Electron-Induced Fragmentation of Methylated Formamides

Zhou Li^{1,2}, M. Michele Dawley¹, Ian Carmichael¹, and Sylwia Ptasińska^{1,2*}

¹Radiation Laboratory, University of Notre Dame, Notre Dame, IN 46556, USA

²Department of Physics, University of Notre Dame, Notre Dame, IN 46556, USA

Abstract

All cationic fragments from 70 eV electron ionization of *N,N*-dimethylformamide (DMF) and *N*-methylformamide (NMF) are detected with a quadrupole mass spectrometer and identified using isotopically-labelled molecules. Ion efficiency curves near the threshold region (5 – 20 eV) for all of the major cationic fragments are measured and fit with a Wannier-type function to determine their appearance energies (AEs). Possible reaction pathways for the formation of each observed cation are proposed, and the corresponding enthalpies (ΔH^0 s) of reaction are evaluated from quantum chemical calculations using the Gaussian09 computational quantum chemistry package. Our results suggest that hydrogen loss for both molecules occurs primarily from the carbonyl carbon. We confirm that hydrogen transfer from the carbonyl site to the nitrogen site occurs during amide bond cleavage. The resultant cations, $\text{C}_2\text{H}_6\text{N}^+$ and CH_4N^+ , and their further fragments, formed by losing an H atom, H_2 , or C_2H_2 , are first identified in mass spectra and further probed by comparing the AEs with calculated ΔH^0 s of all possible formation pathways. Additionally, we suggest that hydrogen transfer also occurs when the N-CH₃ bond breaks in DMF. This detailed study of the simplest molecule with the peptide linkage contributes to our understanding of electron impact on peptide-like molecules.

*Corresponding author: Sylwia.Ptasinska.1@nd.edu.

I. Introduction

FIG. 1. (a) *N,N*-dimethylformamide; (b) *N*-methylformamide (*trans* conformer).

N,N-dimethylformamide (DMF) and *N*-methylformamide (NMF), which are shown in Figure 1, are two derivatives of formamide, which is the simplest molecule that has the N-C=O peptide linkage structure. Formamide has been discovered in the interstellar medium¹⁻⁴ and is considered an important precursor in prebiotic chemistry.⁵⁻¹¹ Besides these astrophysical and prebiotic interests, *N*-methylformamide is recognized as a potential chemosensitizer for combination chemotherapy.¹²⁻¹⁵ NMF can actively enhance the success of radiation treatment on a human colon tumor in nude mice by significantly inhibiting the growth of the tumor.¹⁵ More interestingly, although NMF is a well-known chemosensitizer, DMF can suppress radiation-induced transformations in vitro.¹⁶ Thus, the number of methyl groups on the nitrogen site has a significant impact on the compounds' behavior upon irradiation in the cellular environment. A detailed investigation of interactions between electrons, which are the most abundant secondary products of ionizing radiation, and methylated formamides may be critical to unveiling the essential factors that contribute to their differential antitumor abilities.

Radiotherapy is currently the most widely adopted approach to treat tumors that have become a major health concern in modern society. Because it is well known that radiation can kill cancer cells and simultaneously damage normal cells, improved knowledge of the interaction of high energy radiation with cellular molecules is essential to enhance the efficiency of treatments to better target only the cancer cells. Once high energy radiation interacts with living tissue, a series of complex reaction cascades on the picosecond time scale produces vast amounts of reactive species, such as excited neutrals, ions, free radicals, secondary electrons, etc. Among all of the species, secondary electrons with energy in the range of a few to tens of electronvolts are the most abundant. For example, approximately 5×10^4 electrons can be generated for each energy quantum of a million electron volts deposited.¹⁷⁻¹⁹ The direct radiation damage that is caused by the energy deposition in cellular DNA and in the water molecules near or associated with this DNA only contributes partially to cellular inactivation, mutation, and transformation. Approximately two thirds of the radiation-induced effects are indirect, which are from diffusible free radicals, in particular, the hydroxyl radical.²⁰⁻²² DMF has been shown to have a comparable effect to dimethyl sulfoxide (DMSO) in decreasing cell mortality under γ -irradiation.¹⁶ The radioprotective property of DMSO is generally attributed to its extremely strong ability to scavenge hydroxyl radicals. Because DMF is not as effective as DMSO in scavenging hydroxyl radicals, there must be other factors that account for the similar radioprotective effect of DMF. A detailed study of the interaction between DMF and secondary electrons and the consequent products can help to explain this phenomenon.

Several structural studies on bioactive compounds have suggested that *N*-alkyl moieties have a significant influence on the antitumor activities of drugs.²³⁻²⁶ In addition, the saturated amine group is known for its unusual ability to direct the fragmentation pattern of the molecule.^{27,28} Thus, for compounds containing the group, $-\text{NC}_n\text{H}_{2n+2}$, such as methylated formamide, methylated acetamide, methylated ureas, etc., ions with the formula of $\text{C}_n\text{H}_{2n+2}\text{N}^+$ are

usually the most dominant peaks in the mass spectra. Loudon and Webb²⁹ investigated the appearance energy (AE) of $\text{C}_2\text{H}_6\text{N}^+$ and CH_4N^+ that are formed by the electron impact fragmentation of DMF and NMF. Because these AEs were lower (1.30 eV for $\text{C}_2\text{H}_6\text{N}^+$, >1.9 eV for CH_4N^+) than the lowest energies for forming $\text{C}_2\text{H}_6\text{N}^+$ and CH_4N^+ , they confirmed that the hydrogen relocated during the (C=O)-N bond dissociation, and also that the ions, $\text{C}_2\text{H}_6\text{N}^+$ and CH_4N^+ , were stabilized by isomerizing to $\text{NHCH}_3\text{CH}_2^+$ and NH_2CH_2^+ , respectively.

Here we report results from an electron ionization mass spectrometric study of NMF and DMF in the electron energy range of 5-20 eV. The cationic species of all of the major peaks in the mass spectra were identified by comparing them with the mass spectra of the deuterated compounds. The ion efficiency curves of the cations are reported, and the appearance energies were determined by fitting to a Wannier-type formula.³⁰ Possible reaction pathways that lead to the formation of the cations are discussed by comparing the appearance energies with estimates of the reaction enthalpies that were calculated using Gaussian09 software³¹. These calculations employ perhaps the most accurate composite method, W1BD,³² available in the package. Vertical ionization energetics were captured from high-level CCSD(T)³³ calculations at geometries obtained from Density Functional Theory (DFT) using the B3LYP functional³⁴ and a moderately flexible basis set, denoted 6-311+G(d,p).³⁵

