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Collision induced dissociation of protonated N-nitrosodimethylamine by ion trap mass spectrometry: Ultimate carcinogens in gas phase

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ABSTRACT

Collision induced dissociation of protonated N-nitrosodimethylamine (NDMA) and isotopically labeled N-nitrosodimethyl-d6-amine (NDMA-d6) was investigated by sequential ion trap mass spectrometry to establish mechanisms of gas phase reactions leading to intriguing products of this potent carcinogen. The fragmentation of (NDMA + H^+) occurs via two dissociation pathways. In the alkylation pathway, homolytic cleavage of the N–O bond of N-dimethyl, N′-hydroxydiazenium ion generates N-dimethyldiazenium distonic ion which reacts further by a CH_3 radical loss to form methanediazonium ion. Both methanediazonium ion and its precursor are involved in ion/molecule reactions. Methanediazonium ion showed to be capable of methylating water and methanol molecules in the gas phase of the ion trap and N-dimethyldiazenium distonic ion showed to abstract a hydrogen atom from a solvent molecule. In the denitrosation pathway, a tautomerization of N-dimethyl, N′-hydroxydiazenium ion to N-nitrosodimethylammonium intermediate ion results in radical cleavage of the N–N bond of the intermediate ion to form N-dimethylaminium radical cation which reacts further through α-cleavage to generate N-methylmethylenimmonium ion. Although the reactions of NDMA in the gas phase are different to those for enzymatic conversion of NDMA in biological systems, each activation method generates the same products. We will show that collision induced dissociation of N-nitrosodiethylamine (NDEA) and N-nitrosodipropylamine (NDPA) is also a feasible approach to gain information on formation, stability, and reactivity of alkylating agents originating from NDEA and NDPA. Investigating such biologically relevant, but highly reactive intermediates in the condensed phase is hampered by the short life-times of these transient species.

1. Introduction

N-Nitrosodimethylamine (NDMA) is the smallest compound and one of the most potent carcinogens among dialiphatic nitrosamines. Since the first report on hepatocarcinogicity of NDMA [1], much research has been conducted into the occurrence [2,3], chemical properties [4], and metabolism of this compound [5–7]. Human exposure to NDMA is of concern since this compound has previously been detected in food products, cosmetics, tobacco products, air, and drinking water [2,3]. NDMA can often be formed in reactions of amines with nitrate in foods such as beer, malt barley, cured meet, and fish products [2]. There are several sources of NDMA in drinking water including direct contamination from industrial waste e.g., the liquid rocket fuel industry [3]. NDMA is also a by-product of water treatment involving chloramine and chlorine disinfection [3].

One important area of research deals with the metabolism of NDMA and investigation of its mode of carcinogenic action. The metabolism of NDMA is viewed as a competition between at least two enzymatic pathways. The α-hydroxylation by cytochrome P450-related enzymes is the major metabolic pathway [8] to produce formaldehyde and extremely reactive methanediazonium ion ($CH_3N_2^+$). Methanediazonium ion, a strong methylating agent, is presumed to be the ultimate carcinogen which reacts with the bases of DNA to form products like N^7-methylguanine [9] and promutagenic O^6-methylguanine [10,11]. Metabolic conversion of NDMA via an alternative radical denitrosation pathway [12,13] produces nitric oxide and the imine ($CH_3N{=}CH_2$) which hydrolyzes rapidly to give formaldehyde and methylamine. Denitrosation is considered to be a detoxification metabolic pathway of NDMA [12]. Formation of highly reactive intermediates in the metabolic pathways of NDMA has been proposed based on experimental evidence related to secondary metabolites, i.e., formation of formaldehyde and methylamine in metabolizing tissues [14], evolution of nitrogen gas [15], and the nature of the DNA adduct [9–11], because these stable products can be detected and quantified. Transient species such as the methanediazonium ion are extremely

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difficult to detect experimentally. Kinetic studies have shown that the methanediazoniun ion may have a half-life of ~0.3 s at 25 °C [16].

NDMA can also be activated at non-oxidative conditions via simple protonation of this compound. Olah et al. carried out protolytic dissociation studies of NDMA in acidic media [4]. The authors monitored the formation of products by nuclear magnetic resonance (NMR) after heating NDMA in HSO_3F:SbF_5 solution for 4–8 days at 140 °C. They observed the same products as those generated in the enzymatic denitrosation pathway, but they did not detect methanediazonium ion. Direct observation of methanediazonium ion by NMR [17] was reported in $HFSO_3$:SO_2ClF solution at −120 °C following protonation of diazomethane by $HFSO_3$. Heating the solution to −85 °C resulted in total decomposition of methanediazonium ion and formation of methylfluorosulphate (CH_3OSO_2F) which is the product of methylation of fluorosulphate by methanediazonium ion. Although the existence of methanediazonium ion in solution at low temperatures is well established, its short life-time creates uncertainties surrounding its formation through the electrophilic activation of NDMA at elevated temperatures in strong acids. Protonolysis of NDMA is not a significant pathway in the biological metabolism, but is believed [4] to generate the same products as the enzymatic metabolic pathways.

Formation and subsequent reactions of methanediazonium ion are amenable to direct studies in gas phase by mass spectrometry. Electron ionization of azomethane has been shown to be a convenient method for generating methanediazonium ion and monitoring ion/molecule reactions of methanediazonium ion with ammonia and methylamine in an ionization source by ion cyclotron resonance mass spectrometry [18]. Gas phase ion/molecule reactions of methanediazonium ion with a wide range of nucleophiles have been investigated in a pulsed electron high-pressure mass spectrometry by producing dimethylfluoronium ion ($CH_3FCH_3^+$) and using this precursor ion as the primary reagent in the reaction of methyl transfer to N_2 to produce methanediazonium ion [19].

Stability of methanediazonium ion in the gas phase suggests that gas phase protonolysis of NDMA should be an attractive method for investigating highly reactive intermediates in NDMA chemistry. NDMA has been analyzed using mass spectrometry with a number of separation, ionization, and mass detection techniques. Spectral information from several studies on tandem mass spectrometry detection of NDMA [20–23] indicates that the protonated NDMA is the precursor of methanediazonium ion detected at *m*/*z* 43. It is important to note that the reported tandem mass spectrometry methods are utilized for detecting NDMA at extremely low concentrations in environmental samples, and they do not focus on elucidating products and mechanisms of protolytic dissociation of NDMA. Reaction pathways for gas phase protolysis of NDMA are not well understood, mainly because they proceed via a number of reactive intermediates which have been identified in our study.

The aim of our work was to establish gas phase chemistry of protonated NDMA and its dissociation products using positive mode electrospray ionization (ESI) coupled with sequential tandem mass spectrometry, on a quadrupole ion trap instrument. An isotopically labeled, deuterated analogue (NDMA-d6) was used to confirm the validity of proposed reaction mechanisms. We will show that a quadrupole ion trap can be used for investigating, at the same time, both classical gas phase ion dissociation reactions and ion/molecule reactions involving reactive ions and solvent molecules. Studying such ion trap ion/molecule reactions can also provide an important link between gas phase and solution chemistry for transient species which cannot not be easily detected in solution.

