	4.6
27		49	4.9
32		60	6.0
37		63	6.3
42		70	7.0
∞ 1200	x 64 x 100	50	10.0

Injection sample 1 µl

1 µl 5.0 x 10^{-3} M N-methyl-N-nitrosopiperazine gave peak height at attenuation x 3200 of 50, 50 on 8% carbowax + 2% KOH column on F33 under standard operating conditions (v. Experimental 2.4.III b).

3.3.c.III. Typical Reactions.

[KI. = 10^{-2} M, [HCℓ] = 2 x 10^{-2} M, [N-methylpiperazine] = 10^{-2} M in 25%(v/v) aqueous-EtOH at 25°C. Saturated NO ca. 1.2 x 10^{-2} M.

Time min.	Attenuation	Peak Height	10^3[N-methyl-N-nitrosopiperazine]/M
1	x 1600	15	2.4
5		24	3.8
9		37	5.9
13		42	6.7
18		30	
22		50	7.9
26		51	8.1
32		57	9.0
37		59	9.4
42		54	
49		62	9.8
∞		63	10.0

Injection sample = 1 µl

1 µl 10^{-2} N-methyl-N-nitrosopiperazine gave peak height at attenuation x 1600 of 63, 63, 63 on 8% carbowax + 2% KOH column on F33 under standard operating conditions (v. Experimental 2.4.III (b)).

3.3.c.IV. Typical Reactions.

$[ZnI_2] = 4.66 \times 10^{-3}$ M, [piperidine] = 2×10^{-2} M in EtOH at 25°C.

Initial saturated [NO] ca. 2.2×10^{-2} M.

Time min.	Attenuation	Peak Height	10^3[N-nitrosopiperidine]/M
1	x 64	8.5	1.1
5		11	1.4
12		15	1.9
20		18	2.3
27		18	2.3
60		18	2.3

Injection sample = 1 µl

1 µl 5.0×10^{-3} M, N-nitrosopiperidine gave peak height at attenuation x 64 of 39, 40 on 8% carbowax + 2% KOH column on F33 under standard operating conditions (v. Experimental 2.4.III (b)).

CHAPTER 4

THE FORMATION OF N-NITROSAMINES IN SOLUTION FROM GASEOUS NITRIC OXIDE IN THE PRESENCE OF SILVER(I) SALTS.

4.1 Introduction.

Silver salts have found some application as oxidants in organic chemistry and these reactions have been the subject of several reviews.[136,137] Only comparatively recently, however, has it been realised that the active entity in these reactions is either Ag^{II} or Ag^{III}, and much recent work has focussed on this aspect of the reactions. The standard oxidation potentials for the formation of Ag^{II} [138] and Ag^{III} [139] (Equation 4.1 and 4.2) are reasonably high, so the states are not readily attained.

$$Ag^{I} \longrightarrow Ag^{II} + e^{-} \qquad E_o = 1.98v \ (4MHC\ell O_4) \quad .... \quad 4.1$$

$$Ag^{II} \longrightarrow Ag^{III} + e^{-} \qquad E_o = 0.7\text{-}0.9 \ v \ (MeCN, \ HC\ell O_4) \quad .... \quad 4.2$$

$Ag^{II}(picolinate)_2$, however, is well known and it has found many applications as an oxidant [140-143], Ag^{II} and Ag^{III} would be expected to oxidise NO to NO^{+}, so their ability to catalyse N-nitrosamine formation from NO might be expected. The interaction of Ag^{I} salts with amino compounds has been examined. Hydroxylamine, for example, is known to react directly with Ag^{I}, by an autocatalytic pathway to give Ag^{0} and N_2 (Equation 4.3).[144] Many authors[145-147] have observed

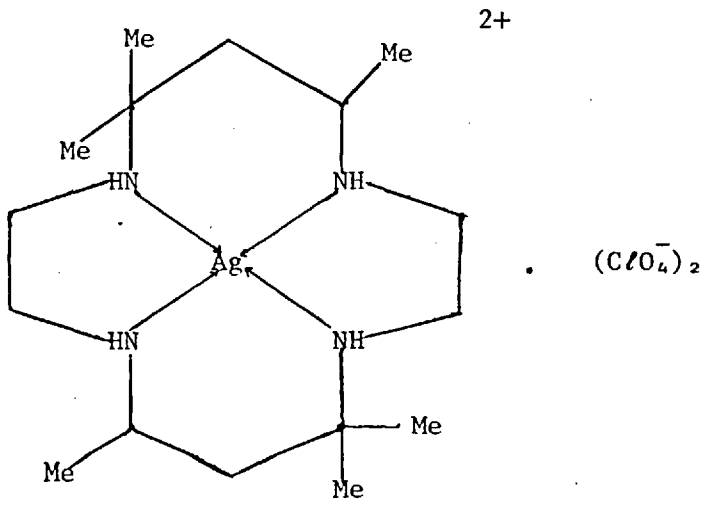
$$2Ag^{I} + 2NH_2OH \longrightarrow 2Ag^{0} + N_2 + 2H^{+} + H_2O \quad \ldots 4.3$$

a relationship between amine basicity and complex formation with Ag^{I}. The equilibrium constant for the formation of $[Ag\text{-}(amine)_2]^{I}$ (Equation 4.4) in 50% aqueous-EtOH increases with amine basicity

$$Ag^{I} + R\text{-}R'\text{-}NH \longrightarrow [(RR'NH)_2Ag]^{I} \quad \ldots 4.4$$

for both primary and secondary amines.[146] Ansell showed, by X-ray crystallography, that both piperidine[148] and morpholine[149] react with Ag^{I} to form only 1:1 complexes, when the amine itself was used as solvent. None of these reports, however, gave any indication of the rate of complex formation or the precipitation of Ag^{0}.

It is evident that Ag^{I} ion has a much less tendency to disproportionate in aqueous solution than for example, Cu^{I}. The equilibrium constant for Cu^{I} disproportionation ($2Cu^{I} \longrightarrow Cu^{0} + Cu^{II}$) is ca. 10^{6} at 25°C, whereas for Ag^{I}, the equilibrium has been estimated as ca. 10^{-20} at 25°C.[150] Kestner and Allred[150] reported the first ligand induced dispropotionation of Ag^{I} and their observations were later confirmed by Barefield and Mocella.[139] The Kestner and Allred complex (Structure 4.1) was prepared by the reaction of both $AgNO_3$ and $AgC\ell O_4$ with the macrocyclic ligand, 5,5,7,12,12,14,- hexamethyl-1,4,8,11-tetraazacyclotetradecane (L) in water and 50% (v/v) aqueous-MeOH.



Structure 4.1

They observed the formation of a silver mirror on the surface of the reaction vessel, and that the rate of formation of the complex was dependent on concentration and temperature. The yellow crystalline $[Ag^{II}(L)](C\ell O_4)_2$ complex was isolated and its composition confirmed by e.s.r. ($g = 2.124 \pm 0.005$), magnetic moment (2.14 BM) measurements and microanalysis. The complex was shown to be four-coordinate in a square planar configuration. These findings concur with earlier observations by MacMillan,[138] that Ag^{II} complexes are four coordinate, with g (e.s.r.) = 2.13. Neither this reaction, nor a silver mirror formed in dry MeCN, but a white precipitate was collected and confirmed to be the Ag^{I} (L)-complex. This Ag^{I} (L) complex was found to disproportionate in water and 50%(v/v) aqueous-MeOH according to Equation 4.5, and it was therefore concluded that the initial step

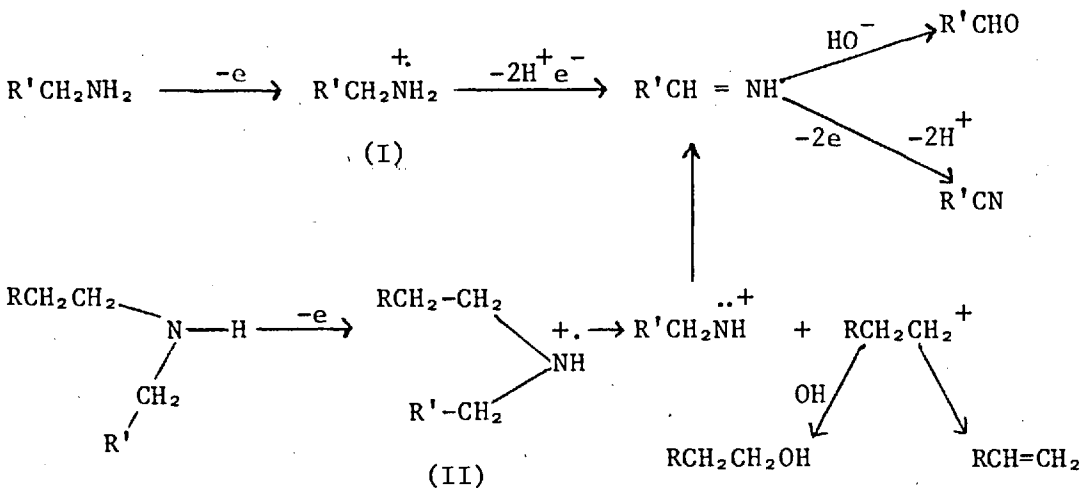
$$2Ag^{I}L \longrightarrow Ag^{0} + L + Ag^{II}L \quad \ldots\ 4.5$$

in the ligand induced disproportionation was the formation of the Ag^{I}-(L)-complex. Kestner and Allred[150] also reported that the macrocyclic ligand underwent oxidation, but did not identify the products. Barefield and Mocella,[139] however, noticed that addition of $NO^{+}C\ell O_4^{-}$ to the Ag^{II}-L-complex reduced NO^{+} to $\dot{N}O$ with the formation of Ag^{III}.

As noted above, the Ag^{II} state can be prepared by the oxidation of Ag^{I} with strong oxidising agents such as, $K_2S_2O_8$ or $(NH_4)_2S_2O_8$. It is now known that the higher oxidation state can be stabilised by many N-bases.[151,152] These products can be isolated if there is an increase in the binding energy, (e.g., picolinate, bipyridyl, 1,10-phenanthroline), and where the ligand is sufficiently stable to withstand the strong oxidising capability of Ag^{II} [153]. Po,[137] has reviewed both the structural and e.s.r. properties of several Ag^{II} and Ag^{III} complexes together with their reactivity.

Ag^{II} is one of the most powerful oxidising agents in organic chemistry. Its power has been demonstrated for AgO and Ag^{II} (picolinate)$_2$ in the conversion of hydrocarbons to alcohols,[148] (e.g., toluene to benzylalcohol), of primary and secondary alcohols to aldehyde and ketones,[141,143] (> 60% yields at 25°C), and of aldehydes to carboxylic acids.[141] Of more direct relevance to the present work, Hampson, et al. have observed that both primary[154] and secondary[155] amines are oxidised electrochemically by AgO in alkali. For primary amines, the products depend on the substitution at the α-carbon; i.e., α-monosubstituted amines give nitriles and aldehydes whereas

α-disubstituted analogues give ketones.[154] Secondary amines, however, are oxidised to alcohols, nitriles and alkenes, e.g., diethylamine gives EtOH, MeCN, $CH_2=CH_2$, di-n-butylamine gives n-PrCHO, n-PrCN, n-BuOH. The rate of reaction was found to depend on the branching e.g., $(n\text{-butyl})_2NH$ reacted 2-3 x faster than n-butyl-NH-t-butyl.[155] All these reactions are believed to involve initial oxidation of the amino group to the radical cation (I) or (II) which then undergoes further reactions as shown in Scheme 4.1.



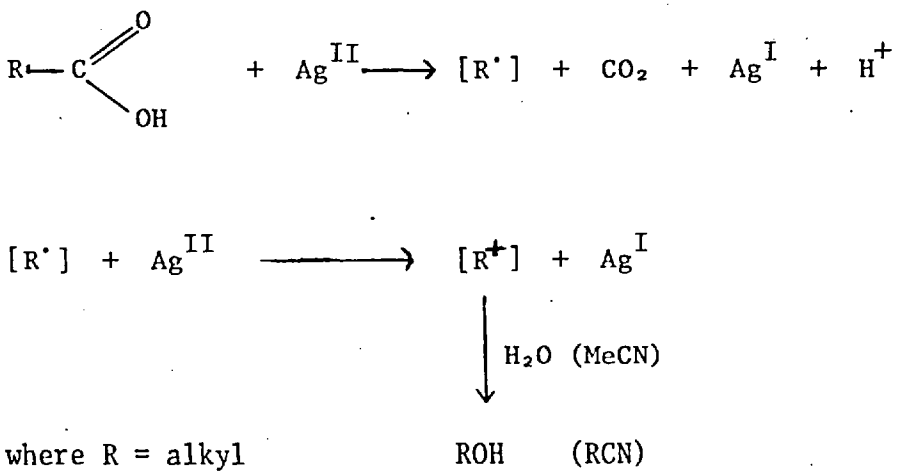
Scheme 4.1

Subsequently, Ortiz [156] showed that either AgO or Ag^{II} $(picolinate)_2$ will oxidise aromatic amines to (free radical) dimerisation products; e.g., aniline gave 20% azobenzene within 30-120 min, depending on the solvent. Hampson, et al.,[140] also reported that primary amines, such as benzylamine were oxidised by Ag^{II} $(picolinate)_2$ to benzonitriles and benzylaldehyde, the maximum yield (> 60%) was observed in ca. 5 min. in DMSO at 40°C,

while N-acetylbenzylamine gave the same yield of benzyladehyde, but required heating at 50°C for 20 min.

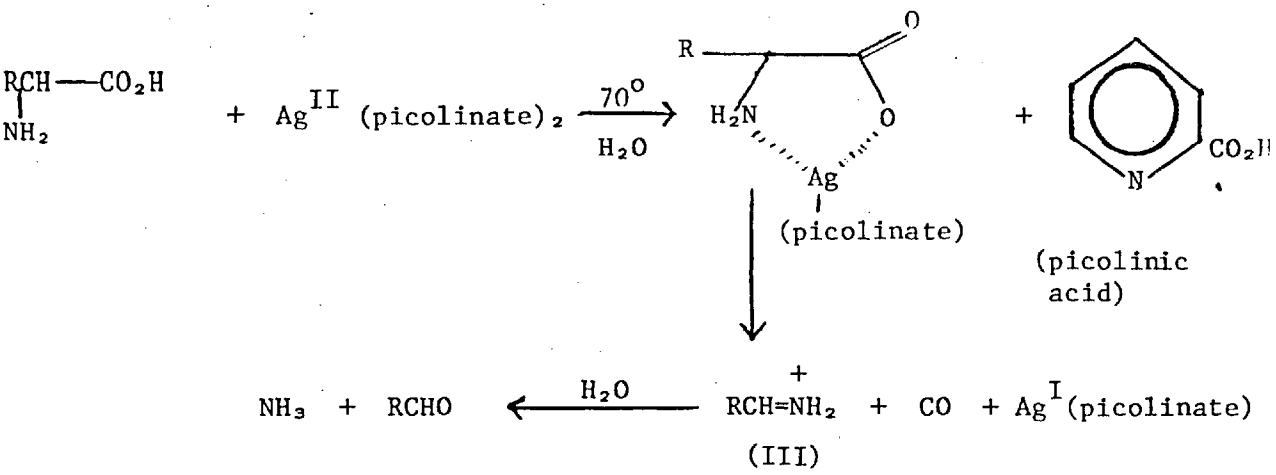
Two trends that are apparent from the above results are, the difference in reactivity of Ag^{II} towards hydroxy and amino compounds, and the dependence of the rate of reaction on substrate structure. Thus, cyclohexylamine and D-glucosamine (2-amino-2-deoxy-D-glucose) react rapidly at room temperature with Ag^{II} (picolinate)$_2$, while cyclohexanol and D-glucose require heating,[143] (the ease of oxidation is dependent on the donating ability of the group). Also, primary amines are oxidised more easily than secondary amines and the more hindered analogues react slower.[141,143]

The Ag^{II} state has also been implicated in the oxidative decarboxylation of aliphatic carboxylic[157-159] and amino acids.[160] When orange Ag^{II} (picolinate)$_2$ and aliphatic acids (formic, acetic, propionic) are heated together at 60-90°C, white [Ag^{I}-picolinate], CO_2, the parent alcohol and side products such as alkenes are formed.[158,159] The ratio of alcohol/alkene depends on the choice of solvent, and the results indicate that 1 mole of CO_2 is formed for 2 moles Ag^{II}-(picolinate)$_2$. This suggests that the initial step is oxidation of the carboxylate group by Ag^{II} (Scheme 4.2), followed by oxidation of the alkyl radical ($R^{\cdot}$) by a second Ag^{II}. The fate of R^{+} depends on the solvent, i.e., gives rise to alcohols by solvation or amides in MeCN.[158] The RH product is formed albeit in low yields, but this suggests that direct H-abstraction (from either the solvent or another acid molecule) by an $R^{\cdot}$ intermediate may be possible.[158]



Scheme 4.2

Amino acids are converted into aldehydes (i.e., DL-α-alanine is converted into acetaldehyde, (88% yield, ca. 26 min. at 70°C in aqueous solution) by Ag^{II} (picolinate)$_2$.[160] The initial step appears to involve complexation of the amino acid with Ag^{II} (picolinate)$_2$, followed by decarboxylation of the bound amino acid to the imine-(III), (Scheme 4.3), which is then hydrolysed to the aldehyde.



Scheme 4.3

4.2.1. Results.

The reactions were carried out at 25°C in carefully degassed ethanol (which contained the Ag^{I} salt) saturated with purified NO and contained under gaseous NO at atmospheric pressure. The equilibrium concentration of dissolved NO was found to be ca. 2.2 x 10^{-2}M (Chapter 2.4.VI) which should be maintained during the reaction by absorption from the gaseous phase. There was no evidence that NO reacted with Ag^{I} salts to give Ag^{0} on standing over 48 hours. The reactions were initiated by injecting neat amine into the solution with a microlitre syringe. After ca. 50 min. the formation of Ag^{0} was apparent by the formation of a silver mirror on the walls of the reaction vessel. A typical plot of the % reaction with time for reaction of 10^{-2} M piperidine in the presence of 10^{-2} M $AgNO_3$ is shown in Figure 4.1. The data refers to both the reduction in unreacted piperidine (II) and the increase in N-nitrosopiperidine product (I). Both plots show a sigmoidal shape indicative of an induction period for the reaction. It is during this induction period that the silver mirror is deposited on the walls of the reaction vessel. After this period, N-nitrosopiperidine formation increases. In the absence of satisfactory kinetic data, the variation with reagent concentration of both the limiting yield of N-nitrosamine, and the time required to reach this limiting yield, was examined. Most of these measurements were carried out using piperidine and $AgNO_3$. By and large, the results fall into two categories.

Thus, with a larger than 2 fold excess of piperidine over $AgNO_3$, the limiting yield of N-nitrosopiperidine forms over a period of ca. 200 min. and ca. 1 mole of N-nitrosopiperidine forms per mole of Ag^{I}

Figure 4.1.

The formation of N-nitrosopiperidine and the loss of piperidine from equimolar (10^{-2} M) $AgNO_3$ and piperidine with NO in anaerobic ethanol at 25°C. Initial saturated [NO] = ca. 2.2×10^{-2} M

(●) (I) Normal reaction $AgNO_3$ + NO $\xrightarrow{\text{piperidine}}$

(▲) (II) Loss of piperidine

(■) (III) Preformed $Ag^{II}(piperidine)_4$ then added NO.

(△) (IV) In the presence of 2.5×10^{-2} M NEt_3

(○) (V) In the presence of 1.33×10^{-2} M 2,2,6,6-tetramethylpiperidine

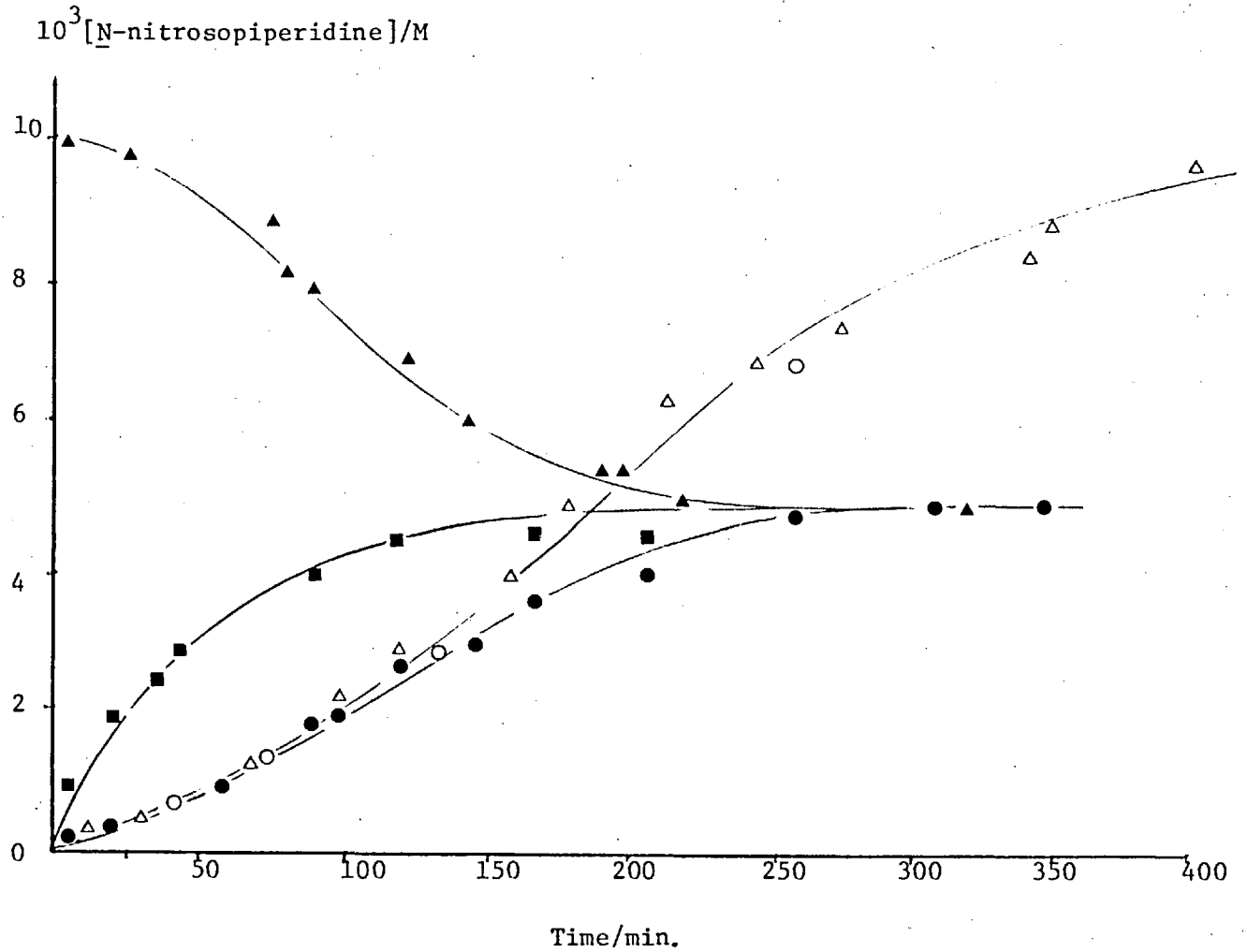


Table 4.1

Effect of initial [piperidine] for the $AgNO_3$ catalysed N-nitroso-piperidine formation in anaerobic EtOH at 25°C. Initial saturated [NO] = ca. 2.2×10^{-2} M.

10^3[$AgNO_3$]/M	10^3[piperidine]/M	10^3[N-nitroso-piperidine]/M	Time/[a] min.	Time/[b] min.
0	10	0.4	2880	
1.0 (1.0)[c]	10	1.2 (1.2)	1200	
2.5 (2.45)[c]	20	2.9 (3.2)	130 (110)	52 (58)
2.50 (2.5)[c]	10	2.9 (2.7)	200 (160)	59
2.86[d] (2.86)[c]	10	3.2	160	60
2.76 (2.32)[c]	5.0 (5.0)	2.15 (2.0)	350	59
2.35[e]	10	2.2	55	
5.0[f]	10	4.5	200	50
5.0 (5.1)[c]	10	4.8 (5.1)	210	54
5.0 [d]	10	4.0	190	62
5.30[e]	10	4.8	135	
9.73	25	9.5	180	55
10.0[e]	10	4.5	90	
9.77	10	4.8	280	40

a Time to reach maximum yield.

b Time taken for Ag^0 to be observed on flask

c Duplicate reactions in parenthesis

d Reaction with $AgClO_4$

e Preformed $Ag^I + R_2NH$, then add NO

f In the presence of 1.25×10^{-3} M N-nitrosopiperidine
1.25×10^{-3} M N-nitrosomorpholine

salt (Table 4.1). With an excess of amine greater than 2:1, the time of formation is about the same (ca. 200 min.) irrespective of [piperidine], but with 2:1 reagent concentration the reaction is clearly slower and less extensive. Also, similar results apply to reactions with $AgC\ell O_4$ in the place of $AgNO_3$. Further, at the conclusion of reaction, all the Ag^I salt had been converted to Ag^0. This was established by observing no precipitation of $AgC\ell$ on addition of $HC\ell$ to the reaction mixture. With equimolar $AgNO_3$ and piperidine, or with excess Ag^I salt (Table 4.2), only ca. 50% of the amine reacted unless addition of base (e.g., NEt_3, or NaOEt) was made as discussed below. Thus, the extent of reaction under these conditions appears to be limited by the availability of unprotonated amine.

Table 4.2

Effect of excess $AgNO_3$ on the yield of N-nitrosopiperidine in anaerobic EtOH at 25°C. Initial saturated [NO] = ca. 2.2×10^{-2} M.

10^3[$AgNO_3$]/M	10^3[piperidine]/M	10^3[N-nitrosopiperidine]/M (Maximum Yield)	Time min.
2.57	2.5	1.2	1200
5.21	5.0	3.0	300
9.77	10.0	4.8	260
10.0	10.0	4.8	260
15.2	10.0	4.8	260
17.7	10.0	4.8	260

All the above reactions showed sigmoidal plots for % reaction versus time, typified by Figure 4.1. However, if the amine were allowed to react first with $AgNO_3$ until a silver mirror was apparent, and then NO was added to the reaction mixture, the usual induction period was not observed (Figure 4.1.III), but in other respects, the reaction was similar to that carried out with the normal procedure. This suggested that the induction period is associated with the formation of Ag^{II}. Independent checks were carried out to establish that the reaction was not autocatalytic. Thus, reaction of 0.01 M piperidine plus 5×10^{-3} M $AgNO_3$ in the presence of 1.25×10^{-3} M N-nitrosopiperidine gave the same rate (Figure 4.1.I) as in its absence. Further, the reaction rate is unaffected by the particle size of the Ag^{I} salt (i.e., ground versus unground) and is the same with homogeneous $AgC\ell O_4$ as with heterogeneous $AgNO_3$ in ethanol (Table 4.1).

As mentioned above, the extent of reaction could be increased for equimolar concentrations of $AgNO_3$ and piperidine if base were added to the reaction solution. Details of these experiments are given in Table 4.3. Bases such as NEt_3 (pK_A 10.65[115]) or 2,2,6,6-tetramethylpiperidine (pK_A 11.07[115]) did not disproportionate $AgNO_3$. The addition of 2.5×10^{-2} M NEt_3 or 1.3×10^{-2} M 2,2,6,6-tetramethylpiperidine to a reaction of 10^{-2} M piperidine and 10^{-2} M $AgNO_3$ did not affect the rate of reaction but increased the yield to ca. 100% (Figure 4.1.IV). The increased yield presumably results from the removal of acid which normally protonates 50% piperidine and hence, inhibits the reaction. Also, there was no evidence that these bases were nitrosated. Further, the addition of ca. $(5\text{-}16) \times 10^{-3}$ M NaOEt increased not only the yield,

but the rate of reaction (Figure 4.2). For example, when 1.62×10^{-2} M NaOEt was added to 10^{-2} M piperidine and 1.05×10^{-2} M $AgNO_3$, a silver mirror was observed on the surface of the reaction vessel after ca. 15 min, and the % N-nitrosopiperidine versus time is exactly the same as that reaction where $AgNO_3$ and piperidine are allowed to react prior to the passage of NO (Figure 4.1), but the yield increases to ca. 65% (Table 4.3).

A vast excess of NaOEt, (e.g., 2.56×10^{-2} M NaOEt) over an equimolar reaction of 10^{-2} M $AgNO_3$ and piperidine, however, inhibited N-nitrosopiperidine formation (ca. 0-5% maximum). The observation suggested that only piperidine coordinated to the Ag salt is able to form N-nitrosopiperidine.

4.2.2. Effect of Amine Structure.

The ability of $AgNO_3$ and $AgC\ell O_4$ to catalyse N-nitrosamine formation from NO was examined in less detail than above, for several other secondary amines. Most of these reactions were carried out using equimolar (0.01 M) $AgNO_3$ and secondary amine. The results in the form of maximum yield of N-nitrosamine and its time of formation are given in Table 4.4. In the case of morpholine and N-methylpiperazine, the influence of $[AgNO_3]$ on the maximum yield was also examined. As with piperidine above, it is clear that the maximum yield of N-nitrosomorpholine is dependent on the $[AgNO_3]$ when the amine is in excess. N-methylpiperazine, however, did not disproportionate Ag^I and very low yields of N-methyl-N-nitrosopiperazine were observed. With aromatic amines, (e.g., N-methyl, N-ethyl-aniline and diphenylamine) disproportionation of Ag^I was observed to give coloured solutions in the absence of NO. The Ag^0 mirror was formed

Figure 4.2.

The effect of added NaOEt on the formation of N-nitrosopiperidine from equimolar (10^{-2} M) $AgNO_3$ and piperidine with NO in anaerobic EtOH at 25°C. Initial saturated [NO] ca. 2.2×10^{-2} M

(●) Normal reaction

(■) In the presence of 5×10^{-3} M NaOEt

(○) In the presence of 5×10^{-3} M NaOEt, reverse addition, piperidine added after EtO^-

(▲) In the presence of 6×10^{-3} M NaOEt

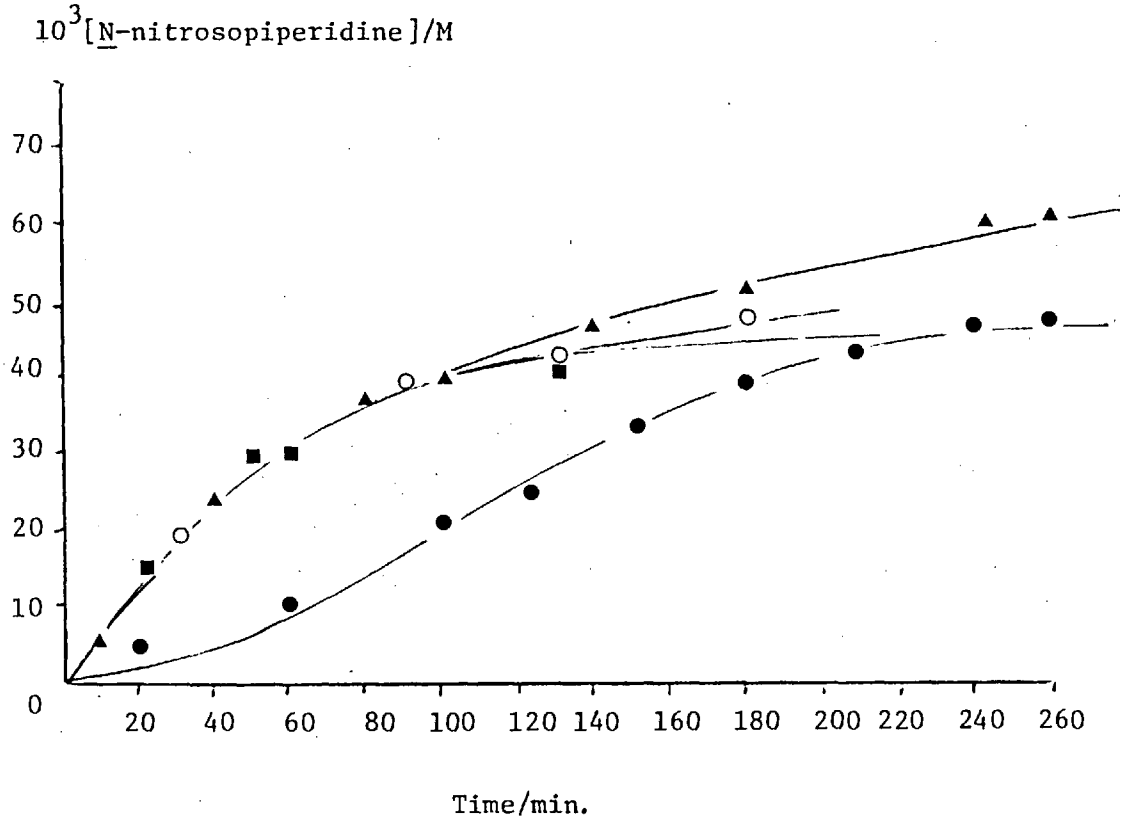


Table 4.3

The Effect of added bases on the yield of N-nitrosamine formation from $AgNO_3$ in anaerobic EtOH at 25°C. Initial saturated [NO] = ca. 2.2×10^{-2} M, [piperidine] = 10^{-2} M.

10^2[$AgNO_3$]/M	10^3[Base]/M	10^4[N-nitrosamine]/M Yield	Time/min.
1.03	0	48	320
1.03	5.4 NaOEt	50 (60)[a]	140 (1200)[a]
1.04	6.0 NaOEt	50 (60)[a]	140 (270)[a]
1.02	10.0 NaOEt	50 (76)[a]	120 (1200)[a]
1.10	16.2 NaOEt	50 (65)[a]	120 (207)[a]
1.05 (1.02)	25.0 NaOEt	0 (5)[a]	240 (1200)
1.10[b]	5.4 NaOEt	50 (70)[a]	(190) 350[a]
1.02[b]	16.2 NaOEt	50 (66)[a]	(180) 350[a]
1.010[b]	25.0 NaOEt	0	225
1.02[c]	4.6 NaOEt	33	205
1.02[c]	16.2 NaOEt	70	1200
1.13	5.0 NaOEt 9.3 NEt_3	100	300
1.0 (1.05)	25 (25) NEt_3	100	360
1.04	15 NEt_3	75[d]	220
1.03	13.3 2,2,6,6-tetraMe-piperidine	75[d]	300

[a] Maximum yield after time in parenthesis

[b] In the presence of 10^{-2} M N-methylpiperazine

[c] In the presence of 10^{-2} M Morpholine

[d] 1×10^{-2} M N-nitrosopiperidine overnight.

Table 4.4

Maximum yield and time of N-nitrosamine formation from the reaction of $AgNO_3$ with secondary amines in anaerobic EtOH at 25°C. Initial saturated [NO] = ca. 2.2×10^{-2}M, [Amine] = 0.01 M.

10^3[$AgNO_3$]/M	Amine	pK_A[a]	Max. % yield[b]	Time/min.[c]
10	Piperidine	11.22	48	300
10	Pyrrolidine	11.27	45	240
10	Morpholine	8.33	45	400
5	Morpholine		55	270
2.5	Morpholine		25	1200
10	N-methylpiperazine	9.8, 5.12	9	1200
5	N-methylpiperazine		8	1200
2.5	N-methylpiperazine		6	1200
9.8	N-methylaniline	4.85	58	150
10	N-ethylaniline	5.11	62	150
10	Diphenylamine	0.79	62	180

[a] Taken from reference 115 . For H_2O at 25°C

[b] % yield based on initial [Amine]

[c] Time to reach maximum yield.

ca. 15 min. after addition of amine and there was no significant induction period for N-nitrosamine formation. These reactions were analysed by u.v. spectroscopy, following the decrease in the absorbance at λ_{max} for the parent amine (v. Experimental, 4.6. III, d(I)).

The reactions are slightly faster for the aromatic amines than piperidine or morpholine, and the maximum yields were obtained after ca. 150-200 min. The rate of reaction seems to correlate with the bulkiness of the amine. For example, equimolar (10^{-2} M) $AgNO_3$ and N-methyl-, N-ethyl-aniline react to give maximum yield ca. 60% , after ca. 150 min. while with diphenylamine, 60% yield was obtained after 180 min. Also, the rate of reaction of 9×10^{-3} M diphenylamine and 10^{-2} M $AgNO_3$ was unaffected by the addition of 10^{-2} M NaOEt. Further, analysis of the coloured solutions by e.s.r., at 77° K [in the absence of NO (v. Experimental 4.6.III d2)] showed the presence of diphenylamino, N-methyl-, N-ethyl-aniline radicals at ca. g = 2.002-2.005 (Figures 4.3-4.5). The g-values agree reasonably well with N- centered free radicals.[161] The trapping of these radicals with the spin trap, t-nitrosobutane proved unsuccessful, even though the radicals were clearly present. Analysis of the reaction solution containing equimolar (10^{-2} M) piperidine and $AgNO_3$ in EtOH by e.s.r., at 77° K in normal e.s.r. cells and at 298° K in e.s.r. flat cells (v. Experimental 4.6. 3c) showed the presence of the Ag^{II} state g ca. 2.12 (Figure 4.6)[138,162], but not of the piperidino radical. This solution, however, at 25°C did decolourise the violet 2,2-diphenyl-1-picryhydrazine (DPPH), which indicates the presence of free radicals.[163] The g-Ag^{II} (piperidine)$_4$ was confirmed by the preparation of Ag^{II}(picolinate)$_2$ where e.s.r. measurement showed g ca. 2.15.

Figure 4.3

The e.s.r. spectra from the reaction of equimolar (10^{-2} M) $AgNO_3$ and diphenylamine in EtOH at 77^{o} K. Resonant magnetic field 3256 G, electron resonant frequencies 9.15 MHz.

