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INVESTIGATIONS INTO A NOVEL ION MOBILITY SEPARATOR COMPRISING A TRAVELLING WAVE ENABLED RF-ONLY STACKED-RING ION GUIDE

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OVERVIEW

Aim

- To characterise the performance of a new type of ion mobility separation device based on a stacked-ring RF ion guide with a travelling voltage wave.

Methods

- A modified Q-ToF has been constructed with a travelling wave based collision cell together with enhanced acquisition software to provide arrival time data of the ions at the ToF analyzer.

Results

- The travelling wave based mobility separator exhibits good separation characteristics at operating pressures around 0.2 mbar.
- Separation of both low molecular weight ions and multiply charged protein ions can be achieved.
- The system transmission in mobility mode is comparable to that in the standard MS modes of operation making it significantly more sensitive than existing DC-only mobility spectrometers.

INTRODUCTION

The separation of ion species according to their mobilities in a buffer gas is a technique that has been around for over thirty years. It has been successfully used in portable devices for rapid detection of chemical warfare agents and for the detection of explosives and illegal drugs. More recently the technique has been applied to more fundamental investigations into the structure and conformation of bio-molecules. The classical Ion Mobility Separator uses a static DC voltage gradient to drive ions through the buffer gas. Reported here is a new approach to mobility-based separation of ions using a travelling voltage wave in an ion guide.

OVERVIEW OF THE TRAVELLING WAVE ION GUIDE

The utility of a travelling voltage wave superimposed on the confining RF of a stacked-ring ion guide to propel ions efficiently through a collision cell has been presented previously [1]. With the appropriate choice of wave velocity, wave amplitude and gas pressure, the ions can be made to 'surf' on the wave, reducing their residence time in the cell (see Figure 1(a)). However, as the wave amplitude is decreased or the velocity increased, ions of lower mobility can no longer keep up with the wave and roll over the top (see Figure 1(b)). This process is repeated as successive waves pass through the cell and produces a mobility-based separation of the entrained ions.

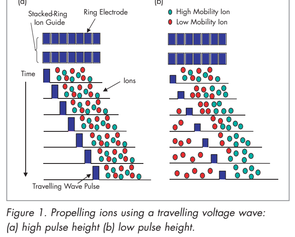

Figure 1. Propelling ions using a travelling voltage wave: (a) high pulse height (b) low pulse height.

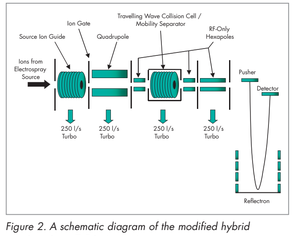

Figure 2. A schematic diagram of the modified hybrid quadrupole oa-ToF instrument.

EXPERIMENTAL

To investigate the performance of the travelling wave based ion mobility separator (TWIMS) a modified Waters hybrid Quadrupole oa-ToF instrument was utilised. A schematic of the instrument is shown in Figure 2. A separate pumping chamber was created around the TWIMS to allow operation at elevated pressures.

The TWIMS cell is based on a Waters Quattro Premier T-Wave collision cell, a photograph of which is shown in Figure 3. The cell has 122 ring electrodes mounted between two printed circuit boards (PCB). Each ring has an internal diameter of 5 mm, a thickness of 0.5 mm and is spaced 1.0 mm from the next. The cell is terminated by DC only lenses with 2 mm diameter apertures.

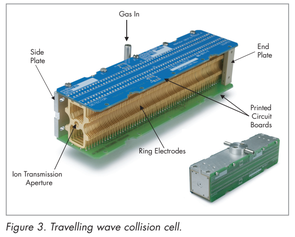

Figure 3. Travelling wave collision cell.

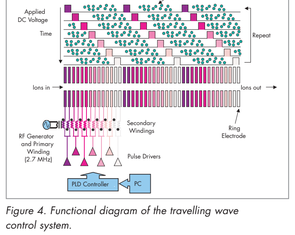

Figure 4. Functional diagram of the travelling wave control system.

