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An automatic variable laser attenuator for IRMPD spectroscopy and analysis of power-dependence in fragmentation spectra

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ABSTRACT

Infrared ion spectroscopy (IRIS) requires reproducibility and standard protocols in order to be fully appreciated as an analytical tool. An important parameter in IRIS measurements is the infrared laser power (energy per pulse), which is often difficult to control precisely on a day-to-day basis. We have developed an automatic variable attenuator to keep the laser power at a well-defined value, independent of IR frequency and time, while maintaining full overlap of the laser beam with the ion cloud in our quadrupole ion trap mass spectrometer. The device has been used to record IR spectra of the metabolite prostaglandin D2 at different laser pulse energies, allowing us to assess the effects on the IRMPD spectra of excessive ion depletion, a threshold pulse energy below which dissociation is not observed, and unobserved fragments due to electron detachment or fragmentation to below the low mass cut-off of the trap.

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1. Introduction

Infrared ion spectroscopy (IRIS) has become a valuable and well-established method for the structural and conformational characterization of (bio)molecular ions in mass spectrometry [1–8]. This technique is based on the successful combination of storage mass spectrometry and infrared laser spectroscopy. Infrared spectra can be obtained for any ion that is trapped and mass-to-charge (m/z) isolated in a mass spectrometer that has been made optically accessible for an IR laser beam. Trapped ions are irradiated with tunable IR radiation from high power lasers such as user facility-based free electron lasers (FELs) and table top optical parametric oscillators/amplifiers. Monitoring the extent of IR-induced dissociation of the ion population, by recording the fragment and precursor ion intensity as a function of IR laser frequency, provides an IR spectrum of the precursor ion. Infrared multiple photon dissociation (IRMPD) spectroscopy [1–4], has been successfully used to record IR spectra of, for example, small peptides and peptide fragments [9–13], macrocycle complexes [14–16] and metal ligand complexes [17–25]. Structural and conformational characterization is then achieved by matching these measured IR-spectra with reference IR-spectra, which are either calculated

using computational chemistry or measured using reference compounds.

While IRIS has become a routine method in ion chemistry, the use of modern commercial mass spectrometry platforms for IRIS [26–30] provides a more solid foundation for the technique to be fully appreciated as an analytical tool. For example, the combination of liquid chromatography and mass spectrometry together with IRIS on a commercial platform [31] allows the application of the same workflows that are already in use in analytical laboratories. Recently, infrared ion spectroscopy has been successfully applied in metabolomics studies [31–36], specifically for the identification of metabolites related to inborn errors of metabolism [32,33]. It has been demonstrated that IRIS can be directly applied in the characterization of small molecules found in complex sample matrices such as body fluids (urine, blood, and cerebrospinal fluid, for example) [33]. Especially isobaric compounds that are hard to distinguish using alternative methods are good candidates for analysis by IRIS and future inclusion of IR spectra in the Human Metabolome Database (HMDB) [37,38] will provide new tools in the field of metabolomics.

An important parameter in the analytical application of IRIS is the stability and reproducibility of the laser pulse energy. For both facility based IR free electron lasers and commercial table top IR lasers, the available laser power is dependent on the IR frequency. Furthermore, the performance of lasers degrades slowly over time, for example due to slow temperature changes in optical materials

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and electronic components (warming up or environmental). Additionally, the performance may vary from day to day as a result of different optimization of the laser parameters by the operator. The detailed appearance of an IR action spectrum, such as the relative intensities of various bands in the spectrum, may depend on the laser power, especially in IRMPD methods. Full control over the laser power is therefore important to obtain spectra that are reproducible and that can serve analytical purposes.

This article describes an automatic variable attenuator for IR laser radiation which is fully implemented (hardware and software) in our IRIS setup. This device maintains a constant laser pulse energy, set at a value defined by the user, over time or as the wavelength is tuned. We demonstrate that the device can be successfully used in recording full range IR spectra of ions under controlled laser power conditions. The IRMPD measurements have been performed on the metabolite prostaglandin D2 in the fingerprint 1000–1800 cm^{-1} range (5.5–10 μm). The dissociation yield as a function of laser pulse energy is examined in detail.

2. Experimental

Infrared spectra were measured in a modified three-dimensional quadrupole ion trap mass spectrometer (Bruker, AmaZon Speed ETD) coupled to the beamline of the infrared free electron laser FELIX. The modifications, optical access to the trapped ions, synchronization of the laser timing to the mass spectrometer and software interfacing, have been described in detail elsewhere [26].

Deprotonated prostaglandin D2 has been selected as a test system. Prostaglandins are a set of physiologically active lipid compounds that are largely isomeric and pose a significant analytical challenge for LC-MS [39]. On the basis of retention time and fragmentation mass spectra, prostaglandin D2 is very difficult to distinguish from its positional isomer prostaglandin E2, as their molecular structures are very similar. The carboxylic acid group of prostaglandin D2 is easily deprotonated, leading to an anion with m/z 351. Ions were generated from a 10^{-7} M solution of prostaglandin D2 (Sigma-Aldrich, 97% purity) in 50:50 methanol:water via electrospray ionization. These ions were transferred to the quadrupole trap, mass-to-charge isolated and irradiated with 2 pulses of the infrared free electron laser FELIX.

When the IR frequency of the laser is on resonance with a vibrational transition of the ion population, multiple IR photons are absorbed leading to an increase in the internal energy of the ions. Aided by intramolecular vibrational redistribution of the energy, the ions dissociate after the internal energy exceeds the lowest energy dissociation threshold. This leads to the appearance of fragment ions in the mass spectrum. An infrared multiple photon dissociation (IRMPD) spectrum is obtained by plotting the frequency-dependent dissociation yield (*vide infra*) as a function of IR frequency. Typically 6 to 8 mass spectra are averaged at each IR setting of the laser.

