Determining Ion Mobility Values using a Travelling Wave Separator

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OVERVIEW

- **Investigation of approaches to determine ion mobilities using a travelling wave separator**
- **Studies undertaken on a modified Synapt HDMS instrument**
- **Ion mobility calibration shown to be an effective approach for determining collision cross-sections. A direct approach to measurement of collision cross-sections is also shown**

INTRODUCTION

Developments in ion mobility–mass spectrometry (IM-MS) instrumentation over the last decade have raised the profile of the technique and many more analysts are now considering the potential benefits for their area of work. Perhaps the most significant developments are those which have increased overall transmission of the IM devices to around 100%, including pre-IM trapping of ions and negating losses from radial ion diffusion during separation. IM offers a further, rapid, separation capability for complex mixtures following liquid chromatography, and, through measurement of ion mobilities, collision cross-sections (CCSs) can be calculated and ion structures inferred by comparison with computational models. Whilst mobilities are directly obtainable from classical uniform electric field IM separators, a more recent design using travelling-wave-based separation has required calibration. Here we outline approaches for obtaining mobilities from a travelling-wave device.

METHODS

Instrumentation

The instrument used in these studies was a modified Synapt HDMS instrument (Waters Corporation), shown in **Figure 1,** which has a hybrid quadrupole/IMS/oa-ToF geometry. Ions are generated using an electrospray ionisation source. The ions pass through a quadrupole mass filter to the IMS section of the instrument which comprises three travelling wave (T-Wave) ion guides. The trap T-Wave accumulates ions whilst the previous mobility separation is occurring, then these ions are released in a packet into the IMS T-Wave in which the mobility separation is performed. The transfer T-Wave delivers the mobility separated ions to the oa-ToF mass analyser. Ion arrival time (AT) distributions are recorded by synchronising the oa-ToF mass spectral acquisitions with the gated release of ions from the trap T-Wave device. The T-Wave mobility separator uses a repeating train of DC pulses to propel ions through the gas-filled cell (see **Figure 2**). The ability of an ion species to keep up with the wave is dependent on its mobility with less mobile species being overtaken by the waves more often than the higher mobility species. Whilst the macroscopic separation characteristic of the T-Wave device differs from a standard drift tube, the microscopic processes are essentially identical.

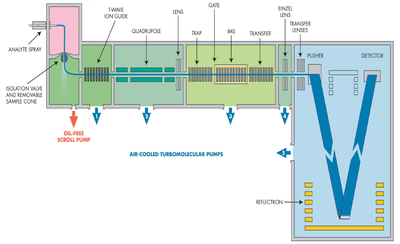

Figure 1 *Diagram of the Synapt HDMS instrument.*

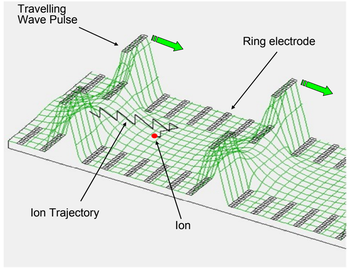

Figure 2 *SIMION picture of the travelling wave device.*

Experiments

Equine myoglobin (mw 16,952) and cytochrome c (mw 12,359), bovine ubiquitin (mw 8,565), gramicidin s, leucine enkephalin and substance p were obtained from Sigma-Aldrich. Yeast enolase digest and bovine serum albumen digest (BSA) were MassPrep standards from Waters. All samples were dissolved in 50:50 ACN:H_2O 0.1% formic acid and infused at 3µL/min to the electrospray source. The IMS T-Wave was operated with He at 2.5 mbar or N_2 at 0.6 mbar.

RESULTS

Ion Mobility Calibration

The first approach to obtaining mobility values from the T-Wave device was to calibrate the system using species of known mobility. **Figure 3** shows plots of the T-Wave arrival times versus 1/CCS for singly charged yeast enolase digest ions (m/z 400-1580) in He and N_2. The CCS values are taken from ref. 1. Also plotted are the calculated ATs for a standard drift tube for comparison. The calibration curves generated have then been used to calculate CCS values for ion species from a BSA digest and several other peptide species (refs 2,3), as shown in **Figure 4**. (NB all literature CCS values are for He).

