Towards matrix-free femtosecond-laser desorption mass spectrometry for in situ space research

Pavel Moreno-García¹*, Valentine Grimaudo¹, Andreas Riedo², Marek Tulej², Peter Wurz² and Peter Broekmann¹

¹Department of Chemistry and Biochemistry, Interfacial Electrochemistry Group, University of Bern, Freiestrasse 3, 3012 Bern, Switzerland
²Physics Institute, Space Research and Planetary Sciences, University of Bern, Sidlerstrasse 5, 3012 Bern, Switzerland

RATIONALE: There is an increasing interest in the quest for low molecular weight biomarkers that can be studied on extra-terrestrial objects by direct laser desorption mass spectrometry (LD-MS). Although molecular structure investigations have recently been carried out by direct LD-MS approaches, there is still a lack of suitable instruments for implementation on a spacecraft due to weight, size and power consumption demands. In this contribution we demonstrate the feasibility of LD-MS structural analysis of molecular species by a miniature laser desorption–ionization mass spectrometer (instrument name LMS) originally designed for in situ elemental and isotope analysis of solids in space research.

METHODS: Direct LD-MS studies with molecular resolution were carried out by means of a Laser Ablation/Ionization Mass Spectrometry (LIMS) technique. Two polymer samples served as model systems: neutral polyethylene glycol (PEG) and cationic polymerizates of imidazole and epichlorohydrin (IMEP). Optimal conditions for molecular fragmentation could be identified for both polymers by tuning the laser energy and the instrument-sample distance.

RESULTS: PEG and IMEP polymers show sufficient stability over a relatively wide laser energy range. Under mild LD conditions only moderate fragmentation of the polymers takes place so that valuable structural characterization based on fragment ions can be achieved. As the applied laser pulse energy rises, the abundance of fragment ions increases, reaches a plateau and subsequently drops down due to more severe fragmentation and atomization of the polymers. At this final stage, usually referred to as laser ablation, only elemental/isotope analysis can be achieved.

CONCLUSIONS: Our investigations demonstrate the versatility of the LMS instrument that can be tuned to favourable laser desorption conditions that successfully meet molecule-specific requirements and deliver abundant fragment ion signals with detailed structural information. Overall, the results show promise for use in similar studies on planetary surfaces beyond Earth where no or minimal sample preparation is essential. Copyright © 2016 John Wiley & Sons, Ltd.


Furthermore, direct LD studies of organic molecules have successfully been carried out without the assistance of a matrix or substrate pretreatment. [13,14,31–33] These investigations are particularly encouraging for in situ space research where minimal or no sample preparation procedures and no spatial disruption of the analyte prior to the analysis are of prime importance. [36–38] In this context, special attention is currently being devoted to the quest for low molecular weight biomarkers of extinct or extant life on planetary objects beyond Earth. [6,39–42] Nonetheless, the few direct LD studies reported so far have employed equipment that is not suitable for implementation on spacecraft, where requirements concerning weight, size and power consumption are very strict. Over the last 15 years, however, laser ablation/ionization mass spectrometry (LIMS) has re-emerged as an analytical technique suitable not only for laboratory, but also for space applications. [43–46] Its improved performance results from using state-of-the-art ultra-short femtosecond (fs) laser sources, [47–51] improved vacuum technology and fast

* Correspondence to: P. Moreno-Garcia, Department of Chemistry and Biochemistry, Interfacial Electrochemistry Group, University of Bern, Freiestrasse 3, 3012 Bern, Switzerland.
E-mail: pavel.moreno@dcb.unibe.ch
Because of its ultimate compactness, simplicity and robustness this is a very suitable analytical tool for elemental, isotope and molecular investigations in space research. In this contribution we demonstrate that our miniature laser mass spectrometer originally designed for in situ chemical analysis of solids in space research can perform desorption and identification of molecular model systems under soft desorption conditions. The figures of merit of LMS have been discussed in detail in previous publications and include sensitive and quantitative elemental analysis down to the 10 ppb level, accurate isotope measurements and 2D elemental imaging of surfaces of heterogeneous materials and high-resolution depth profiling at nm and sub-nm scale. We have performed LD experiments, as a proof of concept, applying low laser pulse energies on two polymers, polyethylene glycol (PEG) and polymerizate of imidazole and epichlorohydrin (IMEP), where parameters such as laser pulse energy, number of single lasershots per composite spectrum and distance between sample and mass analyzer were tuned to establish desorption conditions with reduced molecular fragmentation. Our results confirm the suitability and extended versatility of the LMS instrument to perform not only elemental/isotope analysis with high spatial resolution, but also successful constitutional formula studies of molecules without the need for elaborate sample preparation protocols.

