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Communication: Electron ionization of DNA bases

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No reliable experimental data exist for the partial and total electron ionization cross sections for DNA bases, which are very crucial for modeling radiation damage in genetic material of living cell. We have measured a complete set of absolute partial electron ionization cross sections up to 500 eV for DNA bases for the first time by using the relative flow technique. These partial cross sections are summed to obtain total ion cross sections for all the four bases and are compared with the existing theoretical calculations and the only set of measured absolute cross sections. Our measurements clearly resolve the existing discrepancy between the theoretical and experimental results, thereby providing for the first time reliable numbers for partial and total ion cross sections for these molecules. The results on fragmentation analysis of adenine supports the theory of its formation in space. *Published by AIP Publishing.* [http://dx.doi.org/10.1063/1.4948412]

Various excitation and ionization processes that produce large number of radicals, ions, and secondary electrons within the track of primary radiation are the basis for radiation damage in biological matter.[1–3] The electron ionization processes play a dominant role in this as the number of electrons increases through the cascading process of ionization. The quantitative data on these processes are essential inputs to Monte Carlo particle track structure simulations[4] that are widely used to understand the radiation damage in biological systems and for application to micro-dosimetry, nano-dosimetry,[5,6] and radiation therapy.[7] The data involve a complete set of total and partial cross sections for the respective primary and secondary particles and target molecules. Since no theoretical data and hardly any experimental data on absolute partial cross sections are available for DNA bases, they have not been incorporated in these models yet.

Nucleobases have been detected in the meteorite materials.[8,9] Understanding the origin of these nucleobases and its connection to the origin of life itself is an important astro-chemistry problem today. HCN is an important molecule in this respect. In addition, it is used as an extragalactic tracer of molecules.[10] Oligomerization of HCN in four successive steps in gas-phase reactions during molecular cloud collapse has been proposed[11,12] as a possible path to form adenine ($C_5H_5N_5$) in space.

In spite of their important applications, no accurate experimental data on absolute total and partial ionization cross sections for these species have been reported so far. There have been several theoretical calculations for the total ion cross sections. These include the ones using semi-classical *Deutsch – Mark* formalism,[13] Binary-Encounter Bethe (BEB) formalism,[13–16] improved binary-encounter dipole (iBED) model,[17] spherical complex optical potential (SCOP) model,[18] and quantum mechanical approach.[19] These theoretical results differ between 30% and 40% with respect to each other depending on the energy, but have more or less similar relative shapes. On the experimental side, Burgt *et al.* measured the relative cross sections up to 100 eV and normalized them to absolute values using the average value of various theoretical results at 70 eV.[20–22] So far, there exists only one set of absolute cross section measurements.[23–26] These measurements, carried out up to 200 eV of impact energies, vary from theory by a factor of 0.5–2 depending on the molecule. Even the large difference between the theory and these measurements does not show any consistency, though all of them have been made using the same experimental setup.[23–26] As a general thumb rule, for a given class of molecules like the DNA bases, one expects the cross sections to scale with the size of the molecule or molecular mass. The measured cross sections fail to conform to this thumb rule as they range from 8 A^2 for cytosine (mass = 111 amu) to 32 A^2 for guanine (mass = 151 amu). Our measurements on uracil[29] have also stressed the need for measurements on the DNA bases, as they gave cross sections about a factor of two larger than previously reported.[26] The limited absolute partial cross sections[23–25] that have been reported are also susceptible to similar uncertainties as in the total ion cross sections.

We have measured the partial electron ionization cross sections for all the fragment ions formed from the DNA bases using the well established relative flow technique.[27,28] The partial cross sections are summed to obtain the absolute total ionization cross sections, which are then used to resolve the contradiction between experiment and theory and within various theoretical models. Due to space limitation and considering its importance, in this paper we report the absolute total ionization cross sections along with the representative partial ion cross sections at 100 eV and some important highlights of fragmentation analysis.