Experiments have been performed in the 1000–1800 cm^{-1} range (5.5–10 μm). The FELIX laser pulses have a repetition rate of 10 Hz where each pulse has a duration of about 7 μs and consists of a pulse train of micro-pulses spaced by 1 ns. The radiation is Fourier transform limited by the micropulse duration resulting in a bandwidth of 0.4% of the central frequency. The IR frequency is calibrated using a grating spectrometer.

The output pulse energy of an FEL varies with wavelength and, for a chosen wavelength range, the pulse energy drops at shorter wavelengths. The prostaglandin D2 experiments were carried out with FELIX pulse energies up to 63 mJ. A single pulse of this energy is able to dissociate all ions in the trapped ion cloud when on resonance with one of the more intense vibrational transitions of

the molecule, leading to saturation effects in the IR spectrum. In our lab, five neutral-density IR laser attenuators (of 3, 5, 10, 10, and 10 dB) allow the user to select the desired level of attenuation as 3, 5, 8, 10, 13, ..., 38 dB. Selecting one of these fixed levels of attenuation reduces the IR laser intensity uniformly over the entire wavelength range and does not alter the shape of the frequency-dependent power curve. Here, we report on an automatic variable attenuator that has been constructed to keep the IR pulse energy constant over a wide tuning range; in this device, the level of attenuation is actively adjusted keeping the output power constant.

Similar to most lasers used in ion spectroscopy applications, the FELIX radiation is linearly polarized, so that a polarizer mounted on a rotation stage can be used to attenuate the intensity of the light. Free standing wire grid polarizers are able to cover the full mid-IR range, but they are extremely fragile and therefore need to be mounted in between two IR transparent windows. Less fragile wire grid polarizers fabricated on IR-transparent substrates are commercially available. However, the damage threshold of these polarizers is insufficient to withstand the high peak powers produced by the FELIX FEL (up to 100 MW). Here, we use a free standing wire grid polarizer (InfraSpecs, Germany) placed between two barium fluoride (BaF_2) windows. The BaF_2 windows limit the spectral range in which the variable attenuator can be used from 2 – 250 μm to 2–14 μm . For the device described here, the transmittance of the wire grid polarizer with the two BaF_2 windows is 50–65% (at parallel polarization) in the 1000–1800 cm^{-1} range.

The variable attenuator is constructed by mounting the modified wire grid polarizer onto a stepper motor rotation mount (Thorlabs K10CR1). After passing the attenuator, the beam is guided through the ion trap and directed onto a laser power meter (see Fig. 1). The desired pulse energy is set by the user in the control software that uses the power meter signal as input and that drives the rotation stage. The difference between the measured and desired pulse energy is used as a feedback signal to adjust the angle of the polarizer, such that a constant output pulse energy is obtained independent of the incoming power, which is dependent on wavelength setting and temporal fluctuations.

Fig. 1 presents a schematic of the experimental setup in which the variable attenuator is incorporated. The path of the FELIX infrared laser pulse is indicated by the red line and the data flow is indicated by black lines. Each FELIX pulse is accompanied by a master trigger generated by the laser. The FELIX pulses travel through the variable attenuator where the transmission is reduced by an amount that is dependent on the rotation angle of the stepper motor holding the polarizer. Subsequently, the FELIX pulses go through an optical beam shutter (Thorlabs, SH05) which is synchronized with the ion trap mass spectrometer, opening only during the IRMPD delay in the MS sequence [26]. After passing the shutter, the FELIX pulse is reflected and focused into the ion trap mass spectrometer where the overlap (both the alignment and focus of the beam) of the FELIX pulse and the ion cloud has been optimized to maximize the IRMPD yield. Upon entering and exiting the mass spectrometer the radiation travels through two 3-mm thick KRS-5 windows. Access to the ion cloud is enabled by two 3 mm holes in the ring electrode of the trap. After irradiation of the ion cloud, the FELIX pulse is reflected out of the mass spectrometer and measured by a laser pulse energy meter.

In order to determine the pulse energy correctly it is important that each pulse going through the ion trap mass spectrometer is detected. The oscilloscope (PicoScope 2206B) in Fig. 1 records the IR pulses and is triggered by a signal obtained from an AND-gate using the FELIX trigger and the shutter trigger as inputs. Each laser pulse transmitted through the ion trap is captured and digitized by the oscilloscope. The motorized rotation stage and oscilloscope are

Fig. 1. A schematic of the variable attenuator setup in combination with the quadrupole ion trap mass spectrometer. In this example, two pulses of the FELIX infrared laser are used to irradiate the ions in the trap.

controlled by a Labview program which directly sends the value of the IR pulse energy to the MS to be included in the standard Bruker format data file containing the mass spectra and other instrument parameters as well as the wavelength data. The program maintains a constant pulse energy at a value set by the user. Finally, the variable attenuator can also be used to continuously vary the laser pulse energy (at a fixed wavelength typically) in order to carefully investigate the dependence of the IRMPD yield on pulse energy.

