N-Nitrosamides and Their Precursors in Food Systems. 3. Influence on pH and Temperature on Stability of N-Nitrosamides

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The decomposition of N-nitrosamides was investigated in aqueous and organic solvents as a function of temperature and pH. In aqueous solvent, N-nitrosomethylpropionamide (NOMP) had maximum stability at pH 4.0 over the temperature range 4–50 °C. Further experiments also confirmed that N-nitrosamides are much less stable than volatile N-nitrosamines such as N-nitrosopyrrolidine (N-Pyr) and dimethylnitrosamine (DMN). Thermal decomposition studies utilizing heating conditions commonly encountered in the pan frying of bacon and oven roasting of pork indicated that NOMP was degraded to the extent of 74–97% compared to 3–14% for N-Pyr and DMN. It was tentatively concluded that the major contribution of N-substituted amides, if present in foods, may be as precursors of N-nitroso compounds formed by in vivo N-nitrosation reactions.

It has been previously reported that free amines can readily react with fatty acids and triglycerides in model systems to form N-substituted amides (Kakuda and Gray, 1980a). It was concluded that these reactions could possibly take place in foods during processing and storage, thereby producing another chemical species capable of reacting with nitrite. The kinetics of N-nitrosation of these amides were studied in model systems (Kakuda and Gray, 1980b) and it was reported that the amide is rapidly N-nitrosated in acid environments, thus offering the possibility of in vivo formation of N-nitrosamides.

This paper reports the results of a kinetic study of the decomposition of N-nitrosamides as a function of pH and temperature. The stability of these N-nitroso compounds when subjected to thermal stresses such as encountered in the pan frying of bacon or oven roasting of pork is also reported. These studies were designed to establish whether such compounds could possibly be present in food systems since previous studies have established that N-nitrosamides are unstable under neutral and alkaline conditions (Druckrey et al., 1967).

EXPERIMENTAL SECTION

Materials and Methods. N-Nitrosomethylpropionamide (NOMP) and N-nitrosopentylpalmitamide (NOPP) were prepared and purified as previously described (Kakuda and Gray, 1980a,b). Dimethylnitrosamine (DMN) was obtained from Eastman Kodak Co. (Rochester, NY) and N-nitrosopyrrolidine (N-Pyr) from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals were of reagent grade or better.

Absorbance readings were recorded on a Shimadzu multipurpose spectrophotometer MPS 50 L and pH readings determined on a Model 26 radiometer fitted with a GK 2311 C glass electrode. Gas chromatographic analyses were performed on a Hewlett Packard 402 gas chromatograph equipped with a flame ionization detector and a glass column (6 ft × $^3/_8$ in. i.d.) packed with 3% OV-101 on 80–100 mesh Chromosorb W. The samples were chromatographed isothermally at 90 °C with the flash heater and detector block at 140 and 160 °C, respectively. The nitrogen carrier gas flow rate was 20 mL/min.

Decomposition of NOMP in Aqueous Solvent. *Buffer Systems.* The buffer systems for pH 2.0 and 7.0 were prepared with $NaH_2PO_4 \cdot 2H_2O$. Buffers for pH 3.0 and 5.0 were prepared with citric acid monohydrate. Glacial acetic acid was used for pH 4.0 and $NaHCO_3$ for pH 9.5. The pH of each buffer system was adjusted with either HCl or NaOH and diluted to a final concentration of 0.1 M.

Assay Procedure. The kinetics of decomposition of NOMP were followed by measuring the decrease in absorbance at 237 nm with time. A series of 0.1 M buffer solutions in 25-mL volumetric flasks were preincubated for 5–10 min in a constant temperature water bath. At time zero, 10 μL (0.912 mmol) of NOMP was injected into each flask and immediately and thoroughly mixed by vigorous shaking. During the course of the reaction, 0.5-mL aliquots were withdrawn from each flask and quickly diluted to 25 mL with cold 0.1 M acetate buffer, pH 4.0. The UV absorbance of the diluted samples was measured within 5 min of dilution, although it was observed that the UV absorbance remained constant for at least 1 h after dilution. The reactions were followed for at least 1 half-life and were performed in duplicate. The extinction coefficient for NOMP in cold acetate buffer was 8.86×10^3 M^{-1} cm^{-1}.

