Simultaneous *C*- and *N*-Nitrosation of Synephrine. A Kinetic Study

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A kinetic study of the initial step of the nitrosation of synephrine, *N*-acetylsynephrine and ephedrine by sodium nitrite has been carried out in acid medium at 310 K and ionic strength 0.36 mol dm^{-3}. In order to simplify the reaction, [nitrite] > [amine] has been used. The absorbance changes at 300 nm (synephrine and *N*-acetylsynephrine) and at 332/323 nm (ephedrine) were measured and then analysed according to the initial rate procedure. The results are interpreted on the basis of a simultaneous nitrosation of the amine and phenolic groups in synephrine. Unlike the early reports the global constants for N-nitrosation (0.142 dm^6 mol^{-2} s^{-1}) and C-nitrosation (3.6×10^{-4} dm^3 mol^{-1} s^{-1}) obtained at pH 3 in unbuffered medium indicate that the rate of nitrosation of the amino group is one order of magnitude larger than that for the phenolic group.

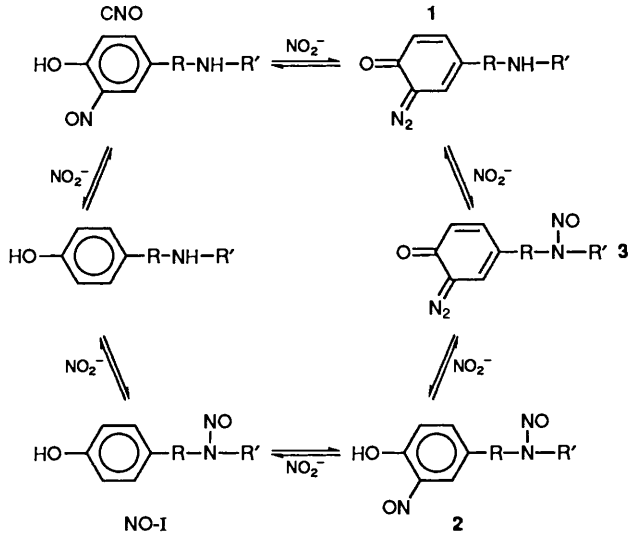
The acute toxicity of nitrosated derivatives is determined basically by its carcinogenic and/or mutagenic activity. Since the mid 1950s, when Barnes and Magee demonstrated for the first time the toxicity of *N*-nitrosodimethylamine, a great number of studies have been devoted to the nitrosation processes and the evaluation of the risks that nitrosated compounds pose to the human being. Nowadays, widely documented evidence exists about the carcinogenic, mutagenic, and teratogenic effects produced by *N*-nitrosamines.[1-11] Several factors, including catalytic and inhibitor agents, can contribute to the formation of *N*-nitrosamines. Phenols are one of the most important agents in N-nitrosation processes due to their environmental diffusion. Thus, some experimental results suggest that phenols (*e.g.*, phenol, resorcinol, catechin) can react with nitrosating agents to produce *C*-nitroso-derivatives which catalyse *N*-nitrosamine formation in acid media.[9,12-18] Work on the inhibition of the amine nitrosation by phenols has also been described.[9,16,19-22] It is also known that the reaction of phenol and derivatives with sodium nitrite causes the appearance of mutagenic diazo-derivatives.[23-27]

Nitrosation of therapeutic drugs has been the aim of many works[28-36] since endogenous nitrosation is a possible means of exposure of humans to the toxic effects of nitrosated compounds. The interaction of therapeutic drugs with nitrosating precursors (nitrites/nitrates) present in the diet or from digestive bacterial synthesis[37,38] could be a real hazard for humans especially when potentially nitrosatable drugs are administered during a long period.

We are interested in the reactivity of a group of molecules with therapeutic activity, which have phenolic as well as secondary amine groups in their structures (phenylhydroxyethylamines),[39,40] with therapeutic activity and which are related to biological molecules such as adrenaline, tyrosine, *etc.* In relation to these compounds, Gillat[41,42] and Walters[43] have reported that phenylephrine and synephrine are easily nitrosatable under standard chemical conditions and also conditions simulating those within the stomach. Furthermore, research concerning the mutagenic activity of the diazo-derivatives of bamethan, etilefrin, synephrine and phenylephrine have proved them to be direct mutagens for TA98 and TA100 strains of *Salmonella Typhimurium*.[44-47]

In the present work, we report the results of a kinetic study of the nitrosation of 4-hydroxy-α-[(methylamino)methyl] benzenemethanol (synephrine) in acid medium (pH 2–4) at 310 K and constant ionic strength (0.36 mol dm^{-3}). The results are compared to those obtained from the nitrosation of α-[1-(methylamino)ethyl]benzenemethanol (ephedrine) and the *N*-acetyl derivative of synephrine.

Kikugawa *et al.*[44,45] have reported that the reaction between sodium nitrite and phenol or substituted phenol (bamethan, etilefrin) results in mainly two products, corresponding to the *N*-nitroso and diazo derivatives, the latter being obtained in excess nitrite. It is presumed that the *C*-nitroso derivative is an intermediate product of this process although it is not included in the global scheme outlined by Kikugawa *et al.* Moreover, since there are two nitrosatable sites in synephrine, a concomitant attack of the nitrosating agents on both nucleophilic centres and the simultaneous formation of *C*- and *N*-nitroso derivatives could be assumed according to the Scheme 1,



Scheme 1

where R = $CHOH-CH_2$ and R' = CH_3. In this scheme, the first step corresponds to the formation of *C*- and *N*-nitroso derivatives (compounds CNO and NO–I), the second step is accomplished in excess nitrite and yields a diazo-derivative (**1**) and the *C*,*N*-dinitroso-derivative (**2**). It should be taken into account that CNO and **2** (*C*-nitroso compounds in Scheme 1) would probably participate as quinone monooximes.[53] Finally, both compounds **1** and **2** give an *N*-nitrosated diazo-derivative (**3**) as a final product.

In principle, for the sake of simplicity no side reactions such as formation of nitro compounds or decomposition of diazo-derivatives are considered in the above Scheme. Neither is the interaction between *C*- and *N*-nitroso-derivatives presently considered. In relation to this it has been reported that the rate of the phenol-catalysed nitrosation process is proportional to

the concentrations of the unprotonated amine, the catalyst (*C*-nitroso-derivative of phenol) and the nitrosating agent. The mechanism starts with an initial and reversible reaction between nitrosophenol and the nitrosating agent followed by a slower reaction in which the amine attacks the intermediate product resulting in the formation of the nitrosamine and the regeneration of the nitrosophenol.[13] Davies *et al.*[12] pointed out that the species which show catalytic activity towards the *N*-nitrosation reaction are those capable of tautomerism to quinonemonooximes or quinonemonooxime imines. This requirement is fulfilled by *p*-nitrosophenol and substituted *p*-nitrosophenols as well as by substituted *o*-nitrosophenols such as those derived from the reaction of synephrine and sodium nitrite.

