Metals in Medicine: Ion Mobility Derived Gas Phase Interaction Radii

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Here we report on the development of an enhanced collision cross-section (Ω) estimating algorithm.

We present gas phase interaction radii for H, C, and the previously uninvestigated O, N, S, F, Cl, Fe, Pt and Ru.

We also demonstrate for the first time that isomeric ruthenium(II) terphenyl anticancer complexes, whose collision cross-section differ by less than 9.0Å², can be separated and characterised by travelling wave ion mobility.

INTRODUCTION

Ion mobility has the potential to separate isomeric species based on differences in gas-phase collision cross-section (Ω) and can provide valuable information on ionic conformation through comparisons with theoretical models. To relate the IM derived Ω of a molecule to its structure accurately, one must obtain prior knowledge of the gas phase radii of the constituent atoms. This is typically carried out by modelling energy minimised trial molecular structures as a sum of overlapping hard spheres of defined radii and their interaction with a buffer gas, thus providing an accurate theoretical Ω. Here we report on an enhanced Ω algorithm and its optimisation and evaluation through the analysis of the metal and halogen containing isomeric organoruthenium anti-cancer complexes.

METHODS

Instrumentation

The instrument used was a SYNAPT HDMS System (Waters Corporation, **Figure 1**) which has a hybrid quadrupole/IMS/oa-ToF geometry. Samples were introduced by a borosilicate glass nanoelectrospray-spray tips and sampled into the vacuum system through a Z-Spray source. The ions pass through a quadrupole mass filter to the IMS section of the instrument. This section 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 is used to deliver the mobility separated ions into the oa-ToF analyser.

Experimental

The Trap/transfer T-Wave regions were filled with SF_6 and the IM T-Wave was filled with Helium, with respective pressures of $2.0e^{-2}$mabr and 3.0mbar. The *m/z* scale was calibrated with a solution of sodium iodode over the range 50-2000. T-Wave ion mobility calibration was carried out using a mixture of a tryptic digest of human haemoglobin, polyglycine and polyalanine, whose Ω values have previously been determined on a standard IMS drift tube instrument[1].

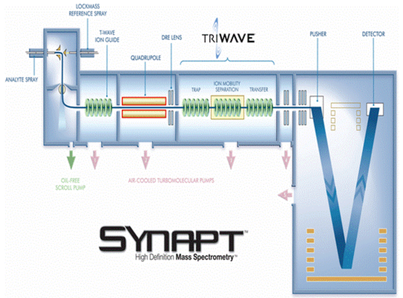

Figure 1. *Schematic of the SYNAPT HDMS System*

Sulphur-Hexafluoride (SF_6) and Helium were obtained from BOC Gases LTD. PDB files obtained for all samples analysed.

The Waters Collision Cross-section Algorithm

The projection approximation (PA) has been used to obtain rotationally averaged cross sections of proposed molecular structures for over 80 years[2]. Historically, a physical model of the molecule was constructed and mounted between a light source and a screen. The area of the shadow was measured for many orientations and then an average calculated. In the approach described here, we have developed a modified projection approximation method. The proposed "models" are supplied as list of PDB files. Approximate areas are obtained as in the historical approach, these estimates are simply averaged. The user also specifies a desired tolerance for the calculated collision cross-section, typically around 1%. By monitoring an ensemble of independent measurements of the collision cross-section, the algorithm terminates when the desired accuracy has been achieved. For the molecules considered here, typical processing times are under one second. The Waters Ω Algorithm values were compared to those derived from the MOBCAL[3] Projection Approximation (PA) and Trajectory Method (TM).