II. Experimental setup and data analysis

The experimental setup for the present study was described in detail previously.³⁶ Briefly, it consisted of a high vacuum chamber equipped with a Hiden 3F Series quadrupole mass spectrometer (QMS) which detected the charged fragments' m/z values and measured their ion efficiencies. The vacuum chamber had a base pressure of $\sim 10^{-9}$ mbar. A filament that was installed at the front of the mass spectrometer was used as the electron source. The resolution of the electron beam was 0.5 eV (full width at half maximum), and the emission current was set to be 4 μA . During the experiment, the vapor of the sample was dosed into the chamber via a gas line connected to a glass vial that contained the liquid sample. The vapor pressure is 5.16 mbar for DMF and 0.33 mbar for NMF at 25 °C. The liquid sample was degassed using the freeze-pump-thaw method prior to experiments. The gas line was linked to the vacuum chamber via a leak valve, which was used to precisely control the dose of the sample vapor. Inside the chamber, the gas inlet, which was a metal tubule, was aimed at the entrance to the QMS. *N,N*-dimethylformamide ($\text{HCON}(\text{CH}_3)_2$, $M = 73$ g/mol) and *N*-methylformamide (HCONHCH_3 , $M = 59$ g/mol) were both purchased from SigmaAldrich with stated purities of 99.8% and 99%, respectively. The deuterated samples, *N,N*-dimethylformamide- d_1 (d_1DMF), *N,N*-dimethyl- d_6 -formamide (d_6DMF), and *N*-methyl- d_3 -formamide (d_3NMF), shown in Figure 2, were purchased from C/D/N Isotopes Inc., and all had an isotopic enrichment of 99 atom % D. All of the samples were purchased as liquids at room temperature. All mass spectra reported in this work were measured at 70 eV. To make sure the mass spectra are solely from the samples, the mass spectra of the background at 70 eV were also measured and subtracted appropriately. Ion efficiencies as a

function of the electron energy were scanned from 5 to 20 eV with signal onsets located in the region of 9 to 15 eV. Argon gas (with the ionization energy of 15.759 eV)³⁷ was used for calibration. All of the ion efficiency curves (for samples and calibration gases) were measured at a pressure of $\sim 1 \times 10^{-6}$ mbar.

FIG. 2. (a) d_1 DMF; (b) d_6 DMF; (c) d_3 NMF.

Wannier extended Wigner's formula^{38,30} and described the cross section as $\sigma = C(E - AE)^n$, which is a function of the electron energy, E . In this formula, C is the slope, AE is the appearance energy and n is an exponent that is only theoretically determined for hydrogen. This n^{th} power rule was first introduced by Märk³⁹ to determine threshold energies, and it thereafter became a widely used method. To extend the equation to the energy region below the threshold, we multiplied the right hand side of the equation with a step function, $\theta(E - AE)$. Then, to take the limited resolution of the electron energy into account, we convoluted it with a Gaussian function, $\exp\{-E^2/(2\rho^2)\}/(2\pi)^{1/2}$, which is an approximation of the electron energy distribution with a resolution of ρ . After adding another constant, B , to remove the effect of the constant background, we obtained the equation to fit the ion efficiency curve, as provided below.

$$\sigma = B + [C(E - AE)^n \theta(E - AE)] \frac{1}{\sqrt{2\pi}} e^{-\frac{E^2}{2\rho^2}}$$

In some cases, the ion efficiency curve manifests two distinct thresholds. Thus, the fitting function was modified as the sum of two threshold fitting functions, as shown below.

$$\sigma = B + [C(E - AE_1)^n \theta(E - AE_1)] \frac{1}{\sqrt{2\pi}} e^{-\frac{E^2}{2\rho^2}} + [C(E - AE_2)^n \theta(E - AE_2)] \frac{1}{\sqrt{2\pi}} e^{-\frac{E^2}{2\rho^2}}$$

The fittings were performed using the OriginPro 8.6 software package with the χ^2 tolerance set to be 10^{-6} . The resolution, ρ , was fixed at 0.5 eV, and the appearance energy, AE , exponent, n , and slope, C , were all optimized by minimizing the mean square deviation during the fitting. All of the R^2 values for the fitting performed in this work are ≥ 0.99 . The energy calibration was performed with Ar^+ , as stated above. The uncertainties of the obtained AE values in Table 1 are a combination of the energy calibration (± 0.1 eV) and the standard errors that were provided by the fitting.

Because the experiment was performed at room temperature (approximately 25 °C), for each possible reaction pathway, the standard enthalpy (heat) of reaction, ΔH^0 , was calculated and then compared with the AE values. The ΔH^0 values were obtained from $\Delta H^0 = \sum \Delta H_f^0(\text{products}) - \sum \Delta H_f^0(\text{reactants})$. The computational quantum chemistry package, Gaussian09,³¹ was used to

optimize the structure of products and reactants, and evaluate the standard enthalpies for these optimized structures. Geometries were first optimized using the DFT approach with the B3LYP functional³⁴ and a moderately flexible basis set denoted 6-311+G(d,p).³⁵ Then the W1BD procedure,³² the most accurate composite method available in the Gaussian 09 package,³¹ was used to calculate the enthalpies, except for H of which the enthalpy was calculated using cbs-qb3 method.⁴⁰ Methyl-substituted amidogen radicals have been reported with diverse ionization energies, for example, the ionization energy of NHCH_3^+ is reported to have an upper and lower bond of 10.6 and 9.9 eV, respectively.⁴¹ The W1BD calculation in this work gives an ionization energy of 9.83 eV for triplet ground state NHCH_3^+ . This result is in good agreement with Wright and Miller's calculation (9.8 ± 0.1 eV)⁴¹. Thus, calculations performed in this work provide reliable theoretical estimates for reaction enthalpies.

N-methylformamide typically exists as a mixture of *cis* and *trans* rotational conformers. These two isomers are separated by a torsional barrier of ca. $70 \text{ kJ}\cdot\text{mol}^{-1}$ around the central C–N bond. According to previous work, the *trans* isomer predominates (92% *trans*, 8% *cis*) at 35°C ,⁴² and the difference in the energy and ionization energy of the two isomers is less than 0.1 eV,⁴³ which lies within the range of the uncertainty due to our calibration method. Thus, the *cis* configuration was ignored in these calculations for simplicity.

III. Results and Discussion

3.1 Mass spectra and appearance energy values

Figure 3 presents mass spectra of DMF and NMF and their deuterated homologs, d_1DMF ($\text{DCON}(\text{CH}_3)_2$), d_6DMF ($\text{HCON}(\text{CD}_3)_2$), and d_3NMF (HCONHCD_3), at an electron energy of 70 eV. The parent cations and the major cationic fragments are marked in the mass spectra and their relative intensities are consistent with those reported previously.⁴⁴

