Kinetics and mechanism of the nitrosation of 2-mercaptopyridine [pyridine-2(1*H*)-thione]

Severino Amado, Andrew P. Dicks and D. Lyn H. Williams *

Department of Chemistry, Durham University, South Road, Durham, UK DH1 3LE

2-Mercaptopyridine (MP) reacts rapidly with nitrous acid in mildly acid aqueous solution (*via* the thione tautomer) to give an unstable *S*-nitroso ion (SNO^+) in a reversible process with an equilibrium constant (K_N) of *ca.* 1×10^5 dm^6 mol^{-2}. SNO^+ is readily detected by two peaks in the UV spectrum at 295 and 240 nm with extinction coefficients 9600 and 9300 dm^3 mol^{-1} cm^{-1} respectively. MP is regenerated when the solution is made alkaline. Kinetic measurements made on the nitrosation reaction give a value of 8200 dm^6 mol^{-2} s^{-1} for the third order rate constant k_3 (defined by rate = k_3 [MP] [HNO_2] [H^+]), which is close to that believed to be the diffusion-controlled limit for attack by NO^+ (or $H_2NO_2^+$). As expected there is marked catalysis by Cl^- and Br^-, and analysis of the kinetic results obtained from variation of measured rate constants with [halide ion] gave values of 3.5×10^9 and 3.7×10^9 dm^3 mol^{-1} s^{-1} respectively for the bimolecular rate constants for NOCl and NOBr reactions with MP, again values close to the diffusion limit. The same experimental results also yielded values of 30 and 2400 dm^3 mol^{-1} s^{-1} for the second order rate constants, for the reverse process of Cl^- and Br^- reaction with SNO^+. Values for K_N of 1.3×10^5 and 7.9×10^4 dm^6 mol^{-2} were obtained from the halide catalysed reactions. In acid solution SNO^+ decomposed to the disulfide (2,2′-dipyridyl disulfide) and NO (measured with a NO-electrode). Quenching of SNO^+ at pH 7.4 gave UV spectroscopic evidence for the neutral deprotonated form (SNO) of SNO^+ and there was a transformation to give mainly MP together with some disulfide. There was clear evidence that SNO^+ (and maybe SNO) can act as an efficient nitrosating species: addition of the thiol *N*-acetylcysteine (at pH 6.15) resulted in the almost instantaneous decomposition of SNO. Addition of *N*-methylaniline (NMA) to an acidified solution of SNO^+ resulted in quantitative *N*-methyl-*N*-nitrosoaniline formation and kinetic measurements of the nitrosation of NMA in the presence of MP showed marked catalysis at low [MP], which disappeared at higher [MP]. These results are quantitatively consistent with nitrosation *via* SNO^+: catalysis disappears at higher [MP] when the nitrous acid is virtually completely converted to SNO^+. A value of 1.7×10^5 dm^3 mol^{-1} s^{-1} was obtained for the bimolecular rate constant for reaction of SNO^+ with the free base form of NMA. MP is thus an excellent catalyst for electrophilic nitrosation. Under somewhat different conditions SNO^+ can then act as a source of HNO_2/NO_2^-, NO or NO^+. The chemistry reported in this paper bears many similarities to that involved in the nitrosation of thioureas, and subsequent reactions of the *S*-nitrosothiouronium ions.

The interest in nitric oxide chemistry continues to grow since the discovery around 1990 that NO is generated *in vivo* and is responsible for a number of crucial body functions. One aspect of this work has been to establish the chemistry of *S*-nitrosothiols (RSNO), with regard to their formation *in vivo*, and the mechanism of both NO release and NO-group transfer, since it is now believed that RSNO species may be involved in the storage and transfer of NO in the body.[1] In addition, RSNO compounds are currently under scrutiny as possible NO donors for medical use to complement and possibly replace the well-known glyceryl trinitrate (nitroglycerine), which has been very widely used for over a century, but which is subject to a tolerance problem. We have established[2] that RSNO species generally, in solution at pH 7.4, release NO in a copper-catalysed process [eqns. (1) and (2)], where the effective catalyst

$$2RS^- + 2Cu^{2+} \longrightarrow 2Cu^+ + RSSR \qquad (1)$$

$$Cu^+ + RSNO \longrightarrow Cu^{2+} + RS^- + NO \qquad (2)$$

is Cu^+, generated by thiolate reduction of Cu^{2+}. Often trace amounts of copper present adventitiously are sufficient to effect reaction, and small amounts of thiolate ion are always present in RSNO samples,[3] because of the slight reversibility of the reaction for RSNO formation from nitrous acid and thiols [eqn. (3)]. Reaction can effectively be halted, either, (a) by the

$$RSH + HNO_2 \longrightarrow RSNO + H_2O \qquad (3)$$

removal of Cu^{2+} with EDTA, or (b) by reduction of the [RS^-] to very low levels by the synthesis in solution of RSNO using a large excess of HNO_2 over RSH. In addition, the exchange reaction [eqn. (4)] is now well-established,[4] which is a specific

$$RSNO + R'S^- \rightleftharpoons RS^- + R'SNO \qquad (4)$$

example of a more general reaction of nucleophilic attack at the nitroso-nitrogen atom: other reasonably powerful nucleophiles, notably amines, azide ion, hydrazine, sulfite and ascorbate have recently been shown to react similarly.[5] At very high thiol concentrations nitrosothiols undergo a quite different reaction, leading to ammonia formation.[6–8]

As part of our structure–reactivity studies of the NO-forming decomposition reaction of *S*-nitrosothiols, we wished to extend our studies to include aromatic and heterocyclic systems, hitherto unexplored. We chose initially to examine the *S*-nitroso species derived from 2-mercaptopyridine (MP). The nitroso compound is not known, but we confidently expected to be able to generate it by simple *S*-nitrosation of MP using nitrous acid.[9] The results of the nitrosation study however turned out rather unexpectedly, and we present those results in this paper, together with the results obtained on the chemistry of the *S*-nitroso species, particularly on its ability to act as an