Experimental

Materials.—Synephrine tartrate and ephedrine hydrochloride were kindly donated by Boehringer Ingelheim. Synephrine base was purchased from Sigma. These compounds were used without further purification. Other reagents were of a high grade of purity (Merck, Sigma).

Instruments.—Spectrophotometric measurements were performed with a Perkin-Elmer Lambda 16 spectrophotometer with a Frigomix 1450 B.Braum thermostatic bath. Acidity was measured using a PHM64 pH-meter with a GK2401c combined electrode calibrated with standard buffer solutions (Crison) of pH 4 and 7. Elemental analysis was carried out with a Perkin-Elmer 240c analyser. ^{1}H NMR spectra of samples dissolved in dimethylsulfoxide were recorded with a Bruker AM-300 NMR spectrometer. Mass spectra (MS) were performed with a Hewlett-Packard HP-5988-A mass spectrometer. Elemental analysis, ^{1}H NMR and MS were carried out by the Technical Services of the University of Granada. TLC were carried out on silica gel 60 PF254 using as eluent chloroform–acetone (1:9, v/v). Spots were visualised by irradiation with UV light at 254 nm. Column chromatography was carried out on silica gel 60 (70–230 mesh ASTM, Merck) and with chloroform–ethyl alcohol (49:1, v/v). HPLC was performed with a Perkin-Elmer LC250 instrument equipped with a diode-array system LC-235 as a detector and an integrator LCI-100. The spectra registered from the peaks in the chromatogram were stored into an Epson PC AX2 computer and the data treatment was carried out with the specific software 2C View (*Medicina Legal* Department, University of Granada). The chromatograph was also equipped with a Spherisorb ODS2 (particle size: 5 μm) column (200 × 4.6 mm). Elutions of samples (10 mm^3) were carried out with acetonitrile–water (10:90) at a flow rate of 0.7 cm^3 min^{-1}. The peaks were detected at 220 nm, although eventually other wavelengths were used. In all cases the analyses were performed on samples with a suitable light protection.

Isolation of N-*Nitrososynephrine.*—*N*-Nitrososynephrine was obtained by reaction at 310 K between a solution of synephrine (5.30 g tartrate) in HCl (2 mmol dm^{-3}; 30 cm^3) and a solution of sodium nitrite (1.032 g) in distilled and deionised water (10 cm^3) ([amine]/[nitrite] = 1.5). The pH was adjusted to pH 3 with HCl. The reaction was kept in darkness and agitated with a magnetic stirrer for 4 h and then the unreacted nitrite was removed by addition of the ammonium sulfamate. After this, the reaction mixture was lyophyllised; then several extractions were carried out with ethyl acetate. The extract was concentrated and applied to a silica gel column and eluted with chloroform–ethyl alcohol (99:1, v/v) and the fractions containing a product which gave only one spot in TLC with R_f 0.65 were separated and evaporated to dryness; usually the quantity of solid obtained was very low (Calc C, 55.10; N, 14.29; H, 6.12. Found: C, 55.4; N, 14.6; H, 6.1%). HPLC of a solution of this solid in water revealed the presence of a group of peaks at retention times 9.28, 9.60 and 10.20 min which have identical absorption spectra (Fig. 1); δ_H 3.00 (3 H, s, CH_3–N), 4.18 and 3.59 (total 2 H, m, CH_2), 4.61 and 4.81 (total 1 H, m, CH), 5.43 and 5.51 (total 1 H, d, CHO*H*), 6.72 and 6.75 (total 2 H, d, phenyl 3,5-ArH); 7.12 and 7.20 (total 2 H, d, phenyl 2,6-ArH) and 9.31 (1 H, s, ArO*H*); *m/z* (%) 196 (M^+, 0.07) and 123 [M^+, −73, CH_2–N(NO)–CH_3, 100]; UV (HCl; 3 × 10^{-3} mol dm^{-3})/dm^3 mol^{-1} cm^{-1}), ε_{300} 18.0 and ε_{323} 14.0 (ethanol), ε_{300} 17.3 and ε_{323} 12.2.

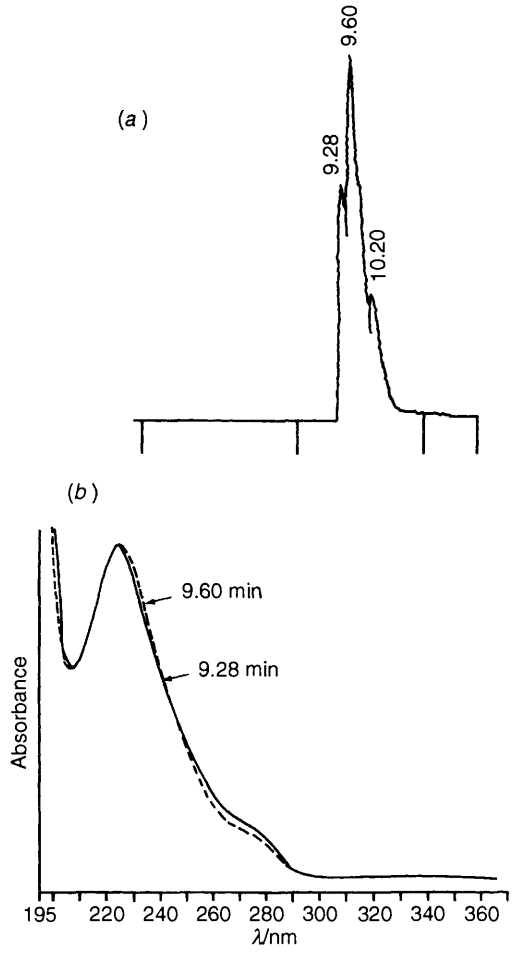


Fig. 1 HPLC of *N*-nitrososynephrine (*a*); spectral analysis of the peaks (*b*)

When the reaction between synephrine and sodium nitrite was accomplished in HCl (3 mol dm^{-3}), a yellowish solid was obtained after saturating the reaction mixture with ethyl acetate. HPLC analysis of this solid showed a group of peaks with a retention time of approximately 5.39 min and another signal at 1.86 min. The absorption spectra corresponding to former peaks are shown in Fig. 2. TLC analysis of the solid revealed the existence of a major component with R_f 0.34. The rapid decomposition of this solid by exposure to air and light and the characteristic absorption at 400 nm (Fig. 3), similar to those exhibited by the diazo-derivatives of bamethan and etilefrin,[44,45] suggests that the main component in the mixture is the 3-diazo-derivative of synephrine. The other component was identified as the unaltered amine, by comparison with the HPLC obtained with pure synephrine. No further characterisation test was carried out with the solid because of its instability.