The major cations' formulas were identified assuming that the deuterated molecules' fragmentation patterns are the same as those for the non-deuterated molecules, i.e., for each cation that results from DMF fragmentation, its deuterated counterpart will be observed at a similar intensity due to the fragmentation of deuterated DMFs. Therefore, by matching the cations in the DMF mass spectrum with those from its deuterated counterparts in the spectra of d_1DMF and d_6DMF , the number of hydrogen atoms in the cations can be determined and assignments reliably made. The peaks at 15, 58, and 73 amu in the DMF mass spectrum were easy to determine as CH_3^+ , $[\text{DMF}-\text{CH}_3]^+$, and DMF^+ , respectively, and their counterparts in the d_1DMF mass spectra are CH_3^+ (15 amu), $[\text{d}_1\text{DMF}-\text{CH}_3]^+$ (59 amu), and d_1DMF^+ (74 amu). In d_6DMF , the above assignments corresponded to CD_3^+ (18 amu), $[\text{d}_6\text{DMF}-\text{CD}_3]^+$ (61 amu), and d_6DMF^+ (79 amu). The peaks at 42 and 44 amu in DMF can be assigned to OCN^+ , H_2CON^+ or $\text{C}_2\text{H}_4\text{N}^+$ and to $\text{C}_2\text{H}_6\text{N}^+$, respectively. Their counterparts are 42 and 45 amu in d_1DMF and 46 and 49 amu in d_6DMF , respectively. Because the masses of the deuterated molecules are increased by 4 and 5 amu in d_6DMF , the two cations contain at least 4 and 5 hydrogen atoms. Thus, the peaks at 42 and 44 amu in the DMF mass spectrum represent $\text{C}_2\text{H}_4\text{N}^+$ and $\text{C}_2\text{H}_6\text{N}^+$, rather than OCN^+ and

H_2CON^+ . In some cases, one single peak in DMF branches into multiple peaks in the deuterated mass spectra, e.g., the peak at 18 amu in DMF. Its corresponding peaks in the d_1DMF mass spectrum are at 18 and 19 amu, and in d_6DMF , they are at 21 and 22 amu. Because the counterparts in d_6DMF are 3 and 4 amu heavier, there are more than two hydrogen atoms in this cation. Thus, the peak at 18 amu does not represent H_2O , but instead NH_4 . The peaks at 28, 29, and 30 amu in the DMF mass spectrum can be CO^+ , HCO^+ and H_2CO^+ or CH_2N^+ , CH_3N^+ and CH_4N^+ . By measuring the deuterated mass spectra, their formulas were determined, as shown in Figure 3(a) and their assignments are explained below. The peak at 28 amu represents both CO^+ and CH_2N^+ , and this is because there are peaks at 28 and 30 amu in d_6DMF that can only be due to CO^+ and CND_2^+ , respectively. The peak at 29 amu represents HCO^+ , and this is because if there was CH_3N^+ , then there would be peaks at 31 (CHD_2N^+) or 32 (CD_3N^+) amu in the d_6DMF mass spectrum; however, these peaks are not present. The peak at 30 amu is attributed to CH_4N^+ , and this is because its counterpart in d_6DMF is at 33 amu, which suggests at least 3 hydrogen atoms in this cation. In addition to all of the major cationic fragments discussed above, another cation species that is present in DMF mass spectra is $[\text{DMF-H}]^+$ (72 amu), which corresponds to $[\text{d}_1\text{DMF-D}]^+$ (72 amu) in d_1DMF and $[\text{d}_6\text{DMF-H}]^+$ (78 amu) in d_6DMF .

The mass spectrum of NMF is shown in Figure 3(b), and it has fewer peaks than DMF. The major cations resulting from the electron-induced NMF fragmentation were also identified. In the mass spectra of NMF, the most intense two peaks are 30 amu (CH_4N^+) and 58 amu (NMF^+) that are formed due to amide bond cleavage and single ionization of the parent molecule, respectively. The deuterated counterparts of CH_4N^+ in the d_3NMF mass spectrum are at 32 amu ($\text{CD}_2\text{H}_2\text{N}^+$) and 33 amu (CD_3HN^+). The fact that the intensity of the peak at 32 amu ($\text{CD}_2\text{H}_2\text{N}^+$) is much higher than 33 amu (CD_3HN^+) indicates that there is a dominant pathway in which a hydrogen atom transfers to a nitrogen site when the $(\text{C}=\text{O})\text{-N}$ bond breaks. The intensity of the peak at 29 amu (HCO^+ , CHDN^+) in the d_3NMF mass spectrum is much higher than the peak at 29 amu (HCO^+ , CH_3N^+) in the NMF mass spectrum. This suggests that CHDN^+ contributes significantly to the peak at 29 amu in d_3NMF . Thus, there is a significant yield of CH_2N^+ in the fragmentation of NMF, and the peak at 28 amu in NMF represents a mixture of CH_2N^+ and CO^+ . The peak at 29 amu in the NMF mass spectrum represents CH_3N^+ and HCO^+ , and the deuterated counterparts are CD_3N^+ (32 amu), CHD_2N^+ (31 amu), CH_2DN^+ (30 amu) and HCO^+ (29 amu) in the d_3NMF mass spectrum. CH_3^+ (15 amu) and $[\text{NMF-H}]^+$ (58 amu) are also shown in the mass spectra of NMF, and their counterparts in d_3NMF are CD_3^+ , $[\text{d}_3\text{NMF-H}]^+$, and $[\text{d}_3\text{NMF-D}]^+$, respectively.

The appearance energies (AEs) of all of the major cations, including uncertainties, were measured and are listed in Tables I and II. The standard enthalpies of reaction, ΔH^0 s (in eV), for the formation of these cations directly from the fragmentation of the molecule ($\text{AB} + \text{e}^- \rightarrow \text{A}^+ + \text{B} + 2\text{e}^-$) via different pathways are shown in the tables. The ΔH^0 values represent the lowest energy requirement for the incoming electrons to trigger the corresponding fragmentation reactions. The predicted reaction pathways leading to each cationic fragment are identified with grey shading in the tables. The reaction pathways are discussed in the following sections.

FIG.3. (a) Mass spectra of DMF and its deuterated isotopologues, d₁DMF and d₆DMF; (b) Mass spectra of NMF and its deuterated isotopologue, d₃NMF.

TABLE I. Appearance energies of cationic fragments from DMF and calculated enthalpies of formation, ΔH^0 , of major reaction pathways.

m/z (amu)	This work (eV)	Other work (eV)	Cationic fragment	Neutral fragment(s)	ΔH^0 (eV)
--------------	-------------------	--------------------	-------------------	------------------------	-------------------