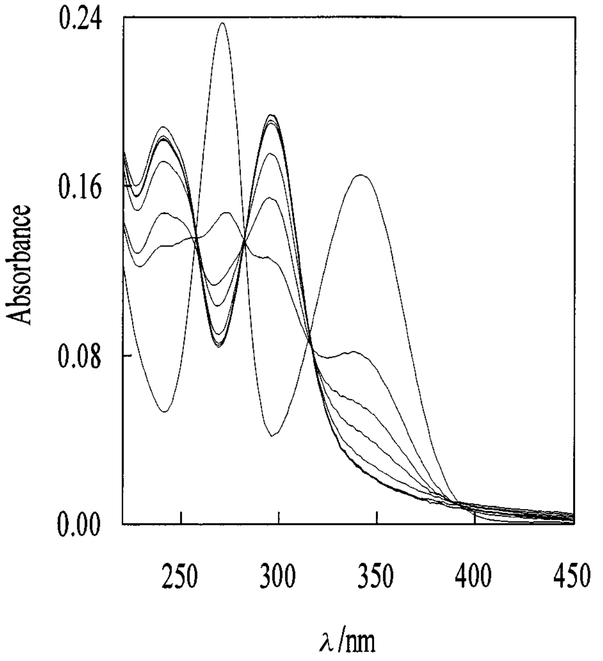


Fig. 1 Spectra of MP (λ_{max} 270 and 340 nm) at 2.02×10^{-5} mol dm^{-3} with HNO_2 (2.02×10^{-4} mol dm^{-3}) and NaBr (2.5×10^{-3} mol dm^{-3}) as a function of [H^+], 0.051, 0.102, 0.204, 0.51, 0.82, 1.02 and 1.36 mol dm^{-3} with decreasing absorbance at 340 nm. The reference cell contained HNO_2, $HClO_4$ and NaBr at the same concentrations as in the measurement cell.

electrophilic nitrosating agent, and also its ability to generate nitric oxide.

Results

Nitrosation of MP

When MP was treated with a ten-fold excess of sodium nitrite in mildly acidic solution, a yellow coloured species was formed. The spectral changes shown in Fig. 1 occurred. The scans are taken immediately after mixing, when the reaction was complete (assisted by a Br^- catalyst), and represent those taken at different acidities ranging from 0.051–1.36 mol dm^{-3} [H^+].

It is clear that the absorbances at 270 and 340 nm due to MP are gradually replaced by those at 295 and 240 nm (extinction coefficients 9600 and 9300 dm^3 mol^{-1} cm^{-1} respectively), as the acidity is raised. The yellow colour arises from the tailing of the 295 nm absorbance into the visible region and the presence of an additional band centred at 563 nm (see inset spectrum in Fig. 3), with a very low extinction coefficient (*ca.* 50 dm^3 mol^{-1} cm^{-1}). This is characteristic of *S*-nitrosothiols, RSNO, and has been assigned to the $n_N \rightarrow \pi^*$ transition,[10] and is clearly visible in the spectra of *S*-nitrosoglutathione and *S*-nitroso-*N*-acetylpenicillamine. These spectral changes are reversible, MP being regenerated as the acidity is lowered. The final product is formed quantitatively when [H^+] is >*ca.* 1 mol dm^{-3}. The same pattern is found if the acidity is maintained at 0.1 mol dm^{-3} and the concentration of nitrous acid is increased from *ca.* 5×10^{-5} to 3.5×10^{-3} mol dm^{-3} (see Fig. 2). Again complete conversion is achieved when [HNO_2] is >*ca.* 3×10^{-3} mol dm^{-3}. All of the solutions (for both figures) contain some added Br^- at 2.5×10^{-3} mol dm^{-3}, since the nitrosation reaction is bromide ion catalysed and this assists the rapid formation of the product with absorbances at 295 and 245 nm. It is clear that we are dealing with an equilibrium process here, forming the *S*-nitroso ion (SNO^+), nitroso[pyridin-2(1*H*)-ylidene]sulfonium as shown in eqn. (5). It is virtually certain that reaction occurs *via* the thione form of MP, since UV studies have shown that this form predominates in aqueous solution,[11] with an estimated equilibrium constant [eqn. (6)] of 49 000.[12] MP should thus be more properly called pyridine-2(1*H*)-thione. It is difficult to get an accurate value for K_N, the equilibrium constant for SNO^+ formation [eqn. (5)], from direct absorbance measurements, since the ion is not sufficiently stable in solution, but it is clear from approximate measurements that K_N has a value of around 1×10^5 dm^6 mol^{-2}.

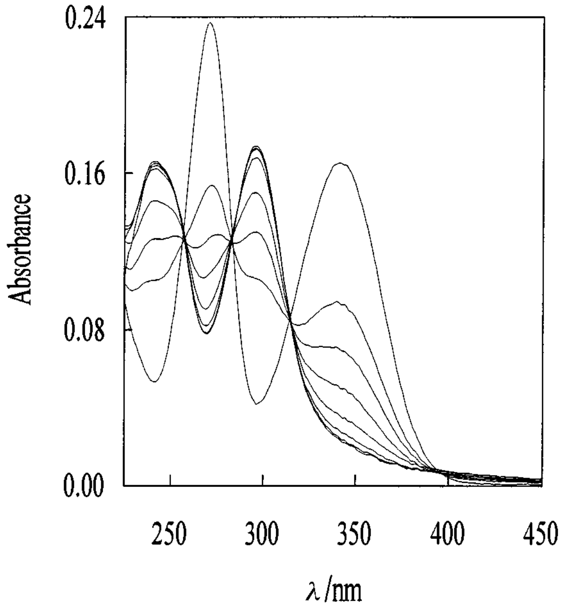
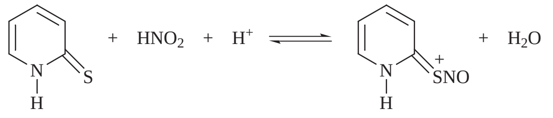