**EXPERIMENTAL**

**Sample preparation**

Two different polymers were investigated as model systems for the LD studies, namely polyethylene glycol (PEG) and polymerizates of imidazole and epichlorohydrin (IMEP). PEG (35 kDa, Sigma-Aldrich, Steinheim, Germany), a linear polymer serving as a proxy for complex and aliphatic molecules identified in the interstellar medium and in comets, was chosen as a neutral model polymer to optimize experimental conditions for the soft LD mode. In addition, IMEP (BASF, Ludwigshafen, Germany) was chosen as a second reference sample due to its more complex molecular structure and its poly-cationic nature and as a proxy for the many nitrogen-containing molecules in the interstellar medium and in comets. As shown in Fig. 1(C), the IMEP polymer contains chloride as counter ion. These polymers are easily available because they are used as additives in industrial Cu electroplating where they tend to be incorporated into the electrodeposited metal films.

Both polymers were dissolved in a well-defined volume of methanol (Sigma-Aldrich) resulting in a stock solution with a polymer mass concentration of 10,000 ppm. Volumes of 400 μL of the PEG- and 1000 μL of the IMEP-containing solutions were stepwise dropcasted (each dose 100 μL) on two blanket Cu wafer coupons (Hionix, San Jose CA, USA, A<sub>PEG-sample</sub> = 117.5 mm<sup>2</sup> and A<sub>IMEP-sample</sub> = 48.9 mm<sup>2</sup>). The methanol was evaporated in a dry Ar atmosphere. Both samples were subjected to vacuum (base pressure in the mid 10<sup>−8</sup> mbar range) prior to their transfer into the main vacuum chamber of the LMS instrument to remove residual traces of the solvent.

**Laser ionization mass spectrometry**

Laser desorption mass spectrometry measurements were conducted using a miniature (160 mm × Φ 60 mm) reflectron-type time-of-flight (TOF) laser ablation/ionization mass spectrometer that was specifically designed for in situ chemical (element/isotope) analysis of solids in space research. The technical details, principles of operation and figures of merits of the instrument were discussed in detail in previous publications. The desorption and

**Figure 1.** Molecular structures of (A) PEG and (C) IMEP. Dashed lines indicate preferential cleavage points of the parent polymer and the arrows designate the m/z values of the most abundant fragmentation products. These masses do not include the Cl<sup>−</sup> counter ion of IMEP in panel (C). (B) Cumulative mass spectra of PEG at three different energy-per-pulse settings (0.21 μJ, 0.32 μJ and 1.08 μJ from top to bottom). To better visualize the relevant fragments, the signals in the upper and middle panels are magnified by a factor of 90× and 20×, respectively. (D) Cumulative mass spectrum of IMEP at mild LD conditions (0.21 μJ). The intensity of the spectra is displayed in linear scale.
ionization of the analytes from the Cu wafer coupons were triggered by irradiation with an ultra-short pulsed laser system (CPA system, Clark-MXR Inc., Dexter, MI, USA, \(\tau \sim 190\) fs, \(\lambda = 775\) nm, laser pulse repetition rate \(\leq 1\) kHz). An optical system outside the vacuum chamber is used for shaping and delivery of the beam towards the mass spectrometer. A doublet lens on top of the instrument is used to focus the laser pulses through the mass analyzer onto the sample surface. The application of laser irradiances at the TW/cm\(^2\) level (ablation mode) results in the formation of craters with a diameter of about 10 \(\mu\)m. The sample was positioned at a vertical separation smaller than 1 mm below the entrance electrode of the mass spectrometer on top of a high-resolution x-y-z translational stage that is controlled remotely with a position accuracy of about 2 \(\mu\)m.\(^{[58]}\) The number of emitted laser pulses, the energy per pulse and the repetition rate of the laser system are adjusted via the computer interface of the laser system. Only positively charged ions can enter the mass analyzer by passing the ion optical system. The latter accelerates, focuses and confines the ions along the ion optical axis towards the field-free drift path, which guides the ions to the ion mirror, where they are reflected backwards to the detector assembly. The ions are recorded sequentially according to their TOF that is proportional to the square root of their mass-to-charge (\(m/z\)) ratio using a multi-channel plate (MCP) detector system (chevron configuration). For each laser pulse a TOF spectrum is acquired by two high-speed ADC cards (8 bit vertical resolution, each card holding two channels) at a sampling rate of up to 4 GS/s over a time period of 20 \(\mu\)s allowing a full mass spectrum to be recorded in the range from hydrogen to lead.\(^{[58]}\) The acquisition and saving of the measurement data are performed on a host computer, which allows the monitoring in real time of the recorded signal during a measurement campaign. Mass spectrometric analyses of the acquired data are conducted subsequently using custom-made analysis software written in MATLAB.