2. Experimental

Ammonium acetate, HPLC grade water, and HPLC grade methanol were all obtained from Fisher Scientific (Nepean, ON). N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), and N-nitroso-n-dipropylamine (NDPA) were purchased from Supelco (Oakville, ON). N-nitrosodimethyl-d6-amine (NDMA-d6) was purchased from Cambridge Isotope Laboratories (Andover, MA). The nitrogen gas (electrospray desolvation gas) and the helium gas (ion trap buffer gas) were obtained from Linde (Guelph, ON). Sample solutions for analysis, containing N-nitrosamines at 2 ppm concentration, were prepared in 0.1 mM ammonium acetate in 90/10 (v/v) HPLC grade methanol/HPLC grade water. Each sample was introduced into the Finnigan LCQ Deca quadrupole ion trap instrument using a syringe pump at flow rates of 3–5 μL/min. The electrospray source was operated in the positive mode and the spray voltage was set to 5.50 kV. The temperature of the capillary was set to 200 °C. In order to transmit ions in the source and minimize ion fragmentation during transmission, the capillary voltage and tube lens offset voltage were set to 0 V. The instrument optics were also optimized for most efficient transmission of ions of interest to the ion trap as follows—multipole 1 offset = −8.0 V, lens voltage = −24.0 V, multipole 2 offset = 11.0 V, multipole RF amplitude = 200.0 Vp–p, entrance lens = −50.0 V.

MS^2, MS^3, and MS^4 experiments were carried out with an isolation window of 1 Th, and an activation time of 30 ms. The q_z parameter and normalized collisional energy (NCE) will be indicated for each spectrum specifically. The ion trap was operated in the low mass setting to allow for detection of low mass ions.

3. Results and discussion

3.1. Sequential tandem mass spectrometry of (NDMA + H^+) and (NDMA-d6 + H^+)

Fig. 1 shows all the spectral data obtained from tandem mass spectrometry experiments on a quadrupole ion trap for protonated N-nitrosodimethylamine (NDMA + H^+) at *m*/*z* 75 and its deuterated analogue (NDMA-d6 + H^+) at *m*/*z* 81. Tandem mass spectra (MS^2) for (NDMA + H^+) and (NDMA-d6 + H^+) are shown in Fig. 1A and B, respectively. Dissociation products of (NDMA + H^+) were detected at *m*/*z* 58, 47, 45, 44, 43, and 33. Dissociation products of (NDMA-d6 + H^+) were observed at *m*/*z* 64, 51, 50, 49, 46, and 36. The first-generation fragmentation products listed above were investigated further by sequential tandem mass spectrometry, and the results are shown in Fig. 1 C–F. The collision induced dissociation (CID) of the first-generation fragment ion at *m*/*z* 58 produced the second-generation products at *m*/*z* 33, 43, 47, and 59 (Fig. 1C). The CID of the isotopically labeled analogue fragment at *m*/*z* 64 produced second-generation products at *m*/*z* 36, 46, 50, and 65 (Fig. 1D). The *m*/*z* 43 ion (Fig. 1E) is the precursor for *m*/*z* 33 and 47, while the *m*/*z* 46 (Fig. 1F) produces the *m*/*z* 36 and *m*/*z* 50 ions. *m*/*z* 59 and 43 for the unlabeled NDMA and *m*/*z* 64 and 46 for the deuterated NDMA-d6 were the only first-generation daughter ions which were successfully examined by sequential tandem mass spectrometry. It was not possible to derive any spectral data from the other first-generation fragments. It was possible to isolate (select) the *m*/*z* 44 fragment ion and its deuterated analogue at *m*/*z* 49, in the ion trap. However, collisional activation of *m*/*z* 44 and *m*/*z* 49 did not yield any detectable dissociation products. The remaining first-generation fragment ions at *m*/*z* 33, 45, 47 (Fig. 1A) and 36, 51, 50 (Fig. 1B) did not survive the isolation (selection) step inside the ion trap. The *m*/*z* 43 ion (Fig. 1C) and its labeled analogue at *m*/*z* 46 (Fig. 1D) were two second-generation daughter ions which were successfully examined in sequential (MS^4) tandem mass spectrometry. The MS^4 mass spectrum of the *m*/*z* 43 ion (not shown) was the

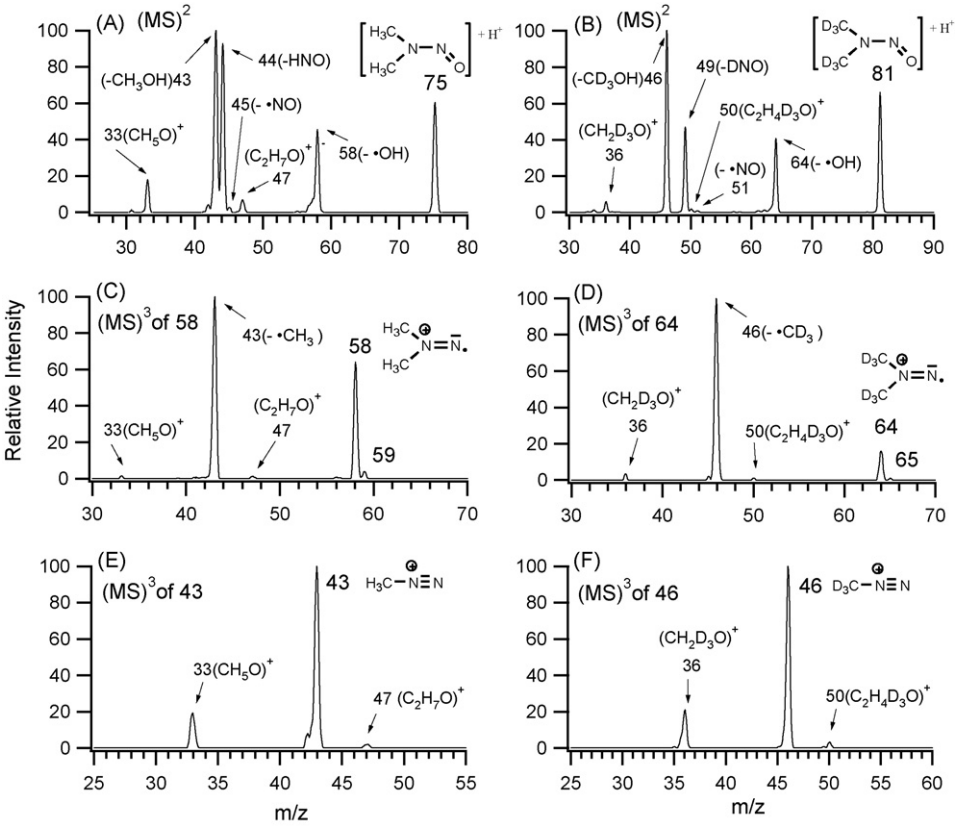


Fig. 1. Sequential tandem mass spectra for protonated N-nitrosodimethylamine (A, C, and E) and N-nitrosodimethyl-d6-amine (B, D, and F). (A) MS^2 spectrum of m/z 75 (NDMA + H^+) at NCE = 29.0 V, q_z = 0.35; (B) MS^2 spectrum of m/z 81 (NDMA-d6 + H^+) at NCE = 29.5 V, q_z = 0.35; (C) MS^3 spectrum of m/z 58 fragment ion at NCE = 29.0 V, q_z = 0.5; (D) MS^3 spectrum of m/z 64 fragment ion at NCE = 25.0 V, q_z = 0.50; (E) MS^3 spectrum of m/z 43 at NCE = 30.0 V, q_z = 0.50; (F) MS^3 spectrum of m/z 46 at NCE = 28.0 V, q_z = 0.50.

