# Inhibition of Nitrosation by the Reaction Medium

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The nitrosation of *N*-methyl-, *N*-ethyl-, *N*-propyl-, and *N*-butylurea in water−acetonitrile mixtures in acid medium was studied. Kinetic monitoring of the reactions was accomplished by spectrophotometric analysis at 249 nm of the *N*-alkyl-*N*-nitrosoureas formed. When the percentage of acetonitrile was increased up to ≈50% (i) the nitrosation rate constant decreased and (ii) the reactivity of different nitrosatable substrates converged. The first of these effects can be rationalized in terms of a mechanism whose slow kinetic step is proton transfer from protonated *N*-alkyl-*N*-nitrosourea to the water of the reaction medium. The second effect can be explained by assuming that the folding of the hydrophobic alkyl group of the substrates decreases when the permittivity of the reaction medium diminishes. The finding of two isokinetic relationships supports the idea of a common reaction mechanism over the whole range of compositions of the solvent mixture. As a conclusion it is shown that, under these circumstances, the nitrosation of *N*-alkylureas can be strongly inhibited by lowering the dielectric constant of the reaction medium.

**Keywords:** *Inhibition of nitrosation; N-alkylureas; N-alkyl-N-nitrosoureas*

## INTRODUCTION

The formation of *N*-nitroso compounds in the human environment has currently received increased attention owing to the carcinogenic, mutagenic, and teratogenic effects of many of them (Mirvish, 1975, 1995; Vidal et al., 1997). Recent discoveries have demonstrated several pathways for the endogenous formation of *N*-nitroso compounds. Ingested or endogenous nitrogenous substrates can react with nitrous acid in the stomach or be nitrosated either there or elsewhere by nitrosating agents arising from the endogenous formation of NO or the bacterial reduction of nitrate (Loeppky and Michejda, 1994).

As a result, many attempts have been made, particularly in the field of food science, to block or inhibit the formation of these pathogenic species (Coultate, 1984; Bao and Loeppky, 1991; Wilcox et al., 1991; Loeppky et al., 1994).

The alkylnitrosoureas have been extensively studied because of their varied organ-specific carcinogenic activity and also because several dialkylnitrosoureas have been used as cancer-therapeutic agents (Lijinsky, 1992; Ueno et al., 1982a,b). For their use as drugs these alkylnitrosoureas have undergone extensive examination with regard to their chemistry and pharmacology (Lijinsky, 1992; Milo et al., 1988). Accordingly, in vivo production of nitrosoureas from food constituents and nitrite might contribute to the aetiology of gastric cancer (Mirvish et al., 1980).

On the other hand, the fact that alkylureas are able to inhibit the infectivity of HIV-1 (Goldstein et al., 1991) has aroused considerable interest in their overall reactivity.

Despite the importance of nitrosoureas, the mechanisms of their formation (first investigated by Mirvish in 1971) have not been studied systematically as has the formation of other nitroso compounds (Casado, 1994).

As part of our ongoing research of blocking nitrosation reactions (González-Mancebo et al., 1997), and prompted by earlier results (Casado et al., 1983, 1996), in this work we attempt to investigate a possible way of inhibiting such reactions. Here, the nitrosation of *N*-methyl-, *N*-ethyl-, *N*-propyl-, and *N*-butylurea in water−acetonitrile mixtures was investigated.

## EXPERIMENTAL PROCEDURES

Solutions of *N*-methylurea (Merck), *N*-ethylurea (Merck), *N*-propylurea (Alfa), and *N*-butylurea (Fluka) were made up by weight from p.a. products with nominal purities of 98−99%. Solutions of sodium nitrite (Merck p.a.) were prepared by direct weighing after drying the product at 343.15 K. Ionic strength (*I*) was maintained with Merck p.a. sodium perchlorate monohydrate solutions made up by weight. Solutions of perchloric acid (Panreac p.a.) were prepared by dilution from the acid at 60% ($\rho$ = 1.52 g cm$^{-3}$). Solutions of acetonitrile were made up from Panreac 99.5% product. *N*-Nitrosomethylurea and *N*-nitrosoethylurea were Sigma p.a. products.

The kinetic study was carried out by measuring the absorbance of the *N*-nitrosoalkylureas formed ($\lambda$ = 249 nm) on a SHIMADZU UV-2101PC double beam spectrophotometer with thermoelectric control with the UV-2101/3101PC program. As a typical example, Figure 1 shows the kinetic runs of two nitrosation reactions.

