Secondary Ion Yield and Fragmentation of Biological Molecules by Employing $^{35}$Cl Primary Ions within the MeV Energy Domain

Boštjan Jenič, † Primož Vavpetič, † Mitja Kelemen, † and Primož Pelicon* †

†Jožef Stefan Institute, Jamova 39, SI-1000 Ljubljana, Slovenia

ABSTRACT: MeV-SIMS is an emerging mass spectrometry imaging method that employs fast, heavy ions to desorb secondary molecules from the analyzed sample. High yields and low fragmentation rates of large molecules, associated with the dominating electronic sputtering process, make it particularly useful in biomedical research, where insight into the distribution of organic molecules is vital. Both yield and fragmentation of desorbed molecules in MeV-SIMS rely on characteristics of the primary ion but may also be impaired by poor instrumental settings. After utilizing secondary ion optics in the linear mass spectrometer at the micro-analytical center of the Jožef Stefan Institute, we demonstrate very efficient detection of secondary ions. As a result, the secondary ion yield, using such settings, solely depends on the species and the characteristics of the primary ion. In order to analyze the yield dependence on the primary ion energy, and the corresponding stopping power within the electronic excitation regime, we used a continuous electron multiplier detector to measure the primary ion current during each measurement of the mass spectra. Secondary ion yield as a function of the primary ion energy and charge is presented as well as fragmentation rates of organic molecules arginine and leu-enkephalin. Other influential instrumental drawbacks are also studied, and their effect on the results is discussed.

KEYWORDS: MeV-SIMS, secondary ion yield, molecular Imaging, heavy swift ions, electronic sputtering, time-of-flight

INTRODUCTION

Frequently used mass spectroscopy imaging (MSI) methods, such as secondary ion mass spectroscopy (SIMS) or matrix assisted laser desorption ionization (MALDI) rely intensely on the efficiency of sputtering secondary ions. A measurable quantity, defined as the number of desorbed ionized particles per each incoming primary ion, is known as the secondary ion yield. In the case of SIMS, the secondary ion yield is strongly correlated to the stopping power of the primary ion within the target. Higher stopping power is associated with higher volume of ejecta from the analyzed sample. Secondary ion yield also heavily relates to the fragmentation rate of sputtered molecules, as well as to the ionization probability of emitted particles, which typically has very low values. In the case of keV primary ion clusters, which are commonly used to desorb heavier molecules with conventional SIMS, the ionization probability is of the order of $10^{-3}$ or lower. Such low values hint the potential need of postionization protocols. However, some postionization techniques, e.g., laser postionization, interfere with the mass analysis of secondary ions and, therefore, are often avoided. Other techniques, such as dynamic reactive ionization, are also being used to enhance the ionization of protonated molecular ions.

SIMS is generally praised within the MSI community for its excellence in imaging resolution as well as proficient mass resolution of the acquired spectra. On the other hand, it experiences difficulties in providing higher yields of heavier, nonfragmented, secondary ions. Difficulties in desorbing heavy secondary ions are partially solved by using large clustered primary ions, such as C$_{60}^+$ and Ar$_n$ ($n = 60–30000$), or by surface modification. Due to the increased employment of MSI methods in biomedicine and similar scientific fields, a novel MSI method, known as MeV-SIMS, emerged in the last decade. Several studies confirmed the enhancement of large molecule yield and lower fragmentation rates when the sample is bombarded with primary ions within the MeV energy domain. By employing fast, heavy ions, the sputtering mechanism shifts from nuclear collisions (collision cascades) to electronic excitations. Additionally, the ionization probability of secondary particles emitted through electronic excitations was found to be of the same order of magnitude.

Electronic sputtering is analytically described by various models, with two most widely adopted being the thermal spike model and the pressure pulse model. Both theories, as well as molecular dynamics based simulations, predict cubic dependence of the yield as a function of the electronic stopping power.