Kinetics of Nitrosation.—The kinetics of nitrosation were performed using spectrophotometric measurements at 300 nm

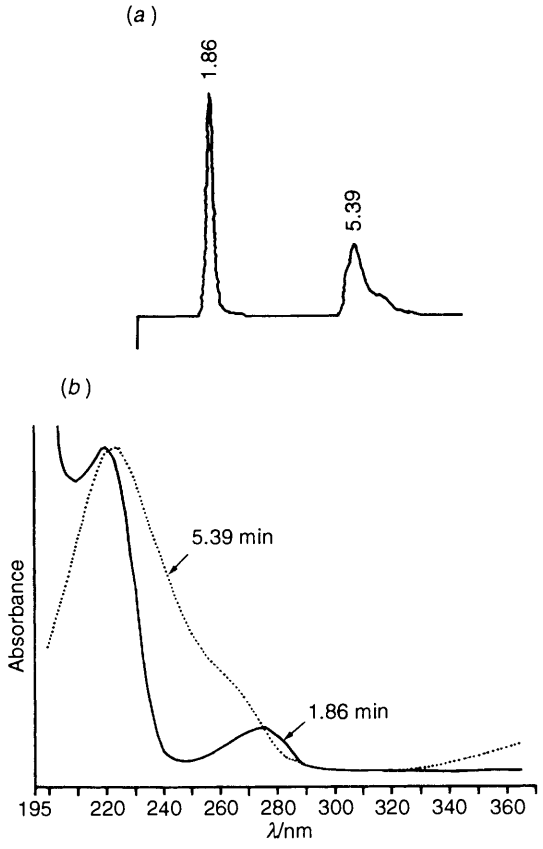


Fig. 2 HPLC of the solid obtained from the reaction HNO_2–synephrine in HCl (3 mol dm^{-3}) (*a*); spectral analysis of the peaks (*b*)

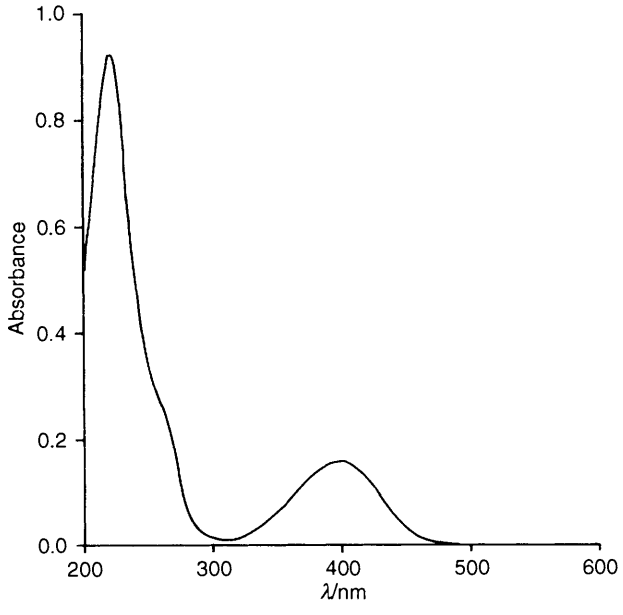


Fig. 3 UV–VIS spectrum of a solution in acetonitrile of the solid obtained from the reaction HNO_2–synephrine in HCl (3 mol dm^{-3})

(synephrine and acetylated derivative) or 323 and 332 nm (ephedrine). Amine and nitrite solutions with different concentrations were used being [nitrite] > [amine] in all cases. Stock solutions of sodium nitrite were prepared daily as required. The temperature was maintained at 310 K and the ionic strength was adjusted with potassium perchlorate at 0.36 mol dm^{-3}. Some experiments were performed in citric/citrate buffered media. pH Measurements were performed before and after each experiment and the variations found were always lower than 1%. The nitrosation reaction was accomplished inside the thermostatted cell compartment of the spectrophotometer by adding either 0.1 cm^3 of amine solution (for synephrine and acetylated derivative experiments) or 1 cm^3 (for ephedrine experiments) to 3 cm^3 of nitrite solution. An identical solution with only nitrite was used as a blank. The reaction mixture was stirred during 10 s and the changes in absorbance due to the nitrosation process were recorded during 95 s in the cases of synephrine and N-acetylated synephrine, and 2 min for ephedrine. From these data, the initial rate procedure was applied for the kinetic analysis. The concentration of nitrite was determined before every experiment using the values of absorbance measured at 323 nm (ε_{323} 11.6 dm^3 mol^{-1} cm^{-1}) corresponding to an isosbestic point in the spectra of NO_2^- and HNO_2 species.[48] Previously, the stability of the instrumental response and the extent of the nitrite decomposition during the total time of the reaction (2 min) were checked. Changes no greater than 0.001 in absorbance units and a 1% variation in absorbance measured at 300 nm with nitrite solutions (0.050–0.0044 mol dm^{-3}) were found, so no significant influence of it is expected on the kinetic data.

Results and Discussion

The absorbance changes obtained in the nitrosation of synephrine at pH 2–4 using different proportions of [nitrite]:[amine] (Fig. 4) reveal that in the region where amine absorption is negligible (> 295 nm) an unexpected product with absorption maximum at about 500 nm is formed when the amine concentration is greater or equal to the nitrite concentration. Neither *N*-nitrososynephrine nor the diazo-derivative show these spectral features showing the possibility of a reaction between synephrine and some intermediate product in the nitrosation process. *para*-Substituted phenols carrying groups activating them for electrophilic substitution can be easily coupled with nitrosonaphthol to form highly coloured products with absorption maxima between 450 and 500 nm. This reaction is well known and has been widely used in pharmaceutical analysis.[49–51] A hypothetical coupling reaction between synephrine and the C-nitrosated derivative could be invoked to explain the spectrophotometric results obtained with relatively high amine concentrations, although no attempt was made to confirm this assumption. The probable interference of a side reaction leads us to perform the kinetic study at [nitrite] > [amine] because, under these conditions, no absorption at about 500 nm is observed when the reaction proceeds for at least 1 h.