72	11.09 ± 0.1	11.35 ^a	[DMF-H] ⁺ (carbonyl site)	H	10.73
			[DMF-H] ⁺ (methyl group)	H	10.76
58	10.98 ± 0.2		HCONCH ₃ ⁺	CH ₃	13.95
			HCONHCH ₂ ⁺	CH ₃	10.46
			OCNHCH ₃ ⁺	CH ₃	10.32
44	10.71 ± 0.1	11.60 ^a , 11.6 $\pm$ 0.1 ^b	N(CH ₃) ₂ ⁺	HCO	13.03
			NHCH ₂ CH ₃ ⁺	CO, H	10.48
				HCO	9.80
43	10.20 ± 0.1 , 16.37 ± 0.2		HCON ⁺	C ₂ H ₆	14.18
				2 x CH ₃	18.10
			CONH ⁺	C ₂ H ₆	11.76
				2 x CH ₃	15.68
			NCH ₂ CH ₃ ⁺	CO, H ₂	10.85
			NHCH ₂ CH ₂ ⁺	CO, H ₂	10.09
42	11.59 ± 0.3		OCN ⁺	H, C ₂ H ₆	17.96
				CH ₃ , CH ₄	17.32
			NCH ₂ CH ₂ ⁺	HCO, H ₂	11.72
				CO, H, H ₂	12.41
			NHCHCH ₂ ⁺	HCO, H ₂	12.80
				CO, H, H ₂	13.48
30	12.08 ± 0.2		NHCH ₃ ⁺	CO, CH ₃	14.04
			NH ₂ CH ₂ ⁺	CO, CH ₃	10.16
29	12.31 ± 0.4	14.50 ^a , 14.3 $\pm$ 0.1 ^b	HCO ⁺	N(CH ₃) ₂	12.22
				NHCH ₂ CH ₃	12.15
			NH ₂ CH ⁺	CO, CH ₄	11.10
			NHCH ₂ ⁺	CO, CH ₄	10.96
28	13.07 ± 0.2		CO ⁺	NH(CH ₃) ₂	14.71
			NCH ₂ ⁺	CO, CH ₃ , H ₂	15.31
				CH ₄ , HCO	14.60
			NHCH ⁺	CO, CH ₃ , H ₂	12.23
				CH ₄ , HCO	11.52
18	12.75 ± 0.2		NH ₄ ⁺	CO, CHCH, H	12.05
				HCO, CHCH	11.36
				CO, CH ₂ CH	10.64
15	10.50 ± 0.3 , 13.90 ± 0.4		CH ₃ ⁺	OCHNCH ₃	13.82

^aData is from reference²⁹.

^bData is from reference⁴⁵.

TABLE II. Appearance energies of the cationic fragments from NMF and calculated enthalpies of formation, ΔH^0 , of major reaction pathways.

m/z (amu)	This work (eV)	Other work (eV)	Cationic fragment	Neutral fragment(s)	ΔH^0 (eV)
58	11.27 ± 0.1	11.20 ^a	[NMF-H] ⁺ (carbonyl site)	H	11.07
			[NMF-H] ⁺ (methyl group)	H	11.21
			[NMF-H] ⁺ (nitrogen site)	H	14.70

30	10.06 ± 0.1	11.65^a	NHCH_3^+	HCO	14.10
			NH_2CH_2^+	HCO	10.22
				H, CO	10.90
29	11.90 ± 0.2	12.40^a	HCO^+	NHCH_3	12.42
				NH_2CH_2	12.11
			NH_2CH^+	CO, H_2	11.87
			NHCH_2^+	CO, H_2	11.73
28	13.71 ± 0.2		CO^+	NH_2CH_3	14.83
			NCH_2^+	HCO, H_2	15.38
			NHCH^+	HCO, H_2	12.29
			NH_2C^+	HCO, H_2	14.51
15	$9.86 \pm 0.3,$ 14.16 ± 0.3		CH_3^+	OCHNH	14.14

^aData is from reference²⁹.

3.2 Ionization energies of NMF and DMF

FIG. 4. Ion efficiency curves for the 73 amu (DMF^+) cation from DMF (left) and 59 amu (NMF^+) cation from NMF (right).

The ionization energy is determined by the minimum energy required to remove one electron from the molecule's highest occupied orbital. The ion efficiency curves near the threshold region for NMF^+ and DMF^+ were measured and fit with a Wannier-type function as shown in Figure 4. The two highest occupied orbitals in DMF and NMF are the carbonyl oxygen lone pair (n_0) and the nonbonding antisymmetric π_2 orbital, which consist of the nitrogen lone pair and carbonyl double bond orbitals, as seen in Figure 5. In a photoelectron spectroscopy study,⁴⁶ the ionization potentials of DMF were determined to be 9.77 eV (vertical) and ≥ 9.40 eV (adiabatic) for the n_0 orbital and 9.25 eV (vertical) and 9.14 eV (adiabatic) for the π_2 orbital. Single point calculations at CCSD(T)/aug-cc-pVTZ level, at the appropriate relaxed geometries obtained by the above-mentioned DFT approach, give ionization energies of 9.81 eV (vertical) and 9.50 eV (adiabatic) for the n_0 orbital and 9.32 eV (vertical) and 9.14 eV (adiabatic) for the π_2 orbital, which agree with the experimental data very well. For NMF, the n_0 orbital ionization potential was measured at 10.05 eV (vertical and adiabatic), while for the π_2 orbital, they were found to be 9.87 eV (vertical) and 9.79 eV (adiabatic).⁴⁶ Our calculations predict 10.07 eV (vertical) and 9.81 eV (adiabatic) for n_0 orbital, and 9.98 eV (vertical) and 9.84 eV (adiabatic) for π_2 orbital. The

ionization energy measured in a prior electron impact ionization study⁴⁷ provided values of 9.45 eV and 10.0 eV for DMF and NMF, respectively, which both lie between the vertical ionization energies of the n_0 and π_2 orbitals. Baldwin et al. argued that the deviation of the measured threshold with the n_0 and π_2 orbital energies is because the measured electron ionization energy is actually the weighted average of two ionization values.⁴⁷ In the current experiment, the electron impact ionization energy for DMF is 9.57 ± 0.1 eV, which is approximately the mean value of the vertical ionization energies of the n_0 and π_2 orbitals. For NMF, the AE is 9.81 ± 0.1 eV, which is closer to the computed π_2 orbital ionization energy of 9.87 eV than that of the n_0 orbital.

FIG. 5. Two highest occupied orbitals of DMF and NMF. (a) π_2 orbital of DMF; (b) n_0 orbital of DMF; (c) π_2 orbital of NMF; (d) n_0 orbital of NMF.

3.3 H atom loss

FIG. 6. Ion efficiency curves for the 72 amu ($[\text{DMF-H}]^+$) cation from DMF (left) and the 58 amu ($[\text{NMF-H}]^+$) cation from NMF (right).

For $[\text{DMF-H}]^+$ and $[\text{NMF-H}]^+$, the AEs are 11.09 ± 0.1 eV and 11.27 ± 0.1 eV (Figure 6), respectively. This result deviates from the values obtained in Loudon and Webb's study which showed that the AE of $[\text{DMF-H}]^+$ (11.35 eV) is slightly higher than that of $[\text{NMF-H}]^+$ (11.20 eV).²⁹ Because our calculation suggests that the ΔH^0 for forming $[\text{DMF-H}]^+$ is lower than for $[\text{NMF-H}]^+$, as seen in Tables I and II, the AEs measured in this work should be more reliable. For NMF hydrogen loss can occur at three different sites: from the carbonyl, from the methyl group, or from the nitrogen. The ΔH^0 s for these three reaction pathways are calculated as 11.07 eV, 11.21 eV and 14.70 eV respectively. Thus, hydrogen loss from the nitrogen site can be excluded since the ΔH^0 of this pathway is more than 3 eV higher than the measured AE. Additionally, since in the mass spectrum of d_3NMF the signal at 61 amu ($[\text{d}_3\text{NMF-H}]^+$) is much

more prominent than that at 60 amu ($[\text{d}_3\text{NMF-D}]^-$), we conclude that $[\text{NMF-H}]^+$ is primarily formed from the loss of a hydrogen on the carbonyl. The estimated enthalpy of reaction (11.07 eV) for this reaction pathway is the lowest among the three reactions mentioned. For DMF hydrogen loss can happen either from the carbonyl site or the methyl group with very similar computed ΔH^0 s, 10.73 eV and 10.76 eV, respectively. However, the mass spectra of isotopically labeled DMFs show that hydrogen loss from the carbonyl ($[\text{d}_1\text{DMF-D}]^-$ from d_1DMF , $[\text{d}_6\text{DMF-H}]^-$ from d_6DMF) is the more prominent than the hydrogen loss from methyl group ($[\text{d}_1\text{DMF-H}]^-$ from d_1DMF , $[\text{d}_6\text{DMF-D}]^-$ from d_6DMF). Therefore, for both DMF and NMF, the formation of the dehydrogenated cations is primarily due to hydrogen loss from the carbonyl sites.