Decomposition of NOPP and NOMP in Organic Solvent. *Solvent System.* The solvent system was composed of 95% ethanol (20 mL) and butanol (30 mL) and various aqueous buffers (60 mL). This solvent readily dissolved the long-chain N-nitrosamide over a pH range from 2 to 5. A 60-mL aliquot of the following aqueous buffer solutions was used in the preparation of the solvent; acetic acid was used for pH 4.0 and 5.0, citric acid for pH 3.0 and phosphoric acid for pH 2.0. The concentration of each buffer was 0.37 M. Each aqueous buffer solution was adjusted to a value below the required pH in order to compensate for the rise in pH when mixed with the organic components. The solvent used for pH 7.0 was 95% ethanol (26 mL), butanol (30 mL), and 0.185 M NaH_2PO_4 (60 mL). In this case, a lower buffer concentration and a higher ethanol content was required to give a homogeneous solution.

Assay Procedure. A stock solution of NOPP was prepared by dissolving 0.32 g of the N-nitrosamide in 5 mL of butanol. The reaction was initiated by pipetting 0.5 mL of the stock NOPP solution into a 25-mL volumetric flask containing the buffered solvents. The flask was thoroughly mixed and returned to a constant temperature water bath. Samples were withdrawn at regular intervals, usually 0.5 mL, and diluted to 25 mL with 0.4 M acetic acid/acetonitrile (1:3, pH 4.0) solution. The absorbance of the diluted

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sample was measured at 245 nm. The reactions were followed for at least 1 half-life and were performed in duplicate. The initial concentration of NOMP was 3.61 $\times 10^{-3}$ M.

The decomposition of NOMP was studied in the same solvent system. The procedure was similar as for NOPP, except that 0.5 mL of butanol was added to the buffered solvent before 10 μL of NOMP was added to initiate the reaction. The initial concentration of NOMP was 3.69 $\times 10^{-3}$ M. The extinction coefficients for NOPP and NOMP in acetic acid/acetonitrile solvent were 7.8 $\times 10^3$ and 7.9 $\times 10^3$ M^{-1}, respectively, both measured at 245 nm.

Stability of *N*-Nitroso Compounds under Various Heating and Cooking Conditions. *Thermal Degradation of NOMP.* Ten-microliter aliquots of NOMP were sealed in 1-mL ampules and heated for specified periods of times over a wide range of temperatures (80–155 °C) in a thermostated oil bath. At the end of each heating period, the contents of each ampule were carefully washed into a 25-mL volumetric flask with cold 0.1 M acetate buffer, pH 4.0. The absorbance of this solution was measured at 237 nm. Unheated vials of NOMP were analyzed in a similar manner and used as a control to calculate percent decompositions.

Bacon Frying. Ten microliters of NOMP was added to four 1-mL ampules and sealed. Each ampule was heated on top of the bacon strips which were fried to crispness in a preheated Presto Teflon coated electric frying pan. The bacon was fried for a total of 8 min, 4 min on each side at 177 °C (350 °F). A mercury thermometer placed on top of the frying bacon registered a maximum temperature of about 100 °C. A second set of ampules containing 10 μL of NOMP was heated directly on the frying pan for 8 min. The temperature of the frying pan was approximately 130 °C. The contents of the ampules were analyzed as described above.

Similarly, 10 μL of N-Pyr and DMN were also sealed in ampules and heated for 8 min in direct contact with the frying bacon. The contents of each ampule were extracted with diethyl ether, concentrated to 1 mL, and then analyzed by GLC. The contents of unheated ampules were extracted and analyzed in a similar manner and used as controls.

Pork Roast. Ten microliters of NOMP, N-Pyr, and DMN were individually sealed in 1-mL ampules. These ampules were firmly attached to the sides of a 5-lb pork roast and baked for 2 h in an oven preheated to 180 °C. The samples were extracted and analyzed for percent decomposition as described above.