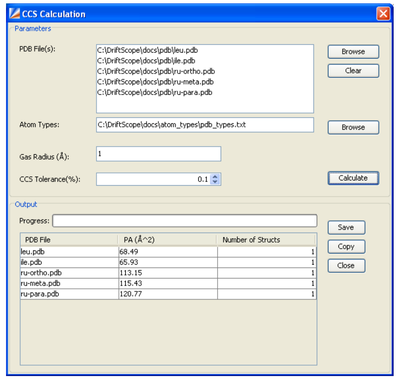

Figure 2. *The User Interface of the Waters Ω calculating algorithm.*

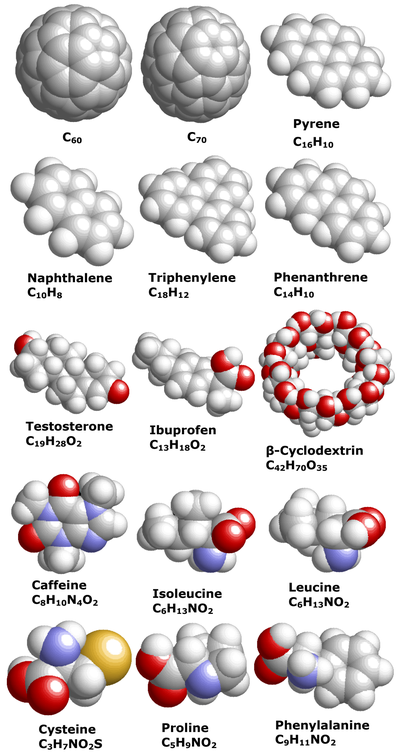

Figure 3. *A selection of some of the compounds used to refine the interaction radii of the Waters Ω calculating algorithm.*

RESULTS

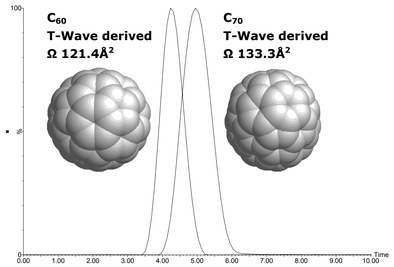

Figure 4. *T-Wave ion mobility chromatogram (msec) of the Buckminster Fullerene structures C_{60} and C_{70} and their T-Wave derived Ω values.*

	T-Wave (Å²)	MOBCAL PA (Å²)	MOBCAL TM (Å²)	Waters Ω (Å²)
C_{60}	121.4	116.8	123.1	121.4
C_{70}	133.3	127.0	135.0	132.4
Pyrene	78.6	80.7	76.6	76.5
Naphthalene	61.7	65.8	60.9	61.7
Triphenylene	89.5	87.2	84.9	87.0
Phenanthrene	74.2	78.0	72.8	75.0
Testosterone	107.6	105.4	104.3	104.0
Ibuprofen	96.3	93.9	92.1	91.6
B-Cyclodextrin	231.3	228.3	237.9	235.3
Caffeine	76.7	78.8	75.5	78.7
Isoleucine	64.5	66.3	62.2	65.9
Leucine	65.5	68.1	64.5	68.5
Cycteine	58.9	63.5	56.2	60.4
Proline	59.5	59.0	55.7	58.6
Phenylalanine	72.3	75.2	72.8	75.0

Table 1. *Comparison of T-wave ion mobility derived Ω values (of some compounds used for the Waters Ω algorithm tuning) with the MOBCAL unmodified code; Projection Approximation (PA) and Trajectory Method (TM) and the Waters Ω algorithm calculations.*

	C	H	O	N	S	F	Cl
Radii (Å)	2.83	1.95	2.95	2.80	3.00	2.70	3.70

Table 2. *The Waters Ω Algorithm inferred gas-phase interaction (with He) radii for carbon, hydrogen, oxygen, nitrogen, sulphur, fluorine and chlorine. Ion Mobility gas: He.*

The Ω for a number of trial compounds were derived by T-Wave IMS. The corresponding PDB files were used to "tune" the gas-phase interaction radii for all the desired atoms. For example, C_{60} and C_{70} and a selection of polyaromatic hydrocarbons were used to derive the C and H-interaction radii (**Figure 3 & 4**).

Table 1 shows the comparison of T-Wave derived Ω, MOBCAL PA, MOBCAL TM and the Waters Ω Algorithm. **Table 2** shows the Waters Ω algorithm inferred gas-phase interaction (with He) radii for C, H and the previously uninvestigated. O, N, S, F and Cl.

