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Kinetic study of nitrosation of methylformamide

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Nitrosation of methylformamide (MFA) has been studied kinetically. The results obtained show that the reaction rate is first order with respect to both the MFA and proton concentration. The absence of catalysis by nucleophilic anions and the observed general base catalysis is interpreted in terms of formation of a protonated nitrosamide intermediate, being the proton transfer the slow step of the process. The primary solvent isotope effect observed corroborates this mechanism. From this study, we were able to obtain the values of the rate constants for the nitrosation process. The catalytic constants in the presence of buffers were also obtained and the analysis of the Brönsted slopes suggests a process with a transition state more similar to the reactants than the products. The absence of electron donating groups in MFA leads to a very low value of the bimolecular rate constant of the nitrosation reaction. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: nitrosation; amides

INTRODUCTION

The chemistry of nitroso compounds has attracted considerable research effort mainly due to their important biological relevance. A wide variety of structurally related compounds possessing the *N*-nitroso-*N*-alkyl functionality have demonstrated a cancer chemotherapeutic potential[1,2] However, the astonishing pace of discovery on the bioregulatory roles of nitric oxide,[3,4] including neurotransmission, hormone secretion, vasodilatation, bacterial cell adhesion and anti-carcinogen properties,[5–9] has greatly increased the interest in nitrosation reactions and nitroso transfer, because nitroso compounds are able to delivery nitric oxide in a controlled manner.[10] Due to this widespread significance of nitrosation reactions, knowledge of their mechanisms and kinetics[11] is of much importance.

There are considerable evidences in the literature to suggest that *N*-nitrosation of amides and ureas in water occurs in a different way from that of amines. The absence of catalysis by X^- species (halides, SCN^-, etc.), that generally speed up amine nitrosation through the formation of efficient nitrosating agents NOX, has been taken as showing that the slow step of the process takes place after the reaction between the nitrosating agents and the amide, the step being proposed to consist of proton transfer of the protonated nitrosamide to the reaction medium.[12–15] The observation of general base catalysis and the fact that the reaction is subject to a primary isotope effect corroborate this mechanism. This mechanism does not reveal the nature of nitrosating agents and it cannot be known if only NO^+ will nitrosate amides, or other, less electrophilic, nitrosating agents (NOX), can nitrosate amides. The answer emerged studying the reaction in water–organic solvent mixtures,[16–19] where a change in the rate limiting step is achieved, providing a direct evidence for the participation of NOX in the nitrosation of amides and ureas.

Amines generally react faster in nitrosation than do amides which are deactivated towards electrophilic attack, largely because the electron withdrawing ability of the CO group. Formamides are among the less nucleophilic substrates to bring about nitrosation at a convenient rate, no doubt due to the absence of any electron donating group bonded to the carbonyl group (see Chart 1). In this work, we extended our studies of nitrosation reactions to formamides, and we report the results of a kinetic investigation in acid media of nitrosation of methylformamide (MFA).

EXPERIMENTAL

MFA was provided by Sigma. All other reagents (from Fluka or Sigma) were of the highest available grade and used without further purification. Kinetic runs were monitored following the decrease in absorbance ($\lambda = 372$ nm) due to the disappearance of nitrous acid using an Agilent 8453 Diode-Array UV–Vis spectrophotometer equipped with a multiple cell carrier thermostated by circulating water. All experiments were carried out at 25.0 °C. In all kinetic experiments, $NaClO_4$ was used to keep the ionic strength of the medium at 1.0 M. All kinetic experiments were performed under pseudo-first-order conditions keeping in deficit the nitrite concentration ($[NO_2^-] = 5 \times 10^{-3}$ M). In all cases, the absorbance–time data fitted accurately the corresponding first-order integrated rate equations. The observed first-order rate

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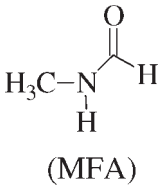


(MFA)

Chart 1.

constants, k_{obs} were reproducible within 5%. The slow process of decomposition of nitrous acid was almost first order with respect to nitrous acid. This decomposition process competed with the nitrosation reaction at low MFA concentrations. Its rate constant was determined under all the experimental conditions and taken into account to calculate k_{obs}. In order to study the effect of buffers, different amounts of buffer solutions were added to mixtures that already contained the amount of acid required to achieve the desired pH.

