Interference by Nitrous Acid Decomposition in the Kinetic Study of Nitrosation Reactions

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ABSTRACT: Under the acidic conditions of the stomach lumen, nitrosation reactions can occur in the human body between nitrite (added to meat because of its antibotulinic properties) and many compounds such as amino acids. From the results obtained, two conclusions can be drawn: (i) In the quantitative study of nitrosation reactions, it is necessary to take into account the competing reaction of HNO_2 decomposition, which in some conditions is the dominant reaction; (ii) two alternative approaches based on the initial rate method are necessary to assess the weight of nitrous acid decomposition using taurine and homotaurine as nitrosatable substrates. In strongly acidic media, the decomposition reaction is dominant: In the case of taurine, the decomposition rate is negligible at pH $\geq$ 3.2. In the pH 3.2−2.5 range, decomposition is lower than nitrosation but not negligible, and at pH $\leq$ 2.5 decomposition is faster than nitrosation. With homotaurine, HNO_2 decomposition is negligible at pH higher than 4.1. In the pH 4.1−2.8 range, nitrosation is faster but decomposition should be considered, and at pH $\leq$ 2.8 decomposition is the dominant reaction. © 2014 Wiley Periodicals, Inc. Int J Chem Kinet 46: 321–327, 2014

INTRODUCTION

N-Nitroso compounds are unique among carcinogenic agents in that they are active in all living species and because there is a broad spectrum of target cells and organs in which they are able to induce cancer [1,2]. Under the acidic conditions of the stomach lumen, nitrosation reactions can occur in the human body between nitrite (added to meat because of its antibotulinic properties) [3] and many compounds such as amino acids [4,5], phenols [6], and bioactive molecules [7].

Biologists have mainly been interested in the pathogenic mechanisms in which nitroso compounds are involved [8,9], whereas chemists have been more interested in their mechanisms of formation [10–12] and hence in ways to block or inhibit them [13,14].

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$$S + Y{-}NO \rightleftharpoons {}^{+}S{-}NO + Y^{-}$$

Scheme 1 The nitrosation reaction.

$$3HNO_2 \rightleftharpoons 2NO + HNO_3 + H_2O$$

Scheme 2 Decomposition of nitrous acid.

Nitrosation reactions (Scheme 1) occur through the attack of a nitrosatable substrate (S) by nitrosating species (Y-NO).

Since nitrosatable substrates are compounds with different nucleophilic sites such as C, O, S, or N-atoms, they conform a large class of substances including amines, thiols, alcohols, and ketones and many others.

Nitrosocompounds themselves such as alkyl nitrites, nitrosamines, and some metal nitrosyl complexes can also act as nitrosating agents by transferring the nitroso group to another substrate [12].

The most commonly used reagent for bringing about electrophilic nitrosation is nitrous acid, generated in aqueous acid solution from a nitrite salt, usually sodium nitrite, and used in situ. Pure nitrous acid has never been isolated (although it has been detected in the gas phase), since decomposition occurs, giving various oxides of nitrogen as final products [15]. This decomposition is usually represented as shown in Scheme 2.

Although the decomposition of nitrous acid is relatively slow at room temperature and at low concentration, when the decomposition rate is not negligible with respect to the nitrosation reaction rate, the results obtained without taking into account the influence of the former reaction can lead to incorrect results and wrong conclusions. This makes relevant the kinetic study of nitrous acid decomposition in different conditions.

To analyze the interference of nitrous acid decomposition on the kinetic quantitative study of nitrosation reactions, here two simple alternative approaches are proposed. To this end, the kinetic study of the nitrosation of two amino acid–like compounds, taurine (2-aminoethane sulfonic acid; Tau) and homotaurine (3-amino-1-propane sulfonic acid; HT) is discussed.

KINETIC BACKGROUND

Nitrosation reactions are often followed by measuring the absorbance of the acidic kinetic nitrosation mixtures (KNM = substrate + nitrite in acidic media) in the UV range of the spectrum. When only the HNO_2/NO_2^- system shows absorption in this range, it can be used as the control species to monitor the nitrosation reaction.

