# Photolysis of N-Nitrosamines in Neutral Media

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## Abstract

The photolysis of N-nitrosamines in solutions of acetonitrile follows a first-order reaction with respect to the concentration of nitrosamine. Quantum yields are very low ( $\approx 0.1$ ) and depend on the concentration of nitrosamine, with the observation of a linear correlation between the reciprocal of quantum yield and the reciprocal of nitrosamine concentration. For all the nitrosamines studied, the final product of the photolysis appears to be unique, alkylidenimine, which in the case of nitroso methyl benzyl amine undergoes a relatively rapid hydrolysis, giving rise to benzaldehyde, following second order kinetics. A provisional model consistent with the experimental results obtained in this work is proposed. © 1993 John Wiley & Sons, Inc.

### Introduction

The dialkyl nitrosamines are markedly stable compounds in comparison with other nitroso derivatives and are widely distributed in the human environment. The interest in these compound lies in their toxic characteristics. The most important biological actions that have been attributed to them are their carcinogenicity, teratogenicity, and mutagenicity [1-3]. Their carcinogenic effects seem to result from alkylation of nucleic acids, although they require metabolic activation before they are able to react with these [4].

The presence of these compounds in a broad range of foods such as meats, fish, vegetables, cheese, and milk is well documented. The appearance of such nitroso compounds in foods is a result of the manipulations and transformations to which they are subjected during their preparation. Additionally, the formation of nitrosamines in polluted urban atmospheres has elicited increased interest in them in order to gain insight into the mechanisms that form them and the consequences of their presence.

The fact that nitrosamines are photolyzed by UV light together with their well known resistance to thermal degradation has led to research aimed at elucidating the photochemical degradation of these compounds with a view to converting them into harmless products. Such photochemical studies have permitted the synthesis of compounds which previously were somewhat difficult to obtain by conventional methods [5].

Studies carried out on photolysis both in the vapor state [6,7] and in acid solution [8–14] are concordant in the sense that the first photochemical step involves breakage of the N - N bond of the nitrosamine, leading to the formation of a dialkylamino (vapor state) or dialkylaminium (acid medium)

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radical and NO, which has been detected in RSE studies of NDMA at 77 K [15,16]. In both cases, mechanisms consistent with the experimental results have been proposed.

Regarding neutral solutions, the studies are much scarcer because for many years nitrosamine were purported to be photostable in solutions and in the absence of acid when both the  $n\pi *$  [8,17,18] and  $\pi\pi^*$  transitions are excited, with the exception in the latter case of nitrosodibenzylamine [5]. The only observation has been a photochemical perturbation of the Z  $\leftrightarrow$  E equilibria through the first excited singlet  $(n\pi)^*$  in asymmetric nitrosamines [19].

In 1978 Gowenlock [20] conducted a study at 367.9 nm on quantum yields of diethylnitrosamine in methanol and cyclohexane media with different concentrations of acid (0-0.15 M in HCl). This author observed that quantum yields are maximum when the acid and nitrosamine are present at equimolar concentrations and that they are higher in solutions of methanol than in cyclohexane. These two effects are perfectly consistent with the mechanisms of photolysis in acid media.

The importance of this kind of study lies in the fact that the authors found that in the absence of acid quantum yield was 0.72 for solutions of methanol and 0.10 in cyclohexane. This points to the occurrence of photolysis in neutral media, although with quantum yield far lower than those obtained with acid media.

In 1984 Michejda et al. [21] performed a study of the photolysis of N-nitrosopiperidine and of N-nitroso diisopropylamine in different mixtures of benzene-methanol. Photolysis was carried out in Pyrex tubes opaque at wavelengths below 290 nm. These authors proposed the following mechanism based on the products of the photolysis:

(I)  

$$\frac{R}{R} N - N = 0 \xrightarrow{hv} R R + NO$$

(II)  $R_2N \cdot + NO + R'H \longrightarrow NONRADICAL PRODUCTS$ 

$$(III) \qquad 2 \text{ NO} + \text{O}_2 \longrightarrow 2 \text{ NO}_2$$

$$(IV) R_2 N \cdot + NO_2 \longrightarrow R_2 NNO_2$$

(V) 
$$R_2 NNO_2 \xrightarrow{hv} NONRADICAL PRODUCTS$$

In the presence of oxygen, the only product obtained in *N*-nitroso dialkylamine, whereas when methanol is present in the solvent, the final products found are amine and formamide.