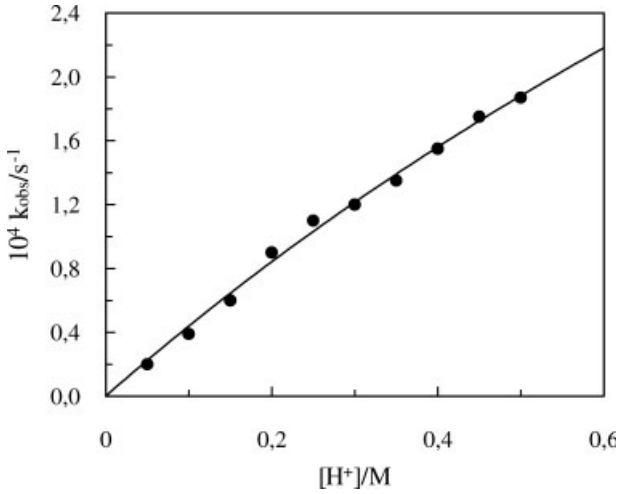


Figure 2. Influence of H^+ concentration upon k_{obs} [MFA] = 0.8 M

RESULTS

The pK_a of the protonated form of MFA in water[20] is −0.38, then MFA exists mainly as the unprotonated form under the experimental conditions used in this study ([H^+] = 0.002–0.5 M).

MFA nitrosation was studied in detail. We analysed the influence of MFA concentration on the reaction rate at different fixed concentrations of [H^+]. In Fig. 1, we can observe that the plots of k_{obs} *versus* MFA concentration pass through the origin (indicating that the overall reaction is an irreversible process) and are not straight lines. This nonlinearity can be attributed to partial protonation of the substrate at the working acidity, which would mean that an increase in MFA concentration would reduce the effective acidity of the medium. Besides, we can also observe in Fig. 1 that the values of the slopes at the origin increase with increasing [H^+]. This result suggests us that the reaction is catalysed by H^+. The effect of acidity on the reaction was also studied detail. Different amounts of perchloric acid were added to the medium and the change in the reaction rate observed. The results (see Fig. 2) show that the plot of k_{obs} *versus* [H^+] passes through the origin and that the dependence slight deviates from linearity at high acidities. This behaviour is indicative of a first-order dependence on the concentration of H^+ and the slight nonlinearity observed may be attributed to the concentration of free MFA being reduced at high acidity by partial protonation. Similar behaviour was observed when studying the nitrosation of methylureas in water–organic solvent mixtures.[17,19]