same as the MS^3 mass spectrum of the m/z 43 ion (Fig. 1E), revealing the formation of two intriguing products at m/z 33 and m/z 47. The MS^4 mass spectrum of the m/z 46 ion (not shown) was identical to the MS^3 mass spectrum of the m/z 46 ion (Fig. 1F) with respect to the formation of product ions at m/z 36 and m/z 50.

3.2. Reaction patterns of (NDMA + H^+) and (NDMA-d6 + H^+)

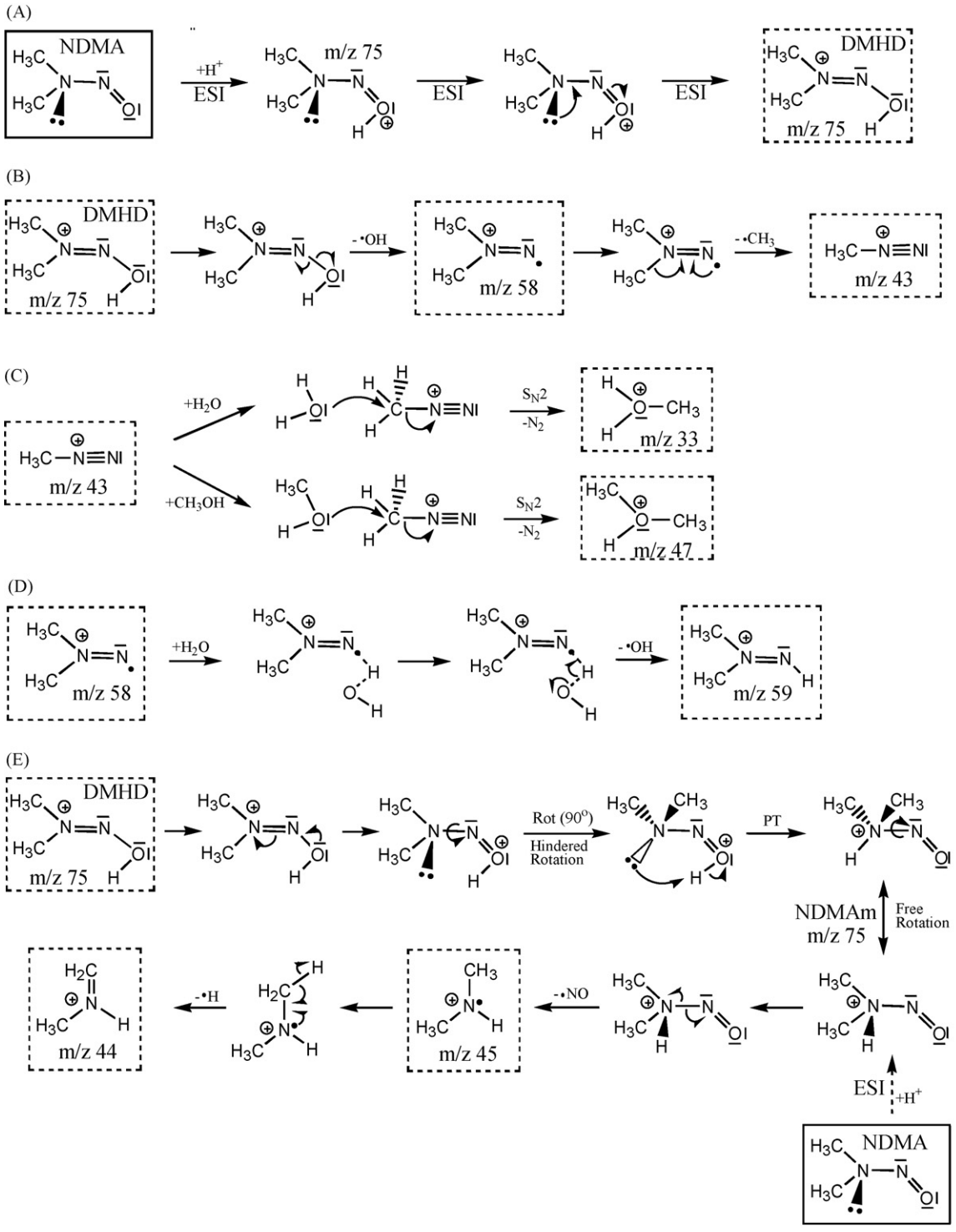
When the spectral data for NDMA and NDMA-d6 is carefully examined, it becomes possible to establish the elemental composition of the dissociation products which are observed in Fig. 1. The elemental composition of the radical and neutral losses/gains which occur during the formation of the dissociation products can also be determined. Based on this information, reaction pathways can be proposed for the dissociation of (NDMA + H^+) and (NDMA-d6 + H^+). Two major dissociation pathways were observed based on the spectral data in Fig. 1.

The first dissociation pattern involves OH radical loss to generate the m/z 58 fragment for (NDMA + H^+). The m/z 58 radical ion dissociates further by the CH_3 radical loss to generate an m/z 43 fragment ion with the elemental composition $[CH_3N_2]^+$. The analogous process for (NDMA-d6 + H^+) generates m/z 64 as a result of OH radical loss, and m/z 46 $[CD_3N_2]^+$ through subsequent CD_3 radical loss. From spectral data, the m/z 43 ion (Fig. 1E) is the precursor for m/z 33 and 47, while the m/z 46 ion (Fig. 1F) has shown to produce m/z 36 and 50 ions. Due to the unusual mass loss (−10) and even more unusual mass gain (+4) for both m/z 43 and m/z 46 precursor ions, it appears that the products at m/z 33, 36, 47, and 50 are not generated by a classical dissociation process. Instead, precursor or product ions must be involved in ion/molecule reactions inside the ion trap. Since both m/z 43 and the deuterated m/z 46 precursor ions react similarly (−10 Da mass loss and +4 Da mass gain), only one reasonable elemental composition of each product ion would satisfy experimental observations. The m/z 33 ion should represent protonated methanol [$CH_3OH + H^+$], while the m/z 47 ion should correspond to protonated dimethyl ether [$CH_3OCH_3 + H^+$]. For the deuterated analogue products, m/z 36 should represent protonated methanol-d3 [$CD_3OH + H^+$] and m/z 50 should correspond to protonated methylmethyl-d3 ether [$CD_3OCH_3 + H^+$]. The composition of the m/z 33, 47, 36, and 50 ions suggests that water and methanol molecules may be involved in gas phase reactions of m/z 43 $[CH_3N_2]^+$ and m/z 46 $[CD_3N_2]^+$ ions inside the ion trap.