3.4 N-CH₃ bond cleavage

FIG. 7. Isomers of $\text{C}_2\text{H}_4\text{NO}^+$: (a) formed by losing a methyl group from the nitrogen site with no hydrogen transfer, (b) hydrogen transfer from the carbonyl site to the nitrogen, (c) hydrogen transfer from the other methyl group to the nitrogen.

N-CH₃ bond cleavage in DMF is indicated by the peak at 58 amu ($\text{C}_2\text{H}_4\text{NO}^+$) in the DMF mass spectrum. The fragmentation process resulting in this cation is provided below.

Considering the simplest case of N-CH₃ bond cleavage, the cation, $\text{C}_2\text{H}_4\text{NO}^+$, should be in the conformation shown in Figure 7(a). The calculated reaction enthalpy is 13.95 eV, which is approximately 3 eV higher than the observed AE value of 10.98 ± 0.2 eV (Figure 8). Thus, to explain the AE value for ONC_2H_4^+ , a more complicated reaction mechanism must be involved. If hydrogen rearrangement is allowed for $\text{C}_2\text{H}_4\text{NO}^+$, there can be three isomers, HCONCH_3^+ , OCNHCH_3^+ , and HCONHCH_2^+ , as shown as Figure 7(a)-(c). For isomers (b) and (c) in Figure 7, the reaction enthalpies calculated are 10.32 eV and 10.46 eV, respectively, which are both slightly lower than the measured AE value. Thus, the breaking of the N-CH₃ bond proceeds along with the transfer of a hydrogen atom from the carbonyl or methyl group. In contrast to DMF, in the mass spectrum of NMF, $[\text{NMF-CH}_3]^+$ (44 amu) was not observed.

FIG. 8. Ion efficiency curve for the 58 amu ($\text{C}_2\text{H}_4\text{NO}^+$) cation from DMF.

FIG. 9. Ion efficiency curves for the 15 amu (CH_3^+) cation from DMF (left) and NMF (right).

CH_3^+ was observed in both the DMF and NMF electron impact spectra. Interestingly, in both cases the ion efficiency curves of CH_3^+ from DMF and NMF show two thresholds. The lower thresholds for CH_3^+ from DMF and NMF are 10.50 ± 0.3 eV and 9.86 ± 0.3 eV (Figure 9), respectively, which are much lower than the formation enthalpies of CH_3^+ (13.82 eV and 14.14 eV) from the fragmentation of DMF and NMF, respectively. These two values match much better with the ionization energy of the CH_3 radical (9.84 ± 0.05 eV⁴⁵). Thus, in the region below the second threshold, the CH_3^+ does not come from the target molecule but from the ionization of the CH_3 radical, a secondary product of the electron-molecule interaction. CH_3 radicals can be from either simply N- CH_3 bond scission or ionizing fragmentation upon electron-scattering. Although, as seen in Table I, CH_3 is the most abundant byproduct for the ionizing fragmentation of DMF, this does not apply to NMF. As seen in Table II, CH_3 is not a byproduct in the ionizing fragmentation of NMF. Therefore, it is more plausible the CH_3 radicals observed are results of electron induced N- CH_3 bond scission in DMF and NFM. In addition, the energies required for N- CH_3 bond scission in DMF and NMF calculated with W1BD procedure are 4.0 eV and 4.3 eV, below the ionizing threshold of CH_3 by far, while the ionizing fragmentation for both molecules starts at around 10 eV. In this way, in the region around 10 eV, the CH_3 radical yield due to N- CH_3 scission is much more abundant than ionizing fragmentation. Due to the relatively low abundance of CH_3 , compared with the DMF and NMF, the intensity of the signal is very low after the first threshold. The second (higher) thresholds for DMF and NMF are 13.90 ± 0.4 eV and 14.16 ± 0.3 eV, respectively, which both agree well with the

formation enthalpies of CH_3^+ from direct electron-induced fragmentation of DMF (13.82 eV) and NMF (14.14 eV), as shown in Tables I and II.

3.5 $\text{C}_2\text{H}_6\text{N}^+$ (44 amu) from DMF and its further decay products: $\text{C}_2\text{H}_5\text{N}^+$ (43 amu), $\text{C}_2\text{H}_4\text{N}^+$ (42 amu), NH_4^+ (18 amu)

The work by Loudon and Webb²⁹ suggests that, because the AE value is approximately 1.3 eV smaller than the predicted ΔH^0 value for $\text{N}(\text{CH}_3)_2^+$, the cation, $\text{C}_2\text{H}_6\text{N}^+$, detected in the electron impact fragmentation of methylated formamide should be due to the isomer, $\text{NHCH}_2\text{CH}_3^+$, as shown in Figure 10.

FIG. 10. Structure of $\text{NHCH}_2\text{CH}_3^+$.

They further suggested that the $(\text{C}=\text{O})\text{-N}$ bond cleavage is followed by hydrogen transfer from the carbon to the nitrogen site, which is stabilized by the formation of a partial double $\text{N}=\text{CH}_2$ bond. The isotopic study in this work provides direct support for this postulate. Additionally, our isotopic mass spectra confirm that C-H bond cleavage during this process primarily originates from the carbonyl site ($(\text{C}=\text{O})\text{-H}$) rather than from the CH_3 group. The most intense peak at 44 amu ($\text{C}_2\text{H}_6\text{N}^+$) is shifted to 45 amu ($\text{C}_2\text{H}_5\text{DN}^+$) in the d_1 DMF mass spectra and to 49 amu ($\text{C}_2\text{HD}_5\text{N}^+$) in the d_6 DMF spectra. This can only be explained as follows: the H/D atom on the carbonyl site is transferred to nitrogen as the amide bond breaks. The AE for $\text{C}_2\text{H}_6\text{N}^+$ from DMF is 10.71 ± 0.1 eV, which is lower than the reported value 11.60 eV,²⁹ but well matches the calculated enthalpy of reaction of 10.48 eV, as shown in reaction (2), in which CO and H are the byproducts. The reaction enthalpy for the other possible pathway, which is shown in reaction (3) and leads to the isomer $\text{CH}_3\text{NHCH}_2^+$, was also calculated. The ΔH^0 of 9.80 eV is approximately 1 eV lower than the AE for $\text{C}_2\text{H}_6\text{N}^+$. The neutral product, HCO, is not likely to form along with $\text{CH}_3\text{NHCH}_2^+$, supporting the postulate that the hydrogen transfers from the carbonyl group to the nitrogen site to produce the $\text{CH}_3\text{NHCH}_2^+$ isomer.