Experimentally measured yields of specific secondary protonized molecules by utilizing MeV primary ions were reported to be approximately $10^{-3}$–$10^{-123}$ on reference samples. The total secondary ion yield on these samples was in the range between 0.1 and 1, depending on the characteristics

Received: July 23, 2019
Accepted: October 14, 2019
Published: November 20, 2019
of the primary ion beam.\textsuperscript{25} Our own measurements of the arginine and leucine amino acid yields have provided an estimation of protonized molecular peak yield in the range of $10^{-3}$ for 5.8 MeV \textsuperscript{35}Cl\textsuperscript{6+} primary ions.\textsuperscript{26} However, aforementioned values were related to the effective yield of detected ionized molecules. Deficiencies of the experimental procedure, such as the absence of secondary ion optics and the limited efficiency of the particle detector, heavily reduced the effective secondary ion yield. After we equipped the linear mass spectrometer at Jožef Stefan Institute (JSI) with an einzel lens, which collects the ions with close to 100% efficiency,\textsuperscript{27} we were also able to measure the efficiency of the MCP detector in relation to the secondary ion’s mass and kinetic energy. Considering all additional factors, we estimated the secondary ion yield within the physical constrains of available primary ion species.

\section*{EXPERIMENTAL METHODS}

MeV-SIMS molecular imaging is executed at the 2 MV tandem accelerator at the Microanalytical center of JSI. Detailed operational principles are described in the work of Jeromel et al.\textsuperscript{28} Given the limits of the primary ion energy and the available focusing power of the standard Oxford Microbeams triplet lens, MeV-SIMS commonly uses \textsuperscript{35}Cl ions with charge states between 4+ and 7+ and energies between 2 and 10 MeV.\textsuperscript{29}

Time-of-flight (TOF) is measured by pulsing the chlorine beam with 20-50 ns wide pulses, generated by the beam deflection system. Desorbed secondary ions are afterwards accelerated into the linear, 1 m long, TOF spectrometer with the acceleration voltage between 500 and 5000 V, and are once more postaccelerated in front of the MCP detector with a potential of $-2200$ V. The einzel lens is positioned at the entrance of the spectrometer, and its middle electrode is optimally biased to approximately 1/2 of the acceleration voltage, e.g., 2500 V for 5 keV secondary ions, as was the kinetic energy of desorbed ions within this work. In order to validate our estimations based on monitoring the count rate of secondary ions, we installed a position-sensitive TimePix detector,\textsuperscript{30} which enabled us to observe the secondary ions detection in real time.\textsuperscript{27} Using the optimal einzel lens settings, the secondary ion beam was focused to a size of less than a millimeter on the detector, which reflects approximately 35 micrometers on the analyzed sample. Combining an einzel lens and a TimePix detector can therefore result in stigmatic imaging with MeV-SIMS; however, current generation of position-sensitive particle detectors does not enable high enough timing precision for sufficient mass resolution of the acquired spectra.

We measured secondary ion yields on reference samples of arginine (m/z = 174.2) and leu-Enkephalin (m/z = 555.6). Both substances were dissolved in water (1 mg/mL) and spin-coated on a silicon wafer. After we deposited the samples into a vacuum chamber (pressure $5 \times 10^{-7}$ mbar), we employed a selected primary ion beam, focused to at least $30 \times 30$ μm\textsuperscript{2} (in most cases $10 \times 10$ μm\textsuperscript{2}), to bombard an area of 200 x 110 μm\textsuperscript{2}. Since the primary ions were bombarding the sample at an angle of 55\textdegree, the dimension of the x-axis was correspondingly larger, when primary ion beam was scanned through an area of 110 x 110 μm\textsuperscript{2} on a plane, perpendicular to the beam axis. Each measurement lasted 10 min on randomly selected area of aforementioned dimensions at primary ion currents between 2000 and 5000 s\textsuperscript{-1}, depending on the acceleration yield of the selected ion species. The corresponding beam fluence did in any case not exceed $10^{10}$ ions/cm\textsuperscript{2}, whereas the static limit in conventional SIMS is commonly accepted as $10^{12}$ primary ions/cm\textsuperscript{2}.\textsuperscript{31} According to our previous measurements on leucine and arginine samples,\textsuperscript{26} the damage cross section of bombarding the sample with 5.8 MeV \textsuperscript{35}Cl\textsuperscript{6+} ions was 8 nm\textsuperscript{2}. Such value of the damage cross section means no significant damage (order of $10^{-4}$) on the analyzed samples during each measurement with aforementioned duration and primary ion currents. Three sets of independent measurements were acquired on each sample with each of the selected primary ion beams.