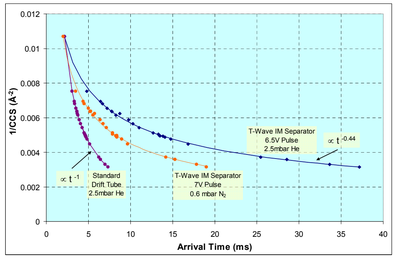

Figure 3 *AT vs 1/CCS plot for the ion species from the yeast enolase digest (300m/s wave)*

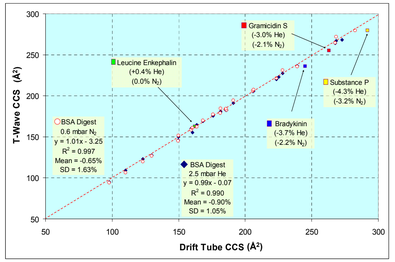

Figure 4 *Comparison of CCSs measured using the calibrated T-Wave with those from drift tubes, differences shown in brackets.*(max. E/N(He)=20.2 Td, max. E/N(N_2)=90.5 Td)

An equivalent study was carried out using the multiply charged envelope of myoglobin ions to calibrate the T-Wave IMS with He. **Figure 5** shows the CCS values for cytochrome c and ubiquitin calculated from the myoglobin-calibrated T-Wave data plotted against the measured drift tube values (ref. 4).

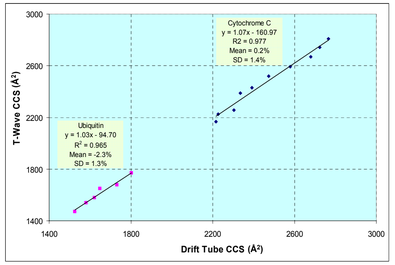

Figure 5 *Comparison of CCSs measured using the myoglobin-calibrated T-Wave with measurements from drift tube systems (2.5mbar He, 300m/s wave at 8V.* max. E/N=24.9 Td).

Ion Mobility Scaling

The second approach to obtaining mobility values from the T-Wave device is to use the separation characteristics of the device as a function of pulse height. **Figure 6(a)** shows a plot of the ATs of the yeast enolase digest ions as a function of pulse height. The forms of the curves generated are essentially equivalent and only differ by a vertical scaling factors which are related to differences in CCS. **Figure 6(b)** shows the same plot as **Figure 6(a)** but with vertical scaling factors applied to bring the curves in line with the m/z 478 species. Calculated CCS values are given in **Table 1**.

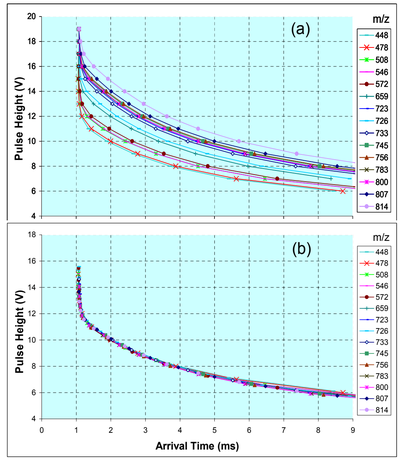

Figure 6. (a) *AT vs pulse height for selected yeast enolase digest ions (2.5mbar He, 300m/s wave)* ***(b)*** *vertical scaling of curves to align with m/z 478.*

m/z	Factor	Calc. CCS Å²	Lit. value[1] Å²	Diff. %
448	1.02	143.9	144.0	-0.1
478	1	146.8	146.8	0.0
508	0.94	156.1	157.1	-0.6
546	0.94	156.1	157.6	-1.0
572	0.91	161.3	162.6	-0.8
659	0.84	174.7	176.8	-1.2
723	0.75	195.7	198.6	-1.5
726	0.82	179.0	184.2	-2.8
733	0.77	190.6	195.3	-2.4
745	0.74	198.3	202.9	-2.3
756	0.73	201.0	205.0	-1.9
783	0.74	198.3	201.8	-1.7
800	0.74	198.3	201.0	-1.3
807	0.72	203.8	210.0	-2.9
814	0.68	215.8	223.9	-3.6

Table 1 *Calculated CCS values for yeast enolase using scaling factors.*

m/z (z)	Factor	Calc. CCS Å²	Lit. value[4] Å²	Diff. %
772(22)	1.24	3809	3815	-0.2
808(21)	1.21	3726	3792	-1.8
849(20)	1.18	3638	3682	-1.2
893(19)	1.13	3609	3570	1.1
943(18)	1.12	3450	3489	-1.1
998(17)	1.08	3379	3384	-0.1
1061(16)	1.05	3271	3313	-1.3
1131(15)	1	3220	3220	0.0
1212(14)	0.95	3164	3143	0.7
1305(13)	0.9	3101	3136	-1.1
1414(12)	0.85	3031	3044	-0.4

Table 2 *Calculated CCS values for myoglobin using scaling factors.*

An equivalent scaling approach was carried out for the multiply charged ions of myoglobin, as shown in **Figure 7.** Calculated CCS values are given in **Table 2**.

