THE COMBINATION OF ION MOBILITY WITH ORTHOGONAL TOF MASS SPECTROMETRY TO IMPROVE THE LIMITS OF DETECTION FOR MALDI ANALYSIS

Daniel Kenny, Jeff Brown, Kevin Giles, Steve Pringle, Jason Wildgoose, Bob Bateman
Waters Corporation, MS Technologies Centre, Manchester, M23 9LZ, UK

OVERVIEW

- **Experiments were carried out on experimental instrumentation incorporating IMS and TOF MS with a vacuum-MALDI ion source.**
- **Ions emerging from the rear of a travelling wave mobility separator are partitioned into packets using a second travelling wave device.**
- **The orthogonal accelerator runs synchronously with the ion packets, but with a delay time matched to the m/z values emerging from the mobility separator.**
- **This technique allows the duty cycle of the orthogonal-TOF to be increased across the whole m/z range.**

INTRODUCTION

MALDI axial TOF mass spectrometers have a high duty cycle and sensitivity when used to record full spectra. In comparison, MALDI orthogonal TOF mass spectrometers have a lower sampling duty cycle, typically between 10% and 25%, and consequently lower sensitivity.

One method used to maximise duty cycle consists of trapping and releasing ions and timing the orthogonal accelerator pulse to coincide with the arrival of ions of interest. Another method reported previously[1] consists of synchronising the orthogonal accelerator with a travelling wave used to partition a continuous stream of ions into packets. Both methods, however, only improve the duty cycle over a limited mass range.

To extend the duty cycle improvement over a wider m/z range an additional stage of separation prior to the partitioning travelling wave device is required. This extra stage of separation restricts the m/z range in each packet of ions. For each individual packet, the synchronisation between the release of the packet of ions and the orthogonal accelerator push may then be adjusted to the m/z range in the packet. In this work we describe how ion mobility may be used as the separation stage to increase the duty cycle of the orthogonal TOF and how this results in an improvement in detection limits.

EXPERIMENTAL

Instrumentation

The experiments were carried out on experimental instrumentation incorporating IMS and TOF MS (Figure 1). The mobility separator (based on T-Wave technology) is a stacked ring electrode device with opposite phases of RF applied to adjacent electrodes to provide radial ion confinement. A DC voltage pulse pattern is applied to the ring electrodes so as to provide travelling voltage 'waves' which propel ions through the gas-filled device. Through appropriate choice of the 'wave' parameters mobility separation can be achieved as less mobile ions roll over the 'wave' more often than the high mobility ions[2].

The travelling wave ion mobility seperator (TWIMS) comprises three T-Wave devices, the first device (accumulation T-Wave) accumulates ions and releases them in a short pulse (100µs) into the next device (IMS T-Wave) in which the mobility separation is performed. The final device (transfer T-Wave) is used to partition and transport the separated ions into the oa-ToF for subsequent analysis.

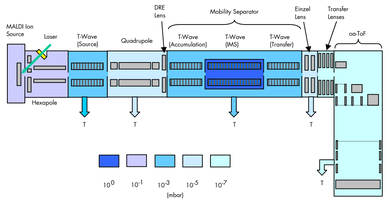


Figure 1. Schematic diagram of the test bench instrument.

To obtain a mobility plot, the oa-Tof push number is recorded with push 1 being the first push after release of ions into the TWIMS. After 200 pushes have been recorded, the process is repeated until the acquisition is completed. Each block of 200 pushes are summed so that each push number corresponds to a particular drift time, where the drift time is calculated from the push number multiplied by the pusher period. Mass chromatograms can then be produced showing the drift time profiles for each ion.

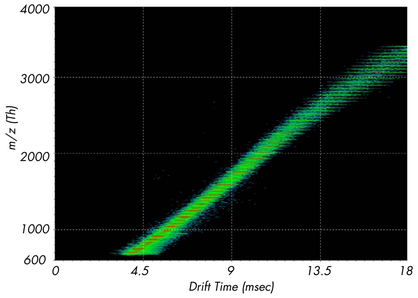


Figure 2. 2-D Mobility plot for a Mixture of PEG 600,1000, 2000 and 3000.

An example of a typical mobility seperation experiment is shown in Figure 2. This mobility plot charts drift time along the x-axis versus m/z on the y-axis. Intensity is depicted using a colour scale from black for zero through blue, green and finally red to indicate 100% intensity. For the mixture of PEGs used in this experiment, all of the ions are singly charged and follow an approximately linear correlation between m/z and drift time.