The primary ion current was measured directly by the CEM detector from DrSjuts, biased to 2400 V, which was positioned behind the sample holder. Since the samples were coated on thick (100) silicon wafers, transmission of the primary ions was not possible. Therefore, we only measured the beam current before and after the spectra acquisition, when samples were removed from the primary ion’s trajectory. However, all measurements were executed under steady primary ion current conditions, and there were no significant fluctuations of beam currents before and after the acquisition of the spectra. Beam intensity was measured for 1 min and saved in listmode file. Five second averages of primary ion count rates deviated less than 5 % from the average value, and the average count rates before and after each measurement deviated less than 2% from eachother.

The energy of primary ions was varied between 2.6 and 10.0 MeV, and charge states were 4+, 5+, 6+, and 7+. Besides the effect of primary ion energy, our interest was also on the influence of the charge state, which had previously been measured as significant.\textsuperscript{32-34} However, large variations of the charge state for a fixed energy were prevented by focusing demands, since we were constrained with the values of the acceptable magnetic rigidity. Therefore, only two ion species with different charges and similar energy were observed.

\section*{RESULTS AND DISCUSSION}

Electronic excitation largely dominates the sputtering process within the MeV energy domain of chlorine ions. Nuclear collisions, on the other hand, only contribute a small fraction of the total stopping power (Figure 1). Since the electronic stopping...
stopping power is approximately linear as a function of energy within the employed range, we present our results in relation to the primary ion energy (see Figure 2 for comparison).

The total ion yield of the secondary ions exhibits linear dependence on the primary ion energy (Figure 2). Typically, each primary ion on average produces approximately 0.2–0.7 secondary ions. The distribution of desorbed particles per individual primary ion was extracted from listmode files and is shown in Figure 3. We can see that the probability of each additional desorbed ion is greatly diminished. Therefore, such numbers are below values, which would allow employing hydrogen or some other light charged particle as a start signal for time-of-flight measurements. By our estimations, each primary ion would need to desorb approximately 10 secondary ions in order to obtain a reliable start signal with light secondary ions. Within the MeV energy domain, such an approach could therefore be used only by post-ionizing secondary molecules, a technique named (MeV) secondary neutral mass spectrometry (SNMS). Similar is also the case with electrons, where values of 0.3–0.4 secondary electron/primary ion were reported. Although these values already enable utilization of secondary electrons as a trigger signal, the strong background, confinement to negative spectra, as well as weak mass resolution, associated with significant initial energy spread of the electrons, do not justify such approach in our case.

The total secondary ion yield exhibits linear correlation with primary ion energy, more profoundly, with electronic stopping power. While the total desorption of secondary molecules typically increases cubically with electronic stopping power, secondary ion production was previously found to follow a linear regime. It is also important to notice that the pressure pulse and thermal spike models define yield as a quantity proportional to the ejected volume (mass) instead of number of sputtered particles. Since the models do not anticipate fragmentation of molecules, the number of emitted ions is proportional to the ejected volume. In our case, however, this equivalence does not hold. Simultaneously, we are only interested in secondary ions, whereas theoretical models deal with desorbed particles, both ionized and neutral.

The spectrum of leu-enkephalin is shown in Figure 4, as is the comparison between intensity of molecular peaks when primary ions of different energies (and charge states) are employed. Mass resolution dm/m of the spectra for m/z = 120 was between 1/250 and 1/500 and for the molecular peak between 1/150 and 1/400, depending on the primary ion. Typically, primary ions with charge states 5+ and 6+ yielded better mass resolution. Mass resolution of MeV-SIMS at JSI can be further increased by going from “pulsed primary ion mode” (used in this work) to “continuous primary ion mode”, where dm/m values of 1/1800 were reported. Simultaneously, a lateral resolution of approximately 800 × 800 nm² is employed.

Figure 2. Total secondary ion yield of the leu-enkephalin and arginine samples as a function of electronic stopping power (above) and primary ion energy (below). Linear dependence on the stopping power can be observed, while total ion yields are within the 0.1–1 secondary ion/primary ion range.