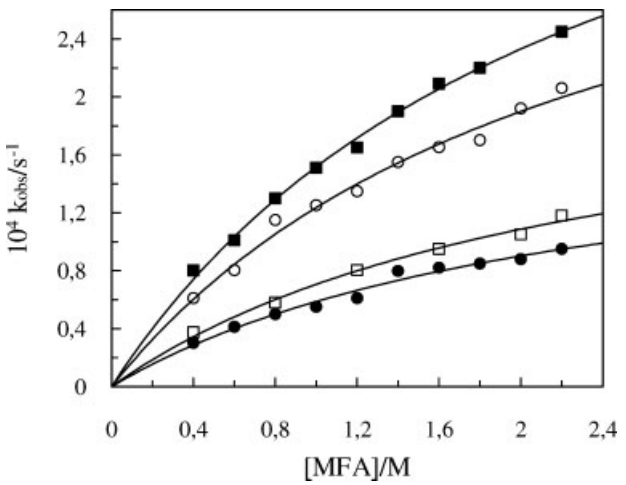


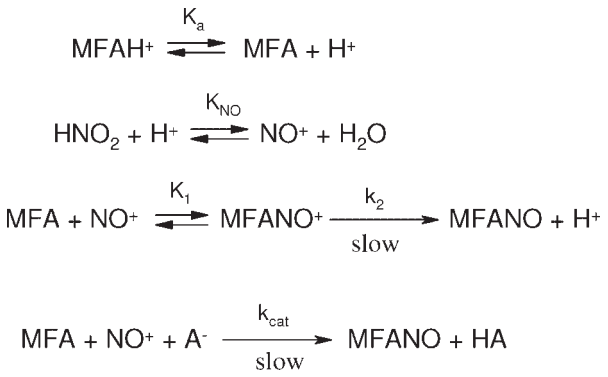
Figure 1. Influence of methylformamide concentration upon k_{obs}, (●) [H^+] = 0.1 M, (○) [H^+] = 0.2 M, (■) [H^+] = 0.3 M and (□) [H^+] = 0.2 M in D_2O

The effect of nucleophiles on the reaction rate is one of the aspects in which nitrosation of amides and amines differ. As we mentioned in the introduction section, halides considerably accelerate the rate at which the amines nitrosate[11] by providing high concentrations of new and effective nitrosating agents (ONCl, ONBr). However, nitrosation of amides and related compounds[12–15] is not susceptible to this type of catalysis. Table 1 show the effect of the addition of X^- to the reaction media on the rate constant for MFA nitrosation. As can be observed, there is no trace of catalysis. Halide ions at these concentrations produce substantial catalytic effects in the nitrosation of amines.[11] This result seems to rule out a mechanism for the nitrosation of MFA similar to that which operates in the case of amines, i.e. a mechanism whose slow step is the reaction between the nitrosable substrate and the nitrosating agent.

The mechanism for the nitrosation of formamides is shown in Scheme 1. The first step, the pre-equilibrium formation of the nitrosating agent (NO^+) through protonation of nitrous acid (K_{NO}), is followed by a fast equilibrium reaction between the

Table 1. Influence of X^- concentration on k_{obs} for the nitrosation of methylformamide, [H^+] = 0.15 M, [MFA] = 0.8 M

[X^-]/M	$10^5\ {}^{Br}k_o/s^{-1}$	$10^5\ {}^{Cl}k_o/s^{-1}$
0.05	6.27	6.67
0.10	6.53	6.04
0.20	6.69	6.04
0.30	6.36	6.03
0.40	6.28	5.91
0.50	6.42	6.22



Scheme 1.

nitrosating agent and MFA (K_1), leading to the formation of a protonated intermediate ($MFANO^+$). The final step is a reversible rate limiting transfer of a proton from ($MFANO^+$) to the reaction medium to give the nitrosomethylformamide (MFANO). This mechanism leads to the next expression for the observed first-order rate constants (Eqn 1), which explains the influence of concentration of MFA and [H^+] on the reaction rate, and the absence of catalysis by X^-. The bimolecular rate constant, for the nitrosation is $k_n = k_2K_1$.

$$k_{obs} = k_2K_1K_{NO}[MFA][H^+] = k_nK_{NO}[MFA][H^+] \quad (1)$$

The nonlinear plots observed when studying the influence of MFA and H^+ on k_{obs} can be explained if we take into account that protonation of MFA could become significant at the acidities used in this study (refer Scheme 1). The effective H^+ concentration will be lowered as the concentration of MFA is increased. For free MFA much in excess of protonated MFA, the expression for k_{obs} will be given by Eqn 2, where k_nK_{NO} stands for the rate constant with free MFA and K_a is the acidity constant for protonated MFA.