3. Results and discussion

3.1. Performance of the variable attenuator: constant laser pulse energy

The dark green trace in the top panel of Fig. 2 shows the FELIX pulse energy as a function of IR frequency. This trace has been recorded with a 5 dB fixed attenuation (in order to be on the same

Fig. 2. Top: the pulse energy of the radiation as a function of wavenumber. Dark green: pulse energy recorded with the variable attenuator fixed at maximum transmission (see text). Other traces have been recorded with the variable attenuator actively adjusting the pulse energy to values of 2.5, 5, 7.5, 10 and 12.5 mJ. Bottom: the corresponding automatically adjusted rotation angle of the variable attenuator as a function of wavenumber.

scale as the other traces) and with the variable attenuator fixed at maximum transmission ($\theta = 0^\circ$, see lower panel); the non-attenuated pulse energies are indicated by the y-axis on the right. In the range between 1000 and 1800 cm^{-1} the non-attenuated pulse energy varies between 18 and 63 mJ. Actively controlling the variable attenuator, the pulse energy can be stabilized to any desired value as long as it is lower than the minimum laser pulse energy in the wavelength range of interest. In practice, in order to compensate for slow degradation of the performance of the FEL over time, the set value needs to be slightly below the minimum. Fig. 2 shows the pulse energy and the rotation of the variable attenuator as a function of wavenumber for five user set values for the pulse energy: 2.5, 5, 7.5, 10 and 12.5 mJ. Note that a rotation angle of 0° corresponds to maximum transmission, and 90° to minimum transmission.

Fig. 2 demonstrates that the automatic variable attenuator is able to produce a constant pulse energy as a function of IR frequency. Lower set values for the pulse energy and higher available (i.e. non-attenuated) pulse energies require more attenuation as is seen from the corresponding larger rotation angle of the wire grid polarizer. The relative pulse-to-pulse energy fluctuations are the same for each trace in the upper panel, indicating that the motorized attenuator does not increase the fluctuations. In the 2.5 mJ measurement, two strong dips in the pulse energy are visible, which are a result of short dropouts of the FEL. No feedback to the motorized polarizer is given during such dropouts.

3.2. Performance of the automatic variable attenuator: overlap with the ion cloud in the mass spectrometer

The waist of the laser beam at the position of the ion cloud is approximately 1.0–1.5 mm [26]. A single pulse of FELIX can dissociate more than 98% of the trapped ions, which we have verified by irradiating deprotonated prostaglandin D2 at its strongest vibrational transition (just below 1600 cm^{-1} , *vide infra*) with the maximum available FELIX pulse energy.

The motorized variable attenuator contains optical components (polarizer and two windows) which upon rotation may change the direction or position of the laser beam. It is important to establish whether or not rotation of the polarizer alters the overlap of the infrared laser beam with the small ion cloud in the quadrupole ion trap. Experimentally, this can be tested by using one of the fixed attenuators together with the variable attenuator to adjust the pulse energy to the same final value; recording IR spectra with the two attenuators and correspondingly different values of the rotation angle of the variable attenuator should then give the same spectrum.

Three IR spectra of deprotonated prostaglandin D2 are measured at the same final pulse energy of 10 mJ, but with a different fixed attenuation of the FELIX radiation before the variable attenuator for each measurement: 0 dB (0% attenuation), 3 dB (50% attenuation) and 5 dB (70% attenuation). The rotation angle of the variable attenuator is then set such as to reach the same output pulse energy of 10 mJ. The IR spectra are displayed in the top panel of Fig. 3. The vertical axis provides the IRMPD yield defined as the cumulative intensity of the fragment ions divided by the cumulative intensity of the fragment plus precursor ions:

$$\text{Yield} = \frac{\sum_{\text{Frag}} I}{\sum_{\text{Frag}} I + I_p} \quad (1)$$

The normalized fragmentation yield as defined in equation (1) is insensitive to fluctuations in the precursor ion production and can take on values between 0 and 1.

Fig. 3 shows the results of the experiment described above. The experiment has been repeated on a second day where the FELIX pulse energy curve deviated. The yield, pulse energy and rotation of the polarizer are shown as a function of IR frequency. At each day, three distinct fixed beam attenuators (0 dB, 3 dB and 5 dB) are used to alter the incoming FELIX pulse energy. This results in a different rotational dependence as a function of wavenumber (bottom panel). Fig. 3 shows that even though the degree of rotation is different for each measurement, the pulse energy of the radiation entering the ion trap is constant at 10 mJ. This shows that even when the FELIX pulse energy as function of the IR frequency changes from day to day, the automatic variable attenuator is able to provide a constant output pulse energy.

The infrared spectra, displayed in the upper panel of Fig. 3, are very similar. The yield for the most intense vibrational band is the same within 15%, which is actually surprisingly good given fluctuations of more than 10% in the IR pulse energy. For the other bands

the deviations between the measurements is larger, although there seems to be no correlation with the polarizer angle in the automatic variable attenuator. The deviations are likely caused by short and long term fluctuations in the spectral composition of the FELIX radiation, both in central frequency, in width and in spectral shape. This also explains the deviations in the ‘valley’ around 1337 cm^{-1} which are relative large when comparing data recorded at two different days. Most importantly, it can be concluded that the rotation of the wire grid variable attenuator does not alter the overlap of the infrared laser beam and the ion cloud in the trap. Therefore, together with the ability to maintain a constant laser pulse energy, we conclude that the automatic variable attenuator can be used reliably to perform IRIS experiments under well-defined laser pulse energy conditions.

3.3. IRMPD yield as a function of IR pulse energy

The automatic variable attenuator allows for convenient measurements of the IRMPD yield as a function of IR pulse energy. We have recorded IR spectra of deprotonated prostaglandin D2 in the $1000\text{--}1800\text{ cm}^{-1}$ range for five pulse energies in the range of $2.5\text{--}12.5\text{ mJ}$ (*vide infra*). It is also possible to scan the pulse energy continuously while keeping the IR frequency constant. This is illustrated in Fig. 4A, where the yield at 1300 cm^{-1} is plotted as a function of pulse energy. At this frequency just in between two relatively weak bands, no extensive dissociation occurs at energies below 10 mJ (see Fig. 3), which allows us to perform measurements over a wide range of pulse energies (here up to 45 mJ).