Instead of being used to acquire mobility type data, the orthogonal accelerator may also be arranged to run synchronously with the second travelling wave device but with a delay time that progressively increases so as to track the m/z values of ions emerging from the TWIMS. In this manner, the duty cycle across the whole mass range may be increased.

Figure 3 displays mobility profiles for the same PEG dataset as in Figure 2. The top (red) trace plots the total ion count (TIC) whereas the bottom traces (blue) plot drift profiles for selected ions. By plotting the centre of each drift profile vs m/z the exact correlation between drift time and m/z can be determined for this known compound. It is thereby possible to calibrate the synchronisation between the release of each packet of ions from the TWIMS and the orthogonal accelerator pusher to the m/z range in each packet.

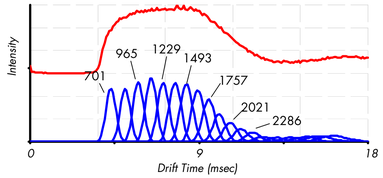


Figure 3. Mass chromatograms for a Mixture of PEGs. Top (red) is a TIC trace. Bottom (blue) individual drift profiles for selected ions.

Sample Preparation

For the duty cycle experiments, a dilution series of a standard tryptic digest (Glycogen Phosphorylase B) was mixed with α-cyano-4-hydroxycinnamic acid (CHCA) matrix at a concentration of 3.3 mg/mL and spotted onto a standard stainless steel target plate. The quantities spotted were 5 fmol, 2 fmol, 1 fmol, 500 attomol, 250 attomol and 125 attomol. No additives were included in the matrix.

Data Acquisition

The laser of the MALDI ion source was focussed to a spot diameter of approximately 250 µm and operated at a repetition rate of 200 Hz. For the duty cycle experiments, data were acquired for each sample quantity in both the normal mode of operation with the standard duty cycle and with ion mobility separation and increased duty cycle by ablating a stripe of material through the centre of each sample spot. Each acquisition took approximately 1 minute to complete. The data were processed using ProteinLynx Global SERVER (Waters Corp.) and database searched using Mascot (Matrix Science).

RESULTS

Figure 4 shows a mobility plot for a tryptic digest of Glycogen Phosphorylase B. The majority of the ions are singly charged and lie within the same mobility band. However, doubly and higher charge states lie in separate bands as marked in the figure. Data from within these bands may be isolated and reformed into mass spectra containing peaks of only one particular charge state. An example of this is shown in Figure 5.

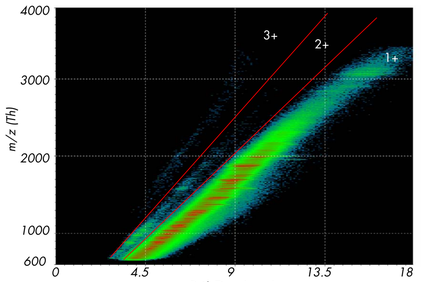


Figure 4. 2-D Mobility plot for Glycogen Phosphorylase B tryptic digest.

The top (red) spectra in figures 5 (a) and (b) are mass spectra acquired without using IMS and so contain ions of all charge states. The bottom (purple) data contain only doubly charged ions and were acquired using IMS. These doubly charged spectra were produced by isolating and combining the data between the two red lines marked in Figure 4.

In Figure 5 (a), two very strong doubly charged ions are visible in the IMS separated spectrum. Figure 5 (b) is an expanded view of the ions around 943 Da. It is clear by comparing the upper (red) trace with the lower (purple) trace that without the ability to perform mobility separation it would have been difficult to identify the doubly charged ion because of the strong singly charged ion which appears within a similar mass range. The same is also true of the doubly charged ion at mass 845 Da.

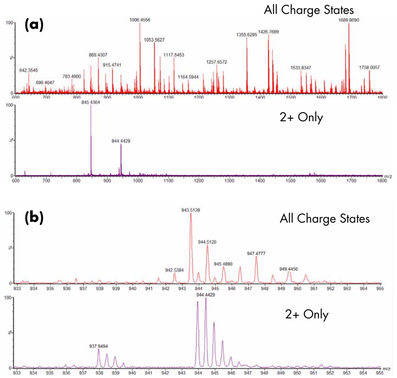
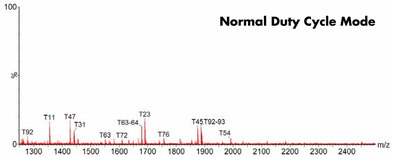


Figure 5 (a) and (b). Top (red) spectra contain ions of all charge states. Bottom (purple) spectra contain only 2+ ions.