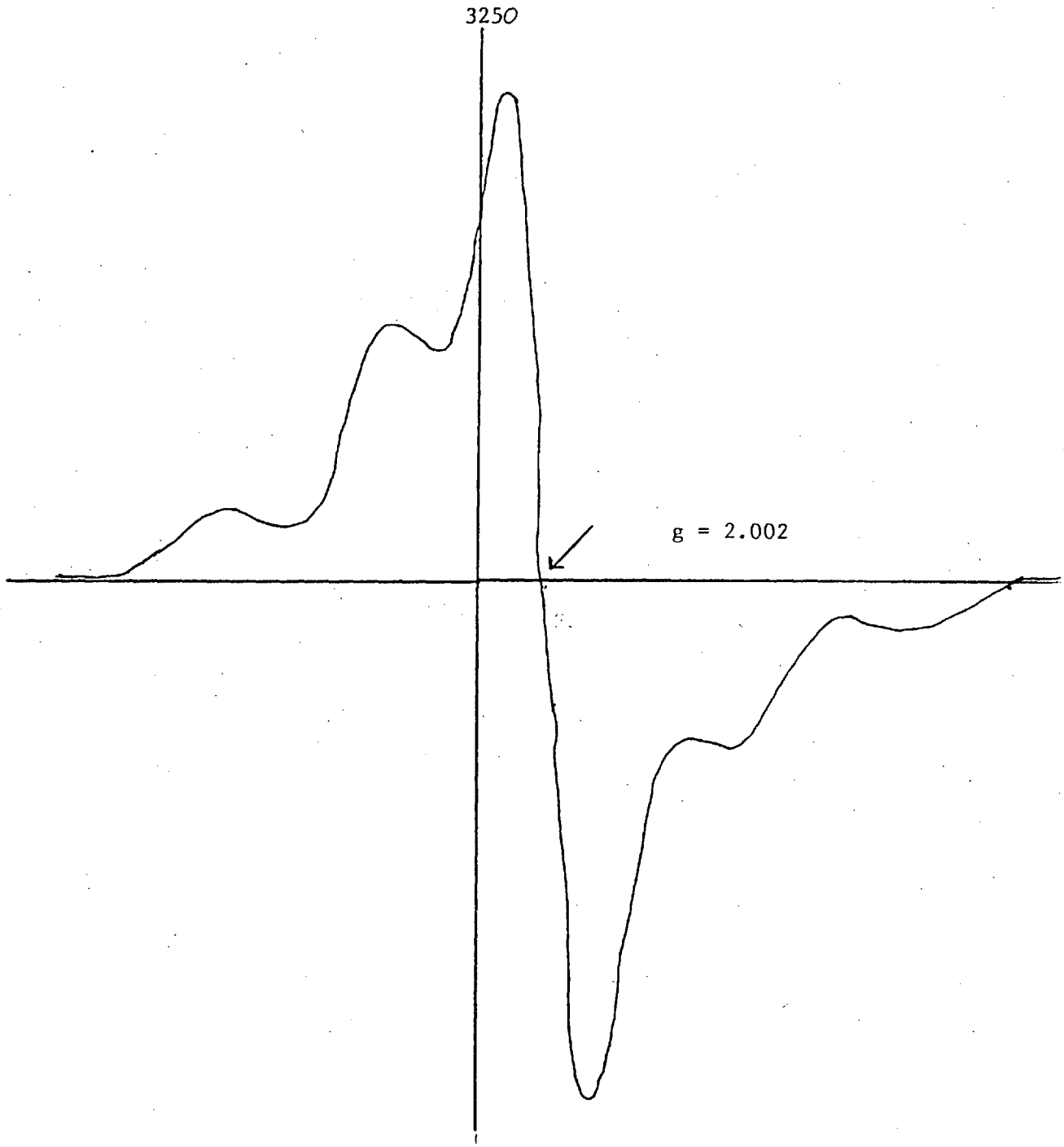


Figure 4.4

The e.s.r. spectra from the reaction of equimolar (10^{-2} M) $AgNO_3$ and N-methylaniline in EtOH at 77^{o} K. Resonant magnetic field strength 3262 G, electron resonant frequency 9.15 MHz

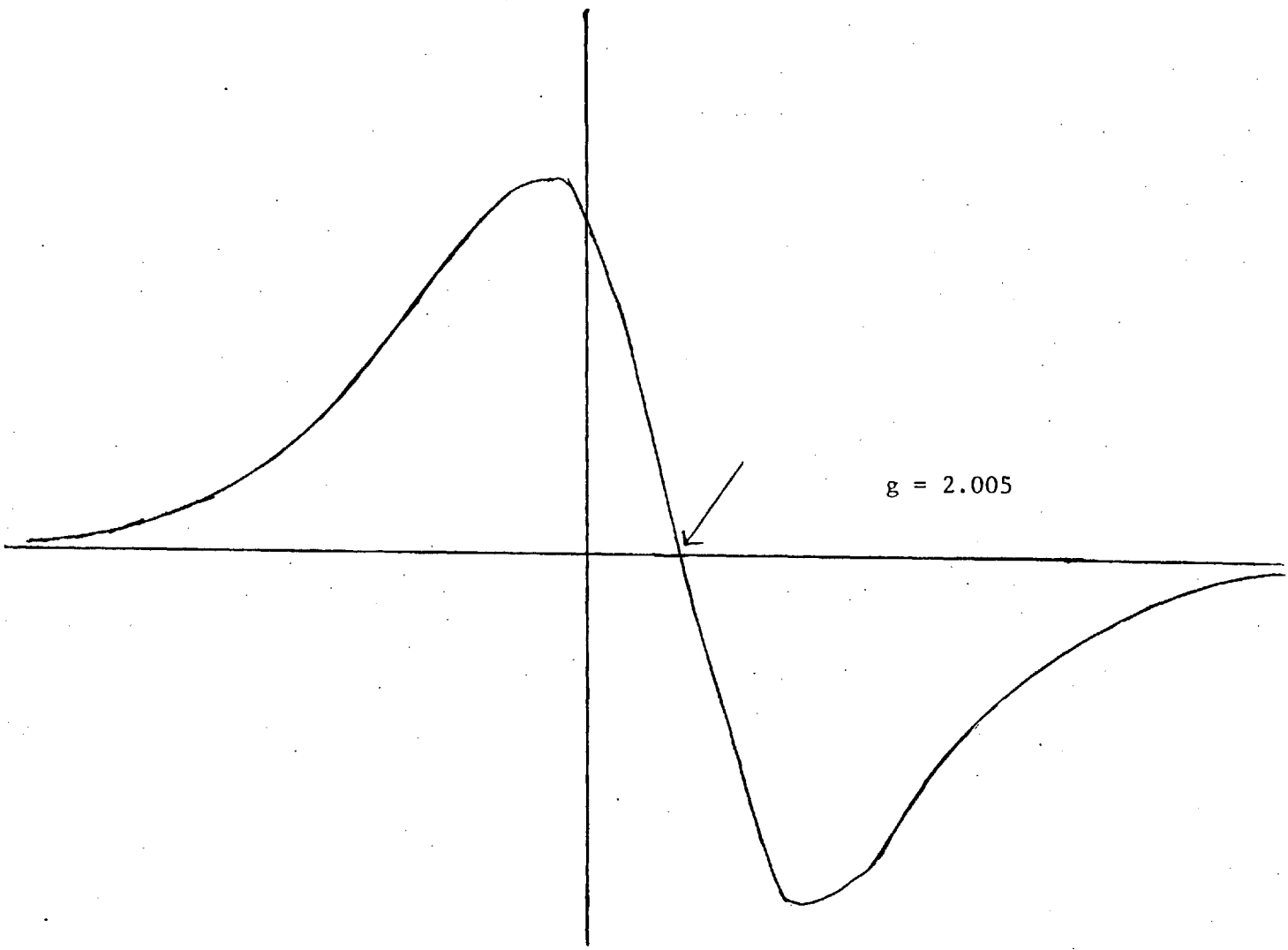


Figure 4.5 and 4.6

4.5. E.s.r. spectra from the reaction of equimolar (10^{-2} M) $AgNO_3$ and N-ethylaniline in EtOH at 77°K.

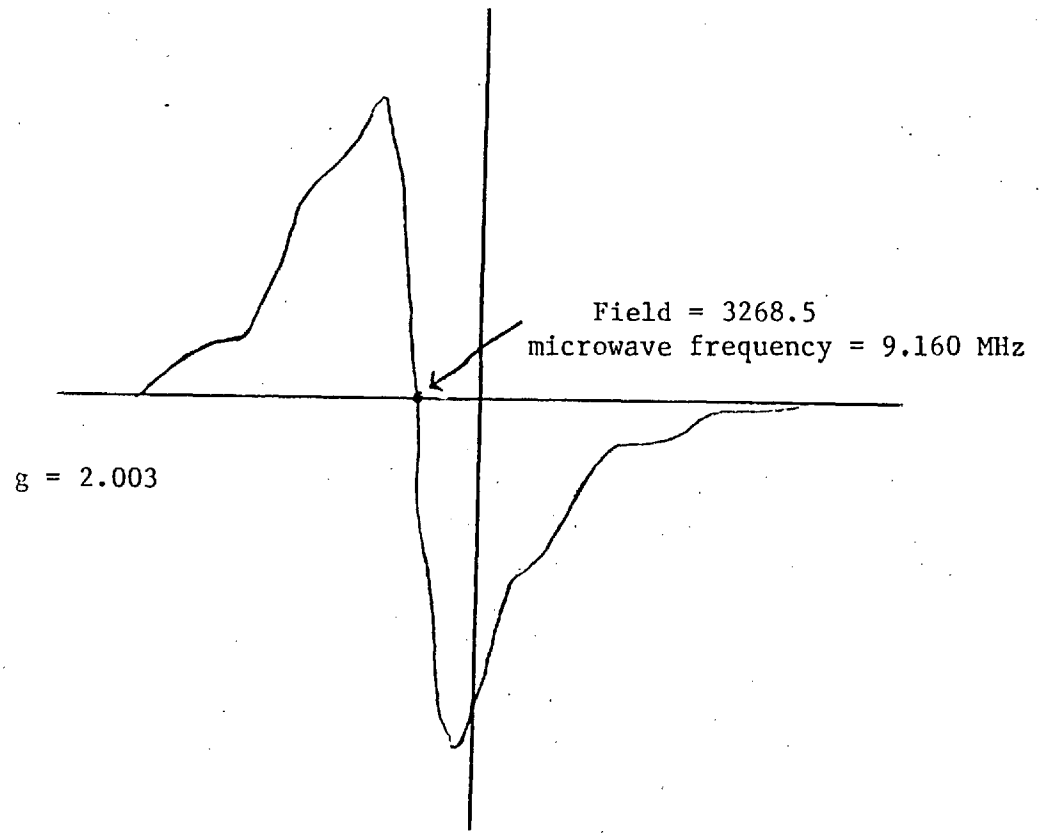


Figure 4.5

4.6. E.s.r. spectra of $Ag^{II}(piperidine)_2$ from the reaction of equimolar (10^{-2} M) $AgNO_3$ and piperidine in EtOH at 77°K

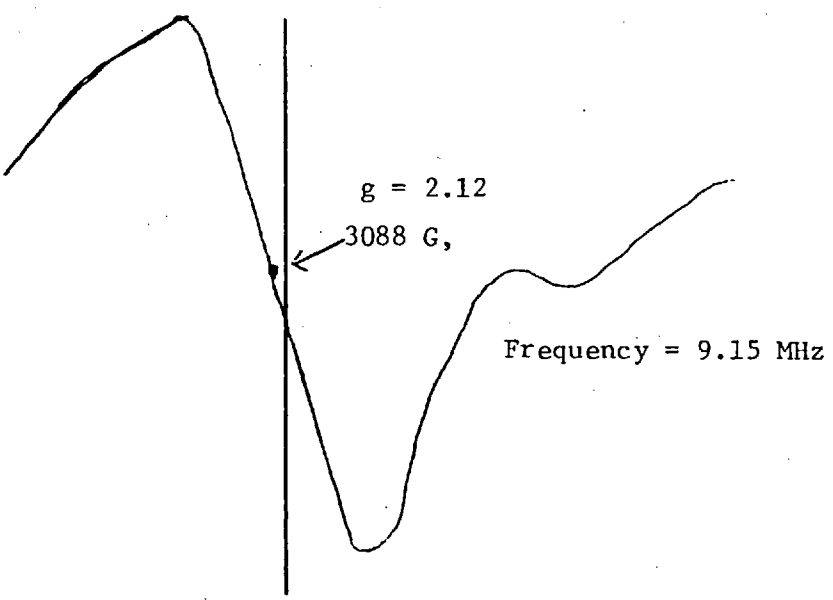
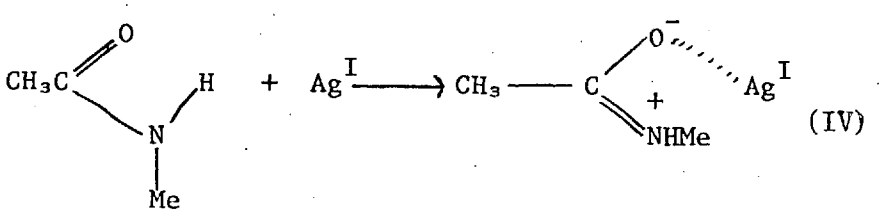


Figure 4.6

One interesting aspect of these results is that neither the yield nor time of N-nitrosamine formation is strongly dependent on the amine basicity. This suggests that the reaction mechanism does not simply involve reaction of amine with an electrophilic nitrosating agent. Also, the low yield obtained with N-methylpiperazine is interesting, and as discussed below probably relates to the fact that this amine is dibasic.

4.2.3. Amides.

Attempts were made to nitrosate amides with NO and $AgNO_3$, but no disproportionation was apparent and no N-nitrosamide could be detected. From the reaction of 10^{-2} M $AgNO_3$ and 10^{-2} M N-methylacetamide in the presence of NO, a precipitate was isolated that was confirmed by authentic material to be the Ag^{I}-N-methylacetamide complex (IV). These complexes are believed to involve O-coordination to Ag^{I} [164] (Equation 4.6) and this was confirmed by observing the



.... 4.6

decrease ν_{max} (CO stretch) from 1640 cm^{-1} in N-methylacetamide to 1605 cm^{-1} in the complex (III). Also, ν_{max} (N-H stretch) increased from 3250 cm^{-1} to 3280 cm^{-1} on coordination, with ν_{max} (C=N-C stretch), ca. 1550 cm^{-1}.

4.3.1. Discussion.

The above observations suggest that the overall stoichiometry for Ag^{I} promoted reactions is given by Equation 4.7.

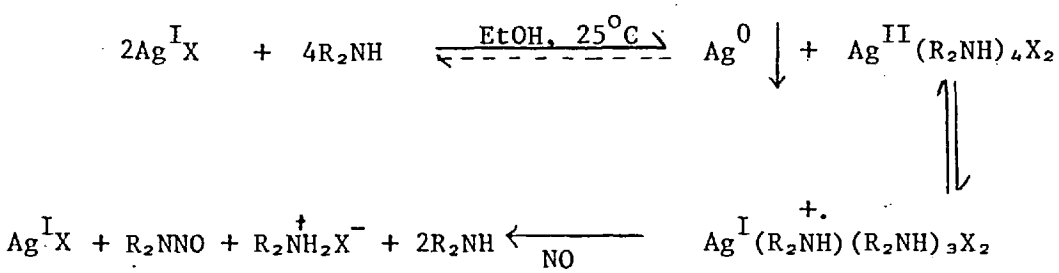
$$Ag^{I} + 2R_2NH \xrightarrow{NO} Ag^{0} + R_2NNO + R_2\overset{+}{N}H_2 \quad \ldots. 4.7$$

Thus, Ag^{I} is consumed, and its concentration limits the yield of N-nitrosamine with excess amine. With excess Ag^{I}, however, protonation of the amine substrate limits the yield. This explains why added base (NEt_3, or 2,2,6,6-tetramethylpiperidine) increases the yield of product.

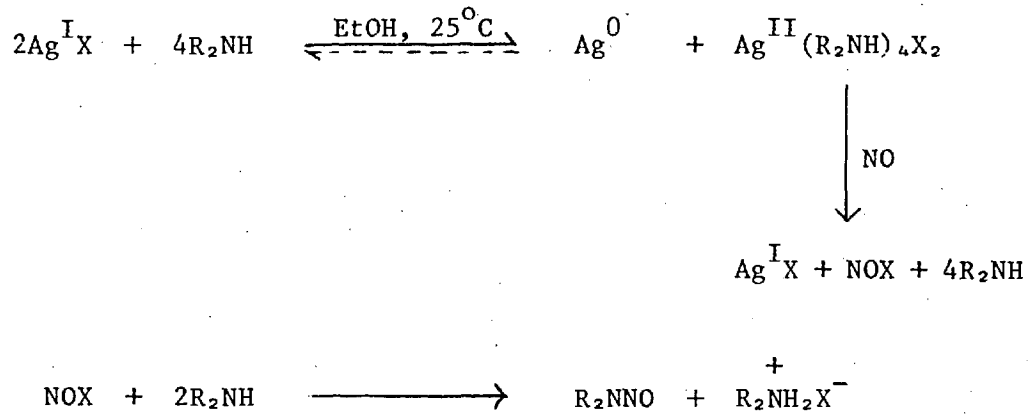
Two mechanisms involving the disproportionation of Ag^{I} and amine to $Ag^{II}(R_2NH)_4$ and Ag^{0} can be envisaged (Scheme 4.4). Pathway (I) involves the oxidation of the amine by Ag^{II} to the amino radical and Ag^{I}, followed by coupling of the amino radical with NO to give N-nitrosamine. Pathway (II) involves the oxidation of NO by $Ag^{II}(R_2NH)_4$ to give Ag^{I} and NO^{+}, which then reacts with the amine to give the product. In both, an induction period associated with the formation of the Ag^{II}-$(R_2NH)_4$ complex would be observed and H^{+} released on forming the product.

Ag-nitrosyl complexes that involve NO^{+} formation are unknown so it seems unlikely that these are involved. Ag^{II}-amine complexes, however, are known and their involvement is implied by the initial disproportionation to form Ag^{0}. With few exceptions, the structures of these Ag^{II}-amines have not been characterised. The coordination of four secondary amines with Ag^{II} for the reactive intermediate in Scheme 4.4. has not been established, but from previous work,[138,151-153,165] this seems likely. The dibasic N-methylpiperazine did not disproportionate Ag^{I} and little N-nitrosamine formed from this amine (Table 4.4). The

(I)



(II)



Where X = NO_3^-, $C\ell O_4^-$

SCHEME 4.4

reason for this difference is not fully understood, but other tertiary amines (Et_3N) and hindered secondary amine (2,2,6,6-tetramethylpiperidine) also failed to disproportionate Ag^I so it may be a steric effect. Significantly, addition of 1.62×10^{-2} M NaOEt to equimolar (10^{-2} M) $AgNO_3$ plus N-methylpiperazine resulted in reaction with 65% N-methyl-N-nitrosopiperazine over ca. 350 min. Independent observations showed that bases such as NaOEt effected the disproportionation of Ag^I in the absence of these amines. With excess (2.5×10^{-2} M) NaOEt, however, no N-methyl-N-nitrosopiperazine was obtained from the reaction of equimolar (10^{-2} M) $AgNO_3$ and N-methylpiperazine. Further, the same results were obtained for the reactions of piperidine (Table 4.3). Closer scrutiny of the Table 4.3 shows that the time for 50% N-nitrosopiperidine is approximately constant (ca. 120-140 min) for additions of up to 1.62×10^{-2} M NaOEt, but with excess, 2.5×10^{-2} M NaOEt, no N-nitrosopiperidine was formed. It seems likely that NaOEt will bind more strongly to Ag^{II} than these amines. If so, this implies that only complexed amines react to form N-nitrosamines. An alternative explanation that NaOEt traps out all the available nitrosating agent is disproved by the observation that little 1×10^{-3} M EtONO (estimated by Shinns procedure, Chapter 2.4), was formed from the reaction of equimolar (10^{-2} M) $AgNO_3$ plus piperidine and 2.52×10^{-2} M NaOEt with initial saturated (ca. 2.2×10^{-2} M) NO in EtOH at 25°C on standing overnight.

Several observations apart from the above lead us to favour Pathway I for the mechanism of these reactions. Thus, only 5×10^{-4} M ethylnitrite (5%) was detected during the course of a reaction of 10^{-2} M $AgNO_3$ with 10^{-2} M piperidine in EtOH at 25°C (v. Experimental 4.6.IIIb), which suggests that oxidation of NO to NO^+ does not occur.

The [ethylnitrite] was estimated by h.p.l.c. as discussed in Chapter 2.4.V. A low concentration, ca. 4×10^{-3} M ethylnitrite was formed in the absence of Ag^{I}, [which probably arises from the oxidation of NO to NO_2 by adventitious oxygen (v. Chapter 2.4.V)], and the excess in the presence of Ag^{I} was determined by difference. This usually amounted to less than 5% of the Ag^{I} salt.

Other evidence in the favour of Pathway I is the observation of aromatic free radicals by e.s.r.. Our inability to trap these radicals may arise because the radicals are held in a solvent cage [$Ag^{II}...R_2NH \rightleftharpoons Ag^{I}...R_2\overset{+\bullet}{N}H$]. This conclusion is supported by the absence of rearranged or dimeric products expected for dialkylamino radicals.

As noted above, the induction period can be associated with the formation of Ag^{0} and Ag^{II} $(R_2NH)_4$. The rate-limiting step of the reaction, however, is unknown. It is clear that Ag^{0} is formed prior to N-nitrosamine formation. The electron transfer is unlikely to be slow. Also, proton transfer is expected to be rapid and this is confirmed by observations that added base does not affect the rate. Danen[166] has reported that the dimethylamino radical cation has a higher acidity (pK_A, 7.05) than the parent amine (pK_A, 10.7), and Chow[167] states that the combination of NO is more energetically favourable for the amino radical ($R_2\dot{N}$) rather than the radical cation ($R_2\overset{+\bullet}{N}H$). It is, therefore, probable that proton transfer takes place prior to N-nitrosamine formation. By elimination, these considerations imply that the interaction of NO with $R_2\dot{N}$ is probably rate-limiting.

The rate of reaction shows some correlation with the basicity of the amine. For example, the time taken to reach the limiting yield shows that piperidine reacts faster than morpholine (Table 4.4). Aromatic

amines react slightly faster but this probably relates to the ease of formation and high stability of the aromatic radicals.

4.3.2. Reactions of $NO^+BF_4^-$ in EtOH and n-BuOH.

To distinguish further between the alternative mechanisms in Scheme 4.4 the reaction of $NO^+BF_4^-$ with piperidine was examined in EtOH and n-BuOH. It was considered that this would approximate to reactions via pathway II of Scheme 4.4. The reactions were carried out under nitrogen in a glove box and solid $NO^+BF_4^-$ was added to the solution of piperidine in alcohol. The total $NO^+BF_4^-$ added was assayed by Shinns procedure and, in some cases, the alkylnitrite estimated directly by h.p.l.c. analyses. The results are summarised in Table 4.5.

Table 4.5

The reaction of $NO^+BF_4^-$ with piperidine in anaerobic alcohols at 25°C.

$10^2[NOBF_4]/M$[a]	Solvent	10^2[piperidine]/M	10^5[N-nitroso-piperidine]/M[b]	10^2[RONO] M
1.12	EtOH	5.0	8.5	1.20
1.63	"	1.0	2.75	
1.70	"	3.75	8.5	1.70
4.66	"	1.0	3.0	
1.14	n-BuOH	5.0	0	1.33
1.35	"	6.25	0	1.40

[a] Determined by Shinns procedure (v. Experimental 4.6.IIIb)

[b] Estimated by h.p.l.c. (v. Experimental 4.6.IIIb)

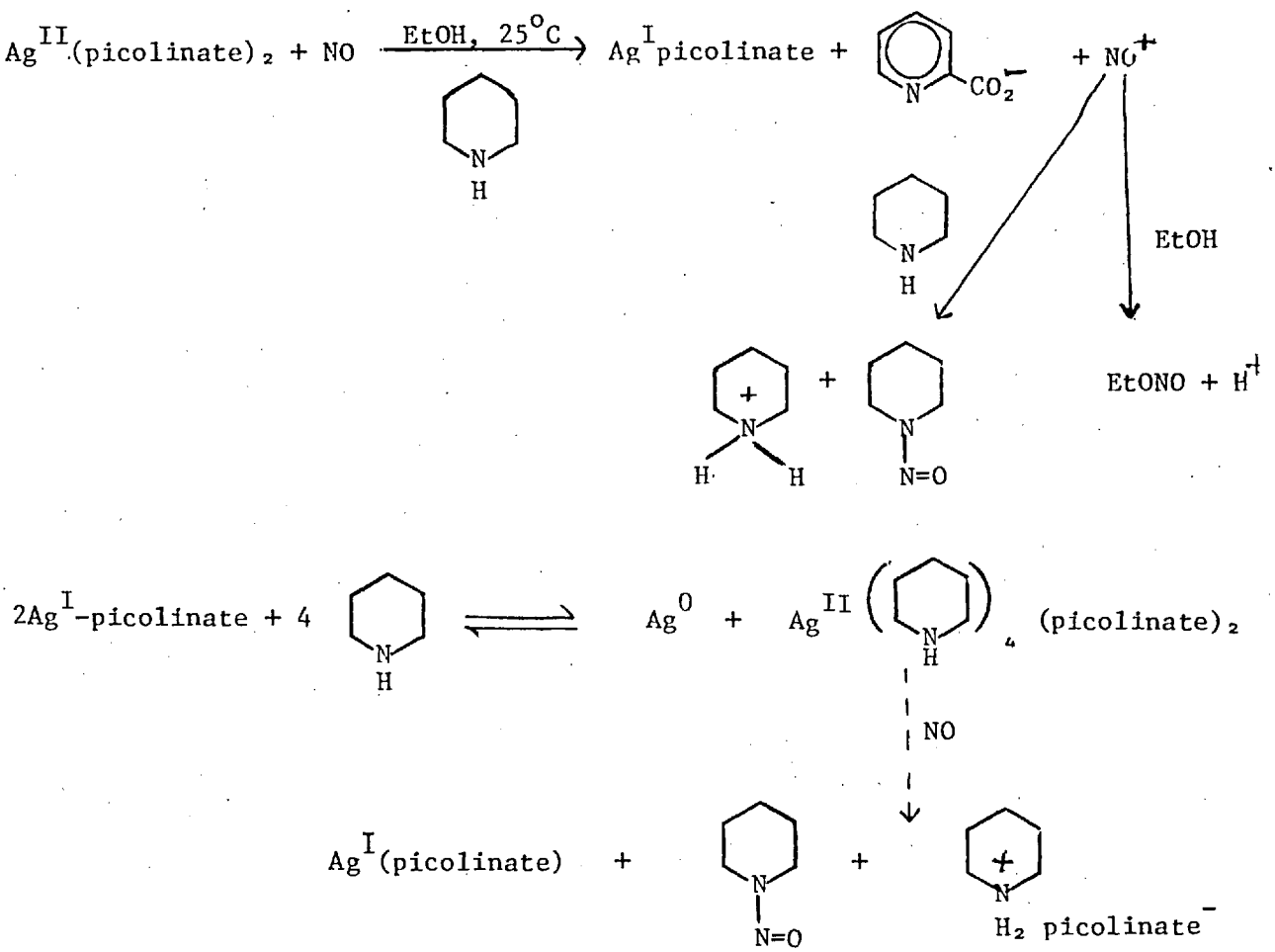
It is evident that $NO^+BF_4^-$ gives almost quantitative yields of alkylnitrite but very little N-nitrosopiperidine. This suggests that NO^+ is not the reagent in our reactions and therefore, supports Pathway I of Scheme 4.4.

4.3.3. Reactions of $Ag^{II}(picolinate)_2$.

Since Ag^{II} complexes appear to be involved in these reactions, and it is possible that Ag^{II} oxidises NO to NO^+, the ability of Ag^{II}-$(picolinate)_2$ to catalyse N-nitrosamine formation was also examined.

When NO was bubbled through an orange solution of 5.09×10^{-3} M $Ag^{II}(picolinate)_2$ in ethanol at 25°C, the solution becomes turbid. On addition of 10^{-2} M piperidine the turbidity cleared and a white precipitate settled. On examination by g.l.c., the yield of N-nitrosopiperidine was only 8×10^{-4} M (i.e., 8% reaction based on initial [piperidine]). On standing this solution overnight, however, a silver mirror formed and the yield of N-nitrosopiperidine increased to 4×10^{-3} M (i.e. 40% reaction). It was found that passage of NO into $Ag^{II}(picolinate)_2$ in ethanol gives high concentrations of ethylnitrite. For example, 1.47×10^{-2} M $Ag^{II}(picolinate)_2$ in the absence of piperidine gave ca. 1.6×10^{-2} M EtONO (as determined by Shinn's procedure), on passage of NO. The white precipitate produced in these reactions was characterised by i.r. spectroscopy as the Ag^I-picolinate salt (ν_{max} (CO) = 1630 cm^{-1} [143]). Addition of HCℓ to the solution resulted in the formation of AgCℓ, and free picolinic acid was identified in solution by i.r. (ν_{max} (CO) = 1710 cm^{-1}).[143] Neither acetaldehyde nor acetic acid from the oxidation of the solvent could be detected by g.l.c. using a 15% polypropylene glycol solvent column.

The results in Table 4.6 suggest that while $Ag^{II}(picolinate)_2$ is capable of oxidising NO to NO^+, the NO^+ is rapidly solvolysed to alkylnitrite even in the presence of piperidine (prior to NO addition), and consequently little N-nitrosopiperidine is formed (Scheme 4.5).



SCHEME 4.5

Table 4.6

Reactions of Ag^{II}(picolinate)$_2$ with piperidine and saturated(ca. 2.2×10^{-2} M)NO.

10^3[Ag^{II}(picolinate)$_2$/M	10^3[RONO]/M[a]	10^2[piperidine]/M	10^3[N-nitrosopiperidine]/M	Time/min.	Solvent
5.7		1.0	0.8	60	EtOH
			4.0[c]	1200	
5.3		1.0	1.5	15	$CHC\ell_3$
5.09	5.7	2.5	0.14	100	EtOH
			3.8	1200[c]	
6.83	8.0	8.75	0.8	20	EtOH
			7.0	1200[c]	
7.0	6.5				EtOH
14.7	16.3				EtOH
13.5	7.6[b]	1.0	1.3	235	EtOH
7.39		2.0	3.2	1200	EtOH
10.3	3.7[b]	1.33	8.09	1	Dioxan

[a] Estimated by Shinn's procedure

[b] NO_2^- estimated by Shinn's procedure at end of reaction

[c] Silver mirror formed overnight

Significantly, where the NO^+ cannot react with the solvent (e.g., in dioxan), the combination of $Ag^{II}(picolinate)_2$ and NO readily produces N-nitrosopiperidine at 25°C (Table 4.6). This product was formed within *ca.* 1 min; probably because dioxan does not compete with piperidine for NO^+. Although the full significance of these findings has not been studied, $Ag^{II}(picolinate)_2$ and NO appears to be an effective reagent for N-nitrosamine formation in non-nucleophilic solvents.

4.4 N-nitrosation and Decarboxylation of L-Proline by NO in the Presence of $AgNO_3$.

We have shown that pyrrolidine (pK_A, 11.27) disproportionates Ag^I and forms N-nitrosopyrrolidine in the presence of NO (Table 4.4). This N-nitrosamine is found in cooked bacon,[10] and is believed to form from the thermal decarboxylation of N-nitrosoproline at elevated temperatures, ca. 180°C.[10] The decarboxylation of N-nitrosoproline has been reported in alkali, and believed to be mediated by bacteria[8] and is catalysed by $Pb(OAc)_4$ (via a free radical pathway) to ultimately give α-acetoxy-N-nitrosopyrrolidine.[168] Apart from these reports, little is known about the decarboxylation of N-nitrosoproline. Ag^{II}, however, has been reported to decarboxylate both aliphatic carboxylic acids[157-159] and amino acids.[160] This suggests that proline may be converted directly to N-nitrosopyrrolidine by NO in the presence of Ag^I salts.

A solution of L-proline in EtOH did not disproportionate Ag^I at 25°C and no N-nitrosoproline was found on passage of NO (Table 4.7). When treated with 1.1 equivalent of sodium hydroxide, however, both disproportionation and N-nitrosoproline formation were evident. Examination of the reaction solution by h.p.l.c. revealed two products, corresponding to N-nitrosoproline and N-nitrosopyrrolidine With 10^{-2} M $AgNO_3$ and 2 x 10^{-3} M L-proline in EtOH at 25°C, the yields were ca. 80% N-nitrosoproline and 20% N-nitrosopyrrolidine over 1200 min. The fact that both N-nitrosopyrrolidine and N-nitrosoproline are found, suggests, but does not prove, that N-nitrosation precedes decarboxylation. In the presence of NaOEt with excess Ag^I, the yield

Table 4.7

The formation and decarboxylation of N-nitrosoproline from L-proline, $AgNO_3$ in the presence of NO under anaerobic conditions in EtOH at 25°C. Initial saturated [NO] = 2.2×10^{-2} M.

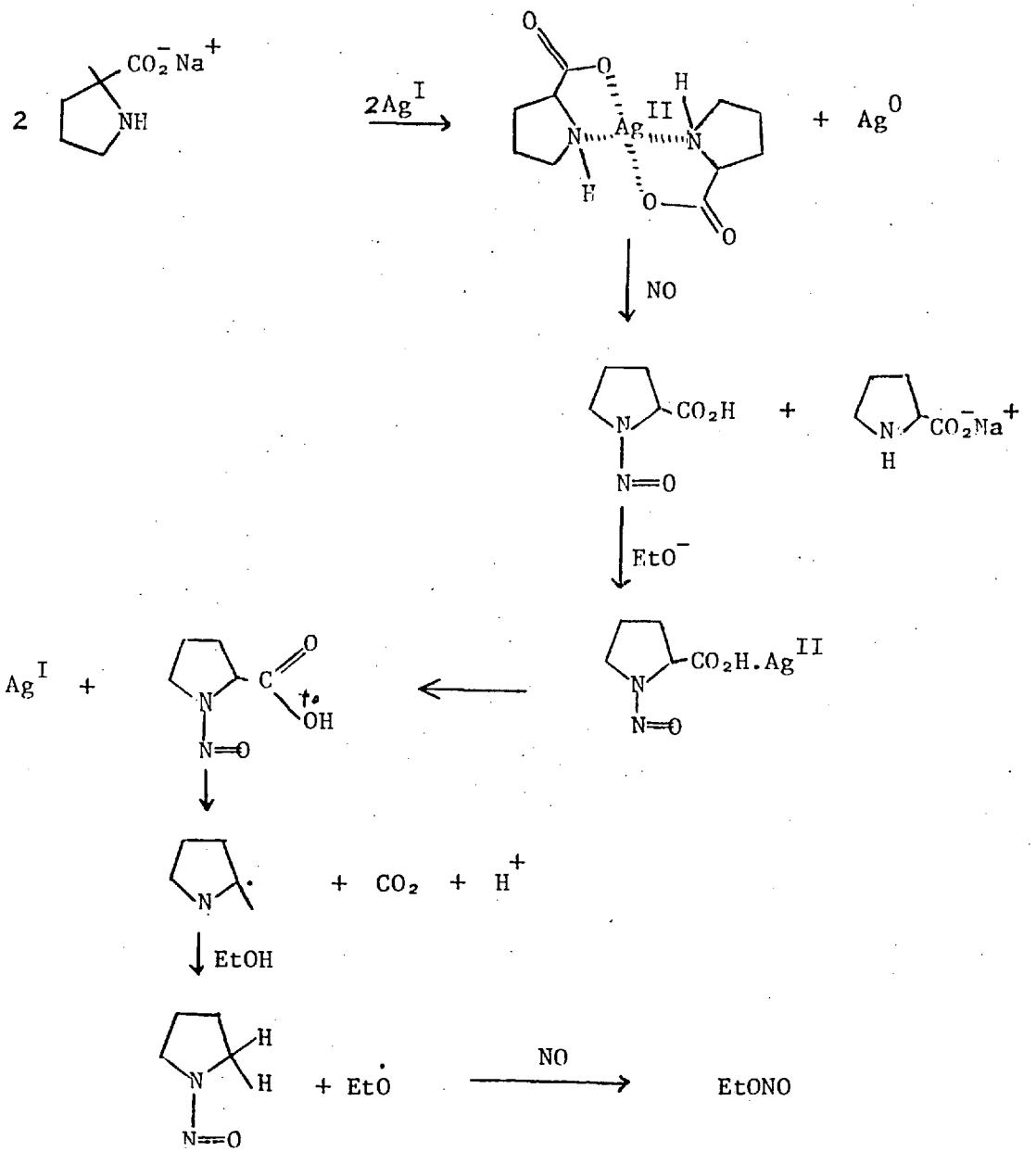
10^2[$AgNO_3$]/M	10^2(L-Proline]/M	10^2[Added Base]/M	% N-nitrosoproline[a]	% N-nitrosopyrrolidine	Time/ min.
1.0	0.2	0	0	0	1200
1.0	0.2	0.22 (NaOH)	88 (81)[b]	12 (19)	1200
1.29	1.0	1.25 (NaOH)	78	22	1200
2.59	1.15	1.25 (NaOH)	59	41	1200
2.59[c]	1.15	1.25 (NaOH)	59	41	1200
2.0[c]	1.0	1.0 (NaOH)	62	38	1200

[a] Yield based on initial [L-Proline]

[b] Duplicate reactions in parenthesis

[c] At 40°C

N-nitrosoproline to N-nitrosopyrrolidine was ca. 1:1 (Table 4.7). Heating to 40°C for 48 hours did not increase the amount of N-nitrosopyrrolidine. These reactions require further study, but the observation that N-nitrosoproline may undergo decarboxylation in the presence of metal salts at 25°C is significant to N-nitrosopyrrolidine formation in food. A tentative mechanism for the formation and decarboxylation of N-nitrosoproline is shown in Scheme 4.6.