Fig. 2 Spectra of MP (2.02 mol dm^{-3}) in the presence of $HClO_4$ (0.10 mol dm^{-3}), NaBr (2.5×10^{-3} mol dm^{-3}) and HNO_2, 5.56×10^{-5}, 1.11×10^{-4}, 2.22×10^{-4}, 5.56×10^{-4}, 1.11×10^{-3}, 2.22×10^{-3} and 3.48×10^{-3} mol dm^{-3} with decreasing absorbance at 340 nm. The reference cell contained HNO_2, $HClO_4$ and NaBr at the same concentrations as in the measurement cell.



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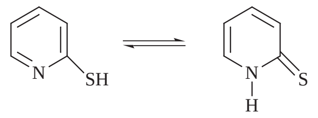


(6)

The kinetics of MP nitrosation were examined with initial [MP] ⪢ initial [HNO_2], in two ways, (a) at constant [H^+] varying [MP], and (b) at constant [MP] varying [H^+]. The results are given in Table 1. In both cases excellent first order behaviour was found and plots of k_0 (defined by rate = $k_0[HNO_2]$) *vs.* either [MP] or [H^+] were good straight lines with small positive intercepts. If we assume the equilibrium as in eqn. (5), then the expression for k_0 is given by eqn. (7), where k_3 is the third order

$$k_0 = k_3\,[MP]\,[H^+] + k_{-1} \qquad (7)$$

rate constant for the forward reaction, k_{-1} is the first order rate constant for the reverse reaction, and K_N is given by k_3/k_{-1}. Values of k_3 were obtained as 8000 ± 300 and 6800 ± 100 dm^6 mol^{-2} s^{-1} from the data in Table 1. The intercepts of both plots were too small and the errors too large to obtain reliable values for k_{-1} and hence K_N. The intercept of the k_0 *vs.* [H^+] plot was deemed to be more reliable (with a much smaller standard deviation, ~10% compared with ~30%), and this gave a crude value of 5.2×10^{-2} s^{-1} for k_{-1} and hence a value of *ca.* 1.3×10^5 dm^6 mol^{-2} for K_N, but this must be regarded as an approximate value. Further results were obtained at much higher concentrations of MP and HNO_2. Under these conditions the intercept was not experimentally detectable (since k_0 values were

Table 1 Values of the measured first order rate constant k_0, at 295 nm, for the nitrosation of MP with initial [MP] ⩾ initial [HNO_2]

[MP]/10^{-4} mol dm^{-3}	[H^+]/mol dm^{-3}	k_0/s^{-1}
2.00	0.100	0.187[a]
2.46	0.100	0.216[a]
2.87	0.100	0.262[a]
3.28	0.100	0.286[a]
3.69	0.100	0.321[a]
4.10	0.100	0.353[a]
4.15	0.025	0.125[b]
4.15	0.030	0.141[b]
4.15	0.035	0.157[b]
4.15	0.040	0.172[b]
4.15	0.045	0.179[b]
4.15	0.050	0.195[b]
4.15	0.060	0.221[b]
4.15	0.080	0.270[b]
4.15	0.100	0.327[b]
4.15	0.150	0.471[b]
4.15	0.200	0.630[b]

[a] [HNO_2] = 5.06 × 10^{-6} mol dm^{-3}. [b] [HNO_2] = 4.15 × 10^{-4} mol dm^{-3}.

much larger), but three values of k_3 were obtained (at different acidities) as 8700, 9000 and 8400 dm^6 mol^{-2} s^{-1}, in quite good agreement with the earlier results. When experiments were attempted under conditions where initial [HNO_2] ⩾ initial [MP], there was significant deviation from first order behaviour in the individual kinetic runs. This is probably due to the intervention of a reaction pathway involving N_2O_3 (probably rate-limiting formation of N_2O_3, which has been noted in other cases, *e.g.* in the nitrosation of the thiosulfate ion[13] and some enols[14]). We have not attempted a full kinetic analysis here. When small amounts of chloride ion or bromide ion were added under these conditions (generating NOCl and NOBr as the effective reagents), then the kinetics reverted to being fully first order, in accord with our interpretation.

We also examined the kinetics of MP nitrosation in the presence of both chloride and bromide ion (X^-) catalysts. The experimental results, which were obtained with initial [HNO_2] ⩾ [MP], are given in Tables 2 and 3 for the variation of k_0 with [H^+] at constant [X^-], and also with [X^-] at constant [H^+], for both halides. Catalysis is evident as expected, with that for bromide ion being the more pronounced. The results are readily analysed in terms of reaction *via* equilibrium concentrations of the corresponding nitrosyl halides, NOX, generated *in situ*,[15] as in eqns. (8) and (9). The expression for k_0, the measured first order rate constant, is given in eqn. (10). Here K_{NOX} is the equilibrium constant for NOX formation [eqn. (8)], k_2 is the second order rate constant for reaction of MP with NOX [forward reaction of eqn. (9)], and k_{-2} is the second order rate constant for the reaction of X^- with SNO^+ [the reverse reaction of eqn. (9)]. The overall equilibrium constant K_N is then given by k_2K_{NOX}/k_{-2}. All of the kinetic experiments in this section showed excellent first order behaviour, and all the plots of k_0 *vs.* [X^-] at constant [H^+], and of k_0 *vs.* [H^+] at constant [X^-] were clearly linear. The plot of k_0 *vs.* [Cl^-] showed a small intercept due to the uncatalysed reaction, which was not apparent in the Br^- plot because of the larger rate constants. The small but clearly significant intercepts for the [H^+] plots arise from the reversibility of the nitrosation as predicted by eqn. (10).