RESULTS AND DISCUSSION

The decomposition of short-chain (NOMP) and long-chain (NOPP) *N*-nitrosamides was investigated in aqueous and organic solvents as a function of pH and temperature. The first-order rate constants for the decomposition of NOMP and NOPP were determined graphically. A logarithmic plot was constructed and the slope of the best fit line drawn with a straight edge calculated. A typical logarithmic plot of NOMP decomposition at pH 4.0 and 30 °C is shown in Figure 1. Results of this decomposition study indicated that in aqueous solvent, NOMP had maximum stability at pH 4.0 over the entire temperature range studied (Table I). The rate constants varied only slightly between pH 2 and 5, but increased markedly under neutral and, in particular, alkaline conditions. For example, at pH 9.5 the first-order rate constants are 300–600 times greater than at pH 4.0. The instability of *N*-nitrosamides in alkaline media has also been reported in detail by Druckrey et al. (1967), who showed that the rate

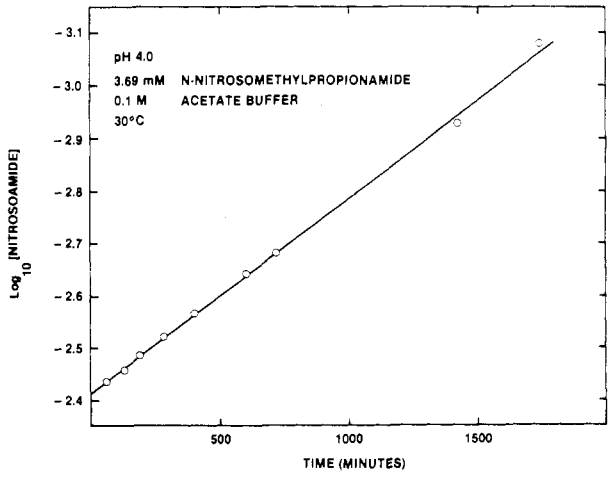


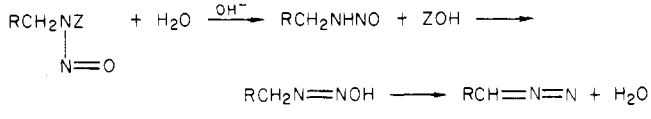
Figure 1. First-order rate plot for the decomposition of *N*-nitrosomethylpropionamide in an aqueous system.

Table I. Effects of pH and Temperature on the Rate of Decomposition of *N*-Nitrosomethylpropionamide in an Aqueous Solvent

	k (min^{-1}) $\times 10^3$				
	temperature, °C				
pH	4	19	30	40	50
2	0.098[a]	0.48	1.22	2.52	6.51
3	0.072	0.37	0.89	0.91	4.73
4	0.069	0.34	0.85	1.83	4.52
5	0.10	0.49	1.33	2.64	6.60
7	0.29	1.86	6.34	15.31	40.00
9.5	19.21	130	470	1200	[b]

[a] Average of two determinations. [b] Too rapid to follow.

of decomposition increases with increasing pH and varies with amide structure. At pH 9, the order of stability was found to be *N*-nitrosourea < *N*-nitrosamide < *N*-nitrosourethane < *N*-nitrososulfonamide < *N*-nitrosoguanidine. At this pH, *N*-nitrosamides decompose rapidly to diazoalkanes (Druckrey et al., 1967; Magee et al., 1976; Lobl, 1972; Douglas et al., 1978). Berry and Challis (1972)



studied the acid-catalyzed decomposition of *N*-nitrosobutylacetamide and showed that decomposition of the *N*-nitrosamide to its starting materials (amide and nitrous acid) was favored at high acidities. Rearrangement via the diazo ester to give nitrogen and carboxylic acid ester was the most important mode of decomposition at low acidities (< M perchloric acid).

Fan and Tannenbaum (1972) determined the first-order rate constants for the decomposition of *N*-nitrosamines and *N*-nitrosoamino acids over a similar pH range. Their experiments were conducted at 110 °C and values ranging from 10^{-3} to 10^{-6} min^{-1} were obtained. The *N*-nitrosamides are obviously much more labile when compared to *N*-nitrosamines. Using an activation energy of 16 kcal/mol (Table II), the rate constant of *N*-nitrosamide decomposition at pH 4.0 should be theoretically about 0.2 min^{-1} at 110 °C. This represents a (2.8×10^3)-fold difference in stability when compared to DMN or N-Pyr at pH 4.0.