A typical HPLC chromatogram of the reaction mixture (Fig. 5) presents three groups of signals. The first one can be assigned to synephrine (1.98 min) and nitrite (2.57 min) by comparison with the signals obtained with the corresponding pure compounds. The second group of signals with a retention time about 5.81 min is formed by several superimposed peaks. The spectral analysis at different heights of the peaks indicates that the main component is the diazo-derivative as can be verified by comparison with the chromatographic results obtained with the solid isolated from the synephrine–nitrite reaction mixture saturated with ethyl acetate. Finally, the third group of signals (retention time around 10.63 min) can be attributed to the *N*-nitrosated derivative since the spectral analysis, features and relative retention times of the peaks agree very well with the corresponding data in the chromatogram of pure *N*-nitroso-synephrine (see Fig. 1). It should be noted that when the time of reaction is longer than 1 h, a new group of signals appears with a retention time of approximately 16 min although the origin of these signals remains unknown. As a summary, these results could be justified considering that, under the experimental conditions used, the synephrine–nitrite reaction gives

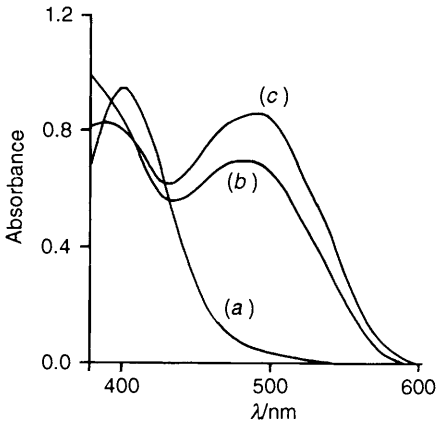


Fig. 4 Absorption spectra corresponding to the synephrine–nitrite reaction (pH = 3.5; T = 310 K; time of reaction = 1 h) [nitrite]:[synephrine]: (*a*) 40:1; (*b*) 1:1; (*c*) 1:40

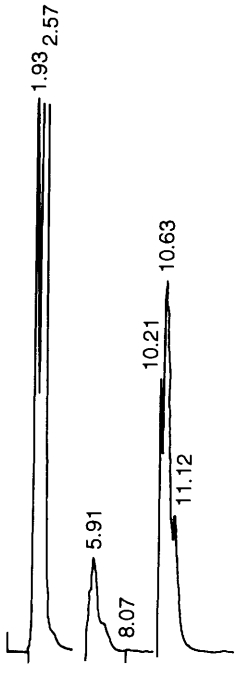


Fig. 5 HPLC of the reaction HNO_2–*N*-synephrine (pH = 3; T = 310 K) [nitrite] > [amine]

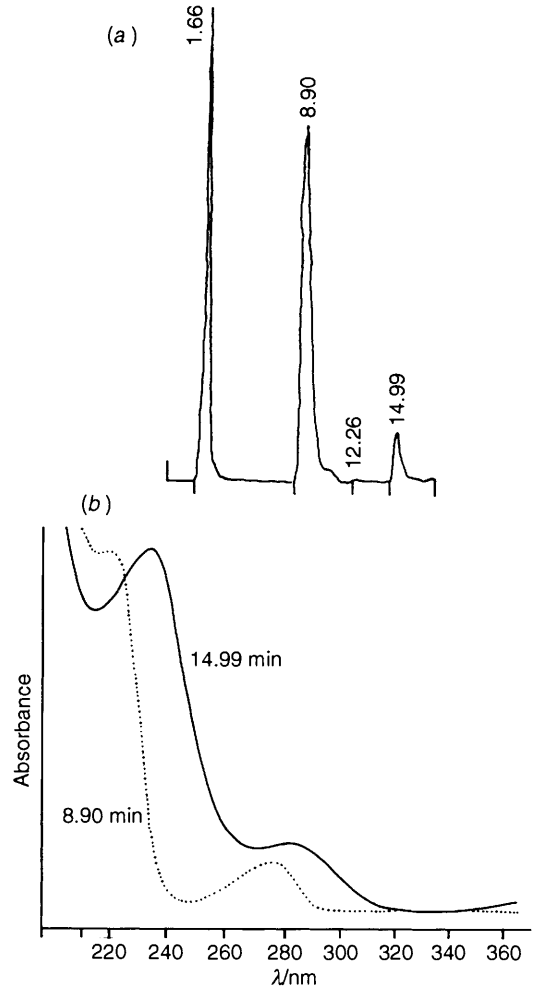


Fig. 6 HPLC of the reaction HNO_2–*N*-acetylsynephrine (pH = 3; T = 310 K) [nitrite] > [*N*-acetylsynephrine] (*a*); spectral analysis of the peaks (*b*)

rise to two products in a major proportion which can be identified as the diazo and *N*-nitroso derivatives of synephrine in good agreement with the results reported for other phenyl-(hydroxyethyl)amines.[44,45] It should be indicated that there is no obvious explanation for the multiplicity of the peaks in the HPLC chromatograms of the two derivatives (further studies are in progress). However, a number of possible causes may be considered: (i) the occurrence of several compounds (for example, compounds **1** and **3** in Scheme 1 could give the signal centred at 5.81 min) and/or (ii) the existence of isomers. In relation to this, it is important to note that although no doubling of the *N*-methyl proton signal was detected in the NMR spectrum, multiple signals were observed for the phenyl and hydroxymethylene protons (see the Experimental section). These data accord well with those reported for *N*-nitroso derivatives etilefrin and bamethan,[44,45] the HPLC traces and NMR spectra of which also displaying doubling of the signals attributed to the *syn–anti* isomerism about the oxygen–nitrogen bond.[52]

On the other hand, we have not been able to detect *C*-nitrososynephrine in the HPLC of the reaction synephrine–nitrite probably because under the conditions of our study, the peak of *C*-nitroso derivative is masked in the chromatogram. However, the HPLC of *N*-acetylsynephrine–nitrite reaction (Fig. 6) shows the peaks corresponding to nitrite (2.66 min), *N*-acetylsynephrine (8.90 min), and another with a retention time of 14.99 min which could be attributed to a product of the reaction. The absorption spectrum associated with this peak presents a shape different to those recorded from *N*-nitroso and diazo-derivatives of synephrine. Although no definitive evidence has been reached from the HPLC analysis, it is interesting to note that it is chemically possible to detect the formation of the *C*-nitroso derivative. Thus, when the nitrosation reaction of synephrine or *N*-acetylsynephrine (stopped with ammonium sulfamate 1 min after mixing the reactants) is treated with aqueous copper sulfate (1×10^{-3} mol dm^{-3}), a red coloured solution is obtained and its absorption spectrum shows the characteristic bands (with maxima at 336 and 530 nm) of the *o*-nitrosophenols: Cu^{II} complexes (Fig. 7).[54]

In order to gain deeper insight into the nitrosation of synephrine we have studied kinetically the initial step of the reaction. The wavelength chosen to monitor the reaction (300 nm) is justified by the low absorption of the synephrine and its *N*-nitroso and diazo derivatives as well as of the species derived from nitrite. In all cases, the kinetic curves have positive slopes and represent small changes in absorbances, but great enough to be differentiated from the instrumental noise, instability in the instrumental response or nitrite loss during the total time of reaction (Fig. 8). Therefore, the kinetic data should be related