The prostaglandin D2 ions were irradiated with 2 IR laser pulses (red trace in Fig. 4A). No dissociation is observed below 7 mJ. The absorption cross section is relatively low at 1300 cm^{-1} and collisional cooling by the helium buffer gas in the quadrupole ion trap likely prevents the ions from reaching the dissociation energy [26,40]. The threshold laser energy at which the ions start to dissociate depends on the IR frequency. The strongest vibrational band of prostaglandin D2 has a dissociation threshold below 0.5 mJ (*vide infra*). At 1300 cm^{-1} , the yield increases linearly with pulse energy between 10 and 20 mJ, after which saturation sets in. However, the yield does not reach its theoretical maximum value of 1 at the highest pulse energy of 45 mJ. Irradiation at the same frequency, but with five times as many IR pulses (10 instead of 2) shows that a yield of 1 is obtained at a pulse energy of 35 mJ, indicating that all ions have been dissociated.

The yield as defined by equation (1) is related to the laser induced photo-dissociation rate k and the irradiation time t via [41,42]:

$$\text{Yield} = 1 - e^{-k t} \quad (2)$$

Assuming the rate-limiting step in the IRMPD process to be the single-photon absorption rate inducing the first vibrational transition, the process can be regarded to follow first-order kinetics. We denote the product kt as the ‘‘fragment fluence’’, which scales linearly with the number of laser pulses (*i.e.* the irradiation time t) and, under the above assumption, also linearly with IR pulse energy and absorption cross section of the vibrational transition in the precursor ion. The fragment fluence is given by

$$\begin{aligned} \text{Fragment fluence} &= -\ln(1 - \text{Yield}) \\ &= -\ln\left(\frac{I_p}{\sum_{\text{Frag}} I + I_p}\right) \end{aligned} \quad (3)$$

Fig. 3. Top: the yield as a function of wavenumber for three fixed attenuation values. Middle: the pulse energy as a function of wavenumber. Bottom: the rotation of the variable attenuator as a function of wavenumber. Day 1 and day 2 indicate the two distinct days at which these measurements have been performed.

In Fig. 4B, the fragment fluence is plotted for the same data sets

Fig. 4. IRMPD of deprotonated prostaglandin D2 ions at 1300 cm⁻¹. Panel A: the yield as a function of pulse energy for irradiation with 2 and 10 IR laser pulses. Panel B: the fragment fluence as a function of pulse energy. Panel C: the precursor, sum of the fragments and individual fragments intensities as a function of pulse energy for irradiation with 10 IR laser pulses. Panel D: the measured yield as a function of the yield which would be measured if there were no charges lost from the ion trap for 0, 25, 50 and 75% of fragment ions that are not detected because their *m/z* value is below the mass cut-off of the quadrupole ion trap or because electron detachment occurs (see text).

as in Fig. 4A. Additionally, the trace for 2 pulses is multiplied by 5 to show that indeed the fragment fluence scales linearly with the number of laser pulses (irradiation time). Note that a yield of 1 corresponds to $I_p = 0$ and hence to an infinite fragment fluence, explaining the increased scattering of data points at energies higher than 30 mJ. Furthermore, as expected, the fragment fluence scales linearly with pulse energy over an extended range (10–30 mJ) as compared to the yield (10–20 mJ).

Fig. 4C shows the intensity of the precursor ion, the sum of the fragment ions and the individual fragment ions as a function of pulse energy measured with 10 laser pulses at 1300 cm⁻¹. A striking observation is the fact that the traces corresponding to the precursor ion (*m/z* 351) and the summed fragment ions are not complementary, that is, the sum of the two traces is not constant. This indicates that at energies above 20 mJ, the majority of fragments are not detected. Looking at the individual fragment ions, we see that at low pulse energies only the *m/z* 333 fragment is formed; at increasing pulse energies, ever more smaller fragment ions appear through sequential dissociation processes (actually, there are more fragment ions included in the summed fragment ion intensity than those listed in Fig. 4C, all having very low intensities). For several fragment ions (*m/z* 189, 233, 271 and 315) we have verified that they absorb at 1300 cm⁻¹ and dissociate into smaller fragment ions, showing that sequential dissociation processes play a role in IRMPD of prostaglandin D2 at high IR pulse energies. This suggests that there may also be fragment ions below the low-mass cut-off (LMCO) of the quadrupole ion trap, which was *m/z* 95 for the experiments reported in Fig. 4. In fact, the calculated collision induced dissociation (CID) mass spectrum for prostaglandin D2 reported in the HMDB suggests the formation of a major fragment at *m/z* 59, probably corresponding to the acetate anion. Moreover, the anionic precursor and fragment ions may undergo electron detachment upon multiple-photon excitation [43–45], which has the same effect as fragmentation below the LMCO: a net loss of charges from

the trap. In the current experiments we cannot establish which of these two processes is dominant in causing the reduced total ion count at higher IR laser pulse energy.

The formation of fragment ions below the LMCO and the formation of neutral molecules as a result of electron detachment affect the intensities in the IRMPD spectra because the values of the yield or fragment fluence as defined in equations (1) and (3) depend on whether or not all charged fragments are included in the sum. This effect is illustrated in a simulation shown in Fig. 4D. Here the value of the yield is determined for cases where a specific fraction of the fragment ions cannot be detected. The derived yield is then lower than its value without loss of charges from the trap. Perhaps counterintuitive, the largest discrepancies arise for intermediate values of the yield (around 0.5). The graph also shows that at complete depletion of the precursor ion, the yield always reaches a value of 1, independent of whether charges are lost or not. This is also obvious from an evaluation of equation (1). As such, the observation of a yield reaching 1 is not indicative for having no fragmentation below the LMCO. The precursor ion depletion curve in Fig. 4C gives an experimental value for the yield-without-loss by realizing that

$$\text{Yield without loss} = 1 - I_p/I_{p,0} \quad (4)$$

where I_p represents the intensity of the precursor ions and $I_{p,0}$ is the precursor ion intensity without IR irradiation (i.e. at 0 mJ). A plot of the measured yield versus the yield-without-loss obtained via equation (4) is shown in Fig. 4D. At low yield, i.e. for pulse energies below 10 mJ, the data points are in between the 0 and 25% cut-off lines, indicating that already at low pulse energy, fragments are formed below the LMCO and/or detachment occurs. At higher yield values, corresponding to pulse energies above 10 mJ, the data points appear to roughly follow the 25% cut off line.