The low rate of photolysis in neutral media can be accounted for by the reversibility of the first photochemical process. This recombination of the primary radicals, possibly responsible for the low quantum yields obtained, has been foreseen in several studies both in the gas phase [7] and in solution, where it would be favored by the "cage" effect of the solvent.

The primary recombination is also highly favored in the absence of radical trapping reagents, such as oxygen, which could partly explain why for many years the photostability of *N*-nitrosamines was upheld and why the works

referred to in this study are the only ones found in the literature dealing with this field.

#### Experimental

The following nitrosamines were used: N-nitroso dipropylamine (NDPA) and N-nitroso dibutylamine (NDBA) provided by Sigma Chem. Comp. (St. Louis) and N-nitrosodiisopropylamine (NDIPA), N-nitroso diamylamine (NDAA), and N-nitroso methylbenzylamine (NMBEA), synthesized at our laboratory from the corresponding amines (Fluka Chemie AG, Switzerland) according to the method described by Zimmer et al. [22]. The benzaldehyde was purchased from Merck and the remaining reagents were RPA.

As solvents, methanol and acetonitrile (RS-HPLC) from Carlo Erba (Milano, Italy), acetic acid (Panreac, Barcelona, Spain), and deionized water twice distilled in glass (the first time in the presence of potassium permanganate) filtered through a 0.2  $\mu$ m pore nylon 66 filter (Gelman Sciences, Inc.) were used.

Spectrophotometric studies were performed on a Shimadzu UV-240 double beam spectrophotometer.

Quantitative measurements of the concentrations of nitrosamine at different times of photolysis were carried out on a Konik KNK-500-A HPLC chromatograph endowed with a KNK-029-375 gradient programmer, a column oven and a Rheodyne manual injection port equipped with a 20  $\mu$ m loop. The chromatograph was coupled to a Kratos 757 UV-Vis double beam variable wavelength detector whose signal was processed by a Spectra Physics SP4290 integrator.

Two columns were used (Scharlau, Barcelona, Spain): a Spherisorb CN 5  $\mu$ m (12 × 4.6 mm) fitted with a precolumn (3.5 × 4.6 mm) of the same characteristics and Nucleosyl 120 C18, 5  $\mu$ m (12 × 4.0 mm) to which a precolumn of the same characteristics was fitted (3 × 4. mm).

Chromatographic analyses were performed at a temperature of  $25^{\circ}$ C at the maximum wavelength of each nitrosamine, obtaining the concentrations of these from the corresponding calibration as the mean value of the results of three consecutive analyses.

Photochemical studies were conducted with a TNN 15-32 15 W low pressure mercury immersion lamp from Heraeus Instruments. The photochemical reactor was mounted on a thermostat at  $25.0 \pm 0.5$  °C. A teflon tube permitted entry of the gases into the reactor (O<sub>2</sub> and, occasionally, N<sub>2</sub>) and to keep the reactor stirred. A second closed circuit, also of teflon, allowed filling through a 10 ml Hamilton syringe from a flow cuvette (Hellma, 10 mm optic pathway and 390  $\mu$ l volume) installed in the spectrophotometer. Spectrophotometric monitoring of the photochemical reactions was made through this. The same circuit permitted sample collection of the photolyzates for chromatographic analysis.

For HPLC monitoring, samples of the photolyzate were taken at time intervals of 5 to 15 min, depending on the reactions. These samples were kept in darkness, at low temperature, until the time of HPLC analysis. In all cases the initial concentrations of the nitrosamines were determined from the chromatographic and spectrophotometric analyses obtained at time zero.



Figure 1. Spectra obtained at different times (min) during the photolysis of 1.27  $10^{-4}$  M NDBA ( $\lambda = 236$  nm) in acetonitrile solutions.

For each nitrosamine different photochemical studies were conducted, keeping all parameters constant except for the initial concentration of nitrosamine. In all cases the reaction volume was  $500 \text{ cm}^3$  and the solvent used was acetonitrile, chosen in view of its excellent transparency in the UV-Vis zone of the spectrum. The reactions were followed until at least 60% of the initial concentration of the nitrosamine in question had disappeared; in most cases, this was 90%.