$$HNO_2 + X^- + H^+ \rightleftharpoons NOX + H_2O \qquad (8)$$

$$MP + NOX \rightleftharpoons SNO^+ + X^- \qquad (9)$$

$$k_0 = k_2K_{NOX}\,[HNO_2]\,[H^+]\,[X^-] + k_{-2}\,[X^-] \qquad (10)$$

For the Cl^- catalysed reactions we obtained values of 3.5 ± 0.3 × 10^9 dm^3 mol^{-1} s^{-1} for k_2 and 30 ± 3 dm^3 mol^{-1} s^{-1} for k_{-2} giving a value of K_N of 1.3 ± 0.5 × 10^5 dm^6 mol^{-2}. Similarly the Br^- catalysed reactions yielded corresponding values of 3.7 ± 0.2 × 10^9 and 2400 ± 200 dm^3 mol^{-1} s^{-1} and 7.9 ± 1.6 × 10^4 dm^6 mol^{-2}. All of these values were determined from k_0 *vs.* [H^+] plots. We used these values to calculate the slopes of the two k_0 *vs.* [X^-] plots and obtained values of 107 ± 11 dm^3 mol^{-1} s^{-1} for the Cl^- catalysed reactions and 6200 ± 300 dm^3 mol^{-1} s^{-1} for the Br^- catalysed reactions. These compare quite well with the experimentally measured values of 89 ± 2 and 5700 ± 100 dm^3 mol^{-1} s^{-1} respectively.

Table 2 Values of k_0 for the chloride ion catalysed nitrosation of MP; [HNO_2] = 2.0 × 10^{-4} mol dm^{-3}; [MP] = 1.97 × 10^{-5} mol dm^{-3}

[Cl^-]/10^{-2} mol dm^{-3}	[H^+]/mol dm^{-3}	k_0/s^{-1}
0.50	0.100	0.80
0.80	0.100	1.09
1.00	0.100	1.25
1.50	0.100	1.71
2.00	0.100	2.13
2.00	0.050	1.39
2.00	0.080	1.84
2.00	0.100	2.13
2.00	0.120	2.47
2.00	0.150	2.94

Table 3 Values of k_0 for the bromide ion catalysed introsation of MP; [MP] = 2 or 4 × 10^{-5} mol dm^{-3}

[Br^-]/10^{-2} mol dm^{-3}	[H^+]/10^{-2} mol dm^{-3}	k_0/s^{-1}
0.25	20	14.6[a]
0.50	20	29.2[a]
0.80	20	46.0[a]
1.00	20	57.4[a]
1.50	20	85.4[a]
2.00	20	114[a]
0.05	1.28	1.51[b]
0.05	1.79	1.75[b]
0.05	2.55	2.16[b]
0.05	4.08	2.82[b]
0.05	6.12	3.70[b]
0.05	8.16	4.46[b]
0.05	10.2	4.92[b]
0.05	15.3	7.02[b]
0.05	20.4	8.75[b]

[a] [HNO_2] = 2.0 × 10^{-4} mol dm^{-3}. [b] [HNO_2] = 4.0 × 10^{-4} mol dm^{-3}.

Decomposition of SNO^+

The yellow colour attributed to SNO^+ fades over a period of several minutes (typically the half-life is about 10 min), which makes it difficult to obtain a reliable value for K_N from direct absorbance measurements. Fig. 3 shows the spectra (recorded every 5 min) of the decomposition of SNO^+ in 0.1 mol dm^{-3} H^+, generated using excess nitrous acid over MP. The final spectrum is identical with that of a commercial sample of 2,2′-dipyridyl disulfide (at the same acidity), at a concentration exactly half that of the original [MP]. This indicates that the decomposition to the disulfide is quantitative. Measurements with the NO-electrode, when the decomposition was carried out under nitrogen gave an NO yield of just over 100%. The small excess probably comes from the decomposition of some of the large excess of nitrous acid used—necessary to generate a high SNO^+ yield.

When SNO^+, generated with a ten-fold excess of MP over HNO_2, is allowed to decompose in normal aerated solution, spectral studies (not shown) reveal that all of the MP is transformed to the disulfide, and not only that amount corresponding to the concentration of the limiting reactant HNO_2. This is because the NO generated is converted quantitatively back to HNO_2 by oxidation and reaction of NO_2 with NO giving N_2O_3, which is hydrolysed to HNO_2. This is a known reaction.[16]

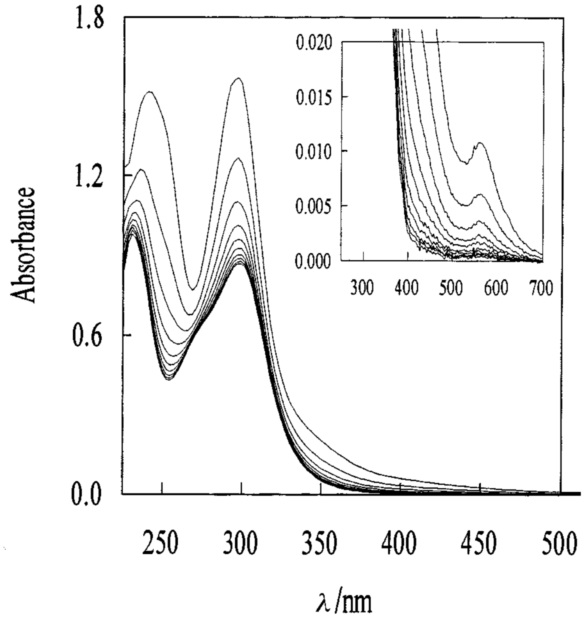


Fig. 3 Spectra recorded every 5 min of the decomposition of SNO^+ (decreasing absorbance) at 2.0×10^{-4} mol dm^{-3} in the presence of HNO_2 (2.0×10^{-3} mol dm^{-3}) and $HClO_4$ (0.1 mol dm^{-3})