When fragmentation below the LMCO is caused by sequential dissociation (IR absorption by fragment ions that undergo secondary dissociation), one may reduce the number of laser pulses and/or the laser pulse energy. A careful analysis of the data is always needed in order to make the correct interpretation of the relative intensities in the IR spectra.

3.4. IRMPD spectra as a function of IR pulse energy.

Infrared spectra of deprotonated prostaglandin D2 in the 1000–1800 cm^{-1} range have been recorded for five pulse energies in the range of 2.5–12.5 mJ as displayed in Fig. 5. The top panels show the yield (equation (1)), fragment fluence (equation (3)), and the precursor depletion spectrum, defined in analogy with equations (3) and (4) as:

$$\text{Precursor Depletion} = -\ln(I_p/I_{p,0}) \quad (5)$$

Since $I_{p,0}$ is taken as a constant value for all frequencies, fluctuations in the precursor ion production are present in the depletion spectrum (Fig. 5E), as is, for example, clearly visible around 1100 and 1500 cm^{-1} . The lower panels are power corrected versions of the upper panels. Here, the y-axis has been scaled with a factor $12.5/\text{energy}$ to account for the fact that lower pulse energies give lower dissociation yields. In the ideal case, that is, in the absence of a threshold pulse energy to dissociation and in the absence of fragmentation below the LMCO, the power corrected fragment fluence spectra in panel D should all be identical. As is discussed above, and displayed in Fig. 4, excitation of the ions at 1300 cm^{-1} shows a threshold pulse energy of 7 mJ, in line with the data shown in Fig. 5. At the most intense band around 1600 cm^{-1} , a high fragment fluence is observed already at a pulse energy of 2.5 mJ, indicating that the threshold is significantly below 2.5 mJ at this frequency. Fig. 5 demonstrates that the threshold pulse energy is frequency dependent and that at low pulse energies some of the weaker vibrational bands are not observed.

The threshold pulse energy can be reduced by removing the helium buffer gas responsible for collisional cooling of the ions from the quadrupole ion trap. Of course, this comes at the cost of a lower ion signal intensity (by several orders of magnitude), due to

the reduced trapping efficiency [26,40]. Removing the helium in the case of deprotonated prostaglandin D2 gives a very low ion intensity, which did not allow us to record an IRMPD spectrum with decent signal-to-noise ratio. Moreover, even in the absence of IR irradiation, the precursor ion undergoes dissociation (yield of 0.1) as a consequence of its internal energy that is not being quenched by collisional cooling. A pulse energy scan with and without helium was recorded, similar to that in Fig. 4A, at an IR frequency of 1370 cm^{-1} in order to observe more pronounced dissociation; the threshold pulse energy reduces from 3 mJ to less than 0.5 mJ upon removal of helium (not shown). Comparison of the power-corrected fragment fluence spectra (Fig. 5D) shows that a linear power scaling works reasonably well for energies in between 5 and 12.5 mJ. Large intensity deviations are visible for the most intense vibrational band just below 1600 cm^{-1} indicating extensive formation of fragments below the LMCO (and/or extensive detachment). Not being influenced by charge loss from the trap, the power corrected precursor depletion spectra (Fig. 5F) show much better agreement across the different pulse energies despite the poor signal to noise ratio of the measurements.

Fig. 6A displays the fragment fluence and precursor depletion spectrum of prostaglandin D2 recorded at 12.5 mJ in the same graph, better illustrating the mismatch in intensity at the strongest band as a result of loss of fragments due to fragmentation below the LMCO or electron detachment. Here, the values on the vertical axis indicate that about 75% of the charge is lost (value obtained from a similar analysis as shown in Fig. 4D). For comparison, panel B shows analogous spectra for the triphenylamine radical cation with m/z 245 (see Ref. [40] for details). The intensity of the precursor ion is very stable, leading to a precursor depletion spectrum with good signal-to-noise ratio. Upon IRMPD, the major fragment ion appears at m/z 244 by loss of atomic hydrogen. Additionally, a minor fragment at m/z 166 was observed (subsequent loss of a benzene ring). All fragment ions have m/z values above the LMCO, and moreover electron detachment obviously does not play a role here, so that the fragment fluence spectrum is identical to the precursor ion depletion spectrum (see Fig. 6B).

Using the fragment fluence (equation (3)) as an IRMPD intensity instead of the yield has the advantage that the intensities can be readily corrected for changes in IR laser pulse energy and irradiation time and that the IRMPD spectra can be directly compared to

Fig. 5. IRMPD spectra of deprotonated prostaglandin D2. Top panels: yield (A), fragment fluence (C) and precursor depletion (E) IR-spectra of the same data. Bottom panels: the linearly power corrected versions of the IR-spectra displayed in the top panels.