To calculate the quantum yields of the different nitrosamines, the potassium ferrioxalate actinometer was used, as described by Hatchard and Parker [23].

#### **Results and Discussion**

The spectra obtained during the time of photolysis of the nitrosamines studied show the same kind of behavior (Fig. 1). This consists in the disappearance of the band corresponding to the nitrosamine ( $\lambda = 234-236$  nm) and the appearance of a new band at wavelengths lower than 200 nm, with the exception of NMBEA, in which case the band appears at 272 nm (Fig. 2). This is not surprising in view of the electronic structure of NMBEA.

This result discloses the photolysis of the *N*-nitrosamines in this reaction medium. The absorbance-time data at each wavelength were analyzed for each nitrosamine by a factor analysis method, giving as a result the existence of only two factors (Table I). This seems to indicate the presence of a single reaction product with an appreciable extinction coefficient in the wavelength range (190-260 nm).

The HPLC analyses of the photolyzates at different times, carried out at the wavelength of maximum absorption of the nitrosamine, only reveals the existence of two major compounds. One of these corresponds to the nitrosamine. In the case of NMBEA, a third signal appears; this was identified as benzaldehyde by comparison with the retention time of the pure compound and by its maximum wavelength in the UV (244 nm). These findings suggest the formation of a major product of photolysis.

Nitrosamine	NDPA	NDIPA	NDBA	NDAA	NMBEA
Factor 1	0.82	0.60	0.66	0.60	0.84
Factor 2	0.17	0.39	0.33	0.39	0.16
Factor 3	7.2E-5	5.3E-4	1.95E-3	1.96E-3	2.3E-4

TABLE I. Factor analysis for N-nitrosamine photolysis spectra. Proportion of original variance.

However, they do not rule out the possible existence of reaction products with extinction coefficients in the UV-V is zone of the spectrum that are sufficiently low for their contribution to the total absorbance to be negligible; thus, they would not be detected either in the spectrophotometric or in the chromatographic analyses using the same detection method.

In all cases the disappearance kinetics of nitrosamine follow a first-orderrate equation with respect to the concentration of the compound, as may be seen in Figure 3. Table II shows the observed rate constants, all of them of the order of  $10^{-4}$  s<sup>-1</sup>, with a mean half-life of nitrosamine ranging between 1.0 h for NDAA and 2.1 h for NMBEA.

The quantum yields (Table II) depend on the nitrosamine concentrations and have very low values. These are concordant with those reported by Gowenlock [20] for the photolysis of NDEA in cyclohexane and in the absence of acid and methanol ( $\phi = 0.1$ ). These values of  $\phi$  necessarily require the existence in the photolysis mechanism of a cyclic stage that will regenerate the reagent, nitrosamine, thus, not producing any net reaction. This stage, very likely consisting of recombination of the primary radicals to regenerate the nitrosamine has been contemplated both by Geiger [7], in the photolysis NDMA in the vapor phase, and by Burgess [24], in solution, where recombination in solvent cage could be responsible for the apparent low quantum yield of photodecomposition.

The recombination of primary radicals was observed by Geiger only when the NDMA is excited in the  $n\pi^*$  band (363.5 nm since irradiation in the  $\pi\pi^*$ band at 248.1 nm produces radicals with a large excess of vibrational energy. Since losses by intermolecular collisions are not favored in gas phases, these radicals decay by another path before undergoing primary recombination.



Figure 2. Spectra obtained at different times (min) during the photolysis of 1.06  $10^{-4}$  M NMBEA ( $\lambda = 236$  nm) in acetonitrile solutions.



Figure 3. Logarithmic plot of the relative concentration of NDBA (N/No) against time of photolysis. Initial concentration of NDBA  $1.27 \ 10^{-4}$  M.