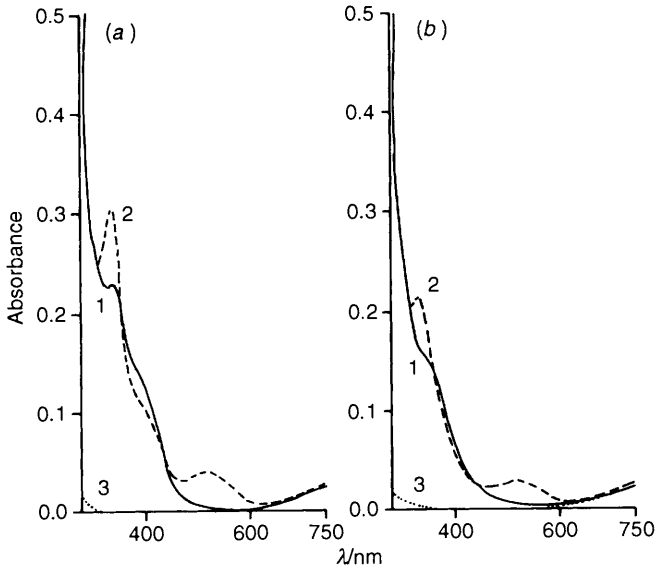


Fig. 7 Absorption spectra registered with the synephrine–nitrite (*a*) and *N*-acetylsynephrine–nitrite (*b*) reactions, (1) without copper sulfate, (2) after adding copper sulfate (pH = 2.5; T = 310 K). The reactions were stopped with ammonium sulfamate prior to adding the copper sulfate (time of reaction = 1 min). The curves (3) correspond to the copper sulfate solution.

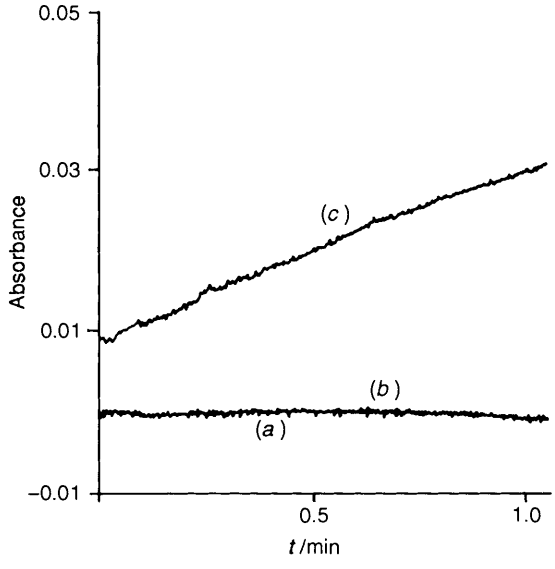


Fig. 8 Plots of the absorbance values measured at 300 nm at different times with (*a*) water; (*b*) aq. $NaNO_2$ (0.029 mol dm^{-3}) using an identical solution as a blank; (*c*) synephrine (1.78 × 10^{-3} mol dm^{-3}) with nitrite (0.029 mol dm^{-3}) reaction (pH = 2.5; T = 310 K)

to the appearance of one or more products resulting in the nitrosation process.

Plots of initial rate of change in the values of absorbance measured at 300 nm $[(dA^{300}/dt)_{t\to 0}]$ *versus* initial concentration of nitrite ($[nitrite]_0$) for the reaction at pH 3 (keeping constant initial concentration of synephrine at 1.78 × 10^{-3} mol dm^{-3}) in the presence and the absence of citrate buffer are shown in Fig. 9. Plots. of $(dA^{300}/dt)_{t\to 0}$ *versus* $[nitrite]_0^2$ (not shown) were also performed but a poor adjustment of the data was obtained.

The mechanism for the nitrosation of secondary amines and phenols is well known and it is expected that the experimental data fit the general eqn. (1), where v_0 represents the initial rate.

$$v_0 = (k[\text{nitrite}]_0 + k'[\text{nitrite}]_0^2)[\text{amine}]_0 \qquad (1)$$

Similar equations are usually employed to analyse experimental data of the nitrosation of secondary amines, considering that

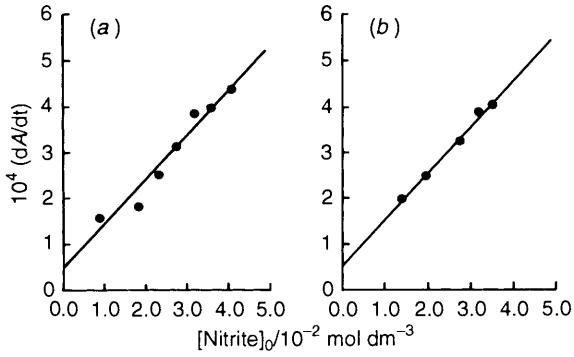
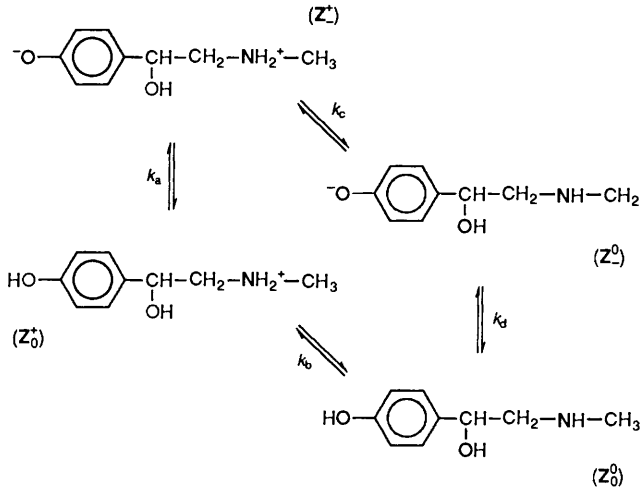


Fig. 9 Plot of $(dA^{300}/dt)_{t\to 0}$ *vs.* $[nitrite]_0$ for the reaction HNO_2–synephrine at pH 3 in (*a*) unbuffered and (*b*) buffered media

the second order term with respect to the nitrite is concerned with a rate controlling step involving attack by N_2O_3 on the unprotonated amine group, and the single term with respect to the nitrite is related to the attack by NO^+ on the same substrate.[55,56] Eqn. (1) can be also used if there is a concomitant nitrosation of the phenol group since electrophilic aromatic substitution in the phenol is brought about by the NO^+ species and consequently, its nitrosation rate is described as a function of the initial concentration of the nitrosating agent.