The second dissociation pattern of (NDMA + H^+) at m/z 75 (Fig. 1A) begins with loss of NO radical to form m/z 45 ion. m/z 45 then eliminates H radical to produce m/z 44. The same pathway for (NDMA-d6 + H^+) at m/z 81 (Fig. 1B) leads to m/z 51 (NO radical loss) followed by elimination of D radical to produce m/z 49.

3.3. Protonation site of NDMA

Before the gas phase chemistry of protonated NDMA can be discussed, the protonation of the neutral NDMA molecule in electrospray ionization (ESI) must be considered. NDMA (Scheme 1A) has a trigonal planar structure with bond angles of approximately 120° at the amino nitrogen atom [24]. Due to extensive delocalization of the amino nitrogen lone pair electrons into the π-electron system of the nitroso (N=O) group, the N–N bond length (134 pm) of NDMA is intermediate between those for a typical double N=N (125 pm) and single N–N (145 pm) bond. The N–O bond length (123 pm) of NDMA is also intermediate between those for a typical double N=O (114 pm) and single N–O (136 pm) bond. Consequently, free rotation about the N–N bond is hindered (a rotational



Scheme 1. Proposed mechanisms for the collision induced dissociation of protonated N-nitrosodimethylamine (NDMA + H^+). (A) O-protonation of NDMA during electrospray ionization; (B) alkylation dissociation pathway of (NDMA + H^+); (C) ion/molecule reactions of methanediazonium ion with a solvent molecule in the ion trap; (D) ion/molecule reaction of N-dimethyldiazenium distonic ion with a water molecule in the ion trap; (E) denitrosation dissociation pathway of (NDMA + H^+).

barrier of 96 kJ/mol [24]), but also NDMA is a very weak base. NDMA is ~10^{10} times less basic than the corresponding amine and it undergoes protonation only in strong acids or "superacids" [24]. In principle, there are three possible protonation sites of NDMA – the amino nitrogen atom, the nitroso nitrogen atom and the nitroso oxygen atom. However, the oxygen atom has been established as the preferred site of proton attachment [25]. The O-conjugated acid of NDMA is the only conjugate acid that was detected by NMR in strong acids or "superacids" [4]. It can be suggested that the chemical conditions inside a small highly charged electrospray droplet are similar to chemical conditions in solution chemistry when dealing with a strong acid or "superacid". During electrospray ionization, a proton should also be accommodated at the nitroso oxygen atom of NDMA.

The O-protonation of NDMA is expected to alter structure and electronic arrangement of NDMA. From X-ray data [24] it is known

that stable electrophilic attachment of Lewis acids such as $CuCl_2$ at the nitroso oxygen shortens the N–N bond of NDMA from 134 pm (partial double bond character) to 126 pm (essentially double bond character). It reflects greater delocalization of lone pair electrons of the amino nitrogen towards the electron-deficient nitroso oxygen at the attachment site. It can be expected that protonation of NDMA at the nitroso oxygen would have a similar effect on the chemical structure of the resulting O-conjugated acid. Consequently, a product of the O-protonation of NDMA should represent essentially a double bond character of the nitrogen–nitrogen bond and resemble the structure of an N-dimethyl, N′-hydroxydiazenium (DMHD) ion. Scheme 1A illustrates the O-protonation of NDMA and the formation of *m*/*z* 75 N-dimethyl, N′-hydroxydiazenium (DMHD) ion in the liquid phase, during electrospray ionization. Electrospray facilitates the ionization of NDMA, and then transfers the DMHD ion into the gas phase. The gaseous *m*/*z* 75 DMHD ion is the precursor for the gas phase protolysis reactions which we observed in our tandem mass spectrometry studies of NDMA.

3.4. Alkylation reaction pathway of (NDMA + H^+)

The *m*/*z* 75 DMHD ion is proposed to react according to two dissociation pathways. The first pathway was termed the "alkylation" pathway and is presented in Scheme 1B and D. The first step of the alkylation pathway (Scheme 1B) involves radical cleavage of the N–O bond of DMHD to eliminate OH radical, and generate the N-dimethyldiazenium radical ion at *m*/*z* 58. The *m*/*z* 58 is a distonic ion because the charge site and radical site are located on two different atoms. Distonic ions are more stable than corresponding classical radical ions which accommodate both the charge and the radical site on the same atom. The *m*/*z* 58 ion is stabilized by separation of the radical and charge site, and can be detected at a high spectral intensity. However, it does dissociate further (Scheme 1B) by radical site induced α-cleavage to eliminate a CH_3 radical and form methanediazonium ion at *m*/*z* 43. *M*/*z* 43 methanediazonium ion is the most abundant dissociation product of (NDMA + H^+). It is formed as a result of the consecutive loss of OH radical and CH_3 radical from the DMHD precursor ion. Further evidence for the sequential radical losses during the formation of methanediazonium ion is the MS^3 spectrum of *m*/*z* 58 (Fig. 1C), which shows that *m*/*z* 43 can be formed from the *m*/*z* 58 distonic ion.

It is important to note that the detection of methanediazonium ion in MS^2 of *m*/*z* 75 (Fig. 1A), MS^3 of *m*/*z* 58 (Fig. 1C) and activation of methanediazonium ion in MS^4 (Fig. 1E) is always associated with the presence of product ions at *m*/*z* 33 and *m*/*z* 47. The detection of *m*/*z* 33 and *m*/*z* 47 can be linked to ion/molecule reactions of the methanediazonium ion with water and methanol, respectively. Scheme 1C shows the proposed mechanism for the formation of *m*/*z* 33 and 47. It occurs via an S_N2 reaction where the oxygen atom of a solvent molecule carries out a backside nucleophilic attack of a solvent molecule on the methyl carbon atom of the methanediazonium ion leading to inversion of the methyl group. This results in the displacement of molecular nitrogen and the formation of methylated water (*m*/*z* 33) and methylated methanol (*m*/*z* 47). The same mechanism was postulated before for gas phase methyl transfer reactions of methylating agents with a variety of nucleophiles [19]. The methanediazonium ion plays a very important role in the mutagenicity and carcinogenicity of NDMA. In biological systems, methyl transfer from the methanediazonium ion intermediate to the O^6-site of guanine and perhaps O^4- or O^2-sites of thymine are believed to be critical mutagenic events [26]. In the gas phase environment of ion trap, one can think of water and methanol molecules as being analogous to the nucleophilic sites of DNA, in the way they react with the methanediazonium ion.