## RESULTS AND DISCUSSION

In previous work (Casado et al., 1983, 1996) it was observed that, in aqueous medium, as the slow kinetic step the reaction mechanism of the nitrosation of *N*-alkylureas displays proton transfer from the protonated *N*-alkyl-*N*-nitrosourea to the water solvent (Scheme

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**Table 1.  Values of Experimental Enthalpy of Activation, $\Delta H^{\#}$, and Entropy of Activation, $\Delta S^{\#}$, for Nitrosation of *N*-Alkylureas**

| percent acetonitrile | *N*-methylurea | | *N*-ethylurea | | *N*-propylurea | | *N*-butylurea | |
|---|---|---|---|---|---|---|---|---|
| | $\Delta H^{\#}$, kJ mol$^{-1}$ | $-\Delta S^{\#}$, J mol$^{-1}$ K$^{-1}$ | $\Delta H^{\#}$, kJ mol$^{-1}$ | $-\Delta S^{\#}$, J mol$^{-1}$ K$^{-1}$ | $\Delta H^{\#}$, kJ mol$^{-1}$ | $-\Delta S^{\#}$, J mol$^{-1}$ K$^{-1}$ | $\Delta H^{\#}$, kJ mol$^{-1}$ | $-\Delta S^{\#}$, J mol$^{-1}$ K$^{-1}$ |
| 0 | 40.2 ± 0.9 | 114 ± 3 | 44.7 ± 0.8 | 111 ± 2 | 43.4 ± 0.5 | 115 ± 1 | 42.3 ± 0.7 | 118 ± 2 |
| 8.9 | 36 ± 1 | 128 ± 4 | 44.8 ± 0.8 | 110 ± 1 | 46.2 ± 0.5 | 109 ± 1 | 42.8 ± 0.7 | 118 ± 2 |
| 19.6 | 39.0 ± 0.5 | 121 ± 2 | 43 ± 1 | 117 ± 3 | 45.5 ± 0.3 | 113 ± 1 | 44.8 ± 0.4 | 114 ± 1 |
| 29.4 | 44.6 ± 0.5 | 105 ± 2 | 47.4 ± 0.9 | 107 ± 3 | 50.6 ± 0.7 | 98 ± 2 | 48 ± 1 | 104 ± 3 |
| 39.7 | 46.3 ± 0.3 | 102 ± 1 | 51.4 ± 0.9 | 96 ± 2 | 52.3 ± 0.5 | 95 ± 1 | 51.9 ± 0.8 | 96 ± 2 |
| 51.2 | 49.6 ± 0.7 | 92 ± 2 | 54.5 ± 0.5 | 86 ± 1 | 54.9 ± 0.5 | 88 ± 1 | 54 ± 1 | 91 ± 3 |
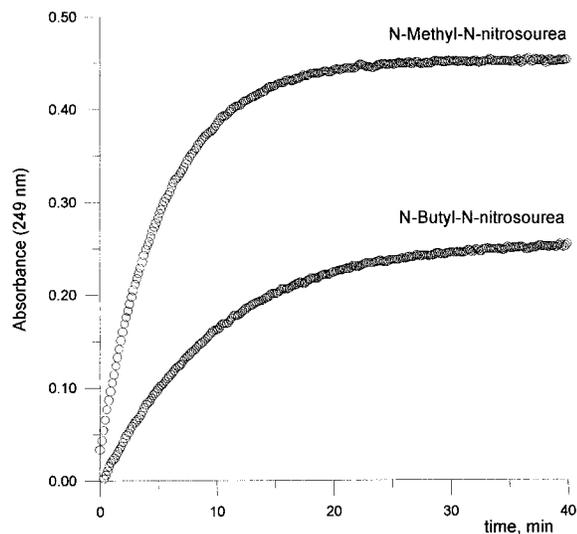


**Figure 1.** Typical kinetic runs of the nitrosation of [*N*-methylurea] = $1.0 \times 10^{-2}$ M and [*N*-butylurea] = $2.5 \times 10^{-2}$ M in a water−acetonitrile mixture (19.6% acetonitrile): $T = 298.15$ K; [nitrite] = $1.0 \times 10^{-4}$ M; [H$^{+}$] = 0.020 M; $I = 1$ M.