For a nitrosation reaction occurring between the substrate S and nitrite, the experimental rate equation can be written as

$$r_N = -\frac{\mathrm{d}}{\mathrm{d}t}([\text{nitrite}]) = k_{\text{exp}}[\text{S}]^{\text{a}}[\text{nitrite}]^{\text{b}} \qquad (1)$$

Since nitrous acid is in equilibrium with the nitrite ion, and their molar absorption coefficients (ε_{HNO_2} and $\varepsilon_{NO_2^-}$, respectively) are different, Eqs. (2)–(4) can be written, where A is the absorbance and l the cuvette light path,

$$K_{\text{a}} = \frac{\left[NO_2^-\right]\left[H^+\right]}{[HNO_2]} \qquad (2)$$

$$[\text{Nitrite}]_0 = \left[NO_2^-\right] + [HNO_2] \qquad (3)$$

$$\begin{aligned} A_{\text{Nit}} &= A_{NO_2^-} + A_{HNO_2} \\ &= l\varepsilon_{NO_2^-}\left[NO_2^-\right] + l\varepsilon_{HNO_2}[HNO_2] \end{aligned} \qquad (4)$$

Equation (5), expressing the relationship between the absorbance (A) of the KNM with the total concentration of nitrite, is easily achieved from the combination of Eqs. (2)–(4), where ε_{ap} is the apparent molar absorption coefficient of the nitrite system:

$$\begin{aligned} A &= l\frac{\varepsilon_{NO_2^-}K_{\text{a}} + \varepsilon_{HNO_2}\left[H^+\right]}{[H^+] + K_{\text{a}}}[\text{Nitrite}]_{\text{o}} \\ &= l\varepsilon_{\text{ap}}[\text{Nitrite}]_{\text{o}} \end{aligned} \qquad (5)$$

Applying the initial rate method (IRM) [16], and using Eqs. (1) and (5), Eq. (6) is easily achieved, where $v_{\text{o,N}}$ is the initial rate of nitrosation:

$$v_{\text{o,N}} = -\frac{1}{l\varepsilon_{\text{ap}}}\left[\frac{\mathrm{d}A_o}{\mathrm{d}t}\right]_{t=0} = k_{\text{exp}}[\text{S}]_{\text{o}}^{\text{a}}[\text{Nitrite}]_{\text{o}}^{\text{b}} \qquad (6)$$

Since the nitrosation reaction occurs simultaneously with that of nitrous acid decomposition, the observed initial rate ($v_{\text{o,T}}$) is the sum of the nitrosation ($v_{\text{o,N}}$) and decomposition ($v_{\text{o,D}}$) reaction rates (Eq. (7)):

$$v_{\text{o,T}} = v_{\text{o,N}} + v_{\text{o,D}} \qquad (7)$$

Taking into account that the percentage of the reaction followed was not more than 2%, a linear plot should be obtained when the absorbance over the reaction time, in fact, was observed (Fig. 1). From the

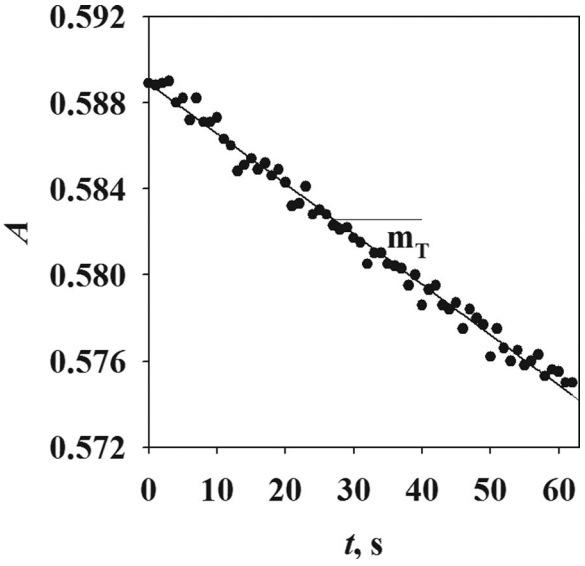


Figure 1 Typical kinetic run of a nitrosation reaction. $T = 25.0°C$; pH 2.97; $[Tau]_o = 0.30$ M; $I = 0.50$ M.