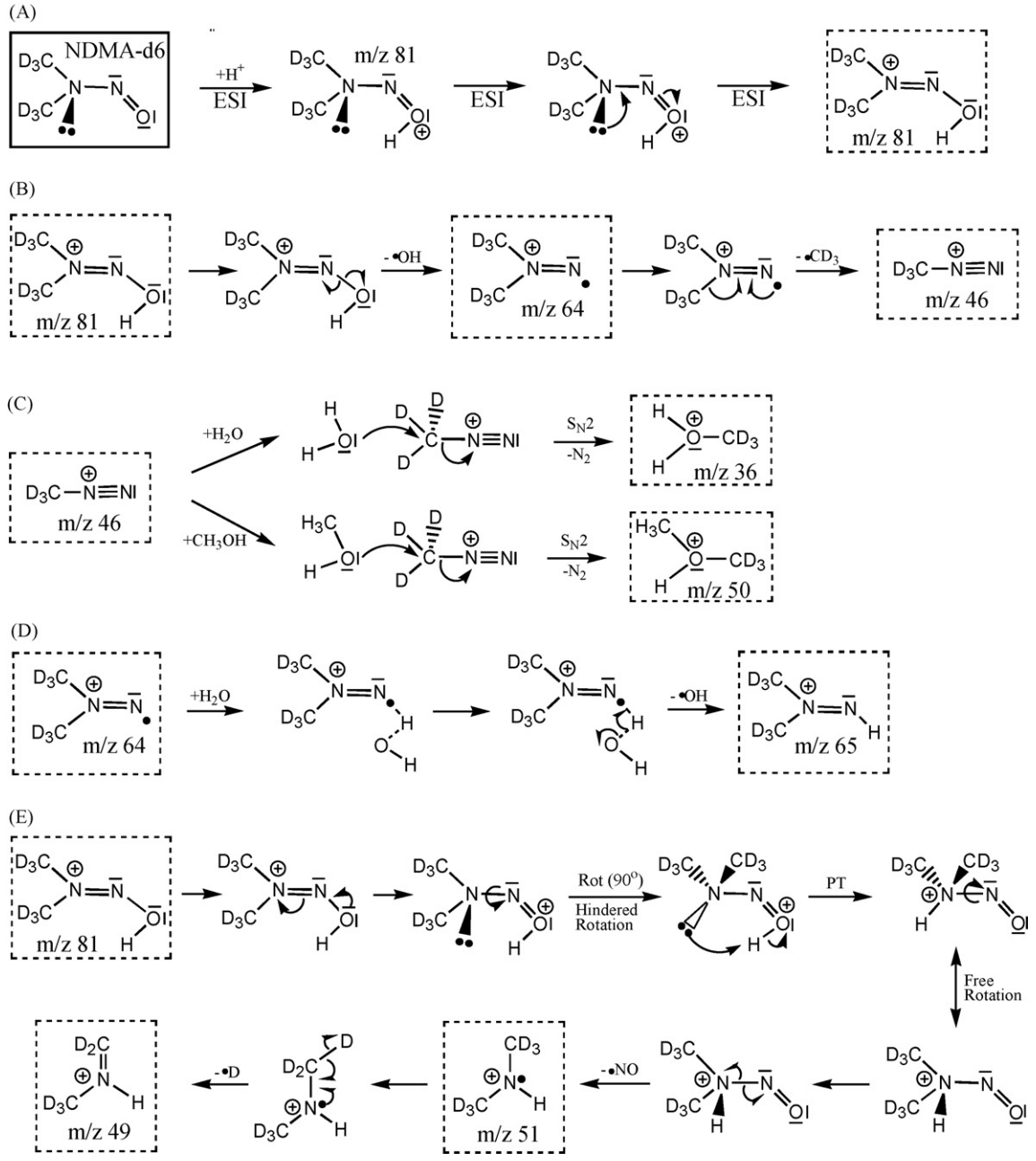
The methylation of water and methanol by methanediazonium ion is not the only example of ion/molecule reactions which was observed in the alkylation pathway of NDMA. When the *m*/*z* 58 N-dimethyldiazenium distonic ion was isolated and collisionally activated in MS^3 (Fig. 1C), one of its products was detected at *m*/*z* 59 (one *m*/*z* unit higher than the precursor ion). Such an unusual mass gain is postulated to represent a radical reaction which involves hydrogen abstraction by the *m*/*z* 58 ion. Scheme 1D shows the hydrogen atom transfer from a water molecule to the radical site on the *m*/*z* 58 N-dimethyldiazenium distonic ion to form the N-dimethyldiazenium cation at *m*/*z* 59 and a hydroxyl (OH) radical. The hydrogen abstraction step is illustrated for water, but could potentially occur with any protic species within the ion trap. The fact that this reaction occurs, helps to demonstrate how reactive the *m*/*z* 58 distonic ion is. The alkylation pathway of (NDMA + H^+) generates at least two reactive products—at *m*/*z* 43 and *m*/*z* 58.

3.5. Denitrosation reaction pathway of (NDMA + H^+)

Another dissociation pathway of (NDMA + H^+) involves initial loss of NO radical and thus is termed the denitrosation pathway. The denitrosation pathway generates the *m*/*z* 45 and 44 product ions which are seen in the spectrum in Fig. 1A. As with the alkylation pathway, the denitrosation pathway is proposed to originate from the same *m*/*z* 75 N-dimethyl, N′-hydroxydiazenium (DMHD) precursor ion which is the product of O-protonation of NDMA during electrospray ionization. The first step in the denitrosation pathway, illustrated in Scheme 1E, is tautomerization of the DMHD ion to a corresponding N-nitrosodimethylammonium (NDMAm) ion. The tautomerization is proposed to proceed through initial charge redistribution within the structure of DMHD. In the process, the localization of the lone pair electrons on the amino nitrogen atom increases, the bond order of the N=N bond decreases, and the bond order of the N–O bond increases. Essentially, the product ion resulting from the charge redistribution has the same structure as the O-conjugate acid of NDMA. It is postulated that the charge redistribution described is energetically unfavorable and occurs in the ion trap during collisional activation of the *m*/*z* 75 ion. As a result of the activation, the rotational barrier is overcome, and a 90° rotation about the N–N bond can generate the rotational conformation of the *m*/*z* 75 ion (O-protonated conjugate acid of NDMA) which allows for a proton transfer (PT) from the oxygen atom to the amino nitrogen atom. The final product of the tautomerization step is the *m*/*z* 75 N-nitrosodimethylammonium (NDMAm) ion, in which rotation about the N–N bond is unhindered. NDMAm is the intermediate precursor ion for the dissociation products of the denitrosation pathway (Scheme 1E). The *m*/*z* 75 NDMAm ion is postulated to undergo homolytic cleavage of the N–N bond. This results in the loss of NO radical and generation of *m*/*z* 45. The *m*/*z* 45 N-dimethylaminium radical cation is a classical radical cation with both the radical and charge site located on the same nitrogen atom. Such an ion is not very stable and prone to further dissociation through α-radical cleavage of a C–H bond. As a result an H radical is eliminated, and *m*/*z* 44 N-methylmethylenimmonium ion is formed. The *m*/*z* 44 immonium ion is the major product of the denitrosation pathway of (NDMA + H^+) in the gas phase. The same ion was identified as the main product of NDMA in acidic media [4].