The electrodes in the cell are divided into repeat sections of seven pairs with interconnections made on the supporting PCBs. Each pair of electrodes are connected to separate DC amplifiers which supply both the travelling wave voltage and any DC offset. The confining phase/anti-phase RF voltages for each pair of electrodes are superimposed onto the DC voltages via secondary windings of an RF transformer. The travelling wave pulse pattern is controlled via a programmable logic device (PLD) connected to a PC. Figure 4 shows a functional diagram of the TWIMS control system with the electrode grouping illustrated using matching colours.

Ion mobility spectra are acquired by storing ions in the source ion guide and gating them periodically to the TWIMS using the ion gate. Temporal acquisition of the mobility separated ions is provided by the ToF data acquisition system. This has been modified such that following a trigger from the ion gate, successive 'pushes' or mass spectra are recorded separately in sequence until 200 have been acquired; then the next gate pulse starts the process again, with the new set of 200 spectra added to the previous 200 and so on. The timings are shown in Figure 5.

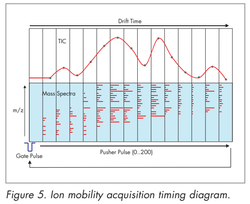

Figure 5. Ion mobility acquisition timing diagram.

In all of the studies undertaken, ions were generated using electrospray ionisation with a sample infusion rate of 5 µL/min.

RESULTS

System Performance Evaluation

Preliminary investigations indicated that the cell operated optimally with an applied RF of 350 V pk-pk at 2.7 MHz, these values were used throughout together with an ion gate pulse width of 200 µs. Unless otherwise stated a wave velocity of 300 m/sec has been used.

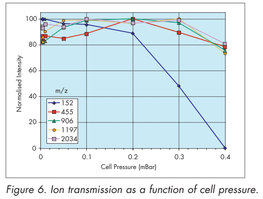

Figure 6. Ion transmission as a function of cell pressure.

Figure 6 shows the ion transmission properties of the cell over a pressure range of 0.005 to 0.4 mbar of Argon for several ion species from m/z 152 to 2034 (from a solution of 4-acetamidophenol, Verapamil, PEG 1000 and Triacetyl ß Cyclodextrin). At each pressure the instrument was tuned for best transmission. Generally, high transmission efficiency is maintained up to 0.2 mbar. Note that with 0.2 mbar in the cell, a pressure of 0.0013 mbar was maintained in the surrounding chamber generating pressures in the adjacent chambers close to those found in normal MS/MS mode.

To evaluate the mobility separation capabilities of the cell, a mixture of Gramicidin-S (m/z 571 2+) and Leucine Enkephalin (m/z 556 1+) in 1:1 Water:Acetonitrile 1% acetic acid was infused and the drift times recorded as a function of cell pressure and travelling wave pulse height. Figure 7 shows the results at cell pressures of 0.025, 0.075 and 0.2 mbar. As can be seen, the separation capability improves dramatically with increased pressure. Below 0.025 mbar, little or no separation was achieved. Note, the drift time shown includes ion flight time from the gate to the cell and from the cell to the ToF.

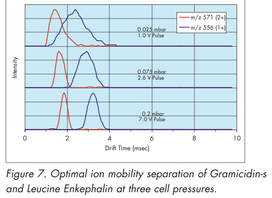

Figure 7. Optimal ion mobility separation of Gramicidin-s and Leucine Enkephalin at three cell pressures.

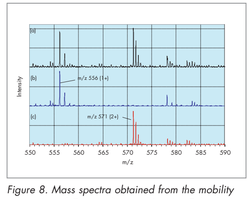

Figure 8. Mass spectra obtained from the mobility separation of Gramicidin-s and Leucine Enkephalin at 0.2 mbar (a) no separation (b) m/z 556 mobility peak and (c) m/z 571 mobility peak from Figure 7.

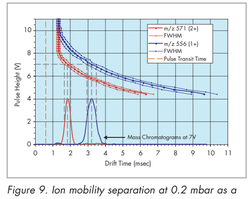

Figure 9. Ion mobility separation at 0.2 mbar as a function of travelling wave pulse height.