$$k_{obs} = \frac{k_nK_{NO}K_a[MFA][H^+]_{total}}{K_a + [MFA]} \quad (2)$$

This hypothesis can be easily checked since Eqn 2 predicts that $1/k_{obs}$ should be a linear function of $1/[H^+]$. This double reciprocal plot is showed in Fig. 3, yielding good straight lines, and the slopes and intercepts of these lines afford values of 2.0 ± 0.2 M for K_a (in good agreement with the literature values),[20] and $(9.0 \pm 1.1) \times 10^{-4}\ M^{-2}\ s^{-1}$ for k_nK_{NO}. The value of the bimolecular rate constant ($k_n = 3000\ M^{-1}\ s^{-1}$) was calculated taking a value of $3 \times 10^{-7}\ M^{-1}$ for K_{NO}.[21] This value for the nitrosation of MFA is very far from the encounter controlled limit ($7 \times 10^9\ M^{-1}\ s^{-1}$).[11] Amides generally react more slowly in nitrosation than do amines, no doubt due to the presence of the strongly electron withdrawing carbonyl group. MFA reacts with NO^+ more than 10^5 times slower than that do 2-imidazolidone (the most reactive amide),[12] 10^4 times slower than methylurea[22] and is 6 times less reactive than methylacetamide[12] and slight lower than that of methylbenzamide.[22] The absence of electron donating groups in the case of MFA leads to lowest values of the bimolecular rate constant for *N*-nitrosation reactions.

Nitrosation of amides is subject to general basic catalysis. To study this catalysis in the case of MFA, buffers of monochloroacetic (MCA), dichloroacetic (DCA), trichloroacetic (TCA) and trifluoroacetic acid (TFA) were used. Figure 4 shows the influence of the total concentration of DCA, TCA and TFA on the observed

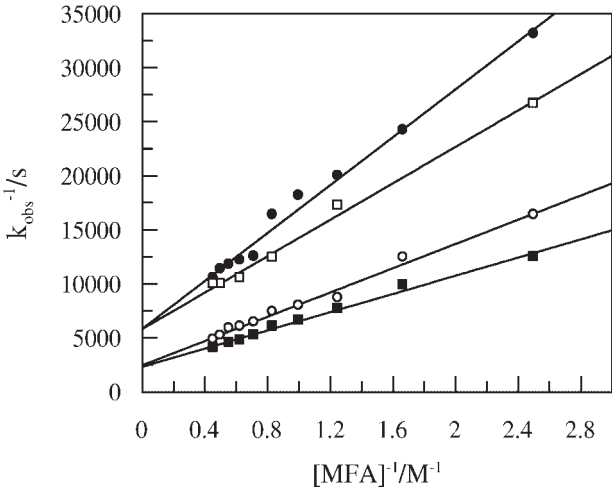


Figure 3. Plots of $1/k_{obs}$ *versus* 1/[MFA] for the data of Fig. 1. (●) $[H^+] = 0.1$ M, (○) $[H^+] = 0.2$ M, (■) $[H^+] = 0.3$ M and (□) $[H^+] = 0.2$ M in D_2O

rate constant, k_{obs}, for the nitrosation of MFA. We can see that the reaction rate increases as buffer concentration is increased (similar results were obtained when studying the influence of MCA buffer, but were not included in Fig. 4 for clarity reasons). The results obtained are indicative of significant buffer catalysis, showing that the reaction is subject to a general base catalysis, and strongly support the mechanism outlined in Scheme 1, in which a slow proton transfer is the rate determining step. From Scheme 1, it is easy to obtain the following rate equation (Eqn 3) in the presence of buffers, where k_{cat} is the catalytic constant for MFA nitrosation. In absence of buffers Eqn 3 can be simplified to Eqn 1.

$$k_{obs} = k_nK_{NO}[MFA][H^+] + k_{cat}K_{NO}[MFA][H^+][A^-] \quad (3)$$

From Eqn 3 and taking into account that K_{AH} is the dissociation constant of the buffers used, we obtain Eqn 4, which is in terms of