Accurate determination of target density, electron beam current and the overlap integral between the two beam profiles, which is required to measure the absolute partial cross sections, is very difficult in a cross-beam experiment. Therefore relative flow technique (RFT)[27] is widely used

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to normalize the cross sections to absolute values in such experiments. The basic principle of RFT is to compare the intensity of the species of interest with that of a standard species of known cross section under identical experimental conditions. The molecules effusing out of a capillary under molecular flow will have a specific angular distribution independent of the nature of the gas. The only change will be a constant multiplier which depends on the pressure behind the capillary, which can be measured accurately. The cross section can be determined using the following equation:

$$\sigma_u = \sigma_s \frac{N_u}{N_s}\frac{I_s}{I_u}\frac{F_s}{F_u}\left(\frac{M_s}{M_u}\right)^{\frac{1}{2}}\frac{K_s}{K_u}. \quad (1)$$

Here, u represents the unknown species and s represents the standard species, N is the intensity of each ion, F is the flow rate of individual gases, M the molecular weight of each gas, I the time averaged electron beam current, and K the detection efficiency for the ions. This equation can be further simplified since $F \cdot M^{1/2}$ is proportional to pressure P behind the capillary under molecular flow conditions as

$$\sigma_u = \sigma_s \frac{N_u}{N_s}\frac{I_s}{I_u}\frac{P_s}{P_u}\frac{K_s}{K_u}. \quad (2)$$

DNA bases have very low vapour pressure and they exist in solid form at room temperature. They have to be heated to 180–250 °C in order to obtain sufficient vapour pressure to carry out measurements. Measuring the pressure at such elevated temperatures is technically not possible due unavailability of suitable manometers. We overcome this difficulty by using the vapor pressure data as a function of temperature for DNA bases, measured elsewhere,[30–32] as pressure behind the capillary.

The experimental setup is described in detail elsewhere.[29,33] Briefly, it consists of a magnetically collimated and pulsed electron gun, an effusive molecular beam, a Time of Flight Mass Spectrometer (ToFMS), a pair of micro-channel plates (MCPs) in chevron configuration, a Faraday cup, and the associated electronics.

The molecule under investigation was prepared as a beam by heating the commercially available (Sigma Aldrich) powder sample in the oven and allowing the sublimated molecules to effuse through a capillary directly into the interaction region. The temperature of the oven was slowly raised over a period of a few days to the required value, while monitoring the water vapour emanating from it by using the mass spectra. This procedure ensured uniform heating of the sample to thermal equilibrium as well as eliminating the water vapour from the sample. The uniformity of the temperature was ensured by monitoring it close to the sample and at the end of the capillary by different well calibrated thermocouples. The temperature of the sample was maintained at a particular value to obtain reasonable target densities, but low enough pressure to ensure molecular flow regime. The temperatures and vapor pressure used in the present measurements are shown in Table I. The molecular flow was ensured by passing the gas through a fine capillary (0.2 mm diameter) and keeping the pressure low enough (<50 mTorr) for the sample gas so that the ratio of mean free path to the capillary diameter was ensured to be >>1. The cations resulting from the electron–molecule collisions were extracted from the collision region by a pulsed extraction field applied immediately after the electron pulse. The ions were detected by the MCP detector mounted at the end of the flight tube and operated in the pulse counting mode. Use of large pulsed extraction field, optimization of the ion optics in the ToF mass spectrometer, and appropriate use of bias on the large area (50 mm diameter) detector ensured that no discrimination against initial kinetic energy and mass to charge ratio of the ions was present in the measurements. Uniform detection efficiency was ensured by two means. To begin with, the uniformity was ensured by changing the bias voltage combination on the front and back of the MCP detector plates and looking for relative variation of intensity of the highest mass peak to that one below 40 amu/e. The operating voltages were fixed in the range where this ratio showed saturation. The uniformity of the detector efficiency up to mass charge to ratio of 130 amu/e was checked by carrying out measurements on Ar and Xe and comparing the ratios of Ar^{2+} to Ar^{+}, Xe^{2+} to Xe^{+}, and Xe^{3+} to Xe^{+} with those reported in the literature. These were found to be in agreement within the experimental uncertainties.

A multi-hit ToFMS card was used to acquire the data. This was controlled by a General Purpose Interface Bus (GPIB) based software which allows storage of the mass spectra and electron current as a function of electron energy. The ion yield curves obtained from the mass spectra collected as a function of electron energy were converted to absolute cross sections by the relative flow technique using the cross section for Ar^{+} from Ar at 100 eV. We have used 2.51×10^{-16} cm^2 as the Ar cross section.[34] The measurements for Ar were carried out by flowing the gas through the same oven and the capillary tube used for making measurements on the sample molecules. The pressure of Ar behind the capillary was measured using a capacitance manometer, while the pressure for the sample molecules was determined by measuring the temperature accurately and using the vapour pressure data of the samples given in Table I. Argon measurements were done immediately before and after for each of the DNA bases under similar experimental conditions, except for the oven temperature. During Ar measurements the oven was kept at room temperature in order to prevent the interference from the DNA base sample. An independent set of measurements on argon at different temperatures were carried out with an empty oven to see any systematic error arising from the difference in temperature. These measurements showed no effect on the argon data with temperature. Total ionization cross sections for each sample were obtained by summing the respective partial cross sections. Details of the measurement procedure used to obtain the ion yield curves as well as absolute cross sections and the estimation of errors in the

TABLE I. Vapor pressure used in the measurements.