SCHEME 4.6

Independent measurements established that 10^{-2} M N-nitrosoproline does not catalyse the reaction and hence decarboxylation is unlikely to proceed via a radical chain process. The intermediate N-nitroso-pyrrolidine α radical presumably abstracts $\dot{H}$ from the solvent which then reacts with NO to form EtONO, to act as a chain terminator. It is also possible that the intermediate radical (V) reacts directly with NO to form N-nitroso-2-nitrosopyrrolidine.

4.5 Summary.

The above results show that Ag^{I} salts in combination with NO are effective promoters for N-nitrosamine formation under neutral anaerobic conditions. The reactions are much faster than N-nitrosamine formation from both dissolved NO ($t_{\frac{1}{2}}$, ca. 8 days[83]) and for the more basic amines, from aqueous HNO_2 (with $[HNO_2] = 2 \times 10^{-2}$ M, $t_{\frac{1}{2}}$ (piperidine) = ca. 34 days at pH 3-3.4[7]).

Interestingly, the silver promoted reaction also applies to proline which can be nitrosated and decarboxylated to N-nitrosopyrrolidine under very mild conditions at 25°C.

The most likely mechanism of the Ag^{I} promoted reaction (Scheme 4.4) involves initial ligand induced disproportionation of Ag^{I} into Ag° and $Ag^{II}(amine)_4$, followed by the oxidation of the amine substrate, rather than NO, by the Ag^{II}-amine complex. The mechanism is supported by results obtained from the reactions of $AgCl O_4$ and $AgNO_3$, where no dependence was found on counter anion, and also from the reactions of $Ag^{II}(picolinate)_2$, $NO^{+}BF_4^{-}$ in alcohol. The study revealed that oxidation of NO to NO^{+} was possible with Ag^{II}, but in the presence of alcohols, high concentrations of alkylnitrite and very little N-nitrosamine were found. The inhibitory effect is related to the rapid solvolysis of released NO^{+} which under our conditions does not nitrosate further. Very little alkylnitrite was detected by both h.p.l.c. and by Shinns procedure during the course of reaction of equimolar (10^{-2} M) $AgNO_3$ and piperidine with saturated (ca. 2.2×10^{-2} M) NO (v. Experimental 4.6.IIIb). The proposed mechanism is also supported by the identification of free radicals by e.s.r. for aromatic amino substrates.

4.6. EXPERIMENTAL

4.6.I. Reagents, Substrates and Products.

The purification of amines, NO, the preparation and purification of authentic n-butylnitrite, N-nitrosamines has been described in Chapter 2.4. II . Reagent grade 1,4-dioxan was passed down a grade 1 alumina column to remove traces of peroxide and checked by adding acidic iodide to a small sample. In the absence of peroxides (no purple colouration due to I_2) the solvent was refluxed over Na metal overnight and distilled over Na metal.

Purified 1,4-dioxan was stored under nitrogen at $0^{o}C$. AnalaR EtOH, $AgNO_3$, $AgC\ell O_4$, t-nitrosobutane, 2,2-diphenyl-1-picrylhydrazine, chromatographically pure L-Proline were used without further purification. Reagent grade NEt_3 (b.p. $89^{o}C$/760 mm), 2,2,6,6-tetramethylpiperidine (b.p. $152^{o}C$/760 mm), pyrrolidine (b.p. $88^{o}C$/760 mm), N-methylaniline (b.p., $196^{o}C$/760 mm), N-ethylaniline (b.p., $205^{o}C$/760 mm), N-methyl-acetamide (b.p. $205^{o}C$/760 mm) were dried over KOH and fractionally distilled. Reagent grade diphenylamine was recrystallised from ether. Sodium ethoxide was prepared daily from sodium metal in EtOH; its concentration was estimated by titrating standard volumes of NaOEt in water with volumetric standard $HC\ell$. $NO^{+}BF_4^{-}$ (BDH 99%) was stored under vacuum and handled in a glove box under nitrogen. The purity (> 85%) was checked by dissolving pre-dried $NO^{+}BF_4^{-}$ in water and analysis by Shinns procedure (Chapter 2.4).

Preparation of Ag^{II}(picolinate)$_2$.[169]

To a solution of $AgNO_3$ (1.7 g, 50 ml.H_2O) was added picolinic acid (3.7 g, 50 ml H_2O). The white precipitate was dissolved with anhydrous sodium carbonate, followed by the addition of $K_2S_2O_8$ (2 g, 60 ml.H_2O). The orange precipitate was filtered, washed with a 10 ml.H_2O then dried under *vacuo* over $CaC\ell_2$. The purity of Ag^{II}-(picolinate)$_2$ (*ca.* 95%) was estimated by the volumetric titration of I_2 with thiosulphate from the reaction of acidic potassium iodide and Ag^{II}(picolinate)$_2$.

Preparation of Ag^{I}-N-Methylacetamide.[164]

Silver nitrate dissolved in excess N-methylacetamide was heated at 35°C for 60 min. Anhydrous diethylether (30 ml) was added to the dark brown solution and an oil separated, which, on further washing, gave pink crystals. The crystals were collected by vacuum filtration and dried in *vacuo* over $CaC\ell_2$ at 25°C.

[Ag(N-methylacetamide)] m.p. 55°C, i.r., (Nujol mull, cm^{-1}), 3280 (N-H), 1605 (C=O), 1550 (C=N-C).

Preparation of N-nitrosoproline.

N-nitrosoproline was prepared by a modified literature procedure.[170] L-proline (1 g, 8.7 mM) was dissolved in c. $HC\ell$ (3 ml) and water (20 mL), then chilled in an ice bath for *ca.* 30 min. Sodium nitrite (0.7 g, 9 mM) was slowly added to the solution ensuring that the temperature did not rise above 5°C, and left to stand for 120 min. The product N-nitroso-proline was extracted with $CHC\ell_3$, dried over $MgSO_4$ and concentrated by a stream of nitrogen. The pale yellow precipitate was collected and

recrystallised from $CH_2C\ell_2$. The white crystals were collected, and dried under vacuum at 25°C.

Yield, 0.8 g (74%), m.p., : 98-100°(d) (lit[170] m.p., 95-103°C); i.r., $\nu_{max}^{CHC\ell_3}$, 1730 (CO), 3400 ($-CO_2H$), 1430 cm^{-1} (NO).

Microanalysis: N-nitrosoproline $C_5H_8N_2O_3$ requires C 41.55%, H 5.55%, N 19.45%. Obtained C 41.73%, H 5.63%, N 19.38%.

N-nitrosopyrrolidine was prepared by D.E.G. Shuker following a literature procedure.[171] N-nitrosopyrrolidine b.p., 69°C, 1.5 mm (lit[171] b.p. 99°C / 15 mm).

4.6. II. Reaction Methods.

The reaction apparatus and procedure has been described in Chapter 2.4. III. Here, after initiation of the reaction by neat amine, bases such as NEt_3, 2,2,6,6-tetramethylpiperidine, NaOEt, NaOH were added immediately to the reaction solution via a microlitre syringe, but there was no apparent difference if this part of procedure was reversed.

4.6.III . Analysis of Products.

4.6.III.a(I). N-nitrosamines and Piperidine.

The analysis of N-nitrosamines were carried out mainly on a Perkin-Elmer F11 instrument, fitted with Anatarox column under standard conditions described in Chapter 2.4. III. The R_F N-nitrosopyrrolidine was 3.4 min.

Piperidine was assayed on the same F11 instrument fitted with a 2 metre, $\frac{1}{8}$" o.d. stainless steel column packed with 15% polypropylene glycol (LB 550-X) on chromosorb W (80-100 mesh) (solvent column) at

column temperature 120°C, injection temperature ca. 200°C, nitrogen, hydrogen, air at 30 p.s.i. The retention time under these conditions was 1.5 min. It was not possible using this procedure to distinguish between complexedpiperidine (i.e., Ag^{II}-(piperidine)$_4$) and free amine in solution, and hence only total loss of piperidine could be followed.

4.6.III.a(II) Analysis of N-nitrosoproline and N-nitrosopyrrolidine from the reaction of $AgNO_3$, proline and initial saturated (ca. 2.2×10^{-2} M) NO in EtOH at 25°C.

The reaction products were assayed by using h.p.l.c., using the same instrument described in Chapter 2, fitted with 69 cm x 2.1 mm. i.d., stainless steel column packed with Bondapak C_{18}/Porasil B and eluting solvent, degassed 10% - aqueous-MeOH, and u.v. detection at 230 nm. At a flow rate of 1.6 ml/min., R_F N-nitrosoproline was 1.2 min, and N-nitrosopyrrolidine 1.7 min. The column was calibrated and the peak heights were linearly related to concentration, however,calibration was required daily due to fluctuationin pump efficiency.

4.6.III(b) Analysis of Alkylnitrite.

The concentration of alkylnitrite was estimated mainly by Shinn's procedure[130] (described in Chapter 2.4.V) and in some cases confirmed by h.p.l.c. For the reactions of $NO^+BF_4^-$ in alcohol typical results were:-

NO^+BF_4 added to 3.75×10^{-2} M piperidine in 40 ml. EtOH gave only 8.5×10^{-5} M N-nitrosopiperidine. 0.1 ml. of the reaction solution gave an absorbance of 0.92 at λ_{max} 551 (ε = 54,000). Hence $[n\text{-BuONO}]_{Shinns}$ = 1.70×10^{-2} M.

Direct analysis of this reaction solution by h.p.l.c., under the same conditions described above, gave peak heights of 43, 42, 43 on 0.1 scale. Standard 1.3×10^{-2} M n-BuONO was prepared by diluting 50 µl-BuONO in 40 ml n-BuOH. 0.1 ml. of this solution in 100 ml, after following Shinn's procedure gave absorbance 0.70. Under the same h.p.l.c. conditions, this standard gave peak heights of 30, 32, 33, 32. Therefore [EtONO] from NO^+BF_4 is ca. 1.70×10^{-2} M, which is in good agreement with the value by the Shinn's method.

4.6.III(c) I Estimation of [EtONO] during the reaction of 10^{-2} M $AgNO_3$, piperidine with initial saturated (ca. 2.2×10^{-2} M NO in EtOH at 25°C.

Analysis of EtONO was carried out by h.p.l.c. method, using the same column and conditions as described above for the analysis of N-nitrosoproline. The [EtONO] was estimated using the normal procedure adopted for analysis of N-nitrosamines by g.l.c., the direct injection of 1-4 µl samples onto the column at time t. min. The problems associated with the formation of EtONO in the absence of catalyst was again apparent (discussed in Chapter 2.4.V.), and also problems with the pump performance, and hence data was corrected for this.

Sample = 4 µl, eluting solvent, 10% aq-MeOH. U.v. detection 230 nm, flow rate 1.6 ml/min, 0.1 scale, 25°C.

Time/min.	Peak Height			
	(a)	(b)	(c)	10^4[EtONO]/M[d]
0	19	50 (50)		
67	-	62	26	3.9
79	-	63	26	3.8
109	-	58	26	5.5
118	-	58	26.5	8.2
185	-	55	18	4.0
196	-	55	24	6.95
205	-	55	17	3.8
1200	-	55	20	4.5

[a] Peak height of alkylnitrite before addition of 10^{-2} M piperidine

[b] Peak height of 1.24×10^{-2} M n-BuONO standard (estimated by Shinn's procedure).

[c] Peak height of EtONO during course of reaction.

[d] [EtONO] from the reaction, calculated from:-

$$\left(\frac{\text{Peak height c}}{\text{Peak height b}} - \frac{\text{Peak height a}}{\text{Peak height b}}\right) \times 1.24 \times 10^{-2}\ \text{M}$$

The corresponding yield of N-nitrosopiperidine by g.l.c. showed that 50% N-nitrosamine had formed after ca. 300 min.

Employing the same technique, other analyses showed the presence of ca. 3×10^{-4} M EtONO after 26 hours. For reactions with Ag^{II}(picolinate)$_2$ and NO, the alkylnitrite was estimated by Shinn's procedure.[130]

4.6.III.c(II) Analysis of the Product from the Reaction of Aromatic Amine and $AgNO_3$.

The extent of reaction was estimated following the loss of amine absorption at the corresponding λ_{max} ArNHMe (λ_{max}^{EtOH} 245 nm, log ε = 40), ArNHEt,(λ_{max}^{EtOH} 247, 294 nm, log ε = 4.12, 3.35), Ar_2NH (λ_{max}^{EtOH} 285 nm log ε = 4.30). After the reaction was initiated by the addition of amine to $AgNO_3$ with saturated (ca. 2.2×10^{-2} M) NO in EtOH at 25°C, 30-50 µl aliquots of the reaction solution were withdrawn and quenched in degassed-EtOH (3.0 ml), (contained in a 1.0 cm pathlength cuvette, fitted with a Subaseal stopper) and the u.v. spectrum recorded, with EtOH as reference.

The yield of N-nitrosamine using this quenching procedure is prone to errors, because NO reacts rapidly with O_2 to form $N_2O_3(N_2O_4)$, and may account for greater amounts of product formation with respect to the expected yield.

4.6.III.d(I). E.S.R. Measurements.

E.s.r. spectra were recorded on a Varian E9 X-band spectrophotometer by Dr. J. Gibson (Department of Inorganic Chemistry, Imperial College). Samples for e.s.r. measurements were prepared by transferring the reaction solution of $AgNO_3$ plus amine (in the absence of NO, under anaerobic conditions) to a Quartz e.s.r. cell (under vacuo) via a stainless steel transfer needle, previously purged with argon. The

spectrum recorded at 77° K. The reaction solution was transferred to a flat e.s.r. cell[161] using the same procedure for spectra at room temperature.

For spin trap experiments, 0.1 g t-nitrosobutane was placed in the e.s.r. cell and degassed, and the reaction solution transferred as described above.

The g-value was estimated from the standard expression[172]

$$g = \frac{h}{\beta} \cdot \frac{\nu}{H} = 0.71448 \times \frac{\nu_e}{H} \quad \frac{(MHz)}{(G)}$$

where h = Planck's constant, β = Bohr magneton, ν_e = electron resonant frequency - absorbed electromagnetic radiation (MHz), H = the magnetic field (G)

There was no evidence of free radical formation from parent amine in the absence of $AgNO_3$.

The g-value of diphenylamino radical ($Ar_2\dot{N}$) is reported to be ca. 2.003 and the diphenylamine nitroxide ($Ar_2N\text{-}\dot{O}$) ca. 2.006.[173] Our value of g = 2.002 is in good agreement with the $\dot{N}$ radical from the reaction of $AgNO_3$ and diphenylamine. The experimental g-values for aryl nitroxides are ca. 2.006-2.007,[161] smaller g-values (ca. 2.003) are normally consistent with $\dot{N}$-centered radicals.[161]

4.7.I. Typical Reaction.

$[AgNO_3]$ = 2.81 x 10^{-3} M, [piperidine] = 10^{-2} M in EtOH at 25°C.

Initial saturated [NO] = ca. 2.2 x 10^{-2} M.

Time/min.	Attenuation	Peak Height	10^3[N-nitrosopiperidine]/M
2	x 200	5	0.4
12		8	0.66
20		11	0.91
34		13	1.07
45		15	1.24
68[a]		18	1.49
78		31	2.56
85		29	2.4
90		36	3.0
98		42	3.5
103		33	2.73
109		36	3.0
124		44	3.64
139		46	3.8
162		43	3.5
∞ 1200		36, 36	3.0

[a] Silver mirror formed on surface of reaction vessel

Injection sample = 3 µl.

3 µl 4.8 x 10^{-3} M N-nitrosopiperidine gave, at attenuation x 200 57, 60, 58, on F11 fitted with Antarox column under normal operating conditions (Chapter 2.4).

4.7. II. Typical Reactions.

$[AgNO_3] = 10^{-2}$ M, [piperidine] = 10^{-2} M in EtOH at 25°C. Initial saturated [NO] = ca. 2.2×10^{-2} M.

Time/min.	Attenuation	Peak Height	10^3[N-nitrosopiperidine]/M
3	x 500	5	0.36
10		14	0.1
20		23	1.6
30		22	1.6
50		14	1.0
60[a]		20	1.42
70		25	1.78
90		31	2.2
100		33	2.35
130		37	2.64
165		60	4.28
200		65	4.64
250		69	4.93
316	x 1000	35	5.0
322		35	5.0
327	x 500	68	

[a] Silver mirror formed on reaction vessel

Injection sample = 3 µl

3 µl 5.0×10^{-3} M N-nitrosopiperidine gave at attenuation 500 of 70, 70, 69, 72 ; attenuation x 100 35, 35 on F11 fitted with Antarox column under normal conditions (Chapter 2.4).

4.7. III. Typical Reactions.

$[AgNO_3] = 1.04 \times 10^{-2}$ M, [piperidine] = 10^{-2} M in presence of 2.5×10^{-3} M NEt_3 in EtOH at 25°C. Initial saturated [NO] = ca. 2.2×10^{-2} M.

Time/min.	Attenuation	Peak Height	10^3[N-nitrosopiperidine]/M
19	x 200	7	0.28
24		18	0.72
30		26	1.04
36		21	0.84
46		34	1.36
51		32	1.28
55		31	1.24
60[a]		48	1.92
65		51	2.04
78	x 500	22	2.2
87		25	2.5
94		28	2.8
130		34	3.4
142		39	3.9
160		45	4.5
184		67	6.7
200		72	7.2
244		73	7.3
280		76	7.6
340		86	8.6
360	x 1000	44	8.8
Overnight	x 1000	49, 48	10.0

[a] Silver mirror formed on surface of reaction vessel.

Injection sample 3 µl

3 µl 4.8×10^{-3} M N-nitrosopiperidine gave at attenuation x 500 48, 49, 48 on F11 fitted with Antarox column under normal operating conditions (Chapter 2.4).

CHAPTER 5

FORMATION OF N-NITROSAMINES IN SOLUTION FROM GASEOUS NITRIC OXIDE IN THE PRESENCE OF METAL SALTS.

5.1 Introduction.

5.1 (1) Catalysis by Copper Salts.

Early work by Drago and his colleagues[81] suggested that in the absence of metal salts, N-nitrosodiethylamine formed slowly in MeOH from NO and diethylamine with the subsequent formation of the salt $[Et_2NH_2]^+[Et_2N\text{-}N_2O_2]^-$. Brackman and Smit[82] showed that this reaction was catalysed by $CuC\ell_2$ and suggested a complex mechanism (Chapter 1.2) to explain their kinetic observations. Subsequently, Challis, Edwards and Humna[174] showed that similar reactions apply to heterocyclic amines (e.g., piperidine, morpholine) and that both Cu^I and Cu^{II} salts have a catalytic effect. Also, the reaction of primary amines coordinated to $Cu^{II}C\ell_2$ with the copper nitrosyl $[Cu^{II}(NO)]C\ell_2$ has been examined by Doyle and his colleagues[109] (discussed in Chapter 1.2). Otherwise, relatively little is known about the reactivity of copper bound amine ligands apart from the $Cu^{II}C\ell_2$ mediated oxidation of aromatic amines via radical intermediates (Equation 5.1)

$$2PhNH_2 + 4Cu^{II}C\ell_2 \longrightarrow [Ph\overset{+\cdot}{N}H_2] \longrightarrow Ph\text{-}N{=}N\text{-}Ph + 4Cu^{I}C\ell + 4HC\ell \quad \ldots\ldots 5.1$$

and the Cu^I catalysed oxidation of diazonium ions (Equation 5.2).[175]

$$PhN_2^+ + Cu^{I}C\ell_2^- \longrightarrow Ph^{\cdot} + Cu^{II}C\ell_2 + N_2 \quad \ldots\ldots 5.2$$

The interaction of NO with transition metal salts is well known and has been reviewed in Chapter 1.2. With cupric halides, NO is believed to form an unstable complex in both EtOH and MeCN (Chapter 1.2).

5.1 (2) Catalysis by Iron Salts.

Fe^{III} salts, such as $Fe^{III}C\ell_3$, are known to combine with NO to form nitrosyl complexes, where the Fe:NO ratio is less than unity.[176] These complexes (other than nitroprusside, e.g., $[Fe(CN)_5NO]^{2-}$), are generally too unstable to be isolated, but ν_{max} for the coordinated NO is within the range 1775-1945 cm^{-1}. They are therefore regarded as $[Fe^{II}-(NO^+)]$ derivatives. The nitrosyl complexes from Fe^{III}-halides are decomposed by water.[176] Aqueous solutions of Fe^{II} salts, such as $FeSO_4$ also react with NO to give nitrosyls where ν_{max}(NO) lies within the range 1730-1850 cm^{-1}. They are therefore regarded as $[Fe^{I}(NO^+)]$ derivatives.[176]

There is good evidence that NO coordinated to iron is capable of nucleophilic attack by amines and alcohols. Maltz,[108] for example, showed that the nitroprusside ion (ν_{max}(NO) 1945 cm^{-1}) gave N-nitroso-diethylamine on reaction with diethylamine in alkaline media up to pH 12.7. Also, it has been claimed that Fe^{II} and Fe^{III}-porphyrins react with NO to give nitrosyls, which under suitable conditions will nitrosate both diphenylamine[177] and methanol.[178] Bonner[177] prepared the octa-ethyl-iron(II)-porphyrin–nitrosyl complex (A), (ν_{max}(NO) 1670 cm^{-1}) from both NO and acidified nitrite. Magnetic susceptibility measurements

indicated the presence of one unpaired electron and so the structure was formally regarded as $[Fe^{I}\text{-}NO^{+}]$, although $\underline{\nu}_{max}$(NO) is low for this entity. Complex A reacted with diphenylamine to give N-nitrosodiphenylamine in T.H.F. (tetrahydrofuran) on heating under reflux at 60°C over 60 min. Also, Wayland and Olson[178] showed that $[Fe^{III}(TPP)C\ell]^{2+}$ (where TPP = tetraphenylporphyrin) reacted with NO in toluene at 25°C to give $[Fe^{II}(TPP)C\ell(NO)]$ ($\underline{\nu}_{max}$(NO) 1880 cm^{-1}). Addition of methanol to the nitrosyl complex gave $[Fe^{II}(TPP)]$, MeONO and $HC\ell$ (Equation 5.3). In the presence of excess NO, the $[Fe^{II}(TPP)]$ complex

$$[Fe^{III}(TPP)C\ell] + NO \underset{\text{toluene, } 25^{\circ}C}{\rightleftharpoons} [Fe^{II}(TPP)C\ell(NO)] \xrightarrow{MeOH}$$

$$[Fe^{II}(TPP)] + MeONO + HC\ell \qquad \ldots\ldots \quad 5.3$$

reacted further with 2 moles of NO to give the dinitrosyl complex (I) (Equation 5.4), believed to contain both bent- ($\underline{\nu}_{max}$(NO) 1690 cm^{-1}) and

$$[Fe^{II}(TPP)] + NO \underset{\text{toluene, } 25^{\circ}C}{\rightleftharpoons} [Fe^{I}(TPP)(\overset{+}{NO})] \xrightarrow{NO} \underset{(I)}{[Fe^{II}(TPP)(\overset{+}{NO})(NO^{-})]}$$

$$\ldots\ldots \quad 5.4$$

linear- ($\underline{\nu}_{max}$(NO) 1870 cm^{-1}) coordinated NO. The reaction of (I) with nucleophiles were not investigated.

5.2. Results and Discussion.

5.2.1. Catalysis by Copper Salts.

As mentioned above, N-nitrosamine formation from NO is very slow in the absence of metal salts. This was demonstrated for the reaction of 10^{-2} M piperidine, morpholine and N-methylpiperazine with saturated solutions of NO in EtOH, dioxan and MeCN at 25°C where, overnight, only 2.5-5% reaction was observed.

Preliminary experiments established that relatively small amounts of Cu^{I} and Cu^{II} salts catalysed N-nitrosopiperidine formation in dioxan at 25°C. The reactions were truly catalytic with 8×10^{-4} M $CuC\ell_2$ converting ca. 95% of 10^{-2} M piperidine to N-nitrosopiperidine in 55 min. Also, evidence below suggests that under certain circumstances, diffusion of NO into solution may become rate-limiting. Further, the reactions were much faster in dioxan than in EtOH and faster than N-nitroso-piperidine formation from acidified nitrite.[7] For example, 10^{-2} M piperidine with 4.75×10^{-3} M $CuC\ell_2$ gave 100% reaction over 9 min. in dioxan, whereas with 10^{-2} M $CuC\ell_2$ in EtOH produced 32% reaction over 100 min. The faster rate in dioxan could arise from a higher concentration of the copper-nitrosyl complex (II) due to the fact that in EtOH, the complex reacts with the solvent as well as with amine (Equation 5.5). These preliminary measurements suggested that catalysis

$$Cu^{II}X_2 + NO \rightleftharpoons \underset{(II)}{[Cu^{I}(\overset{+}{NO})]X_2} \overset{ROH}{\rightleftharpoons} Cu^{I}X + RONO + HX \quad \ldots\ 5.5$$

by copper salts could be profitably examined in solvent dioxan.

When cupric salts are suspended in dioxan and NO bubbled through, a violet solution is obtained. On addition of a secondary amine, the

violet colour is lost immediately to give a pale green or colourless solution. As the N-nitrosation proceeds, the violet colour returns and by visual assessment, approaches its original intensity at the end of reaction.

To gain insight into the reaction mechanism, the effect of reagent concentrations on the reaction rates were examined for catalysis by $CuC\ell_2$ and $CuC\ell$. For reactions carried out with a saturated solution of NO in dioxan maintained under an atmosphere of NO (v. Experimental 5.4.V), plots of % reaction *versus* time were curved, but became linear and approximately parallel, irrespective of the reagent concentrations as reaction proceeded. The plots obtained are exemplified by Figure 5.1. Similar kinetic behaviour was observed for catalysis by HI in aqueous-EtOH (see Chapter 3), and was related to rate-limiting diffusion of NO into solution.

Since independent measurements showed that the concentration of dissolved NO was low (*ca.* 1.6×10^{-2} M), the same explanation may apply to catalysis by copper salts. This was confirmed by observing that when NO was bubbled through the solution as reaction proceeds, no tailing off in the amount of N-nitrosamine was apparent (Figure 5.1). The initial rate, however, is very similar to that obtained with the initially saturated NO solutions.

For reactions where NO is bubbled continuously, the % reaction *versus* time are in most circumstances linear (Figure 5.2). Thus, the reaction follows a pseudo zeroth order kinetic expression (Rate = k_o). Evidence summarised in Table 5.1 shows that for reaction of 0.01 M piperidine, $t_{\frac{1}{2}}$ is virtually the same for both $CuC\ell$ and $CuC\ell_2$.

Figure 5.1

The dependence on NO on the nitrosation of 0.01 M piperidine in the presence of $CuCl_2$ in anaerobic dioxan at 25°C.

(○) $[CuCl_2] = 4.75 \times 10^{-3}$ M, initial saturated [NO] ca. 1.6×10^{-2} M

(●) $[CuCl_2] = 5 \times 10^{-3}$ M, [NO] excess

(□) $[CuCl_2] = 2.5 \times 10^{-3}$ M, initial saturated [NO] ca. 1.6×10^{-2} M

(■) $[CuCl_2] = 2.5 \times 10^{-3}$ M, [NO] excess.

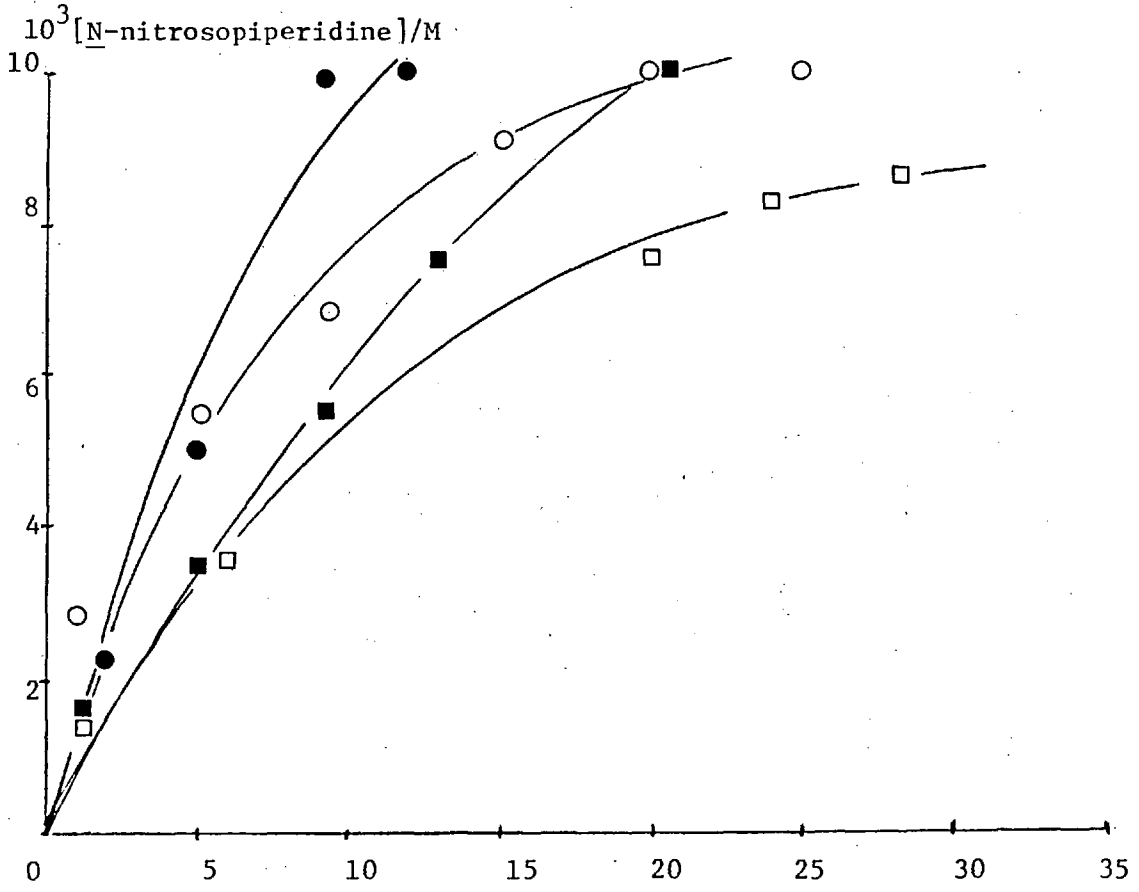


Figure 5.2

Dependence of the rate of N-nitrosopiperidine formation on [piperidine] (5×10^{-3} M (▲), 1×10^{-2} M (■), 2×10^{-2} M (●) 4×10^{-2} M (○)) for $CuCl_2$ catalysis in anaerobic dioxan at 25°C. [$CuCl_2$] = 8×10^{-4} M, [NO], excess.

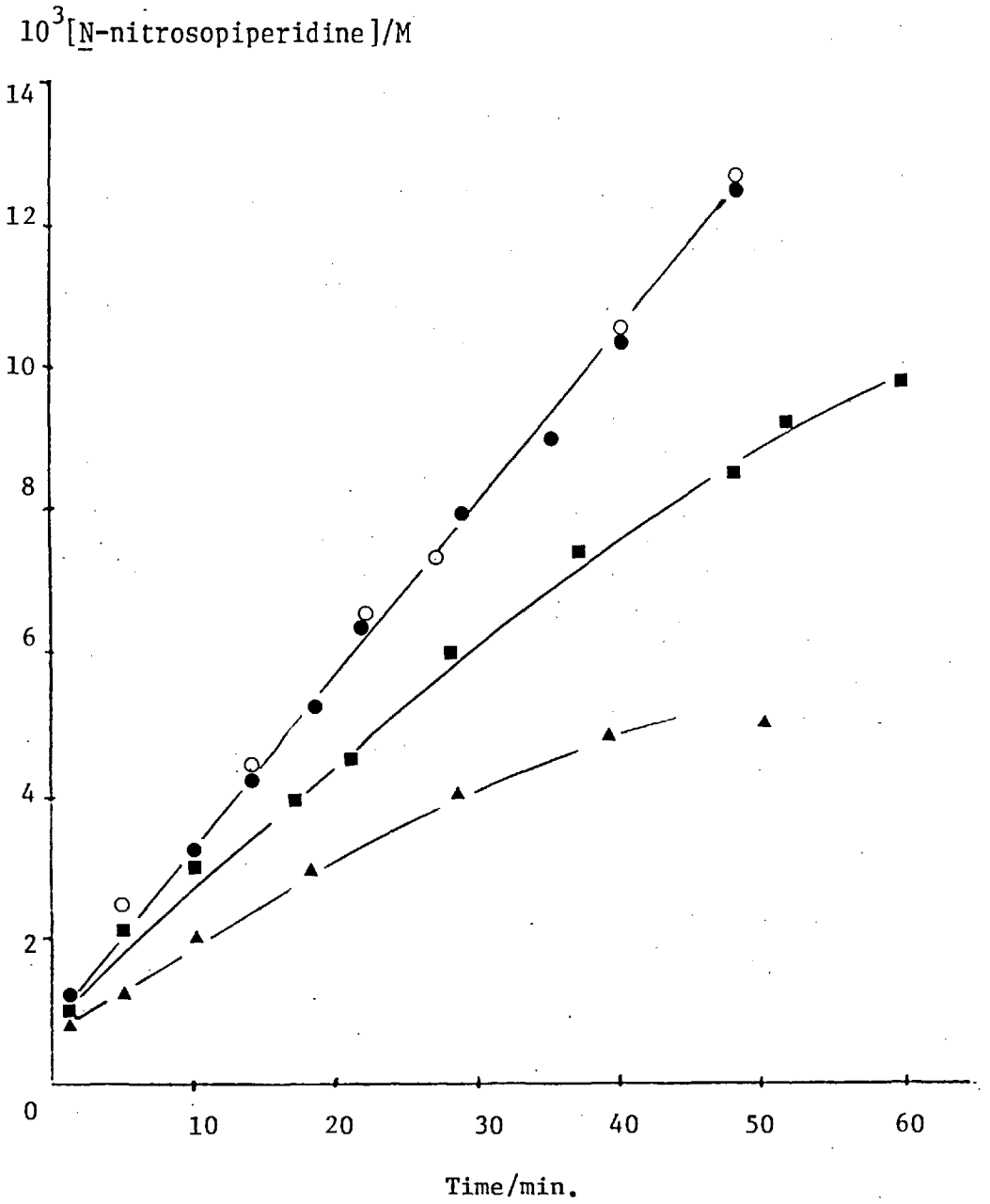


Table 5.1

Dependence of N-nitrosopiperidine (NNP) formation on [$CuC\ell_2$] and [$CuC\ell$] in anaerobic dioxan at 25°C.

[NO] = excess.

10^3[Metal Salt]/M	10^2[Piperidine/M	% Yield[a]	Time min.[b]	$t_{\frac{1}{2}}$ min.
$CuC\ell_2$				
0	1.0	5	1200	
4.75	1.0	100	12	4.6
2.50	1.0	100	20	8.5
0.9	1.0	75	35	24.2
0.8	.05	100	38	16
0.8	4.0	25	38	38[e]
0.8	2.0	50	38	38
0.8 (0.8)	1.0	70 (80)	42 (46)	30.2 (26)
0.8[c]	1.0	50	52	52
0.8[d]	1.0	50	55	55
0.5	1.0	50	42	42
0.23	1.0	20	50	47[e]
0.23	2.0	25	50	50[e]
0.23	4.0	12.5	50	50[f]
1.0	2.0	50	27	27
$CuC\ell$				
2.50	1.0[d]	70	34	23
2.50 (2.67)	1.0	100	25 (21)	9 (8.5)
1.87	1.0	100	42	12.5
1.87	0.5	100	26	9
1.85	1.0	100	40	12
1.80	2.0	50	11	11
1.80	4.0	25	11	11[e]
1.70	1.0	90	40	14
1.0	1.0	85	34	21
0.5	1.0	57	52	46

[a] Based on initial [Amine]

[b] Time to reach yield

[c] 0.01 M Morpholine

[d] 0.01 M N-methylpiperazine

[e] $t_{\frac{1}{4}}$ min.

[f] $t_{\frac{1}{8}}$ min.

