International Journal of Mass Spectrometry 306 (2011) 196–203

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Two-dimensional FT-ICR/MS with IRMPD as fragmentation mode

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ARTICLE INFO

Article history:
Received 27 August 2010
Received in revised form 22 October 2010
Accepted 27 October 2010
Available online 12 November 2010

Keywords:
FT-ICR
2-Dimensional
IRMPD
FTMS
Pulse sequence
Double-frequency

ABSTRACT

In 1988, Gäumann et al. introduced a pulse sequence for two-dimensional FT-ICR/MS correlating parent ions and fragment ions without the need for ion isolation. The improvement in computer technology makes this pulse sequence analytically useful in order to obtain structural information on complex samples. The pulse sequence can be applied to all cyclotron radius-dependent fragmentation modes, including gas-free fragmentation modes like IRMPD, which do not affect sensitivity and resolving power like the pulsing of a gas into the ICR cell does. This study shows the feasibility of 2D FT-ICR/MS and lays the groundwork to turn this method into a viable analytical tool.

1. Introduction

Two-dimensional FT-ICR/MS [1] was first introduced by Gäumann et al. in 1987 in order to study ion-molecule reactions and fragmentations in an ICR cell [2–5]. It was based on an experiment by Marshall et al. in 1984 [6] proving that it is possible to de-excite ion packets in the ICR cell. The 2D FT-ICR/MS experiment was modeled after NOESY spectroscopy in 2D-NMR [7].

By comparing the relative ICR magnitude after an increasingly long excitation pulse and a pulse with a phase inversion after 1 ms, Marshall's experiment demonstrated for the first time that coherent ions may be de-activated in an ICR cell by inverting the phase of the excitation voltage. Gäumann et al. applied this finding to excitation pulses over a wide mass range by inserting an incremental delay between two excitation pulses.

The resulting pulse sequence is shown in Fig. 1. P_1 and P_2 are two identical excitation pulses and t_1 the delay between them. During the application of P_1, ions are excited and continue to orbit in phase with the voltage of P_1 during t_1. The phase difference between the ion motion and the radiofrequency voltage of P_2 depends on their cyclotron frequency and t_1. When ions are in phase with the radiofrequency voltage during P_2 ($\Delta\varphi = 2k\pi$), they are excited to a higher radius, but when they are out of phase with P_2 ($\Delta\varphi = 2(k+1)\pi$), ions are focused back to the center of the ICR cell, which is consistent with Marshall's experiment.

Gäumann showed that, for a given m/z ratio, the phase difference between the ion motion and the excitation voltage is a linear function of the cyclotron frequency and t_1:

$$\Delta\varphi = (\omega_{ion} - \omega_{ref}) \times t_1 + \varphi_0 \quad (1)$$

$\Delta\varphi$ is the phase difference between the ion motion and the excitation voltage, ω_{ion} is the cyclotron frequency of the ion, ω_{ref} is a reference frequency (in practice, it corresponds to the highest frequency of the pulse, i.e., the cyclotron frequency of the lowest m/z ratio of the mass range, and is due to the fact that the experiment was performed in heterodyne mode in the second dimension), and φ_0 is a constant term. As a result, if the experiment is repeated at incremental values of t_1, the signal magnitude of the ions is a periodic function of t_1 and its frequency is the ions' cyclotron frequency offset by a reference frequency [8].

During the subsequent mixing interval τ_m, fragmentations and ion-molecule reactions are triggered. In Gäumann's first experiments, a reacting gas was introduced in the ICR cell during τ_m for that goal [2–5], but it was also achieved by pulsing a laser in the ICR cell [5]. The abundance of fragment ions or reaction products depends on the cyclotron radius of the parent ions: in the case of a laser pulse, ions with a high cyclotron radius are outside the laser beam and therefore do not fragment. Only ions that have been de-excited and are therefore in the path of the laser beam can be fragmented. In the case of a reacting gas, the collision frequency

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doi:10.1016/j.ijms.2010.10.034

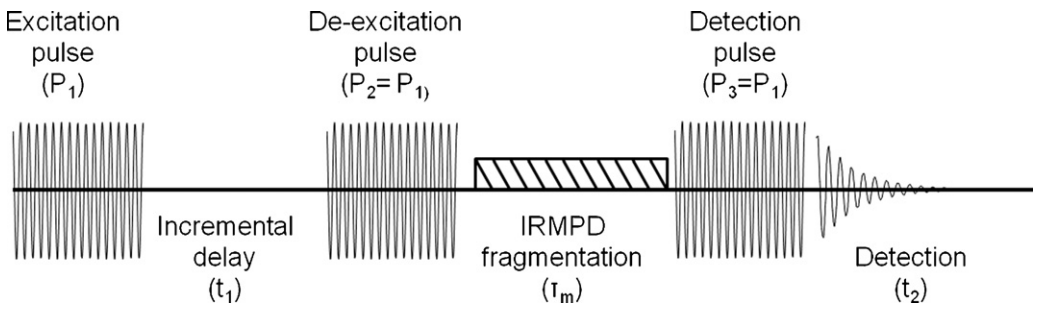

Fig. 1. Pulse sequence for a two-dimensional mass spectrometry experiment.

and energy, which determine the reaction yield, depend on the cyclotron radius of the parent ions: the kinetic energy of the ions depends on their cyclotron frequency, and therefore fragmentation rates depend on it, too. In both cases, the abundance of fragment ions depends on the radius of parent ions. The signal magnitude of fragment ions has therefore the same dependency on t_1 as the signal magnitude of parent ions.