**Scheme 1.  Proton Transfer as the Slow Kinetic Step in the Nitrosation of *N*-Alkylureas**
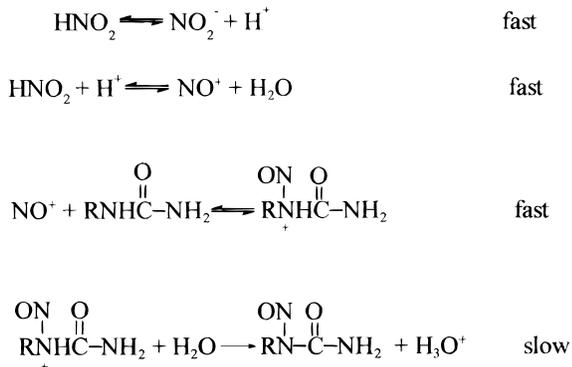


1).  On studying the nitrosation of *N*-alkylureas in aqueous medium, the following sequence of reactivities was found: *N*-methylurea ≫ (*N*-ethylurea ≈ *N*-propylurea ≈ *N*-butylurea) ≫ *N*-allylurea.  This order can be explained in terms of the capacity of the protonated *N*-alkyl-*N*-nitrosourea to form a hydrogen bond with the water molecule to which the proton will be transferred and the degree to which the formation of such bonds is hindered by the hydrophobic alkyl chain of the *N*-alkylnitrosourea.

On the basis of this scheme, it is logical to speculate that the progressive substitution of the water of the reaction medium by a solvent with a lower dielectric constant should hinder proton transfer, thus hampering the formation of the *N*-alkylnitrosoureas.  On working
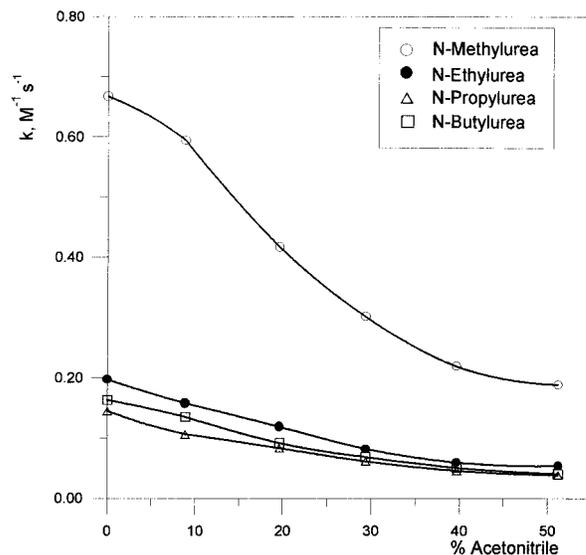


**Figure 2.** Variation in the rate constant in the nitrosation of *N*-alkylureas with the percentage of acetonitrile in the reaction medium:  $T = 298.15$ K; [H$^{+}$] = 0.020 M; $I = 1$ M; [nitrite] = $(1.00 \pm 0.01) \times 10^{-4}$ M; [MU] = $(1.00 \pm 0.01) \times 10^{-2}$ M; [EU] = $(1.50 \pm 0.01) \times 10^{-2}$ M; [PU] = $(2.00 \pm 0.01) \times 10^{-2}$ M; [BU] = $(2.50 \pm 0.01) \times 10^{-2}$ M.

with water−acetonitrile mixtures with up to ≈50% of the latter, the rate equation was always

$$\text{rate} = k[\text{nitrite}][N\text{-alkylurea}]$$

Three replicas were made of each kinetic experiment, obtaining $k$ (experimental rate constant) values with a deviation of less than 3%.  Figure 2 shows the variation in $k$ with the composition of the medium.

To determine the activation parameters, experiments were carried out at eight temperatures in the 285.15−303.15 K range.  Table 1 shows the results obtained.

To check whether proton transfer was the rate controlling step under the working conditions used, with no disturbance from other influences such as the reaction between the nitrosating agent and the nitrosatable substrate (as occurs in the nitrosation of amines), a series of experiments were carried out in the presence of Cl$^{-}$ ions.  The absence of catalysis by Cl$^{-}$ in the concentration range [NaCl] = $(0.00−2.00) \times 10^{-2}$ M shows that the slow step of the process takes place after the reaction between the nitrosating agent and the *N*-alkylurea.

As is known (Senent, 1986; Exner, 1988), the existence of an isokinetic relationship can serve to defend the argument that the reactions of a series share a common mechanism.