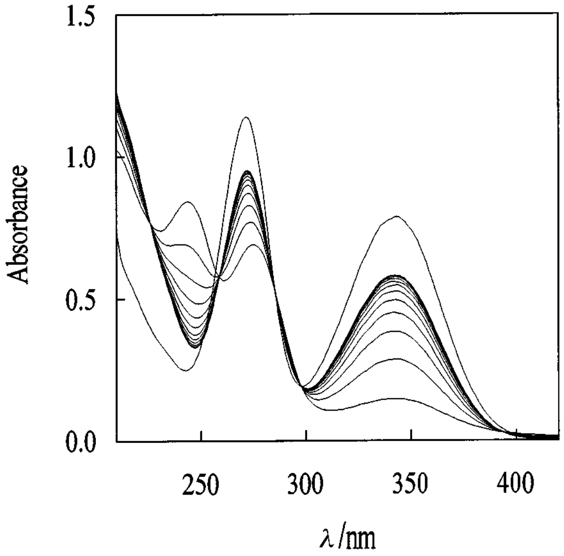


Fig. 4 Spectra recorded at 2 min intervals (increasing absorbance at 340 nm) of SNO^+ (prepared from MP, 1.09×10^{-4} mol dm^{-3} and HNO_2, 1.09×10^{-4} mol dm^{-3} in $HClO_4$, 0.4 mol dm^{-3}) quenched in phosphate buffer pH 7.31. The spectrum of MP (1.09×10^{-4} mol dm^{-3}) is also shown for comparison.

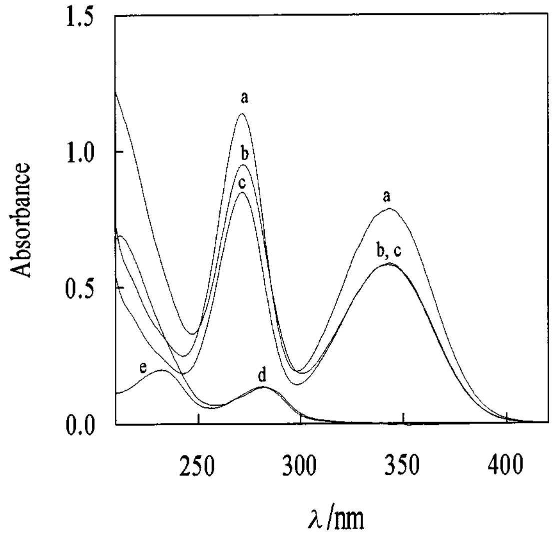
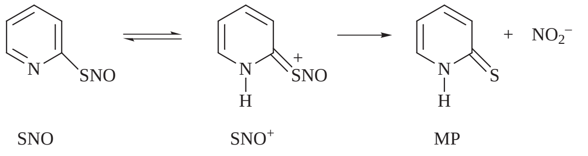


Fig. 5 Spectra showing the final products (75% MP and 25% disulfide) of the decomposition of SNO^+ in buffer pH 7.31. See text for full description.

When a freshly prepared solution of SNO^+ is quenched at pH 7.4, there is a first order transformation to MP. However the initial spectrum is very different from that of SNO^+. There is an absorbance at around 240 nm, but without the shoulder at around 250 nm present in SNO^+. More striking however is the total absence of the substantial absorbance at 295 nm (see Fig. 4). The reasonable explanation is that at this high pH, SNO^+ is deprotonated to give the fully aromatic 2-thionitroso compound which does revert to MP by protonation and water-promoted denitrosation as outlined in eqn. (11), or possibly by a direct reaction, without the intermediate formation of SNO^+. The overall reaction has a half-life of about 4 min. As expected this reaction is also bromide ion catalysed.



(11)

Reaction is not however quantitative since MP is formed in only *ca.* 75% of the theoretical yield, as is shown in Fig. 5. Spectrum (a) is that of MP at a concentration which would correspond to 100% reaction; spectrum (b) is the final spectrum of the decomposed SNO^+; spectrum (c) is that of MP at 75% theoretical yield; spectrum (d) is the subtraction of spectra (b) and (c). This shows clearly the absorbance at 281 nm due to the disulfide at a concentration corresponding to 12% yield, and spectrum (e) is authentic disulfide at the same concentration. Unfortunately the peak at 232 nm is obscured in (d) by the absorbance due to the nitrite ion product. The NO electrode detected *ca.* 20% nitric oxide product, which is consistent with the spectral analysis.

SNO^+ as a nitrosating species

We have examined the possibility that SNO^+ once generated, can act directly *in situ* as an electrophilic nitrosation reagent, without intermediate formation of NO^+, or something derived from it, *i.e.* as outlined in eqn. (12), for transfer to a general

$$SNO^+ + A \longrightarrow A^+\text{–}NO + MP \qquad (12)$$

nucleophilic species A. We worked with *N*-methylaniline (NMA), since the nitroso product is well-known with a large UV absorbance centred at 269 nm. Fig. 6 shows the repeat scan spectra at 5 min intervals for the nitrosation of NMA (4.23×10^{-4} mol dm^{-3}) with nitrous acid (2.02×10^{-5} mol dm^{-3}) at 0.1 mol dm^{-3} $HClO_4$, in the presence of a very low concentration of MP (8.2×10^{-6} mol dm^{-3}). There is a clear build up of the nitrosamine. In the absence of added MP the same reaction still occurs but at orders of magnitude more slowly (half-life several hours). The extent of catalysis is clearly spectacular, even at the low [MP] used. The inset spectra in Fig. 6 show (a) the final spectrum of the catalysed reaction, with the spectrum of MP subtracted out, and (b) the final spectrum (after many hours) of the uncatalysed nitrosation of NMA. The two spectra are virtually identical, and correspond to quantitative *N*-nitrosamine (NMA–NO) formation.