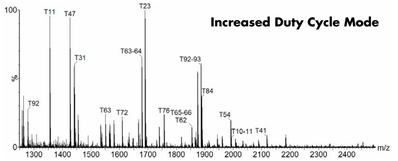


Figure 7. Mass spectra of 1 fmol Glycogen Phosphorylase B showing the difference in signal intensity obtained between normal and increased duty cycle modes.

Figure 7 shows data obtained from 1 fmol of a tryptic digest of Glycogen Phosphorylase B with the instrument running in both normal and increased duty cycle mode.. It is immediately obvious that the use of ion mobility to increase the duty cycle has increase the signal level several fold.

Figure 8 is a comparison of the duty cycle obtained using this method as a function of mass for the fragment ions of Glufibrinopeptide. Across the whole mass range the duty cycle has been improved to between 80% and 97%. This is equivalent to a gain factor of 4 at higher mass and >10 at low mass.

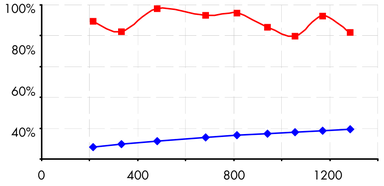


Figure 8. Plots of normal duty cycle (blue) and increased duty cycle (red) for fragments of Glufibrinopeptide.

To further investigate the improvement in duty cycle and hence the improvement in detection limits at low levels, a dilution series ranging from 5 fmol to 125 attomol of a tryptic digest (Glycogen Phosphorylase B) were analysed. Data were again acquired for each quantity in both the normal mode of operation with the standard duty cycle and with ion mobility separation with increased duty cycle.

For the two highest sample concentrations, three repeat acquisitions were made. For the remaining lower concentrations six acquisitions at each concentration were made to reduce the effect of statistical scatter in the protein identification scores.

After the data were acquired it was database searched using Mascot using standard search parameters. Figure 9 shows the average Mascot score for the six different sample concentrations.

At 1 fmol and above the enhanced sensitivity gained from running with increased duty cycle made only a small improvement in the Mascot score (although the Mascot score is logarithmic and so even this small improvement is still significant). Below 1 fmol the improvement in Mascot score is marked. This is clearly highlighted at 125 attomol as the average score of 31 is well below the score of 67 required for a confident identification at the 95% confidence level. Moreover, at 125 attomol, three of the six repeat measurements were unable to provide any identification for the protein.

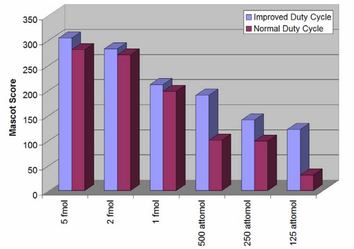


Figure 9. Average Mascot scores for different concentrations of a tryptic digest of Glycogen Phosphorylase B in normal and improved duty cycle modes.

CONCLUSION

- **Novel experimental instrumentation incorporating IMS and TOF MS.**
- **IMS allows different charge state MALDI ions to be isolated.**
- **The use of ion mobility in this instrument allows the duty cycle of the orthogonal-TOF to be increased across the whole m/z range.**
- **The extra sensitivity gained from the increase in duty cycle allows confident protein identifications to be made from sample concentrations as low as 125 attomol.**

References

1. "A comparison of methods of improving the duty cycle on orthogonal tof mass analysers" Jason Wildgoose, Steven Pringle, Kevin Giles, Robert Bateman, Steve Bajic. Presented at the 53rd ASMS Conference, San Antonio, USA 2005.
2. "Travelling Wave Ion Propulsion in Collision Cells" K. Giles, S.Pringle, K. Worthington and R. Bateman. Presented at the 51st ASMS Conference, Montreal, Canada 2003. The travelling wave device described here is similar to that described by Kirchner in US Patent 5,206,506 (1993).

720001703EN

TO DOWNLOAD A COPY OF THIS POSTER VISIT WWW.WATERS.COM/POSTERS