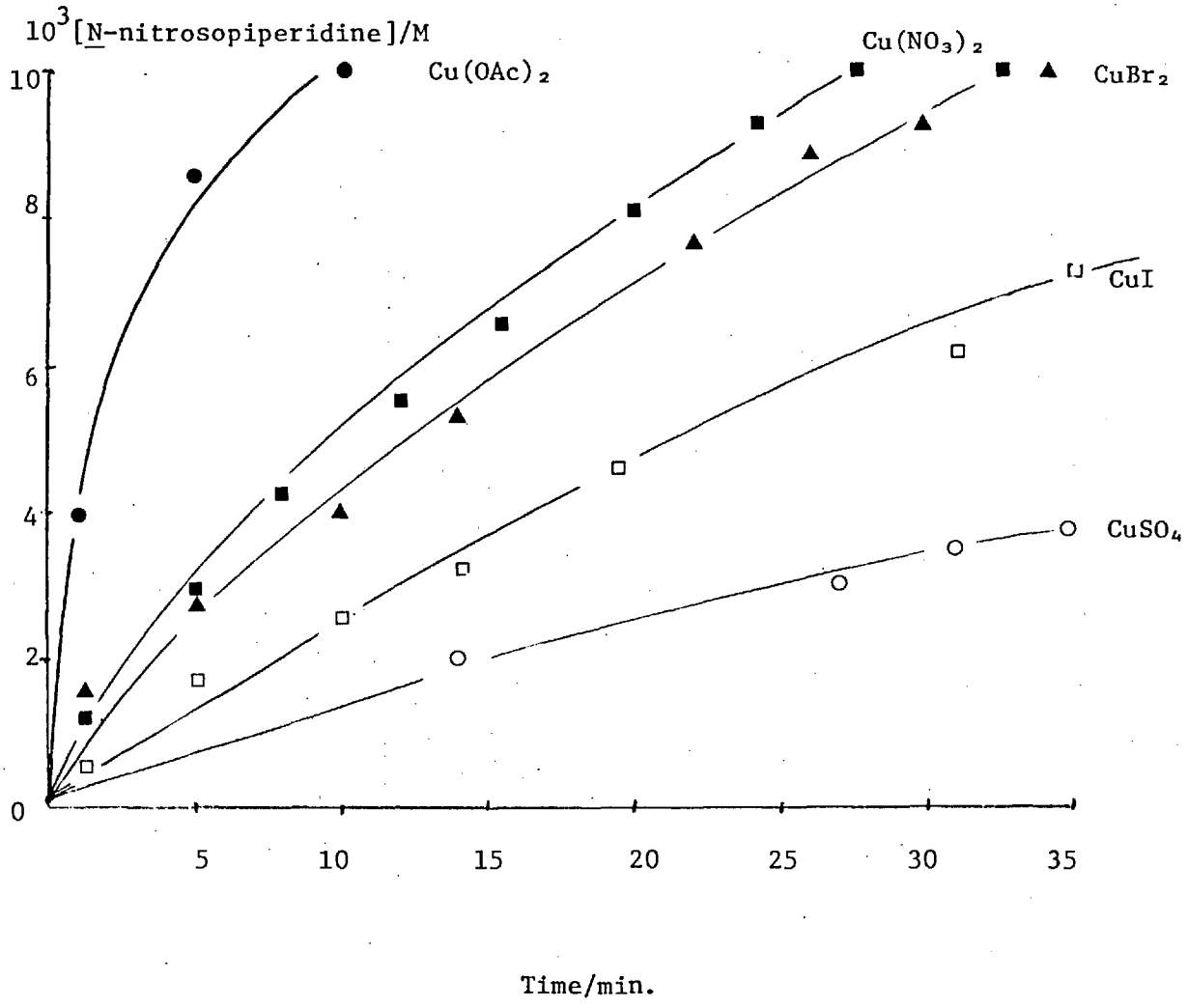
The observation that $t_{\frac{1}{2}}$ varies with the copper salt concentration yet the reaction follows pseudo zeroth order kinetics shows that the copper salts are true catalysts. Further, the catalytic efficiency of $Cu^{I}C\ell$ and $Cu^{II}C\ell_2$ is almost identical.

Other results summarised in Figure 5.3 show that the reaction rate for 0.01 M piperidine is dependent on the nature of the catalyst anion. The order of catalytic activity is $Cu^{II}(OAc)_2 > Cu^{II}(NO_3)_2 > Cu^{II}Br_2 > Cu^{II}C\ell_2 = Cu^{I}C\ell > Cu^{I}I \approx Cu^{II}SO_4$. All of these reactions however, proceeded to completion.

The observation of linear % reaction versus time plots (Figure 5.2) implies that the kinetic order in amine is zero or very close to zero (i.e., Rate = $\underline{k}_2[NO][MX_2]$). Examination of the effect of initial [amine] on the reaction rate partially supports this deduction. The results are reported for the reaction of piperidine with 8×10^{-4} M $CuC\ell_2$ (Figure 5.2) and 1.8×10^{-3} M $CuC\ell$ in Table 5.1. For the $CuC\ell$ catalysed reaction it can be seen that the reaction rate is not independent of the initial [Amine] despite the above conclusion. In fact, $t_{\frac{1}{2}}$ suggests an approximate first order dependence for [piperidine] $< 2 \times 10^{-2}$ M, but a zeroth order dependence for [piperidine] $> 2 \times 10^{-2}$ M. For the $CuC\ell_2$ catalysed reactions, the rate for [piperidine] $\lesssim 2 \times 10^{-2}$ M also increases with [piperidine] but the kinetic order is much less than one. Further, as with $CuC\ell$ catalysis, a zeroth order dependence is clearly apparent for [piperidine] $> 2 \times 10^{-2}$ M. Thus, both Cu^{I} and Cu^{II}-catalysed reactions have a complicated kinetic dependence on [Amine]. This suggests that the amine is involved in pre-equilibrium processes rather than a simple slow reaction with a nitrosating agent.

Figure 5.3

The dependence of the copper salt anion on the rate of formation of N-nitrosopiperidine from 0.01 M piperidine in anaerobic dioxan at 25°C. [NO] = excess. $[Cu(OAc)_2] = 1 \times 10^{-3}$ M (●), $[Cu(NO_3)_2]$ (■), $[CuBr_2]$ (▲) = 9.70×10^{-4} M, [CuI] (□) = 2.5×10^{-3} M, $[CuSO_4]$ (○) = 1×10^{-3} M.



The effect of amine basicity on the reaction rate was examined for $CuC\ell_2$ catalysis and these results are included in Table 5.1. The more basic piperidine reacts faster than morpholine or N-methyl-piperazine but the difference is small.

Additional experiments carried out to determine the nature of the rate-limiting step, involved the effect of added azide and bromide salts and tertiary amines. Unfortunately, inorganic salts were found to be insoluble in dioxan and it was therefore, necessary to use tetra-n-butylammonium-azide and bromide . Results in Table 5.2 show that both have an inhibiting effect. This outcome is expected for the azide salt which is known to react rapidly and irreversibly with nitrosating agents[48] ($N_3^- + NOX \rightarrow N_2O + N_2 + X^-$). The same effect

Table 5.2

Effect of added $(n\text{-Bu})_4\overset{+}{N}\overset{-}{N_3}$, $(n\text{-Bu})_4\overset{+}{N}\overset{-}{Br}$ on the rate and maximum yield of N-nitrosopiperidine (NNP) formation from piperidine in the presence of $Cu^{II}C\ell_2$ and $Cu^{II}Br_2$ in anaerobic dioxan at 25°C. Initial saturated [NO] ca. 1.6×10^{-2} M.

$10^3[Cu^{II}C\ell_2]$/M	$10^3[(n\text{-Bu})_4\overset{+}{N}\overset{-}{N_3}]$/M	10^2[piperidine]/M	Yield 10^3[NNP]/M	Time/ min.
1.0	0	1.0	5.5 (10.0)[a]	22 (1200
1.0	7.5	1.0	0 (6.5)[a]	80 (1200
1.0	25.0	1.0	0 (0.6)[a]	200 (1200
$10^3[Cu^{II}Br_2]$/M	$10^3[(n\text{-Bu})_4\overset{+}{N}\overset{-}{Br}]$/M			
1.0	0	2.0	10 (20)[a]	18 (1200
1.0	1.0	2.0	2.5 (10)[a]	28 (2400

[a] Maximum Yield after time t in parenthesis.

for bromide salts is more difficult to explain because nucleophilic anions (such as Br^-) usually catalyse nitrosation *via* NOX formation. It was observed however, that $(n\text{-Bu})_4N^+Br^-$ discharged the violet colour associated with Cu-nitrosyl complex (II), (Equation 5.3). One possible explanation is that $CuBr_2$ and $(n\text{-Bu})_4N^+Br^-$ themselves complex. The addition of tertiary amines (e.g., triethylamine) had a complicated effect on the reaction. For 2×10^{-2} M piperidine, 10^{-3} M $CuC\ell_2$ and constant excess of NO, the rate (k_0 = slope of zeroth order plot of % reaction *versus* time) decreased for $[NEt_3] < 2 \times 10^{-3}$ M, but thereafter, increased (Table 5.3). Above 10^{-2} M NEt_3, the rate reached a limiting value. The changes are more readily seen from a plot of k_0 *versus* $[NEt_3]$ in Figure 5.4. The significance of all these findings are disscussed below.

Brackman and Smit[82] reported that nitrous oxide (N_2O) was a by-product of the $CuC\ell_2$ catalysed *N*-nitrosamine formation in MeOH. I.r., analysis of the gaseous reaction products (v. Experimental 5.4. III) confirmed its formation with solvent dioxan for both $Cu^{II}C\ell_2$ and $Cu^{I}C\ell$ catalysis. The amount is substantial, our own semi-quantitative analyses indicating a maximum N_2O *ca.* 55-60% of the initial [Amine]. One factor contributing to the rate of reaction when NO is not continuously bubbled through the solution, may be the displacement of NO by N_2O, which preferentially dissolves in dioxan.

Frazer[111] also reported that the violet $[Cu^{II}(NO)]C\ell_2$ nitrosyl when prepared in EtOH dissociated to give ethyl nitrite and $Cu^{I}C\ell$ (Chapter 1.2) and this was later confirmed by both Brackman and Smit (for reaction in MeOH to give MeONO) and by our own present studies. Further, the nitrosation of MeOH by $[CoC\ell_2.NO]$ nitrosyl has been reported.[105]

Table 5.3

The effect of added NEt_3 on the rate of N-nitrosopiperidine (NNP) formation from initial 2×10^{-2} M piperidine in the presence of $Cu^{II}C\ell_2$ in anaerobic dioxan at 25°C. [NO] = excess.

$10^3[Cu^{II}C\ell_2]$/M	$10^3[NEt_3]$/M	% Yield[a] NNP	Time[b] min.	10^5 k_o mol^{-1} l^{-1} $min.^{-1}$[c]
1.1	0	50 (100)[d]	27	3.7
1.0	1.0	32 (75)[d]	27	2.4
1.0	2.0	18 (75)[d]	27	1.3
1.0	5.0	27.5 (75)[d]	27	2.0
1.0	10.0	42.5 (100)[d]	27	3.0
1.0	17.0	42.5 (100)[d]	27	3.0
1.0[e]	0	50 (100)[d]	27	3.7
1.0[e]	2.0	12.5 (71)[d]	27	0.93

[a] Based on initial [piperidine]

[b] Time to reach % yield

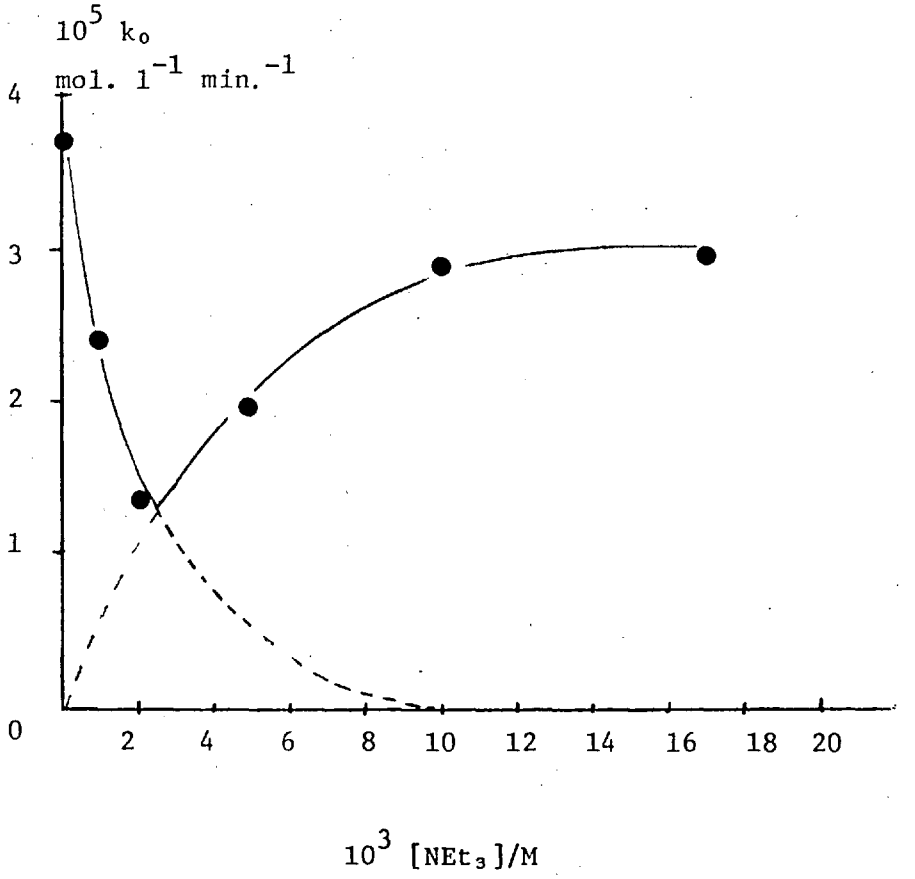
[c] k_o calculated from zeroth order plot $\frac{d\ NNP}{dt} = k_o$

[d] Maximum yield on standing overnight, after % yield reached after time t. NO flow stopped, i.e., further reaction with saturated [NO]

[e] With $Cu^{I}C\ell$ in place of $CuC\ell_2$

Figure 5.4.

The effect of added NEt_3 on the rate (k_o) of N-nitrosopiperidine formation from 2×10^{-2} M piperidine, in the presence of 10^{-3} M $Cu^{II}C\ell_2$ in anaerobic dioxan at 25°C. [NO] = excess.



5.2.2. Discussion.

Clearly, both Cu^{I}- and Cu^{II}-salts catalyse N-nitrosamine formation from secondary amines and NO. Relatively small amounts of these salts accelerate the reactions and produce quantitative yields of N-nitrosamines. The kinetic results point to some well defined, albeit unexpected dependencies. These salient features are:-

1) First order dependence on [CuCℓ] and [$CuCℓ_2$].

2) CuCℓ and $CuCℓ_2$ have a similar catalytic effet.

3) The degree of catalysis depends on the counter ion. The observed order is $AcO^- > NO_3^- \approx Br^- > Cℓ^- > I^- \approx SO_4^-$.

4) These reactions are truly catalytic i.e., 100% yield of N-nitrosamine irrespective of initial metal salt concentration. This important finding shows that overall, the reaction does not produce acid, since this would lead to the formation of protonated amine and hence less than quantitative amounts of N-nitrosamine.

5) The kinetic dependence on amine is low and under certain circumstances is zeroth order.

6) The reaction rate for $CuCℓ_2$ and CuCℓ catalysis is not strongly dependent on amine basicity.

7) N_2O is formed as a co-product. This shows that a redox process is involved.

8) The reactions are inhibited by $(n\text{-Bu})_4\overset{+}{N}Br^-$, (irreversibly) by $(n\text{-Bu})_4\overset{+}{N}N_3^-$ and both inhibited and catalysed by tertiary amines, depending on concentration.

As noted previously, the interaction of NO with Cu^{II} salts is considered to give a violet complex $[Cu^{II}(NO)]X_2$.[110] Addition of amine discharges the violet colour immediately, presumably by forming a different complex. This entity cannot be isolated, so its structure is conjectural, but three {III, IV, V} seem likely.

$[Cu^{II}(amine)_n]X_2$ (III) $[Cu^{I}(amine)_n]X$ (IV) $[Cu^{II}(amine)_nNO]X_2$ (V)

Structure (III) and (IV) can probably be disregarded because lightly coloured khaki complexes are produced by adding either $Cu^{II}C\ell_2$ or $Cu^{I}C\ell$ to piperidine in the absence of NO. Both, however, give a colourless solution on passage of NO, similar to that produced by adding piperidine to the violet $[Cu^{II}(NO)]C\ell_2$. Further, structure (IV) is inconsistent with other observations for transnitrosation by Ph_2NNO (v. below), which show that the yield of N-nitrosopiperidine per mole of Ph_2NNO is higher for $Cu^{II}C\ell_2$ than $Cu^{I}C\ell$. This implies that NO is consumed in converting $Cu^{I}C\ell$ to $Cu^{II}C\ell_2$ and, inter alia, that reaction arises from $CuC\ell_2$. Thus, our findings will be rationalised in terms of an initial formation of Structure (V), which may react by the release of NOX (Scheme 5.1)* or by nitrosation of the bound amine (Scheme 5.2).

---

* Footnote.

Reaction by a very low steady-state concentration of $[Cu^{II}NO]X_2$ is ruled out by the kinetic amine dependence. Application of steady-state kinetics show that the process would lead to an inverse amine dependence.

$$Cu^{II}\text{-}X_2 + NO \rightleftharpoons [Cu^{II}(NO)]X_2 \xrightarrow{nR_2NH} [Cu^{II}(R_2NH)_nNO]X_2 \quad (V)$$

$$k_1 \downarrow \quad \uparrow \underline{k}_{-1}$$

$$R_2NNO + HX \xleftarrow[\underline{k}_2]{R_2NH} [NOX] + [Cu^{I}(R_2NH)_n]X$$

SCHEME 5.1

Scheme 5.1 accommodates the observed amine dependence if $\underline{k}_2$ is slow for [piperidine] $\leq$ 0.02 M but $\underline{k}_1$ is slow for [piperidine] $>$ 0.02 M. It would also explain the kinetic dependence on the anion of the Cu^{II} salts, and inhibition by $(\underline{n}\text{-Bu})_4N^+N_3^-$. It is not consistent however, with the effect of tertiary amines. The fact that these inhibit implies that only amine bound to the Cu^{II}-salt is reactive, as in Scheme 5.2.

$$Cu^{II}X_2 + NO \rightleftharpoons [Cu^{II}(NO)]X_2 \xrightarrow{nR_2NH} [Cu^{II}(R_2NH)_n(NO)]X_2 \quad (V)$$

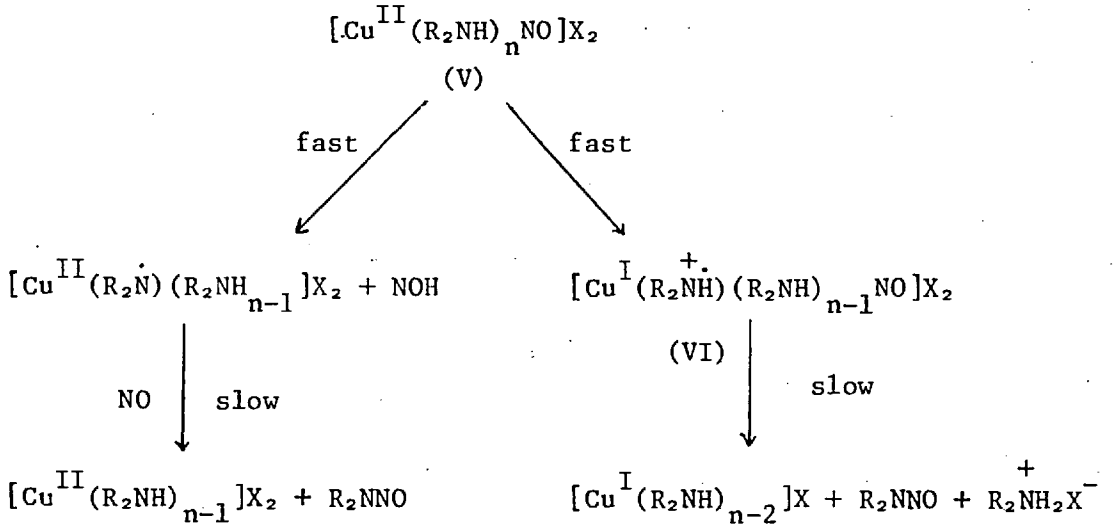
$$\downarrow$$

$$Cu^{II}X_2 + R_2NNO + (NOH)$$

SCHEME 5.2

The mechanism is therefore preferred, with the proviso that (V) must be formed quantitatively in order to explain the low kinetic dependence on amine. The inhibition by $(\underline{n}\text{-Bu})_4N^+N_3^-$ and NEt_3 relates to the preferential formation of complexes other than (V), involving N_3^- and NEt_3. It is more difficult to explain the kinetic dependence on the anion of the Cu^{II}-salt, but this could be important in the conversion of

(V) to products. Several redox mechanisms for this step can be envisaged (Scheme 5.3), but that involving formation of an amino



SCHEME 5.3

radical cation and the release of NOX is supported by the effect of NEt_3. The unusual shape of Figure 5.3 whereby the effect of added NEt_3 is discontinuous suggests that N-nitrosamine forms by two pathways. Thus, in the absence of added NEt_3, reaction proceeds by (VI). Addition of NEt_3 reduces the concentration of (VI) in preference for the analogous $[Cu^{I}(\overset{+\cdot}{N}Et_3)(NEt_3)_{n-1}NO]X_2$. This explains inhibition by NEt_3. This complex may decompose in a similar fashion to (VI) to yield $Et_3\overset{+}{N}NO$ (or NO-X) which nitrosates piperidine. Thus, with high $[NEt_3]$ all the CuX_2 is bound to NEt_3, the reaction of released $Et_2\overset{+}{N}NO$ (or NOX from the reaction of $Et_3\overset{+}{N}$ NO + X^-) with piperidine is the formation pathway. This corresponds to the constant rate observed in Figure 5.3 at $[NEt_3] > 10^{-2}$ M. The internal oxidation of NEt_3 by $Cu^{II}C\ell_2$ to $Cu^{I}C\ell$ and $\overset{+\cdot}{N}Et_3$ has been reported.[179]

The catalysis by Cu^{I} salts without apparent formation of Cu^{o} implies reaction involving a $Cu^{I} \rightleftharpoons Cu^{II}$ redox process. This can only be accomplished by concurrent reduction of NO or amine. We favour the former because there is some evidence for this process (see below) with iron salts, although in principle, this involves the formation of the very unstable NO^{-} entity. However, evidence for transnitrosation by Ph_2NNO (below) shows that 2 moles of NO are required per mole of N-nitrosamine with $Cu^{I}C\ell$ salts, and N_2O (via $2(NOH) \longrightarrow N_2O + H_2O$) is a co-product of our reactions. A mechanism embracing these requirements which proceeds through an intermediate similar to (V) is shown in Scheme 5.4. Tentative support for this type of process comes from a recent report showing

$$Cu^{I}X + 2NO \longrightarrow [Cu^{I}(NO)_2]X \xrightarrow{nR_2NH} [Cu^{II}(NO)(R_2NH)n]XNO^{-}$$

$$\downarrow$$

$$Cu^{I}X + R_2NNO + NOH$$

SCHEME 5.4

that Cu^{I} salts react with gaseous NO and NO derived from the reduction of acidic nitrite by ascorbate to give $Cu^{II}(NO^{-})$ identified by change in e.s.r. spectra.[180] Thus, the reaction pathways effectively amount to redox cycles involving either Cu^{II}- or Cu^{I} (Equation 5.6 and 5.7).

$$Cu^{II}X_2 + 2NO + R_2NH \longrightarrow Cu^{II}X_2 + R_2NNO + (NOH) \quad .... \quad 5.6$$

$$Cu^{I}X + 2NO + R_2NH \longrightarrow Cu^{I}X + R_2NNO + (NOH) \quad .... \quad 5.7$$

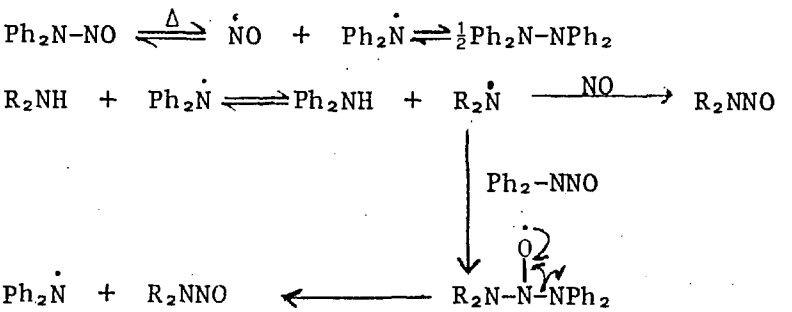
5.2.3. Transnitrosation.

5.2.3 (I) Introduction.

The transfer of the nitroso group from one N-nitrosamine to another amine can proceed both directly or indirectly (v. Chapter 1.1.3.). Most of these reactions refer to acidic conditions and involve the nitrosonium ion (NO^+) carriers such as $Ph_2\overset{+}{N}HNO$ or HNO_2. Under neutral conditions, N-nitrosodiphenyl amine (Ph_2NNO) undergoes homolysis to release $\dot{N}O$ (Equation 5.8). Challis and Osborne[45] briefly investigated this reaction in dioxan and suggested that reaction

$$Ph_2N\text{-}NO \overset{\Delta}{\rightleftharpoons} Ph_2\dot{N} + \dot{N}O \qquad \ldots\ 5.8$$

under anaerobic conditions might proceed via a nitroxide intermediate (VI) as shown in Scheme 5.5.



(VI)

SCHEME 5.5

It was decided to investigate the copper catalysed reactions further using Ph_2NNO as a source of NO. This allows control of reagent concentrations, unlike the situation with gaseous NO.

5.2.3.2. Results and Discussion.

5.2.3.2. (I) Reactions in the absence of metal catalysis.

The reactions were carried out in both anaerobic and aerobic dioxan at 53°C. Equimolar (ca. 0.05 M) Ph_2NNO and piperidine under anaerobic conditions gave 0.01 M and 0.014 M N-nitrosopiperidine after 42 and 73 h., respectively (Table 5.4).

Table 5.4

The yield and reaction time of N-nitrosopiperidine (NNP) from initial 0.05 M piperidine with N-nitrosodiphenylamine (N-DPA) in dioxan at 53°C under anaerobic and aerobic conditions.

	Yield		10^3[NNP]/M	
10^2[N-DPA]/M	10^3[N NP]/M	Time/h.	Max. Yield	Time/days
Anaerobic				
2.91	5.0	42	13.6	21
4.8	8.0	40	34.0	21
5.03	10.03	44	14.3	3
5.06	5.5	40	34.0	21
Oxygen saturated.				
2.58	14.3	43	14.3	21
5.0	24.7	42	24.7	21
5.12	26.0	42	26.0	21
5.24	20.3	44	26.0	21

Thus, N-nitrosopiperidine forms slowly and after 21 days the yield is only ca. 70% of the initial [Ph_2NNO]. Under aerobic conditions (i.e., oxygen saturated dioxan) however, reaction is much faster: e.g., with equimolar (0.05 M) Ph_2NNO and piperidine, 0.025 M N-nitrosopiperidine

forms after 42 h (Table 5.4). The yield of N-nitrosopiperidine, however, appears to be limited to ca. 50% of the initial [Ph_2NNO]. At the conclusion of the experiment, no Ph_2NNO could be detected by qualitative T.L.C. analysis (1 mm SiO_2, benzene eluent, R_F Ph_2NNO = 0.50). Since no limiting yield is apparent for reactions under anaerobic conditions, a different mechanism probably applies. The limiting 50% yield in the presence of oxygen is not unexpected as oxygen reacts rapidly with NO to form $\dot{N}O_2$. Thus, transnitrosation to piperidine via N_2O_3 (Scheme 5.6) is expected. The intermediacy

$$Ph_2NNO \underset{}{\overset{\text{dioxan } 53^{\circ}C}{\rightleftharpoons}} Ph_2\dot{N} + \dot{N}O$$

$$NO + \tfrac{1}{2}O_2 \longrightarrow \dot{N}O_2 \xrightarrow{NO} N_2O_3$$

$$N_2O_2 + R_2NH \longrightarrow R_2NNO + HNO_2$$

SCHEME 5.6

of $Ph_2\dot{N}$ is confirmed by the identification of Ph_2N-NPh_2 (qualitative T.L.C. under the same conditions as above, R_F = 0.71) at the end of reaction. The reaction under anaerobic conditions may proceed via the nitroxide intermediate (Scheme 5.5) suggested by Challis and Osborne.[45]

5.2.3.2 II $Cu^{II}C\ell_2$ and $Cu^{I}C\ell$ Catalysed Transnitrosation in Anaerobic EtOH at 60°C.

N-nitrosopiperidine formation is faster when Ph_2NNO and piperidine are heated in anaerobic EtOH at 60°C in the presence of either $Cu^{II}C\ell_2$ or $Cu^{I}C\ell$ than in their absence. Typically, with equimolar (10^{-2} M) Ph_2NNO and piperidine only 4.5% N-nitrosopiperidine is found after

1200 min. compared to 25% N-nitrosopiperidine after ca. 250 and 430 min, in the presence of 10^{-2} M $Cu^{II}C\ell_2$ and $Cu^{I}C\ell$, respectively. Most of the work reported below was carried out by heating the metal salt and Ph_2NNO in anaerobic EtOH for 40 min. at 60°C and then initiating reaction by the addition of neat amine.

Typical plots of % transnitrosation versus time for $CuC\ell_2$ catalysis are shown in Figure 5.5 and 5.6. For both $Cu^{II}C\ell_2$ and $Cu^{I}C\ell_2$, linear plots were obtained when the dead space above the reaction solution was large (ca. 25 ml). With smaller dead space (ca. 5 ml) the plots were linear but curved as the reaction slowed towards completion. An obvious explanation for the difference is that the dead space volume influences the [NO] in solution. Examination of Figure 5.4 shows that the initial formation of N-nitrosopiperidine is fast but subsequently slows down and becomes independent of $[Cu^{II}C\ell_2]$. This conclusion is evident from the times for 25% reaction given in Table 5.5 (For reaction with 2 x 10^{-2} M $Cu^{II}C\ell_2$, and equimolar (10^{-2} M) Ph_2NNO and piperidine, the time is quoted for 40% reaction which incorporates the initial fast formation of 15% N-nitrosopiperidine). The initial rapid formation of N-nitrosopiperidine is dependent on $[CuC\ell_2]$. Thus, with 2 x 10^{-2} M, 10^{-2} M, 5 x 10^{-3} M, 1 x 10^{-3} M $CuC\ell_2$ and equimolar (10^{-2} M) Ph_2NNO and piperidine, 15.5, 6, 4.5, 3% N-nitrosopiperidine is formed after only 10 min., respectively. The most likely explanation is that NO released before injection of secondary amine combines with $CuC\ell_2$. The violet colouration, indicative of $[Cu^{II}(NO)]C\ell_2$, is not apparent, however, presumably because of masking by the dark brown-green solution of Ph_2NNO and $Cu^{II}C\ell_2$.

Figure 5.5.

The dependence of [$CuC\ell_2$] on the transnitrosation reaction between initial equimolar (10^{-2} M) N-nitrosodiphenylamine and piperidine in anaerobic EtOH at 60°C. [$CuC\ell_2$] = 1 x 10^{-3} (△), 5 x 10^{-3} (●), 1 x 10^{-2} (■), 2 x 10^{-2}M(▲).

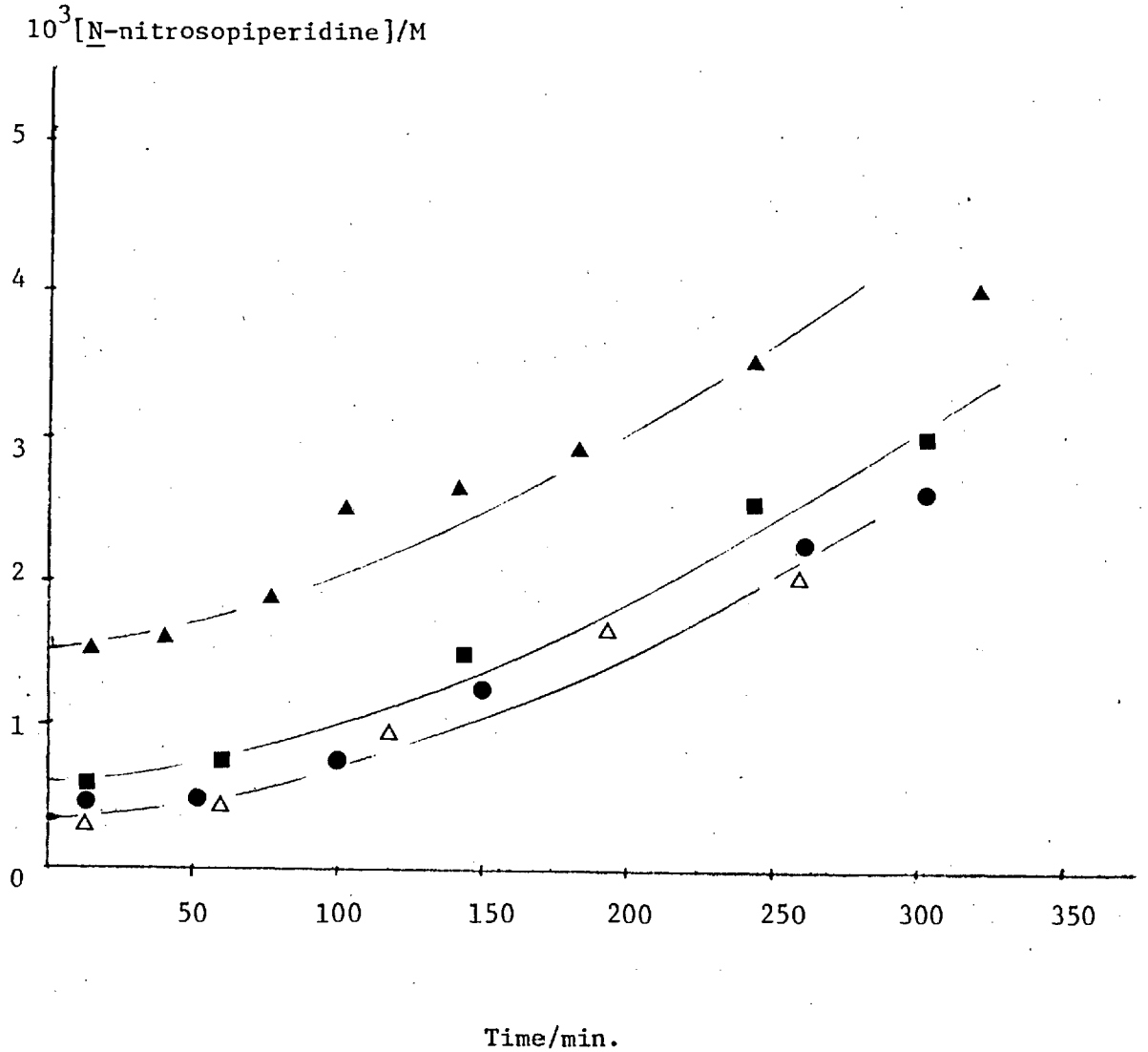


Figure 5.6.

The dependence on initial [Ph_2NNO] on the rate of transnitrosation to 10^{-2} M piperidine in the presence of 10^{-2} M $CuC\ell_2$ in anaerobic EtOH at 60°C. Initial [Ph_2NNO] = 10^{-2} M (■), 2×10^{-2} M (●), 4×10^{-2} M (▲).

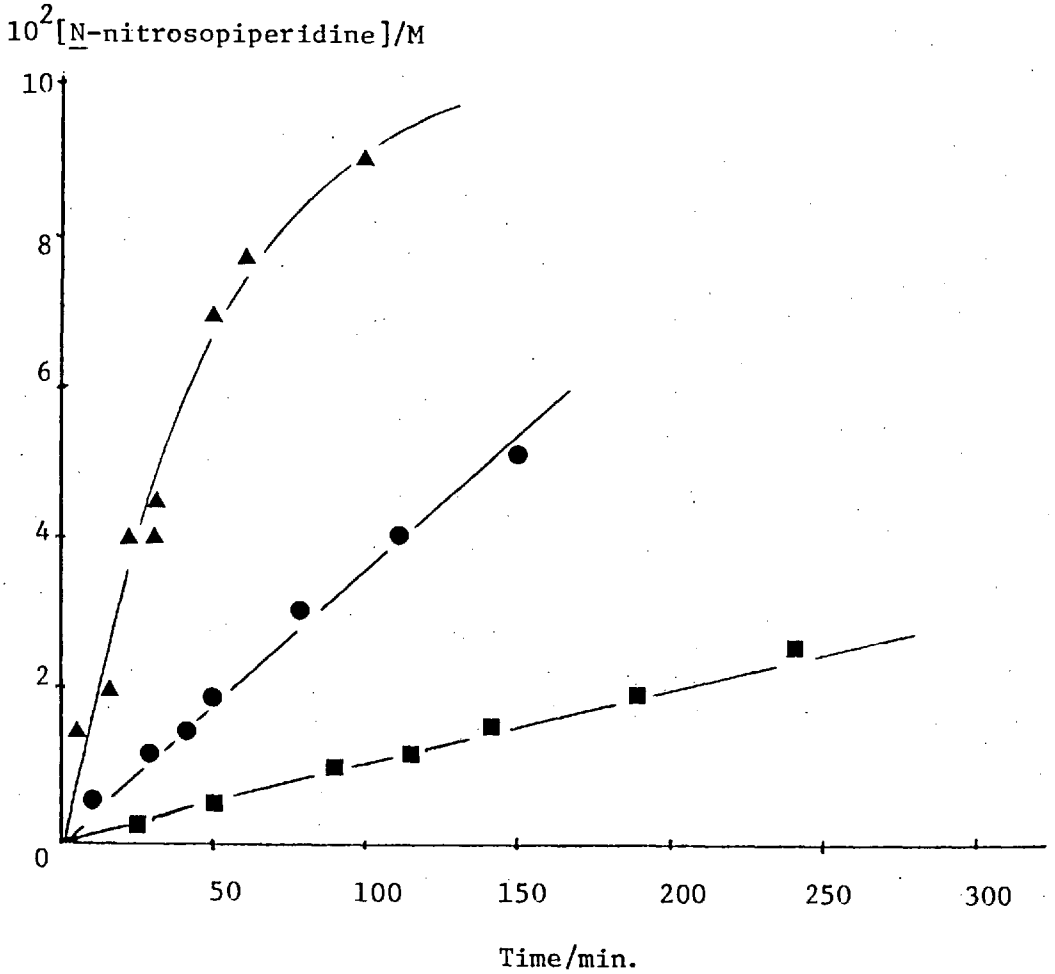


Table 5.5

The yield and reaction times of N-nitrosopiperidine (NNP) formation from the reaction of N-nitrosodiphenylamine (N-DPA) and piperidine in the presence of $CuC\ell_2$ in anaerobic EtOH at 60°C.