Similarly the addition of *N*-acetylcysteine to a solution of MP and HNO_2 (added to buffer pH 6.15) resulted in a virtually instantaneous disappearance of the absorbance due to SNO^+. It is almost certain that transnitrosation has occurred, but we have not here demonstrated the formation of *S*-nitroso-*N*-

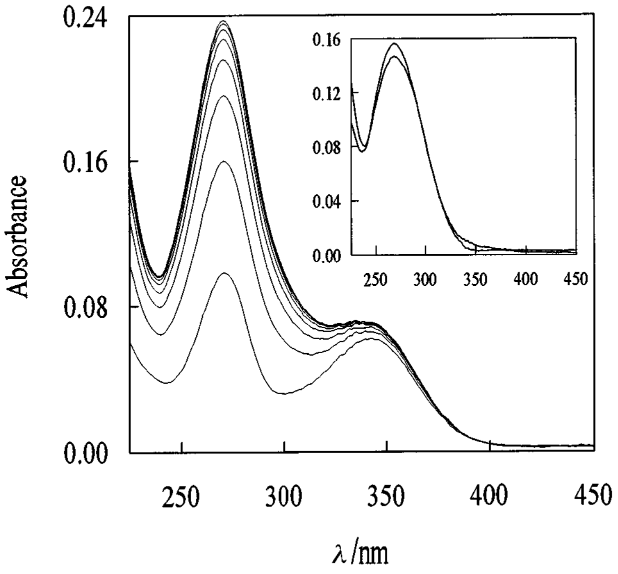


Fig. 6 Nitrosation of NMA (4.23×10^{-4} mol dm^{-3}) by HNO_2 (2.02×10^{-5} mol dm^{-3}) containing MP (8.2×10^{-6} mol dm^{-3}) and $HClO_4$ (0.1 mol dm^{-3}). Spectra (increasing absorbance) were recorded every 5 min. The inset spectrum gives the final spectrum (with that of MP subtracted out), compared with the final spectrum of the same reaction without added MP.

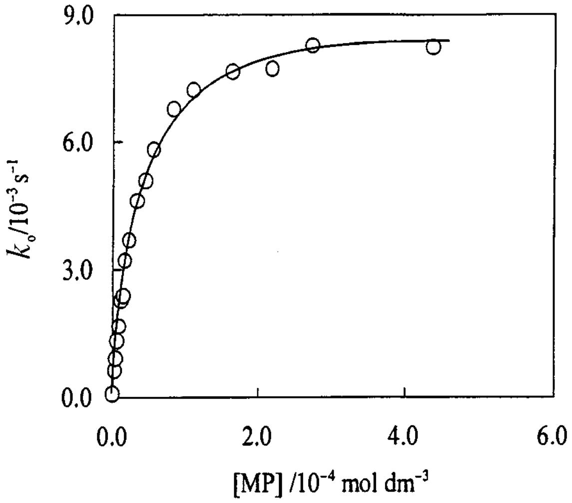


Fig. 7 Values of k_0 for the nitrosation of NMA (4.23×10^{-4} mol dm^{-3}) by HNO_2 (2.02×10^{-5} mol dm^{-3}) in $HClO_4$ (0.1 mol dm^{-3}) as a function of added MP

acetylcysteine (SNAC) formation because of the major overlap of absorbances in the 350 nm region for MP and SNAC.

Demonstration of MP catalysis of NMA nitrosation is more vividly demonstrated in Fig. 7, which shows the increasing k_0 values for initial concentrations of NMA (4.23×10^{-4} mol dm^{-3}) and HNO_2 (2.02×10^{-5} mol dm^{-3}) in 0.1 mol dm^{-3} $HClO_4$, with increasing [MP]. At low [MP] the plot is approximately linear, but catalysis levels off at higher [MP]. This could be due to effectively complete conversion of HNO_2 to SNO^+ at the higher [MP]. More quantitatively, if we accept that reaction occurs as indicated by eqns. (13) and (14), and that at

$$MP + HNO_2 + H^+ \rightleftharpoons SNO^+ + H_2O \quad K_N \qquad (13)$$

$$SNO^+ + NMA \longrightarrow NMA\text{–}NO + MP + H^+ \quad k_2 \qquad (14)$$

this acidity, the total stoichiometric concentration of NMA is effectively that of the protonated form, with nitrosation taking place *via* the free base form (acid dissociation constant K_a), then