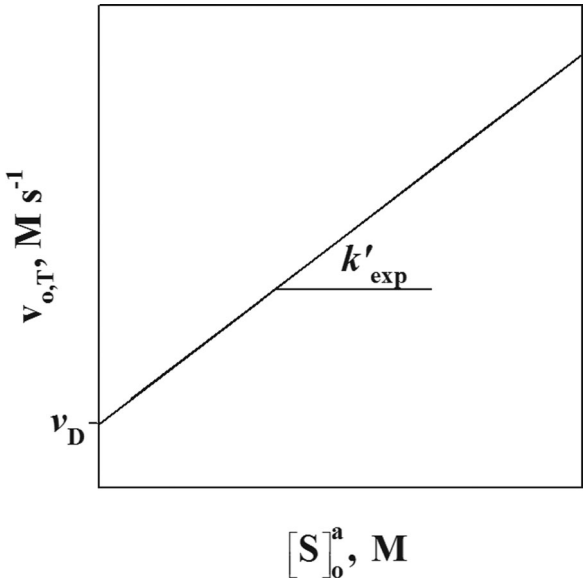


Figure 2 Initial reaction rate of nitrosation as a function of $[S]_o^a$.

slope (m_T) of this straight line, the initial rate ($v_{o,T}$) can be obtained:

$$m_T = \frac{dA_o}{dt} = -v_{o,T} l \varepsilon_{ap} \quad (8)$$

To determine $v_{o,N}$ and $v_{o,D}$, two approaches can be used as follows.

Approach 1

From a set of experiments with different initial concentrations of substrate $[S]_o$, the values of $v_{o,T}$ are obtained (Fig. 1). Plotting the $v_{o,T}$ values against those of $[S]_o^a$, a straight line is obtained (Eq. (9) and Fig. 2) whose slope is the rate constant of nitrosation, k'_{exp} ($k'_{exp} = k_{exp}[\text{nitrite}]_o^b$), and intercept is the initial rate of decomposition, $v_{o,D}$. The difference, $v_{o,T} - v_{o,D}$, is the value of $v_{o,N}$:

$$v_{o,T} = k'_{exp}[S]_o^a + v_{o,D} \quad (9)$$

Approach 2

Only two experiments are required. The first experiment is performed in the absence of substrate, and hence the reaction rate obtained from the slope of the straight line (Eq. (10)) gives the $v_{o,D}$ value (Fig. 3). The second experiment should be performed in the presence of substrate, and hence the reaction rate obtained is $v_{o,T}$ (Fig. 3 and Eq. (11)). The difference $v_{o,T} - v_{o,D}$ is the $v_{o,N}$ value (Eq. (12)).

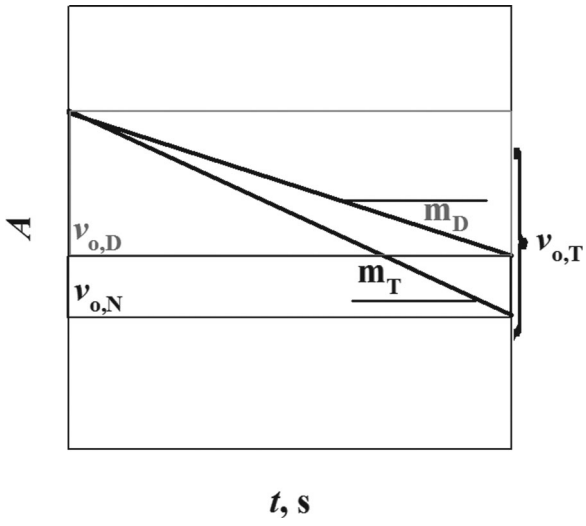


Figure 3 Variation in nitrite absorbance along time in the presence ($v_{o,T}$) and in the absence ($v_{o,D}$) of substrate.

Applying Eq. (7) to the nitrous acid decomposition reaction and to the simultaneous nitrosation/decomposition reactions, Eqs. (10) and (11) respectively, are obtained:

$$m_D = \frac{d_{A_D}}{dt} = -v_{o,D} l \varepsilon_{ap} \quad (10)$$

$$m_T = \frac{dA_T}{dt} = -v_{o,T} l \varepsilon_{ap} \quad (11)$$

Substitution of Eqs. (10) and (11) in Eq. (7) gives Eq. (12):

$$v_{o,N} = v_{o,T} - v_{o,D} = -\left(\frac{m_T}{l\varepsilon_{ap}} - \frac{m_D}{l\varepsilon_{ap}}\right) = -\left(\frac{m_T - m_D}{l\varepsilon_{ap}}\right) \quad (12)$$

EXPERIMENTAL

The above-mentioned reactions were monitored by measuring the absorbance of KNM (pH range ≈ 2–5). Since only the HNO_2/NO_2^- system shows absorption in the UV spectrum (maximum at $\lambda = 371$ nm), nitrite was used as the control species to monitor the nitrosation reaction. KNM was made up by the addition of 0.1 mL of a sodium nitrite solution to a cuvette containing 3.0 mL of a substrate solution, both of them made up by weight. The ionic strength and pH were controlled with $NaClO_4$ and $HClO_4$ solutions. In the experiment, in the absence of substrate, nitrite sodium solution was added to a solution with no substrate but containing $NaClO_4$ and $HClO_4$. No buffer solution was required because the IRM was used.