10^2[$CuC\ell_2$]/M	10^2[N-DPA]/M	10^2[Piperidine]/M	% Yield[a] NNP	Time[b] min
0	1.0	1.0	4.5	1200
0.1	2.0	1.0	25 (95)[c]	300
0.1	1.0	1.0	25 (38)[c]	300
0.15	1.0	1.0	25 (45)[c]	300
0.50	1.0	1.0	25 (50)[c]	320
1.05	1.0	1.0	25 (51)[c]	260
2.0	1.0	1.0	40 (50)[c]	360
1.0[d]	1.0	1.0	22.5 (45)[c]	90
1.0[e]	1.0	1.0	22.5 (45)[c]	160
1.0	1.0	0.1	50 (100)[c]	300
1.0	1.0	0.25	50 (100)[c]	360
1.0	1.0	0.5	50 (100)[c]	220
1.0	2.0	1.0	11 (88)[c]	30
1.0	4.0	1.0	50 (101)[c]	30
2.14	4.03	4.0	(60)[g]	
1.0[f]	1.0	1.0	2.5 (27.5)[c]	200

[a] Yield based on initial [Amine]

[b] Time to reach % yield

[c] Maximum yield on standing overnight

[d] 0.01 M morpholine

[e] 0.01 M N-methylpiperazine

[f] In presence of [KN_3] = 5.40×10^{-3}M

[g] Maximum yield standing 7 days

Other data in Table 5.5 and Figure 5.6 show that the reaction is dependent on $[Ph_2NNO]^2$ but largely independent of [piperidine]. These observations suggest that the rate expression for $CuC\ell_2$ catalysed transnitrosation is given by Equation 5.9. Results in

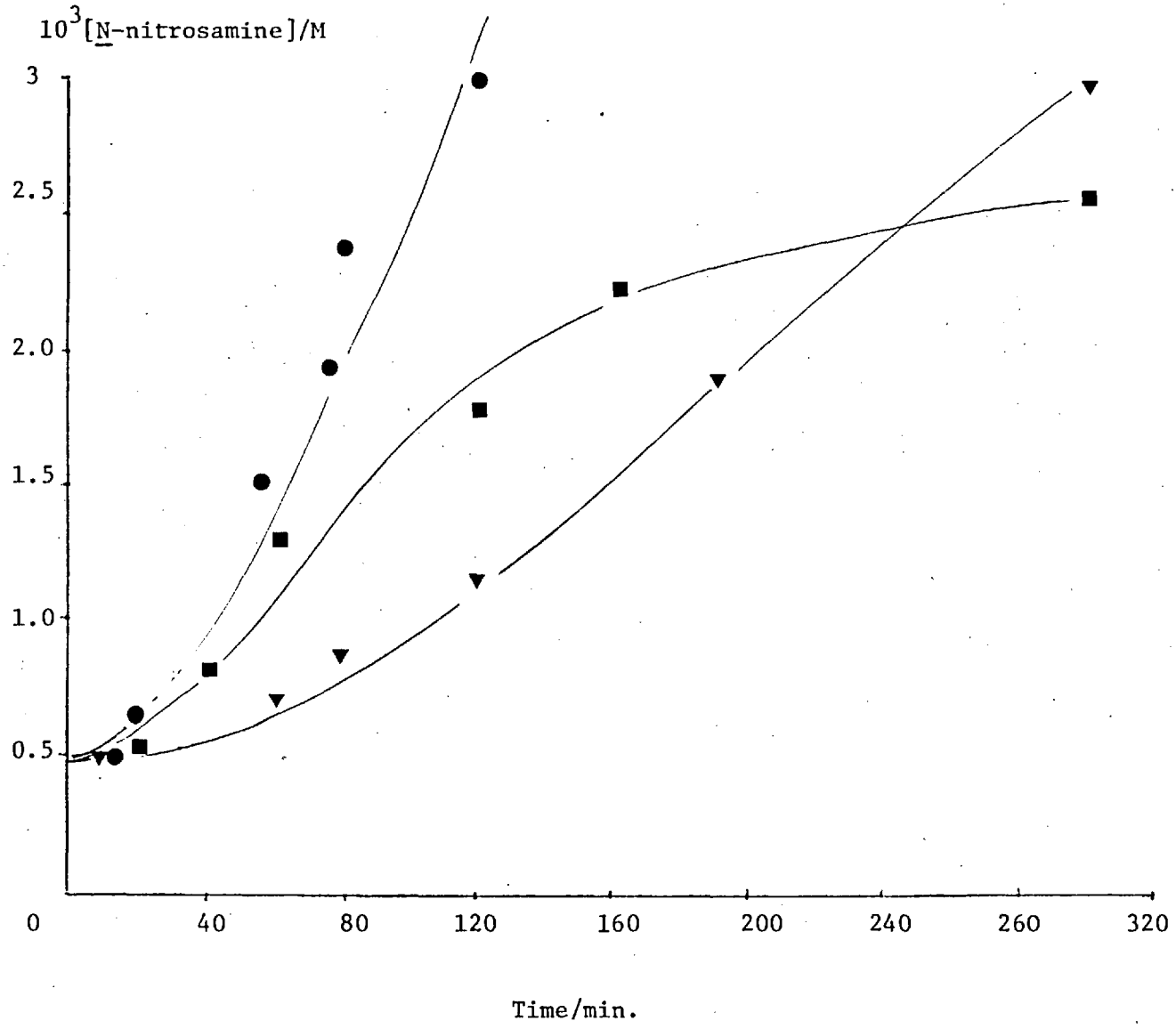
$$\text{Rate} = k\ [Ph_2NNO]^2 \qquad \ldots\ldots \quad 5.9$$

Table 5.5 also shows that the maximum yield of N-nitrosopiperidine is dependent only on $[Ph_2NNO]$. Thus, with equimolar (10^{-2} M) Ph_2NNO and piperidine, 50% N-nitrosopiperidine forms, but with greater than 2×10^{-2} M Ph_2NNO, quantitative yield was obtained. Hence, the reaction in the presence of $CuC\ell_2$ are catalytic (as found for gaseous NO) which implies that acid is not produced and does not inhibit the reaction (Scheme 5.2). This was confirmed by observing no Ph_2NNO (by T.L.C.) at the conclusion of experiments with equimolar (10^{-2} M) Ph_2NNO and piperidine. Further, N_2O was identified as a reaction product although the recovery was very low. Independent experiments showed that authentic N_2O escaped *via* the Subaseal stopper and inlet tubes on heating under reaction conditions overnight (v. Experimental 5.4.III).

Reaction of 10^{-2} M morpholine or N-methylpiperazine with 10^{-2} M Ph_2NNO and $CuC\ell_2$ showed that the reaction is sensitive to the structure of the amine (Figure 5.7). The dependence on amine structure is clearly not simple as morpholine reacts much faster than N-methylpiperazine or piperidine. Although maximum yield is similar for piperidine and morpholine, it is not known why reaction of 10^{-2} M N-methylpiperazine slows substantially after *ca.* 25% N-nitrosamine formation.

Figure 5.7.

Dependence of amine structure piperidine (▼), morpholine (●), N-methyl-piperazine (■), on the rate of transnitrosation in the presence of initial 10^{-2} M Ph_2NNO and 10^{-2} M $CuC\ell_2$ in anaerobic EtOH at 60°C. Initial [Amine] = 10^{-2} M.



Additional experiments showed that both the rate and limiting yields of N-nitrosopiperidine are inhibited by azide. Thus, with 5.40×10^{-3} M KN_3, 10^{-2} M $CuC\ell_2$, piperidine and Ph_2NNO, only 5% reaction was observed after 200 min. compared with 20% in its absence. The limiting yield drops from ca. 52% to 27.5% in the presence of KN_3 (Table 5.5). Azide ion is known to react irreversibly with NOX reagents.[48] The concurrent reduction of reaction rate may indicate that KN_3 preferentially binds to $CuC\ell_2$ and hence, displaces piperidine.

Data in Table 5.6 show that $Cu^{I}C\ell$ also catalyses transnitrosation between equimolar (10^{-2} M) Ph_2NNO and piperidine in anaerobic EtOH at 60°C. The rate is almost half that for $CuC\ell_2$ catalysis, but this is not unexpected if the initial step is the oxidation of $Cu^{I}C\ell$ to $Cu^{II}C\ell.NO^{-}$ by NO. The data in Table 5.6 also show that the rate bears a first order dependence on [$CuC\ell$], but the kinetic dependence on [Ph_2NNO] and [Amine] is not straightforward. Thus, increasing [Ph_2NNO] from 10^{-2} to 2×10^{-2} and 4×10^{-2} M increases the rate by factors of only 1.54 and (1.54 x 1.23), respectively. Further, the rate is independent of [piperidine] $< 10^{-2}$ M but increases by a factor of 1.8 from 10^{-2} to 2×10^{-2} M. The latter observation points to total complexation of the amine with copper salt at low [Amine]. As for $CuC\ell_2$ catalysis, the maximum yields of N-nitrosamine are limited by [Ph_2NNO], the reactions are inhibited by KN_3 (Table 5.6) and traces of N_2O are found as co-products.

5.2.3.2. III $Cu^{II}C\ell_2$ and $Cu^{I}C\ell$ Catalysed Transnitrosation in Anaerobic Dioxan at 53°C.

N-nitrosopiperidine formation from equimolar (10^{-2} M) reagents is faster in dioxan at 53°C than in EtOH at 60°C as found for gaseous

Table 5.6

The yield and reaction time of N-nitrosopiperidine (NNP) formation from the reaction of N-nitrosodiphenylamine (NDPA) and piperidine (P) in the presence of CuCℓ in anaerobic EtOH at 60°C.

10^2[CuCℓ]/M	10^2[NDPA]/M	10^2[Amine]/M	% Yield[a] NNP	Time[b] min.
0	1.0	1.0	4.5	1200
0.12	1.0	1.0	4 (36)[c]	420
0.55	1.0	1.0	19 (38)[c]	420
1.03	1.0	1.0	25 (50)[c]	430
2.0	1.0	1.0	25 (50)[c]	170
1.06[d]	1.0	1.0	22.5 (45)[c]	160
1.05[e]	1.0	1.0	22.5 (45)[c]	300
1.0	1.0	0.25	50 (100)[c]	100
1.05	1.0	0.50	50 (100)[c]	360
1.07	1.0	2.0	12.5 (25)[c]	160
1.05	2.0	1.0	36 (72)[c]	240
1.0	4.0	1.0	46 (92)[c]	260
2.19	3.92	1.0	59	1200
1.0[f]	1.0	1.0	5 (30)[c]	200

[a] % yield based on initial [Amine]

[b] Time taken to reach % yield

[c] Maximum yield on standing overnight

[d] In the presence of 0.01 M morpholine in place of piperidine

[e] In the presence of 0.01 M N-methylpiperazine in place of piperidine

[f] In the presence of 4.80×10^{-3} M KN_3

NO. This applies to both $Cu^{II}C\ell_2$ and $Cu^{I}C\ell$ catalysis.

Data in Table 5.7 show that the reaction is independent of $[CuC\ell_2]$, but dependent on $[Ph_2NNO]$ and [Amine]. For example, with equimolar (10^{-2} M) $CuC\ell_2$ and piperidine for 10^{-2}, 2×10^{-2} and 4×10^{-2} M Ph_2NNO, respectively, $t_{\frac{1}{2}}$ = 250, 155 and 85 min. The kinetic dependence on piperidine is not so straightforward being approximately first order below 10^{-2} M, but zeroth order above 10^{-2} M. (With equimolar (10^{-2} M) $CuC\ell_2$ and Ph_2NNO, $t_{\frac{1}{2}}$ = 250, 150, 135 min. for 5×10^{-3}, 1×10^{-2}, 2×10^{-2} M piperidine, respectively). A similar dependence applied to reactions in EtOH.

The reaction rate shows no strong dependence on $[CuC\ell]$ with $t_{\frac{1}{4}}$ = 215 and 135 min. for 10^{-3} and 10^{-2} M $CuC\ell$ in the presence of equimolar (10^{-2} M) Ph_2NNO and piperidine. Also, the $CuC\ell$ catalysed reactions were independent of $[Ph_2NNO]$ ($t_{\frac{1}{2}}$ = 240, 250 min) for 4×10^{-2} and 10^{-2} M Ph_2NNO with 10^{-2} M $CuC\ell$ and piperidine, but dependent on [piperidine] ($t_{\frac{1}{2}}$ = 250, 120, 70 min. for 10^{-2}, 2×10^{-2}, 4×10^{-2} M piperidine respectively, (Table 5.8)).

Since sensible kinetic dependencies could not be obtained, the extent of reaction with reagent concentration was examined. The results are summarised in Table 5.7 and 5.8 and show that as for reactions in EtOH, the limiting yield depends principally on the Ph_2NNO : amine ratio. For $CuC\ell_2$, the limiting yield varies between 50-75% of the initial $[Ph_2NNO]$ with equimolar Ph_2NNO and piperidine, with higher yields applying to the higher $[CuC\ell_2]$. The results can be rationalised by assuming that $CuC\ell_2$ produces only 50% N-nitrosamine formation because of concurrent amine protonation (Equation 5.10) and that further

Table 5.7

The yield and time of N-nitrosopiperidine (NNP) formation from N-nitroso-diphenylamine (NDPA) in the presence of $CuC\ell_2$ in anaerobic dioxan at 53°C.

10^3[$CuC\ell_2$]/M	10^2[NDPA]/M	10^3[Piperidine]/M	% Yield[a]	Time[b] min.
0	10	10	5	1200
1	10	10	25 (50)[c]	220
2	6.48	6.6	25 (50)[c]	310
5.4	10	10	25 (50)[c]	140
6.7	6.4	3.3	50 (100)[c]	160
6.97	6.4	6.6	37 (75)[c]	360
6.97	25.6	6.6	50 (100)[c]	260
7.62	8.11	8.33	31 (62)[c]	280
8.2	8.99	8.33	45	1200
8.6	9.0	1.0	53	1200
10	10	10	37.5 (75)[c]	250
10	20	10	50 (100)[c]	155
10	20	10	90	1200
10	10	10	50 (100)[c]	85
10	10	40	6.25 (12.5)[c]	140
10.9	10	10	46	1200

[a] % Yield based on initial [Amine]

[b] Time taken to reach % yield

[c] Maximum yield after 1200 min.

Table 5.8

The Yield and times of N-nitrosopiperidine formation from N-nitrosodiphenylamine in the presence of CuCℓ in anaerobic dioxan at 53°C.

10^3[CuCℓ]/M	10^3[NDPA]/M	10^3[Piperidine]/M	% Yield[a]	Time[b] min
1	10	10	12.5 (50)[c]	215
2.3	6.5	6.6	45	1200
5.0	10	10	12.5 (50)[c]	215
5.4	10.2	10	54	1200
6.8	6.5	6.6	52	1200
6.8	25.48	6.6	100	1200
8.1	6.5	3.3	100	1200
8.5	8.2	8.3	49	1200
10	10	10	25 (50)[c]	250
10	10	20	12.5 (25)[d]	120 (260
10	10	40	6.25 (12.5)[d]	70 (200

[a] % Yield based on initial [Amine]

[b] Time taken to reach % yield

[c] Maximum yield on standing overnight

[d] Maximum yield after time in parenthesis

$$CuC\ell_2 + Ph_2NNO \xrightarrow[2R_2NH]{53^oC,\ dioxan} R_2NNO + \overset{+}{R_2NH_2}C\ell^- + CuC\ell + Ph_2\dot{N} \quad \ldots \quad 5.10$$

nitrosation requires neutralization by the $CuC\ell \rightleftharpoons CuC\ell_2$ redox process (Equation 5.11).

$$Cu^{I}C\ell + Ph_2NNO \xrightarrow[-Ph_2\dot{N}]{dioxan,\ 53^oC} Cu^{II}(NO^-)X \xrightarrow{\overset{+}{R_2NH_2}C\ell^-} Cu^{II}C\ell_2 + R_2NH + NOH \quad \ldots\ 5.11$$

For low concentration of $Cu^{II}C\ell_2$ the catalysis approximates to the $CuC\ell$ reaction, the redox cycle requiring 2 moles of NO per mole of N-nitrosamine formation should be theoverriding importance.

For $CuC\ell$ catalysis the maximum yield is 50% with equimolar Ph_2NNO and piperidine which is consistent with the disproportionation of 2NO into NO^- and NO^+. With a 2 fold excess of Ph_2NNO, quantitative yields of N-nitrosamine are obtained. The NO^- would be evolved as NOH and significantly N_2O is identified as a co-product of both $CuC\ell_2$ and $CuC\ell$ reactions.

5.2.4 Discussion.

The obscure kinetic dependencies observed for the transnitrosation reactions in both anaerobic EtOH and dioxan suggest that either homolysis of Ph_2NNO to give NO or the diffusion of NO into solution is partially rate-limiting. The most important quantitative observations, however, are that the maximum yield of transnitrosated product is dependent on the initial [Ph_2NNO], and that $CuC\ell_2$ catalysis is faster than $CuC\ell$. The mechanism of amine nitrosation is probably the same as that discussed for gaseous NO in the preceding sections.

5.2.5. Other Metal Salts.

5.2.5. I Results and Discussion.

The effect of several metal salts other than copper on the formation of N-nitrosopiperidine from piperidine and saturated (ca. 2.2×10^{-2} M) NO in anaerobic EtOH are given in Table 5.9. For most reactions, the solutions were analysed over a period of time and the amount of product steadily increased. In Table 5.9, however, only the yield of N-nitrosopiperidine at the time the experiment was concluded is cited. Usually, when this 'final' yield was estimated, the reaction had virtually ceased and N-nitrosopiperidine was increasing very slowly with time.

Formation of N-nitrosopiperidine at 25°C is significant only in the presence of $CoSO_4$, $PdC\ell_2$ and $PtC\ell_4$. No reaction occurred with $NiC\ell_2$, $CdC\ell_2$, $HgC\ell_2$ and $Hg(OAc)_2$ unless the solution was heated to 60°C. This heating may cause disproportionation of the salts. With $FeC\ell_3$ and $Fe(NO_3)_3.9H_2O$ very little reaction occurred in EtOH even on heating over 48 hrs at 40-50°C. Much faster reactions were observed in solvents other than EtOH and these are discussed further below.

The reactions in the presence of $PdC\ell_2$ and $PtC\ell_4$ proceeded more rapidly than the remainder so these were examined further.

Passage of NO through a brown suspension of $PdC\ell_2$ in anaerobic EtOH at 25°C gave a red solution indicative of the formation of a $[PdNO]C\ell_2$ complex.[87] The colour discharged instantaneously on the addition of 0.01 M piperidine, but little N-nitrosopiperidine formed over a period of ca. 4 h . On standing overnight, however, the red colour reappeared and substantial amounts of N-nitrosopiperidine (as

Table 5.9

The yield and reaction times of N-nitrosopiperidine (NNP) in the presence of metal salts in anaerobic EtOH. Initial saturated [NO] = ca. 2.2×10^{-2} M, [piperidine] = 10^{-2} M.

10^3[catalyst]/M		Temp. °C	% Yield NNP[a]	Time min.
5	$CoSO_4$	25	45	95
5	$NiCl_2$	60	32	360
5	$CdCl_2$	60	15	260
5	$HgCl_2$	60	48	1300
10	$Hg(OAc)_2$	60	10	150
5.3	$Fe(NO_3)_3.9H_2O$	45	3	1200
10	$FeCl_3$	45	1	1200
2.64	$PdCl_2$	25	22	1200
4.75	$PdCl_2$	25	50	1200
13.2[b]	$PdCl_2$	25	62.5	1200
14.8	$PdCl_2$	25	45	1200
5.95	$PtCl_4$	25	31	30
10.70	$PtCl_4$	25	4	55
4.63[c]	$PdCl_2$	25	12	1200
5.34[d]	$PdCl_2$	25	5	1200

[a] % Yield based on initial [piperidine]

[b] In the presence of 1.5×10^{-2} M piperidine

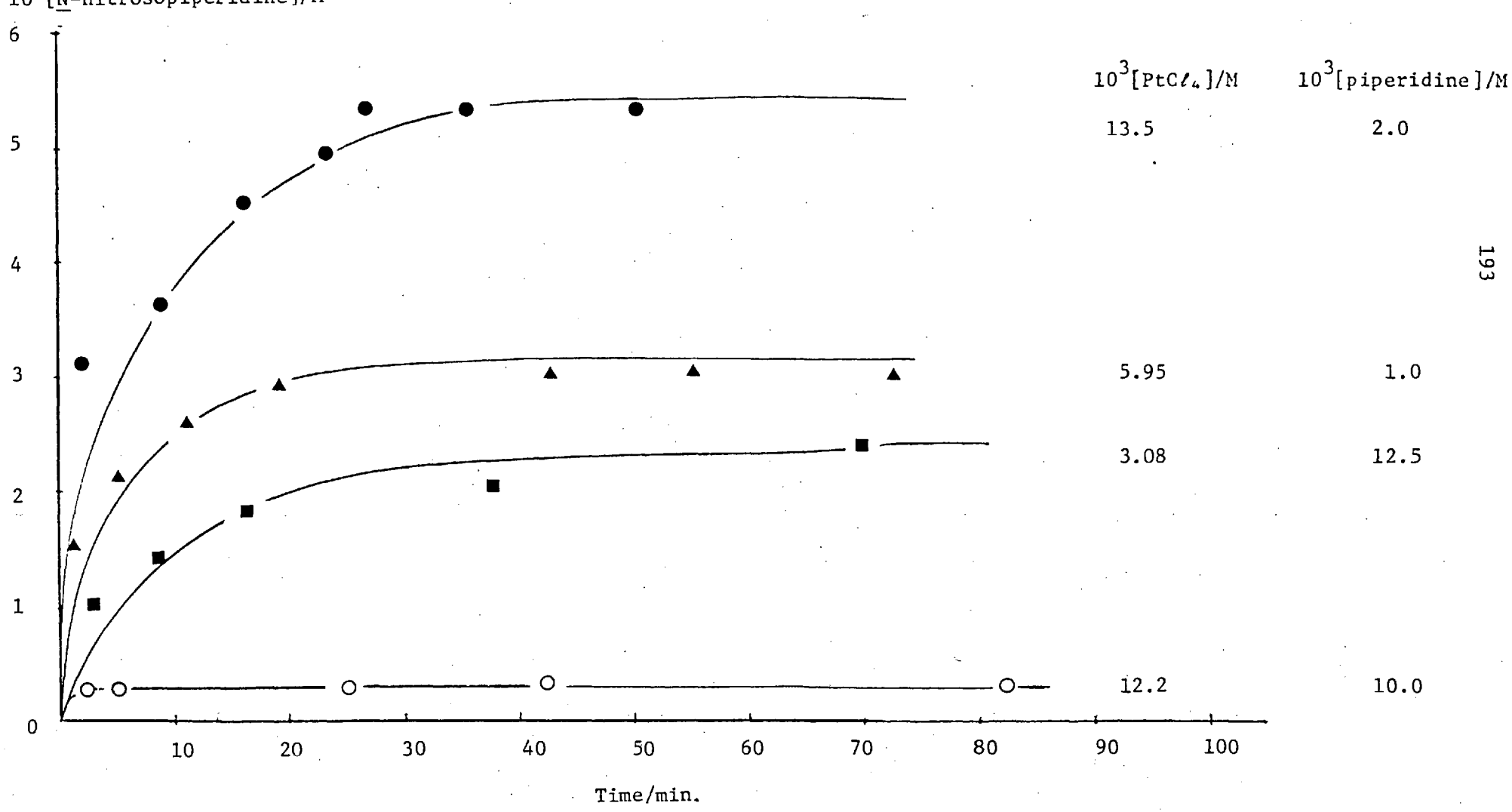
[c] In the presence of 10^{-2} M N-methylpiperazine in MeCN

[d] In the presence of 10^{-2} M N-methylpiperazine.

cited in Table 5.9) were formed. These reactions show some similarities with those in the presence of $AgNO_3$ (Chapter 4). Thus, the reaction has an induction period, and the yields of N-nitrosopiperidine appears to be dependent on the $PdC\ell_2$/piperidine ratio. With excess piperidine, the yield is roughly proportional to $[PdC\ell_2]$ but with equimolar piperidine the yield is about 50%. Also, no reaction was evident with N-methylpiperazine in place of piperidine. However, precipitation of Pd^0 was not apparent, so a simple redox analogue to the Ag^I reaction is unlikely. Also, it seems very unlikely that the $[PdNO]C\ell_2$ complex is not involved.

No reaction was evident on passing NO into solutions of $PtC\ell_4$ in EtOH at 25°C. Addition of 0.01 M piperidine, however, resulted in rapid N-nitrosopiperidine formation and the concurrent formation of a yellow precipitate which remained throughout the reaction. The precipitate was isolated and washed thoroughly with EtOH and dried under vacuo. Elemental analysis (C, 23.35 ; H, 4.77 ; N, 4.79%) suggested the composition $[Pt(piperidine)_2]C\ell_4$ but it is also possible that the complex is $Pt(piperidine.HC\ell)_2C\ell_2$ (C, 23.35 ; H, 4.77 ; N, 5.5%). Its significance to the reaction is not known. Variation in the amount of N-nitrosopiperidine with time in Figure 5.8 gives a clear indication of both the rapidity of these reactions and some of the kinetic dependencies. In particular, the extent of reaction appears to be dependent on the $PtC\ell_4$: piperidine ratio. Thus, with equimolar concentration of these reagents little N-nitrosopiperidine forms over ca. 60 min, unless another base (e.g., NEt_3) is added when N-nitroso-piperidine is produced rapidly ($t_{\frac{1}{2}}$ ca. 2 min) and quantitatively. With excess piperidine, however, ca. 25% of the amine reacts unless NEt_3 is

Figure 5.8. The dependence of $PtCl_4$: piperidine ratio on the rate and maximum yield of N-nitrosopiperidine in anaerobic EtOH at 25°C. Initial saturated [NO] ca. 2.2×10^{-2} M.



added when the yield again increases. Further, the rate of N-nitrosamine formation is dependent on the structure of the amine. For example, piperidine (pK_A 11.22)> morpholine (pK_A 8.33)> N-methylpiperazine (pK_A 9.8, 5.11).

A crucial factor appears to be the formation of a Pt^{IV}-amine complex which, in principle, could oxidise both NO or R_2NH. Surprisingly, the reaction is catalysed by added KN_3. For example, with 6×10^{-3} M $PtC\ell_4$, 4×10^{-3} M piperidine and saturated NO in EtOH, no N-nitroso-piperidine formation was evident at 25°C, but addition of 5.5×10^{-3} M KN_3 gave quantitative (92%) N-nitrosopiperidine, ca. 17 min. This can be tentatively rationalised by formation of a Pt^{IV}-N_3^- complex which oxidises like the Pt^{IV} amine complex.

5.2.5.II Reactions with Iron Salts.

Anhydrous ferric chloride and hydrated ferric nitrate were observed to form green and red nitrosyl complexes, respectively, on contact with NO in EtOH at 25°C. Addition of 10^{-2} M piperidine to the complex, obtained from either 10^{-2} M $FeC\ell_3$ or 5.3×10^{-3} M $Fe(NO_3)_3.9H_2O$ did not discharge the colour or produce N-nitrosopiperidine. The nitrosyl complexes appeared to be very stable, with no apparent change on heating to 40-50°C for 48 h.

Significant reaction was observed, however, using MeCN or dioxan in place of EtOH as solvent (Table 5.10) . For example, 5.24×10^{-3} M $Fe(NO_3)_3.9H_2O$ and 10^{-2} M piperidine gave 100% N-nitrosopiperidine at 25°C after ca. 8 min in either dioxan or MeCN compared to ca. 5% over 300 min. in the absence of $Fe(NO_3)_3.9H_2O$. The results in Table 5.10) show several characteristic features:-

Table 5.10

The yield and time of formation of N-nitrosopiperidine (NNP) from piperidine in the presence of Iron salts in anaerobic MeCN at 25°C. Initial saturated [NO] = 1.4×10^{-2} M.

10^3[Catalyst]/M		10^2[Piperidine]/M	% Yield[a] NNP	Time[b] min.
0		1.0	5	300
5.24[c] (5.10)	$Fe(NO_3)_3.9H_2O$	1.0	100 (100)[d]	8[c] (8)
1.92	"	1.0	100	100
0.74 (0.69)[d]	"	1.0	45[d] (59)[e]	50
0.67 (0.64)	"	0.5	95	100
0.429	"	2.0	50[e]	1200
6.46	$FeBr_3$	1.0	50 (80)[e]	3
5.45	$FeCl_3$	1.0	50 (80)[e]	200
10.9	$Fe(ClO_4)_3$	1.0	4[e]	1200
11.2	$FeCl_2$	1.0	50	70
10.3	$FeCl_2$	1.0	100	1200
3.3	$FeCl_2$	1.0	30 (77)[e]	80
10.4	$FeSO_4$	1.0	37 (75)[e]	120
0		1.0[f]	5	300
2.14	$Fe(NO_3)_3.9H_2O$	1.0[f]	100	90

[a] % yield based on initial [Amine]

[b] Time to reach yield

[c] In anaerobic dioxan, 25°C

[d] Duplicate reactions in parenthesis

[e] Maximum yield on standing overnight

[f] In the presence of 10^{-2} M N-methylpiperazine

1) The reaction rate is enhanced by both Fe^{II} and Fe^{III} salts.

2) The degree of enhancement is dependent on the counter ion, e.g., $FeBr_3$ > $Fe(NO_3)_3$ ~ $FeCl_3$ >> $Fe(ClO_4)_3$ and $FeCl_2$ > $FeSO_4$. This suggests that the nucleophilic reactivity of the anion is important.

3) The relationship between reaction rate and limiting yield is not straightforward. The limiting yield is higher than the initial salt concentration, which suggests that the salt acts as a catalyst (as for Cu^{I}, Cu^{II}) rather than a promoter (as in the case of Ag^{I} salts).

4) Analysis of the gaseous products by i.r. showed N_2O at concentrations ca. 20% relative to the final yield of N-nitroso-piperidine.

5.2.5.III. Discussion.

The iron catalysed N-nitrosamine formation can be accommodated within the reports of the oxidation of NO to NO^+ and NO^- by Fe^{III} and of the disproportionation of 2NO into NO^+ and NO^- by Fe^{II} porphyrins. The intermediacy of NOX (and inter alia NO^+) seem to be required by the dependence on the counter ion. This can be accommodated for Fe^{III} salts by formation of an $[Fe^{II}\text{-}NO^+]$ complex. There is no apparent dependence on the structure of the amine which may imply that the solvent MeCN (and possibly dioxan) displaces NO^+ to form the complex and to give NOX which is stable in the non-nucleophilic solvent (Equation 5.12). The ability of MeCN to displace arene

$$Fe^{III}X_3 + NO \longrightarrow [Fe^{II}\text{-}NO^+]X_3 \rightleftharpoons^{MeCN,\ 25^oC} [Fe^{II}(MeCN)_n]^{2+} + NOX \quad \ldots\ 5.12$$

diazonium intermediates has been reported in connection with Ru-nitrosyl reactions with aromatic amines.[102]

To account for 100% yields with both Fe^{III} and Fe^{II} salts and the evolution of N_2O as a co-product, both added Fe^{II} salts and the Fe^{II} complex generated in equation 5.12, above, could induce reductive disproportionation of NO into NO^+ and NO^- (Equation 5.13). Displacement of NO^+ by MeCN would give NOX and

$$Fe^{II}X_2 + 2NO \longrightarrow [Fe^{II}(NO)(NO^-)]X_2 \quad .... \quad 5.13$$

NO^-, with N-nitrosamine and N_2O formation arising from the reaction of NOX and NO^-, respectively. Disproportionation of NO by Fe^{II} species rather than Fe^{III} (Equation 5.14) is preferred because the reaction of $Fe(NO_3)_3.9H_2O$ and $FeC\ell_2$ with piperidine gave a dark red

$$Fe^{III} + 2NO \longrightarrow [Fe^{III}(NO^+)(NO^-)] \quad .... \quad 5.14$$

precipitate after passage of NO that <u>did not</u> oxidise KI to I_2, whereas under the same test conditions, $FeC\ell_3$ and KI gave yellow FeI_2 and I_2.

Thus, the preferred reaction pathways for the nitrosation of secondary amines are those shown in Scheme 5.7.

$$Fe^{III}X_3 + NO \xrightarrow{MeCN,\ 25^oC} [Fe^{II}(NO^+)]X_3 \xrightarrow{R_2NH} R_2NNO + HX + FeX_2$$

$$FeX_2 + 2NO \xrightarrow{MeCN,\ 25^oC} [Fe^{II}(NO^+)(NO^-)]X_2$$

$$\downarrow R_2NH$$

$$[Fe^{II}(MeCN)n]X_2 + R_2NNO + NOH$$

$$\downarrow$$

$$\tfrac{1}{2}N_2O + \tfrac{1}{2}H_2O$$

SCHEME 5.7

Both are analogous to those proposed for catalysis by Cu^{I} and Cu^{II} salts.

5.3. Conclusion.

We have shown that Cu^{I} and Cu^{II} salts catalyse N-nitrosamine formation from gaseous NO and secondary amines in anaerobic dioxan at 25°C. The reactions are fast, for example, 8×10^{-4} M $CuC\ell_2$ give quantitative yields of N-nitrosamine within ca. 60 min. The reaction appears to involve the metal bound amine ligand and not the copper nitrosyl $[Cu^{II}(NO)]X_2$ but several mechanistic details are unclear. Support for the reaction via the bound amine comes principally from the inhibition by NEt_3, which appears to complex preferentially with the metal salt. Further, the reactions appear to involve a redox cycle $Cu^{I} \rightleftharpoons Cu^{II}$ which amounts to the reduction of NO to NO^{-}, which ultimately is evolved as N_2O ($2NOH \longrightarrow N_2O + H_2O$). Transnitrosation reactions using Ph_2NNO in place of NO in the presence of Cu^{I} and Cu^{II} salts also leads to rapid N-nitrosamine formation. These studies supported the conclusion that NO is reduced by Cu^{I} salts, but otherwise were of little help because either homolysis of Ph_2NNO (to give $Ph_2\dot{N}$ and $\dot{N}O$) or diffusion of NO into solution is rate-limiting.

Other metal salts also catalysed N-nitrosamine formation under suitable conditions. For most the mechanism could not be deduced, but much of the evidence points to nitrosation by NOX reagents. Fe^{II} and Fe^{III} salts, however, appear to catalyse N-nitrosamine formation by similar mechanisms to those for Cu^{I} and Cu^{II} salts.

5.4. EXPERIMENTAL

5.4.I. Reagents, Substrates, and Products.

Amine substrates, MeCN, 1,4-dioxan, NO, the preparation and purification of authentic N-nitrosamine has been described in Chapter

2.4.II. AnalaR tetra-ethylammonium bromide was used without further purification. Reagent grade $CdCl_2$, $CoSO_4$, $CuBr_2$, $Cu(NO_3)_2$, $Cu(OAc)_2$, $CuSO_4$, $FeBr_3$, $FeCl_3$, $FeCl_2$, $Fe(ClO_4)_3$, $Fe(NO_3)_3.9H_2O$, $FeSO_4$, $HgCl_2$, $Hg(OAc)_2$, $NiCl_2$, $PdCl_2$, $PtCl_4$ were used without further purification. AnalaR $CuCl_2$ was dried at 110°C and stored in vacuo. CuCl was prepared from anhydrous $CuCl_2$ (9 g) by heating with glycerol (30 ml) at 180-200°C for 10-15 min.[134] The white crystals were filtered, washed thoroughly with EtOH and vacuum dried. The purity was checked by adding acidic KI to a solution of CuCl in EtOH. There was no evidence of I_2 formation, indicative of $CuCl_2$. CuI was prepared as described in Chapter 3.1. Tetra-n-butylammonium azide was prepared following a literature procedure.[181] The white hygroscopic crystals were handled in a glove box filled with argon.

N-nitrosodiphenylamine was prepared by the action of nitrous acid on diphenylamine,[127] and purified by recrystallisation from MeOH. N-nitrosodiphenylamine, pale yellow solid, m.p., 67°C, lit[127] 68°C.

5.4 (II) Reaction Methods.

The apparatus and reaction procedure for studying the metal catalysed N-nitrosamine formation with gaseous NO was described in Chapter 2.4. .

Transnitrosation.

N-nitrosodiphenylamine and the catalyst was placed in either the 3-neck reaction flask or a 50 ml. conical flask, the appropriate solvent added and solution degassed as previously described. For reactions carried out in the conical flask, the system was sealed with a Subaseal

stopper and degassed with purified nitrogen using two 5" hypodermic needles as inlet and outlet.

The reaction vessel was then placed in a thermostatted bath at 60°C or 53°C (± 0.2°C) and left to equilibrate for 40 min. After this period, the reaction was initiated by injecting neat amine via the Subaseal stopper and N-nitrosamine formation analysed by g.l.c. The same procedure was adopted for reactions in the absence of metal catalysts, the solutions were degassed as above or oxygenated by bubbling oxygen (B.O.C.) for 30 min.

5.4. (III) Product Analysis.

5.4. III a. N-nitrosamine Formation.

The yield of N-nitrosamine was assayed by g.l.c. on Perkin-Elmer F33 and Perkin Elmer F11 instruments. The columns, operating conditions and the retention times of N-nitrosamines are listed in Chapter 2.4.III.b .

5.4. III b. N_2O Formation.

The $[N_2O]$ released from the reaction of $Cu^{II}C\ell_2$, $Cu^{I}C\ell$, $Fe^{III}(NO_3)_3.9H_2O$ and $Fe^{II}C\ell_2$ was estimated using the same apparatus following the procedure described in Chapter 3.3.3 . b .2 . From the reaction of 2.5×10^{-3} M $CuC\ell$, 10^{-2} M N-methylpiperazine and saturated NO in dioxan at 25°C, 10^{-2} M N-methyl-N-nitrosopiperazine formed. The gaseous product was extracted into the i.r. cell and gave peak heights of 122 at i.r. stretching frequency 2240 cm^{-1}, characteristic of N_2O .[135] The recovery of N_2O was estimated by comparison with standard additions of authentic N_2O in the i.r. cell. Additions of

1.0, 2.0 ml N_2O gave peak heights 58, 58, 60 and 96, 98, 100, respectively. Since 5×10^{-3} M (≡ 4.46 ml) N_2O is expected for 10^{-2} M N-nitrosamine formation under the reaction conditions, (i.e., 40 ml), the % recovery was estimated as:-

$$\frac{122 \times 2.0}{4.46 \times 98} \times 100 = 55.8\%$$

(N_2O volume was not corrected to S.T.P.).