$\text{C}_2\text{H}_6\text{N}^+$ can further dissociate into $\text{C}_2\text{H}_5\text{N}^+$ or $\text{C}_2\text{H}_4\text{N}^+$ by losing a hydrogen atom or H_2 , respectively.²⁸ In the mass spectrum of DMF, there are peaks at 43 amu and 42 amu. The peak at 42 amu ($\text{C}_2\text{H}_4\text{N}^+$) is at a much higher intensity than the peak at 43 amu; thus, $\text{C}_2\text{H}_6\text{N}^+$ is much more likely to decay by losing H_2 than a hydrogen atom. The two AEs for 43 amu are 10.20 ± 0.1 eV and 16.37 ± 0.2 eV, as shown in Figure 11. The first threshold at 10.20 eV represents $\text{C}_2\text{H}_5\text{N}^+$ because the enthalpies of reaction for all the possible pathways that form HCON^+ are higher than this value. The values for the reaction enthalpies that forms the isomers, $\text{NCH}_2\text{CH}_3^+$ and $\text{NHCH}_2\text{CH}_2^+$, are 10.85 eV (reaction (4)) and 10.09 eV (reaction (5)),

respectively. Because ΔH^0 is still higher than the AE value, $C_2H_5N^+$ is more likely to have the structure $NHCH_2CH_2^+$.

The second threshold of 43 amu from DMF at 16.37 eV should represent $HCON^+$ or $CONH^+$ and reactions (6)-(9) are possible fragmentation pathways. Among these fragmentation pathways, reaction (8) is most likely because its enthalpy of reaction is most similar to the detected AE value. The deviation between the calculated ΔH^0 and the AE value should be owing to the fact that the reaction (8) involves multiple bond dissociation and hydrogen atom transfer; thus, it is very likely that some transient states increase the energy threshold for the reaction to proceed.

FIG. 11. From top to bottom, ion efficiency curves from DMF at 44 amu ($C_2H_6N^+$), 43 amu ($C_2H_5N^+$ / $CONH^+$), 42 amu ($C_2H_4N^+$), and 18 amu (NH_4^+).

The peak at 42 amu in the mass spectrum of DMF occurs at a much higher intensity than that at 43 amu. As discussed above, this peak is due to $C_2H_4N^+$. The AE of the mass 42 was determined to be 11.59 ± 0.3 eV. The calculated ΔH^0 of OCN^+ is at least 17.32 eV, which is almost 6 eV higher than the observed value. This further supports our identification of mass 42 as $C_2H_4N^+$. The ΔH^0 of the following two isomers $N(CH_2)_2^+$ and $NHCHCH_2^+$ were estimated to

be 11.72 eV and 12.80 eV, respectively. Thus, $C_2H_4N^+$ has the structure $N(CH_2)_2^+$. This is also consistent with our study with isotopically-labeled DMF in which a major peak was observed at 42 amu ($N(CH_2)_2^+$) from d_1 DMF and at 44 amu ($N(CD_2)_2^+$) from d_6 DMF.

In a study by Levsen and McLafferty,²⁸ $C_2H_6N^+$ was also shown to decay to NH_4^+ by losing C_2H_2 . Such a reaction pathway explains the 18 amu peak in our mass spectra. The AE of NH_4^+ was determined to be 13.75 ± 0.2 eV. From the enthalpies of reaction, all of the following three pathways, reaction (12-14), can contribute to the formation of the NH_4^+ ion. However, the ΔH^0 of reaction (12) is closest to the experimentally determined AE. Thus, it is reasonable to conclude that the pathway shown as reaction (12) is the most probable. However, CO and H are the most likely byproducts released when $C_2H_6N^+$ is formed; thus, reaction (12) can be interpreted as reaction (2) followed by $C_2H_6N^+$ losing C_2H_2 , which further justifies the postulation that NH_4^+ decays from $C_2H_6N^+$.²⁸

3.6 CH_4N^+ (30 amu) and its further decay products: CH_3N^+ (29 amu) and CH_2N^+ (28 amu)

The peak representing CH_4N^+ (30 amu) is prominent in both the mass spectra of DMF and NMF with the AE values at 12.08 ± 0.2 eV and 10.06 ± 0.1 eV (Figure 12), respectively. The cleavage of (C=O)-N bond in NMF to form $NHCH_3^+$, shown as reaction (15), requires a reaction enthalpy of 14.10 eV which is around 4 eV higher than the measured AE value (10.06 ± 0.1 eV). Thus, this reaction pathway can be excluded. As suggested in reference [29], $NH_2CH_2^+$ should be the stable isomer of CH_4N^+ rather than $NHCH_3^+$. Measurement of AE for CH_4N^+ from NMF in this work supports this point. The enthalpies for forming $NH_2CH_2^+$ from NMF, seen in reaction (16), (17), match much better with the AE 10.06 ± 0.1 eV. The fact that the AE for CH_4N^+ from NMF is closer to the ΔH^0 of reaction (16) indicates that HCO is the byproduct. Thus, when the amide bond ((C=O)-N) dissociates, in the case of NMF, a hydrogen atom can transfer from either the carbonyl site or the methyl group to the nitrogen site. This is different from the case of amide bond cleavage in DMF in which the hydrogen transfer from the carbonyl group to the nitrogen occurs simultaneously with amide bond cleavage. Thus, DMF fragments into the cation, $C_2H_6N^+$, and the other byproducts of CO and H, rather than HCO as the byproduct. This postulate is also supported by the huge difference of the intensity ratio of mass 32 amu ($CD_2H_2N^+$) to 33 amu (CD_3HN^+) in the d_3 NMF mass spectrum and mass 49 amu ($C_2D_5HN^+$) to 50 amu ($C_2D_6N^+$) in the d_6 DMF mass spectrum. In the d_3 NMF mass spectrum, 33 amu (CD_3HN^+) is approximately half

of the height of 32 amu ($\text{CD}_2\text{H}_2\text{N}^+$), whereas in the d_6DMF mass spectrum, 50 amu ($\text{C}_2\text{D}_6\text{N}^+$) is at least one order of magnitude lower than 49 amu ($\text{C}_2\text{D}_5\text{HN}^+$) and is almost invisible.