In Figure 8, the mass spectra obtained from the data acquired at 0.2 mbar and 7 V pulse height (Fig. 7) are presented indicating good discrimination capability. Figure 9 shows the separation capability of the TWIMS as a function of travelling wave pulse height.

In Figure 10, the separation of the m/z 571 and 556 peaks were recorded at 0.2 mbar using a constant pulse height of 7 V and varying the wave velocity from 90 to 600 m/sec. At 90 m/sec the peaks have the same drift time which then reduces with increasing wave velocity up to 180 m/sec. At this point the lower mobility m/z 556 ion starts to roll over the wave and its drift time increases, whereas the more mobile m/z 571 drift time continues to reduce up to a wave velocity of 240 m/sec when it also starts to roll over the top of the wave.

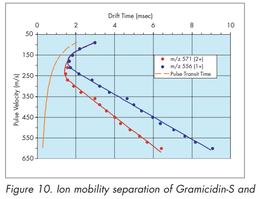

Figure 10. Ion mobility separation of Gramicidin-S and Leucine Enkephalin at 0.2 mbar, 7 V pulse height and various travelling wave velocities.

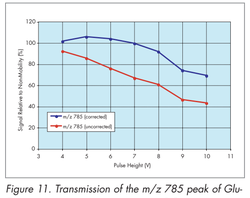

Figure 11. Transmission of the m/z 785 peak of Glu-Fibrinopeptide in mobility mode at 0.2 mbar compared with transmission in non-mobility mode. The corrected data account for dead-time effects in the acquisition system.

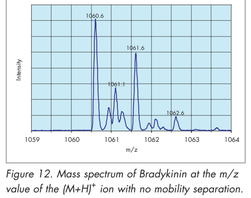

Figure 12. Mass spectrum of Bradykinin at the m/z value of the $(M+H)^+$ ion with no mobility separation.

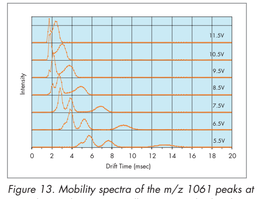

Figure 13. Mobility spectra of the m/z 1061 peaks at 0.2 mbar and various travelling wave pulse heights.

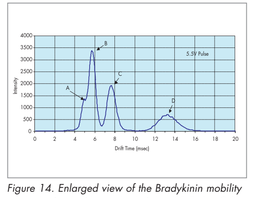

Figure 14. Enlarged view of the Bradykinin mobility peak obtained with a pulse height of 5.5 V at 0.2 mbar. Four peaks are apparent.

The data presented in Figure 6 indicate that ion transmission at 0.2 mbar is as efficient as at 0.005 mbar without ion gating and mobility separation. The data presented in Figure 11 shows the transmission of the doubly charged ion of Glu-Fibrinopeptide (m/z 785) (100 fmol/µL in 1:1 Methanol: Water 1% acetic acid) in mobility separation mode compared to that with no travelling wave/ion gating. The data indicate that there is essentially no loss in sensitivity when using the mobility mode compared to standard operation with pulse voltages up to 8 V.

Bradykinin Mobility Separation

Previous DC-only drift tube mobility studies have been undertaken on the Bradykinin ion at the nominal mass-to-charge ratio of the singly protonated precursor ion, m/z 1061, where four mobility peaks were observed and attributed to multimers of the form $(M_n+nH)^{n+}$ n=1 to 4 [2]. Bradykinin (0.1 mg/mL in 1:1 Water:Acetonitrile) was infused into the TWIMS system operating at a cell pressure of 0.2 mbar and the mass spectrum obtained around m/z 1061 with no mobility separation is shown in Figure 12. The main $(M+H)^+$ ion at 1060.6 and its isotopes are apparent, along with other peaks indicating the presence higher charge state species.