By the same procedure, 2.57 ml, N_2O was recovered from the reaction of 9×10^{-4} M $CuC\ell_2$, 10^{-2} M piperidine and saturated NO in dioxan at 25°C. After g.l.c., analysis showed that 10^{-2} N-nitrosopiperidine had formed. The % recovery was estimated as ca. 57% based on 5×10^{-3} M N_2O maximum.

N_2O (ca. 1.8 ml, 40% based on final yield of N-nitrosamine formation was also recovered from the reactions of 5×10^{-3} M $Fe(NO_3)_3.9H_2O$, 10^{-2} M $FeC\ell_2$ and 10^{-2} M piperidine with saturated NO in MeCN at 25°C.

N_2O was also identified from the transnitrosation reactions involving $Cu^{II}C\ell_2$ and $Cu^{I}C\ell$ in dioxan at 53°C. There was no evidence of N_2O formation in the absence of the metal salts. However, the recovery was very low, with peak height of N_2O at 2240 cm^{-1} of 3-6, which corresponds to ca. 0.1 ml N_2O. It was shown, however, that N_2O was lost from the reaction vessel presumably via the Subaseal or inlet, outlet joints. For example, 10 ml, N_2O in dioxan at 25°C gave an off-scale reading at i.r. stretching frequency 2240 cm^{-1}, whereas on heating at 53°C overnight, gave peak height of 5. Attempts were made to seal the joints using Teflon sleeves, but the recovery of N_2O did not increase significantly.

5.4.(IV) Analysis of Reaction Products from the Transnitrosation Reactions.

After g.l.c. analysis had shown that the yield of transnitrosated product had reached a maximum, the reaction solution was analysed by T.L.C. on (1 mm silica plates, 20" x 20", developed with benzene) R_F Ph_2NNO = 0.5, Ph_2NH = 0.6, Ph_2N-N-Ph_2 = 0.71 was determined with authentic materials.

In the absence of metal catalyst under anaerobic conditions, three spots were identified to be unreacted Ph_2NNO, Ph_2NH and Ph_2N-NPh_2. The major products were Ph_2NH, Ph_2NNO and traces of Ph_2N-NPh_2. Analyses of the reaction solution after 21 days, carried out in aerobic dioxan at 53°C showed only two spots corresponding to Ph_2N-NPh_2 and Ph_2NH. Only Ph_2NH was identified from the reactions involving $Cu^{II}C\ell_2$ and $Cu^{I}C\ell$, there was no evidence of Ph_2N-NPh_2 formation. It has been reported, however, that on heating the colourless Ph_2N-NPh_2 in toluene at 90°C a brown-green solution was obtained that did not give an e.s.r. signal for the diphenylamino radical ($Ph_2\dot{N}$), and it was concluded that $Ph_2\dot{N}$ may have polymerised to 5,10-diphenyl-5,10-dihydrophenazine and diphenylamine.[182] There was no evidence of this polymeric material in the transnitrosation reactions, but its formation may explain the continual presence of Ph_2NH in the analyses.

5.4. (V). Estimation of Saturated [NO] in Dioxan at 25°C.

The concentration of saturated NO in dioxan at 25°C was estimated by a method described by Armor[183] and by a more direct u.v. method (Chapter 2.4.VI).

In the method by Armor, 1-5 ml. saturated NO in dioxan was injected into oxgyen-saturated water (80 ml) and stirred vigorously. Nitric oxide is oxidised rapidly and quantitatively to NO_2^- with excess oxygen. The method of analysis involved the reaction of NO_2^- with acidic sulphanilamide and N-1-naphthylethylene diamine dihydrochloride.[130] The concentration of dissolved NO by this method gave [NO] ca. 1.85×10^{-2} M. This value agrees reasonably well with the direct U.V. method which gave [NO] ca. 1.6×10^{-2} M.

5.4.VI a. Typical Reactions.

$[CuC\ell_2]$ = 2.50 x 10^{-3} M, [piperidine] = 10^{-2} M, in anaerobic dioxan at 25°C. Initial saturated [NO] = ca. 1.6 x 10^{-2} M

Time min.	Attenuation	Peak Height	10^3 [N-nitrosopiperidine]/M
1	x 128 x 10	8	1.33
6		21.5	3.6
9		32	5.3
13		39	6.5
20		45	7.5
24		49	8.2
28		51.5	8.6
∞		60	10.0

Injection sample = 1 µl

1 µl 10^{-2} M N-nitrosopiperidine at attenuation x 128 x 10 gave peak height 60, 60 on Perkin-Elmer F33 under standard operating conditions (Experimental 2.4.IV).

5.4.VI. b Typical Reactions.

$[CuC\ell_2] = 2.5 \times 10^{-3}$ M, [piperidine] = 10^{-2} M, in anaerobic dioxan 25°C. [NO] = excess.

Time min.	Attenuation	Peak Height	10^3 [N-nitrosopiperidine]/M
1	x 128 x 10	8.5	1.4
5		20.5	3.4
9		30	5.0
13		45	7.5
17		60	10.0
21		60	10.0

Injection sample = 1 µl.

1 µl 10^{-2} M N-nitrosopiperidine at attenuation x 128 x 10 gave peak height 60, 60, 60 on Perkin-Elmer F33 under standard operating conditions (Experimental 2.4.IV).

5.4.IV. c Typical Reactions.

$[CuC\ell] = 2.5 \times 10^{-3}$ M, [piperidine] = 10^{-2} M in anaerobic dioxan at 25°C. [NO] = excess.

Time min.	Attenuation	Peak Height	10^3 [N-nitrosopiperidine]/M
1	x 128 x 10	4	0.7
5		20	3.3
9		30.5	5.1
14		47	7.8
18		54.5	9.1
21		60	10.0
26		60	10.0

Injection sample = 1 µl

1 µl 10^{-2} M N-nitrosopiperidine at attenuation x 128 x 10 gave peak height 60, 60 on Perkin-Elmer F33 under standard operating conditions (Experimental 2.4.IV).

5.4.IV. d Typical Reactions.

$[Fe(NO_3)_3.9H_2O] = 7.0 \times 10^{-4}$ M, [piperidine] = 10^{-2} M in anaerobic MeCN at 25°C. Initial saturated [NO] ca. 1.4×10^{-2} M.

Time min.	Attenuation	Peak Height	10^3 [N-nitrosopiperidine]/M
2	x 500	15	1.36
7		22	2.0
12		28	2.54
16		27	24.5
20		28	
25		27	
29		28	
42		35	3.2
52		39	3.5
57		40	3.6
75		45	4.1
96		59	5.36
137		67	6.1
∞ (1200)	x 1000	55	10.0

Injection sample = 3 µl

3 µl 4.5×10^{-3} M N-nitrosopiperidine gave at attenuation x 500 50, 49, 50, 49 on Perkin-Elmer F11 under standard operating conditions (Experimental 2.4. IV).

CHAPTER 6

THE FORMATION AND DECOMPOSITION OF N-NITROSAMINES BY γ-RADIATION.

6.1. Introduction.

6.1.1 The Radiolysis of Aqueous-Solutions.

The passage of high energy, ionising radiation, such as γ-rays from a ^{60}Co source, through aqueous solutions gives initially electrons, positively charged water ions and excited water molecules[184-188] (Equation 6.1). The electrons lose energy

$$H_2O \xrightarrow{\gamma} e^-, H_2O^{+\cdot}, H_2O^* \qquad \ldots\ldots \quad 6.1$$

in approximately 10^{-11} seconds by further collisions and when they reach a thermal energy of 4×10^{-21} Joules at room temperature, become solvated[185] $e^-_{aq.}$; $H_2O^{+\cdot}$ is unstable and decomposes in 10^{-13} seconds to $HO^{\cdot}$ and H^+. Excited water molecules (H_2O^*) decompose to $\dot{H}$ and $\dot{HO}$.[185] The entities $e^-(aq)$, $\dot{H}$, $\dot{HO}$ are formed in proximity so they react together giving the molecular products[185,188] H_2O_2, H_2, shown in Scheme 6.1.

$$H_2O \xrightarrow{\gamma} \begin{cases} e^- \longrightarrow e^-(aq) \\ + \\ H_2O^{+\cdot} \longrightarrow \dot{HO} + H^+ \\ \uparrow \\ H_2O^* \longrightarrow \dot{HO} + \dot{H} \end{cases} \Bigg\} \; H_2O,\ H_2O_2,\ H_2$$

SCHEME 6.1

The hydrated electron is the simplest nucleophilic reagent. It is highly selective and reacts at diffusion controlled rates with strong oxidising agents, NO_3^-, unhydrated aldehydes and ketones.[188-192] Generally, it will add to organic compounds bearing a partial positive charge.[189]

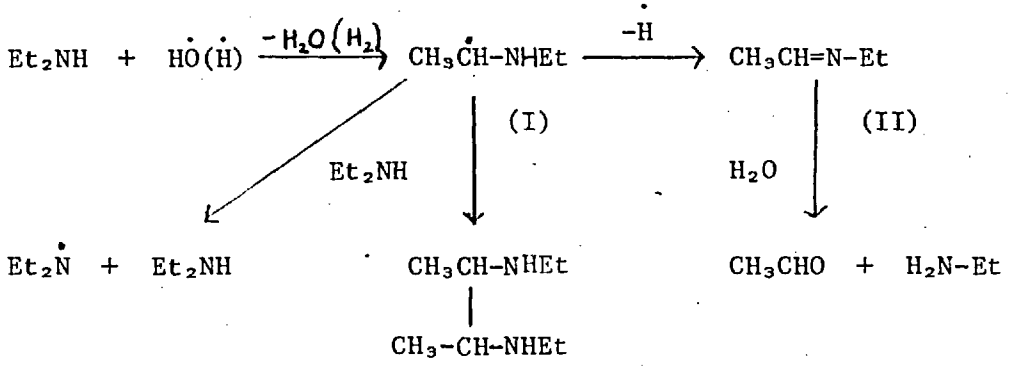
The main reaction of $\dot{H}$ is H-abstraction, although double bond additions are known.[189] H-abstraction occurs more readily α- to -OH or $-CO_2H$ (Equation 6.2 and 6.3 respectively) groups and from methine and methylene rather than methyl groups.[189]

$$RCH_2\text{-}OH + \dot{H} \longrightarrow \dot{R}CH\text{-}OH + H_2 \quad \ldots\ldots \quad 6.2$$

$$RNH_2\text{-}CH_2\text{-}CO_2H \longrightarrow RNH_2\text{-}\dot{C}H\text{-}CO_2H + H_2 \quad \ldots\ldots \quad 6.3$$

The oxidising species $\dot{HO}$ can undergo four types of reaction: (I) charge transfer, e.g., $X^- + \dot{HO} \longrightarrow HO^- + X$,[193] with a variety of anions, halides, thiocynate ; (II) H-abstraction, e.g., $CH_3OH + \dot{HO} \longrightarrow \dot{C}H_2\text{-}OH + H_2O$;[189] (III) addition, particularly when π-electrons are present[190,193] and (IV) displacement, e.g., $RI + \dot{HO} \longrightarrow ROH + I^{\cdot}$.[194]

The reaction of the radicals produced by the radiolysis of water with organic compounds such as alcohols,[190] amino-acids,[195] acids, aldehydes and ketones[190] has been reviewed and will not be discussed here. Both $\dot{HO}$ and $\dot{H}$ are reported to react with amines in aqueous solution,[196-198] but the chemical yields are very low. The initial step as shown for Et_2NH in Scheme 6.2 is H-abstraction, α- to the amino group to give (I), followed by rearrangement to the imine (II), and subsequent hydrolysis to aldehyde, and amino compounds. (I) can undergo concurrent radical dimerisation.



SCHEME 6.2

A direct H-abstraction from the parent amine to give $Et_2\dot{N}$ occurs only slightly but $Et_2\dot{N}$ is generated by a secondary reaction of (I) with Et_2NH (Scheme 6.2). The one exception to this general rule is that primary aromatic amines react with e^-_{aq} and $H\dot{O}$ to give the amino radical cation.[199]

6.1 (2). Radiolysis of Alkali Metal Nitrates and Nitrites.

The radiolysis of aqueous nitrate solutions has received much attention,[186,189,200-204] but speculation remains about the finer details of the mechanism. The products of the reaction of NO_3^- with the reducing radicals, however, are well established,[204,205] (Equation 6.4).

$$NO_3^- \longrightarrow NO_2^- + \tfrac{1}{2} O_2 \qquad \ldots\ldots \quad 6.4$$

NO_3^{2-}, $\dot{N}O_2$ and $\dot{N}O_3$ intermediates have been identified by e.s.r.,[186,201-202]. The most popular mechanism involves attack by $\dot{H}$ [205] (Equation 6.5) and e^-_{aq} [206] (Equation 6.6) to give $N_2O_4 (\rightleftharpoons 2NO_2)$,

$$NO_3^- + \dot{H} \longrightarrow {}^-OH + \dot{N}O_2 \rightleftharpoons \tfrac{1}{2} N_2O_4 \quad \ldots\ldots 6.5$$

$$NO_3^- + e_{aq}^- \longrightarrow NO_3^{2-} \xrightarrow{H_2O} \dot{N}O_2 + 2\,{}^-OH \quad \ldots\ldots 6.6$$

which is then hydrolysed to NO_2^- [186] (Equation 6.7). However, NO_2^-

$$N_2O_4 + 3H_2O \longrightarrow NO_2^- + NO_3^- + 2H_3O^+ \quad \ldots\ldots 6.7$$

formation has also been attributed to the hydrolysis of N_2O_5 [204] (Equation 6.8).

$$\dot{N}O_2 + \dot{N}O_3 \rightleftharpoons N_2O_5 \xrightarrow{H_2O} 2NO_2^- + 2H_3O^+ + O_2 \quad \ldots\ldots 6.8$$

The central theme of NO_3^- reduction by γ-radiation is the formation of N_2O_x intermediates which give NO_2^- on hydrolysis. The radiation chemical yield[185] (G, = the number of NO_2^- molecules formed per 100 eV) is dependent on the amount of radiation, the concentration and the counter cation of the nitrate salt.[205]

Nitrite is also reduced by $\dot{H}$ [206] (Equation 6.9), e_{aq}^- [206] (Equation 6.10) and oxidised[206] by $\dot{H}O$ (Equation 6.11); this complicates the estimation of $G(NO_2^-)$ from NO_3^-.

$$NO_2^- + \dot{H} \longrightarrow NO_2^{2-} + H^+ \quad \ldots\ldots 6.9$$

$$NO_2^- + e_{aq}^- \longrightarrow NO_2^{2-} \xrightarrow{H_2O} NO + 2\,{}^-OH \quad \ldots\ldots 6.10$$

$$NO_2^- + \dot{H}O \longrightarrow \dot{N}O_2 + {}^-OH \quad \ldots\ldots 6.11$$

Nitric oxide produced by (Equation 6.9, 6.10) is also oxidised by $\dot{HO}$ to NO_2^- [207] (Equation 6.12).

$$NO + \dot{HO} \longrightarrow NO_2^- + H^+ \quad \ldots\ 6.12$$

The bimolecular rate constants[208] for the attacking radicals on nitrite and nitrate are summarised in Table 6.1

Table 6.1

Attacking Species	k. 1. mol^{-1} sec^{-1}.	
	NO_2^-	NO_3^-
e_{aq}^-	4.6×10^9	$0.8 - 1.1 \times 10^9$
$\dot{H}$	$6. \times 10^8$	2.4×10^9
$\dot{HO}$	2.5×10^9	

Eberhwilt[209] claims chemical evidence for the formation of $\dot{NO}_2$ from the radiolysis of $NaNO_3$, by observing the nitration of benzene.

The rates of recombination of $\dot{NO}_2$ (Equation 6.13) and the

$$\dot{NO}_2 + \dot{NO}_2 \rightleftharpoons N_2O_4 \quad , \quad k = 4.5 \times 10^8 \text{ 1 mol}^{-1} \text{ sec}^{-1} \quad \ldots\ 6.13$$

reaction of $\dot{NO}_2$ and $\dot{NO}$ (Equation 6.14) in water at 20°C has been

$$\dot{NO}_2 + \dot{NO} \rightleftharpoons \dot{N}_2O_3 \quad , \quad k = 1.1 \times 10^9 \text{ 1 mol}^{-1} \text{ sec}^{-1} \quad \ldots\ 6.14$$

measured by Grätzell, et al.,[207,210] in the pulse radiolysis of aqueous nitrite and NO respectively. The relevant dissociation constants are

$K_{N_2O_3}$ = 7.5×10^{-5} mol l^{-1} [207] and $K_{N_2O_4}$ = 1.53×10^{-5} mol l^{-1} [210] under the same conditions. They also found that the formation of N_2O_4 in equilibrium with N_2O_3 (Equation 6.15) lies well over to the left in water at 20°C.[207]

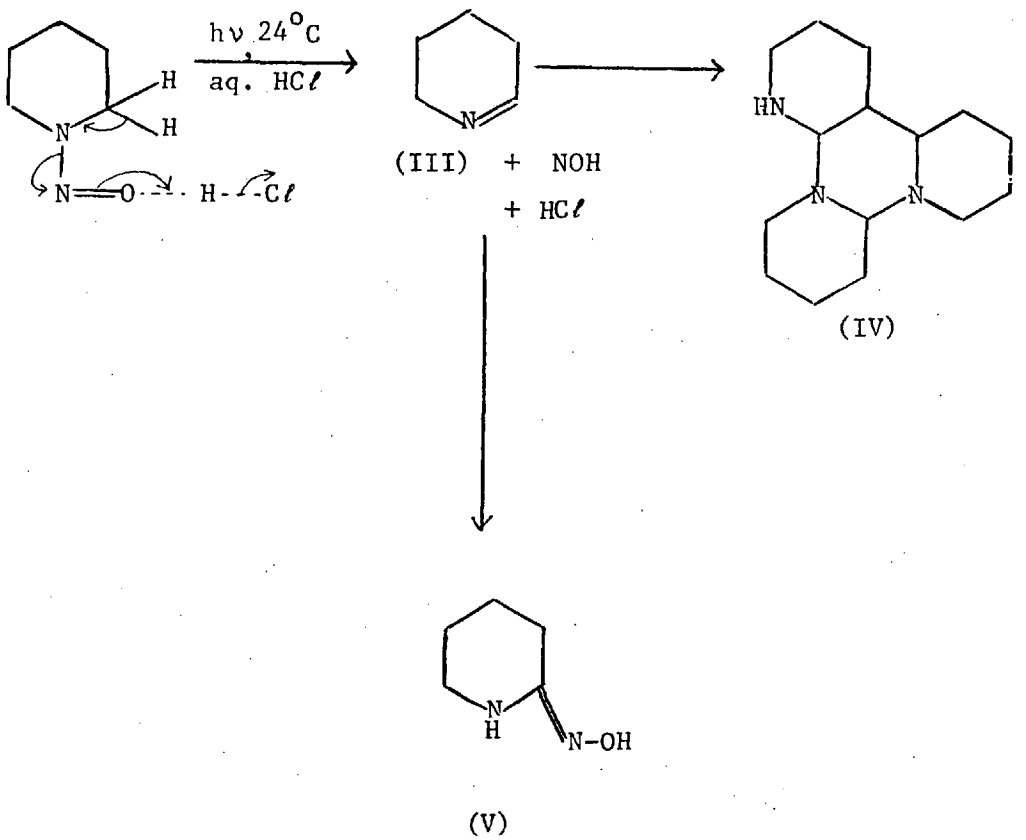
$$2N_2O_3 \rightleftharpoons N_2O_4 + NO, \qquad K = 3.5 \times 10^{-4} \text{ mol } l^{-1} \qquad \ldots. \; 6.15$$

There are many other forms of ionising radiation, e.g., Hg-photolysis,[211,212] X-rays,[213] radioactive isotopes[214] that reduce nitrate to nitrite, and in each case the presence of $\dot{N}O_2$ has been shown by e.s.r. Enzymatic denitrificants also reduce nitrate to nitrite,[33] only NO has been observed by e.s.r., but $\dot{N}O_2$ formation is suspected.[215] The electrochemical reduction of nitrate *via* $\dot{N}O_2$ in dimethylformamide has also recently been reported.[216]

6.1 (3). Concluding Remarks.

Since both N_2O_3 and N_2O_4 are known to form *N*-nitrosamines,[40] it follows that γ-radiation of aqueous nitrate may also produce *N*-nitrosamines. This deduction required verification however, because X-rays[217] and Hg-photolysis,[167,218-220] also decompose *N*-nitrosamines. Gowenlock and his colleagues[220] have recently reported that *N*-nitrosamines are decomposed under neutral conditions by photolysis. The extent of decomposition, the quantum yield was estimated by the evolution of NO, although the origin of NO was not discussed. Earlier, Chow[78] reviewed the photochemistry of *N*-nitrosamines and reported that *N*-nitrosopiperidine could only decompose photolytically in acidic media. Acid was thought to weaken the N-N bond of *N*-nitrosopiperidine by complexation (Scheme 6.3). The products obtained by Hg-photolysis

of N-nitrosopiperidine in 0.24 N HCℓ at 24°C were isotripiperidien (IV) and 2-piperidinoxime (V). To account for these products Chow assumed that the initial step involved photoelimination of NOH with the formation of the alkyldieneimine (III), which then polymerised to give (IV) or underwent nucleophilic attack at the 2-position by released NOH, to give (V).



SCHEME 6.3

6.2 Results and Discussion.

The experiments were carried out by passing γ-radiation from a ^{60}Co source (1.173 and 1.33 MeV, 1 M rad/h) through aqueous anaerobic solutions at 25°C. Preliminary results showed that N-nitrosamines formed from secondary amines and $NaNO_2$, and both N-nitrosamines and N-nitroamines from secondary amines and $NaNO_3$. For example, with 0.1 M $NaNO_2$ and 2 x 10^{-3} M morpholine, 100% N-nitrosomorpholine was obtained after 60 min. γ-irradiation, whereas with 0.1 M $NaNO_3$ and 2 x 10^{-3} M morpholine, ca. 20% N-nitroso- and 7.5% N-nitromorpholine were formed over the same period. However, because γ-radiation is likely to decompose N-nitrosamines, it was desirable to briefly examine these reactions first.

6.2 (I) Decomposition of N-nitrosamines and N-nitroamines by γ-Radiation.

Results listed in Table 6.2, show the extent of decomposition of N-nitrosopiperidine in aqueous anaerobic solution at ambient temperature as a function of time of γ-irradiation. A salient feature is that the extent of decomposition is independent of the initial [N-nitrosopiperidine] and depends only on the γ-dose. The corresponding plot of the extent of decomposition versus dose (Figure 6.1) is reasonably linear. Its slope gives a value of G(-N-nitrosopiperidine) = 6.18 (for dilute solutions, 1 M rad changes G by 1.03 x millimoles product,[195] hence 6 x 1.03 = 6.18). The G value is consistent with the attack by $\dot{HO}$ and e^-_{aq} on N-nitrosopiperidine and short chain radical process (G values > 10 are normally indicative of radical chain process[185]).

Table 6.2

The decomposition of N-nitrosopiperidine (NNP) with respect to dose absorbed γ-radiation (1 M rad/hr) in aqueous anaerobic solution at ambient temperature.

Initial 10^2[NNP]/M	Time/min.	10^2[NNP]/M Decomposed[a]	Additives
1.0	2	0.05	
1.0	6	0.07	
1.0	15	0.16	
1.0	30	0.275	
1.0	45	0.55	
1.0	60	0.61	
1.0	120	0.83	
0.2	6	0.048	
0.5	6	0.07	
0.5	15	0.12	
0.4	60	0.30	
2.5	120	0.834	
2.0	240	0.76	
2.0	990	1.32	
1.0	30	0	0.1 M $NaNO_2$
1.0	30	0.09	0.1 M $NaNO_3$
1.0	30	0.105	0.05 M $NaNO_2$
1.0	30	0.19	0.025 M $NaNO_2$
1.0	30	0.26	0.010 M $NaNO_2$
1.0	30	0.32	0.001 M $NaNO_2$
1.0	30	0.26	0.05 M $NaNO_3$
1.0	30	0.29	0.025 M $NaNO_3$
1.0	30	0.32	0.01 M $NaNO_3$
1.0	30	0.32	0.001 M $NaNO_3$
1.0	30	0.18	0.1 M piperidine
1.0	30	0.19	0.02 M piperidine
1.0	30	0.26	0.01 M piperidine
1.0	30	0.28	0.01 M HCℓ
1.0	30	0.33	0.10 M HCℓ
1.0	30	0.33	0.50 M HCℓ
1.0	30	0.27	0.10 M NaOH

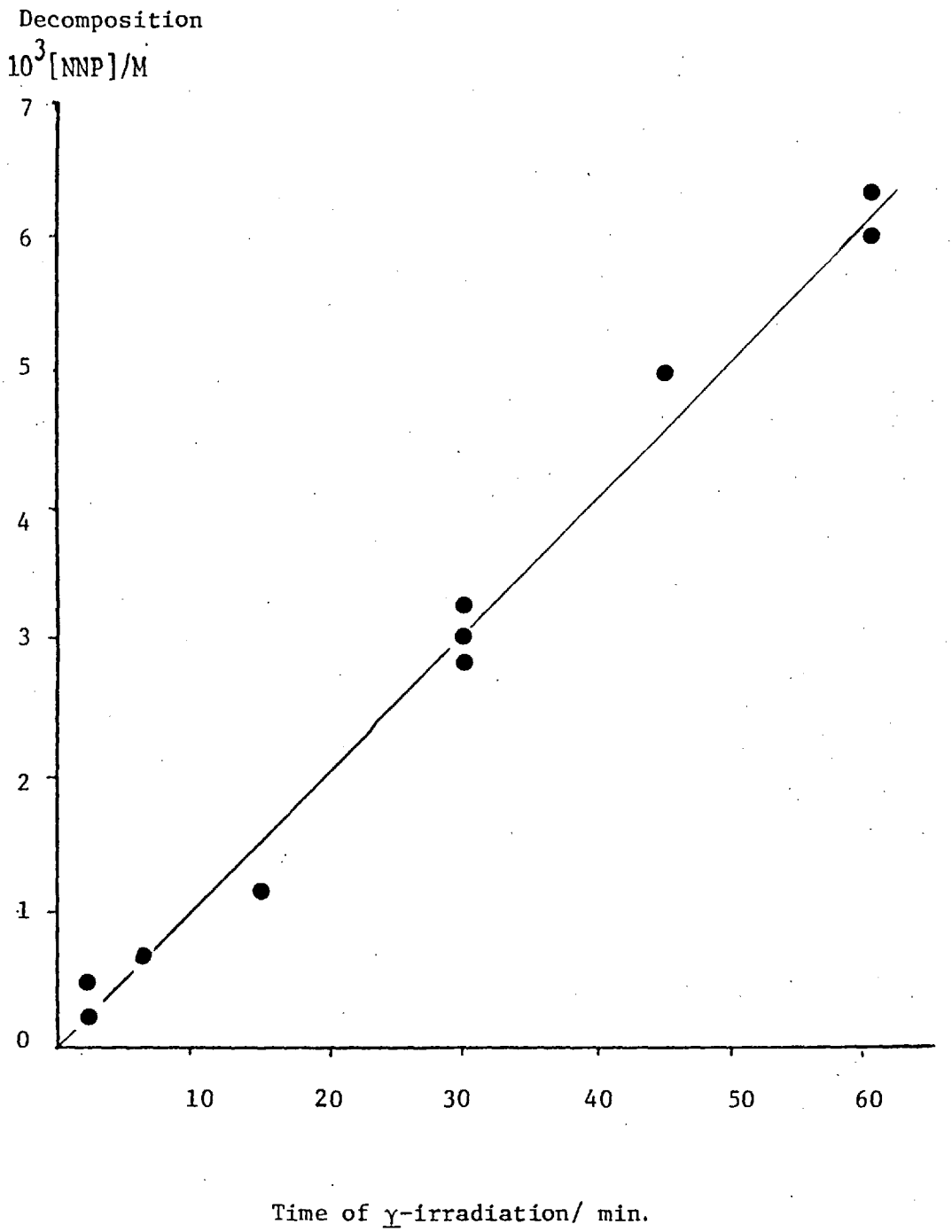
Table 6.2 (cont.)

Initial 10^2[NNP]/M	Time/min.	10^2[NNP]/M Decomposed[a]	Additives
1.0	30	0.15	pH 5.11
1.0	30	0.29	pH 6.99
1.0	30	0.29	pH 9.20

[a] Estimated from difference between initial [NNP] and final [NNP].

Figure 6.1.

The decomposition of N-nitrosopiperidine (NNP) by γ-radiation in aqueous anaerobic solution at ambient temperature. γ-dose 1 M rad/h , Initial [NNP] = 10^{-2} M.



Other data in Table 6.2 shows that neither acid nor base has any substantial effect on the amount of N-nitrosopiperidine decomposed. Significantly, however, the decomposition is inhibited by both 0.02 M piperidine and 0.1 M $NaNO_3$ and totally prevented by 0.1 M $NaNO_2$. Further, the inhibition is related to the concentration of added $NaNO_2$ or $NaNO_3$, and at low ca. 10^{-3} M $NaNO_3$, no protection was evident (Table 6.2). Protection is probably due to competition for the radiolytic radicals by the added salt and N-nitrosamine.

The decomposition of N-nitroso-, N-nitro-morpholine, N-methyl-N-nitroso-, and N-methyl-N-nitro-piperazine was briefly examined, and these results are summarised in Table 6.3. For N-nitrosamines, it is evident that the substrate has only a small effect on the extent of decomposition, and that the decomposition is inhibited, as found for N-nitrosopiperidine by additions of excess $NaNO_3$ and $NaNO_2$. The decomposition of N-nitroamine, however, does show dependence on the structure and for N-methyl-N-nitro-piperazine, on the concentration used. The inhibition by added $NaNO_3$ for N-nitrosamines is not as pronounced as the corresponding reactions with N-nitrosamines, and this is discussed further below.

6.2.2. (a). Reaction Products from the Decomposition of N-nitrosopiperidine.

Analysis of the products from the γ-irradiation of N-nitrosopiperidine was made on both the aqueous solution by g.l.c. and on an organic fraction by T.L.C. after extraction with $CH_2C\ell_2$ (v. Experimental 6.4.VI).

6.6.2. (b). Analysis of Aqueous Solution.

G.l.c. analyses showed the presence of four peaks, confirmed with authentic materials to be acetone, isopropanol, methanol, and ethanol. The yield of these compounds, however, did not bear any relation to the extent of decomposition: typically, when 2.5×10^{-2} M N-nitrosopiperidine was irradiated for 120 min, 1.52×10^{-3} M acetone, 9.5×10^{-4} M EtOH, traces of MeOH, i-PrOH and 1.66×10^{-2} M unreacted N-nitrosopiperidine

Table 6.3

The decomposition of N-nitrosamines and N-nitro-amines with respect to dose γ-irradiation (1 M rad/h) in aqueous anaerobic solution at ambient temperature. N-nitrosomorpholine (N-NM), N-nitropiperazine (N-NO_2-M), N-methyl-N-nitrosopiperazine (N-NMP), N-methyl-N-nitro-piperazine.

Initial 10^2[NNM]/M	Time/min.	10^2[Amount Decomposed]/M[a]	Additives
1.0	30	0.25 (0.30)[b]	
1.0	120	0.58	
1.0	990	1.0	
1.0	30	0	0.1 M $NaNO_2$
1.0	30	0.1	0.05 M $NaNO_2$
1.0	30	0.1	0.1 M $NaNO_3$
1.0	30	0.17	0.05 M $NaNO_3$
10^2[N-NO_2-M]/M			
0.5	30	0.25	
1.20	30	0.36 (0.40)[a]	
1.20	30	0.27	0.1 M $NaNO_3$
1.20	30	0.27	0.05 M $NaNO_3$
1.36	30	0.40	
1.36	30	0.38	0.05 M $NaNO_3$
1.36	30	0	0.1 M $NaNO_2$
2.38	30	0.40	
10^2[N-NMP]/M			
0.75	30	0.2	
0.75	30	0	0.1 M $NaNO_2$
0.75	30	0.09	0.5 M $NaNO_2$
0.75	30	0.1	0.1 M $NaNO_3$
0.75	30	0.16	0.05 M $NaNO_3$

Table 6.3 (cont.)

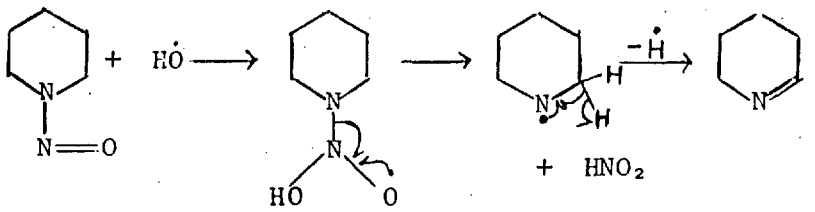
Initial 10^2[N-NO_2-MP]	Time/min.	10^2[Amount Decomposed]/M[a]	Additives
1.30	30	0.27 (0.3)[b]	
1.30	30	0.24	0.1 M $NaNO_3$
1.30	30	0.24	0.05 M $NaNO_3$
2.46	30	0.88 (0.9)[b]	

[a] Difference between initial and final concentration.

[b] Duplicate reactions in parenthesis.

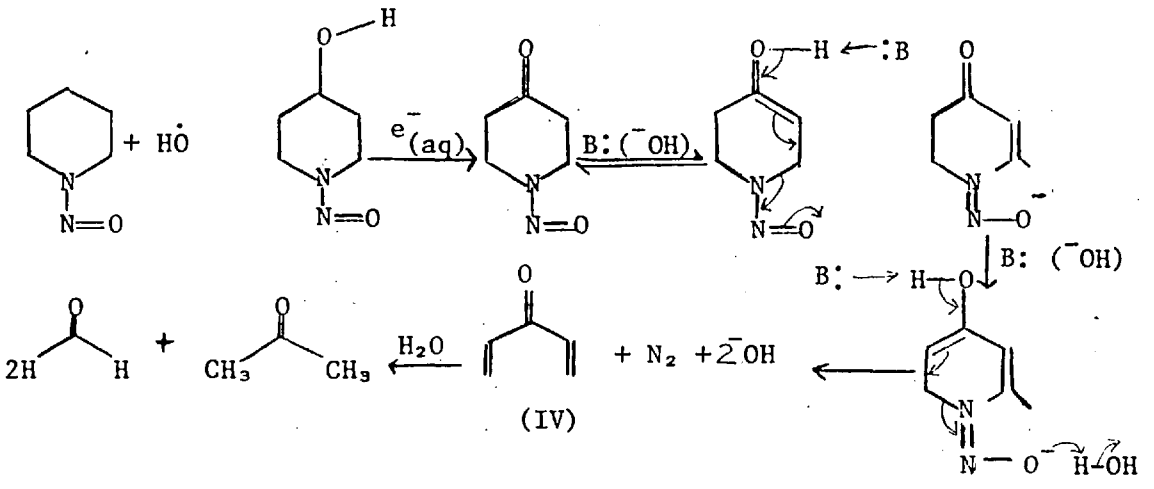
were found; irradiation of 10^{-2} M N-nitrosopiperidine for the same period, 5.15 x 10^{-4} M acetone, 2.4 x 10^{-4} M EtOH were obtained along with 1.66 x 10^{-3} M unreacted starting material.

Acetone was further characterised by its DNP-derivative (m.p., 123.5° lit[221] 126° ; M^+ 238). Nitrite was not detected by Shinn's test[130] and hence it is unlikely that HO· attacked the nitroso group of N-nitrosopiperidine as shown in Equation 6.16. There was no evidence of other carbonyl products associated with α- hydroxylation or the hydrolysis of the imine formed (Equation 6.16).



.... 6.16

Acetone could form, however, from an initial 4-hydroxylation followed by oxidation by e^-_{aq} (Scheme 6.4). Enolisation and base-catalysed rupture of the C-N bond of N-nitrosopiperidone would lead to the dieneone IV, which would be expected to hydrolyse to acetone and formaldehyde.



SCHEME 6.4

Formaldehyde was not detected by g.l.c., however, but its decomposition to CO_2 on γ-irradiation has been previously reported.[188,190] Methanol was identified, and this could arise from the reduction of formaldehyde.[195] Isopropanol formation is probably due to attack of the hydrated electron on acetone for which there is much evidence.[186] The formation of ethanol is not clear.

6.2.2 (c) Analyses of Organic Fraction.

An organic extract of the N-nitrosopiperidine γ-irradiated solutions on T.L.C. (2 mm silica) showed three bands with R_F = 0.80, 0.40, 0.05 on development with 80/20 (v/v) pet-ether (40-60)/acetone. The major band at R_F = 0.80 was collected and confirmed to be unreacted N-nitrosopiperidine. The band at R_F = 0.40 showed i.r. absorbances ($CHC\ell_3$) at 1710 (C=O), 1465, 1430 and 1360 cm^{-1} (N-NO stretch). This product on sublimation gave 0.3 mg pale yellow crystals. Mass spectra indicated a molecular weight of 326. The compound may contain the carbon fragment of three N-nitrosamine molecules, as identified by Chow.[78] The band at R_F = 0.05 gave strong i.r. bands ($CHC\ell_3$) at 1710, 1675 and 1360 cm^{-1} and a weak absorption at 1430 cm^{-1}.

6.2.2 (d) Concluding Remarks.