Fig. 6. The fragment fluence and precursor depletion IR-spectra of (A) deprotonated prostaglandin D2 and (B) radical cation of triphenylamine. Fragments included for prostaglandin are those indicated in Fig. 4C and for triphenylamine include the fragment ions at m/z 244 and 166.

calculated IR absorption spectra. Nevertheless, the assumption that the fragment fluence (and photodissociation rate) scales linearly with the IR laser pulse energy is only valid in cases where the dissociation threshold is low. Similar conclusions have been reported by Williams and co-workers [42]. Precursor depletion spectra suffer from fluctuations in ion intensity, but are very valuable for identifying whether the fragment fluence is underestimated as a result of electron detachment and/or fragmentation below the LMCO of the mass spectrometer.

4. Conclusions

Infrared ion spectroscopy has become a valuable tool in identifying molecules in complex mixtures. Although the appearance of spectral features in action spectra recorded on different instruments (mass spectrometers and IR lasers) may be slightly different, they are usually sufficiently similar [24,40,46–49], so that they can be employed for identification purposes across different IR/MS platforms. Obviously, it is important to accurately report relevant parameters, such that experiments can be repeated under the same conditions, at the very least on the same instrument. In a way, this is comparable to the reporting of MS/MS spectra in databases: different MS platforms may give somewhat different results, but reporting relevant parameters such as electron energy for ionization or CID parameters make these database of general use.

For the analytical application of IR ion spectroscopy, a decent reproducibility of the IR spectra recorded on a specific setup is imperative. We have demonstrated a device that maintains the laser pulse energy of the IR laser constant over time and with changing of the IR laser frequency. IRMPD spectra recorded on different days using this device indeed show good reproducibility. This is an important step in an effort to measure reference IR spectra of metabolites under well-defined conditions for inclusion in databases such as the HMDB.

Having full control over the IR laser pulse energy, we present a detailed study of the power dependence of the intensities in the IR spectrum of a representative analytical target ion, the metabolite prostaglandin D2 in its deprotonated form. At high laser pulse energies, this ion forms fragments that are not visible in the mass spectrometer either because they are below the low-mass cut-off (LMCO) of the ion trap or because they undergo electron detachment to form neutral species. An analysis is presented of how this affects the intensities in the IRMPD spectrum. Finally, we investigate how intensities scale with laser pulse energy, and illustrate the effects of a threshold pulse energy below which dissociation does not occur, as well as of strong precursor ion depletion, electron detachment, and fragmentation below the LMCO. Although many studies reporting IRMPD spectra quickly dismiss deviations

between intensities in IRMPD spectra and (computed) linear IR spectra as “artefacts of the IRMPD process”, we show here that these artefacts can often be understood and corrected for if a proper analysis is performed.

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References

- [1] J. Oomens, B.G. Sartakov, G. Meijer, G. Von Helden, Gas-phase infrared multiple photon dissociation spectroscopy of mass-selected molecular ions, *Int. J. Mass Spectrom.* 254 (1–2) (2006) 1–19.
- [2] J. Lemaire, P. Boissel, M. Heninger, G. Maclaure, G. Bellec, H. Mestdagh, A. Simon, S. Le Caer, J. Ortega, F. Glotin, Gas phase infrared spectroscopy of selectively prepared ions, *Phys. Rev. Lett.* 89 (27) (2002) 273002.
- [3] N.C. Polfer, Infrared multiple photon dissociation spectroscopy of trapped ions, *Chem. Soc. Rev.* 40 (5) (2011) 2211–2221.
- [4] J.R. Eyler, Infrared multiple photon dissociation spectroscopy of ions in Penning traps, *Mass Spectrom. Rev.* 28 (3) (2009) 448–467.
- [5] N.C. Polfer, J. Oomens, Vibrational spectroscopy of bare and solvated ionic complexes of biological relevance, *Mass Spectrom. Rev.* 28 (3) (2009) 468–494.
- [6] N.C. Polfer, J. Oomens, Reaction products in mass spectrometry elucidated with infrared spectroscopy, *Phys. Chem. Chem. Phys.* 9 (29) (2007) 3804–3817.
- [7] L. MacAleese, P. Maitre, Infrared spectroscopy of organometallic ions in the gas phase: from model to real world complexes, *Mass Spectrom. Rev.* 26 (4) (2007) 583–605.
- [8] T.D. Fridgen, Infrared consequence spectroscopy of gaseous protonated and metal ion cationized complexes, *Mass Spectrom. Rev.* 28 (4) (2009) 586–607.
- [9] L.J. Kempkes, J. Martens, G. Berden, J. Oomens, Spectroscopic characterization of an extensive set of c-type peptide fragment ions formed by electron transfer dissociation suggests exclusive formation of amide isomers, *J. Phys. Chem. Lett.* 9 (22) (2018) 6404–6411.
- [10] K.H.B. Lam, J.K.-C. Lau, C.-K. Lai, I.K. Chu, J. Martens, G. Berden, J. Oomens, A.C. Hopkinson, K.W.M. Siu, Loss of water from protonated polyglycines: interconversion and dissociation of the product imidazolone ions, *Phys. Chem. Chem. Phys.* 20 (27) (2018) 18688–18698.
- [11] J. Martens, J. Grzetic, G. Berden, J. Oomens, Structural identification of electron transfer dissociation products in mass spectrometry using infrared ion spectroscopy, *Nat. Commun.* 7 (2016) 11754.
- [12] O. Hernandez, B. Paizs, P. Maître, Rearrangement chemistry of an ions probed by IR spectroscopy, *Int. J. Mass Spectrom.* 377 (2015) 172–178.
- [13] E. Nicol, C. Clavaguéra, G. Ohanessian, Vibrational spectroscopy of deprotonated peptides containing an acidic side chain, *Int. J. Mass Spectrom.* 435 (2019) 42–50.
- [14] B. Martínez-Haya, J.R. Avilés-Moreno, F. Gámez, G. Berden, J. Oomens, Preferential host-guest coordination of nonactin with ammonium and hydroxylammonium, *J. Chem. Phys.* 149 (22) (2018) 225101.
- [15] A. Filippi, C. Fraschetti, L. Guarcini, C. Zazza, T. Ema, M. Speranza,