**Measurement procedure**

To investigate in particular the evolution of molecular fragmentation upon laser irradiation several measurement campaigns were carried out on both polymer samples where the laser pulse energies were systematically varied between 0.16 \(\mu\)J and 1.68 \(\mu\)J. The sample surfaces were first kept at the focal point of the laser and then gradually displaced outwards in single steps of 50 \(\mu\)m. This approach allowed for mass spectrometric studies with high resolution of applied laser irradiances. In the case of PEG, campaigns 1–2, 3–9, 10–12 and 13–14 were conducted with pulse energies of 0.21 \(\mu\)J, 0.32 \(\mu\)J, 1.08 \(\mu\)J and 1.68 \(\mu\)J, respectively (Fig. 2(A)). For IMEP the energy per pulse was set to 0.16 \(\mu\)J, 0.21 \(\mu\)J, 0.32 \(\mu\)J, 0.43 \(\mu\)J, 0.65 \(\mu\)J, 0.76 \(\mu\)J and 1.08 \(\mu\)J for campaigns 1–3, 4–5, 6–12, 13–14, 15–17, 18–19 and 20–21, respectively (Fig. 2(B)).

The difference between the campaigns with the same energy per pulse was the increasing distance of the sample relative to the focal point, thus changing the laser irradiance on the sample. Each measurement campaign was conducted on a fresh and untreated sample position and consisted of 100 and 10 cumulative mass spectra for PEG and IMEP, respectively. Each of these mass spectra was built by signal accumulation of 2000 single laser shots.

**RESULTS AND DISCUSSION**

Figures 1(A) and 1(C) show the molecular structures of both model polymers. The expected fragment ions and their corresponding masses are indicated on these structures, and they will be discussed in the context of the measured mass spectra in more detail.

Previous investigations using the LMS instrument in laser ablation mode (at high applied laser irradiances, TW/cm\(^2\)) have determined the accurate quantitative elemental composition of Cu films electrodeposited in the presence of both model polymers (Figs. 1(A) and 1(C)) which tend to get partially embedded into the deposit upon growth.\(^{[61,62]}\) Aiming at performing structural analysis, in this work we used significantly lower laser irradiances – close to the desorption threshold – to prevent strong fragmentation and atomization of the analytes. This was achieved by using an ultra-short fs-laser as clean desorption/ionization source for the sample material.

Figure 1(B) shows measurements of the PEG reference sample at three distinct irradiance conditions. The sample mass spectra were built from 100 files (each an accumulation
of 2000 single laser shot mass spectra) for three different laser pulse energy values (0.21 μJ, 0.32 μJ and 1.08 μJ from top to bottom). At laser pulse energies just above the desorption threshold (0.21 μJ), only a few fragment ions were detected with significant signals (upper panel in Fig. 1(B)).

The peak at m/z 45 is the most intense feature in the mass spectrum (highlighted yellow in Figs. 1(A) and 1(B)) and it can be assigned to a protonated monomer species [M + H]+, which forms through two symmetric C–C bond cleavages in the parent PEG polymer chain at the α positions next to the oxygen atoms. Further but less abundant fragment ions are observed at m/z 31, 43, 59, 73 and 87, and these can be, respectively, attributed to [CH2O+H]+, [CHCH2]+, [CH2OCH2CH2+H]+, [CH2CH2OCH2CH2+H]+ and [OCHCH2OCH2CH2]+.