Molecule	Temperature (K)	Vapor pressure (mTorr)
Cytosine	505.9	14.0[30]
Thymine	439.9	5.26[30]
Adenine	474	45.82[31]
Guanine	491.8	5.53[32]

technique have been discussed for the case of uracil.[29] The estimated maximum uncertainty in the total ion cross sections is 12%. The main sources of this uncertainty are from the vapour pressure data (4%), uncertainty in the detection efficiency for ions over the range of interest (10%), and the cross section for Ar used for normalization to absolute scale (5%).

The partial ionization cross sections for various ions formed by 100 eV electrons are given in the form of bar charts in Fig. 1 for adenine ($C_5H_5N_5$, m/e = 135) and guanine ($C_5H_5N_5O$, m/e = 151) and in Fig. 2 for cytosine ($C_4H_5N_3O$, m/e = 111) and thymine ($C_5H_6N_2O_2$, m/e = 126), respectively. Apart from the absolute magnitude of the cross sections, they also provide details of the fragmentation pattern of the molecules. One interesting feature that can be noticed from the two figures is the unique pattern seen in adenine data. In this we observe prominent fragment peaks corresponding to ions $C_nH_nN_n^+$ (n = 5, 4, 3, 2, 1) at m/e of 135 ($C_5N_5H_5^+$), 108 ($C_4N_4H_4^+$), 81 ($C_3N_3H_3^+$), 54 ($C_2N_2H_2^+$), and 27 (HCN^+). The ratios of partial ionization cross sections for these ions to that of the parent ion at 31%, 11.2%, 23%, and 14%, respectively, are considerably high. Recently, Dawley *et al.*[35] have shown that these ions are formed by loss of one, two, three, and four HCN units from the adenine molecule. Similar loss of HCN from adenine on fragmentation has been reported using deuterated adenine[36] and using ^{15}N*–labeled* adenine.[37] This successive elimination of HCN units with such large cross sections is exclusively observed only in the case of fragmentation of adenine. The relatively large intensity of $C_nH_nN_n^+$ (with n = 1–4) ions shows their larger stability. This stability and the reversibility of reactions indicate that adenine is a pentamer of HCN and it can be formed in interstellar space by oligomerization of HCN.[11,12]

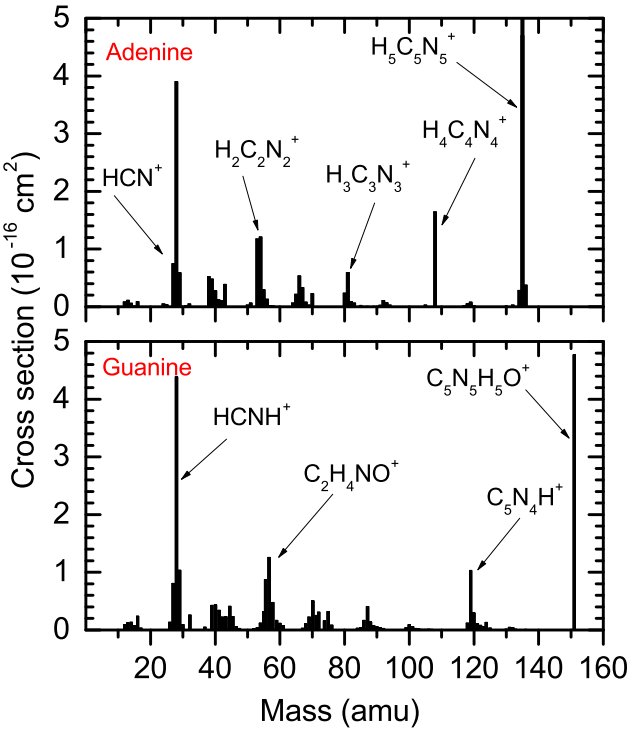

FIG. 1. Partial electron ionization cross sections for cytosine and thymine at 100 eV.