One important point to note about the denitrosation dissociation pathway is that the N-nitrosodimethylammonium precursor ion may be generated in two ways. The first way involves the described tautomerization of the N-dimethyl, N′-hydroxydiazenium ion. The second way has not yet been discussed, but it may involve direct protonation of NDMA at the amino nitrogen during electrospray ionization. Amino nitrogen protonation of NDMA is thermodynamically less favorable than O-protonation. The O-conjugate acid is stabilized through resonance. Protonation on the amino nitrogen does not lead to resonance stabilization of the conjugate acid, and is thus expected to be of higher energy

[25]. However, previous studies carried out on NDMA [4] in acidic media assumed the presence of N-nitrosodimethylammonium ion in solution in small undetectable amounts (less than 5% of the O-conjugate acid). This assumption was made in order to explain formation of denitrosation products which must originate from N-nitrosodimethylammonium ion. However, our spectral data does not agree with this assumption, and indicates that the direct protonation of NDMA at the amino nitrogen is not a significant source of N-nitrosodimethylammonium ion. This can be concluded based on spectral intensities of dissociation products of NDMA (Fig. 1A) from the two established fragmentation pathways. The *m*/*z* 43 (methanediazonium ion) is the major product of the alkylation pathway associated with the fragmentation of the O-protonated precursor ion. The *m*/*z* 44 (N-methylmethylenimmonium ion) is the major product of the denitrosation pathway associated with the fragmentation of the precursor ion protonated at the amino nitrogen. The spectral intensities for *m*/*z* 43 and *m*/*z* 44 in the MS^2 spectrum of (NDMA + H^+) in Fig. 1A are not consistent with a ratio of 95% O-protonated/5% N-protonated NDMA. If that were the case, the intensity of the *m*/*z* 44 peak (product of N-protonation) would be much smaller. Based on this observation it is postulated that the N-nitrosodimethylammonium ion originates mainly from the tautomerization of the N-dimethyl, N′-hydroxydiazenium ion. Such tautomerization is proposed to be a reversible process which occurs in the gas phase, and determines the relative abundances of the tautomers. At low ion energy, the DMHD (O-protonated) tautomer is more abundant because its formation is favored thermodynamically. However, ion activation could shift the distribution toward the N-protonated (NDMAm) tautomer. In the gas phase, ion activation is achieved through collisions of the ion with helium gas.



Scheme 2. Proposed mechanisms for the collision induced dissociation of protonated N-nitrosodimethyl-d6-amine (NDMA-d6 + H^+). (A) O-protonation of NDMA-d6 during electrospray ionization; (B) Alkylation dissociation pathway of (NDMA-d6 + H^+); (C) ion/molecule reactions of methane-d3-diazonium ion with a solvent molecule in the ion trap; (D) Ion/molecule reaction of N-dimethyl-d6-diazenium distonic ion with a water molecule in the ion trap; (E) denitrosation dissociation pathway of (NDMA-d6 + H^+).

The tautomerization of N-dimethyl, N′-hydroxydiazenium ion to N-nitrosodimethylammonium ion may also occur in solution where ion activation could be achieved by heating the solution. The denitrosation of NDMA in acidic media was observed only at elevated temperatures [4], which may be related to the formation of the N-nitrosodimethylammonium ion through the described tautomerization process in liquid phase.

All of the gas phase reactions, which we proposed for (NDMA + H^+) and its dissociation products, were based on the tandem mass spectrometry information obtained for both (NDMA + H^+) and its deuterated analogue (NDMA-d6 + H^+). All of the pathways described for (NDMA + H^+) are also valid for (NDMA-d6 + H^+). The reaction pathways for the deuterated NDMA analogue are presented in Scheme 2. It is important to note that formation of radical ions and occurrence of ion/molecule reactions are not commonly observed phenomena for protonated ions which are generated in electrospray. As such, studying the deuterated analogue in addition to NDMA was vital in clarifying the identity of the unique products of (NDMA + H^+). The fact that the reactions occur similarly for (NDMA + H^+) and (NDMA-d6 + H^+) helps to verify the proposed dissociation pathways.

3.6. Alkylating products from the dissociation of (NDEA + H^+) and (NDPA + H^+)

The intriguing aspect of our NDMA studies is the detection of products of ion/molecule reactions on a conventional quadrupole ion trap. In numerous applications of ion/molecule reactions in mass spectrometry [27,28], a volatile reactant is usually admitted to an ion trap for the reactions to occur. In our experiments, water and methanol are introduced to the trap only as ultra-trace contaminants of the helium buffer gas [29,30]. Since water and methanol are present in very small amounts, ion/molecule reactions can be observed only for very reactive ions [29,30]. Consequently, not only classical ion gas phase dissociation reactions, but also ion/molecule reactions of highly reactive dissociation products with solvent molecules can be observed. Ion/molecule reactions can provide a link between gas phase and solution chemistry for transient species which cannot easily be investigated in solution. Tandem mass spectral data for larger aliphatic nitrosamines are presented in Fig. 2 to illustrate this point.

The ion trap tandem mass spectrum for protonated N-nitrosodiethylamine (NEMA) in Fig. 2A shows the detection of ethanediazonium ion at m/z 57. The products of gas phase ion/molecule reactions of the m/z 57 ethanediazonium ion with water and methanol are also detected at m/z 47 and m/z 61, respectively. Ion/molecule reactions of ethanediazonium ion proceed in a similar fashion to those presented for the m/z 43 methanediazonium analogue (Scheme 1C), and produce ethylated solvent ions at m/z 47 and m/z 61 (Fig. 2A). The tandem mass spectrum for protonated N-nitrosodipropylamine (NPMA) in Fig. 2B shows the detection of m/z 43 propenylium ion (propyl carbocation), but products of ion/molecule reactions for this m/z 43 propylating agent with water (m/z 61) or methanol (m/z 75) are not observed. In contrast to the formation of diazonium ions from NDMA and MDEA, propanediazonium ion ($C_3H_7N_2^+$) at m/z 71, was not detected in the spectrum of (NDPA + H^+). The dissociation product of (NEMA + H^+) at m/z 75 and (NDPA + H^+) at m/z 89 are also related to key intermediates in the chemistry of aliphatic nitrosamines. N-ethyl, N-hydro, N′-hydroxydiazenium ion at m/z 75 (Fig. 2A) and N-propyl, N-hydro, N′-hydroxydiazenium ion at m/z 89 (Fig. 2B) are N-protonated analogues of alkanediazoic acids (alkyldiazohydroxides). Alkanediazoic acids are believed to be alkylating agents and precursors for alkanediazonium and/or alkenylium ions in metabolic pathways of aliphatic nitrosamines. Alkanediazoic acids are extremely reactive species which decompose instantly in aqueous media. It is important to note that the detected products of NEMA and NDPA (Fig. 2) have been postulated in theoretical studies [31–34] as highly reactive metabolites in enzymatic pathways. Investigating their chemistry in condensed phase is a challenging task [35]. m/z 75 as well as m/z 89 and m/z 43 have been detected in tandem mass spectrometry [23]. To our best knowledge, the detection and S_N2 ion/molecule reactions of ethanediazonium ion have not been reported before. However, a reasonable theoretical basis for S_N2 reaction pathways of ethanediazonium ion has been postulated before [26]. The S_N2 mechanism is enforced if ethyl carbonium ion derived from the loss of the leaving group (N_2) is too unstable to exist as an independent entity