Experiments carried out at lower laser pulse energies showed no laser-desorbed ions in the measurable mass range. However, this observation does not exclude the desorption of neutral species, which have a lower desorption threshold,[65] and do not enter the instrument operated in positive ion mode. At moderate laser pulse energies (0.32 μJ) the above-mentioned features increase in intensity and a few more fragments appear, as illustrated in the middle panel of Fig. 1(B). By further increasing the laser pulse energy we fully enter into laser ablation, causing destruction of molecules up to complete atomization of the removed material. Under these conditions the 12C+ signal overwhelms all others indicating major fragmentation and atomization of the analyte. Moreover, under these conditions, additional molecules and clusters are generated in the ablation plume, whose signals become dominant and decrease the resolution of the overall spectrum (Fig. 1(B), lower panel).

Figure 1(D) shows measurements of the IMEP polymer. The mass spectrum is obtained at the lowest applied energy per pulse (0.21 μJ). All other acquisition conditions were the same as in Fig. 1(B). A peak at the mass of the monomer with its Cl− counter ion (m/z 160) was not observed, nor was there any evidence for the Cl− isotopes. We assume that even the lowest energy necessary to desorb the analyte is high enough to remove the Cl− anion from the IMEP backbone. Therefore, the main fragments observed other than the positively charged monomer (m/z 125) were those ions comprising the heteroatomic ring (C3N2H3, m/z 67, denoted hereinafter Im): [CH2Im]+, [CH2ImCH2]+, [ImCH2CHOH]+ and [CH2ImCH2CHOHCH2]+. Less intense peaks were also observed at lower masses, and these might represent protonated aliphatic fragments. Suggested fragment ions and corresponding features in the mass spectra are highlighted by the same colour code in both the polymer structures and the mass spectra in Fig. 1.

At the lowest applied laser pulse energies, the rate of the desorption process was not uniform and not every laser pulse removed detectable material from the sample by means of continuous desorption of the analyte, which is typical for laser desorption near the threshold energy.[65] This is why we represent the mass spectra in a cumulative fashion. This approach provides solid data sets for further interpretation.

Figure 2 shows the dependence of the integrated intensities for selected PEG and IMEP fragment ions as a function of the applied laser pulse energy. The plot clearly illustrates the existence of characteristic thresholds in the applied laser pulse energy for the desorption of the two monomer units (m/z 45 and m/z 125 for PEG and IMEP, respectively) and their fragments.[65] Generally, the dependence of the intensity on the laser energy can be subdivided into four sections which are highlighted white, grey, cyan and green in Fig. 2: (i) at low pulse energies, below the desorption threshold, there is no measurable intensity at all; (ii) at mild pulse energies, just above the desorption threshold, the abundance of relevant fragment ions marginally increases whereas the 12C+ intensity remains at zero; (iii) passing a certain level of the applied pulse energy leads to a drastic increase in the intensities of polymer-related fragment ions and 12C+, the intensities reach fragment-specific plateaux; and (iv) applying even higher laser pulse energies leads to a decrease in the intensities of fragment ions whereas the 12C+ intensity continues to increase. The latter observation is indicative of the more severe fragmentation and atomization of the model polymers at the highest pulse energies.

It should be noted that the characteristic thresholds in the applied pulse energy for the desorption of fragment ions are polymer-specific quantities, which are higher in the case of the neutral PEG and significantly lower for the charged IMEP polymer.

As expected from matrix-free laser desorption, both polymers fragment to some extent even under the mildest experimental conditions (in terms of laser energy) and desorption of positive ions from the analyte can be observed. However, our results clearly demonstrate that useful molecular and structural information can be obtained from these measurements. This result shows promise for the future identification of low molecular mass organic molecules serving as biomarkers beyond Earth, e.g., amino acids.[66] These results also show improved performance compared with previous desorption studies on glycine.[56]

CONCLUSIONS

The increasing interest in the detection of bio-markers in space research is driving the current development of dedicated instruments for space investigations based on LD-MS. The LD measurements discussed in this report and conducted on two different model polymers, PEG and IMEP, demonstrate the versatility of our miniature laser mass spectrometer designed for in situ space research. Under optimal laser desorption conditions, mass spectra with detailed structural information of the model polymers can be obtained allowing for the clear identification of the parent monomer molecules. Overall, the results show promise for similar studies being performed on planetary surfaces beyond Earth where no or minimal sample preparation is a requirement for operation. The performance of this laser mass spectrometer should be improved by the implementation of a UV fs-laser source, which would enhance the ionization efficiency and reduce fractionation effects.[66] This instrument is currently being developed.

Acknowledgement

Financial support of this work by the Swiss National Science Foundation (SNSF) is gratefully acknowledged.
REFERENCES