The subsequent excitation pulse brings parent and fragment ions to a high cyclotron radius and the time transient is detected at the incremented dates t_2. The Fourier transform according to t_2 gives the spectrum of the cyclotron frequencies of the ions, and the Fourier transform according to t_1 gives the modulation frequencies of the abundance of the ions. The resulting two-dimensional spectrum has a characteristic self-correlation line, which reflects the mass spectrum of the sample. The MS/MS parent ion spectrum of each ion species can be read horizontally. The MS/MS fragment ion spectrum of each fragment or product ion can be read vertically. This method was applied by Gäumann et al. for fragmentations by IRMPD and ion-molecule reactions by pulsing a reacting gas during τ_m. They published results for ions of low m/z ratios (<200 amu) using the heterodyne mode in the second dimension and with a narrow mass range (m/z 10–300) and with low resolution in both directions of the spectra: 256 × 1024 data points.

Other methods to record two-dimensional mass spectra have been proposed. Stored-Waveform Ion Radius Modulation (SWIM) was developed by Ross et al. in 1993 in order to modulate ion radii sinusoidally for 2D spectra using CID [9,10]. Hadamard transform FT-ICR/MS, in which a Hadamard comb was applied to an excitation pulse [11–14], was introduced by Williams and McLafferty.

IRMPD is a fragmentation mode that is used extensively in FT-ICR/MS for in-cell MS/MS [15–17]: it is routinely installed on commercial instruments, it does not require gas to be pulsed and therefore does not affect the vacuum in the ICR cell and it is simple to use. While IRMPD does not completely mimic CID, it is efficient, both for odd-electron ions and even-electron ions, and the resulting fragments mostly follow known fragmentation paths.

In this study, we show that the 2D FT-ICR/MS experiment performed by Gäumann et al. with IRMPD is feasible with an external ionization source, with the mass range and mass resolution that have become standard in mass spectrometry in the last 20 years, and with a resolution in the second dimension that had hitherto never been obtained in any 2D FT-ICR/MS experiment. We performed the experiment on a peptide and a peptide mixture and we show that 2D FT-ICR/MS has the potential to become a fully-fledged analytical method that can be used to get extensive structural information on complex samples.

2. Experimental methods

Experiments were performed on a 9.4 T ApexQE FT-ICR/MS from Bruker Daltonics (Bremen, Germany) with a positive nanoESI ion source. The peptides used in this study were angiotensin I, substance P and fragments 1–8 of bradykinin purchased from Sigma–Aldrich (Saint Louis, MO, USA) and dissolved in a 50:50 methanol/water mixture with 0.1% formic acid.

The first sample was composed of angiotensin I alone at 1 pmol/μL, and the second sample was a mixture of all thee peptides at 1 pmol/μL each. The methanol and formic acid were obtained from Merck (Darmstadt, Germany) and the water was deionized with a MilliQ water filter system purchased from Millipore (Billerica, MA, USA). The samples were injected in the nanoESI ion source at a 200 nL/min flow rate using a 100 μL syringe (Hamilton, Bonaduz, Switzerland) and an automated syringe pump (Cole Parmer, Vernon Hills, Ill, USA). Ions were accumulated for 1.0 s in the accumulation octopole before being transferred to the ICR cell.

The event sequence and the acquisition of spectra were controlled with the Apex Control software, in LC mode, using a modified pulse program reflecting the event sequence shown in Fig. 1. For the purpose of this study, all pulses were identical in duration, amplitude and frequency range. They were built using the Apex Control (Bruker, Bremen, Germany) pulse generator. Pulse amplitude attenuation was 7.0 dB (i.e., identical to the default pulse amplitude attenuation of excitation pulses) and pulse length was 1.0 μs per frequency (the default excitation pulse length is 20.0 μs per frequency). The frequency list had a 2.0 kHz increment.

Ions were excited and detected over a m/z 86.67–2000 mass range. The length of the time transients was 32,768 data points, which is relatively short for FT-ICR/MS experiments. This choice was imposed by data processing considerations: the programs used in order to calculate the Fourier transforms, being designed for NMR, were not able to handle very large data files due to its 32 bit implementation.

The two-dimensional mass spectra presented in this study were obtained with IRMPD as a fragmentation mode using a continuous CO_2 laser with a 10.6 μm wavelength and 25 W power. The laser power and the pulse length were computer-controlled. Experiments were conducted at 50% laser power and τ_m = 100 ms. The delay between the two first pulses varied with a 0.3 μs increment, giving a Nyquist frequency of 1667 kHz in the second dimension. 2048 transients of 32,768 data points were acquired and stored as a binary file, allowing the same m/z range (86.67–2000) to be detected in both dimensions. The receiver that processed the acquired ion signal had a 3 kHz–10 MHz frequency range.

The data files were imported and Fourier transformed using NPK (NMR Processing Kernel), a program developed for the data analysis of NMR data [18]. The Fourier transform was adjusted to FT-ICR/MS experiments: no phase correction was performed on the data, and the modulus was applied to the result. The spectra were plotted using NMRNotebook 2.60, a software purchased from NMRTec (Illkirch-Graffenstaden, France) and developed to process, visualize and analyze 1D and 2D NMR spectra.

A simulation of the 2D parent ion spectrum of m/z 433 (f = 333 kHz) was undertaken using the Fast Fourier Transform