All kinetic runs were performed in triplicate.

A Shimadzu UV-2401PC spectrophotometer with a thermoelectric six-cell holder temperature system was used. A Metrohm pH lab 827 pH meter was used. The solution temperature was kept constant (±0.05°C) with a Lauda Ecoline RE120 thermostat. Water was deionized with a Wasserlab Ultramatic-ecomatic system. Numerical treatment of the data was performed using Sigma Plot 10.0 software.

Taurine (>99%) and homotaurine (97%) were obtained from Sigma-Aldrich (St. Louis, MO); $NaNO_2$ and $NaClO_4$ were obtained from Merck (Darmstadt, Germany); $HClO_4$ and NaOH were purchased from Panreac (Barcelona, Spain).

RESULTS AND DISCUSSION

Application of Approach 1 to the Nitrosation Reaction of Taurine

Since for the nitrosation of taurine the orders with respect to $[Nitrite]_o$ and $[Tau]_o$ were found to be 2 and 1, [17] respectively, the initial reaction rate was

$$v_{o,N} = k_{exp}[Tau]_o[Nitrite]_o^2 \quad (13)$$

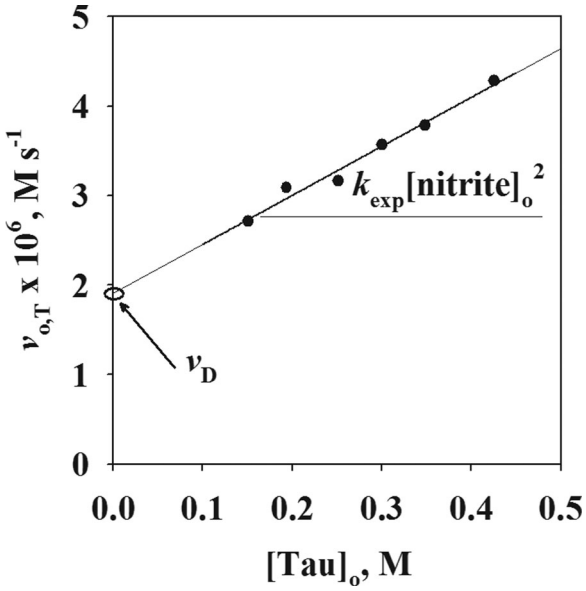


Figure 4 Initial rate of taurine nitrosation as a function of $[Tau]_o$. $[Nitrite]_o = 0.010$ M; $T = 25.0$°C; $I = 0.50$ M; pH 2.6.

From Eqs. (8) and (13), Eq. (14) is obtained:

$$v_{o,T} = v_{o,N} + v_{o,D} = k_{exp}[Tau]_o[Nitrite]_o^2 + v_{o,D} \quad (14)$$

Figure 4 shows the good fitting of the values of $v_{o,T}$ and $[Tau]_o$ to Eq. (14). The $v_{o,D}$ value was obtained from the intercept, and k_{exp} was obtained from the slope.

The above experiment was repeated at different pHs, and the k_{exp} values obtained are depicted in Fig. 5 together with those that would have been obtained if nitrous acid decomposition had been neglected. The strong error affecting the results (especially at low pHs) when nitrous acid decomposition was neglected can be observed.

The fitting of the experimental values of k_{exp} at different pHs to the theoretical rate equation deduced for the mechanism proposed previously for the nitrosation reaction [17] of taurine (Scheme 3 and Eq. (15)) is also shown in Fig. 5. The good fit of experimental data to the theoretical equation confirms the mechanism and permits the k_{Nit} value to be calculated from the parameter α of Eq. (15):

$$v_{o,N} = \alpha \frac{[H^+]^2}{([H^+] + K_{II})(K_a[H^+])^2}[Tau]_o[Nitrite]_o^2 \quad (15)$$