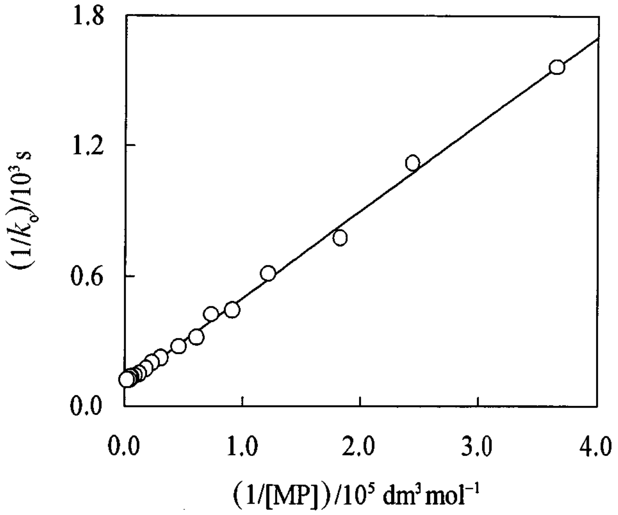


Fig. 8 Double reciprocal plot for the data given in Fig. 7

the expression for the measured first order rate constant is as in eqn. (15), where $[NMA]_t$ is the total stoichiometric concentration of NMA. It is easy to see that at high [MP] when K_N[MP][H$^+$] becomes $\gg 1$, catalysis will disappear. The double reciprocal plot of $1/k_0$ *vs.* 1/[MP] should then be linear with a positive slope and intercept. This is borne out by the experimental results as shown by the graph in Fig. 8. We get a value for K_N of $2.5 \pm 0.3 \times 10^5$ dm^6 mol^{-2} (from intercept/slope [H$^+$]), and a value of $1.7 \pm 0.2 \times 10^5$ dm^3 mol^{-1} s^{-1} for k_2 the bimolecular rate constant for reaction of SNO^+ with NMA. This value of k_2 predicts a limiting value for k_0 at high [MP] of 10×10^{-3} s^{-1}, which looks a reasonable extrapolated value for Fig. 7.

$$k_0 = k_2 K_N K_a\,[NMA]_t[MP]/(1 + K_N[MP][H^+]) \qquad (15)$$

Similar results were obtained at low [MP] when HNO_2 was used in large excess over NMA. The plot of k_0 *vs.* [MP] was an excellent straight line with a significant positive intercept, almost certainly due to the uncatalysed reaction, which now makes a significant contribution to the rate of the reaction, at high [HNO_2].

Discussion

Nitrosation of MP

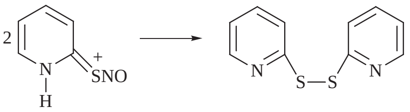
All of the evidence in the literature supports the idea that MP exists almost entirely in solution,[11,12] and also in the solid state,[17] in the thione form. Our results are all consistent with equilibrium formation of the *S*-nitrosated species SNO^+ as described by eqn. (5). The free thiol form of MP is at such a low concentration as to make it impossible that it is the reactant here—all derived rate constants would be massively in excess of the diffusion controlled limit. In water, MP is resistant to aerial oxidation (half-life for disulfide formation is hundreds of hours[18]), no doubt because the concentration of the thiol form is so low. The pK_a for deprotonation of the *S*-protonated form is −1.38.[11] It did not prove possible to get a reliable value for K_N from our direct absorbance measurements because of the instability of SNO^+, but the value is approximately 1×10^5 dm^6 mol^{-2}.

The system bears a striking similarity to that of the *S*-nitrosation of thiourea, a reaction studied initially by Werner,[19] and later put on a firm mechanistic basis by Stedman and coworkers.[20,21] In that case, nitrosation also generated an unstable yellow species, identified as the *S*-nitroso ion, with an equilibrium constant of 5000 dm^6 mol^{-2}. Similarly the yellow, unstable, *S*-nitroso anion[13] developed from nitrous acid and thiosulfate ion has an equilibrium constant of 1.66×10^7 dm^3 mol^{-2}. The thione form of MP is structurally very similar to thiourea, and it is possible that the significantly larger value of K_N in the MP case may arise from a hydrogen bond stabilisation in SNO$^+$. Studies with 4-mercaptopyridine, which also exists predominantly in the thione form, should throw some light on this.

Our third order rate constant k_3 [eqn. (7)], with an average value of 8200 dm^6 mol^{-2} s^{-1}, is very similar to the value obtained for the nitrosation of thiourea (6960 dm^6 mol^{-2} s^{-1}),[20] and indeed to the values obtained for the nitrosation of a range of structurally different but very reactive species,[22] including alkyl thioureas, aniline derivatives, some thiols and nitronic acids. Given the relative constancy of k_3 over such a range it has been argued[23] that this represents the diffusion controlled limit for attack by the NO$^+$ (or $H_2NO_2^+$) reagent, and it is no surprise that this is the case for the thione derived from 2-mercaptopyridine. A similar conclusion is reached from the results of kinetic experiments with added Cl$^-$ and Br$^-$, where the derived bimolecular rate constants for both NOCl and NOBr reactions are again close to the calculated diffusion limit. We were able to extract values of K_N from the results of both halide catalysed reactions. In principle these should be more reliable than those measured by direct absorbance measurements because of the decomposition problem, and the kinetic experiments here are on the stopped-flow timescale, and so decomposition should be minimal. However the analysis of k_0 *vs.* [H$^+$] plots relied on the measurement of a rather small intercept, in both cases. This necessarily resulted in rather large standard errors, and we were disappointed that the two values obtained, 1.3×10^5 and 7.9×10^4 dm^6 mol^{-2} were not a little closer. However there was acceptable agreement between the calculated and measured slopes for the k_0 *vs.* [halide] plots, using our derived K_N and rate constant values.

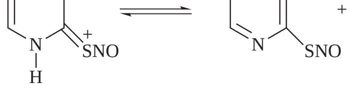
Analysis of the catalysed reaction results also gave values of 30 and 2400 dm^3 mol^{-1} s^{-1} for k_{-2} [eqn. (10)], the bimolecular rate constants for the halide ion promoted denitrosation of SNO$^+$. This reflects the relative nucleophilicities of the two halide ions in water. The rate constant ratio Br$^-$/Cl$^-$ of 80 compares well with the corresponding value of 55 for the denitrosation of *N*-methyl-*N*-nitrosoaniline,[24] showing that the two reactions have similar sensitivities to the nucleophiles.