The MOBCAL PA assumes that the gas-phase interaction radii of O and N are identical to C. Equally, S is identical to Si. The TM was included in this comparison (**Table 1**); it should provide a more complete model for determining Ω values because it utilises Lennard-Jones potential values and scattering angles between the incoming and departing buffer gas atom trajectory. However, it is assumed that the Lennard-Jones potentials for O, N and S are the same as C and S respectively.

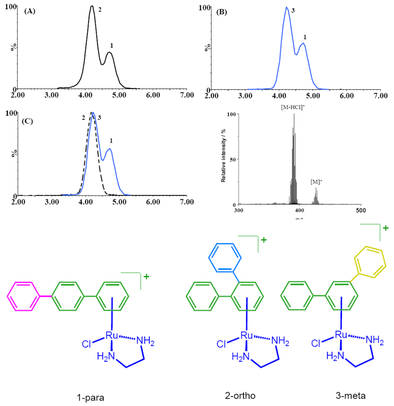

Figure 5. *T-Wave ion mobility drift times (msec) of; (A) mixture of complexes 2 and 1; (B) IM drift times of complexes 3 and 1; (C) IM drift times of the complexes 3 and 1 (solid line) overlaid with IM drift time of complex 2 (dotted line); (D) ESI mass spectrum of a 1 ng/mL (methanol) solution of complex 1.*

Here we have shown for the first time that low-molecular-weight isomeric ruthenium(II) terphenyl anticancer complexes can be differentiated as a function of their structures in the gas phase using ion mobility spectrometry. The drift times obtained for the *m/z* 427.1 species from a mixture of complexes **2** and **1** and the mixture of complexes **3** and **1** showed significant differences, while similarities in the conformations of **2** and **3** render them more difficult to separate under the present experimental conditions (**Figure 5**). The T-Wave mobility-derived Ω measurements were in excellent agreement with theory (**Table 3**). The differences in shape and mobility correlate with the reported differences in anti-cancer activity. Complexes 2 and 3 show similar but reduced anti-cancer activity compared to the more potent complex 1[4].

Complex	$[M]^+$ calculated CCS (Å²)				
	T-Wave (± 0.5%)	MOBCAL Projection Approx.	MOBCAL Trajectory Method	MOBCAL Exact Hard Sphere	Waters CCS Algorithm
1	120.6	121.5	121.3 (±8.8)	130.0	120.8
2	113.0	112.2	112.9 (± 3.9)	121.8	113.1
3	113.6	116.1	117.2 (± 5.5)	125.1	115.4

Complex $[M-HCl]^+$	$[M-HCl]^+$ calculated CCS (Å²)				
	T-Wave (± 0.5%)	MOBCAL Projection Approx.	MOBCAL Trajectory Method	MOBCAL Exact Hard Sphere	Waters CCS Algorithm
1	115.3	119.3	118.1 (± 8.2%)	126.7	114.9
2	106.6	109.3	108.2 (± 3.6%)	117.7	104.2
3	110.0	114.3	114.9 (± 4.7%)	122.4	109.7

Table 3. *Comparison of the Ω values of complexes **1-3** and associated HCl neutral loss species obtained using T-wave ion mobility measurements, MOBCAL (unmodified code) and the Waters Ω algorithm calculations.*

CONCLUSION

We have developed and validated an improved collision cross-section (Ω) estimating algorithm based on the Projection Approximation method. The new algorithm is located in the DriftScope Software and is Windows based.

Comparing T-Wave derived Ω with the Waters algorithm theoretical Ω, we can infer gas phase interaction radii for H, C, and the previously uninvestigated O, N, S, F & Cl. Atoms such as Fe, Pt and Ru are located internally within the investigated molecules and as a consequence, have negligible impact on the Ω value.

We have also demonstrated for the first time that isomeric ruthenium(II) terphenyl anticancer complexes, whose Ω values differ by less than 9.0Å², can be separated and characterised by travelling wave ion mobility.

1. http://www.indiana.edu/~clemmer/Research/research.htm
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3. Mesleh, Hunter, Schvartsburg, Schatz & Jarrold; *J. Phys. Chem.* 1996, **100**, 16082-16086.
4. Williams *et al*, *J. Am. Soc. Mass Spectrom.* 2009, **20**, 1119-1122

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