The members may differ in the identities of a functional group, the length of a side chain, the composition of the solvent, and so on.  In this work we considered
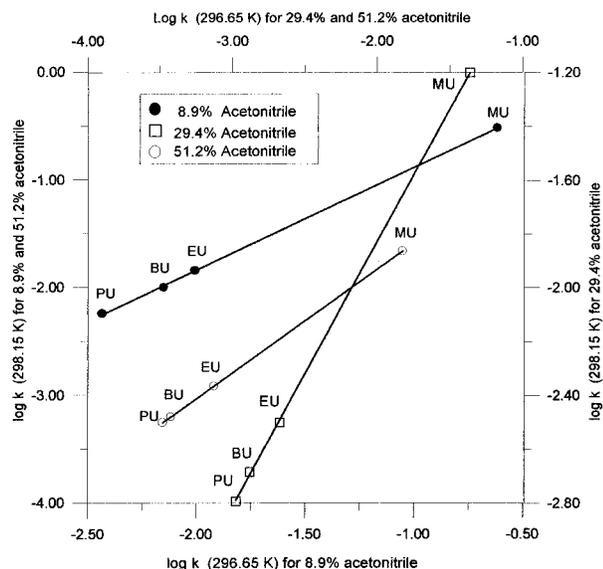
**Figure 3.** Isokinetic relationship in the nitrosation of *N*-alkylureas with the substituent as the variable factor [MU = *N*-methylurea; EU = *N*-ethylurea; PU = *N*-propylurea; BU = *N*-butylurea]: [H$^+$] = 0.020 M; $I$ = 1 M; [nitrite] = (1.00 ± 0.01) × 10$^{-4}$ M; [MU] = (1.00 ± 0.01) × 10$^{-2}$ M; [EU] = (1.50 ± 0.01) × 10$^{-2}$ M; [PU] = (2.00 ± 0.01) × 10$^{-2}$ M; [BU] = (2.50 ± 0.01) × 10$^{-2}$ M.



**Figure 4.** Isokinetic relationship in the nitrosation of *N*-alkylureas with the solvent as the variable factor: [H$^+$] = 0.020 M; $I$ = 1 M; [nitrite] = (1.00 ± 0.01) × 10$^{-4}$ M; [MU] = (1.00 ± 0.01) × 10$^{-2}$ M; [EU] = (1.50 ± 0.01) × 10$^{-2}$ M; [PU] = (2.00 ± 0.01) × 10$^{-2}$ M; [BU] = (2.50 ± 0.01) × 10$^{-2}$ M. Percent acetonitrile: (a) 0.0; (b) 8.9; (c) 19.6; (d) 29.4; (e) 39.7; (f) 51.2.

two variable factors: (i) the substituent of the nitrosatable substrate and (ii) the solvent, i.e., the percentage of acetonitrile in the reaction medium. A mathematical formulation of the isokinetic effect is the linear relationship between two series of log $k$ values measured at two temperatures $T_1$ and $T_2$:

$$\log k(T_2) = a + b \log k(T_1)$$

It should be pointed out that the meaning of the isokinetic relationship is the existence of a *compensation effect* between values of enthalpy, $\Delta H^{\#}$, and entropy of activation, $\Delta S^{\#}$, so that the Gibbs free energy of activation, $\Delta G^{\#}$, is approximately constant.

The results shown in Figures 3 and 4 support the idea of a common mechanism. From the results obtained the following may be inferred:

(1) In the acetonitrile concentration range employed, the increase in size of the alkyl group of *N*-alkylurea renders it less susceptible to nitrosation.

(2) In the same concentration range, the rate of nitrosation decreases considerably with the increase in the percentage of acetonitrile in the reaction medium.

(3) The values of the rate constants of the nitrosation reactions of the substrates studied tend to converge as the proportion of acetonitrile in the reaction medium increases.