It is clear that SNO$^+$ generated in acid solution decomposes with a half-life of *ca.* 10 min, giving quantitatively 2,2′-dipyridyl disulfide. Nitric oxide was detected as a product using the NO-electrode system. It is very likely that the decomposition is as in eqn. (16). The *S*-nitroso ions derived from thiourea and tetramethylthiourea behave similarly, and their decomposition mechanism has been studied by Stedman and co-workers.[25,26] Two parallel pathways have been identified, one involving a bimolecular process between two *S*-nitroso ions, and the other involving the reversible formation of a radical cation intermediate. It is likely that our SNO$^+$ species behaves similarly, but we have not examined the reaction further.



+ 2NO + 2H$^+$ (16)

When a solution of SNO$^+$ is quenched in buffer pH ~7, there is a measurable rate of return to MP in a first order process (Fig. 4). The initial spectrum however suggests that deprotonation of SNO$^+$ has occurred [eqn. (17)]. It would appear from a crude spectral analysis that the pK_a for this dissociation is of the order of 2–4. Since the pK_a of the *N*-protonated form of MP is 9.8,[11] our estimate is in the right area, given the expected electron withdrawing effect of the -NO group. This return to MP, which will be irreversible, since the nitrous acid generated will become nitrite ion, competes with the spontaneous decomposition of SNO$^+$, to give the disulfide, as demonstrated by the spectral analysis in Fig. 5 and by the nitric oxide determination.



+ H$^+$ (17)

SNO$^+$ as a nitrosating species

We have shown that SNO$^+$ will readily nitrosate the secondary amine NMA to give a quantitative yield of the *N*-nitrosamine, and almost certainly *S*-nitrosates a thiol, although we have not looked for the product in this work. This raises the possibility that MP can act as a nucleophilic catalyst in nitrosation, by the generation of a nitrosyl species (NOX), which is present in higher concentration than (or in principle is more reactive than) the reagent generated by acidic solutions of nitrous acid. Non-basic nucleophiles which have demonstrated catalysis for a wide range of substrates include: Cl$^-$, Br$^-$, I$^-$, SCN$^-$, $SC(NH_2)_2$, $SC(NR_2)_2$, $S_2O_3^{2-}$ and $(CH_3)_2S$. The catalytic reactivity for a given concentration of catalyst, is governed by the product of the equilibrium constant for NOX formation, and the rate constant for NOX reaction with the substrate. The former is generally the dominant factor: for example, although NOCl reacts with aniline derivatives at the diffusion limit, K_{NOCl} is very small (1.1×10^{-3} dm^6 mol^{-2}), and so catalytic activity is modest, whereas although $(NH_2)_2CS^+NO$ is powers of ten less reactive in the same reactions, the equilibrium constant for its formation is very much larger (5000 dm^6 mol^{-2}), and so thiourea is a much more efficient catalyst than is chloride ion.[27]

We have shown that MP (at very low concentrations) is a spectacular catalyst for NMA nitrosation. This arises principally because of the large value for K_{NOX} (~1×10^5 dm^6 mol^{-2}), but also because of its reasonably large value for the rate constant for its reaction with NMA. At higher [MP] values catalysis disappears since there is virtually complete conversion of HNO_2 to SNO$^+$. This happens also with $S_2O_3^{2-}$ as a catalyst at all concentrations since K_{NOX} is so large (1.67×10^7 dm^6 mol^{-2}). However the negatively charged *S*-nitroso ion is not a very efficient nitrosating agent,[28] and so overall $S_2O_3^{2-}$ is not such an efficient catalyst as is thiourea for example.

In this work we have not made a direct comparison between MP and thiourea for catalytic activity, but the product $K_{NOX}\,k_2$ is 2×10^{10} dm^9 mol^{-3} s^{-1} for SNO$^+$ in its reaction with NMA, and is 6×10^9 dm^9 mol^{-3} s^{-1} for $(NH_2)_2CS^+NO$ in its reaction with aniline.[29] This makes MP the slightly better catalyst, if we assume that aniline and *N*-methylaniline have comparable reactivities.

Experimental

All materials used were commercial samples of the highest purity grade available. Spectra were obtained on conventional UV–VIS spectrophotometers, which were also used for some of the kinetic measurements. Rate constants for the faster reactions were obtained on an Applied Photophysics stopped-flow spectrophotometer. All of the reactions were carried out in water at 25 °C. Most were followed at fixed wavelength, using the time-drive mode, whilst for some, a section of the spectrum was scanned at appropriate time intervals. In all cases the data were analysed on a PC interfaced with the spectrophotometers, using the Enzfitter software program designed for rate constant determination. The vast majority of the experiments gave excellent first order behaviour, and the individual rate constants were reproducible to better than ±3%. Nitric oxide was determined using a World Precision ISO-NO specific electrode, which was calibrated using an ascorbic acid–sodium nitrite mixture. Measurements were made under nitrogen.

Conclusions

We have shown that the thione tautomeric form of MP undergoes nitrosation in water at rates close to the diffusion controlled limit, to give the *S*-nitroso ion in an equilibrium reaction, with an equilibrium constant ~1×10^5 dm^6 mol^{-2}. Nitrous acid or nitrite ion and MP are rapidly regenerated by increasing the pH. In acid solution SNO$^+$ decomposes to give the disulfide and NO. SNO$^+$ acts as an effective nitrosating agent, and MP has been shown to be a very efficient catalyst of nitrosation carried out by HNO$_2$–H$^+$. There is evidence that *S*-nitroso-2-mercaptopyridine does exist in solution, but is unstable with respect to MP and disulfide formation. Solutions of SNO$^+$ can be regarded as sources of HNO$_2$ or NO$_2{}^-$, NO and NO$^+$, depending on the pH and on the presence of a nitrosatable species.

Acknowledgements

We thank the EPSRC for a research studentship to A. P. D., and for an equipment grant, and the Rectorado of the University of Santiago de Compostela, Spain for a travel/study grant to S. A.

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Paper 8/03858I
Received 21*st May* 1998
Accepted 18*th June* 1998