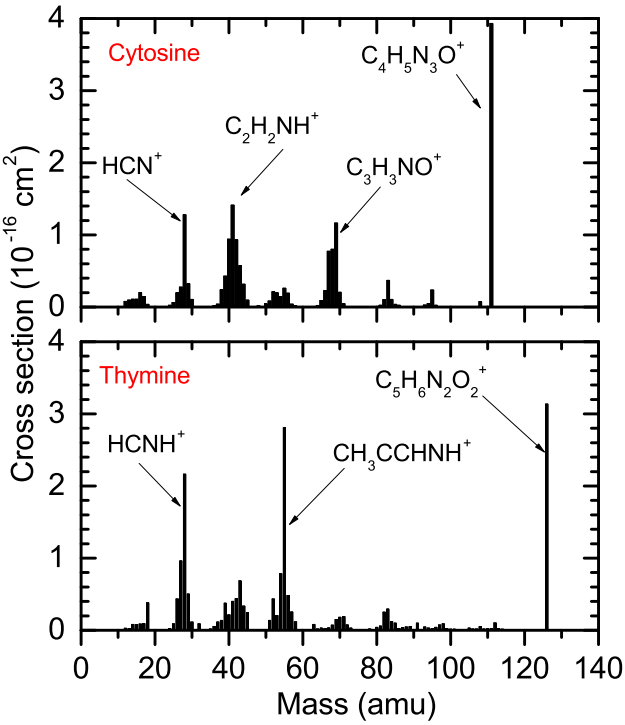

FIG. 2. Partial electron ionization cross sections for adenine and guanine at 100 eV.

Fig. 3 shows experimentally determined total ionization cross sections from threshold to 500 eV. The available theoretical[13–19] and measured[20–26] cross sections are also shown in the figure. The measurements[23–26] used a crossed beam method and the absolute cross sections were obtained directly by measuring the total ion current, the target beam density, and the volume overlap between the beams using the geometrical details of the setup. The most striking part of the results is the difference between the present and the previous measurements. In the case of cytosine the present data are more than a factor of two larger than what have been reported by Shafranyosh *et al.*[23] at the peak. The results of Shafranyosh *et al.* are also considerably smaller than the theoretical values. For thymine, the previous measurements[24] are lower by about 35% as compared to the present results at the peak energy of 100 eV. For adenine and guanine the previous measurements[25] are considerably larger (about 30% each) than what we have obtained at the peak energies. The relative shape of the cross sections as a function of energy also is quite different between the present set and the previous measurements. The latter shows relatively faster rise for the cross sections from the threshold as compared to the present set for all the molecules. Though the measurements in Refs. 20–22 used theoretical values for normalization, the relative shape of their ion yield curves available up to 100 eV for cytosine, adenine, and thymine are in good agreement with the present measurement.

All theoretically calculated cross section curves exhibit typical shape with a maximum energy around 80 eV and a gradual decline towards higher energies for all the four molecules. There is a noticeable spread among the available