The acid dissociation of synephrine occurs simultaneously in the phenolic and amine group since the ratio of the dissociation constant is < 100. As a result, a parallel dissociation scheme (Scheme 2) where four different species coexist, can be considered in order to explain the individual ionisation steps.



Scheme 2

Microscopic constants k_a, k_b, k_c and k_d are determined using the macroscopic constant, *i.e.* K_1 and K_2, as calculated from potentiometric measurements and by means of known relationships

$$K_1 = k_a + k_b$$
$$K_2^{-1} = k_c^{-1} + k_d^{-1}$$
$$K_1K_2 = k_ak_c = k_bk_d$$

The values obtained[57] at 298 K and with ionic strength of 0.02 mol dm^{-3} for k_a, k_b, k_c, k_d, K_1, and K_2 are 5.9 × 10^{10}, 4.8 × 10^{-10}, 1.4 × 10^{-10}, 1.7 × 10^{-10}, 1.07 × 10^9 and 7.4 × 10^{-11} mol dm^{-3}, respectively.

Admitting the nitrosation of both substrates, *i.e.* phenol and amine and, using the notation introduced in Schemes 1 and 2, a well known set of reactions have been considered as steps in the mechanism of the synephrine nitrosation;

$$HNO_2 \overset{K_N}{\longleftrightarrow} H^+ + NO_2^-$$

$$HNO_2 + H^+ \overset{K_{N1}}{\longleftrightarrow} NO^+ + H_2O$$

$$2\,HNO_2 \overset{K_{N2}}{\longleftrightarrow} N_2O_3 + H_2O$$

$$[(Z_0{}^0) + (Z_-{}^0)] + N_2O_3 \xrightarrow{k_1} (NO\text{–}I)$$

$$[(Z_0{}^0) + (Z_-{}^0)] + NO^+ \xrightarrow{k_2} (NO\text{–}I)$$

$$[amine] + NO^+ \underset{k_{-3}H^+}{\overset{k_3}{\longleftrightarrow}} (D) \xrightarrow{k_4} (CNO)$$

where (D) is a dienone intermediate as suggested by Challis and Lawson.[58] By application of stationary state approximation to the dienone, the initial rate can be expressed as

$$v_0 = \left(\frac{d[NO\text{–}I]}{dt}\right)_{t\to 0} + \left(\frac{d[CNO]}{dt}\right)_{t\to 0}$$

$$= (k_1[N_2O_3] + k_2[NO^+])([Z_0{}^0] + [Z_-{}^0]) + \frac{k_4k_3[NO^+][amine]}{(k_{-3}[H^+] + k_4)}$$

and considering that $[nitrite]_0 = [NO^-] + [NO_2H]$ and $[amine]_0 = [Z_0{}^+] + [Z_0{}^0] + [Z_-{}^+] + [Z_-{}^0]$ the following equation is obtained,

$$v_0 = \left(\frac{k_1K_{N2}[nitrite]_0[H^+]^2(k_b[H^+] + K_1K_2)}{([H^+] + K_N)^2} + \frac{k_2K_{N1}[H^+]^2(k_b[H^+] + K_1K_2)}{([H^+] + K_N)} + \frac{k_4k_3K_{N1}[H^+]^2([H^+]^2 + K_1[H^+] + K_1K_2)}{(k_{-3}[H^+] + k_4)(K_N + [H^+])}\right) \times \frac{[nitrite]_0[amine]_0}{[H^+]^2 + K_1[H^+] + K_1K_2}$$

but taking into account the small value of the acid dissociation constants K_1 and K_2 the equation above can be simplified to give

$$v_0 = \left(\frac{k_1K_{N2}[nitrite]_0[H^+]k_b}{([H^+] + K_N)^2} + \frac{k_2K_{N1}[H^+]k_b}{([H^+] + K_N)} + \frac{k_4k_3K_{N1}[H^+]^2}{(k_{-3}[H^+] + k_4)(K_N + [H^+])}\right) \times [nitrite]_0[amine]_0$$

which is equivalent to eqn. (1).

On the other hand, changes in absorbance at 300 nm can be expressed by the equation

$$\frac{1}{b}\frac{dA^{300}}{dt} = \varepsilon_C\frac{d[CNO]}{dt} + \varepsilon_N\frac{d[I\text{–}NO]}{dt} + \varepsilon_{NO_2H}\frac{d[NO_2H]}{dt} + \varepsilon_{NO_2^-}\frac{d[NO_2^-]}{dt} + \varepsilon_A\frac{d[A]}{dt}$$

where b represents the cell pathlight (1 cm). It has been also considered in the eqn. that $[nitrite] = [NO_2H] + [NO_2^-]$ and the absorbance measured at 300 nm is the sum of the absorption of synephrine (A); its N-nitroso-derivative (NO–I); C-nitroso-derivative (CNO) and the species derived from nitrite. Considering that the data in Table 1 seem to establish a linear relationship between $(dA^{300}/dt)_{t\to 0}$ and $[nitrite]_0$, it could be assumed that there is no contribution of amine nitrosation to the changes of absorbance observed in the first step of the reaction. Taking this into account, the last equation can be written as

$$\frac{1}{b}\left(\frac{dA^{300}}{dt}\right)_{t\to 0} = \varepsilon_C\frac{d[CNO]}{dt} + \varepsilon_{NO_2H}\frac{d[NO_2H]}{dt} + \varepsilon_{NO_2^-}\frac{d[NO_2^-]}{dt} + \varepsilon_A\frac{d[A]}{dt}$$

thus, from the kinetic scheme we get

$$\frac{1}{b}\left(\frac{dA^{300}}{dt}\right)_{t\to 0} = \varepsilon\frac{k_4k_3K_{N1}[H^+]^2[nitrite]_0[amine]_0}{(K_N + [H^+])(k_{-3}[H^+] + k_4)}$$

where

$$\varepsilon = \varepsilon_C - \varepsilon_{NO_2H} - \varepsilon_{NO_2^-} - \varepsilon_A$$

being $\varepsilon_C = 15\,000$, $\varepsilon_{NO_2H} = 2$ and $\varepsilon_{NO_2^-} = 9$ dm^3 mol^{-1} cm^{-1} as estimated from the literature[48,59] and $\varepsilon_A = 8$ dm^3 mol^{-1} cm^{-1}, calculated by us.

It can be easily derived from the former equations that the global constant k in eqn. (1) is

$$k = \frac{k_4k_3K_{N1}[H^+]^2}{(K_N + [H^+])(k_{-3}[H^+] + k_4)}$$

and

$$v_0 = k[nitrite]_0[amine]_0 \qquad (2)$$

From the values of the slopes, extinction coefficients, and the initial amine concentration, the global constant k (see Table 1) could be obtained. At pH 3 the value found for k (3.6×10^{-4} dm^3 mol^{-1} s^{-1}) is approximately two orders lower than those obtained in the reaction of phenol and nitrite by Challis,[60] 0.0109 dm^3 mol^{-1} s^{-1}, at pH 1.5 and 298 K and by Virk and Issenberg,[17] 0.0148 dm^3 mol^{-1} s^{-1}, at pH 3 and 310 K.