FIG. 12. From top to bottom, ion efficiency curves for the 30 amu (CH_4N^+), 29 amu (left, HCO^+ , right, CH_3N^+), and 28 amu (CH_2N^+) cations. Right column shows cations from DMF; left column shows cations from NMF.

$$\Delta H^0 = 14.10 \text{ eV} \quad (15)$$

$$\Delta H^0 = 10.22 \text{ eV} \quad (16)$$

$$\Delta H^0 = 10.90 \text{ eV} \quad (17)$$

The AE value for CH_4N^+ from DMF is $12.08 \pm 0.2 \text{ eV}$, which is approximately 2 eV lower than the formation heat of the isomer, NHCH_3^+ (14.04 eV), suggests that hydrogen transfer must also be involved in this fragmentation pathway. Meanwhile, the formation heat of the isomer, NH_2CH_2^+ , is only 10.16 eV, as shown in Table I. Thus, NH_2CH_2^+ is more likely to represent the

cation at 30 amu. The reason the detected threshold is higher than the calculation may be because the reaction to form NH_2CH_2^+ consists of two steps: 1) the breaking of the N-CH₃ bond and the rearrangement of one hydrogen atom (the threshold for this step, as discussed above, is approximately 11 eV), and 2) the breaking of the (C=O)-N bond and the transfer of another hydrogen atom to the nitrogen site further increases the threshold to approximately 12 eV.

CH_4N^+ can decay by losing a hydrogen atom or H_2 (30 amu $\rightarrow$ 29 amu or 28 amu).²⁹ In NMF, the AE values of mass 29 amu and 28 amu were determined to be 11.90 ± 0.2 eV and 13.71 ± 0.2 eV, respectively. The computed formation enthalpies of HCO^+ (12.42 eV and 12.11 eV) are higher than the AE for the 29 amu cation (11.90 ± 0.2 eV), as shown in Table II. Associating the AE with the formation of NH_2CH^+ or NHCH_2^+ better explains the mechanism because the ΔH^0 values for forming these two cations are 11.87 eV and 11.73 eV, which are slightly lower than 11.90 ± 0.2 eV. For mass 28, again the calculation suggests that CO^+ requires a higher AE value of at least 14.83 eV. Thus, the experimental AE value is due to the formation of CH_2N^+ rather than CO^+ . Based on the calculated ΔH^0 s of NCH_2^+ (reaction (18)), NHCH^+ (reaction (19)), and NH_2C^+ (reaction (20)), which are 15.38 eV, 12.29 eV, and 14.51 eV, respectively, reaction (19) is the only pathway with a ΔH^0 that is lower than 13.71 ± 0.2 eV. Thus, the cation at 29 amu represents NHCH^+ . The slight disagreement between the AE value and the theoretical threshold (the AE is approximately 1.5 eV higher than the ΔH^0) is explainable because the enthalpy of reaction depends only on the final and initial states and the real determinants of the AE are the transition states in the pathway, which may be higher than the ΔH^0 .

For DMF, the AE values for masses 29 and 28 were determined to be 12.31 ± 0.4 eV and 13.07 ± 0.2 eV, respectively. As discussed in the section on the mass spectrum of DMF, the peak at 29 amu is primarily due to HCO^+ , and that at 28 amu is due to both CO^+ and CH_2N^+ . The AE value of mass 29 agrees with the calculated formation enthalpy value (12.22 eV), considering the fragmentation is a simple bond cleavage, as shown in reaction (21). If hydrogen transfer is allowed, as shown in reaction (22), the formation heat can be even lowered to 12.15 eV. Because hydrogen transfer occurs in the formation of $\text{C}_2\text{H}_6\text{N}^+$ as mentioned in the above discussion on amide bond cleavage, it is very likely that this process also occurs when amide bond cleavage results in HCO^+ . Thus reaction (21), (22) are both possible for forming the 29 amu cation. For the other possibility of 29 amu that represents CH_3N^+ , the formation heat is calculated to be lower than 11 eV, as shown in Table 1. Thus, HCO^+ should be the main component of the peak at 29 amu from DMF.

For 28 amu, the computed ΔH^0 of CO^+ is 14.71 eV, and for NHCH^+ , depending on the byproducts, the ΔH^0 values are 12.23 eV (reaction (23)) and 11.52 eV (reaction (24)), which are lower than the measured AE value of 13.07 eV. As stated in the section on $\text{C}_2\text{H}_6\text{N}^+$, hydrogen transfer should occur from the carbonyl site. Thus, HCO is not likely to be a byproduct of this reaction. Reaction (23) is the pathway associated with the AE value, of which ΔH^0 is actually closer to the AE value of 13.07 ± 0.2 eV. Similar to the other cases discussed above that involve multiple bond dissociation, the experimental AE value tends to be somewhat higher than the calculated estimate of the ΔH^0 .

IV. Conclusions

This work provides a very detailed examination of the cationic mass spectra of DMF and NMF, as well as the deuterated samples, d_1DMF , d_6DMF , and d_3NMF , obtained from electron impact ionization at 70 eV. All of the major cationic fragments from both DMF and NMF were carefully identified by comparing them with the mass spectra of isotopically labelled samples. The ion efficiency curves in the region of 5-20 eV for these cations were measured and reported. By fitting the Wannier cross-section function to the efficiency curves, the AEs for all of the cations were determined. The AE measurements performed in this work provide revised data for values obtained in previous studies.^{48,29} In addition, we report several AEs and mass spectra of deuterated DMF and NMF not previously documented. We also present a detailed analysis of the reaction pathways for the formation of each cation. For example, by comparing with the mass spectra of isotopically-labeled molecules, we confirmed and clarified the hydrogen transfer pathway during the amide bond cleavage. For DMF, the hydrogen atom on the carbonyl site relocates to nitrogen, and for NMF, the hydrogen atom from both the carbonyl site and the methyl group can transfer to the nitrogen. However, transfer from the carbonyl site is preferable. We also discovered that this hydrogen transfer behavior not only occurs when the amide bond breaks but also when the N-CH₃ bond cleaves. The fragmentation patterns of DMF and NMF bear some similarity: amide bond cleavage with hydrogen atom transfer is the most dominant pathway, and the loss of a hydrogen atom primarily occurs from the carbonyl site. However, there are differences: the hydrogen relocation has a greater chance of originating from a methyl group in the case of NMF than from DMF. Overall, this work provides a very detailed report of the ionization and fragmentation behavior of methylated formamides. The data provided in this paper will contribute broadly to understanding the fragmentation patterns of key biomolecules that are relevant in the astrophysical, prebiotic, and radiological fields.

Acknowledgements

This material is based upon work supported by the U.S. Department of Energy Office of Science, Office of Basic Energy Sciences under Award Number DE-FC02-04ER15533. This is contribution number NDRL 5121 from the Notre Dame Radiation Laboratory. We would like to thank John H. Brems and Joanna Kabuye for their lab assistance during this project.