Clearly, N-nitrosopiperidine is destroyed by γ-radiation into essentially harmless products at a rate of ca. 10^{-4} M N-nitrosopiperidine/min. The extent of decomposition is independent of the initial [N-nitrosopiperidine] so the reaction is not bimolecular, and the products do not destroy N-nitrosopiperidine. Also, the rate of decomposition

is independent of pH. Not all the products have been identified, but their yields cannot be increased by increasing the dose so alternative methodsare required to characterise them.

6.2.3 (a) Formation of N-nitrosamines from Secondary Amines and $NaNO_2$ on γ-Irradiation.

With reagent concentrations (ca. 8×10^{-4} M $NaNO_2$ and 2×10^{-3} M piperidine), the yield of N-nitrosopiperidine increased to a maximum of ca. 6×10^{-5} M after 6 min. γ-irradiation (≡ 10^5 rad.) and then decreased. After 45 min. (≡ 7.5×10^5 rad), no N-nitrosopiperidine could be detected. The variation in the yield of N-nitrosopiperidine with respect to time of γ-irradiation is shown in Table 6.4.

Table 6.4

The decomposition of $NaNO_2$ and the formation of N-nitrosopiperidine (NNP) with respect to dose γ-radiation in the absence and presence of 2×10^{-3} M piperidine. γ-dose 1 M rad/h. Initial $[NaNO_2] = 8 \times 10^{-4}$ M.

Time/min.	Residual 10^4 $[NaNO_2]$/M		10^5 [NNP]/M
	NO Piperdine	2×10^{-3} M Piperidine	
2	7.95	7.95	0.5
6	6.6	6.30	6.25
15	6.12	5.93	1.5
30	5.5	3.25	Trace
45	3.3	1.10	0
60	3.65	0	0

Analysis of the reaction solution by Shinn's test[130] after 30 min. showed that $NaNO_2$ had been decomposed by γ-radiation. This was checked by following the decrease in [$NaNO_2$] (By Shinn's test) on γ-irradiation of 8×10^{-4} M $NaNO_2$ in both the presence and absence of 2×10^{-3} M piperidine. The results in Table 6.4 show that $NaNO_2$ loss during γ-irradiation is greater in the presence of piperidine than in its absence, The fact that the difference in not accounted for by formation of N-nitrosopiperidine is consistent with previous observations that N-nitrosopiperidine itself decomposes on γ-irradiation.

It was shown previously (Table 6.1, 6.2) that the decomposition of $0.75\text{-}1.0 \times 10^{-2}$ M N-nitrosamine does not occur in the presence of 0.1 M $NaNO_2$. This suggested that significant yields of N-nitrosamine may be obtained by γ-irradiation of aqueous $NaNO_2$ and secondary amine where the $NaNO_2$ is in excess. Data in Table 6.5 and Figure 6.2 for reaction of 0.1 M $NaNO_2$ with 2×10^{-3} M amine shows the yield of N-nitrosamine has a linear dependency with the radiation dose. After 60 min. γ-irradiation, quantitative yields of N-nitrosamine were obtained for N-methylpiperazine and morpholine compared to only 40% for piperidine (Table 6.5). With the same reagent concentrations in phosphate buffer (pH 6.99) 30 min. γ-irradiation gave 79×10^{-5} M N-methyl-N-nitrosopiperazine and 27×10^{-5} M N-nitrosomorpholine but virtually no N-nitrosopiperidine (Table 6.6). The apparent dependence of the product yields on the basicity of the amine suggests that only unprotonated amine reacts.

Table 6.5

Yields of N-nitrosamines with respect to absorbed γ-radiation (1 M rad/h) for reaction of 0.1 M $NaNO_2$ with initial 2 x 10^{-3} M morpholine, piperidine and N-methylpiperazine in aqueous anaerobic solution at ambient temperature. (N-nitrosomorpholine - N-NM, N-nitrosopiperidine - NNP, N-methyl-N-nitrosopiperazine N-NMP).

Time/min.	10^5 [N-nitrosamine]/M		
	N-NM	NNP	N-NMP
6	44(46)[a]	14	41
15	79	28	80
30	170 (173)[a]	61	130
45		70	153
60	200 (200)[a]	88	200 (200)[a]

[a] Duplicate reactions in parenthesis.

Since quantitative yields can be obtained in the absence of buffer, it is probable that HO^- generated by the radiolysis makes the reaction solutions alkaline. Addition of 0.01 M HCℓ inhibited both N-nitrosomorpholine and N-nitrosopiperidine formation but catalysed N-methyl-N-nitrosopiperazine formation from 7.8 x 10^{-4} M $NaNO_2$ after 6 min. γ-irradiation in the presence of 10^{-3} M amine (Table 6.7). The result for N-methyl-N-nitrosopiperazine is not contradictory because independent measurements show this refers to the normal acid catalysed reaction by HNO_2. Addition of 0.4 M NaOH also severely inhibited N-nitrosomorpholine and N-nitrosopiperidine formation from

Figure 6.2.

The dependence of dose γ-irradiation (1 M rad/h^{-1}) on the yield of N-nitrosamines in aqueous anaerobic solution at ambient temperature. Initial 2 x 10^{-3} M piperidine (▲), morpholine (■), N-methylpiperazine (●), [$NaNO_2$] = 0.1 M.

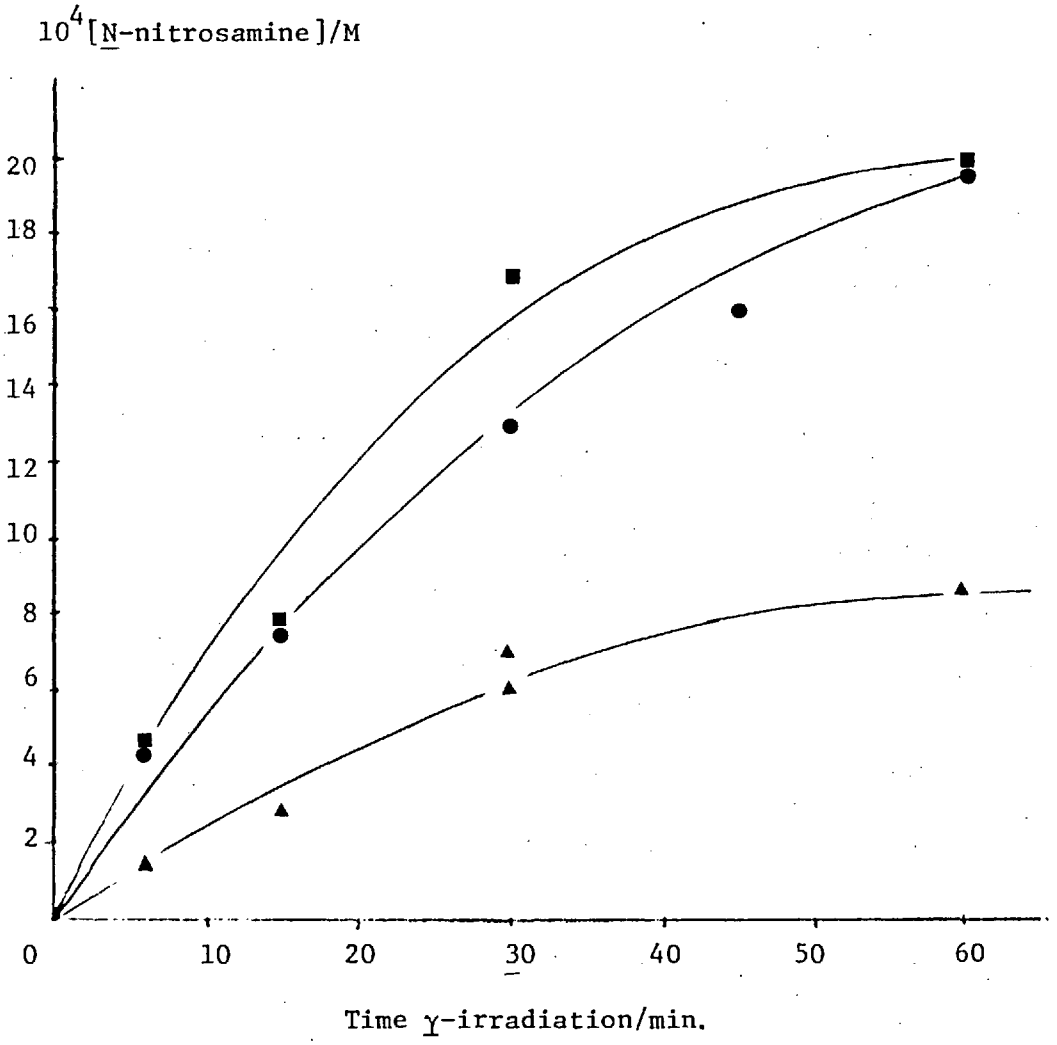


Table 6.6

Effect of pH on the formation of N-nitros- and N-nitro-amines. Initial 2×10^{-3} M piperidine, morpholine, N-methylpiperazine; γ-dose (1 M rad/h) for 30 min., in aqueous anaerobic solution at ambient temperature. (N-nitrosopiperidine, NNP, N-nitrosomorpholine, NNM, N-methyl-N-nitrosopiperazine, N-NMP).

0.1 M $NaNO_2$	10^5 [N-nitrosamine]/M		
pH	NNP	NNM	N-NMP
9.2	17.6	153	132
6.99	0.8	27	79

0.1 M $NaNO_3$	10^5 [Product]/M	
Product	pH 9.2	pH 6.99
NNP	3.6	trace
N-nitropiperidine	7.5	trace
NNM	22	trace
N-nitromorpholine	13.2	trace
N-NMP	6	6
N-methyl-N-nitropiperazine	7.2	7.2

Table 6.7

Effect of added HCℓ on N-nitrosamine formation. Initial 2 x 10^{-3} M piperidine, morpholine, and N-methylpiperazine; γ-dose (1 M rad/h) for 6 min. in aqueous anaerobic solution at ambient temperature. (N-nitrosopiperidine, NNP, N-nitrosomorpholine, NNM, N-methyl-N-nitroso-piperazine, N-NMP).

10^4 [$NaNO_2$]/M	10^3 [HCℓ]/M	10^5 [N-nitrosamine]/M		
		NNP	NNM	N-NMP
7.82	0	2.16	3.0	5.75
7.82	1	0.72	0.86	10.25[a]
7.82	10	0	0	42.0[a]
10^4 [$NaNO_3$]/M				
9.0	0	0.49	2.39	1.75
9.0	1	0	0	3.13[a]
9.0	10	0	0	0

[a] Same yield apparent with no γ-irradiation.

Table 6.8

Effect of added NaOH on yields of N-nitroso- and N-nitro-amines. γ-Dose 1 M rad/h for 30 min. in aqueous anaerobic solution at ambient temperature. Initial [Morpholine], [Piperidine] = 2×10^{-3} M Initial [$NaNO_2$] = 0.1 M.

Amine	10^3 [NaOH]/M	10^5 [N-nitrosamine]/M	10^5 [N-nitroamine]/M
Morpholine	0	172	
Morpholine	2	128	
Morpholine	400	17.8	
Piperidine	0	62.5 (64.5)[a]	
Piperidine	2	50	
Piperidine	400	20	
Initial [$NaNO_3$] = 0.1 M			
Morpholine	0	24 (20)[a]	21 (15)
Morpholine	2	10	7
Morpholine	400	4.8	12
Piperidine	0	7 (6.7)[a]	37 (40)[a]
Piperidine	2	6.7	40
Piperidine	400	5.5	5.3

[a] Reactions in duplicate.

0.01 M $NaNO_2$ after 30 min. γ-irradiation (Table 6.8) for reasons that are not entirely clear. It is possible that either the hydrolysis of N_2O_3 or generation of radicals from $NaNO_2$ become significant.

Other results show that the yield of N-nitrosamine is dependent on the extent of irradiation. The effect of both amine concentration and dose of radiation is shown in Figure 6.3 for morpholine. This shows that the initial rate of N-nitrosomorpholine formation is independent of the [morpholine] and that quantitative yields of N-nitrosomorpholine are obtained for 10^{-3} M and 2×10^{-3} M morpholine after 30 and 60 min., respectively. An obvious explanation of the amine independence is that formation of the nitrosating agent is rate-limiting. (The non-linear dependence at higher concentrations of morpholine probably relates to the relative protective ability of the diminished [$NaNO_2$], and also that the rate of formation of the nitrosating agent is dependent on [$NaNO_2$]). Other data on Table 6.9 show that yields of both N-nitrosomorpholine and N-nitrosopiperidine depend on

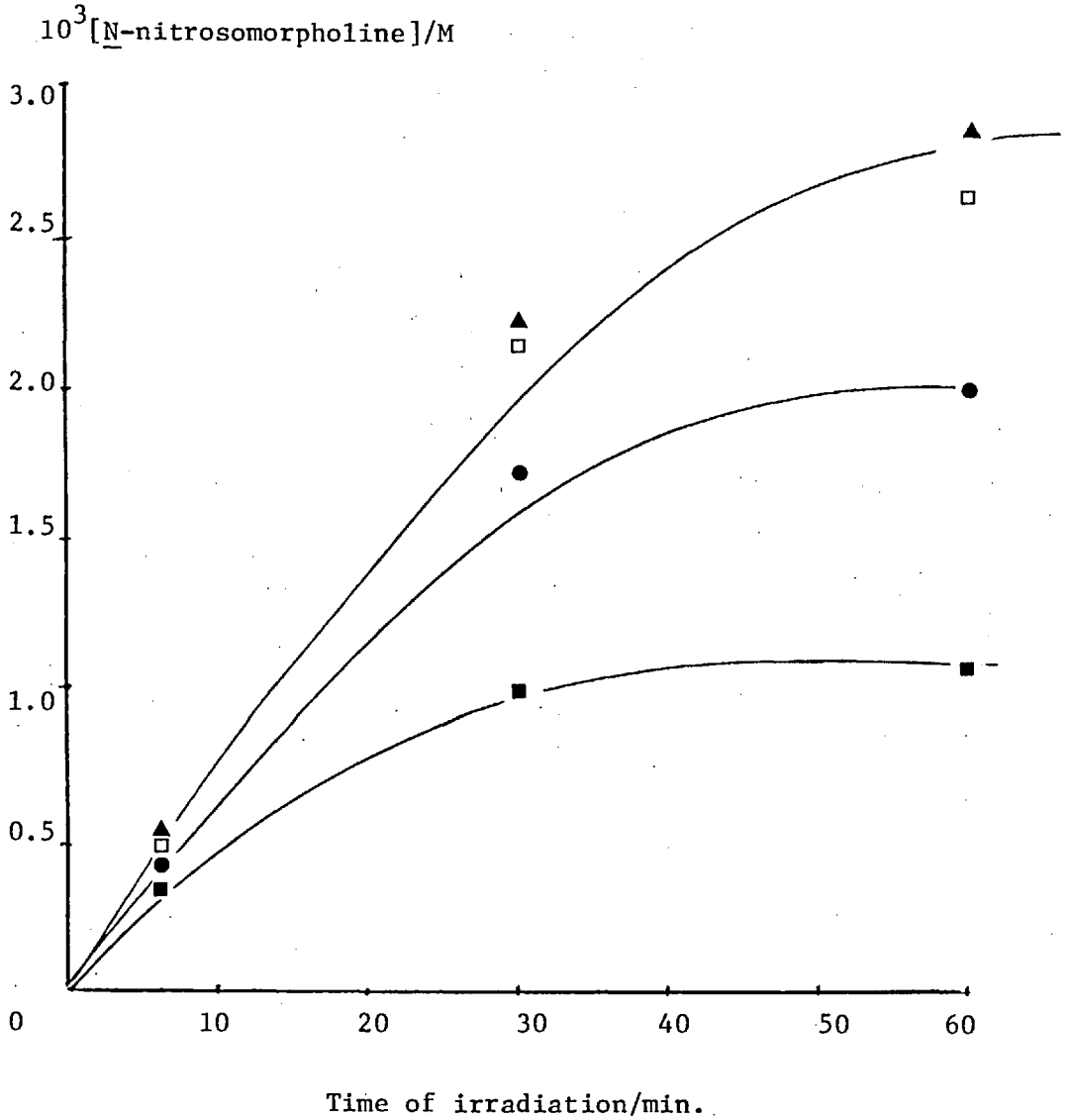
Table 6.9

Variation in N-nitrosamine yield with [$NaNO_2$]. Initial 2×10^{-3} M morpholine, piperidine; γ dose (1 M rad/h) for 30 min. aqueous anaerobic solution at ambient temperature.

10^3 [$NaNO_2$]/M	10^5 [N-nitrosamine]/M	
	N-nitrosomorpholine	N-nitrosopiperidine
1	3	3
5	40	30
10	94	43
50	145	62
100	174	62

Figure 6.3.

Variation in yield of N-nitrosomorpholine with time of γ-irradiation for varying initial [Morpholine] = 1×10^{-3} M (■), 2×10^{-3} M (●), 5×10^{-3} M (□), 1×10^{-2} M (▲). Initial [$NaNO_2$] = 0.1 M, aqueous anaerobic solution at ambient temperature. γ-dose 1 M rad/h .



the initial [$NaNO_2$]. The dependence here is not straightforward and the yield of N-nitrosamine per mole of $NaNO_2$ passes through a maximum. Thus, for high [$NaNO_2$] the amount of N-nitrosamine tends to level off to a maximum value dependent on both the basicity and dose of radiation. For example 2×10^{-3} M morpholine gave 1.72×10^{-3} M, 2×10^{-3} M N-nitrosomorpholine while 2×10^{-3} M piperidine gave only 6.2×10^{-4}, 8.8×10^{-4} M N-nitrosopiperidine after 30 and 60 min. irradiation, respectively (Table 6.9 and Figure 6.2). The limiting yields at high [$NaNO_2$] suggests trapping of the nitrosating agent by the amine is subject to saturation. Analysis of these data gives a clear indication that the yield of N-nitrosomorpholine is dependent on the total dose of γ-irradiation. For example, 0.1 M $NaNO_2$ and 2×10^{-3} M morpholine irradiation for 30 min. at 1 M rad/h (≡ 0.5 M rad) gave similar yields of N-nitrosomorpholine to exposure for 118 hours at 72 rad $min.^{-1}$ (≡ 0.51 M rad), (Table 6.10). The yield of N-nitrosomorpholine bears an approximately linear relationship to the dose, with ca. 3.5×10^{-9} M N-nitrosomorpholine produced per rad (Figure 6.4). At low doses, however, the yield of N-nitrosomorpholine/rad is lower compared to yields at high doses, and this is probably due to the error (± 10-15%) for the estimation of low doses (v. Experimental 6.4.III).

Other results summarised in Table 6.11 show unequivocally that added KN_3 inhibits the formation of N-nitrosamine. For example, with 2×10^{-3} M morpholine and 0.1 M $NaNO_2$, 30 min. irradiation usually gives 1.53×10^{-3} M N-nitrosomorpholine (pH 9.2) but this decreased to 1×10^{-5} M N-nitrosomorpholine in the presence of 4×10^{-3} M KN_3. It is clear that the nitrosating agent preferentially reacts with KN_3 rather than morpholine.

Table 6.10

Variation of N-nitrosomorpholine yield with γ-dose. Initial 0.1 M $NaNO_2$ with 2×10^{-3} M Morpholine in aqueous anaerobic solution at ambient temperature.

Dose rad/h	Time Irradiation /h	Total Dose 10^4 rad.	10^5 [N-nitrosomorpholine]/M
720	3	.216	1.19
180	17	.306	1.41
10^6	0.1	10	43
10^6	0.25	25	78
10^6	0.50	50	158
4320	118	51	145
10^6	1.0	100	200
4320	118	51	8.15[a]
			10^5 [N-nitrosopiperidine]/M
4320	118	51	52.7[b]
4320	118	51	3.28[c]

[a] In the presence of 0.1 M $NaNO_3$ instead of 0.1 M $NaNO_2$

[b] In the presence of 0.1 M $NaNO_2$, 2×10^{-3} M piperidine

[c] In the presence of 0.1 M $NaNO_3$ instead of 0.1 M $NaNO_2$, 2×10^{-3} M piperidine.

Figure 6.4.

The yield of N-nitrosomorpholine from 2 x 10^{-3} M morpholine with respect to dose of γ-irradiation in aqueous anaerobic solution at ambient temperature. Initial [$NaNO_2$] = 0.1 M.
(■) 1 M rad/h dose for 0.1, 0.25, 0.50, 1.0 h. (▲) 12 rad $min.^{-1}$ for 3 h. (□) 3 rad/min. for 17 h. (○) 72 rad/min for 118 h.

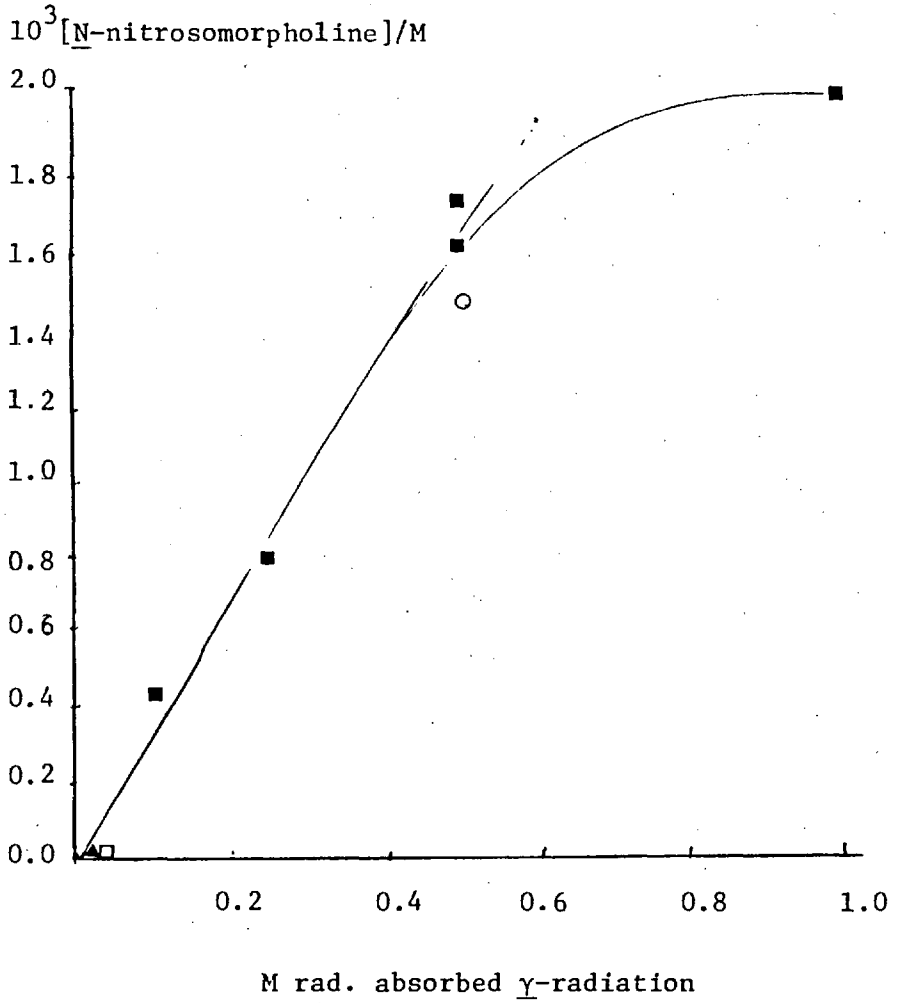


Table 6.11

Inhibition by KN_3. γ-Radiation (1 M rad/h) for 6 min. in aqueous anaerobic solution at ambient temperature for $NaNO_2$ and $NaNO_3$.

10^3 [$NaNO_2$]/M	10^3 [KN_3]/M	Amine	10^3 [Amine]/M	Yield 10^5 [N-nitrosamine]/M	10^5 [N-nitroamine]/M
0.9	0	Morpholine	1	6.25	
0.9	1.0	Morpholine	1	1.02	
100*	0	Morpholine	2	153	
100*	4.0	Morpholine	2	1.0	
0.9	0	Piperidine	1	3.08	
0.9	1.0	Piperidine	1	0.95	
10^3 [$NaNO_3$]/M					
0.9	0	Morpholine	1	2.4	
0.9	1.0	Morpholine	1	0.5	
100*	0	Morpholine	2	22	13.2
100*	2.0	Morpholine	2	2.0	23.4
0.9	0	Piperidine	1	1.1	
0.9	1.0	Piperidine	1	0.5	

* Reaction at pH 9.2 γ-dose (1 M rad/h) for 30 min.

6.2.3 (b) Formation of N-nitrosamines and N-nitroamines from Secondary Amines and $NaNO_3$ on γ-radiation.

As for the reactions using $NaNO_2$ with low concentrations, (e.g., 8×10^{-4} M $NaNO_3$ and 2×10^{-3} M piperidine), formation of N-nitrosopiperidine reached a low maximum after 6 min. irradiation (1.95×10^{-5} M N-nitrosopiperidine) and subsequently decreased with longer exposures (Table 6.12). Also, independent experiments showed that γ-irradiation of $NaNO_3$ leads to the formation of $NaNO_2$ in agreement with previous reports.[200-205] The amount of $NaNO_2$ appears to reach a maximum which is clearly higher in the presence of piperidine (Table 6.12). These results show that there is no simple correlation between the yields of $NaNO_2$ and N-nitrosopiperidine.

Table 6.12

The formation of $NaNO_2$ and N-nitrosopiperidine with respect to dose γ-radiation of initial 8×10^{-4} M $NaNO_3$ in absence and presence of 2×10^{-3} M piperidine, in aqueous anaerobic solution at ambient temperature. γ-dose 1 M rad/h.

Time/min.	10^5 [$NaNO_2$]/M		10^5 [N-nitrosopiperidine]/M
	NO piperidine	2×10^{-3} M piperidine	
2	5	7.5	0
6	7.5	21	1.95
15	8.0	26	0.95
30	11.5	31	Traces
45	7.5	26	0
60	3.5	22.5	0

It was shown previously (see Table 6.2, 6.3) that decomposition of ca. .75-1.0 x 10^{-2} M N-nitrosamine by γ-radiation was reduced in the presence of 0.1 M $NaNO_3$. It follows that N-nitrosamine formation may be significant in the presence of relatively high [$NaNO_3$], as with $NaNO_2$. Radiolysis of the appropriate solutions of $NaNO_3$, however, resulted in the formation of both N-nitrosamines and N-nitroamines. Independent checks established that the N-nitroamine does not result from the oxidation of N-nitrosamine by H_2O_2 or radical oxidants.

Variation in the yields of N-nitroso- and N-nitroamines with time of irradiation are summarised in Table 6.13 and the N-nitrosamine data are plotted in Figure 6.5.

Table 6.13

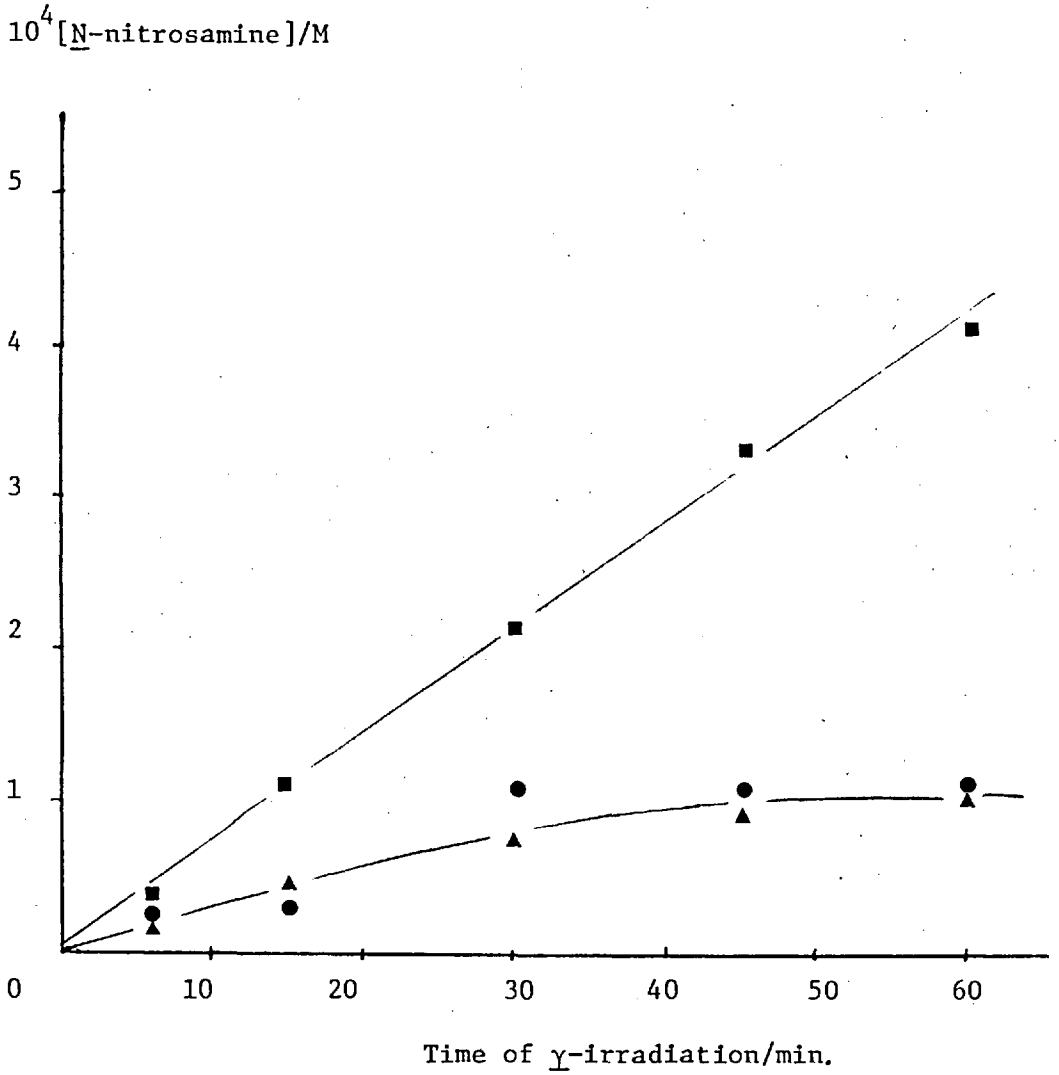
Dependence of N-nitroso- and N-nitroamine formation on the γ-dose (1 M rad/h). Initial 2 x 10^{-3} M piperidine (P), morpholine (M) and N-methylpiperazine (NMP), 0.1 M $NaNO_3$, in aqueous anaerobic solution at ambient temperature.

Time/min.	10^5 [N-nitrosamine]/M			10^5 [N-nitroamine]/M		
	P	M	NMP	P	M	NMP
6	2	4 (3.7)[a]	2	18	2 (1.17)	
15	3	11	4.5	16	10	1
30	10	21.5	7.5	30	11.7	5
45	10	33	9	49	13	6
60	10	42 (40)[a]	10	53	16 (17)	8

[a] Reactions in duplicate

Figure 6.5

Dependence of N-nitrosamine formation with respect to dose γ-irradiation. (1 M rad/h). Initial 2 x 10^{-3} M piperidine (●), morpholine (■), N-methylpiperazine (▲), [$NaNO_3$] = 0.1 M, aqueous anaerobic solution at ambient temperature.



Generally, N-nitrosamine yields are lower than comparable experiments with $NaNO_2$, but other differences are apparent. Thus, the amount of N-nitrosomorpholine is much higher than N-methyl-N-nitrosopiperazine and N-nitrosopiperidine, and does not appear to reach a limiting value after 30-60 min. and the amines appear to exhibit selectivity towards the nitrosating agent, which is not only dependent on the basicity. The yields of N-nitroamines, however, increase linearly with time of irradiation for all three substrates and are dependent on the basicity of the amine (Table 6.13), i.e., piperidine (53×10^{-5} M), > morpholine (16×10^{-5} M) > N-methylpiperazine (8×10^{-5} M) after 60 min. γ-irradiation. As for $NaNO_2$ product formation for piperidine and morpholine is severely inhibited by acid (Table 6.7). The yields of N-nitroso- and N-nitroamines in buffered media are shown in Table 6.6 : at pH 6.99, only N-methylpiperazine reacted. Added NaOH also decreased the amount of both N-nitroso- and N-nitroamine (Table 6.8).

The effect of both [$NaNO_3$] and [morpholine] on the product yields was also examined and these data are given in Table 6.14 and Figures 6.6 and 6.7, respectively. Figures 6.6 and 6.7 suggest that, as with $NaNO_2$, the rate of N-nitrosomorpholine and N-nitromorpholine, formation is virtually independent of [morpholine]. This would be expected if formation of the nitrosating agent is rate-limiting. The influence of [$NaNO_3$] is to increase product yields (Table 6.14) but a saturation effect is apparent above 10^{-2} M $NaNO_3$ for N-nitrosomorpholine formation while N-nitromorpholine increases.

Figure 6.6.

Variation in the yield of N-nitrosomorpholine with time of γ-irradiation for varying initial [morpholine] = 1×10^{-3} M (▲), 2×10^{-3} M (○), 5×10^{-3} M (□), 1×10^{-2} M (△). Initial [$NaNO_3$] = 0.1 M, aqueous anaerobic solution at ambient temperature. γ-dose 1 M rad/h .

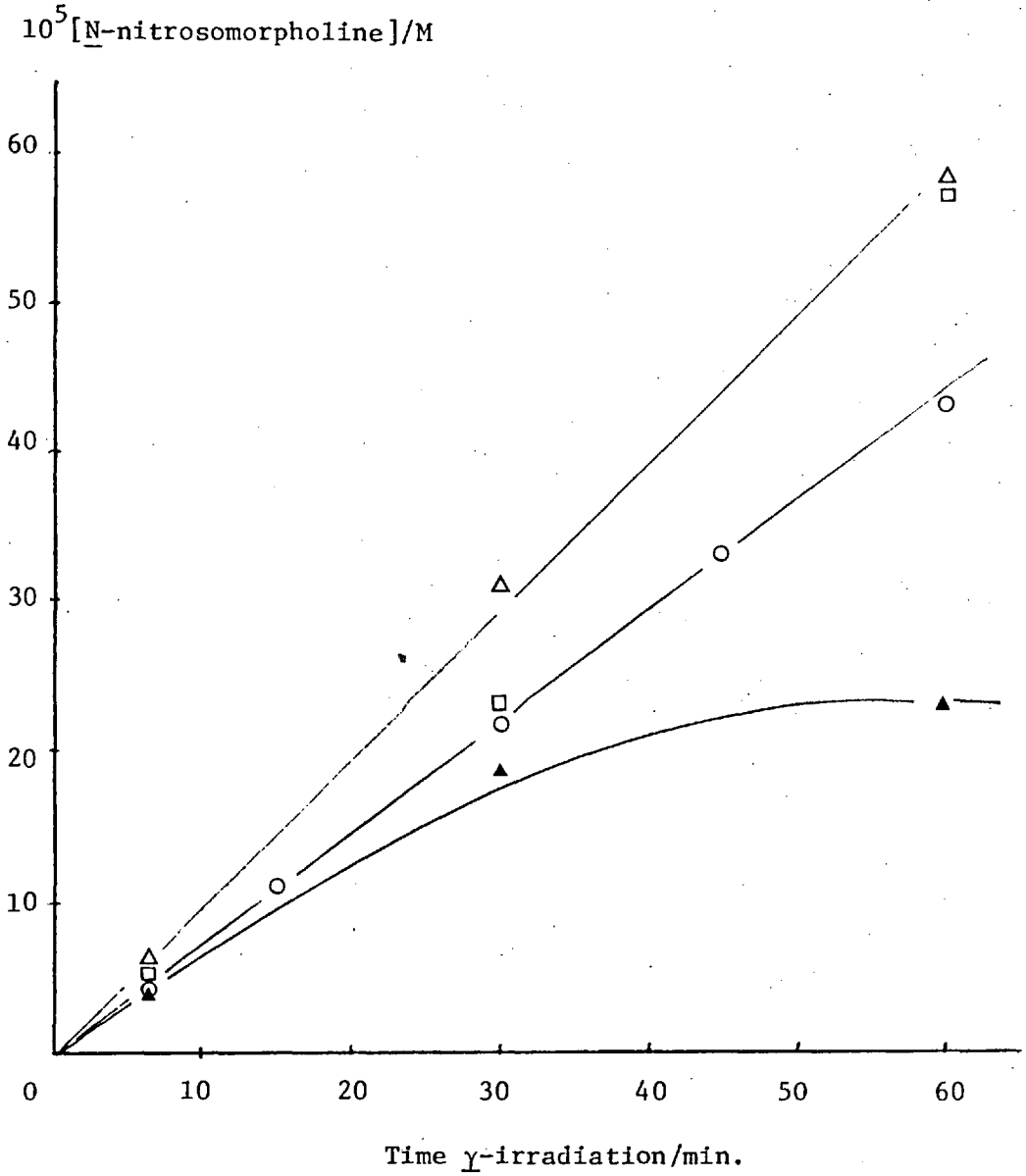


Figure 6.7.

Variation in the yield of N-nitromorpholine with time of γ-irradiation for varying initial [morpholine]= 1 x 10^{-3} M (▼), 2 x 10^{-3} M (○), 5 x 10^{-3} M (□), 1 x 10^{-2} M (△). Initial [$NaNO_3$] = 0.1 M, aqueous anaerobic solution at ambient temperature. γ-dose 1 M rad/h.