An easier comparison can be made between the stabilities of *N*-nitrosamines and *N*-nitrosamides by calculating half-life values. At intermediate pH values (pH 4–7) and 110 °C, DMN and N-Pyr have half-lives ranging from 16

Table II. Activation Energies of Decomposition of *N*-Nitrosomethylpropionamide in Aqueous Solution as a Function of pH

pH	activation energy, kcal/mol
2	16.2
3	15.8
4	15.9
5	16.1
7	18.4

Table III. Effects of pH and Temperature on the Decomposition Half-Life (h) of *N*-Nitrosomethylpropionamide

	temperature, °C				
pH	4	19	30	40	50
2	117.1	24.3	9.6	4.6	1.8
4	167.2	33.1	13.0	6.4	2.5
7	39.4	6.2	1.8	0.8	0.3

Table IV. Rate Constants for the Decomposition of *N*-Nitrosomethylpropionamide and *N*-Nitrosopentylpalmitamide in Organic Solvent at 50 °C

	k (min^{-1}) × 10^3	
pH	NOMP	NOPP
2.0	2.53	2.95
3.0	1.54	2.80
4.0	1.60	2.98
5.0	2.12	2.76
7.0	5.80	3.05

Table V. Calculated Rate Constants for the Decomposition of *N*-Nitrosopentylpalmitamide in Aqueous Solvent at 50 °C

pH	k (min^{-1}) × 10^3
2	7.61
3	7.24
4	7.20
5	8.61
7	20.83

to 55 days (Fan and Tannenbaum, 1972), while the half-lives of NOMP at pH 4.0 are measured in hours (Table III). Near body temperature (40 °C), NOMP has a half-life of 6.4 at pH 4.0 and only 0.8 h at pH 7.0.

The rate constants for the decomposition of NOPP in aqueous solvent could not be obtained due to the insolubility of NOPP in an aqueous medium. However, the rates of decomposition were obtained in organic solvent for the pH range 2 to 7 (Table IV). The stability of NOMP was also investigated in the same organic solvent in order to determine the effects of solvent and chain length on decomposition rates. In acid media, NOPP decomposed at a greater rate than did NOMP; however, at pH 7, NOMP was more unstable.

The results indicate that the organic solvent tends to stabilize the *N*-nitroso compounds. The rate constants for NOMP decomposition in organic solvent were approximately a third of those measured in aqueous solvent over the pH range 2 to 5. At pH 7, the effect was even more pronounced with rate constants differing by a factor of 7 (Tables I–IV). This lack of pH dependence was probably due to the low polarity of the organic solvent and may account for the greatly reduced rates at pH 7.0.

The theoretical rate constants for NOPP decomposition in aqueous solvent were calculated using a conversion factor derived from data obtained for NOMP in organic and aqueous solvents (Table V). The rates so derived for NOPP were slightly larger than the rates for NOMP except at pH 7.0 where the rate was half. In general, the differences in chain length do not appear to have a large effect on the rates of decomposition. The major difference is in solubility, the long chain *N*-nitrosamides being insoluble in nonpolar materials.

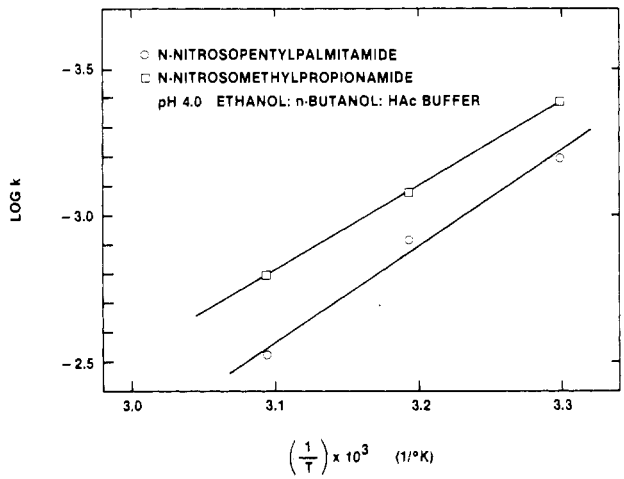


Figure 2. The effect of temperature on the rates of decomposition of *N*-nitrosomethylpropionamide and *N*-nitrosopentylpalmitamide in an organic solvent.