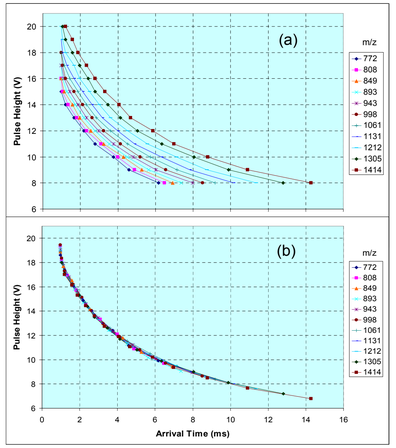

Figure 7 (a) *AT vs pulse height for selected myoglobin ions (2.5mbar He, 300m/s wave)* ***(b)*** *vertical scaling of curves to align with m/z 1131.*

Direct Mobility Measurement

The third approach to mobility measurement uses a knowledge of the T-Wave peak shape together with a measurement of the precise pulse height at which an ion species starts to 'surf' along with the wave. At this voltage, the ion undergoes repeat trajectories on the pulse potential profile which has a Gaussian form due to field penetration and relaxation along the longitudinal axis of the T-Wave. Thus since the electric field function can be determined for this trajectory and the length of time an ion takes to cover the 3mm pulse step distance is known (= pulse period) a mobility can be calculated (see **Figure 8**).

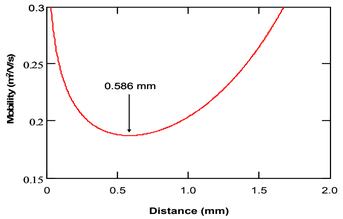

Figure 8 *Mobility vs distance in-front of pulse at which an ion starts its 3mm trajectory (10V pulse, 10µs period)*

From **Figure 8** it is calculated that the ion species travels along a repeat trajectory from 0.586 to 3.586mm in front of the wave. **Figure 9** shows a contour plot of the ATD data obtained for the m/z 556 ion of leucine enkephalin as a function of pulse height. From this plot it is determined that the ions fully 'surf' on a wave at a pulse height of 13.8V . By using the calculation illustrated in **Figure 8**, for a 13.8V pulse, a mobility value of 0.136 $m^2/V/s$ is obtained. Using the He cell pressure of 2.5mbar and room temperature a CCS of 161.2 Å² is calculated, in good agreement with the 162 Å² reported in ref 3. Direct calculations of CCS for other species using this technique are shown in **Table 3**.

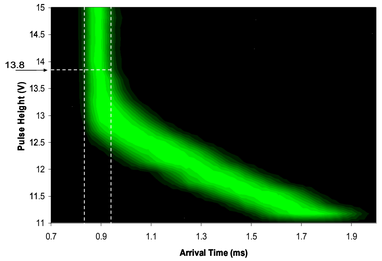

Figure 9 *Plot of the AT vs pulse height for m/z 556 (Leu-Enk). The ions can be seen to surf completely on the wave at a pulse height of 13.8V (300m/s wave 2.5mbar He)*

	m/z	z	Pulse V	Mobility m²/V/s	Calc. CCS Å²	Lit. Value[1-4] Å²	Diff. %
Myoglobin	1305	13	13.4	0.093	3053	3136	-2.6
Myoglobin	772	22	10.4	0.12	4004	3815	5.0
Ubiquitin	1225	7	12.3	0.101	1514	1580	-4.2
Ubiquitin	778	11	9.7	0.129	1862	1802	3.3
Cyt. C	1124	11	11.7	0.107	2246	2303	-2.5
Cyt. C	687	18	8.9	0.14	2808	2766	1.5
Substance P	1348	1	16.2	0.077	284	292	-2.7
Gramicidin S	1141	1	14.7	0.085	257	263	-2.3
Bradykinin	1060	1	13.3	0.094	233	245	-4.9
Leu-Enk	556	1	13.8	0.136	161	162	-0.6
Y. En. Digest	478	1	12.5	0.15	146	147	-0.7
GroEL	11,150	72	16.5	0.81	19400	?	

Table 3 *Direct T-Wave Calculated CCS values* (max. E/N=52 Td)

CONCLUSION

- Three approaches to obtaining mobilities and CCS data from a T-Wave mobility separator have been illustrated
- Calibration of the T-Wave device has been shown to produce CCS measurements to within 5% of literature values for both He and N_2 drift gases
- Relative scaling of arrival time - pulse height plots have also shown to provide CCS values within 5% of reported values.
- A direct method for measuring CCS values in a T-Wave device has been shown to produce excellent results and can be of benefit for particularly large species where appropriate calibrants are not available

REFERENCES

(1) Valentine *et al*, JASMS, **10** (1999) 1188 **(2)** Ruotolo *et al, JASMS, **15** (2004) 870*
(3) Polfer *et al* , *JCP A*, **112** (2008) 1286 **(4)** www.indiana.edu/~clemmer/

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