- Spectroscopic discrimination of diastereomeric complexes involving an axially chiral receptor, *ChemPhysChem* 18 (18) (2017) 2475–2481.
- [16] A. De Petris, B. Chiavarino, M.E. Crestoni, C. Coletti, N. Re, S. Fornarini, Exploring the conformational variability in the heme b propionic acid side chains through the effect of a biological probe: a study of the isolated ions, *J. Phys. Chem. B* 119 (5) (2015) 1919–1929.
 - [17] R.C. Dunbar, J. Martens, G. Berden, J. Oomens, Water microsolvation can switch the binding mode of Ni (II) with small peptides, *J. Phys. Chem. Lett.* 8 (12) (2017) 2634–2638.
 - [18] R.C. Dunbar, G. Berden, J. Oomens, How does a small peptide choose how to bind a metal ion? IRMPD and computational survey of CS versus Iminol binding preferences, *Int. J. Mass Spectrom.* 354 (2013) 356–364.
 - [19] T.E. Hofstetter, C. Howder, G. Berden, J. Oomens, P.B. Armentrout, Structural elucidation of biological and toxicological complexes: investigation of monomeric and dimeric complexes of histidine with multiply charged transition metal (Zn and Cd) cations using IR action spectroscopy, *J. Phys. Chem. B* 115 (43) (2011) 12648–12661.
 - [20] B. Chiavarino, M.E. Crestoni, S. Fornarini, D. Scuderi, J.-Y. Salpin, Interaction of cisplatin with 5'-dGMP: a combined IRMPD and theoretical study, *Inorg. Chem.* 54 (7) (2015) 3513–3522.
 - [21] M.B. Moghaddam, T.D. Fridgen, Structures, unimolecular fragmentations, and reactivities of the self-assembled multimetallic/peptide complexes [mnn (GlyGly-H) 2n-1]+ and [Mnn+1 (GlyGly-H) 2n] 2+, *ChemPhysChem* 17 (14) (2016) 2181–2189.
 - [22] P.D. Dau, D. Rios, Y. Gong, M.C. Michelini, J. Marçalo, D.K. Shuh, M. Mogannam, M.J. Van Stipdonk, T.A. Corcovilos, J.K. Martens, G. Berden, J. Oomens, B. Redlich, J.K. Gibson, Synthesis and hydrolysis of uranyl, neptunyl, and plutonyl gas-phase complexes exhibiting discrete actinide–carbon bonds, *Organometallics* 35 (9) (2016) 1228–1240.
 - [23] S. Chakrabarty, M.J. DiTucci, G. Berden, J. Oomens, E.R. Williams, Structural investigation of the hormone melatonin and its alkali and alkaline earth metal complexes in the gas phase, *J. Am. Soc. Mass Spectrom.* 29 (9) (2018) 1835–1847.
 - [24] M.U. Munshi, S.M. Craig, G. Berden, J. Martens, A.F. DeBlase, D.J. Foreman, S.A. McLuckey, J. Oomens, M.A. Johnson, Preparation of labile Ni+ (cyclam) cations in the gas phase using electron-transfer reduction through ion–ion recombination in an ion trap and structural characterization with vibrational spectroscopy, *J. Phys. Chem. Lett.* 8 (20) (2017) 5047–5052.
 - [25] D. Corinti, C. Coletti, N. Re, R. Paciotti, P. Maitre, B. Chiavarino, M.E. Crestoni, S. Fornarini, Short-lived intermediates (encounter complexes) in cisplatin ligand exchange elucidated by infrared ion spectroscopy, *Int. J. Mass Spectrom.* 435 (2019) 7–17.
 - [26] J. Martens, G. Berden, C.R. Gebhardt, J. Oomens, Infrared ion spectroscopy in a modified quadrupole ion trap mass spectrometer at the FELIX free electron laser laboratory, *Rev. Sci. Instrum.* 87 (10) (2016) 103108.
 - [27] L. Mac Aleese, A. Simon, T.B. McMahon, J.-M. Ortega, D. Scuderi, J. Lemaire, P. Maitre, Mid-IR spectroscopy of protonated leucine methyl ester performed with an FTICR or a Paul type ion-trap, *Int. J. Mass Spectrom.* 249 (2006) 14–20.
 - [28] L. Hamlow, Y. Zhu, Z.J. Devereaux, N. Cunningham, G. Berden, J. Oomens, M.T. Rodgers, Modified quadrupole ion trap mass spectrometer for infrared ion spectroscopy: application to protonated thiated uridines, *J. Am. Soc. Mass Spectrom.* 29 (11) (2018) 2125–2137.
 - [29] A.F. Rodrigues-Oliveira, F.W. M. Ribeiro, G. Cervi, T. C. Correra, Evaluation of common theoretical methods for predicting infrared multiphotonic dissociation vibrational spectra of intramolecular hydrogen-bonded ions, *ACS Omega* 3 (8) (2018) 9075–9085.
 - [30] B. Schindler, G. Renois-Predelus, N. Bagdadi, S. Melizi, L. Barnes, S. Chambert, A.-R. Allouche, I. Compagnon, MS/IR, a new MS-based hyphenated method for analysis of hexuronic acid epimers in glycosaminoglycans, *Glycoconj. J.* 34 (3) (2017) 421–425.
 - [31] J. Martens, V. Koppen, G. Berden, F. Cuyckens, J. Oomens, Combined liquid chromatography–infrared ion spectroscopy for identification of regioisomeric drug metabolites, *Anal. Chem.* 89 (8) (2017) 4359–4362.