There is little difference in the values of the slopes found in the presence and absence of citrate buffer. This difference is more important as the pH decreases and seems to indicate that citric acid acts as an inhibitor in the nitrosation process. Citrate is a commonly used buffer in nitrosation studies[12–16] and, in principle, although it has not been reported, it should be possible to suppose that the citrate exerts a nitrosating action in a similar way to that reported for the acetate anion. The influence of the anion acetate on the rate of nitrosation of amines has been revised[61,62] and the nitrosyl acetate has been considered as an effective nitrosating agent when the concentration of nitrosatable substrate is high enough to react

Table 1 Values of slopes, intercepts and determination coefficients calculated from the plots of $(dA^{300}/dt)_{t\to 0}$ *vs.* $[\text{nitrite}]_0$; values of global constant k in eqn. (2); $[\text{synephrine}]_0 = 1.78 \times 10^{-3}$; $[\text{nitrite}]_0 = 0.01$–$0.045$ mol dm^{-3}

Medium	pH	10^5 Intercepts/$^{-1}$	10^2 Slopes/dm^3 mol^{-1} s^{-1}	Determ. C	10^4 k/dm^3 mol^{-1} s^{-1}
$HClO_4$	2.0	1.05	2.16	0.97	8.1
$HClO_4$	2.5	1.02	1.66	0.98	6.2
$HClO_4$	3.0	4.39	0.96	0.95	3.6
$HClO_4$	3.5	6.99	0.45	0.98	1.7
$HClO_4$	4.0	4.12	0.27	0.92	1.0
Buffer	2.0	8.13	1.54	0.97	5.8
Buffer	2.5	5.14	1.05	0.97	3.9
Buffer	3.0	4.93	0.98	1.00	3.7
Buffer	3.5	5.86	0.54	0.99	2.0
Buffer	4.0	2.78	0.033	0.92	1.2

with nitrosyl acetate in a comparable rate to that exhibited in the reaction with nitrite. However, at low concentrations of substrate, as in the case of synephrine which at pH 3 has species with the amino group unprotonated (macroscopic dissociation constants[57] $pK_1 = 8.97$; $pK_2 = 10.13$) in very little proportion, the reaction with nitrosyl citrate could be hindered because the equilibrium constant expected for the formation of nitrosyl citrate must also be low (1.4×10^{-8} dm^3 mol^{-1} as estimated by Casado *et al.*[62] for nitrosyl acetate). A plausible explanation could be the existence of a pathway where the reaction between the dienone intermediate and citric acid at low pH and relatively high concentration of citric acid is more effective than the forward reactions for the formation of the *C*-nitroso-derivative. Thus, the scheme of the reactions in buffered media had to be completed by adding the following sequences

$$\text{Citric} \underset{H^+}{\overset{K_{C1}}{\longleftrightarrow}} \text{Citr}^- \underset{H^+}{\overset{K_{C2}}{\longleftrightarrow}} \text{Citr}^{2-}$$

$$[(Z_0^+) + (Z_0^0)] + NO^+ + \text{Citr}^- \underset{k'_{-3}\text{Citric}}{\overset{k'_3}{\longleftrightarrow}} (D) \overset{k'_4}{\longleftrightarrow} \text{CNO}$$

where the dissociation equilibria for the citric acid which can influence the formation of CNO in the range of pH 2–4 ($K_{C1} = 7.0 \times 10^{-4}$ and $K_{C2} = 1.8 \times 10^{-5}$ mol dm^{-3}) have been considered.

Nitrosation of the *N*-acetyl-derivative of synephrine was carried out in an unbuffered medium at pH 3. The results obtained were intercept = 1.42×10^{-4}; slope = 1.13×10^{-2}; determination coefficient = 0.98 (pH adjusted with $HClO_4$). These results are in good agreement with those in Table 1.

Furthermore, an experiment made with a constant initial nitrite concentration (0.03 mol dm^3) and variable synephrine concentration (6.4×10^{-4}–2.1×10^{-3} mol dm^{-3}) at pH 3 adjusted with perchloric acid gave a straight line when $(dA^{300}/dt)_{t\to 0}$ was plotted *versus* the initial concentration of synephrine. An intercept of 1.44×10^{-5}, a slope of 0.152 and a value of 0.99 for the determination coefficient were determined from the adjusted line. A similar plot from the data obtained for an experiment made in buffered medium gave an intercept of 2.06×10^{-5}, a slope of 0.176 and 1.00 for the determination coefficient. From the slope, ε and the initial concentration of nitrite, values of 3.4×10^{-4} and 3.9×10^{-4} dm^3 mol^{-1} s^{-1} respectively, were found for k which are in good agreement with the values 3.6×10^{-4} and 3.7×10^{-4} dm^3 mol^{-1} s^{-1} obtained in the experiments made with variable concentrations of nitrite.

It can be observed in Table 1 that a good linearity and also intercepts distinct from zero are achieved in all cases. The latter could be misunderstood as a spontaneous nitrosation in the absence of nitrite. We have revised some sources of systematic errors such as the value of ε^{323} used to determine the nitrite concentration or the linear adjustment of the kinetic data to calculate $(dA^{300}/dt)_{t\to 0}$, but the extinction coefficient at 323 nm is in very good agreement with the values reported in the literature and the adjustment of the kinetic data were always obtained with determination coefficients higher than 0.99. We have not been able to find an explanation for the relatively large values of the intercepts but the fact that we have performed the same experiment with variable concentrations of amine and obtained an intercept significantly lower (1.44×10^{-5}) whereas the global constant is practically the same, point to the instrumental artefact as a possible source of the high intercepts in Table 1.