The greater reactivity of *N*-methylurea can be explained in terms of the extent to which hydrogen bonding between the protonated *N*-alkyl-*N*-nitrosourea and the water molecules to which the proton will be transferred is hindered by the alkyl chain of the nitrosourea: the larger, more hydrophobic alkyl groups of *N*-ethylurea, *N*-propylurea, and *N*-butylurea would tend to fold back on the rest of the molecules, thereby preventing pretransfer hydrogen bonding. Figure 5 shows a somewhat simplistic scheme of the hindering of proton transfer by the alkyl chain of the protonated *N*-methyl-*N*-nitrosourea and *N*-butyl-*N*-nitrosourea. In
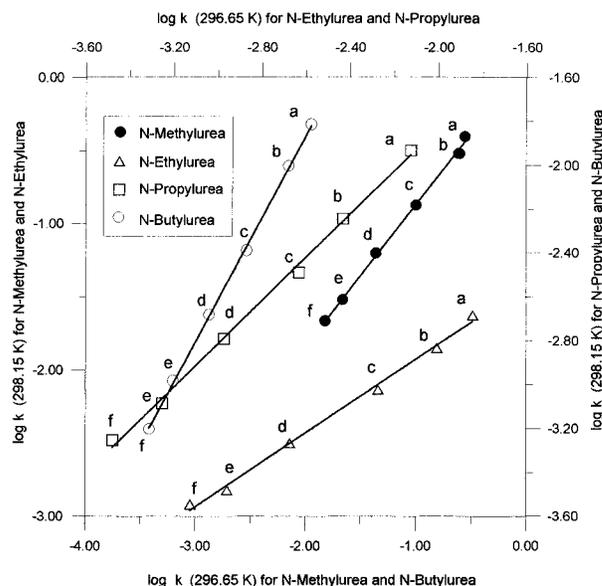


(a)



(b)
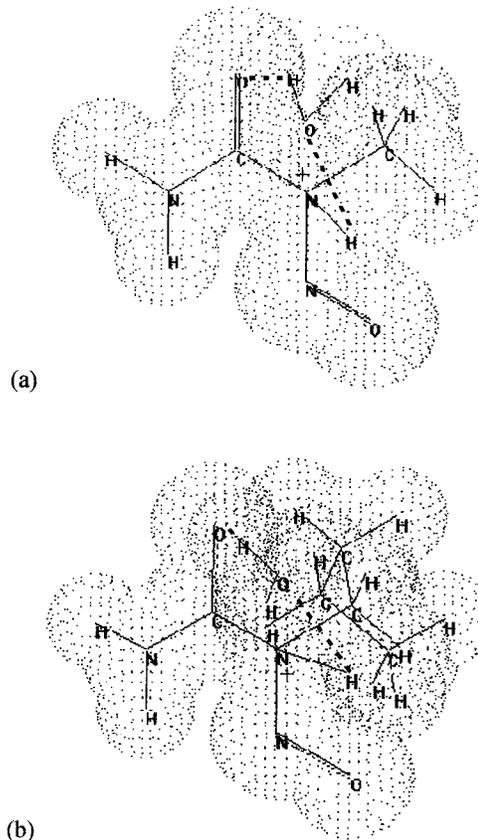
**Figure 5.** Proposed hydrogen bonding between water and protonated *N*-alkyl-*N*-nitrosoureas: (a) *N*-methyl-*N*-nitrosourea; (b) *N*-butyl-*N*-nitrosourea.

addition, when the hydrophobic character of the alkyl chain of the *N*-alkylurea increased, one should observe not only an increase in the enthalpy of activation with the most hindered substrates but also a less ordered transition state. The decrease in the absolute value of $\Delta S^{\#}$ confirms this (Table 1).

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Furthermore, as the percentage of acetonitrile increases in the reaction medium, diminishing its dielectric constant (relative permittivities $\epsilon_{CH_3CN} = 36$; $\epsilon_{H_2O} = 78$), (i) the folding of the alkyl group will have less effect and, as a result, the $k$ values of the different reactions should converge, as is indeed the case and (ii) the enthalpy of activation should increase as a result of the greater difficulty in proton transfer, as in fact has been observed (parallel decreasing of the absolute value of the entropy of activation reveals a *compensation effect* (Figure 4) that supports the idea of a common mechanism over the whole range of composition of the solvent mixture).

## CONCLUSION

Kinetic study of the nitrosation reactions of *N*-alkylureas in water−acetonitrile media up to (50% in acetonitrile shows that in that range (in which the kinetic controlling step is proton transfer from the protonated *N*-alkyl-*N*-nitrosourea to the water of the medium) it is possible to strongly inhibit the reactions by increasing the percentage of the organic component.

This result may be of use when working with hydrophilic/lipophilic media, such as in food science. For instance, the results may be significant in those cases of the presence in the human stomach of mixtures of alcoholic spirits and food containing vegetal oils, such as salads, or food coming from the preserves industry. Since the dielectric constant of these mixtures is lower than that of the water, a slowing down of the in vivo nitrosation of alkylureas would be expected.

## ACKNOWLEDGMENT

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