 - [32] J. Martens, G. Berden, H. Bentlage, K.L.M. Coene, U.F. Engelke, D. Wishart, M. van Scherpenzeel, L.A.J. Kluijtmans, R.A. Wevers, J. Oomens, Unraveling the unknown areas of the human metabolome: the role of infrared ion spectroscopy, *J. Inher. Metab. Dis.* 41 (3) (2018) 367–377.
 - [33] J. Martens, G. Berden, R.E. Outersterp, L.A.J. Kluijtmans, U.F. Engelke, C.D.M. van Karnebeek, R.A. Wevers, J. Oomens, Molecular identification in metabolomics using infrared ion spectroscopy, *Sci. Rep.* 7 (1) (2017) 3363.
 - [34] A.P. Cismesia, M.R. Bell, L.F. Tesler, M. Alves, N.C. Polfer, Infrared ion spectroscopy: an analytical tool for the study of metabolites, *Analyst* 143 (7) (2018) 1615–1623.
 - [35] O. Gorlova, S.M. Colvin, A. Brathwaite, F.S. Menges, S.M. Craig, S.J. Miller, M.A. Johnson, Identification and partial structural characterization of mass isolated valsartan and its metabolite with messenger tagging vibrational spectroscopy, *J. Am. Soc. Mass Spectrom.* 28 (11) (2017) 2414–2422.
 - [36] F. Berthias, B. Maatoug, G.L. Glush, F. Moussa, P. Maitre, Resolution and assignment of differential ion mobility spectra of sarcosine and isomers, *J. Am. Soc. Mass Spectrom.* (2018) 1–9.
 - [37] D.S. Wishart, D. Tzur, C. Knox, R. Eisner, A.C. Guo, N. Young, D. Cheng, K. Jewell, D. Arndt, S. Sawhney, HMDB: the human metabolome database, *Nucleic Acids Res.* 35 (suppl_1) (2007) D521–D526.
 - [38] D.S. Wishart, Y.D. Feunang, A. Marcu, A.C. Guo, K. Liang, R. Vázquez-Fresno, T. Sajed, D. Johnson, C. Li, N. Karu, HMDB 4.0: the human metabolome database for 2018, *Nucleic Acids Res.* 46 (D1) (2017) D608–D617.
 - [39] L.A. Garza, Y. Liu, Z. Yang, B. Alagesan, J.A. Lawson, S.M. Norberg, D.E. Loy, T. Zhao, H.B. Blatt, D.C. Stanton, Prostaglandin D2 inhibits hair growth and is elevated in bald scalp of men with androgenetic alopecia, *Sci. Transl. Med.* 4 (126) (2012) 126ra34.
 - [40] M.U. Munshi, G. Berden, J. Martens, J. Oomens, Gas-phase vibrational spectroscopy of triphenylamine: the effect of charge on structure and spectra, *Phys. Chem. Chem. Phys.* 19 (30) (2017) 19881–19889.
 - [41] B. Lucas, G. Gregoire, J. Lemaire, P. Maitre, F. Glotin, J. Schermann, C. Desfrancois, Infrared multiphoton dissociation spectroscopy of protonated N-acetyl-alanine and alanyl-histidine, *Int. J. Mass Spectrom.* 243 (2) (2005) 105–113.
 - [42] J.S. Prell, J.T. O'Brien, E.R. Williams, IRPD spectroscopy and ensemble measurements: effects of different data acquisition and analysis methods, *J. Am. Soc. Mass Spectrom.* 21 (5) (2010) 800–809.
 - [43] J.D. Steill, J. Oomens, Action spectroscopy of gas-phase carboxylate anions by multiple photon IR electron detachment/attachment, *J. Phys. Chem. A* 113 (17) (2009) 4941–4946.
 - [44] J. Gao, G. Berden, J. Oomens, Laboratory infrared spectroscopy of gaseous negatively charged polyaromatic hydrocarbons, *Astrophys. J.* 787 (2) (2014) 170.
 - [45] M. Haertelt, V.J. Lapoutre, J.M. Bakker, B. Redlich, D.J. Harding, A. Fielicke, G. Meijer, Structure determination of anionic metal clusters via infrared resonance enhanced multiple photon electron detachment spectroscopy, *J. Phys. Chem. Lett.* 2 (14) (2011) 1720–1724.
 - [46] M.Z. Kamrath, E. Garand, P.A. Jordan, C.M. Leavitt, A.B. Wolk, M.J. Van Stipdonk, S.J. Miller, M.A. Johnson, Vibrational characterization of simple peptides using cryogenic infrared photodissociation of H2-tagged, mass-selected ions, *J. Am. Chem. Soc.* 133 (16) (2011) 6440–6448.
 - [47] A.L. Patrick, C.N. Stedwell, B. Schindler, I. Compagnon, G. Berden, J. Oomens, N.C. Polfer, Insights into the fragmentation pathways of gas-phase protonated sulfoserine, *Int. J. Mass Spectrom.* 379 (2015) 26–32.
 - [48] T. Baer, R.C. Dunbar, Ion spectroscopy: where did it come from; where is it now; and where is it going? *J. Am. Soc. Mass Spectrom.* 21 (5) (2010) 681–693.
 - [49] J.C. Poutsma, J. Martens, J. Oomens, P. Maitre, V. Steinmetz, M. Bernier, M. Jia, V. Wysocki, Infrared multiple-photon dissociation action spectroscopy of the b 2+ ion from PPG: evidence of third residue affecting b 2+ fragment structure, *J. Am. Soc. Mass Spectrom.* 28 (7) (2017) 1482–1488.