Nitrosamine	Concentration 10 <sup>4</sup> (M)	$\frac{k_{\rm obs}}{({\rm s}^{-1})}$	φ
NDPA	0.595	1.28	0.049
	0.992	1.45	0.084
	1.48	1.82	0.154
NDIPA	0.334	1.28	0.036
	1.03	1.25	0.076
	1.58	1.08	0.098
NDBA	1.21*	0.04	0.003
	0.87	1.47	0.078
	1.22	1.49	0.106
	1.20	1.52	0.112
	1.49	1.57	0.135
	1.80	1.47	0.152
NDAA	0.364	1.90	0.057
	1.18	1.98	0.137
	1.69	1.74	0.168
NMBEA	1.06	0.92	0.056
	1.37	0.93	0.073

TABLE II. Rates of photolysis  $(k_{obs}, s^{-1})$  and quantum yield measurements for N-nitrosamines.

\* N<sub>2</sub> purged.

In our case, the "cage" effect of the solvent may, in principle, favor this primary combination and, although, as will be discussed below, it is little favored, the conditions under which the reaction was carried out in our work make it competitive with the step involving the formation of the reaction products. Interestingly, when the nitrosamine solution is degassed with nitrogen, the quantum yield decreases some 40-fold ( $\phi_{N_2} = 0.003$ ).

In the photolysis of the nitrosamines there is, therefore, a strong participation by the oxygen, which possibly reacts as a radical-trapping agent for the



Figure 4. Spectra obtained at different times (min) during the hydrolysis of the product ( $\lambda = 272$  nm) resulting from the photolysis of 1,06 10<sup>-4</sup> M NMBEA over 200 min.

NO radicals, thus, preventing primary recombination. This again points to the possible existence of this primary recombination in the reaction medium assaged.

Regarding NMBEA, this compound shows a behavior similar to that of the other nitrosamines studied during their photolysis (Table II). However, it differs strikingly with respect to the behavior of the reaction product since this is hydrolyzed at an appreciable rate, giving rise to a product that absorbs at a wavelength of 244 nm (Fig. 4) together with a new signal in the chromatograms with a retention time coinciding with that of the benzaldehyde. The hydrolysis of the photolysis product of NMBEA follows pseudo-first-order kinetics (Fig. 5) with respect to the concentration of reagent, with an observed constant in turn depending linearly on the concentration of water (Table III, Fig. 6) and, thus, obeying overall secondorder kinetics:

$$r = 1.6 \ 10^{-2} [R] [W]$$

where r is the rate hydrolysis, R is the reagent (product of the photolysis reaction), and W represents the water.

This secondary reaction seems to show that the product of the photochemical reaction is alkylidenimine, whose hydrolysis, in the case of NMBEA, would give rise to benzaldehyde and methylamine.

The formation of alkylidenimine has been one of the steps proposed since early studies on NDMA and NDEA in the vapor phase [6], and this compound (i.e., alkylidenimine) has been detected in studies in acid media [9], where it has been observed that it may undergo hydrolysis or trimerization and even amidoxime may be formed in the presence of NOH [8,9], through an intermolecular mechanism [13]. In the medium used in this work hydrolysis seems to be the most favorable mechanism since benzaldehyde is formed almost quantitatively.

In the light of the foregoing and maintaining as the primary photochemical step the photolysis of the N - NO bond to give rise to NO and a dialkylamino radical, which as mentioned is a process well documented in the literature,



Figure 5. Logarithmic plot of absorbance at 272 nm (product of photolysis reaction of NMBEA) against time. Water concentration  $2.22 \, 10^{-3} \, M$ .

it is possible to postulate a provisional mechanism for the photolysis of the nitrosamines studied. This would be based, at least, on the following steps:

(I) Photodissociation of the nitrosamine producing NO and dialkylamino radicals;

(II) Recombination of the primary radicals and regeneration of the nitrosamine;

(III) Reaction of the dialkylamino radical with the nitrosamine, giving rise to amine and a second radical that, in turn, evolves (IV) to give rise to dialkylidenimine; and

(V) Reaction of the NO with the oxygen dissolved in the reaction medium to give rise to other nitrogen oxides  $(NO_2, N_2O_4)$ .