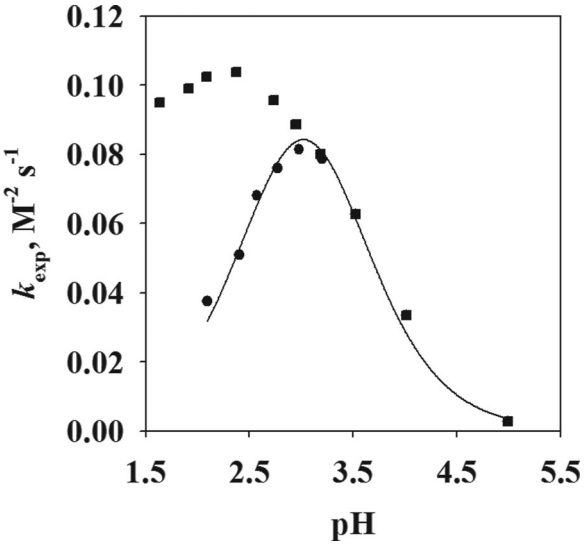


Figure 5 Variation in k_{exp} with pH: ■, observed values; •, corrected values without HNO_2 decomposition. $[Nitrite]_o$ = 0.010 M; $T = 25.0°C$; $I = 0.50$ M.

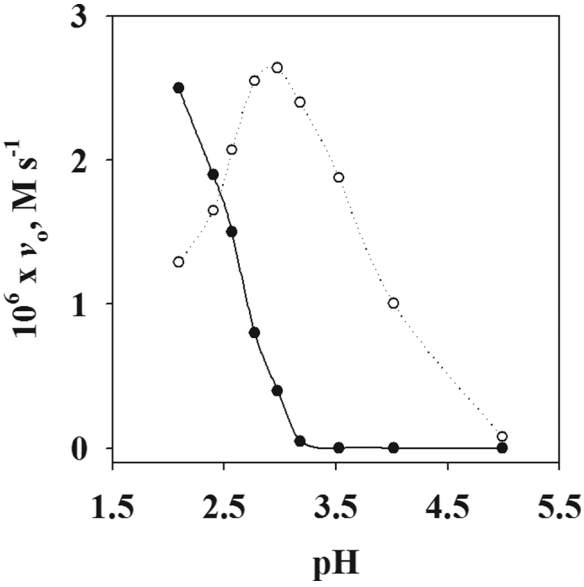


Figure 6 Representation as a function of pH of the (•) initial rate of nitrous acid decomposition, $v_{o,D}$, and the (○) initial rate of taurine nitrosation, $v_{o,N}$. $[Tau]_o$ = 0.30 M; $[Nitrite]_o$ = 0.010 M; $I = 0.50$ M; $T = 25.0°C$.

$$\alpha = k_{Nit} K_2 K_3 K_a K_{II} \quad (16)$$

It should be pointed out that neglecting nitrous acid decomposition means that the k_{exp} values do not fit at low pHs. This fact suggests that at these pHs the mechanism of nitrosation is different from those seen at higher pHs. Another possible interpretation could be to neglect the k_{exp} values corresponding to the lowest pHs, leading to a higher and mistaken value of the nitrosation rate constant, k_{Nit}.

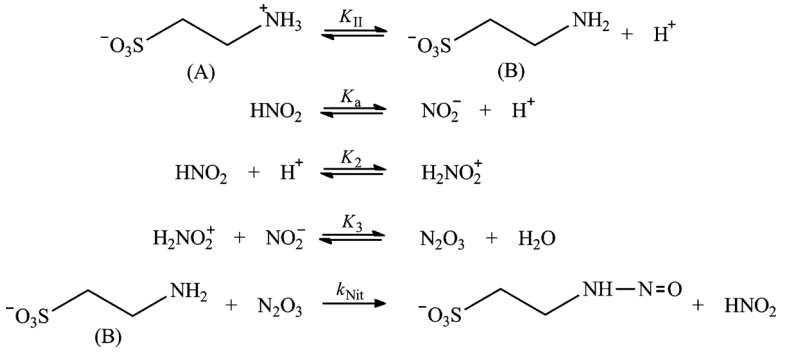
With the values of k_{exp} obtained at different pHs, those of $v_{o,N}$ were calculated. A comparison of $v_{o,N}$ with the $v_{o,D}$ profiles (Fig. 6) shows that at pH $\geq$ 3.2 the decomposition rate was negligible. In the pH 3.2−2.5 range, the decomposition rate was lower than nitrosation but not negligible, and at pH $\leq$ 2.5 decomposition was faster than nitrosation.