Figure 3. Desorption probability distribution for 5 MeV 35Cl5+ primary ions on leu-enkephalin. Results were obtained by analysis of list mode files, which chronologically store data regarding detected particles. Each primary ion on average produces 0.47 secondary ions. 65% of the primary ion hits do not result in any secondary ion, while 27% of primary ions produce 1 secondary ion and 6% desorb two secondary ions. Further on, the numbers are exponentially diminished. The distribution remains similar to primary ions of various energies. The probability of each additional secondary ion is drastically lower.

Figure 4. Spectra of leu-enkephalin (m/z = 555.6) as obtained by 10 MeV 35Cl7+ primary ions. Inset exhibits differences between leu-enkephalin peak relative intensities (normalized to the peak of m/z = 138) in spectra obtained by 10 MeV 35Cl7+ and 2.6 MeV 35Cl4+ ions.
was achieved with a continuous primary ion beam, which enabled molecular imaging in the cellular level.

The yield behavior of the non-fragmented protonated molecules is shown on Figure 5. Both leu-enkephalin and arginine yields still rise roughly linearly with increasing energy of the primary ion. We did not notice any influence of the charge state of primary ions. Since we only employed ions with four different charges, it is possible that the effect might become considerable only on a larger scale, not reached by MeV-SIMS at JSI. Typical values for both arginine and leu-enkephalin yields are several percent.

Although the behaviors of the total ion yield and the nonfragmented molecular yield seem similar, their ratio as a function of primary ion energy (Figure 6) exhibits the increase of nonfragmented molecular peak intensity with higher primary ion energy. Such a ratio might depend on several parameters besides just fragmentation. The amount of the spectral background could be associated with the primary ion species, since the beam deflector might not deflect ions with various charges and velocities with the same efficiency. Additionally, modest variations in count rate of each selected primary ion beam could contribute to such differences. However, the fragmentation rate of the molecular peak still represents significant contribution to higher molecular peak integral in the spectra provided by faster primary ions. Figure 6 also exhibits the higher contribution of signal toward the heavier mass range in spectra for faster primary ions. Spectra of the 2.6 MeV primary ions have a greater share of counts in the low mass (fragments) interval, while the 10 MeV primary ions produce more signal from heavier molecules. (Bottom) Difference between integrals up to a specific mass. Up to approx $m/z = 150$, the difference between spectra from 2.6 MeV and 10 MeV primary ions increases, while afterward the signal becomes stronger on the spectra from 10 MeV Cl ions. Noticeable difference can be observed at $m/z = 556$ (leu-enkephalin) and also $m/z = 1112$ (2 × leu-enkephalin).

Figure 5. Secondary ion yields of the protonated molecules of leu-enkephalin (above) and arginine (below) as a function of primary ion energy and charge. The influence of charge cannot be confirmed, while linear dependence on energy can be observed.

Figure 6. (Top) Ratio between molecular ion yield and total ion yield for leu-enkephalin and arginine samples as a function of primary ion energy. Increase of main molecule detection can be observed with higher energies. (Middle) Rate of detected ions up to a certain mass compared to total ion yield for 2.6 MeV and 10 MeV Cl ion beams. Spectra of the 2.6 MeV primary ions have a greater share of counts in the low mass (fragments) interval, while the 10 MeV primary ions produce more signal from heavier molecules. (Bottom) Difference between integrals up to a specific mass. Up to approx $m/z = 150$, the difference between spectra from 2.6 MeV and 10 MeV primary ions increases, while afterward the signal becomes stronger on the spectra from 10 MeV Cl ions. Noticeable difference can be observed at $m/z = 556$ (leu-enkephalin) and also $m/z = 1112$ (2 × leu-enkephalin).
Additional experimental issue arises due to the limited detection efficiency of the MCP detector, which can result in mass spectra being biased toward the smaller masses. The detection probability decreases with heavier masses, since the velocity of molecules with identical kinetic energy and mass \( m \) decreases as \( m^{-1/2} \). Each MCP detector has its unique properties, related to type and age; thus, we estimated the efficiency function of our existing double-plate MCP detector.