References

- ¹ G.R. Adande, N.J. Woolf, and L.M. Ziurys, *Astrobiology* **13**, 439 (2013).
- ² B.M. Jones, C.J. Bennett, and R.I. Kaiser, *Astrophys. J.* **734**, 78 (2011).
- ³ P. Redondo, C. Barrientos, and A. Largo, *Astrophys. J.* **793**, 32 (2014).
- ⁴ M.M. Dawley, C. Pirim, and T.M. Orlando, *J. Phys. Chem. A* **118**, 1228 (2014).
- ⁵ B. Bhushan, 39th COSPAR Sci. Assem. Held 14-22 July 2012 (2012).
- ⁶ L.L.S. d. Hendecourt, P. de Marcellus, C. Meinert, I. Myrgorodska, L. Nahon, P. Modica, T. Buhse, and U.J. Meierhenrich, *IAU Gen. Assem.* (2015).
- ⁷ A. Kumar, R. Sharma, and Kamaluddin, *Astrobiology* **14**, 769 (2014).
- ⁸ A. Lopez-Sepulcre, A.A. Jaber, E. Mendoza, B. Lefloch, C. Ceccarelli, C. Vastel, R. Bachiller, J. Cernicharo, C. Codella, C. Kahane, M. Kama, and M. Tafalla, *Mon. Not. R. Astron. Soc.* **449**, 2438 (2015).
- ⁹ T.M. Orlando, H. Barks, R. Buckley, G. Grieves, E. Dimauro, and N.V. Hud, *Astrobiol. Sci. Conf. 2010 Evol. Life Surviv. Catastrophes Extrem. Earth Beyond* (2010).
- ¹⁰ R. Saladino, C. Crestini, F. Ciciriello, G. Costanzo, R. Negri, and E. di Mauro, *Proc. Third Eur. Work. Exo-Astrobiology* (2004).
- ¹¹ A.M. Schoffstall, *Orig. Life* **7**, 399 (1976).
- ¹² S.P. Langdon, J.A. Hickman, A. Gescher, M.F. Stevens, D. Chubb, and L.M. Vickers, *Eur. J. Cancer Clin. Oncol.* **21**, 745 (1985).
- ¹³ A. Gescher, N.W. Gibson, J.A. Hickman, S.P. Langdon, D. Ross, and G. Atassi, *Br. J. Cancer* **45**, 843 (1982).

- ¹⁴ P.G. Cooksey, E.N. Gate, A. Gescher, J.A. Hickman, S.P. Langdon, and M.F. Stevens, *Biochem. Pharmacol.* **32**, 3037 (1983).
- ¹⁵ D.L. Dexter, E.S. Lee, S.F. Bliven, A.S. Glicksman, and J.T. Leith, *Cancer Res.* **44**, 4942 (1984).
- ¹⁶ A.R. Kennedy and M.C. Symons, *Carcinogenesis* **8**, 683 (1987).
- ¹⁷ ICRU, Washingt. (1979).
- ¹⁸ J.A. LaVerne and S.M. Pimblott, *Radiat. Res.* **141**, 208 (1995).
- ¹⁹ V. Cobut et al., *Radiat. Phys. Chem.* **51**, 229 (1998).
- ²⁰ P. O'Neill, *Adv. Space Res.* **14**, 221 (1994).
- ²¹ J.D. Chapman, D.L. Dugle, A.P. Reuvers, C.J. Gillespie, and J. Borsa, (1975).
- ²² J.D. Chapman, A.P. Reuvers, J. Borsa, and C.L. Greenstock, *Radiat. Res.* **56**, 291 (1973).
- ²³ E.N. Gate, M.D. Threadgill, M.F.G. Stevens, D. Chubb, L.M. Vickers, S.P. Langdon, J.A. Hickman, and A. Gescher, *J. Med. Chem.* **29**, 1046 (1986).
- ²⁴ D. Newell, A. Gescher, S. Harland, D. Ross, and C. Ruddy, *Cancer Chemother. Pharmacol.* **19**, 91 (1987).
- ²⁵ A.H. Soloway, R.J. Brumbaugh, and D.T. Witiak, *J. Theor. Biol.* **102**, 361 (1983).
- ²⁶ A.L. Ruchelman, P.J. Houghton, N. Zhou, A. Liu, L.F. Liu, and E.J. LaVoie, *J. Med. Chem.* **48**, 792 (2005).
- ²⁷ R.S. Gohlke and F.W. McLafferty, *Anal. Chem.* **34**, 1281 (1962).
- ²⁸ K. Levsen and F.W. McLafferty, *J. Am. Chem. Soc.* **96**, 139 (1974).
- ²⁹ A.G. Loudon and K.S. Webb, *Org. Mass Spectrom.* **12**, 283 (1977).
- ³⁰ E.P. Wigner, *Phys. Rev.* **73**, 1002 (1948).
- ³¹ M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery Jr., J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, Ö. Farkas, J.B. Foresman, J. V Ortiz, J. Cioslowski, and D.J. Fox, *Gaussian 09, Revision E.01* (Gaussian Inc.,

Wallingford, CT, 2009).

- ³² E.C. Barnes, G.A. Petersson, J.A. Montgomery, M.J. Frisch, and J.M.L. Martin, *J. Chem. Theory Comput.* **5**, 2687 (2009).
- ³³ J.A. Pople, M. Head-Gordon, and K. Raghavachari, *J. Chem. Phys.* **87**, 5968 (1987).
- ³⁴ A.D. Becke, *J. Chem. Phys.* **98**, 5648 (1993).
- ³⁵ R. Krishnan, J.S. Binkley, R. Seeger, and J.A. Pople, *J. Chem. Phys.* **72**, 650 (1980).
- ³⁶ M.M. Dawley and S. Ptasińska, *Int. J. Mass Spectrom.* **365-366**, 143 (2014).
- ³⁷ S.G. Lias, in *NIST Chem. WebBook, NIST Stand. Ref. Database Number 69*, edited by P.J. Linstrom and W.G. Mallard (National Institute of Standards and Technology, Gaithersburg MD, 20899, n.d.).
- ³⁸ G.H. Wannier, *Phys. Rev.* **90**, 817 (1953).
- ³⁹ T.D. Märk, *J. Chem. Phys.* **63**, 3731 (1975).
- ⁴⁰ J.A. Montgomery, M.J. Frisch, J.W. Ochterski, and G.A. Petersson, *J. Chem. Phys.* **110**, 2822 (1999).
- ⁴¹ T.G. Wright and T.A. Miller, *J. Phys. Chem.* **100**, 4408 (1996).
- ⁴² L.A. LaPlanche and M.T. Rogers, *J. Am. Chem. Soc.* **86**, 337 (1964).
- ⁴³ J.F. Lu, Y.F. Wu, Z.Y. Yu, and X.J. Lin, *Russ. J. Phys. Chem. A* **85**, 1384 (2011).
- ⁴⁴ NIST Mass Spec Data Center, S.E. Stein, and Director, *Mass Spectra* (National Institute of Standards and Technology, Gaithersburg MD, 20899, n.d.).
- ⁴⁵ J.R. Reeher, G.D. Flesch, and H.J. Svec, *Org. Mass Spectrom.* **11**, 154 (1976).
- ⁴⁶ C.R. Brundle, D.W. Turner, M.B. Robin, and H. Basch, *Chem. Phys. Lett.* **3**, 292 (1969).
- ⁴⁷ M.A. Baldwin, A.G. Loudon, K.S. Webb, and P.C. Cardnell, *Org. Mass Spectrom.* **12**, 279 (1977).
- ⁴⁸ B.G. Gowenlock, P.P. Jones, and J.R. Majer, *Trans. Faraday Soc.* **57**, 23 (1961).