Application of Approach 2 to the Kinetic Study of Homotaurine Nitrosation

The homotaurine nitrosation reaction is similar to the nitrosation of taurine. Since the orders of the reaction with respect to $[Nitrite]_o$ and $[HT]_o$ proved to be 2 and 1, respectively, Eq. (17) can be written as

$$v_{o,N} = -\left(\frac{m_T - m_D}{l\varepsilon_{ap}}\right) = k_{exp}[HT][Nitrite]^2 \quad (17)$$

Figure 7 shows the decrease in the absorbance of KNM and that of a similar solution without homotaurine (only decomposition).



Scheme 3 Mechanism of reaction for taurine nitrosation.

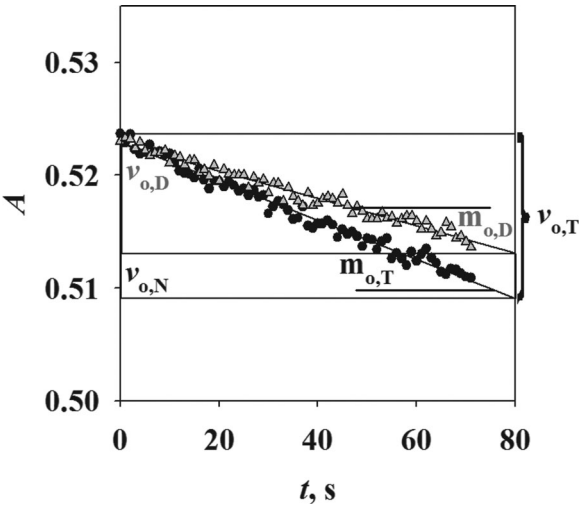


Figure 7 Nitrite absorbance along time: •, in the presence of substrate (v_T). ([HT]$_o$ = 0.30 M); △, in the absence of substrate ($v_{o,D}$). [Nitrite]$_o$ = 0.010 M; pH 1.90; T = 25.0°C.

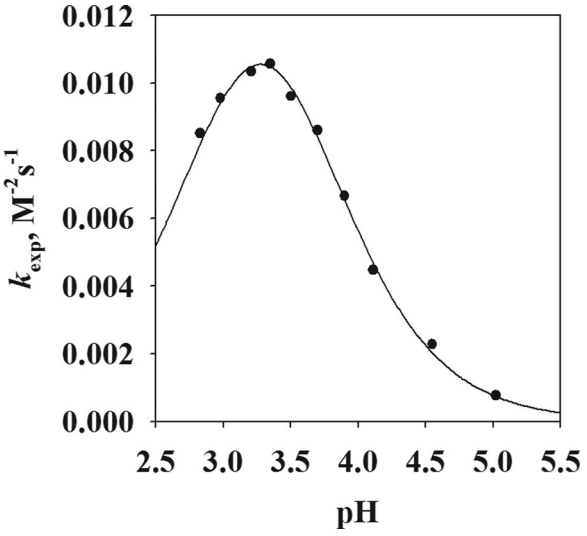


Figure 8 Variation in k_{exp} with pH. [HT]$_o$ = 0.5 M; [Nitrite]$_o$ = 0.010 M; T = 25.0°C.

With the slopes obtained from the fitting of the absorbance/time experimental data, the value of the experimental rate constant for HT can be calculated. The effect of nitrous acid decomposition is quite clear, such that if this reaction is not considered this would lead to values of the nitrosation rate constants higher than the true ones.

Figure 8 shows the values obtained for k_{exp} for the nitrosation of HT at different pHs as well as the fitting to the theoretical rate equation obtained from the proposed mechanism. The good fit again confirms the proposed mechanism.

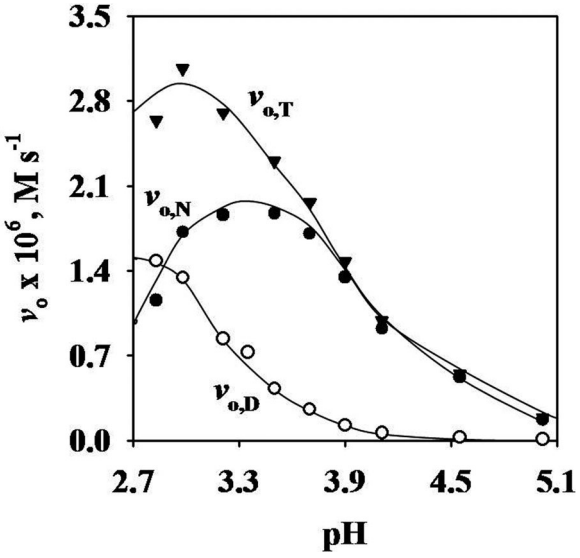


Figure 9 Variation in pH of (•) $v_{o,N}$ (▼) $v_{o,T}$ (○) $v_{o,D}$. [HT]$_o$ = 0.5 M; [Nitrite]$_o$ = 0.010 M; T = 25.0°C.