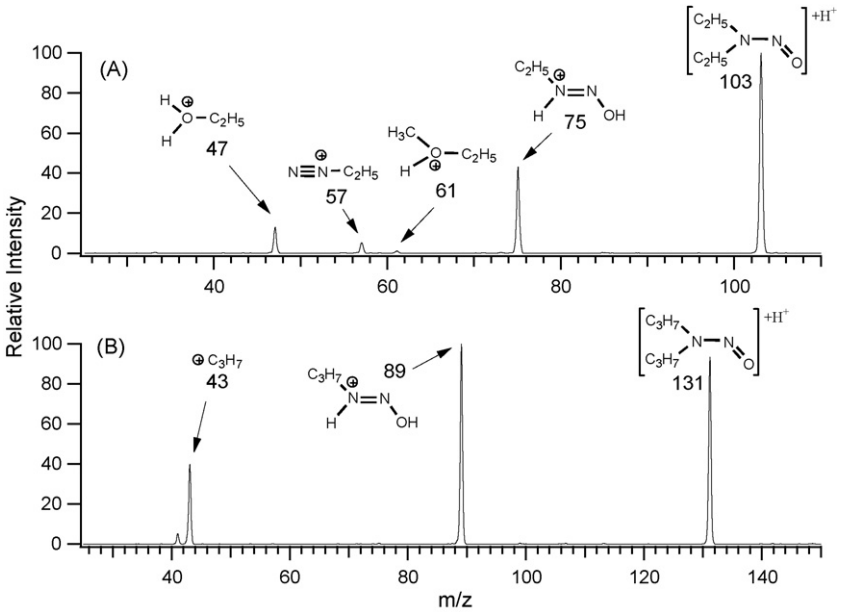


Fig. 2. Tandem mass spectra for protonated N-nitrosodiethylamine (NDEA) and N-nitrosodipropylamine (NDPA). (A) MS^2 spectrum of m/z 103 (NDEA + H^+) at NCE = 20.0 V, q_z = 0.25; (B) MS^2 spectrum of m/z 131 (NDPA + H^+) at NCE = 19.0 V, q_z = 0.20.

in an encounter complex of ethanediazonium ion with a nucleophile (water, methanol). A projected life-time of ethyl carbocation is close to its vibration frequency, which implies that ethyl carbocation is on borderline of stability. Thus, ethanediazonium ion will probably react with water and methanol via S_N2 reaction pathways.

A part of the controversy in the chemistry of aliphatic nitrosamines is the nature and potency of the ultimate carcinogen in the critical alkylation step. Our observations indicate that methanediazonium and ethanediazonium ions are the most potent alkylating agents, as only these two species were capable of alkylating solvent molecules. Our results also show that alkanediazonium ions are associated with decomposition of NDMA and NDEA, but propenylium ion was detected for the NDPA precursor. In order to provide a rational explanation for the formation of different alkylating agents from different nitrosamines, mechanisms of gas phase reactions of nitrosamines have to be established. Larger aliphatic N-nitrosamines dissociate according to different pathways than those presented for NDMA. We are currently working on investigating gas phase chemistry of different classes of nitrosamines to elucidate reactions leading to the formation of alkylating agents and other reactive species which may be associated with DNA damage.

4. Conclusions

A detailed sequential tandem mass spectrometry study of $(NDMA + H^+)$ and $(NDMA\text{-}d6 + H^+)$ has established the mechanisms of gas phase reactions for protolytic cleavage of NDMA. Following its nitroso oxygen protonation, NDMA decomposes via reactive intermediates in two dissociation pathways. In the alkylation pathway, the homolytic cleavage of the N–O bond gave rise to N-dimethyldiazenium distonic ion which showed to abstract a hydrogen atom from a solvent molecule. Further CH_3 radical loss from the N-dimethyldiazenium distonic ion has produced methanediazonium ion which showed to be capable of methylating water and methanol molecules in the gas phase of the ion trap. In the denitrosation pathway, the tautomerization of N-dimethyl, N′-hydroxydiazenium ion to N-nitrosodimethylammonium intermediate ion resulted in the homolytic cleavage of the N–N bond to form relatively unstable N-dimethylaminium radical cation which reacted further through α-cleavage to generate N-methylmethylenimmonium ion. The fragmentation of $(NDMA + H^+)$ has shown a number of interesting features such as unique structures of small ions generated from radical cleavages of chemical bonds and ion/molecule reactivity of products from the alkylation pathway. We believe that similar reactions of $(NDMA + H^+)$ occur in the condensed phase.

We have also established that the protolytic cleavage of NDMA, NDEA, and NDPA in the gas phase generates the same products as those implicated in metabolic decomposition pathways of the corresponding compounds. Investigating such reactive products in the gas phase provides not only the detection of biologically relevant transient species but also insights into their reactivity through gas phase ion/molecule reactions. We believe that tandem mass spectrometry can contribute to a better understanding of chemistry of N-nitrosamines with respect to key intermediates such as alkylating agents, reactive radicals, and perhaps other biologically relevant species which have not been considered before.