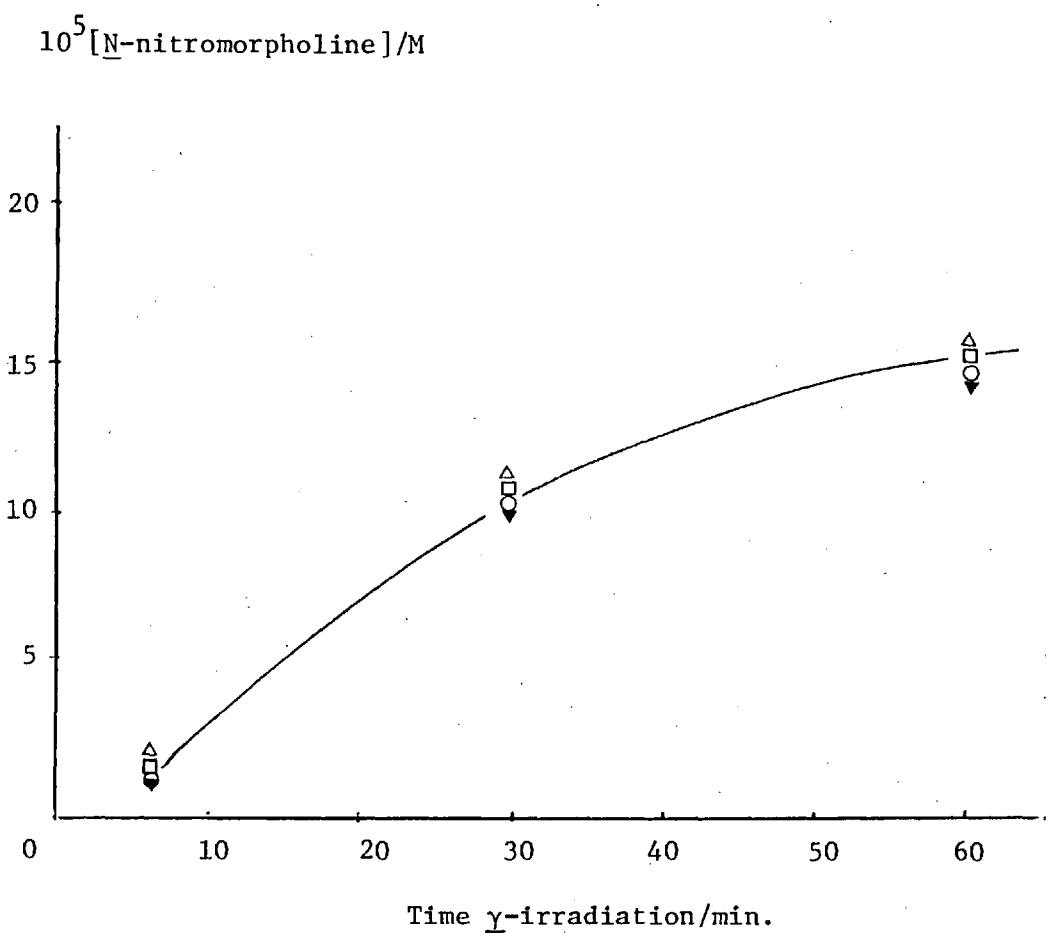


Table 6.14

Effect of [$NaNO_3$] on the formation of N-nitroso- and N-nitro-amines; Initial 2 x 10^{-3} M piperidine (P), morpholine (M), γ-dose (1 M rad/h) for 30 min. in aqueous anaerobic solution at ambient temperature.

10^3 [$NaNO_3$]/M	10^5 [N-nitrosamine]/M		10^5 [N-nitroamine]/M	
	M	P	M	P
1	1.25	.42	4	10.5
5	12.5	4.5	6.5	15
10	19	10	10	26
50	19.5	10	11	29
100	21.5	10	11.7	30

Other results in Table 6.11 show unequivocally that added KN_3 inhibits N-nitrosamine formation from the radiolysis of $NaNO_3$. For example, at pH 9.2 with 2 x 10^{-3} M morpholine and 0.1 M $NaNO_3$, 30 min. γ-irradiation usually gives ca. 22 x 10^{-5} M N-nitrosomorpholine but this is decreased to 2 x 10^{-5} M N-nitrosomorpholine in the presence of 2 x 10^{-3} M KN_3. It is not clear, however, why thereis an apparent increase in the yield of N-nitromorpholine in the presence of KN_3.

6.2.4. N-nitrosamine formation from morpholine and $NaNO_2$, $NaNO_3$ on Hg-photolysis.

The Hg-photolysis of aqueous anaerobic solutions of $NaNO_2$, $NaNO_3$, in the presence of 2 x 10^{-3} M morpholine at ambient temperature was studied briefly. The results (summarised in Table 6.15, and 6.16) show trends similar to those observed from the γ-radiolysis of these

solutions. Thus, the yield of N-nitrosomorpholine is dependent on the [$NaNO_2$] , the length of photolysis, but largely independent on the [morpholine]. Generally, higher yields of N-nitrosomorpholine was observed from the photolysis of $NaNO_2$ than $NaNO_3$ and the reactions of $NaNO_3$ were not examined further. Also, there was no detectable N-nitromorpholine from the photolysis of $NaNO_3$ as found during the γ-radiolysis reactions.

Table 6.15

N-nitrosomorpholine formation from the Hg-photolysis of $NaNO_2$ and morpholine in aqueous anaerobic solution at ambient temperature, time Hg-photolysis, 60 and 120 min.

10^3 [$NaNO_2$]/M	10^3 [Morpholine]/M	10^5 [N-nitrosomorpholine]/M	
		60 min.	120 min.
1.0	2		4.76
1.0	5	1.25	5.35
10.0	2	28.9	22.5
10.0	5	29.8	30
20	2	56	63.2
20	5	56.5	93.5
50	2	59	81.5
50	5	88	114
80	2	100	146
80	5	99.5	146
100	2		174
100	5		176

Table 6.16

N-nitrosomorpholine formation from the Hg-photolysis of $NaNO_3$ and morpholine in aqueous anaerobic conditions at ambient temperature. Photolysis time 120 min.

10^3 [$NaNO_3$]/M	10^3 [Morpholine]/M	10^5 [N-nitrosomorpholine]/M
1	2	1.16
1	5	1.16
10	2	1.16
10	5	1.74
100	2	2.33
100	5	2.9

6.3 Discussion and Conclusion.

Present results show that N-nitrosamines form when aqueous neutral solutions of $NaNO_2$ and $NaNO_3$ are exposed to γ-radiation in the presence of secondary amines. With low (ca. 10^{-3} M) concentration of salt and 2 x 10^{-3} M piperidine, small amounts of N-nitrosopiperidine were detected after ca. 6 min, but this disappears on further irradiation. With excess salt, however, substantial amounts (sometimes quantitative) of N-nitrosamines are obtained over ca. 60 min. Generally, higher concentrations of N-nitrosamines are obtained from $NaNO_2$ than $NaNO_3$, but N-nitroamines form concurrently only from $NaNO_3$.

There is good evidence in the literature, confirmed by these results, that N-nitrosamines are decomposed by ionising radiation.[78,217-220] It is also known that aqueous nitrite and nitrate solutions form N_2O_3 and N_2O_4, respectively on γ-irradiation[201,204] and Hg-photolysis.[211,212] Most of the results can be accommodated within the context of these facts, provided that the decomposition of N-nitrosamine and N-nitroamine is inhibited by both nitrite and nitrate. Evidence cited in Table 6.2 and 6.3 shows that this inhibition occurs for N-nitrosamines and to a lesser extent for N-nitroamines, and that nitrite is a better inhibitor than nitrate.

It is also known that gaseous N_2O_3 and N_2O_4 react with secondary amines in neutral and alkaline aqueous solution to form N-nitrosamines and N-nitroamines,[222] respectively. Thus, the observation that N-nitrosamines form only from γ-irradiation of aqueous nitrite and both N-nitroso- and N-nitroamines from aqueous nitrate concur with conclusions about the generation of N_2O_3 and N_2O_4.

Several other observations provide information about the detailed mechanism of N-nitroso- and N-nitro-amine formation. Reaction by both aqueous nitrite and nitrate is inhibited by decreasing pH. This suggests that the free (unprotonated) amine is reactive. The reaction is also inhibited by added HO^- which may reflect an increased rate of hydrolysis of N_2O_3 and N_2O_4 (Equation 6.17 and 6.18).

$$N_2O_3 + 2HO^- \longrightarrow 2NO_2^- + H_2O \quad \ldots\ldots 6.17$$

$$N_2O_4 + 2HO^- \longrightarrow NO_3^- + NO_2^- + H_2O \quad \ldots\ldots 6.18$$

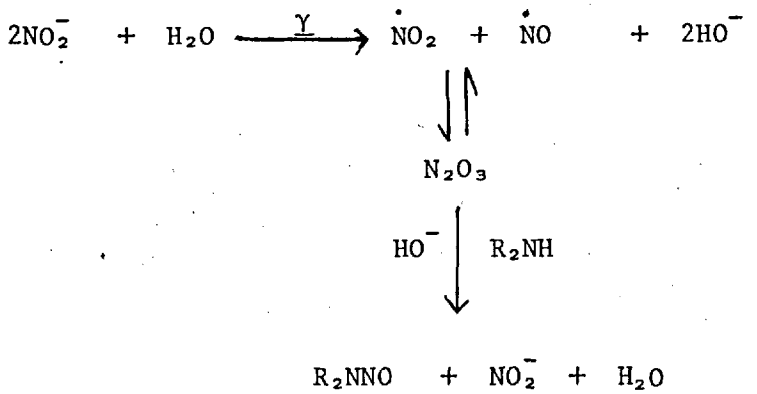
Alternatively, the generation of the nitrosating agent itself, may be inhibited by added HO^-. Further, the rate of formation of N-nitrosamine from aqueous nitrite is dependent on the dose of γ-radiation, and the $[NaNO_2]$, but is virtually independent of [amine]. This suggests that reaction is not a result of the usual acid catalysed nitrosation pathway, that activation of the amino substrate by radical intermediates (e.g., Equation 6.19) is not involved, and that radical initiated formation of N_2O_3 is the rate-limiting step.

$$R_2NH + \dot{H}O \longrightarrow H_2O + R_2\dot{N} \xrightarrow{NO} R_2NNO \quad \ldots\ldots 6.19$$

The inhibiting effect of added NaN_3 supports the conclusion of N_2O_3 formation and shows further, that N-nitrosamine formation occurs in solution and not in the dead space (gaseous-region) above the reaction solution. There is good evidence to show that NaN_3 reacts rapidly with N_2O_3 to give a mixture of N_2O and N_2[48] (Equation 6.20).

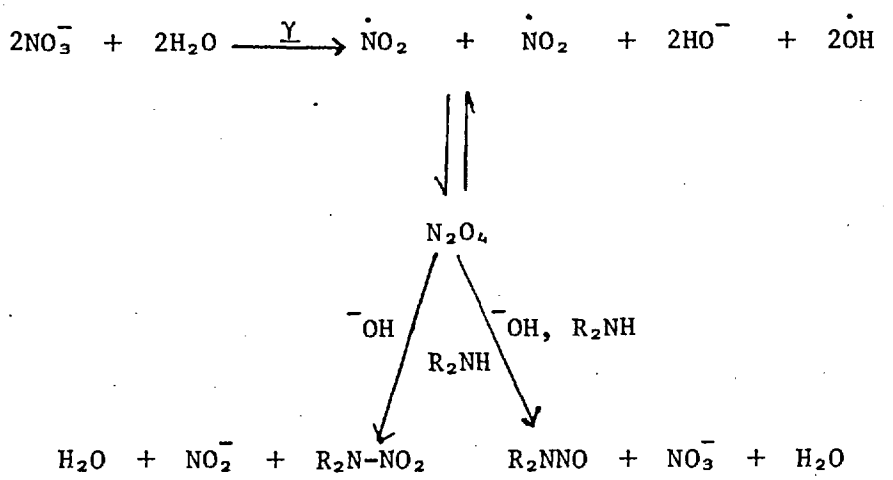
$$N_2O_3 + N_3^- \longrightarrow N_2O + N_2 + NO_2^- \quad .... \quad 6.20$$

On the basis of these observations, the proposed formation of the N-nitrosamine of piperidine, morpholine and N-methylpiperazine follow the mechanism outlined in Scheme 6.5 .



SCHEME 6.5

Similar conclusions apply to N-nitrosamine formation from aqueous nitrate, although these reactions show additional complexities. Like those with aqueous nitrite, they are inhibited by acid, alkali and added KN_3. Further, the rate of N-nitroso- and N-nitro-amine formation is dependent on the dose of γ-radiation and [$NaNO_3$], but is largely independent of the [Amine]. This suggests that reaction follows a similar mechanism to that of Scheme 6.5, except the intermediate formation of N_2O_4 is involved (Scheme 6.6).



SCHEME 6.6

It is more difficult to explain variation in the yields of N-nitrosamine and N-nitroamine products with amine structure. With morpholine and N-methylpiperazine yields of N-nitrosamine were larger than the corresponding N-nitroamine but this dependency was reversed for piperidine. Further, under similar reaction conditions, yields of N-nitrosomorpholine are larger than those of N-nitrosopiperidine or N-methyl-N-nitrosopiperazine. Also, the yield of N-nitropiperidine is larger than N-nitromorpholine or N-methyl-N-nitropiperazine. Thus, the amine basicity does not seem to be an important factor. The simplest explanation is that the stability of the various products towards γ-irradiation varies markedly. Evidence cited in Table 6.3 shows that N-nitrosamine decomposition is independent of structure and that nitrate inhibits decomposition. Further, the extent of decomposition of N-nitromorpholine and N-methyl-N-nitropiperazine (Table 6.3) is not extensively inhibited by added 0.1 M $NaNO_3$, and there is a slight dependence on the structure of the

N-nitroamine towards decomposition. Significantly, N-methyl-N-nitropiperazine decomposition is dependent on concentration. The product of this N-nitroamine may catalyse the breakdown.

Alternatively, formation of N-nitropiperidine may stem from the reaction of $\dot{N}O_2$ rather than N_2O_4. This explanation is unsatisfactory since no N-nitropiperidine formation was observed after γ-irradiation of aqueous $NaNO_2$ solutions. The formation of N-nitrosamines, however, requires reaction via N_2O_4.

N-nitrosomorpholine was found after the Hg photolysis of aqueous $NaNO_2$ and $NaNO_3$ solutions in the presence of morpholine. The rate of N-nitrosomorpholine is dependent on the length of exposure of Hg-photolysis, $[NaNO_2]$ and $[NaNO_3]$, but largely independent of [morpholine]. It is likely therefore, that formation of the N-nitrosamine follows a similar scheme as proposed for γ-radiation. No attempt was made to determine the energy required for the reduction of aqueous $NaNO_2$ and $NaNO_3$, but clearly, this process is not restricted to high energy γ-rays.

6.4 EXPERIMENTAL

6.4 (I). Reagents, Substrates and Products.

The purification of amines, the preparation and purification of N-nitrosamines has been described in Chapter 2.4. Here AnalaR grade $NaNO_2$, $NaNO_3$, KN_3, sulphanilamide, N-naphthylethylene diamine dihydrochloride and volumetric standard N-HCℓ, NaOH were used without further purification. All reaction solutions were made up with deionised, degassed water under a nitrogen atmosphere. Phosphate (pH 6.99) and saturated borax (pH 9.2) buffer solutions were prepared with degassed, deionised water.

6.4. (II). Preparation of N-nitroamines.

N-nitromorpholine was prepared by Mr.B.Li by the oxidation of N-nitrosomorpholine with trifluoracetic anhydride and purified following a literature procedure.[223] (N-nitromorpholine, white solid m.p., 52°C (lit[223] 52°C)).

N-nitropiperidine was prepared by Dr. S. Krytopoulos by oxidation of N-nitrosopiperidine with trifluoracetic anhydride.

N-nitropiperidine, pale yellow oil, b.p., 116-117°C/17 mm.[224]

N-methyl-N-nitropiperazine was prepared by Mr. D.E.G. Shuker following a literature procedure.[225]

N-methyl-N-nitropiperazine, pale orange crystals, m.p., 46-48°C, lit[225] 48-50°C.

6.4. (III) Reaction Methods.

For the decomposition of N-nitrosamines and N-nitroamines, 5 ml of the appropriate solution was pipetted into a pyrex Quickfit test-tube (previously purged with nitrogen) and then sealed under nitrogen. For reactions involving the formation of N-nitroso- and N-nitroamines, 4.5 ml. of freshly prepared stock solutions of $NaNO_2$, or $NaNO_3$ was pipetted into the Quickfit tube and the final volume adjusted to 5.0 ml. by appropriate addition of water, amine or additive. The tube was finally purged with nitrogen and sealed.

γ-irradiation of these samples were carried out in the centre of a ^{60}Co source of dose rate 1 M rad/h for the given time. For low dose rate irradiations the samples were placed at fixed distances from the centre of the source, and the dose estimated using the inverse square relationship. The dose rate (± 10%) at fixed distances from the ^{60}Co source was previously calibrated by Dr. P. Clay, Department of Chemical Engineering, Imperial College and gave fairly good agreement with the inverse square relationship. The total dose absorbed, however, has a larger experimental error (± 10-15%) due to the scattering of γ-rays by the walls of the ^{60}Co chamber and also the diminished energy of the γ-rays on travelling large distances. After γ-irradiation, the solutions were assayed by g.l.c., after standing for ca. 60 min; although the temperature of these solutions (even on longer than 60 min. exposures) did not exceed 30°C. The dead space above the reaction solution was ca. 5 ml.

6.4. (IV) Hg photolysis was performed on samples prepared as above and photolysed using a 125 W Osram high pressure bulb, at a fixed distance

of 13 cm from each sample. No attempt was made to restrict the wavelength of light. The temperature of these solutions was maintained at room temperature by using a fan to circulate cool air.

6.4. (V) Analysis of Products.

N-nitrosamines and N-nitroamines were assayed by g.l.c. on Perkin-Elmer F33 instrument fitted with 8% Carbowax plus 2% KOH column under standard conditions described in Chapter 2.4.III.b, with the exception that column temperature was 190°C. The retention time of these products are shown below:-

Compound	Retention time/min.
N-nitrosopiperidine	2.0
N-nitropiperidine	2.7
N-nitrosomorpholine	2.4
N-nitromorpholine	3.2
N-methyl-N-nitrosopiperazine	2.6
N-methyl-N-nitropiperazine	3.5

The extent of decomposition was estimated by comparing peak heights on g.l.c., of the standard solution of N-nitroso- and N-nitro-amine with those after γ-irradiation. The yields of N-nitrosamine and N-nitroamines formation were obtained and confirmed by direct comparison and spiking with standard solution of authentic material as described in Chapter 2.4.III.

6.4. (VI). Product Analysis from the Decomposition of N-nitroso-piperidine.

After irradiation of N-nitrosopiperidine solution, alcoholic 2,4-dinitrophenylhydrazine (DNP) was added. The only product to form a DNP-derivative was collected and recrystallised from methylated spirit. DNP-acetone, m.p., 123.5°C, lit[221] m.p., = 126°C ; the mass spectra gave molecular ion at 238.

The organic fraction was extracted with $CH_2C\ell_2$ (3 x 25 ml), washed with H_2O, dried over $MgSO_4$ and concentrated under reduced pressure.

The aqueous solution was analysed by g.l.c. on a Perkin-Elmer F11 instrument fitted with 15% polypropylene glycol column, the operating conditions and retention times for the identified organic products are given below.

Perkin-Elmer F11

Column - 2 metre, $\frac{1}{8}$'' i.d., stainless steel column, packed with 15% polypropylene glycol (LB-550-X) on Chromosorb W (80-100 mesh).

Conditions: Column temp. 60°C

Injection temp. 240°C

$N_2 = H_2 = Air = 30$ p.s.i.

Compound	Retention time/min.
CH_3COCH_3	2.1
MeOH	3.0
EtOH	3.6
i-PrOH	4.4

The major product R_F = 2.1 min. was identified as acetone by spiking technique. The presence of isopropanol R_F = 4.4 min. was further characterised by oxidation with dichromate/H_2SO_4 followed by distillation of acetone (b.p., 56°C/760 mm) and derivatisation by DNP-derivative. Methanol and ethanol were confirmed by spiking with authentic materials.

The organic fraction was analysed on t.l.c. plates (60" x 20", 2 mm thick silica, developed with 4:1 or 2:1 v/v pet-ether (40-60)/ acetone). The developed bands were located with a u.v. lamp, the products extracted with $CHC\ell_3$ and concentrated under reduced pressure. The i.r., spectra ($CHC\ell_3$) for bands at R_F 0.80 compared with authentic N-nitrosopiperidine.

i.r., $CHC\ell_3$ 1460, 1430, 1360 cm^{-1} (N-NO st).

The bands at R_F 0.4 and 0.05 are listed below although the products could not be further characterised.

Band R_F 0.04

i.r., $CHC\ell_3$ 1710 (CO), 1465, 1430, 1365 cm^{-1} (N-NO str).

Band R_F 0.05

i.r., $CHC\ell_3$ 1710 (CO), 1675, 1450, 1370, 1265, 1125, 1075, 985.

6.4. (VII) Nitrite Estimation.

Nitrite was estimated following the modified Shinn's procedure as described in Chapter 2.4.III (b). Typically, 1.0 ml. of reaction solution was added to acidic 0.5% sulphanilamide (6 ml) in a 25 ml. flask and after 3 min. the coupling agent added. The concentration of nitrite was estimated as described previously.

6.5. (I) Typical Reaction.

The effect of γ-radiation (1 M rad/hr) on the yield of N-nitrosomorpholine (NNM) in aqueous anaerobic solution at ambient temperature. Initial $[NaNO_2]$ = 0.1 M, [morpholine] = 2 x 10^{-3} M. Analyses by g.l.c., F33 under standard conditions (v. Experimental 6.4.(V)). Injection sample = 1 μl.

Time (γ-radiation) /min.	Attenuation	Peak Height	10^5 [NNM]/M
6	x 16	38, 38	46.2
1 μl 10^{-3} M N-NM at attenuation x 16 gave peak heights 41, 41			
15	x 64	56, 56	79
1 μl 10^{-3} M NNM at attenuation x 64 gave peak heights 71, 71.			
30	x 64	39, 39	173
1 μl 10^{-3} M NNM at attenuation x 64 gave peak heights 45, 45.			
60	x 128	47, 47	200
1 μl 10^{-3} M NNM at attenuation x 64 gave peak heights 47, 47.			

6.5.(II). The effect of γ-radiation (1 M rad/hr) on the yield of N-nitrosomorpholine (NNM) and N-nitromorpholine ($N\text{-}NO_2M$) in aqueous anaerobic solution at ambient temperature. Initial [$NaNO_3$] = 0.1 M, [Morpholine] = 2×10^{-3} M. Analyses as described (6.5.(I)).

Time (γ-radiation)/ min.	Attenuation	Peak Height NNM	Peak Height $N\text{-}NO_2\text{-}M$	10^5 [N-NM]/M	10^5 [$N\text{-}NO_2$-M]/M
6	x 16	3,3	3,3	3.7	1.2
1 µl 10^{-3} M NNM at attenuation x 32 gave peak heights 41, 41.					
1 µl 1.76 x 10^{-3} M $N\text{-}NO_2$-M at attenuation x 32 gave peak heights 22, 22.					
30	x 16	19, 20	4,4	21.5	11.7
1 µl 10^{-3} M N-NM at attenuation x 32 gave peak heights 4.5, 45.					
1 µl 1.76 x 10^{-3} M $N\text{-}NO_2$-M at attenuation x 32 gave peak heights 31, 31.					
60	x 16	76,76	9,9	40.4	17.4
1 µl 10^{-3} M NNM at attenuation x 64 gave peak heights 47, 47.					
1 µl 1.76 x 10^{-3} M $N\text{-}NO_2$-M at attenuation x 32 gave peak heights 45, 46.					

CHAPTER 7

SUMMARY

7.1 Reactions of Nitric Oxide.

Nitric oxide is a common pollutant associated with combustion processes,[56,58] making its ability to convert amino compounds into carcinogenic N-nitrosamine relevant to the aetiology of human cancer. Several previous investigations[82,83,87] and our own studies have shown that N-nitrosamine formation from dissolved NO and secondary amines is slow ($t_{\frac{1}{2}}$ ca. 8 days[83]) unless certain metal catalysts or promoters are present. The best promoter appears to be air (or oxygen) which oxidises NO to $\dot{N}O_2$. This leads to the formation of N_2O_3 and N_2O_4, both of which react rapidly with secondary amines at ambient temperature in both organic[41] or neutral and alkaline media.[40,83]

We have shown that NO in the presence of iodine leads to rapid N-nitrosamine formation ($t_{\frac{1}{2}}$ ca. 3 min) in anaerobic EtOH at 25°C and for the more basic amines, these reactions are faster than the maximum rates of N-nitrosamine formation in aqueous HNO_2 (at pH 3-3.4 with $[HNO_2] = 2 \times 10^{-2}$ M, $t_{\frac{1}{2}}$ (piperidine) ca. 34 days, and $t_{\frac{1}{2}}$ (morpholine) ca. 67 min.[7]). The reaction mechanism involves essentially the oxidation of NO to NO^+ (via nitrosyl iodide formation) and nitrosation by nucleophilic attack of unprotonated amine substrates on this entity. In neutral conditions, iodine acts as a promoter and the yield of N-nitrosamine is dependent on the $[I_2]$. However, in acidic media its effect is catalytic. These reactions also involve nitrosation by nitrosyl iodide but where I_2 is probably derived from the neutralisation of the strong acid HI by NO yielding NOH (evolved as N_2O and H_2O) and NOI. This hypothesis was supported by independent studies with HI itself and HI derived from the solvolysis of metal

iodides. These reactions are fast, and for N-methylpiperazine the rate maximiumis at pH ca. 2.2 which approximates to gastric pH.[7]

Less effective catalysis was observed from the reaction of Ag^{I} salts and dissolved NO in neutral conditions. These reactions are still considerably faster than reactions in the absence of Ag^{I} and with nitrous acid. Silver(I) salts act as promoters, and in conditions of excess amine the maximum yield is strictly controlled by $[Ag^{I}]$. These reactions appear to involve disproportionation of Ag^{I} by the amine to an Ag^{II}-amine complex followed by oxidation to the amino radical ($R_2\dot{N}$) which reacts directly with NO in a rate-limiting radical coupling step.

Silver(II) picolinate oxidises NO to NO^{+} and N-nitrosamine formation results from reaction of the released NO^{+} species and the unprotonated amine in anucleophilic solvents. Other metals such as Cu^{I}, Cu^{II}, Fe^{II}, Fe^{III}, also form nitrosyl complexes but nitrosation appear to involve metal-bound amine ligands, and not direct release of NO^{+}-X species.

The above results can be accommodated within the context of the reported reactivity of NO. Thus, NO itself can lead to N-nitrosamine formation by reaction with dialkylamino radicals or as NO^{+} with unprotonated amines. From our own studies of these reactions, two important observations regarding the maximum yield and the choice of mechanims have become apparent. The maximum yields fall into two categories, either: (I) stoichiometric with respect to [catalyst] (i.e., where the catalyst acts as a promoter), or (II) where the yields are quantitative (where the catalysis is truly catlytic). Both oxidation of amine (to $R_2\overset{+\cdot}{N}H$) or NO, (to NO^{+}) lead to N-nitrosamine formation with the concurrent release of acid (Equation 7.1 and 7.2, respectively).

$$R_2\overset{+\cdot}{N}H + NO \longrightarrow R_2NNO + H^+ \quad .... \quad 7.1$$

$$NOX + R_2NH \longrightarrow R_2NNO + HX \quad .... \quad 7.2$$

Hence the observed yields for the more basic amines (i.e., piperidine, morpholine) under neutral conditions are either 50% (based on initial [Amine]) in conditions of excess catalyst or stoichiometric with respect to [promoter]. For these reactions the limiting yield is controlled by both the concentration of unprotonated amine and the [promoter]. When the maximum yields are greater than 50% or stoichiometric, the reaction scheme must include the removal of liberated acid; we have no evidence that protonated piperidine or morpholine react rapidly with NO. Removal of liberated acid requires the generation of base during the reaction; this may be the reduction of NO to NO^- (evolved as N_2O) or by direct $\dot{H}$-abstraction by NO on a metal-bound amine complex. This latter reaction presumably proceeds if there is an increase in the acidity of N-H bond by complex formation. Both these pathways, however, lead to N_2O formation, and so quantitative yields imply that N_2O be formed as a co-product.

7.2 Human Exposure.

It has been demonstrated that many metal salts, iodine, and HI catalyse N-nitrosamine formation from NO under extremely mild conditions. Both copper and iron salts are dietary additives and are major inorganic minerals [226] (at concentrations ca. 0.08-0.15 mg/litre [227]) required for many metabolic functions in humans. Iodine, and iodide salts are present in saliva and gastric mucosa.[227]

These findings are relevant to recent observations that ascorbic acid may inhibit N-nitrosamine formation where the inhibitory effect is related to the reduction of nitrite to NO.[7] Clearly assumptions that NO does not form carcinogenic N-nitrosamines are incorrect, whenever, metal salts, iodine and iodide salts are present and the results presented here are relevant to the possibility of *in vivo* N-nitrosamine formation.

7.3 γ-Radiation Studies.

γ- Radiation decomposes N-nitrosamine in aqueous anaerobic solution. The preliminary study showed that the extent of decomposition is independent of the structure of N-nitrosamine and pH, but dependent on γ-dose. The decomposition is inhibited by excess $NaNO_2$ and $NaNO_3$, probably by preferential reaction with HO and $e^-_{(aq)}$ produced from the radiolysis of aqueous solutions.

The γ-irradiation of aqueous nitrate and nitrite solutions leads to the generation of N_2O_4 and N_2O_3 intermediates, respectively, and in the presence of secondary amines, N-nitroso- and N-nitro-amines form from the γ-irradiation of aqueous $NaNO_3$ solutions, while N-nitrosamines only form from aqueous $NaNO_2$ solutions.

Although high doses were used in our experiments, it seems unlikely that γ-induced N-nitrosamine formation contributes to human exposure, except where γ-radiation is used to sterilise food: for example, shell fish and cured meats are sterilised by γ-radiation where the reported dosage is *ca.* 0.2 M rad.[185] N-nitrosamine formation might be expected since most meat and fish contain amines and nitrate/nitrite preservatives.[10]

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APPENDIX

Rapid Formation of *N*-Nitrosamines from Nitric Oxide in the Presence of Silver(I) Salts

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Summary *N*-Nitrosamines form rapidly from NO and secondary amines in the presence of Ag^{I} salts *via* amino radical cation intermediates derived from Ag^{II}-amine complexes.

WE have shown[1] that NO is an ineffectual reagent for the *N*-nitrosation of amines in the absence of either oxygen or iodides including iodine itself. Activation by oxygen results from the formation of either N_2O_3 or N_2O_4[1a] and by iodides from the formation of NOI.[1b] Other work has shown that the *N*-nitrosation of amines by NO may also be catalysed by Cu^{II} and Fe^{III} salts.[2] These reactions, which have been the subject of several patents,[3] are generally considered to involve oxidation of NO to NO^{+}, which then reacts with unprotonated amine to give products. We report some new Ag^{+} promoted nitrosations by NO where strong evidence suggests that oxidation of the amine rather than NO is the activating process.

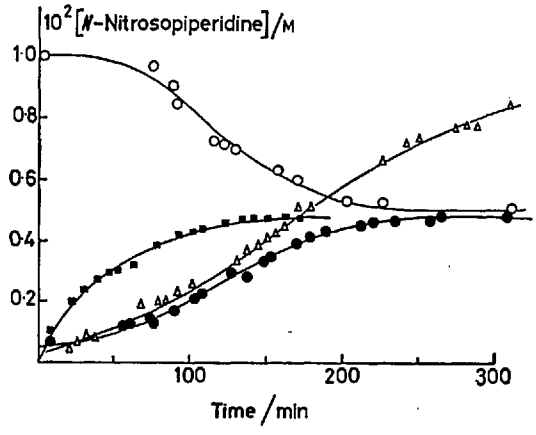


FIGURE. Formation of *N*-nitrosopiperidine from 2×10^{-2} M NO and 10^{-2} M piperidine in EtOH at 25 °C in the presence of 10^{-2} M $AgNO_3$. ○, Variation in residual [piperidine]; ●, variation in [*N*-nitrosopiperidine]; △, variation in [*N*-nitrosopiperidine] in the presence of $1{\cdot}5 \times 10^{-2}$ M Et_3N; ■, variation in [*N*-nitrosopiperidine] where piperidine and $AgNO_3$ reacted prior to addition of NO.

These reactions were carried out at 25 °C in carefully degassed EtOH containing the Ag^{I} salt saturated with purified NO and contained under gaseous NO at atmospheric pressure. The equilibrium concentration of dissolved NO was *ca.* 2×10^{-2} M which should be maintained during reaction by absorption from the gaseous phase. Usually, reactions were initiated by injecting the amine into the flask through a Subaseal stopper with a syringe. Aliquots were removed similarly at timed intervals for prompt g.l.c. assay of *N*-nitrosamine content.

TABLE 1. Formation of *N*-nitrosopiperidine from *ca.* 2×10^{-2} M NO in EtOH at 25 °C in the presence of $AgNO_3$.

10^2[Piperidine]/M	10^2[$AgNO_3$]/M	10^2[*N*-Nitrosopiperidine]/M
1·0	0·10	0·12
1·0	0·25	0·27
1·0	0·50	0·48
1·0	1·0	0·48
0·25	0·25	0·10
0·50	0·52	0·30

Variation of % reaction with time for equimolar (10^{-2} M) piperidine and $AgNO_3$ under various experimental conditions is shown in the Figure. The usual experimental procedure (*vide supra*) gave sigmoid shaped plots for both loss of piperidine and formation of *N*-nitrosopiperidine. During the well defined induction period (*ca.* 60 min) a silver mirror was progressively deposited on the walls of the reaction vessel. With a larger than 2 fold excess of piperidine, the maximum yield of *N*-nitrosamine (apparent after

TABLE 2. Maximum yields and formation times for *N*-nitrosamines from 2×10^{-2} M NO in EtOH at 25 °C in the presence of $AgNO_3$.

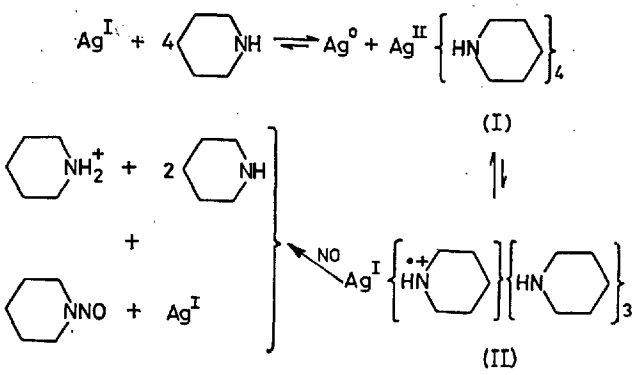
Amine (0·01 M)	10^2[$AgNO_3$]/M	10^2[*N*-Nitrosamine]/M	Reaction time/min
Piperidine	0·25	0·27	130
Pyrrolidine	1·0	0·45	150
Morpholine	0·50	0·50	1300
N-Methylpiperazine	1·0	0·09	300
"	1·0	0·66[a]	150
N-Methylaniline	1·0	0·75	100
Diphenylamine	1·0	0·65	1300

[a] In the presence of $5{\cdot}4 \times 10^{-3}$ M NaOEt

ca. 200 min) was governed by the Ag^I salt concentration in an approximate 1:1 relationship. (Table 1). With equimolar concentrations of Ag^I salt and piperidine, however, *N*-nitrosamine formation was slower and only *ca.* 50% of the amine reacted (Table 1). These observations suggest the overall stoicheiometry is defined by equation (1). Significantly, with equimolar concentration of Ag^I

$$Ag^I + 2\,R_2NH \xrightarrow{NO} Ag^0 + R_2NNO + R_2NH_2^+ \qquad (1)$$

salt and piperidine, the yield of *N*-nitrosopiperidine was increased to 100% by the addition of base such as Et_3N or 2,2′,6,6′-tetramethylpiperidine (Figure). One other important observation was that the induction period noted above was not apparent when the Ag^I salt and piperidine were allowed to react in EtOH (to give a silver mirror) and then NO was passed into the solution (Figure).



SCHEME

The results are consistent with a mechanism (Scheme) involving relatively slow initial disproportionation of the Ag^I salt to give an Ag^{II}-amine complex (I). Related Ag^{II}-complexes are known to be powerful oxidants capable of generating radical cations ($R_2N^{\cdot+}H$) from amines[4] and, presumably, of oxidising NO to NO^+. Thus formation of *N*-nitrosopiperidine could arise from the interaction of either NO with the radical cation (II) or NO^+ with the neutral piperidine. We prefer the former pathway (as in the Scheme) for the following reasons. Reaction of $NO^+BF_4^-$ with 10^{-2} M piperidine in EtOH at 25 °C gives high yields of ethyl nitrite (EtONO) but only 0·25% of *N*-nitrosopiperidine, whereas the reaction with Ag^I salt and NO gives quantitative yields of *N*-nitrosopiperidine and very little EtONO. Further, when disproportionation of the Ag^I salt is effected by excess of NaOEt, the maximum yield of N-nitrosopiperidine decreases to *ca.* 5%. Finally, the addition of $NO^+ClO_4^-$ to macrocyclic tetra-aza-Ag^{II} complexes gives NO plus the corresponding Ag^{III}-complex.[5]

The reactions involving NO and Ag^I salts are not specific to piperidine and maximum yields of *N*-nitrosamine with formation times for other secondary amines are summarised in Table 2. Any correlation between reactivity and amine basicity (pK_a) is not apparent. The dibasic *N*-methylpiperazine gave no reaction without the addition of NaOEt. Most of these reactions were carried out with $AgNO_3$, but similar results were obtained with $AgClO_4$.

These reactions are very much faster than *N*-nitrosamine formation from both dissolved NO alone ($t_{\frac{1}{2}}$ *ca.* 8 days)[1] and, for the more basic amines, from aqueous HNO_2 {with $[HNO_2] = 2 \times 10^{-2}$ M, $t_{\frac{1}{2}}$ (piperidine) = *ca.* 34 days at pH 3—3·4}.[6] The results are, therefore, relevant to assessments of human exposure to carcinogenic *N*-nitrosamines. In particular, they suggest that metal amine solutions may produce *N*-nitrosamines on exposure to NO as has been observed specifically for cutting oils.[7]

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