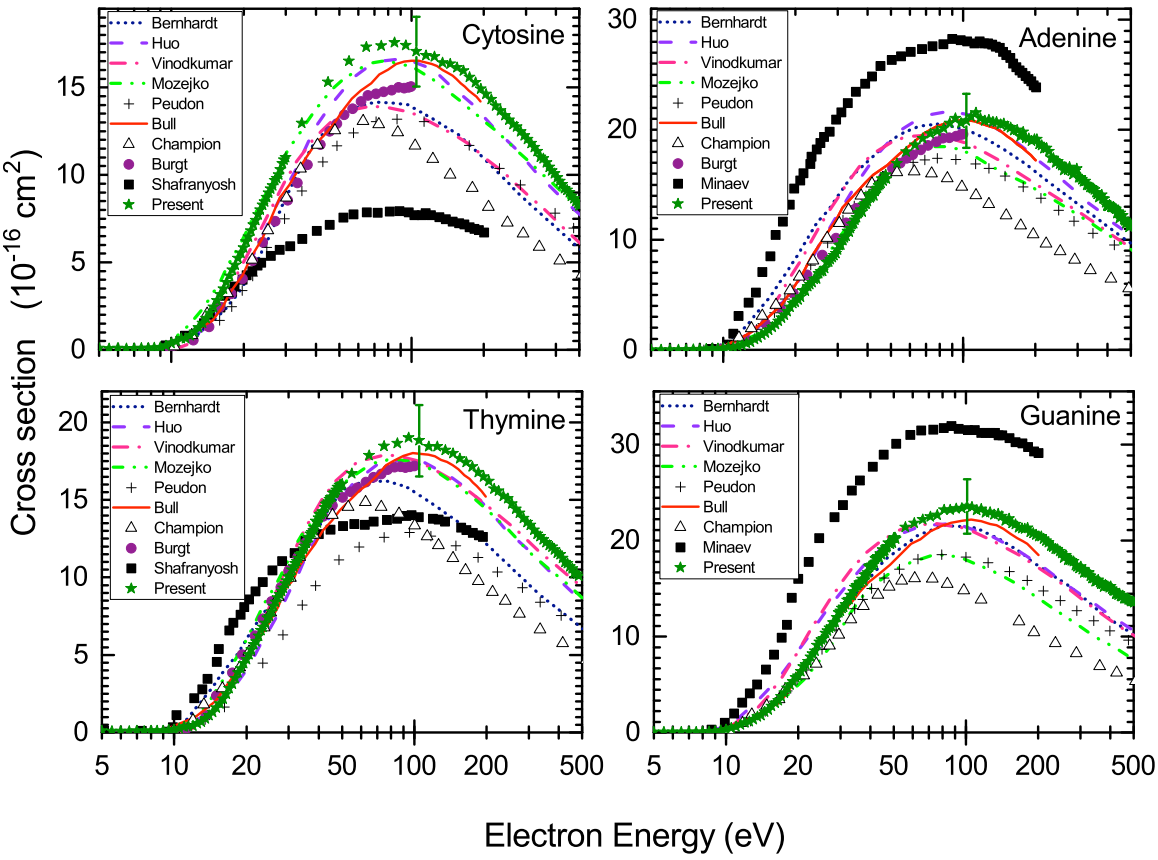

FIG. 3. Total ionization cross sections. dotted—Bernhardt and Paretzke (theory);[13] dashed-dotted-dotted—Mozejko and Sanche (theory);[14] plus—Peudon *et al.* (theory);[15] solid line—Bull *et al.* (theory);[16] dashed—Huo *et al.* (theory);[17] dashed-dotted—Vinodkumar *et al.* (theory);[18] triangle—Champion (theory);[19] circle—Burgt *et al.* (experiment normalized to theory);[20–22] square—Shafranyosh *et al.* (cytosine-experiment),[23] (thymine-experiment),[24] and (adenine- and guanine-experiment);[25] and stars—present data.

theoretical calculations, yet they appear to show similar qualitative features. All these calculations show lower values in comparison to the present measurements above 100 eV, though the difference is within the experimental uncertainties for some of them. For the case of cytosine, the results of Mozejko and Sanche[14] are in very good agreement with the present measurements in the entire energy range, while those given by Huo *et al.*[17] may be considered in fair agreement. In the case of thymine, three of the theoretical results[14,17,18] agree well with the present measurements in the entire energy range, while that given by Bernhardt and Paretzke[13] appears to be in agreement up to 60 eV. In the case of adenine, there appears a shift in the peak position between the experimental and theoretical results. This results in an overestimation of the theoretical values at lower energies, in general. The exception is the one by Mozejko *et al.*,[14] which give almost same values as the present experiment for energies below 70 eV. The results of Huo *et al.*[17] are in better agreement with the present results when one considers the entire energy range. For the case of guanine also, the experimental data show shift in the peak position to a slightly larger value. Yet, on the whole there appears to be very good agreement between the present measurement and three of the theoretical calculations.[13,17,18] Based on the agreement with the present experimental results of all the four bases, it appears that the improved binary-encounter dipole (iBED) model employed by Huo *et al.*[17] may be a more reliable theoretical tool in calculating the total ion cross sections for all molecules in the same category as the DNA bases. However, we also note that the recent work by Bull *et al.*[16] in which they employed an empirical energy dependent correction for the results obtained using BEB model (up to 200 eV) provide even better agreement with our measured values for all the four molecules.

To conclude, we have measured absolute partial ionization cross sections for all the DNA bases and used them to obtain the total ion cross sections. These results have crucial and immediate application in modeling radiation damage in living cells and also provide a benchmark test for various theoretical models for the total ionization cross sections. The present results do not agree with the existing measured values for the four molecules. Among the theoretical calculations available those obtained using the improved binary-encounter dipole (iBED) model employed by Huo *et al.*[17] are in better agreement with the present results, indicating good reliability for that technique for the molecules like the DNA bases. On the other hand, the empirically corrected BEB model[16] appears to provide the best match with our measured data. The observation of successive loss of HCN units and relatively high partial cross sections for the associated ions from adenine base supports the idea that adenine is a pentamer of HCN formed by successive addition of HCN units during molecular cloud collapse or in various other astrophysical environments. As a corollary, we also note that the measurement technique we have employed can be easily extended to measure cross

sections using the relative flow technique to any collision phenomena on atoms and molecules that exist as solids at room temperature, whose vapour pressure data are known or could be measured in an independent experiment.

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