Table VI. Effect of Temperature on the Rate of Decomposition of *N*-Nitrosomethylpropionamide and *N*-Nitrosopentylpalmitamide in Organic Solvent, pH 4.0

		k (min^{-1}) × 10^3	
temp, °C		NOMP	NOPP
30		0.74	0.64
40		0.82	1.22
50		1.60	2.98
60		4.19	9.64
	E_a (kcal/mol)	13.31	15.42

Table VII. Thermal Decomposition of *N*-Nitrosomethylpropionamide[a]

temp, °C	k (min^{-1}) × 10^3	$t_{1/2}$, min
80	7	102
98	40	17
115	98	7
134	272	2.5
150	945	0.7
155	1590	0.4

[a] Average of duplicate determinations.

The effect of temperature on the kinetics of decomposition of NOMP and NOPP in organic solvents is shown in Figure 2. The temperature was varied from 30 to 60 °C. The Arrhenius plot was not linear over the entire temperature range (the 60 °C values were high); however, using three points, the activation energies for NOPP and NOMP decompositions were 15.4 and 13.3 kcal/mol, respectively (Table VI).

As a result of the large differences in the stabilities of *N*-nitrosamines and *N*-nitrosamides, experiments were performed to determine the percent decomposition under heating conditions commonly encountered in the cooking of certain foods. Samples of NOMP in sealed ampules were heated for specified periods of times over a wide range of temperatures (80–155 °C) in a thermostated oil bath (Table VII). This range of temperatures was chosen as it represents temperatures reached during the frying of bacon. Results indicate that NOMP is very unstable at temperatures approaching those likely to be encountered toward the end of the frying process. In another thermal stability experiment, samples of NOMP in sealed ampules were placed along side and on top of strips of bacon in a

Table VIII. A Comparison of the Stabilities of N-Nitrosamines and N-Nitrosamides in Sealed Ampules When Heated under Different Cooking Conditions

cooking condition	% decomp
bacon frying (8 min)	
NOMP (top of bacon, 100 °C)[a]	74
NOMP (on pan, 130 °C)[a]	91
N-Pyr (on pan, 130 °C)	3
DMN (on pan, 130 °C)	14
pork roasting (180 °C, 2 h)[b]	
NOMP	97
N-Pyr	10
DMN	8

[a] Temperatures were measured with a mercury thermometer. [b] Temperature of oven.

frying pan, and the bacon was cooked as previously described. The NOMP samples were degraded to the extent of 74–91% compared to only 3% for N-Pyr and 14% for DMN (Table VIII). Similar trends were observed for samples heated for 2 h under pork roasting conditions. Approximately 97% of the initial NOMP was destroyed compared to 7–10% for N-Pyr and DMN. These results again reflect the differences in stabilities of the various N-nitroso compounds.

CONCLUSIONS

This and the previous two papers have shown that N-substituted amides can be formed in model systems from the interaction of free amines and triglycerides and therefore may be considered potential precursors of N-nitroso compounds in food systems. These compounds are readily N-nitrosated under the appropriate conditions and thus may make a contribution to the total N-nitroso compounds to which man might be exposed. This model system study has also confirmed that N-nitrosamides are unstable under neutral and alkaline conditions and are therefore unlikely to occur in foods. N-Nitrosamides are readily decomposed at temperatures above 100 °C and unlike the volatile N-nitrosamines, DMN and N-Pyr, would not withstand the temperatures normally encountered in the pan frying of bacon; for example, at 150 °C, NOMP has a half-life of only 0.7 min (Table VII).

Although this study has shown that N-substituted amides can be formed readily in model systems, subsequent studies are necessary to establish their formation in meat systems. Furthermore, the stability of the N-nitrosamides should be investigated further by incorporating them into the meat systems before cooking. These studies should provide additional information on precursors of N-nitroso compounds in foods. It was noted by Walker (1977) that while our knowledge of our intake of preformed N-nitrosamines is increasing, very little is known about the N-nitrosatable amines or other amino compounds in food systems. It was also pointed out that in vivo N-nitrosation might be the major source of N-nitroso compounds and that it was very important to place more attention on the amine (or amide) content of our foods. Similar views were expressed by Lijinsky (1977) who stated that, because of the enormous variety of secondary and tertiary amino compounds that are ingested as components of food, food additives, agricultural chemicals as residues in crops, and drugs, the formation of N-nitroso compounds in the stomach could represent the most important human exposure to this group of carcinogens.

NOTE

Because of the hazardous nature of N-nitroso compounds, extreme caution should be exercised in handling these compounds.

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