The nitrosation of ephedrine at pH 3 in buffered and unbuffered media was followed by means of absorbance measurements at 332 and 323 nm. In this case a better adjustment was obtained when the experimental data were plotted against the square of the initial nitrite concentration (Fig. 10). This agrees very well with the expected nitrosation of the secondary amine group and formation of *N*-nitroso-ephedrine (II–NO) by means of a diffusion controlled attack by the nitrosating agent N_2O_3 implying that the reaction is of second order[63] with respect to $[HNO_2]$. Therefore it can be written as eqn. (3). In addition, for the nitrosation of ephedrine, it

$$v_0 = k'[\text{nitrite}]_0^2[\text{amine}]_0 \qquad (3)$$

can be deduced that

$$\frac{1}{b}\left(\frac{dA}{dt}\right)_{t\to 0} = \varepsilon_N \frac{d(\text{II–NO})}{dt} + \varepsilon_{NO_2H}\frac{d[NO_2H]}{dt} + \varepsilon_{NO_2^-}\frac{d[NO_2^-]}{dt}$$

since ephedrine has no absorption at neither 323 nor 332 nm. Using as a reference the reaction scheme outlined for synephrine, the following expression can be derived

$$\frac{1}{b}\left(\frac{dA}{dt}\right)_{t\to 0} = \varepsilon' \frac{k'_1 K_{N2} K'_A [H^+][\text{nitrite}]_0^2[\text{amine}]_0}{(K_N + [H^+])^2}$$

where k_1' is the rate constant for the nitrosation by N_2O_3 of the amine group in ephedrine; K_A' is the dissociation constant for ephedrine (9.36 at 25 °C) and ε' is

$$\varepsilon' = \varepsilon_N - 2\varepsilon_{NO_2H} - 2\varepsilon_{NO_2^-}$$

From the curves reported by Markovits *et al.*[48] the values of ε 11.6 and 18.5 dm^3 mol^{-1} cm^{-1} for NO_2H at 323 and 332 nm respectively and 11.6 and 14.6 dm^3 mol^{-1} cm^{-1} for NO_2^- at the same wavelengths were estimated. For ε_N, the values employed were 55.9 and 77.6 dm^3 mol^{-1} cm^{-1} at 323 and 332 nm respectively.[64]

Table 2 Values of slopes, intercepts and determination coefficients calculated from the plots of $(dA/dt)_{t\to 0}$ *versus* $[\text{nitrite}]_0^2$. Values of global constant k' in eqn. (3); $[\text{ephedrine}]_0 = 0.040$; $[\text{nitrite}]_0 = 0.02$–$0.042$ mol dm^{-3}

Medium	pH	Intercept/min^{-1}	Slopes/dm^6 mol^{-2} min^{-1}	Determ. C	k'/dm^6 mol^{-2} min^{-1}
λ = 332 nm					
$HClO_4$	3.0	1.24×10^{-4}	3.87	0.98	0.142
Buffer	3.0	5.89×10^{-5}	4.26	1.00	0.156
λ = 323nm					
$HClO_4$	3.0	5.94×10^{-5}	3.13	0.99	0.137
Buffer	3.0	1.87×10^{-4}	3.33	1.00	0.146

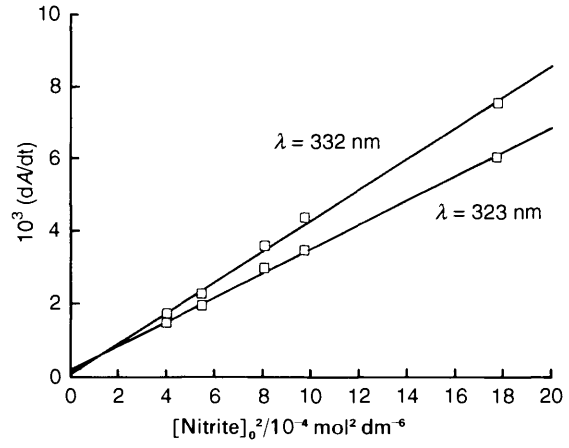


Fig. 10 Plot of $(dA/dt)_{t\to 0}$ *vs* $[\text{nitrite}]_0^2$ for the reaction HNO_2–ephedrine at pH 3 in buffered medium

The parameters of the adjustment of $(dA/dt)_{t\to 0}$ *vs.* $[\text{nitrite}]_0^2$ and the global constants k' calculated from the slopes, ε' and initial concentration of ephedrine (0.040 mol dm^{-3}), are given in Table 2.

The global constant k' can be related to the kinetic rate constants and the hydronium concentration by the equation

$$k' = \frac{k_1' K_{N_2} [H^+] K_A'}{(K_N + [H^+])^2}$$

From the acid dissociation constants of nitrous acid K_N (7.1×10^{-4} mol dm^{-3}) and ephedrine, it is possible to calculate the value for the product $k_1' K_{N_2}$. Thus, the values found were in the order of 9.5×10^5 dm^6 mol^{-2} s^{-1} (*e.g.* 9.51×10^5 dm^6 mol^{-2} s^{-1} for the nitrosation of ephedrine in $HClO_4$ measured at 332 nm). These results are in accordance with those reported in literature for amines of similar pK_a namely morpholine (pK_a = 8.70) which has a value of 6.7×10^5 dm^6 mol^{-2} s^{-1} (at 298 K and ionic strength 0.5 mol dm^{-3}) and methylbenzylamine (pK_a = 9.54) with 5.0×10^5 dm^6 mol^{-2} s^{-1} (at 298 K and ionic strength 2.0 mol dm^{-3}).[63] A similar behaviour could be expected for synephrine since the microscopic constant k_b is close to K_A'.

As pointed out before, the nitrosation reaction has been studied with [nitrite] > [amine], with [nitrite] in the range *ca.* 0.01–0.05 mol dm^{-3} in an attempt to simplify the kinetic scheme. Under these experimental conditions, the kinetic analysis of the nitrosation of synephrine reported in the present paper permit a number of conclusions. The initial step of synephrine nitrosation takes place according to the process outlined in Scheme 1 where nitrite and phenolic group are simultaneously nitrosated. Unlike the early conclusions about the relative rate of nitrosation of secondary amine and phenol reported by Challis,[58,60] extrapolating the results found for ephedrine, it is possible to state that at pH 3 the rate of N-nitrosation is an order of magnitude larger than that for C-nitrosation. These results can be related to the structural features of synephrine which only permit the substitution in the aromatic ring of the nitroso group in position *ortho* to the phenolic hydroxy group. This represents lower yields in the formation of the nitrosated compound as reported in relation with the nitrosation of phenol by sodium nitrite where *o*-nitrosophenol is always obtained in a lower proportion than *p*-nitrosophenol.[18,54,58] However, the initial rate obtained from the spectrophotometric measurements seems to be basically related to the formation of the *C*-nitroso-derivative as a result of its favourable extinction coefficient at 300 nm (15 000 dm^3 mol^{-1} cm^{-1} as estimated from *p*-nitrosophenol) against that exhibited by the *N*-nitroso-derivative (18 dm^3 mol^{-1} cm^{-1}). This conclusion is supported by the results found in the nitrosation of the *N*-acetyl-derivative of synephrine. On the other hand, no catalytic effects due to the *C*-nitroso-derivative have been detected which seems to indicate that this compound is not accumulated in a high enough amount during the reaction. Actually *o*-nitrosophenols are extremely unstable and are rapidly converted to *o*-diazoquinone[18] in excess of sodium nitrite.

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