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References

[1] P.N. Magee, J.M. Barnes, The production of malignant primary hepatic tumours in the rat by feeding dimethylnitrosamine, Br. J. Cancer 10 (1956) 114–122.
[2] W. Lijinski, N-Nitroso compounds in the diet, Mutat. Res. 443 (1999) 129–138.
[3] W.A. Mitch, J.O. Sharp, R.R. Trussell, R.L. Valentine, L. Alvarez-Cohen, D.L. Sedlak, N-Nitrosodimethylamine (NDMA) as a drinking water contaminant: a review, Environ. Eng. Sci. 5 (2003) 389–404.
[4] G.A. Olah, D.J. Donovan, L.K. Keefer, Carcinogen chemistry. I. Reactions of protonated dialkylnitrosamines leading to alkylating and aminoalkylating agents of potential metabolic significance, J. Natl. Cancer Inst. 54 (2) (1972) 465–472.
[5] D.F. Heath, The decomposition and toxicity of dialkylnitrosamines in rats, Biochem. J. 85 (1962) 72–91.
[6] H.M. Godoy, M.I. Diaz Gomez, J.A. Castro, Relationship between dimethylnitrosamine metabolism or activation and its ability to induce liver necrosis in rats, J. Natl. Cancer Inst. 64 (1980) 533–538.
[7] M.I. Diaz Gomez, H.M. Godoy, J.A. Castro, Further studies on dimethylnitrosamine metabolism, activation, and its ability to cause liver injury, Arch. Toxicol. 47 (1981) 159–168.
[8] C.S. Yang, J.-S.H. Yoo, H. Ishizaki, J. Hong, Cytochrome P450IIE1: roles in nitrosamine metabolism and mechanisms of regulation, Drug Metab. Rev. 22 (1990) 147–159.
[9] P.N. Magee, E. Farber, Toxic liver injury and carcinogenesis. Methylation of rat-liver nucleic acids by dimethylnitrosamine in vivo, Biochem. J. 83 (1962) 114–124.
[10] M.C. Archer, G.E. Labuc, Nitrosamines, in: M.W. Anders (Ed.), Bioactivation of Foreign Compounds, Academic Press, New York, 1985, pp. 403–431.
[11] A. Loveless, Possible relevance of O-6 alkylation of deoxyguanosine to the mutagenicity and carcinogenicity of nitrosamines and nitrosamides, Nature 223 (1969) 206–207.
[12] A.J. Streeter, R.W. Nims, P.R. Sheffels, Y.-H. Heur, C.S. Yang, B.A. Mico, C.T. Gombar, L.K. Keefer, Metabolic denitrosation of *N*-nitrosodimethylamine *in vivo* in the rat, Cancer Res. 50 (1990) 1144–1150.
[13] E.S. Burak, G.W. Harrington, R. Koseniauskas, C.T. Gombar, Estimation of the fraction of the dose of *N*-nitrosodimethylamine metabolized to methylamine in rats, Cancer Lett. 58 (1991) 1–6.
[14] N. Venkatesan, J.C. Arcos, M.F. Argus, Differential effect of polycyclic hydrocarbons on the demethylation of the carcinogen dimethylnitrosamine by rat tissues, Life Sci. 7 (18) (1968) 1111–1119.
[15] D.F. Heath, A. Dutton, The detection of metabolic products from dimethylnitrosamine in rats and mice, Biochem. J. 70 (1958) 619–626.
[16] J.F. McGarrity, T. Smyth, Kinetics and mechanism of the acid-catalysed hydrolysis of diazomethane, J. Chem. Soc., Chem. Commun. (10) (1977) 347–348.
[17] D. Berner, J.F. McGarrity, Direct observation of the methyldiazonium ion in fluorosulfuric acid, J. Am. Chem. Soc. 101 (1979) 3135–3136.
[18] M.S. Foster, J.L. Beauchamp, Gas-phase ion chemistry of azomethane by ion cyclotron resonance spectroscopy, J. Am. Chem. Soc. 94 (1971) 2425–2431.
[19] T.B. McMahon, T. Heinis, G. Nicol, J.K. Howey, P. Kebarle, Methyl cation affinities, J. Am. Chem. Soc. 110 (1988) 7591–7598.
[20] J.B. Plomley, C.J. Koester, R.E. March, Determination of N-nitrosodimethylamine in complex environmental matrices by quadrupole ion storage tandem mass spectrometry enhanced by unidirectional ion ejection, Anal. Chem. 66 (1994) 4437–4443.
[21] Y. Zhao, J.M. Boyd, S.E. Hrudey, X.-F. Li, Characterization of new nitrosamines in drinking water using liquid chromatography tandem mass spectrometry, Environ. Sci. Technol. 40 (2006) 7636–7641.
[22] X. Liu, Y. Zhao, K. Chan, S.E. Hrudey, X.-F. Li, J. Li, Analysis of nitrosamines by capillary electrospray-high-field asymmetric waveform ion mobility spectrometry MS with programmed compensation voltage, Electrophoresis 28 (2007) 1327–1334.
[23] Y. Zhao, X. Liu, J.M. Boyd, F. Qin, J. Li, X.-F. Li, Identification of N-nitrosamines in treated drinking water using nanoelectrospray ionization high field asymmetric waveform ion mobility spectrometry with quadrupole time-of flight mass spectrometry, J. Chromatogr. Sci. 47 (2009) 92–96.
[24] B.C. Challis, J.A. Challis, N-Nitrosamines and N-nitrosimines III properties and reactions of N-nitrosamines, in: S. Patai (Ed.), The Chemistry of Amino, Nitroso, and Nitro Compounds and Derivatives. Part 2, John Wiley & Sons, 1982, pp. 1151–1223.
[25] L.K. Keefer, J.A. Hrabie, B.D. Hilton, D. Wilbur, Nitrogen protonation of N-nitrosodimethylamine, J. Am. Chem. Soc. 110 (1988) 7459–7462.
[26] E.D. Loechler, A violation of the Swain–Scott principle, and not S_N1 versus S_N1 reaction mechanism, explains why carcinogenic alkylating agents can form different proportions of adducts at oxygen versus nitrogen in DNA, Chem. Res. Toxicol. 3 (1994) 276–280.
[27] J.S. Brodbelt, Analytical applications of ion-molecule reactions, Mass Spectrom. Rev. 16 (1997) 91–110.
[28] M.N. Eberlin, Structurally diagnostic ion/molecule reactions: class and functional-group identification by mass spectrometry, J. Mass Spectrom. 41 (2006) 141–156.
[29] M. Baker, W. Gabryelski, Collision induced dissociation of deprotonated glycolic acid, Int. J. Mass Spectrom. 262 (2007) 128–135.
[30] J. Sultan, Collision induced dissociation of deprotonated guanine: Fragmentation of pyrimidine ring and water adduct formation, Int. J. Mass Spectrom. 273 (2008) 58–68.

[31] C. Thomson, C.A. Reynolds, A theoretical study of N-nitrosamine metabolites: possible alkylating species in carcinogenesis by N,N-dimethylnitrosamine, Int. J. Quantum Chem. 30 (1986) 751–762.
[32] C.A. Reynolds, C. Thomson, Hydrated carbonium ions as possible metabolites: an ab initio study, Int. J. Quantum Chem. 32 (1987) 123–131.
[33] G.P. Ford, Unimolecular dissociation of primary alkanediazonium ions. Ab initio and semiempirical molecular orbital calculations, J. Am. Chem. Soc. 108 (1986) 5104–5108.
[34] L.L. Lu, Y.D. Liu, R.G. Zhong, Theoretical investigation of mono- and bi-functional alkylating agents transformed from nitrosodimethylamine derivatives, J. Mol. Struct.: Theochem. 893 (2009) 106–110.
[35] J. Ho, J.C. Fishbein, Rate-limiting formation of diazonium ions in the aqueous decomposition of primary alkenediazoates, J. Am. Chem. Soc. 116 (1994) 6611–6621.