(I) 
$$\begin{array}{c} \text{RCH}_2 \\ \text{RCH}_2 \end{array} N \longrightarrow N \stackrel{\text{RCH}_2}{\longrightarrow} N \cdot + \text{NO} \\ \text{RCH}_2 \end{array}$$

(II) 
$$\begin{array}{c} \operatorname{RCH}_2 \\ \operatorname{RCH}_2 \end{array} \operatorname{N} \cdot \begin{array}{c} k_1 \\ \operatorname{NO} \end{array} \begin{array}{c} \operatorname{RCH}_2 \\ \operatorname{RCH}_2 \end{array} \operatorname{N} \cdot \begin{array}{c} k_1 \\ \operatorname{RCH}_2 \end{array} \operatorname{N} - \operatorname{N} \end{array}$$

(III) 
$$\operatorname{RCH}_2$$
 N· +  $\operatorname{RCH}_2$  N· N·  $\operatorname{RCH}_2$  N· N·  $\operatorname{RCH}_2$  N· N·  $\operatorname{RCH}_2$  N· N· NO  $\operatorname{RCH}_2$  N· N·  $\operatorname{RCH}_2$  N· N· NO

(IV) 
$$\overrightarrow{RCH}_N$$
-NO  $\overrightarrow{RCH}$ -RCH=N-CH<sub>2</sub>R + NO

(V) 
$$2 \text{ NO} + \text{O}_2 \longrightarrow 2 \text{ NO}_2 \implies \text{N}_2\text{O}_4$$

[H <sub>2</sub> O] 10 <sup>2</sup> (M)	$k_{\rm obs} \frac{10^4}{(s^{-1})}$
0.222	4.2
0.296	4.7
7.41	15
12.35	24
18.52	33

TABLE III. Observed constants  $(k_{obs})$  obtained as a function of the concentration of water for the hydrolysis of the photolysis product of NMBEA.

The validity of this mechanism can be checked through analysis of the steady state. Assuming that only one fraction,  $\alpha$ , of the intensity of the inciding radiation, 1, gives rise to the formation of the dialkylamino radicals (Step I), its formation rate will be  $\alpha$ I. For the quantum yield of the disappearance of the nitrosamine, treatment of the steady state for the dialkylamine radicals gives an expression, thus:

(1) 
$$\phi = \frac{2\alpha k_2[\mathbf{N}]}{k_1 + k_2[\mathbf{N}]}$$

where [N] is the concentration of nitrosamine. One therefore obtains a linear relationship between the reciprocal of the quantum yield and that of the concentration of the nitrosamine

(2) 
$$\frac{1}{\phi} = \frac{k_1}{2k_2\alpha} \frac{1}{[\mathbf{N}]} + \frac{1}{2\alpha}$$

This result is consistent with our experimental results, as may be seen in Figure 7.

From the slopes and ordinates of the plots of eq. (2), it is possible to obtain the values of a and of the  $k_1/k_2$  ratio. The results (Table IV) give for  $k_2$  a value around  $10^3-10^4$ -fold higher than  $k_1$ , showing that the recombination reaction of the primary radicals is very little favored, despite the "cage" effect of the solvent, when the molecule of nitrosamine in the  $\pi\pi^*$  transition is excited. This finding is consistent with the results of Geiger [7] for the photolysis of



Figure 6. Variation of specific rate  $(k_{obs})$  of hydrolysis of the photolysis product of NMBEA vs. water concentration.

Nitrosamine	$k_1/k_2  10^4$	α	
NDPA	5.88	0.31	
NDIPA	1.76	0.10	
NDBA	10.3	0.50	
NDAA	2.08	0.19	

TABLE IV. Values obtained for  $\alpha$  and the  $k_1/k_2$  ratio (eq. (1)) through the proposed mechanism.

NDMA in the vapor phase and, according to that author, would be due to the excess vibration energy of the dialkylamino radical. However, this does not imply that the rate of process II, primary recombination, will be negligible in our case since the rate of process III depends on the concentration of nitrosamine  $(10^{-4} \text{ M})$  such that it is comparable to the rate of primary recombination.

Regarding the values of  $\alpha$ , these are lower than unity, in concordance with their definition. However, they are not very significant values since they were obtained from the reciprocals of the ordinates at the origin on correlating very few experimental data.

This highly speculative mechanism is the basis of ongoing studies designed to check it by systematically examining, both quantitatively and qualitatively, the photolysis of other nitrosamines.

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Figure 7. Variations of reciprocals of quantum yields vs. reciprocals of concentration of NDBA.

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