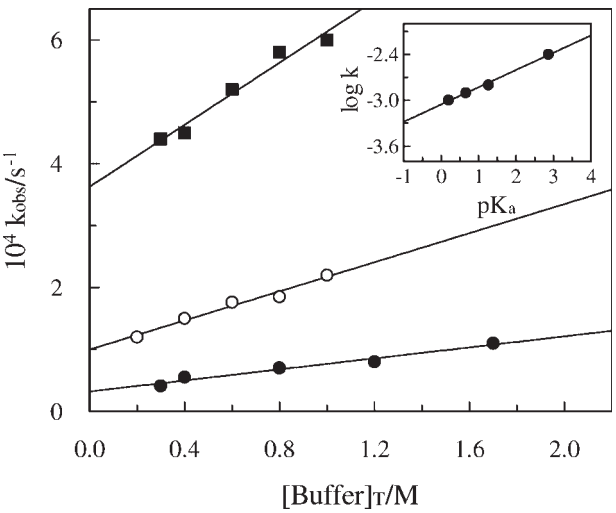


Figure 4. Influence of the total concentration of buffers on k_{obs}, $[MFA] = 0.8$ M (●) DCA, pH = 1.26, (○) TCA, pH = 0.66, (■) TFA, $[H^+] = 0.79$ M. The inset shows the Brönsted plot

the total concentration of buffer and explains the experimental behaviour observed in Fig. 4.

$$k_{obs} = k_n K_{NO}[MFA][H^+] + \frac{k_{cat} K_{NO} K_{AH}[MFA][H^+]}{K_{AH} + [H^+]}[Buffer]_T \quad (4)$$

From the intercepts of the plots of Fig. 4, we can obtain again the value of $k_n K_{NO}$. A mean value of $9.50 \times 10^{-4}\,M^{-2}\,s^{-1}$ was obtained, in good agreement with that obtained from the influences of acidity and methylformamide concentration. The slopes of the straight lines of Fig. 4 let us to obtain the catalytic constants ($k_{cat}K_{NO}$) for the four buffers used. The inset of Fig. 4 shows the Brönsted plot relating the catalytic efficiency and the pK_a of buffers studied. It is clear that this plot is essentially a straight line. From the slope, we can calculate a value of $\beta = 0.23$ for the Brönsted exponent. This value is very similar to that found for nitrosation of guanidines.[23] The practice of identifying the degree of proton transfer in the transition state with the value of the Brönsted slope allows us to estimate that the transition state of the slow process for the nitrosation occurs early along the reaction coordinate ($\beta \approx 0.20$), which means that protonation of nitrosomethylformamide is nearly complete in the transition state.

One last indication about the nature of the slow step was obtained when the reaction was carried out in D_2O and the corresponding solvent isotope effect was measured. The results obtained and its comparison with the reaction in presence of H_2O is shown in Figs 1 and 3. The value obtained for the acidity constant for protonated MFA is 1.4 M. This value means that in D_2O, ΔpK_a is 0.16, in good agreement with the literature values.[12] The observed deuterium isotope effect for the nitrosation reaction $k_n K_{NO}(H_2O)/k_n K_{NO}(D_2O)$ is 1.52. Besides, taking into account the mechanism outlined in Scheme 1, the observed value for the isotope effect for the nitrosation reaction includes the influence of the isotopic substitution on the equilibrium constants K_1 and K_{NO} and on the rate constant for the slow step k_2. Replacement of water by deuteriated water increases the value of K_{NO} 2.55 times.[24] Assuming that there is a negligible isotope effect upon K_1, because it does not involve a proton transfer, then the value of the kinetic isotope effect on the slow step, $k_2(H)/k_2(D)$, can be estimated as 3.9. This result is consistent with a rate-limiting proton transfer. The magnitude of the isotope effect can be related to the degree of symmetry of the transition state.[25] Thus, both values of the Brönsted exponent and of the solvent isotope effect on the slow step suggest a process with a transition state more similar to the reactants than the products.

The study of the nitrosation of methylformamide let us to confirm that nitrosation of formamides in water occurs in a different way from that of amines. The absence of catalysis by X^- species, the existence of general base catalysis and the fact that the reaction is subject to a primary solvent isotope effect indicate us that the slow step is the proton transfer from the protonated nitrosamide to the reaction medium. The analysis of the Brönsted slope and the isotope effect suggest that in the transition state the protonation of nitrosoformamide is nearly complete. The absence of electron donating groups in MFA leads to a very low value of the bimolecular rate constant of the nitrosation reaction.

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