The initial rates of decomposition ($v_{o,D}$) and nitrosation ($v_{o,N}$) were also calculated and compared in Fig. 9, together with the initial rate of disappearance of nitrite ($v_{o,T}$).

A comparison of the these three initial rates allows us to conclude that for the HT nitrosation reaction the decomposition reaction is negligible at pH values higher than 4.1. In the pH 4.1−2.8 range, nitrosation is faster, but decomposition should be considered, and at pH ≤ 2.8 decomposition is the dominant reaction.

CONCLUSIONS

From the results obtained, the following conclusions can be drawn:

i. In the quantitative study of nitrosation reactions, it is necessary to take into account the competing reaction of HNO_2 decomposition, which in some conditions can be the dominant reaction.
ii. Two different approaches based on the IRM were performed to assess the weight of nitrous acid decomposition using taurine and homotaurine as nitrosatable substrates. The results allow us to conclude that in strongly acidic media the decomposition reaction is dominant: In the case of taurine, the decomposition rate is negligible at pH ≥ 3.2. In the pH 3.2−2.5 range, decomposition is lower than nitrosation but not negligible, and at pH ≤ 2.5 decomposition is faster than nitrosation. With homotaurine, HNO_2

decomposition is negligible at pH higher than 4.1. In the pH 4.1−2.8 range, nitrosation is faster, but decomposition should be considered, and at pH $\leq$ 2.8 decomposition is the dominant reaction.

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BIBLIOGRAPHY

1. Magee, P. N.; Barnes, J. M. Br J Cancer 1956, 10, 114–122.
2. Lijinsky, W. Chemistry and Biology of N-Nitroso Compounds; Cambridge University Press: Cambridge, UK, 1992.
3. Engel, R. E.; J Am Vet Med Asso 1977, 171, 1157–1160.
4. García-Santos, M. P.; González Mancebo, S.; Hernández Benito, J.; Calle, E.; Casado, J. J Am Chem Soc 2002, 124, 2177–2182.
5. Gil, R.; Casado, J.; Izquierdo, C. Int J Chem Kinet 1994, 26, 1167–1178.
6. González-Mancebo, S.; García-Santos, M. P.; Hernández-Benito, J.; Calle, E.; Casado, J. J Agric Food Chem 1999, 47, 2235–2240.
7. González-Jiménez, M.; Arenas-Valgañón, J.; Calle, E.; Casado, J. J Org Biomol Chem 2011, 9, 7680–7684.
8. Walters, C. L. Science 1973, 179, 96–97.
9. Mirvish, S. S. Cancer Lett 1995, 93, 17–48.
10. Casado, J. (1994). In Fast Reactions in Solution; Royal Society of Chemistry Annual Meeting, Burgos, Spain.
11. Casado, J.; González Alatorre, G.; Izquierdo, C.; Brunner, C. Int J Chem Kinet 1996, 28, 307–313.
12. Williams D. L. H. Nitrosation Reactions and Chemistry of the Nitric Oxide, 2nd ed.; Elselvier: Amsterdam, 2004.
13. García-Prieto, J. C.; Mateos, R.; Calle, E.; Casado, J. J Agric Food Chem 1998, 46, 3517–3520.
14. González-Mancebo, S.; Calle, E.; García-Santos, M. P.; Casado, J. J Agric Food Chem 1997, 45, 334–336.
15. Abel, E.; Schmid, H. Z Phys Chem 1928, 136, 419–430.
16. Casado, J.; López-Quintela, M. A.; Lorenzo-Barral, F. M. J Chem Educ 1986, 63, 450–452.
17. Arenas-Valgañón, J.; Gómez-Bombarelli, R.; González-Pérez, M.; González-Jiménez, M.; Calle, E.; Casado, J. Food Chem 2012, 134, 986–991.