In Figure 13, mobility separation of the m/z 1061 peaks has been undertaken at various travelling wave pulse heights. With a pulse height of 11.5V, two peaks are seen. As the pulse height is reduced a third peak separates out and finally evidence for a fourth peak as a shoulder appears at 6.5 V. An enlarged picture of the 5.5 V mobility spectrum is shown in Figure 14 with the four peaks labelled. The mass spectra obtained by combining data from each peak are shown in Figure 15 where the presence of the ions $(M_n+nH)^{n+}$ n=1 to 4 is confirmed.

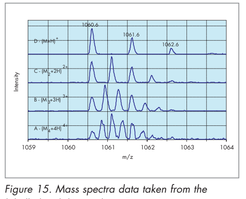

Figure 15. Mass spectra data taken from the labelled mobility peaks in Figure 14.

Myoglobin Mobility Separation

To evaluate the TWIMS separation capability on a species with a significant number of charge states, a solution of Horse Heart Myoglobin (MW=16951) was infused (100 fmol/ul in 1:1 Water:Acetonitrile 0.1% Formic acid). Figure 16 is the mass spectrum obtained at 0.2mbar cell pressure with no mobility separation. Figure 17 shows the mobility separation at various travelling wave pulse heights.

Figure 18 show the mobility spectrum obtained at 7 V pulse height together with the selected mass chromatograms of a range of the ions seen in Figure 16. It is quite apparent that good temporal separation of the different charge states can be achieved using this approach. m/z 616 is the haeme-group and nominal m/z 694 an unknown quadruply charged ion. Figure 19 shows the mass spectra obtained around m/z 694 without mobility separation and with mobility separation combining the data from the m/z 694 mobility peak in Figure 18. This illustrates that the mobility separation can provide efficient background removal.

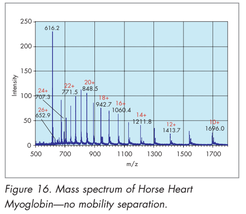

Figure 16. Mass spectrum of Horse Heart Myoglobin—no mobility separation.

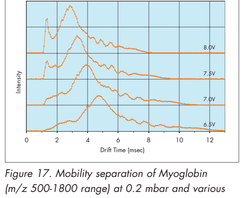

Figure 17. Mobility separation of Myoglobin (m/z 500-1800 range) at 0.2 mbar and various travelling wave pulse heights.

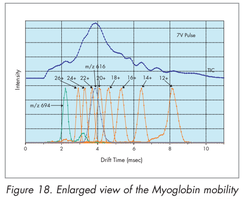

Figure 18. Enlarged view of the Myoglobin mobility peak obtained with a pulse height of 7.0 V at 0.2 mbar. Also shown are selected mass chromatograms for a range of ions seen in Figure 16.

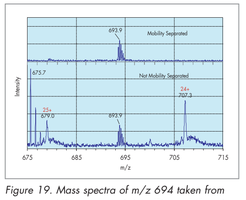

Figure 19. Mass spectra of m/z 694 taken from the non-mobility separated data (Figure 16) and from the mobility peak data in Figure 18.

CONCLUSIONS

A new mobility separation device has been developed which utilises a travelling voltage wave superimposed on the confining RF of a stacked-ring ion guide to provide ion separation.

The device has been shown to provide good mobility separation capabilities when operating at 0.2 mbar pressure for both low molecular weight and high molecular weight species. The utility of this approach over standard DC-only drift tube systems is that the confining RF of the TWIMS together with use of ion accumulation in the source ion guide leads to a very efficient separation device with little or no ion losses over that of the standard, non-mobility, mode of operation.

More studies are required to further characterise this device but the data produced at present indicate that the TWIMS can be an extremely useful tool in the analysis of ion mixtures.

The use of the TWIMS for charge state discrimination will be presented in Poster TPG 119.

REFERENCES

1. "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).
2. A.E. Counterman, S.J. Valentine, C.A. Srebalus, S.C. Henderson, C.S. Hoaglund and D.E. Clemmer, J. Am. Soc. Mass Spectrom., 1998, 9, 743

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