By varying the acceleration voltage between 500 and 5000 V, and measuring the yields of secondary ions with masses between 1 and 1200 Da, we were able to obtain an approximate relation between the velocities of the secondary ions and the probability of detecting them by the MCP detector. Although some previously obtained results indicate that the detection efficiency of the MCP detector relies both on velocity and also weakly on the mass of the secondary ions, our results up to masses of 100 Da exhibited no significant influence of the ion’s mass. For the higher masses, however, we were unable to obtain the full detection due to the acceleration voltage limit at 5 kV. Therefore, our estimation of the efficiency only had to rely on the coincidence with lighter masses. Because of that, small corrections have to be considered for secondary ions with masses above 300 Da.

The measured detection efficiency (Figure 8) suggests that the detected number of the arginine molecules at 5 kV (velocity of approximately \( 9 \times 10^6 \) m/s with post acceleration) is roughly 80% of the actual yield of sputtered ionized arginine molecules. This puts the arginine yield past the 3% barrier for primary ions. Additional opening of the object and collimator slits resolves this problem, although such action negatively affects both lateral and mass resolution of the primary beam. Primary ions with energies between 5 and 7 MeV and charges 5+ or 6+ therefore represent an ideal compromise in the same velocity than we predicted.

Figure 7. Ratio between arginine molecular peak intensity and the intensity of two of its fragments as a function of the primary ion energy (above). The graph below shows the ratio between fragments and molecular peak (inverse of the quantity in the above graph, which demonstrates the ratio between intact molecules and fragments) as a function of nuclear stopping power share. The plots demonstrate the linear increase of the fragmentation rate with increase of the nuclear stopping power contribution.

![Figure 7](image)

**Figure 8.** Measured MCP detection efficiency as a function of secondary ion velocity. Results are reliable for masses up to approximately 200 Da.

CONCLUSION

The secondary ion yield analysis reveals the preference on employing primary chlorine ions on the higher end of the available energy range. The greater number of desorbed ions, as well as lesser fragmentation rates, both favor 10 MeV \( ^{35}\text{Cl}^+ \) ions, which is the fastest chlorine ion beam available on the JSI tandem accelerator. Focusing such ions is still well within the capabilities of the existing ion optics setup provided by Oxford Microbeams coupled with a Micro-beam experimental station. However, the acceleration efficiency of the highly charged primary ion beam is severely diminished; thus, secondary ion yield enhancement is overshadowed by lower currents of primary ions. Additional opening of the object and collimator slits resolves this problem, although such action negatively influences both lateral and mass resolution of the primary beam. Primary ions with energies between 5 and 7 MeV and charges 5+ or 6+ therefore represent an ideal compromise between sufficient secondary ion yield related to fast acquisition of sufficient statistics and the quality of the analysis.

When imaging biological samples, fragmentation of secondary molecules should be thoroughly addressed. The impact of nuclear sputtering mechanism, even at its low relative shares, emphasizes the importance of utilizing swift heavy ions. Further understanding of the fragmentation process could be attained by molecular dynamics simulations of sputtering through both nuclear and electronic sputtering.

MCP detection efficiency is, at the moment, a significant drawback to the MeV-SIMS setup at JSI. Molecular imaging is limited to masses below 3 kDa. Electronic sputtering within the relevant energy domain has previously been found to desorb significantly heavier molecules. In order to overcome this problem, we are aiming to implement a strong (\( \sim 10 \) kV) post-acceleration potential in front of the secondary ion detector.
On the basis of our results, summarized in Figure 8, post-acceleration of such magnitude should markedly enhance the detection efficiency and the upper mass limit of secondary ions, allowing us to achieve sufficient image statistics for molecules with masses in range between 3 and 10 kDa. Simultaneously, we also aim to find an optimal acceleration voltage for secondary ions. While increased acceleration voltage results in better detection, the shorter TOF impairs the mass resolution; therefore, a well thought compromise should be made in order to maximize the benefits of MeV-SIMS molecular imaging.

■ AUTHOR INFORMATION

 Corresponding Author
 *E-mail: primoz.pelicon@jss.si.

 ORCID

 Primož Pelicon: 0000-0003-2469-3342

 Notes

 The authors declare no competing financial interest.

 ■ ACKNOWLEDGMENTS

 Work at Jožef Stefan Institute was supported by the Slovenian Research Agency (ARRS) Grant Nos. J7-9398, N1-0090, P1-0112, and I0-0005 and EU H2020 Project No. 824096 “RADIATE”.

 ■ REFERENCES


